One-Day Intensive Course Workbook

Genomics in Primary Care Medicine

Syllabus
Abstracts
Glossary
BioEthics
Monograph

ACE

ACHIEVING CLINICAL EXCELLENCE

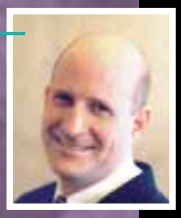
z,gm,ACEgencover,022202

ACE Faculty

R.W. "Chip" Watkins, MD, MPH, FAAFP

Dr. Watkins is a board-certified family physician. He holds academic appointments at a number of the leading medical schools in North Carolina. Dr. Watkins maintains expertise in a wide variety of subjects including advanced prevention strategies, female health and wellness, herbal approaches to Syndrome X and dysinsulinism.

Bradley S. Rachman, DC, DABPM



While in private practice, Dr. Rachman was the director of one of the most progressive and successful Functional Medicine centers in the US. Serving as Medical Science Director for Great Smokies Diagnostic Laboratory, Dr. Rachman offers a unique blend of science-based information and patient-centered clinical experience.

Patrick Hanaway, MD

Dr. Hanaway is a board-certified family physician with a Medical Degree from Washington University and residency training at the University of New Mexico. He serves as the Medical Director for the Family to Family Clinic, and as the Research Program Director for Great Smokies Diagnostic Laboratory.

T. Michael Culp, MA, ND

Dr. Culp received his doctoral degree from Bastyr University and has particular expertise in therapeutic and orthomolecular nutrition. He serves as the Medical Education Director for Great Smokies Diagnostic Laboratory. He has taught graduate courses at Bastyr University and Atlantic University of Chinese Medicine.

ACE

ACHIEVING CLINICAL EXCELLENCE

z,gm,acefac,030702

Schedule

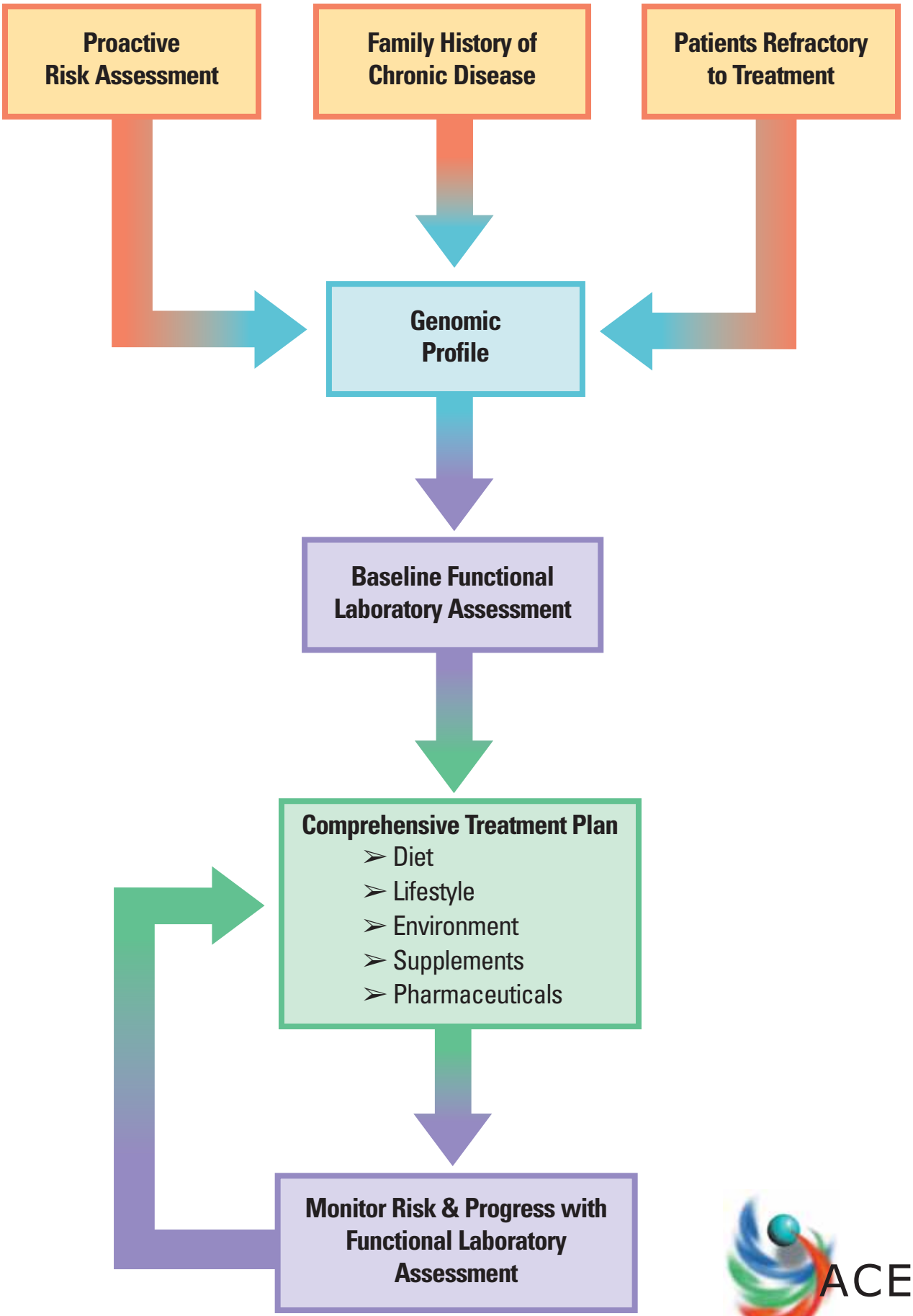
Time	Topic
9:00-10:30	***The Genomic Revolution: Changing the Shape and Practice of Medicine***
10:30-11:00	BREAK
11:00-11:30	***The Origin of Life*** *Genes, Sex, and Evolution* *Adaptive Advantages of Polymorphisms*
11:30-12:00	***New Methodologies – New Frontiers*** • *Polymerase Chain Reaction* • *Invader™ Amplification* • *Pharmacogenetics* • *Proteomics* • *Frankenfoods* • *Cloning*
12:00-12:30	***Predictive Genomics and Functional Medicine***
12:30-1:00	***Osteo Genomics: Polymorphisms Affecting Bone Health***
1:00-2:00	LUNCH
2:00-2:45	***Cardio Genomics: Polymorphisms Affecting Cardiovascular Health***
2:45-3:15	***Immuno Genomics: Polymorphisms Affecting Immunological Health*** • *Allergy* • *Atopy* • *Asthma* • *Auto-Immunity*
3:15-3:45	***Detoxi Genomics: Polymorphisms Affecting Detoxification Pathways*** • *Cancer* • *Fatigue and Fibromyalgia*
3:45-4:15	BREAK
4:15-4:45	***BioEthical Considerations***
4:45-5:00	***Implementation Strategies***

ACE

ACHIEVING
CLINICAL EXCELLENCE

z,ac,sch,030702

Predictive Genomics and Functional Medicine



ACE Genomics
In Primary Care Medicine


Osteo Genomics
Polymorphisms Affecting Bone Health

Presentations


Genomics
in Primary Care Medicine
The Genomic Revolution:
Changing the Shape and Practice
of Medicine

Courtesy Considerations

Please turn cell phones and pagers to vibrate or off

"Swiss Train" Schedule

- Lunch and Breaks on time
- Questions at the breaks and lunch
- Finish by 5pm

Remember to sign in for CEU and CME credits

Be Here Now: Engage & Focus

People are Unique

People are as different on the inside as they are on the outside

Individuality

Some men have constitutions that are like wooded mountains running with springs, others like those with poor soil and little water, still others like land rich in pastures and marshes, and yet others like the bare dry earth of the plain

— Hippocrates
(5th century BC)

Hippocrates' Vision

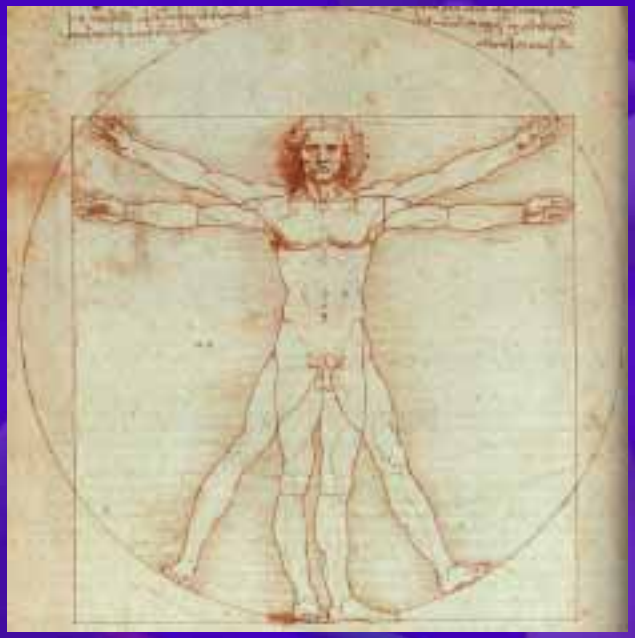
- Humans are governed by the same physical laws as the cosmos
- Nature is the great physician
- Health is a balance of elements and humours within each compartment of the body
- By observing the natural course of an illness, we are able to intervene rationally
- Medicine, then, is empirical

The Four Elements
The Four Humours

FIRE
Yellow Bile
(choleric)

Dry

Hot



EARTH
Black Bile
(melancholy)

AIR
Blood
(sanguine)

Cold

Moist

WATER
Phlegm
(phlegmatic)

Louis Pasteur
The Germ Theory of Disease

Disease is caused by germs external to the individual

Any cure must be objective and external

- Pasteurization
- Fermentation of beer & wine
- Vaccine against rabies

Pasteur-Beauchamps Debate

Germ Theory of Disease

vs.

Host Theory of Disease

Chemical Individuality
Archibald Garrod

Garrod studied alkaptonuria, realized that it behaved like a Mendelian recessive, and postulated that a gene was simply a recipe for a single chemical

"Inborn errors of metabolism are due to a failure of a step in the metabolic sequence due to loss or malfunction of an enzyme"

Altered genes and altered proteins are "the seat of chemical individuality"

Garrod A. Inborn Errors of Metabolism. London, 1909

ACE

Chemical Individuality
Archibald Garrod

"The existence of chemical individuality follows of necessity from that of chemical specificity, but we should expect the differences between individuals to be more subtle and difficult of detection. Indications of their existence are seen…in the quantitative differences in those portions of the end products of metabolism….

These idiosyncrasies may be summed up in the proverbial saying that one man's meat is another man's poison…"

Biochemical Individuality

Roger Williams first coined the term "biochemical individuality" in 1956 to explain genetic variability in disease susceptibility, nutrient needs, and drug responsiveness among otherwise seemingly healthy people

Williams RJ. Biochemical Individuality: The Basis of the Genotropic Concept. New York, NY: John Wiley; 1956.

ACE

Internal and External "Causes" of Disease

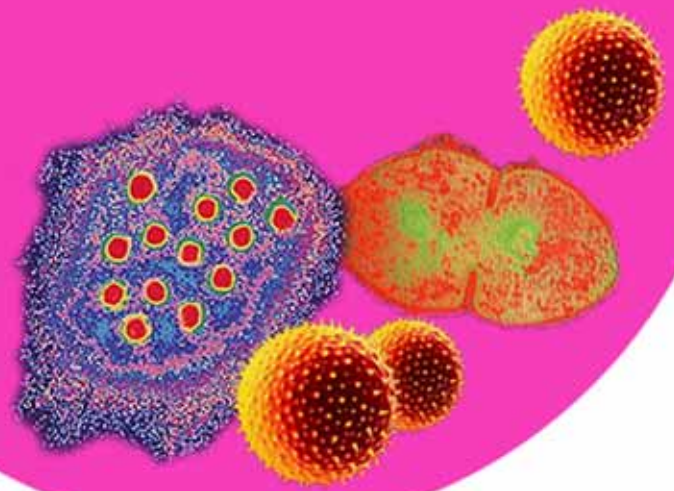
Internal Causes	External Causes
Four Humours	Divine Wrath
Constitution	Weather
Psychosomatic	Habitat
Perceived Stress	Germs
Heredity	Toxins
Genetics	Diet
	Lifestyle

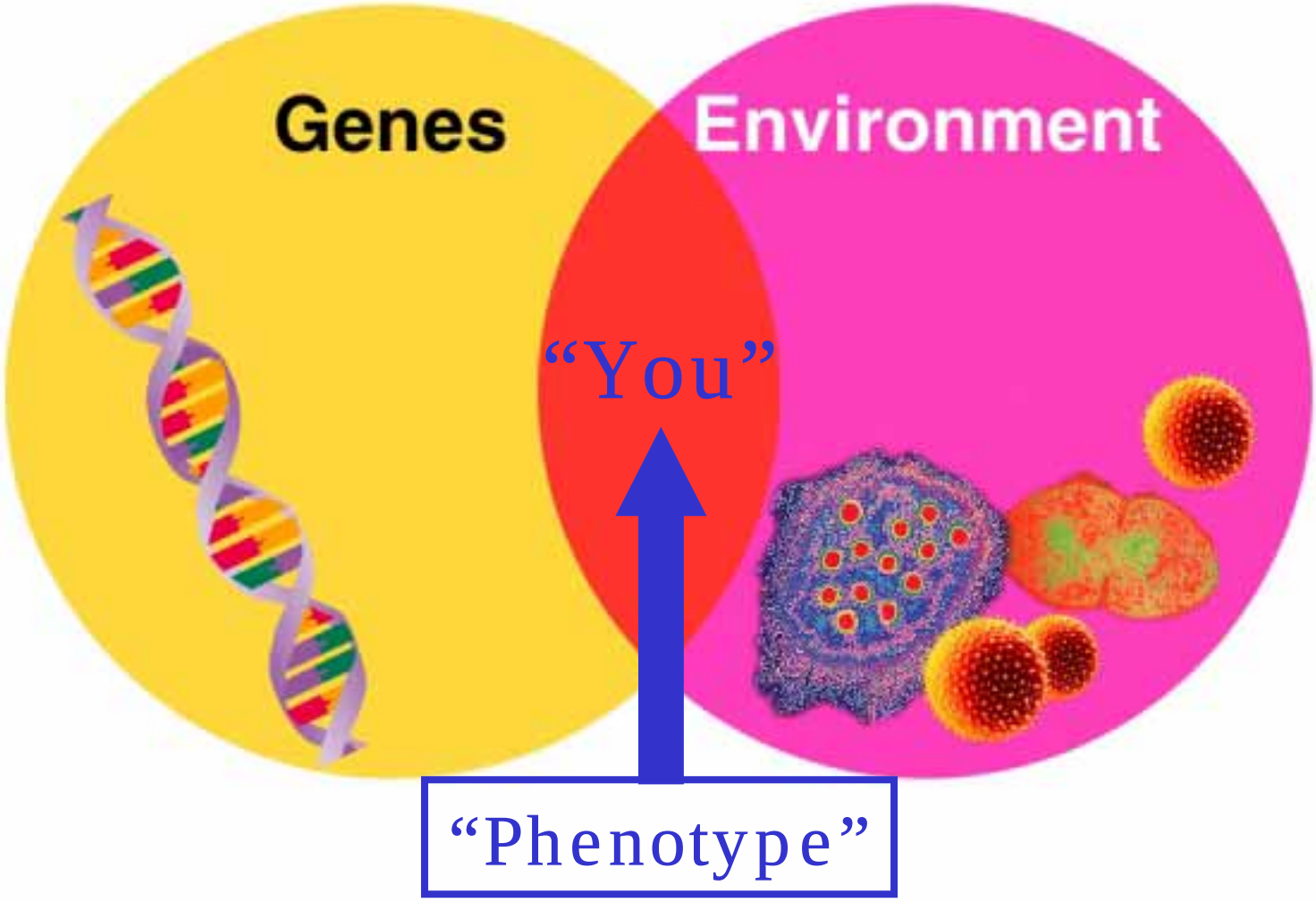
Measurable

ACE

Genes

Environment



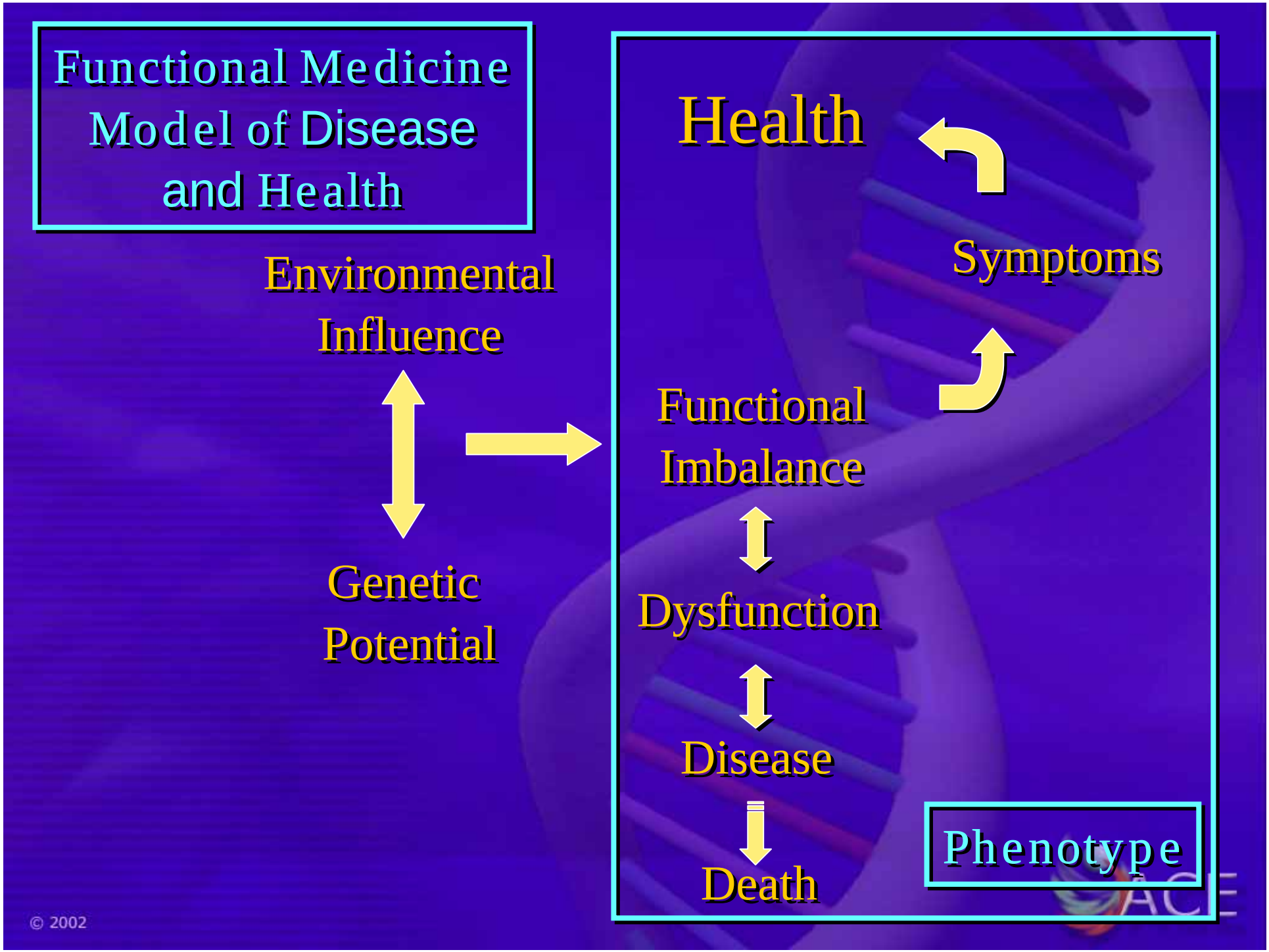

Genes
Environment
"You"
"Phenotype"

All disease is genetic even when it is also something else

Paul Berg

Co-inventor of genetic engineering

Functional Medicine Model of Disease and Health



Functional Medicine:

The clinical discipline designed to

1) promote health
2) anticipate and prevent disease, or
3) to correct an existing disease

by improving physiological function

The primary clinical focus of functional medicine is on the functional integrity of the body's metabolic systems

ACE

Functional Integrity of the Body

1. How well it works (output)
2. Metabolic reserve (potential)

The Goose that Laid the Golden Eggs

Functional Integrity = Balance = Health

Golden Eggs	Goose
Output	Reserve
Action	Potential
Phenotype	Genotype
Energy	Longevity
Physiology	Genetics

Key Components of Functional Medicine

- Treat the whole person, not the disease
- All of the body's functions depend on one another — the whole is only as strong as its weakest link
- Health is a state of balance
- Health is physical, emotional, mental, & social
- Prevention is better than cure
- Genes ***and*** Environment are together responsible for every aspect of health or disease

Genetic Determinism

- The effects of many of our genes are inescapable
- Most Mendelian traits fall into this category: e.g., eye color, blood type, number of fingers
- Numerous genetic diseases also fall into this category: Huntington's disease, Tay-Sach's disease, Thalassemia, Cystic Fibrosis, etc.

Genetics and Disease Expression

Genetics is often cited as an excuse for inaction and lack of responsibility on the part both of the physician and of the patient, because of the underlying belief that our genes are unchangeable

The Three Fates
Daughters of Zeus and Themis (Necessity)

Clotho
Spinner

Lachesis
Weaver

Atropos
Cutter

"George Burns" Syndrome

Why do some people live a healthy life and die young while others live like George did and live to be 100?

Environmental Exposure and Disease Risk?

Exposure	Disease Risk
↓↓	Low Risk
↓↓	High Risk
↑↑	Low Risk
↑↑	High Risk

Environmental Exposure and Disease Risk?

Exposure	Genetic Susceptibility	Disease Risk
↓↓	↓↓	Low Risk
↓↓	↑↑	High Risk
↑↑	↓↓	Low Risk
↑↑	↑↑	High Risk

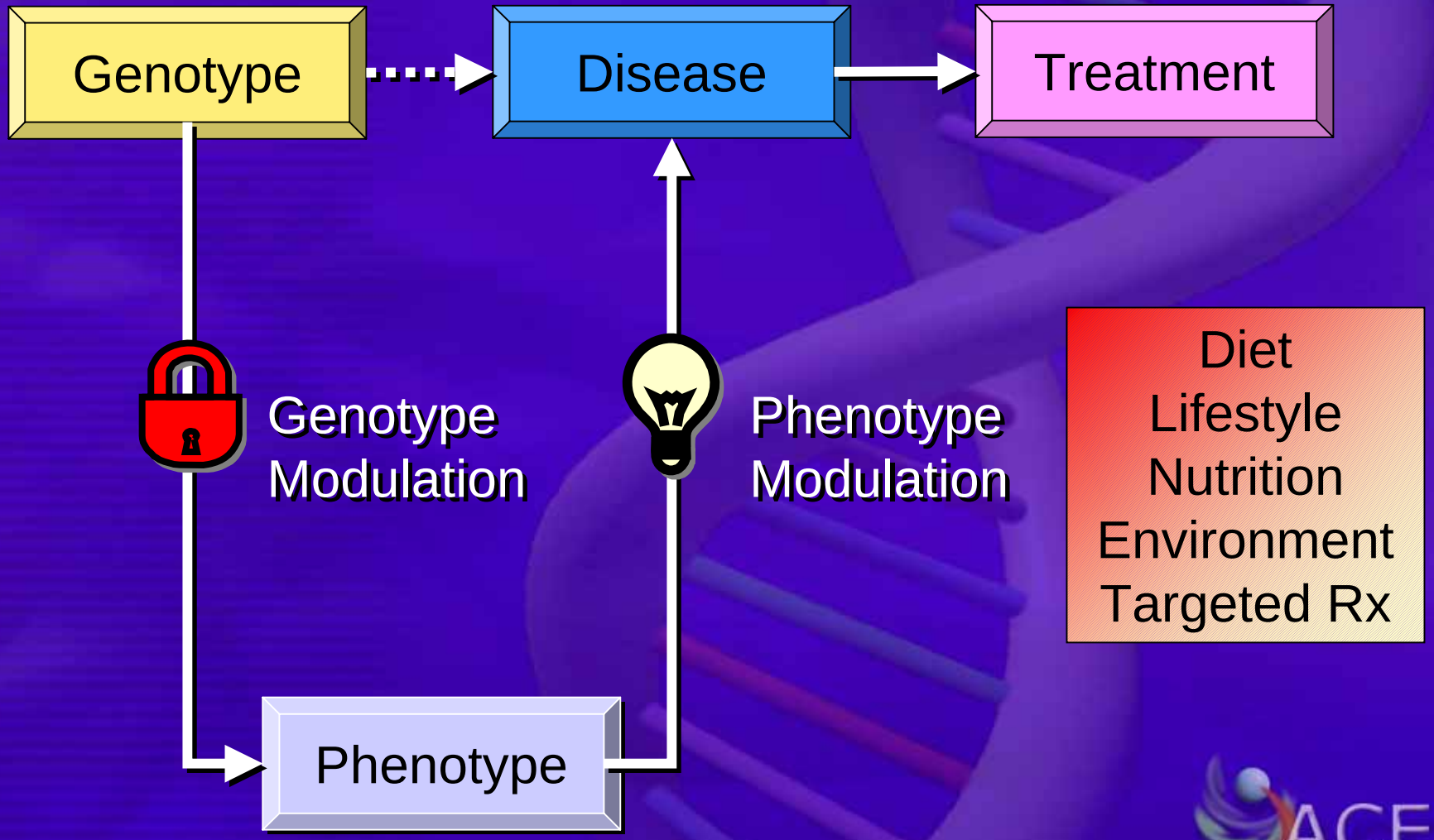
Genes Do Not Cause Disease

Genes have functions

Genes may predispose us to disease, but genes are not our fate

Environment and genes together cause disease (phenotype)

Gene-Environment-Disease Visual Model



Functional Medicine Perspective

- The expression of any gene is modified by the environment we subject it to
- The stronger the genetic component in a disease, the greater the individual responsibility to utilize environment, diet, and lifestyle to modify the expression of that genetic material

Intelligence as a Model for Gene-Environment Interaction

No study of the causes of intelligence has failed to find substantial heritability

ACE

IQ Test Correlations

Same person tested twice	87%
Identical twins reared together	86%
Identical twins reared apart	76%
Fraternal twins reared together	55%
Siblings reared together	47%
Parents & children together	40%
Parents & children apart	31%
Adopted children together	0%
Unrelated people apart	0%

Scarr S. Developmental theories for the 1990s: development and individual differences. *Child Development* 1992;63:1-19.

ACE

The Environment of the Womb

- Why should fraternal twins have a much higher correlation in intelligence tests than biological siblings?
- The answer appears to be the womb, which accounts for ~20% of the similarity in intelligence of twins
- The influence of the womb environment on our intelligence appears to be ~3 times as great as anything our parents did to us or for us after birth

Daniels M, et al. Of genes and IQ. In *Intelligence, genes and Success.* Ed. Devlin B, et al. Copernicus; New York, 1997.

ACE

Genetic Influences on Intelligence Increase With Age

- IQ changes with age and, remarkably, so does its heritability
- The heritability of IQ in childhood is ~45% — in late adolescence it rises to ~75%
- As you grow up, you gradually express your own innate intelligence by selecting environments to suit your innate tendencies, not vice-versa

Heritability Is Mutable

- Genetic influences are not frozen at conception
- Environmental influences are not inexorably cumulative

Silly Cocktail Factoid

People with higher IQs have more symmetrical bodies than people with low IQs: ears, finger length, foot, ankle, wrist, elbow breadth, etc.

The magnitude of this “fluctuating asymmetry” appears to be a sensitive measure of the amount of stress the body was under while developing *in utero*

IQ and Stress

People with higher IQs appear to have been under less stress while developing in the womb

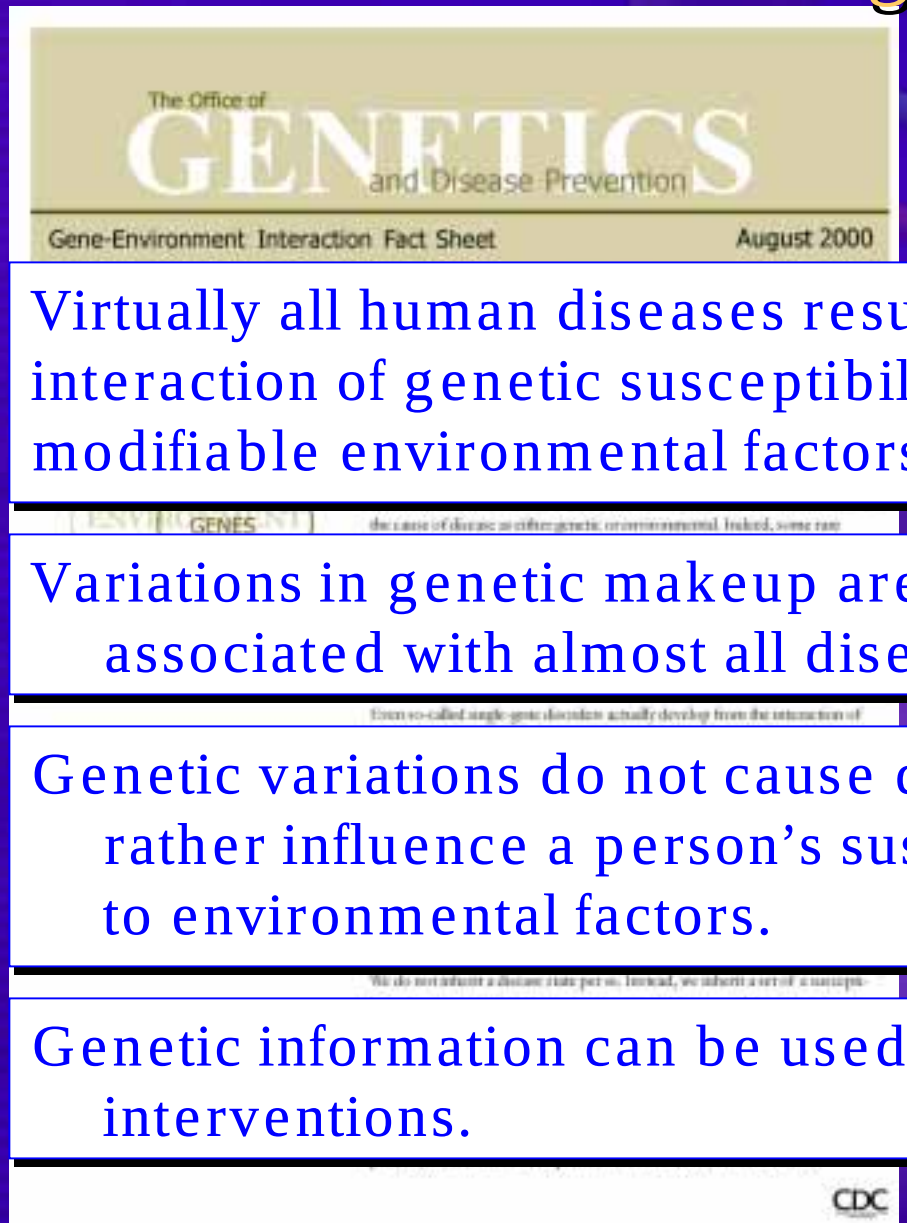
Or perhaps they were genetically more resistant to such stresses

It may be that you not only inherit IQ but also that you inherit an ability to develop your IQ by adapting better to environmental stress

Furlow FB, et al. Fluctuating asymmetry and psychometric intelligence. *Proc Royal Soc London, Series B* 1997;264:823-9.

ACE

Gene-Environment Interaction Fact Sheet Centers for Disease Control — August 2000



Pulling People From the River

Theodore Gericault, The Raft of the Medusa

**“You’re not ill yet, Mr. Blendell,
but you’ve got potential.”**


The Origins of Genetics

Greek Theories of Genetics

Offspring were made by combining different elements from each parent

Elements from various animals could simply be combined:

Camel + Leopard = Giraffe

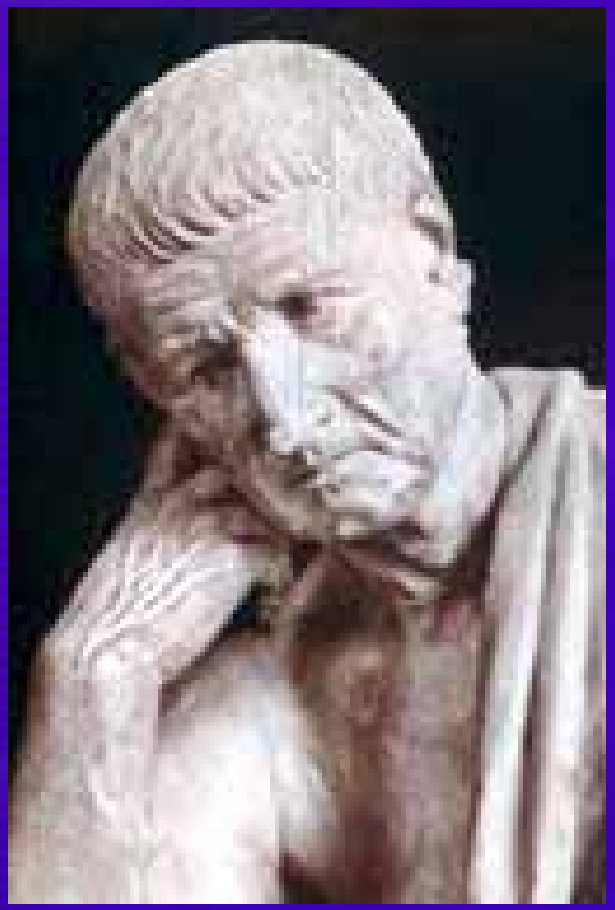
Human + Horse = Centaur

Eagle + Lion = Griffin

Hippocrates and Genetics

- The male contribution to his children is carried in the semen
- Women too must produce a similar fluid
- Fluids (the four humours) are made throughout the body and the specific combination of fluids differentiates tissues and gives a person individuality
- The traits of offspring were determined by the combination of fluids from each parent

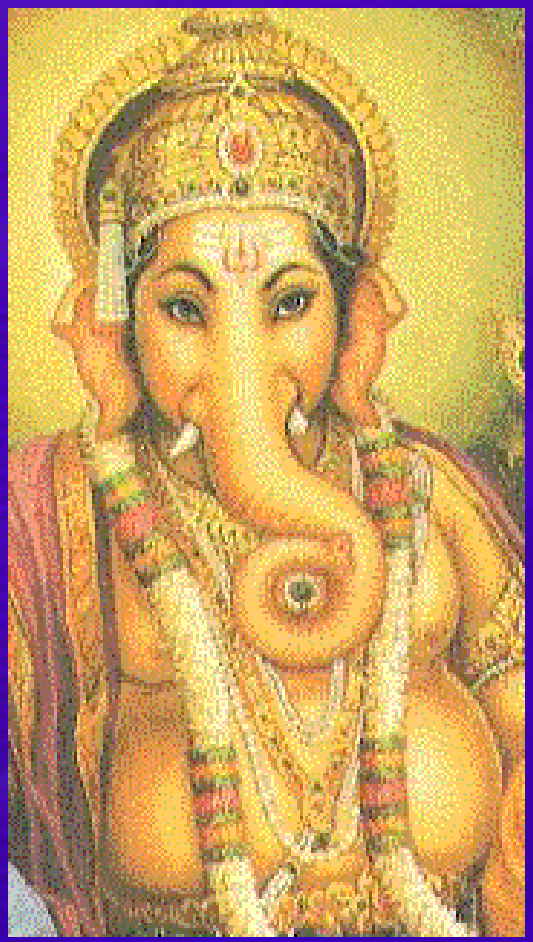
Aristotle



Particles of “blood” were passed from one generation to the next via semen, itself the thickest part of blood

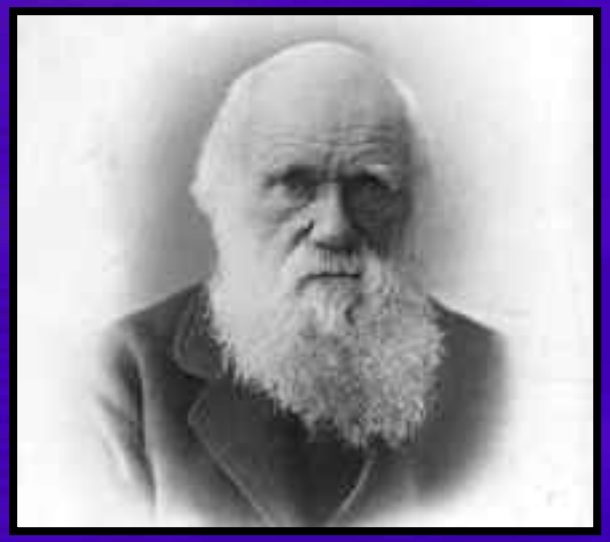
- “bloodline”
- “blood relative”

India and Genetics



- Early Hindus observed that diseases ran in families and eventually came to believe that children inherit their parent's characteristics
- The Laws of Manu state, "A man of base descents can never escape his origins
- Eventually the caste system emerged from this line of thinking

Darwin and Genetics



Darwin predicated the Theory of Evolution on the notion that change happened gradually

He assumed that heredity involved the mixing of “blood”

However, he was perplexed how “ancestral traits” could disappear altogether and then reappear in later generations

Origin of Modern Genetics: Gregor Mendel

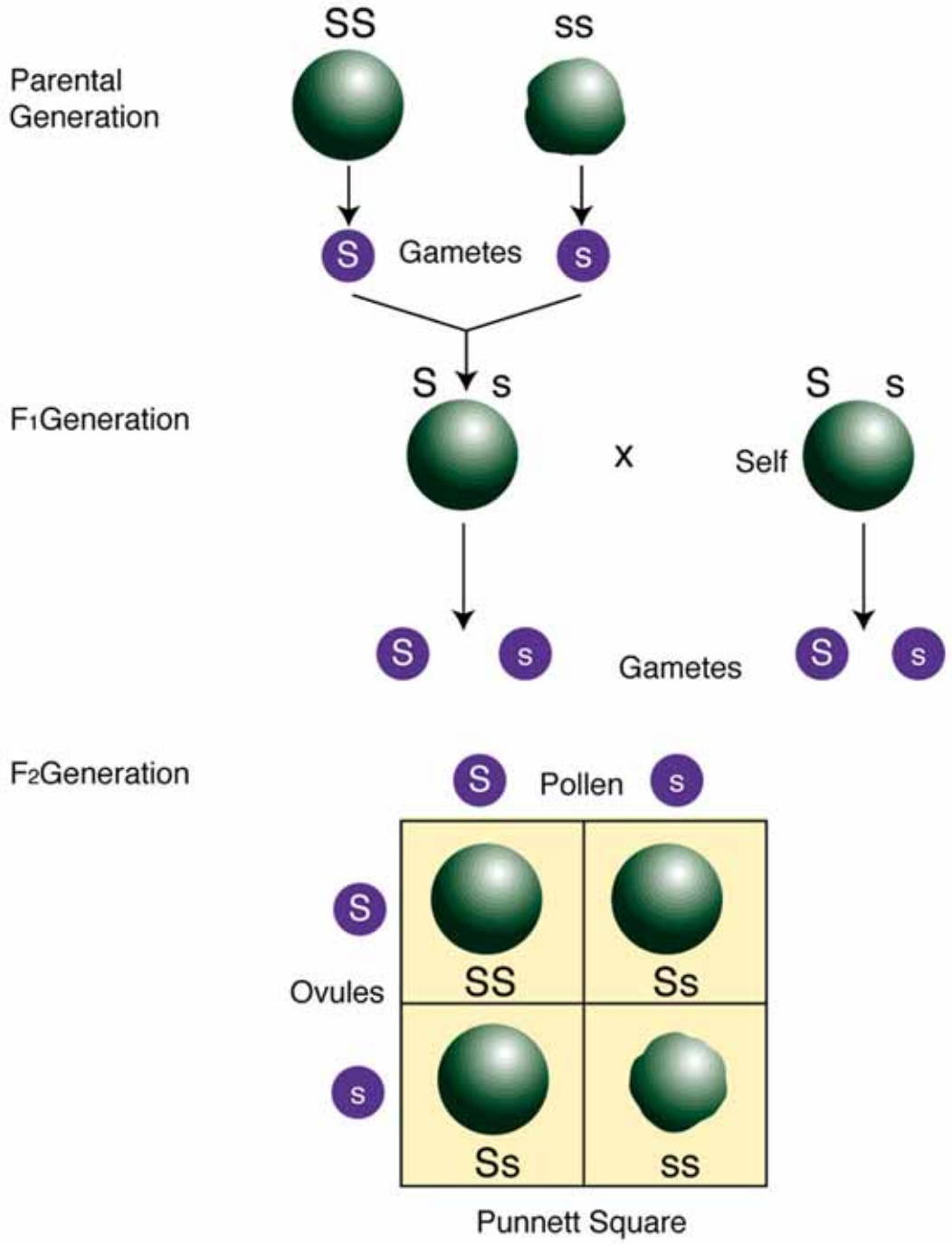
Mendel and Heredity

- The genius of Gregor Mendel's work was that he contradicted the accepted view of his day that characteristics "mix" by a mingling of blood or fluids
- Traits do not mix, they are hard, indivisible, and particulate
- The only reason inheritance seems to blend is because most traits involve multiple genes at work

The Seven Traits of Highly Effective Pea Plants

Seed Color	Yellow or Green
Seed Shape	Smooth or Wrinkled
Flower Color	Purple or White
Stem Length	Tall or Short
Color of Pods	Green or Yellow
Position of Flowers	Terminal or Opposite
Shape When Ripe	Inflated or Constricted

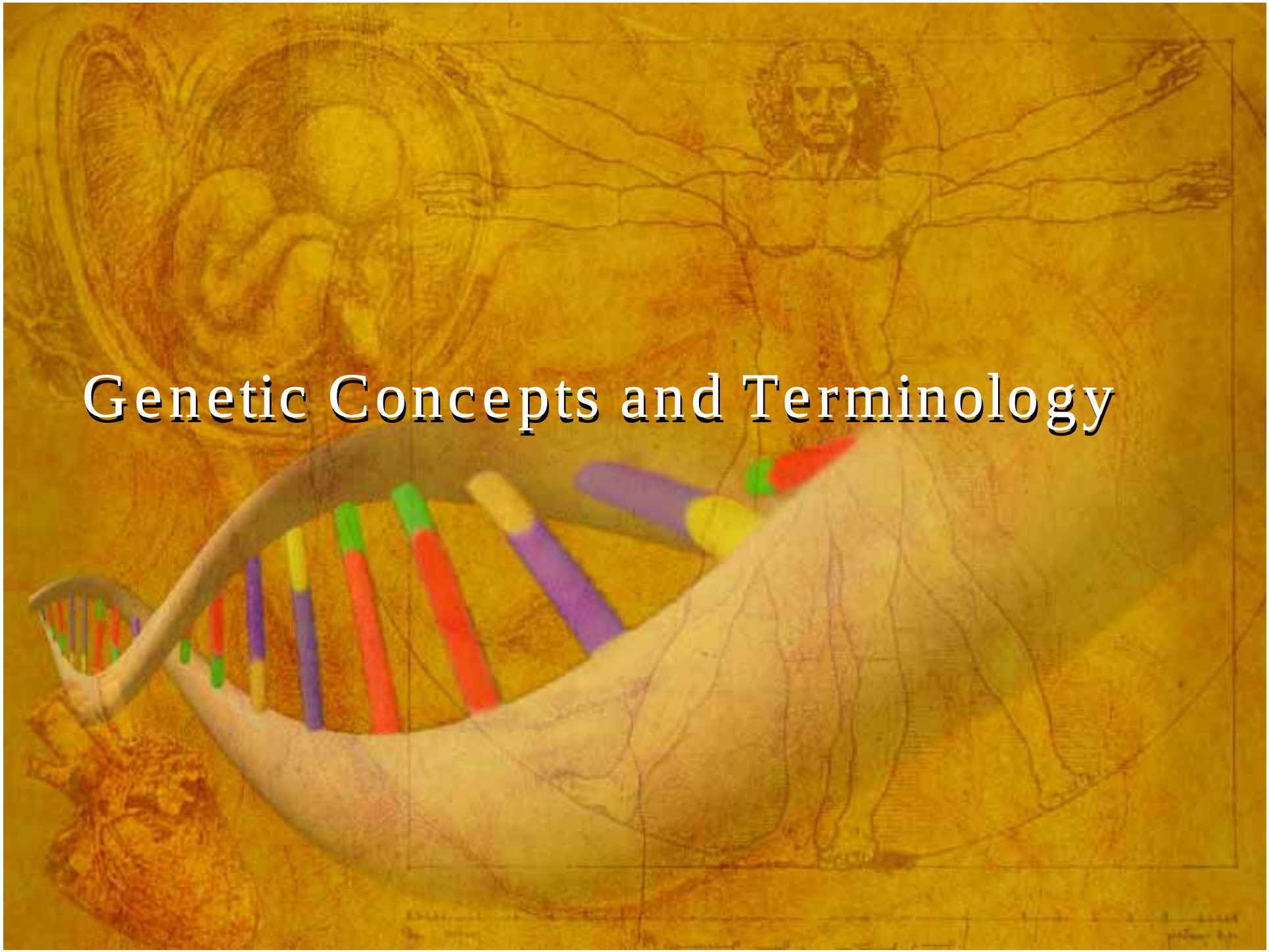
Mendel's Peas



Gregor Mendel — 1865
"Experiments in Plant Hybridization"

The Laws of Heredity

- Each physical characteristic corresponds to a separate gene
- Genes come in pairs
- Only one gene of each pair is passed on from each parent
- It is equally probable that either gene will be passed on
- Some characteristics are dominant while others are recessive

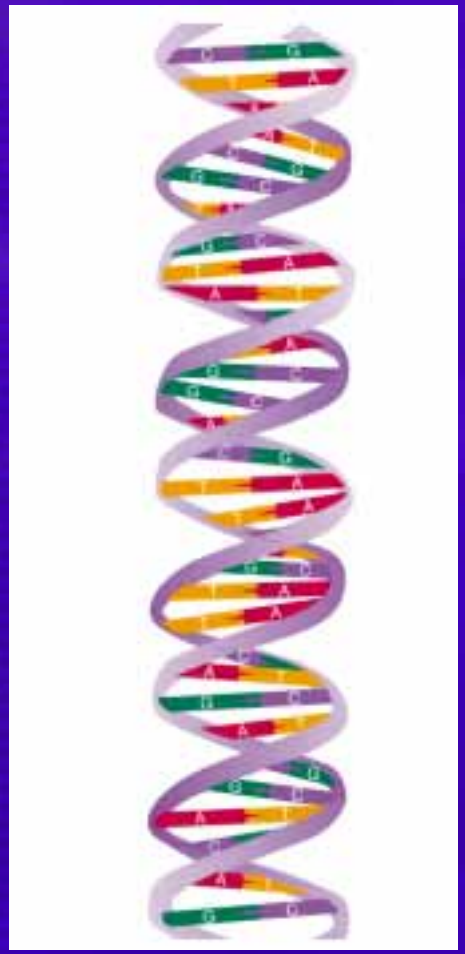

Genetic Concepts and Terminology

Genetics and Genomics

- Genetics encompasses all aspects the scientific study of heredity
- Genomics is the study of genomes, or the totality of the DNA of a single species
- Genomics examines the totality of all our genes as a dynamic system, influencing and being influenced by our biochemistry and physiology

ACE

DNA
Deoxyribose Nucleic Acid



- Is the assembly and operational guide for all living creatures on this planet
- Digital information – four letters: A T C G

Functions of DNA

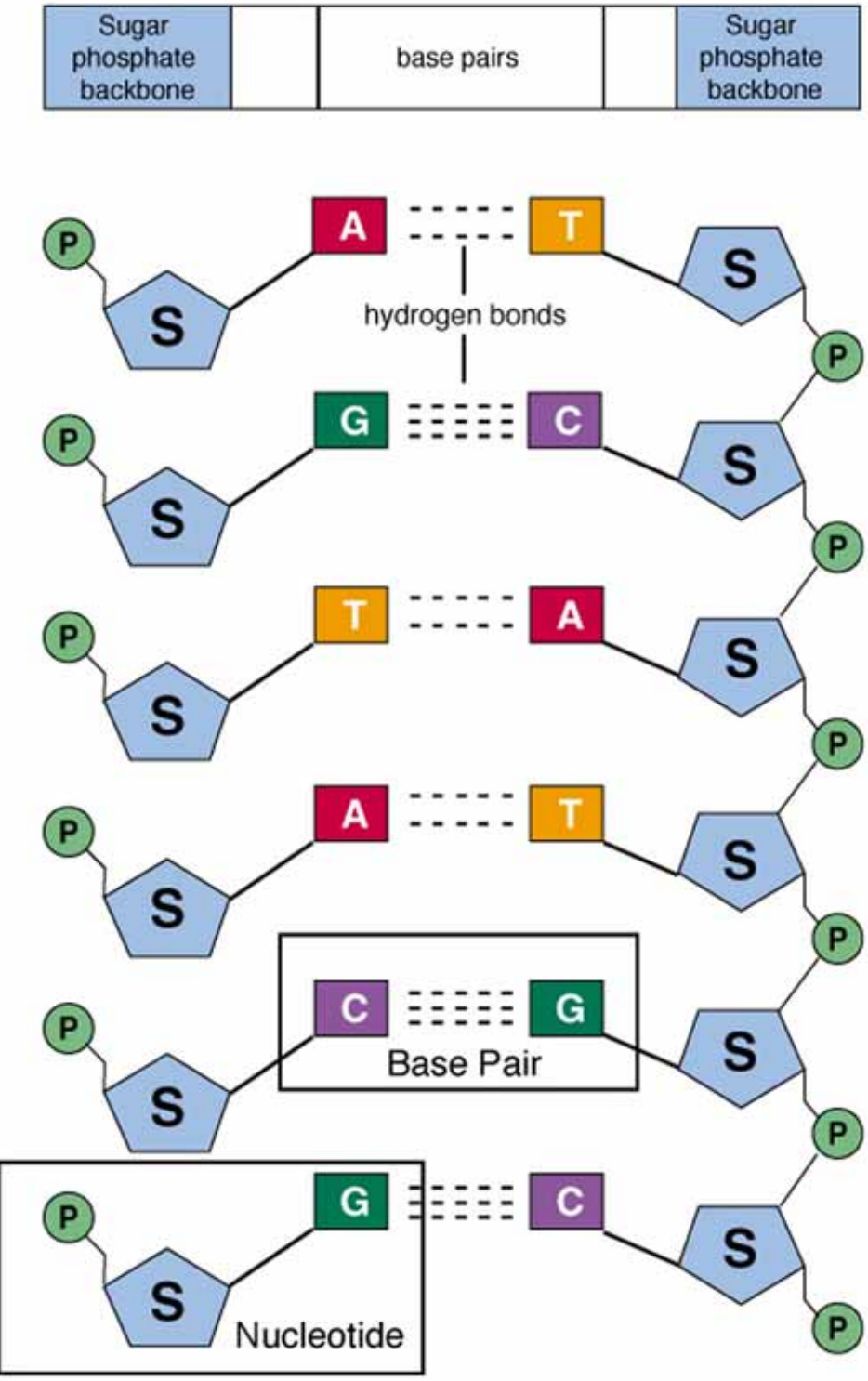
1. DNA replicates itself
2. DNA codes for RNA which in turn codes for proteins
3. DNA regulates gene expression, allowing for
   - Cell growth
   - Cell differentiation
   - Cell replication
   - Cell death

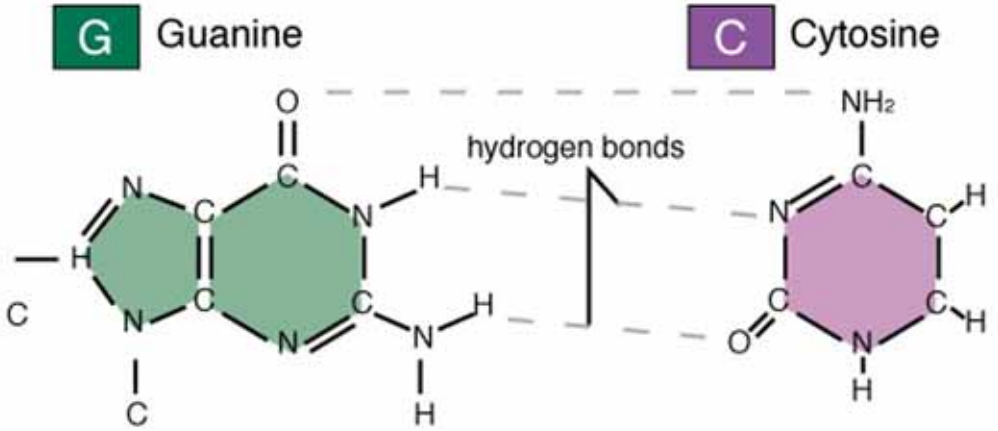
DNA
Deoxyribonucleic Acid

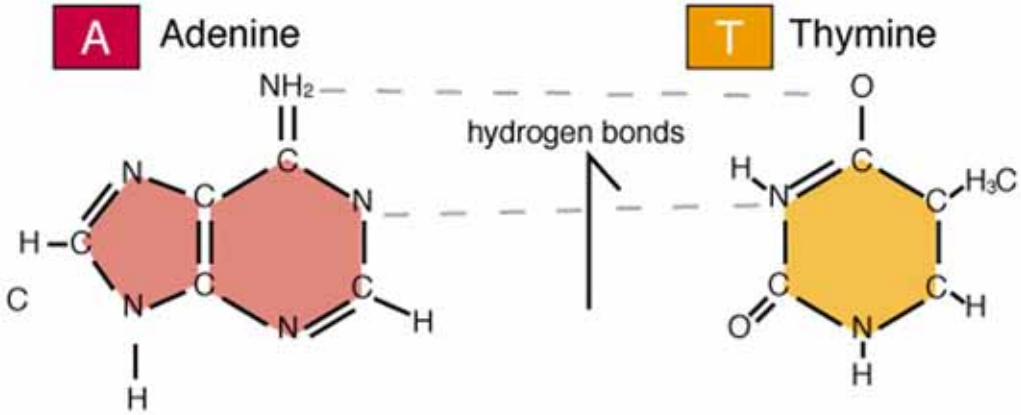
- If all the DNA of a single cell were unraveled into a straight line, it would be ~ 6 feet long
- If all the DNA in all your cells were unraveled into a straight line, it would reach to the sun and back…a thousand times

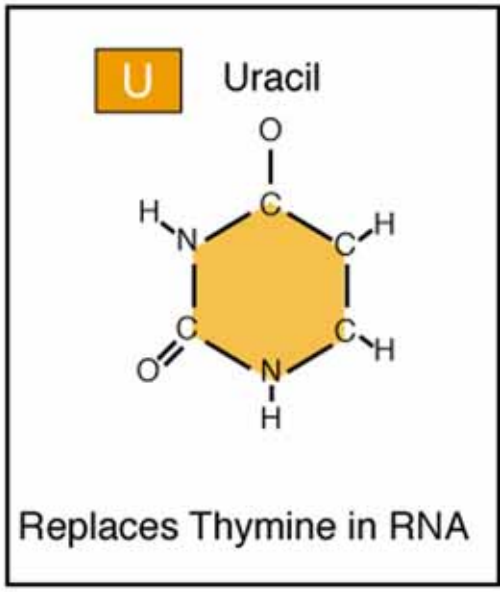
The Structure of DNA

- The Structure of DNA is complimentary: two long strands of sugar-phosphate polymers cross-linked by two bases, one a purine, the other a pyrimidine, like rungs on a ladder
- Adenine always linking with Thymine
- Guanine always linking with Cytosine


Sugar phosphate backbone
base pairs
Sugar phosphate backbone
hydrogen bonds
Base Pair
Nucleotide
P
S
A
T
G
C


G Guanine
C Cytosine
hydrogen bonds

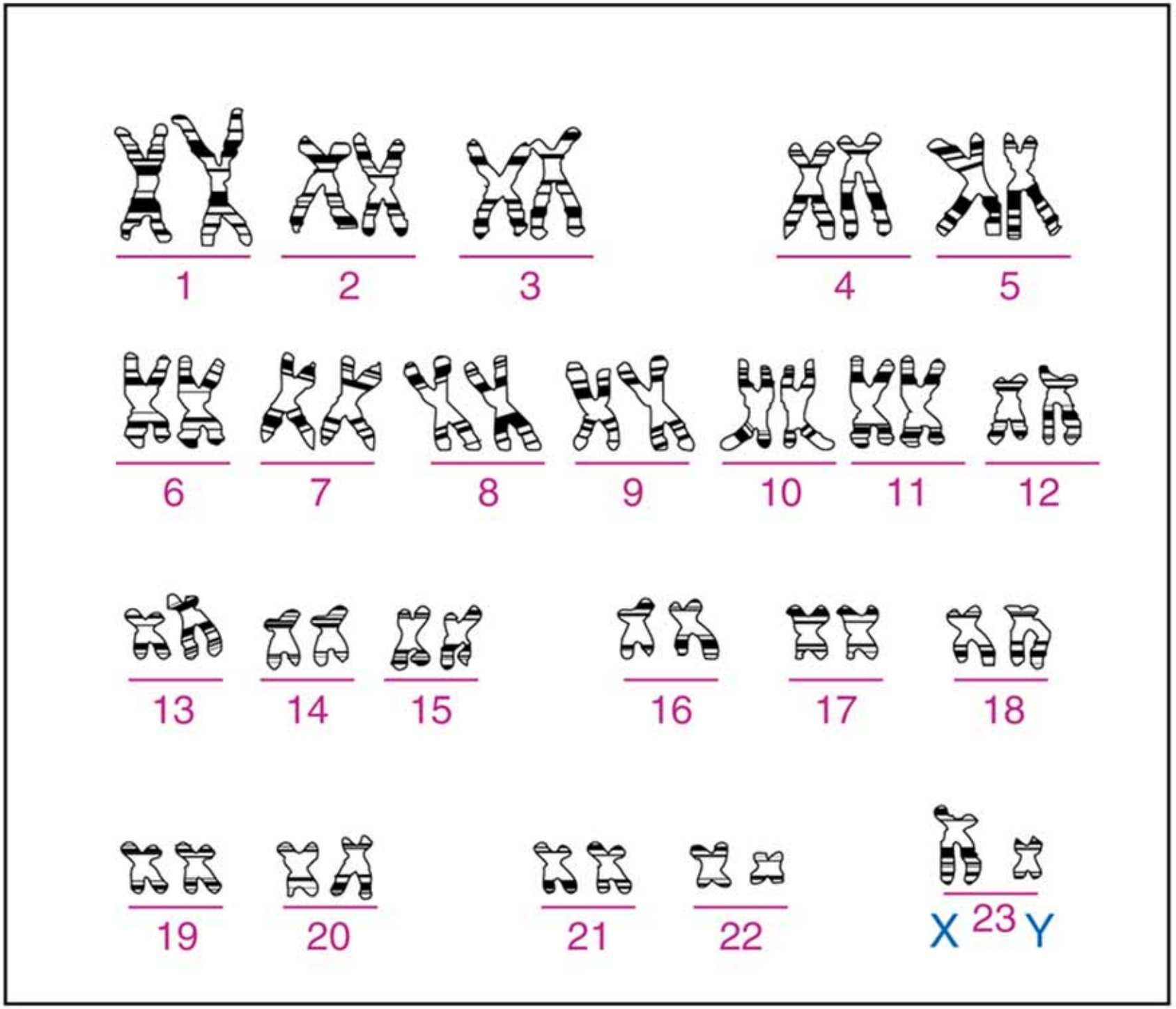

A Adenine
T Thymine
hydrogen bonds

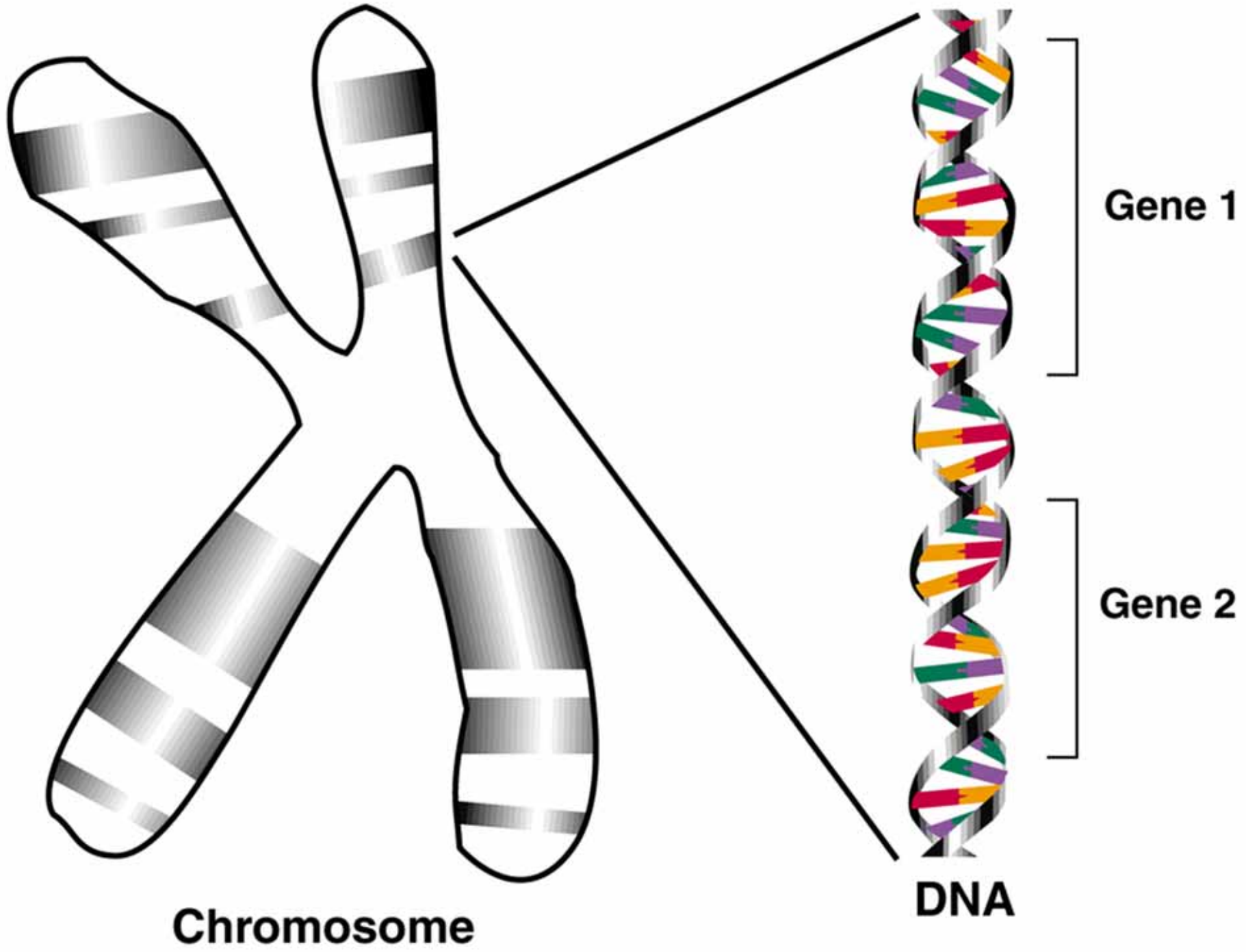

U Uracil
Replaces Thymine in RNA

Complimentary Structure

The complimentary binding of the DNA bases allows it to

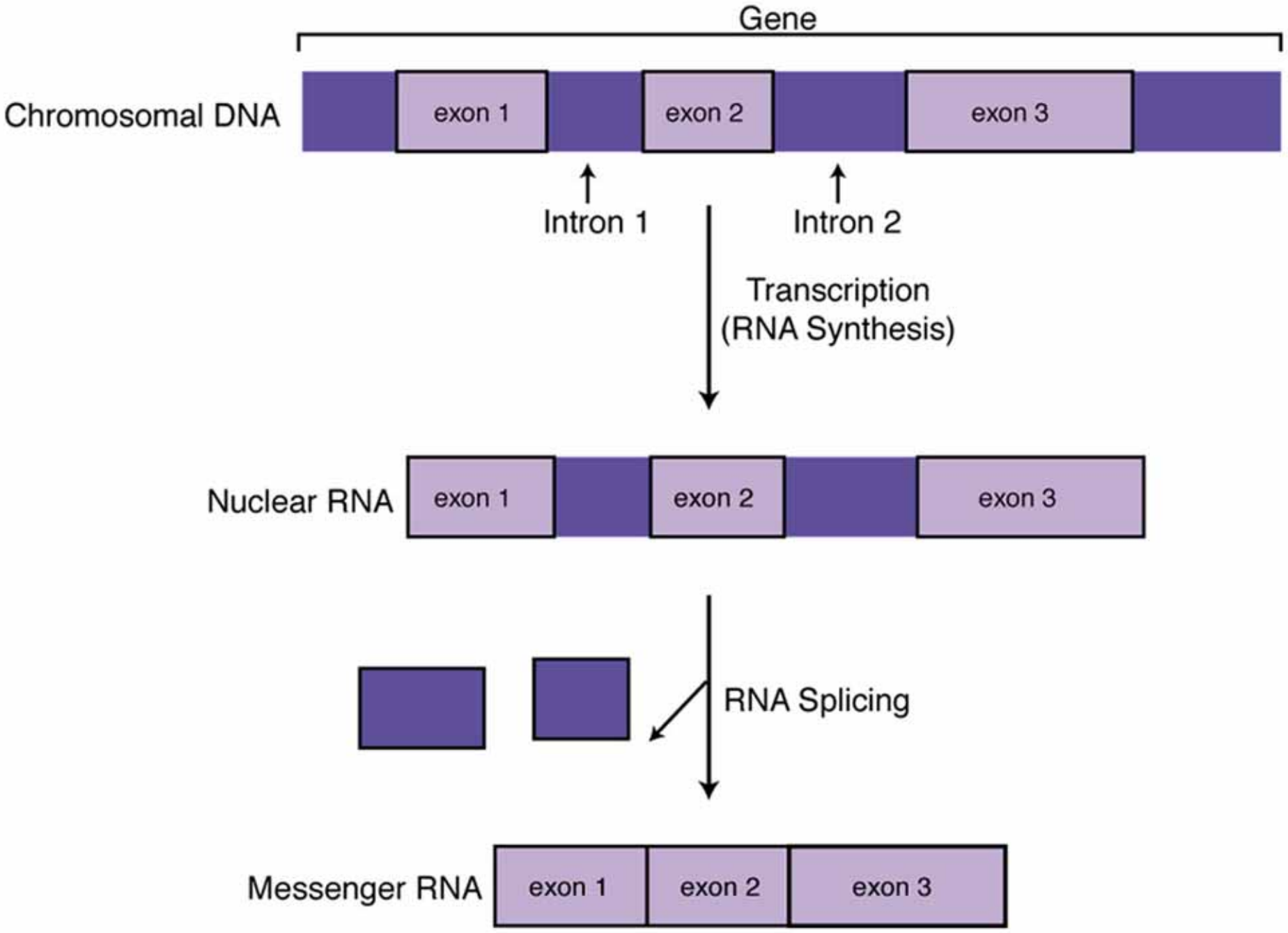
1. Split apart and make two perfect copies of itself, and
2. Transcribe its stored information to RNA in order to translate that information into proteins


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X 23 Y



Genes

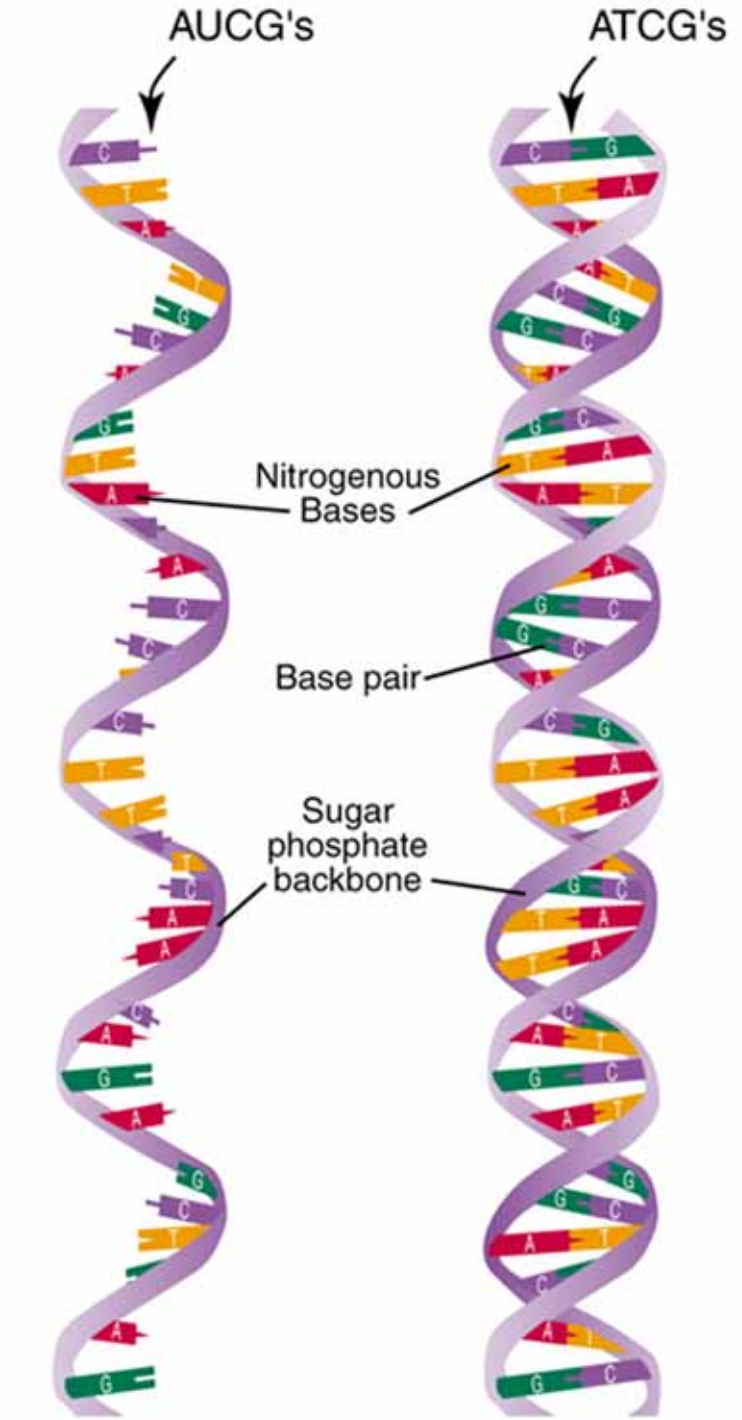
RNA Synthesis and Processing

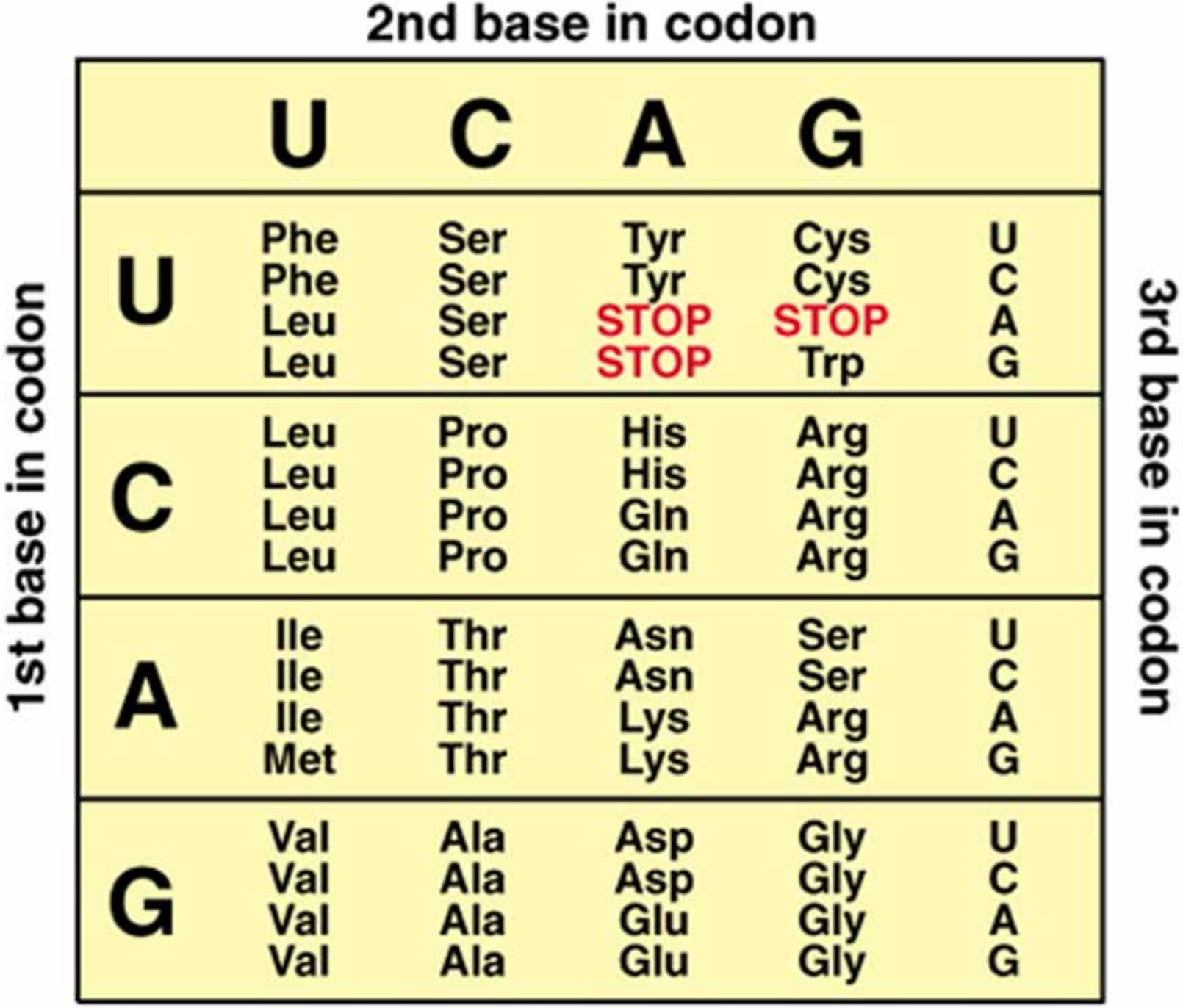


Ribonucleic Acid (RNA)

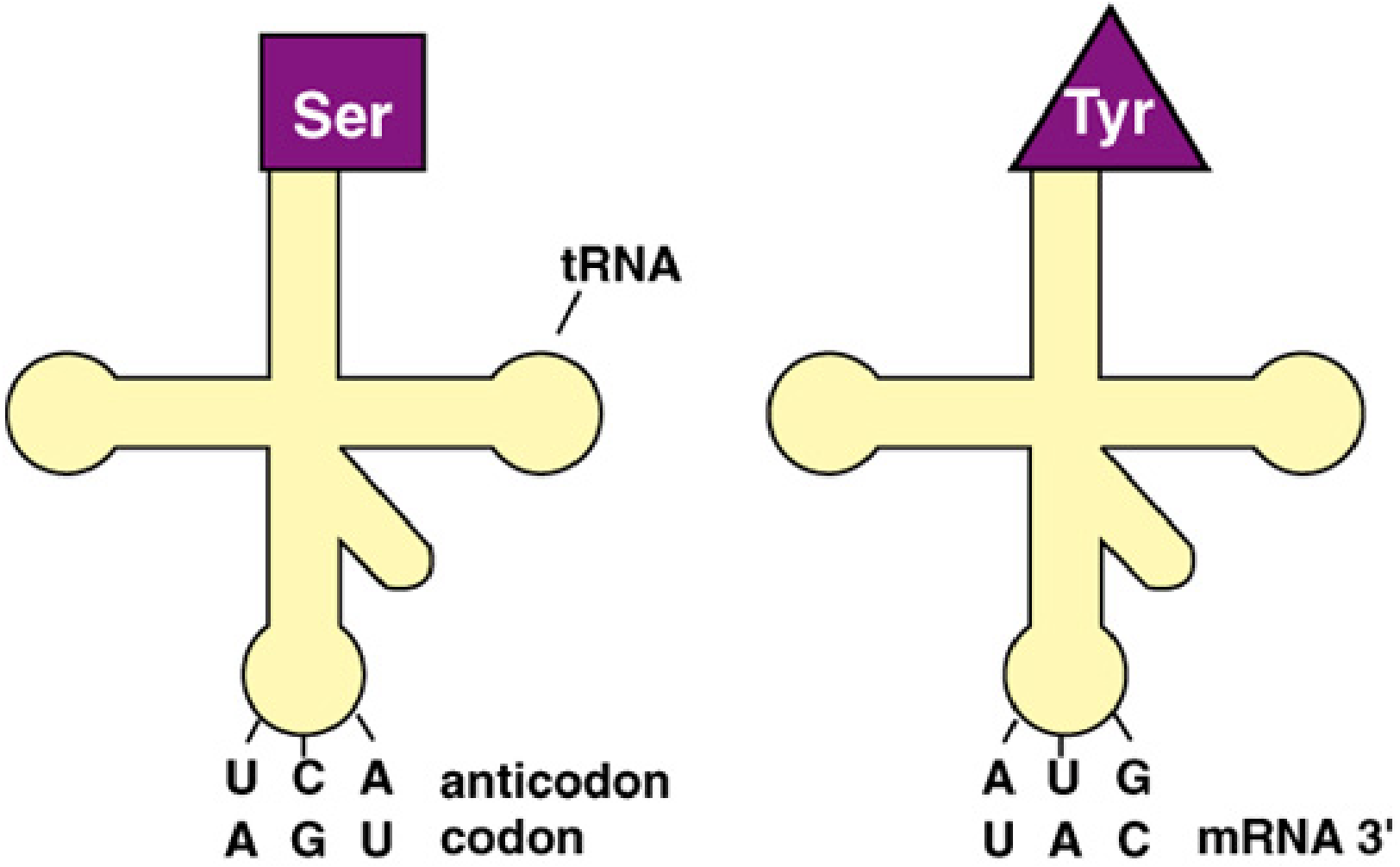
RNA is structurally similar to DNA except that,

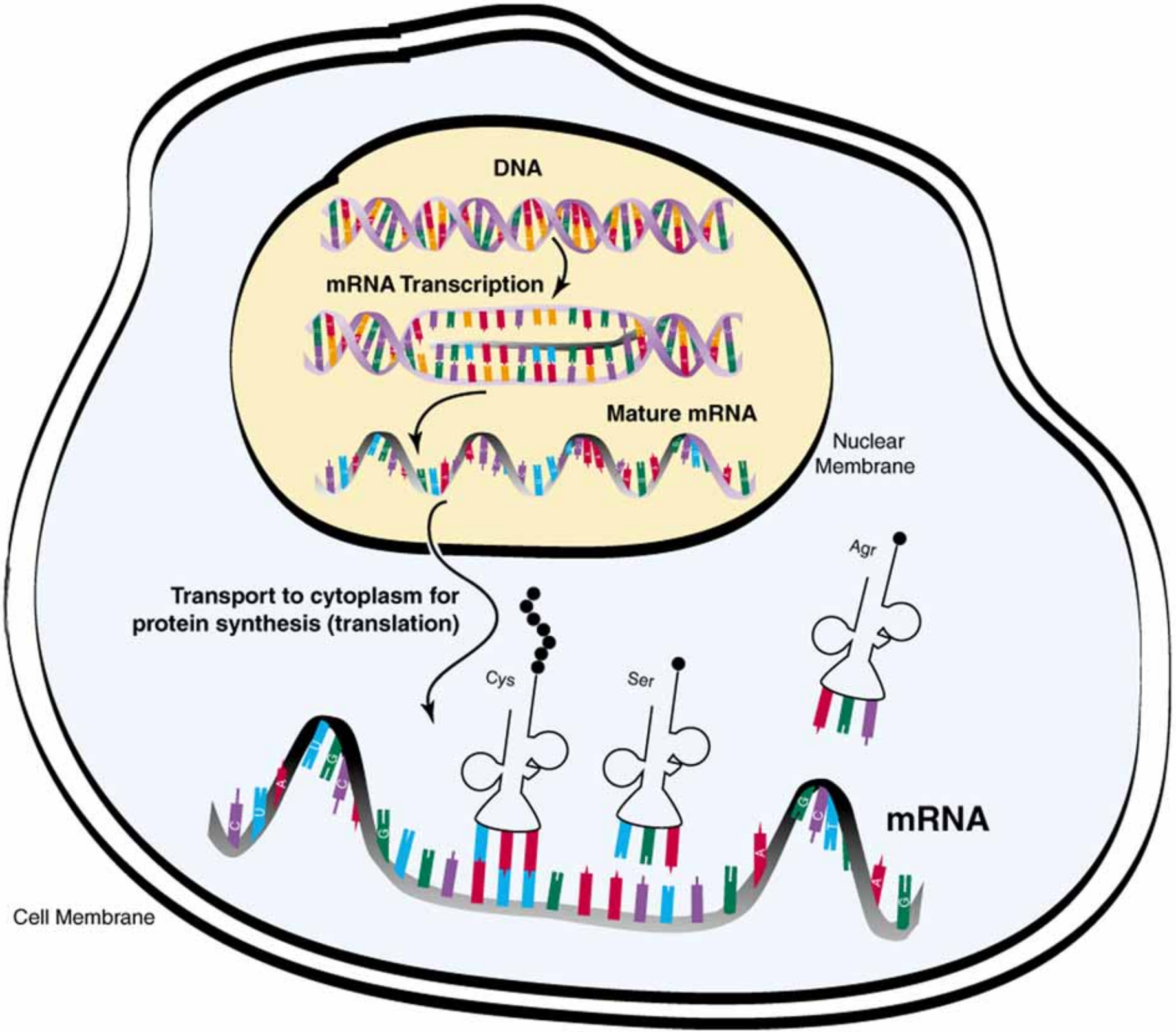
1. RNA is single stranded,
2. RNA uses the nucleotide uracil (U) in the place of thymine (T), and
3. RNA's 3-nucleotide codons (think of them as "3-letter words") code directly for specific amino acids, allowing for the synthesis of proteins in ribosomes


AUCG's
ATCG's
Nitrogenous Bases
Base pair
Sugar phosphate backbone
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid

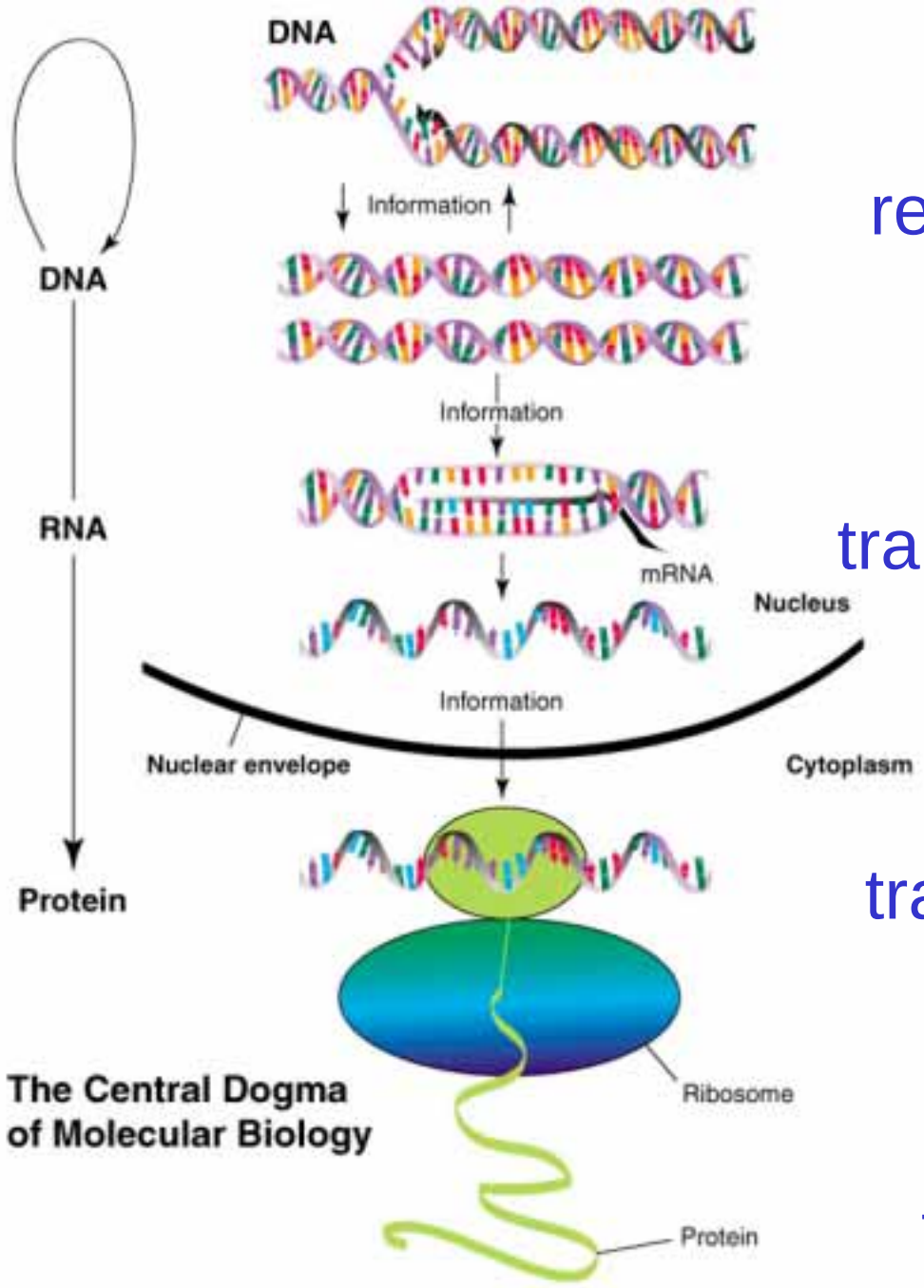

2nd base in codon
1st base in codon
3rd base in codon
U C A G
U
Phe Ser Tyr Cys U
Phe Ser Tyr Cys C
Leu Ser STOP STOP A
Leu Ser STOP Trp G
C
Leu Pro His Arg U
Leu Pro His Arg C
Leu Pro Gln Arg A
Leu Pro Gln Arg G
A
Ile Thr Asn Ser U
Ile Thr Asn Ser C
Ile Thr Lys Arg A
Met Thr Lys Arg G
G
Val Ala Asp Gly U
Val Ala Asp Gly C
Val Ala Glu Gly A
Val Ala Glu Gly G

Codons, Transfer RNA, Amino Acids




DNA
mRNA Transcription
Mature mRNA
Nuclear Membrane
Transport to cytoplasm for protein synthesis (translation)
Agr
Cys
Ser
mRNA
Cell Membrane

Information

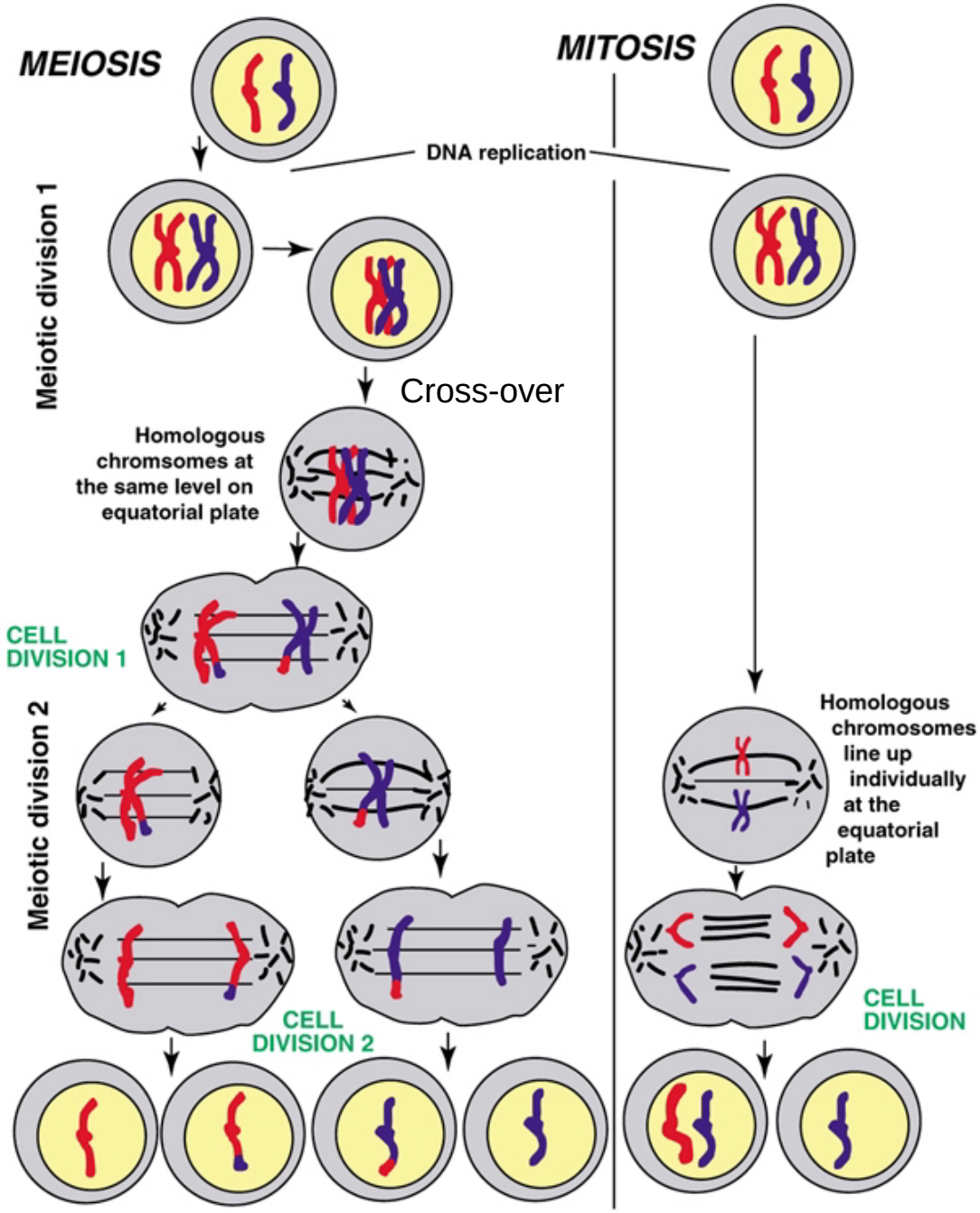


replication

transcription

translation

function


MEIOSIS
MITOSIS
DNA replication
Meiotic division 1
Cross-over
Homologous chromsomes at the same level on equatorial plate
CELL DIVISION 1
Meiotic division 2
Homologous chromosomes line up individually at the equatorial plate
CELL DIVISION 2
CELL DIVISION

Imagine that the genome is a book

There are twenty-three chapters, called CHROMOSOMES

Each chapter contains several thousand stories, called GENES

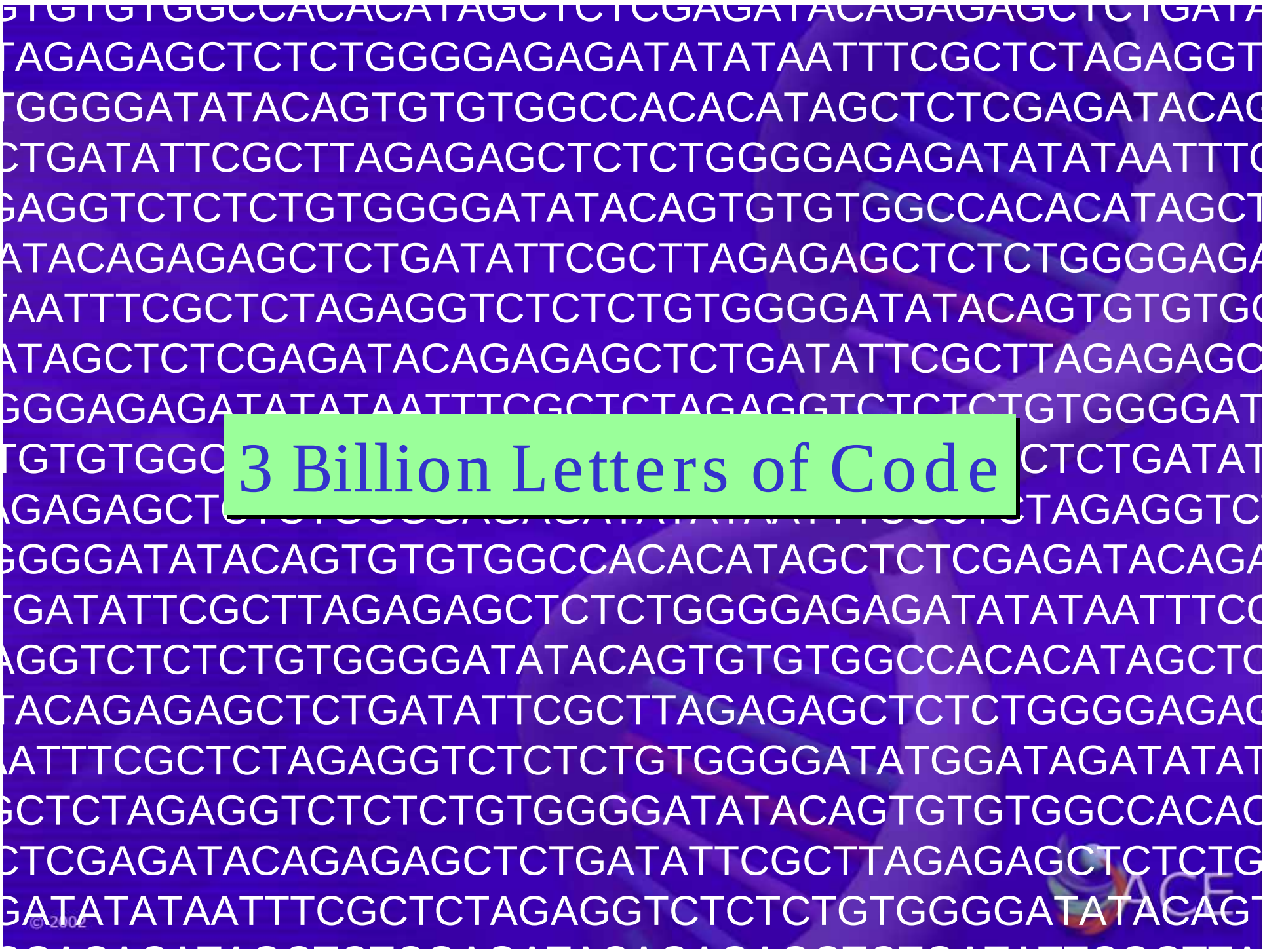
Each story is made up of paragraphs, called EXONS, which are interrupted by advertisements, called INTRONS

Each paragraph is made up of 3-letter words, called CODONS

Each word is written in letters,called BASES

Matt Ridley. Genome:The autobiography of a species in 23 chapters. Perennial; New York; 1999.

ACE


3 Billion Letters of Code

Relative Size:
Keeping DNA in Perspective

ACE

Earth

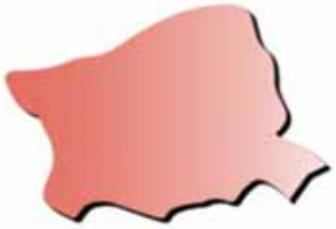
Cell

Country

Chromosome

State

Chromosome Fragment



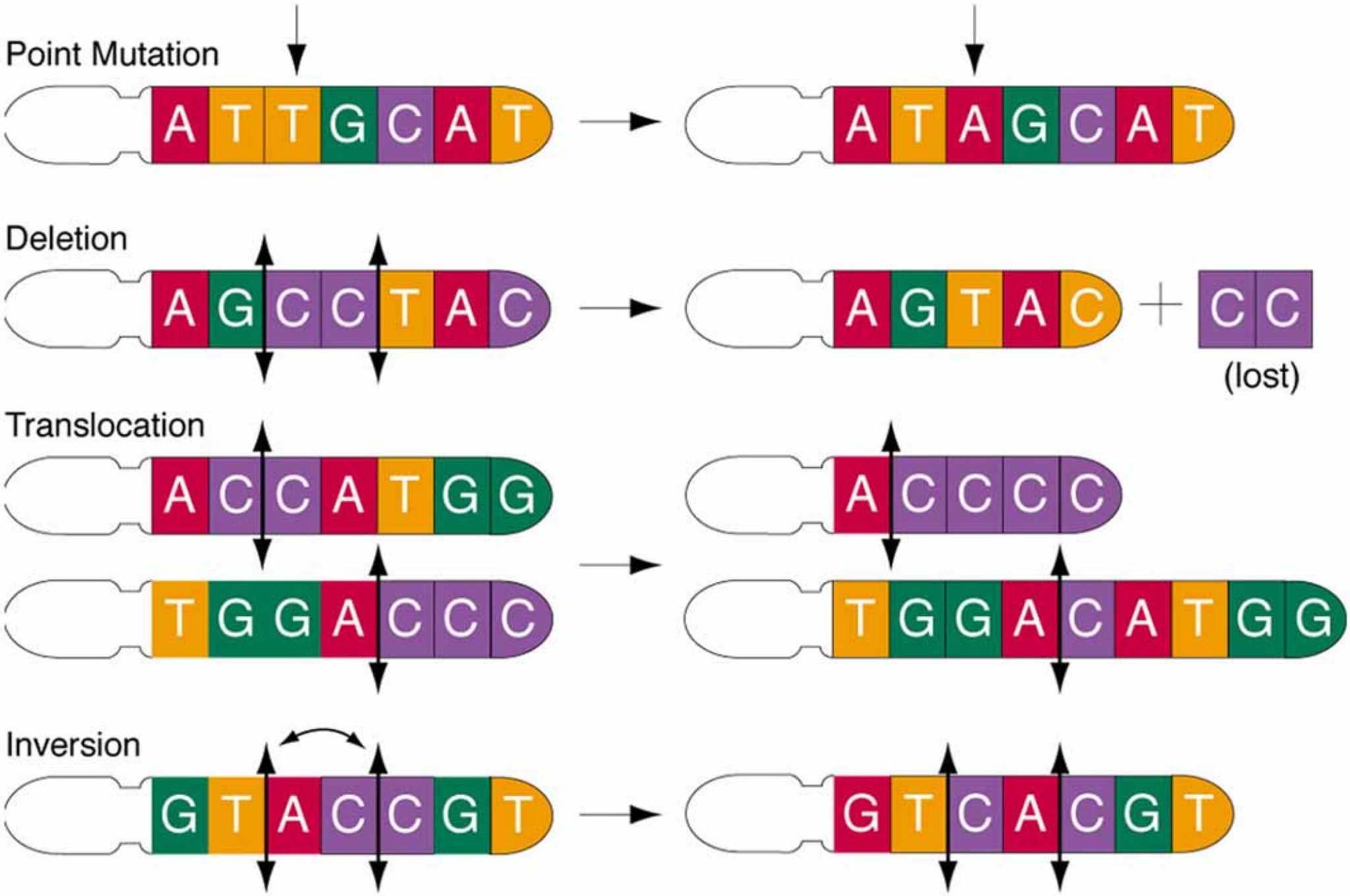
City

Gene

People

Nucleotide Base Pairs

Mutations of Chromosomes



What you say and what people hear are never the same thing

What people think when they hear the word "mutation"

Polymorphisms

- Variations in the genetic code of individuals are known as polymorphisms: “many shapes”
- Polymorphisms are largely responsible for our biochemical individuality

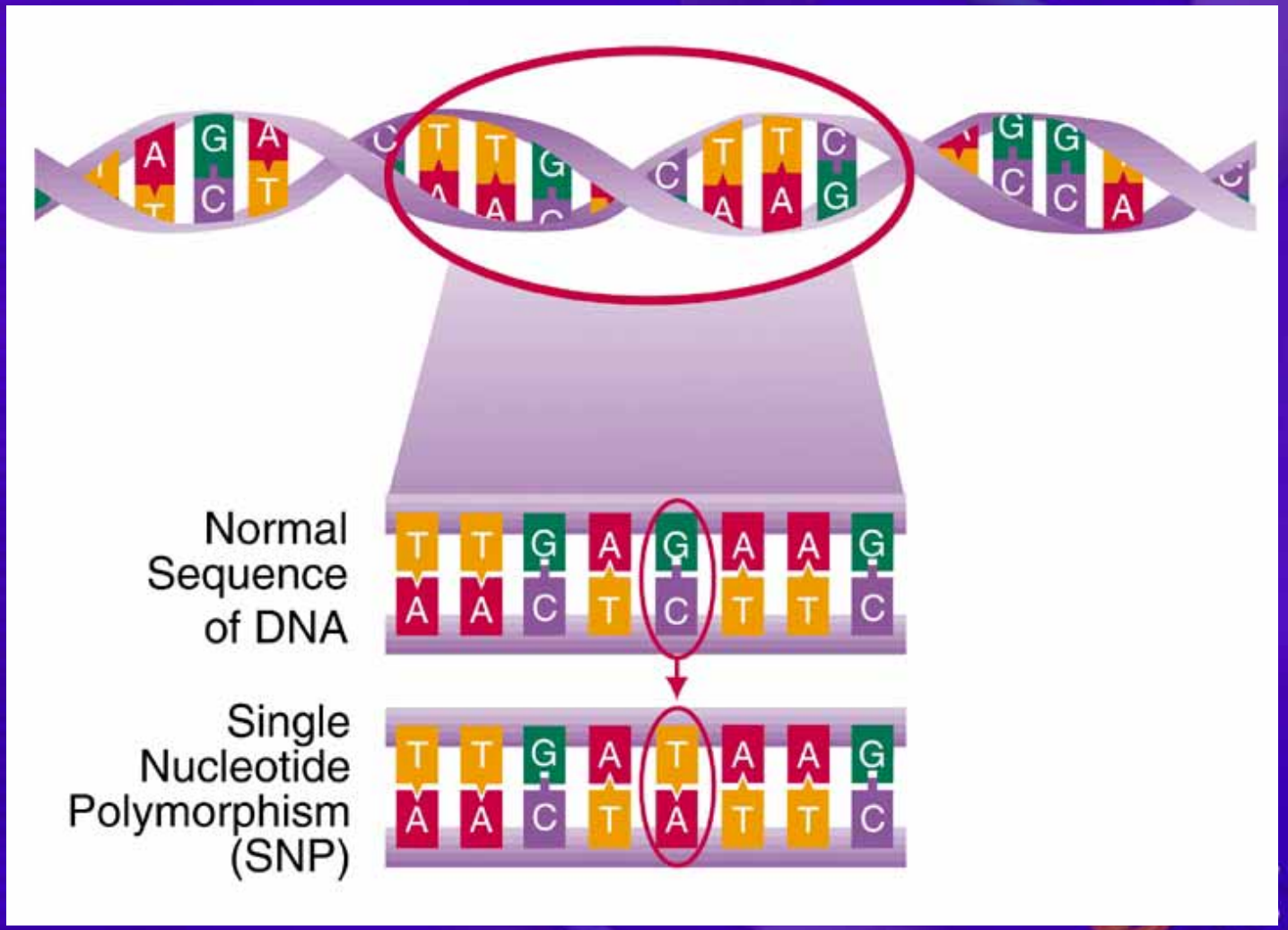
Facts About Polymorphisms

- They are not rare – up to 50% of the population may have a specific polymorphism
- Not all polymorphisms are "bad", some make us more resistant to specific disease, etc.
- The effects of most polymorphisms are modifiable through diet, lifestyle and specific medical intervention

Facts About Polymorphisms

- In many cases, polymorphisms can allow us to identify those people who are more susceptible to developing chronic disease like coronary artery disease, cancer, rheumatoid arthritis, osteoporosis, or chronic inflammation

Single Nucleotide Polymorphism SNP



The Emerald

- Emeralds always have within them inclusions
- Known as “jardins”, these imperfections give emeralds their unique character, identity, and beauty
- No two emeralds are alike

Some DNA Facts

- There are somewhere between 40,000 and 80,000 genes in the human genome
- We have at least a vague idea of the function of ~20,000 of them
- ~100,000 SNPs have been identified, but there are likely to be millions in the genome

To Be Clinically Significant, Polymorphisms Must Be:

Relevant

SNPs must affect clinically important conditions

Prevalent

A substantial percentage of people must be carriers

Modifiable

The expression of the gene must be modifiable

Measurable

Functional laboratory testing must be available to measure therapeutic effectiveness

ACE

Understanding the Genomics Revolution in the Context of the New Economy

The New Economy

Today, it is not the rare that is valuable but the commonplace

One fax machine is useless

But, for every additional fax machine in operation, your fax machine becomes more valuable

More...Cheaper...Faster

Device	Cost at Introduction	Cost Today
Calculator	$120 in 1972	$5
Color TV	$1000 in 1954	$180
VCR	$1400 in 1978	$80
Cell Phone	$4200 in 1984	Free

ACE

The Genomic Revolution

- By 1991, the entire scientific world had mapped <2,000 genes
- In 1995 alone, Celera Corporation mapped 35,000 genes
- By 2000, Celera had gene maps equivalent to the information in six Libraries of Congress

The Genomic Revolution

Cost of sequencing a single gene

1974: $150 million

1998: $150

2000: $50

2002: $5

The only certainty is that where we are today is not where we will be tomorrow

Knowledge is exploding
Costs are collapsing

The Genomic Revolution Is Now

The great thing in this world is not so much where we stand but in what direction we are moving

Oliver Wendell Holmes


The Origin of Life

All forms that perish other forms supply,
(By turns we catch the vital breath and die)
Like bubbles on the sea of matter borne,
They rise, they break, and to that sea return

Alexander Pope

An Essay on Man

Variety Is the Spice of Life

- Variety is also the driving force of natural selection and evolution
- All life on this planet “speaks” the same language, viz., DNA
- The same four “letters” write the code for all life on Earth
- In this sense, at least, all life on Earth is one

Evolution and Genetics

- The more diverse a species' gene pool, the more likely the species will survive and also the more likely one segment of that species will evolve into an entirely new species
- Alterations in the genetic code is the mechanism by which evolution can occur

The Origin of Life

Life is a rather difficult thing to define, but minimally exhibits two distinct skills:

- The ability to replicate
- The ability to create order

The key to both of these features of life is information

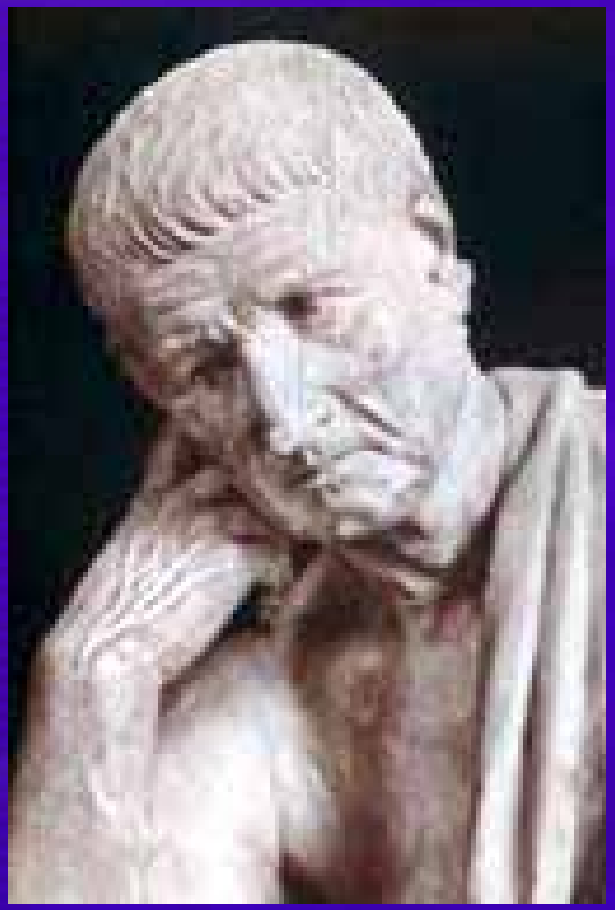
Claude Shannon

- Information and entropy are two sides of the same coin
- The more entropy, the less inherent information in an object – more information, less entropy
- Entropy and information are linked through energy: a continuum of harnessing and releasing energy

Campbell J. Grammatical Man: information, entropy, language, and life. Allen Lane, London, 1983

ACE

Aristotle



- The idea that information is the key to living beings is not new
- Aristotle argued that the "concept" of a chicken must be implicit in an egg
- An acorn is somehow, literally, "informed" by the plan of an oak tree

First and Second Creation

All things that are created must have first a creation of intent, of mind, of plan, of logos, of Word

Only then can the second, physical creation occur

Life, too, must have a template before it manifests on the physical plane

In the beginning was the Word and the Word was with God and the word was God. The same was in the beginning with God... And the Word was made flesh....

The Gospel of John, 1:1-9

The Origin of Life

- The primordial information that originally coded for life cannot be DNA
- It takes proteins to regulate DNA, and it takes DNA to code for proteins
- Neither DNA nor protein can exist without the other

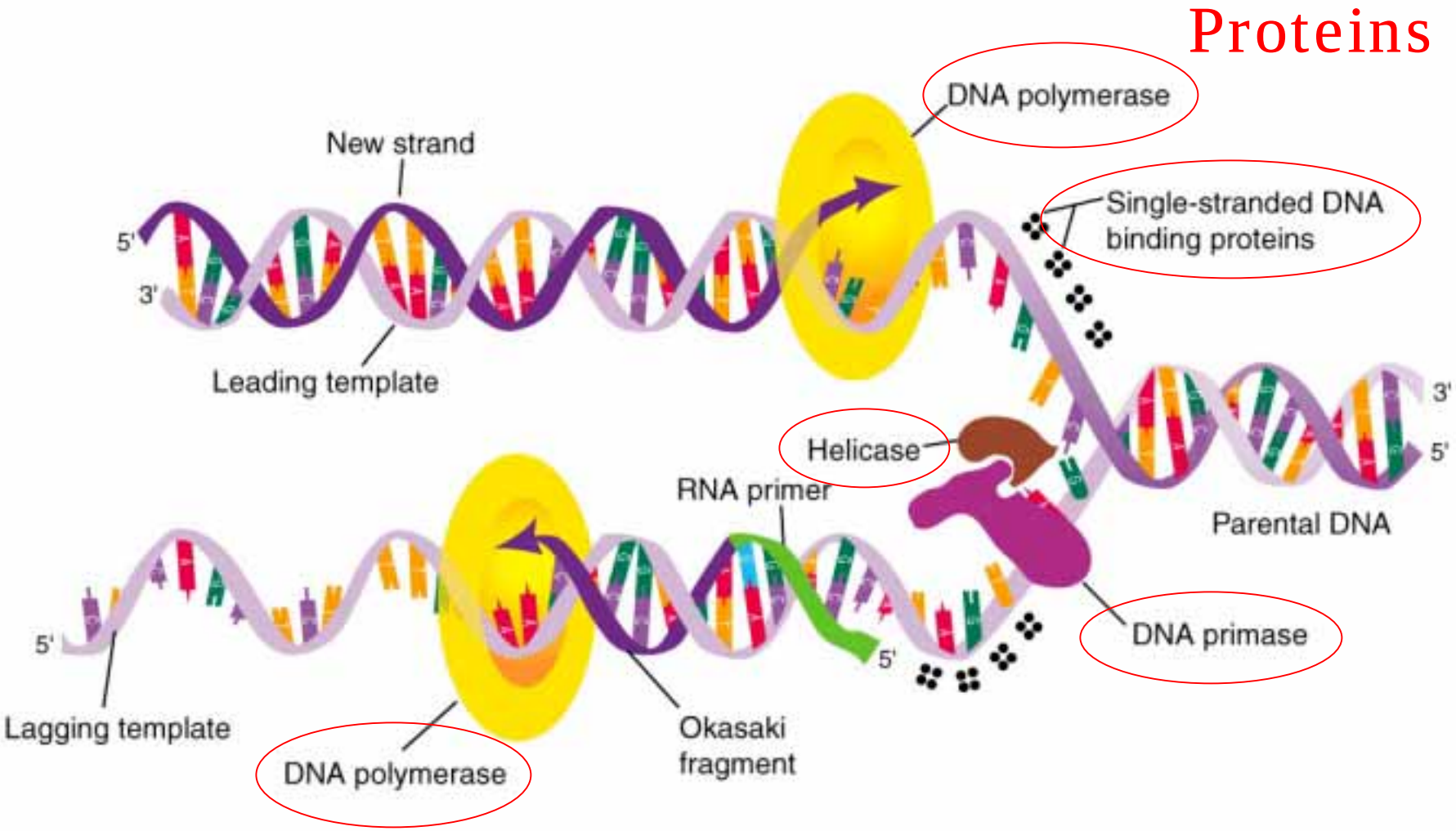
ACE

Chicken and Egg Conundrum

Protein is phenotype: metabolism, body, action, the proverbial chicken

DNA is genotype: information, code, potential, the proverbial egg

Protein – DNA Interface

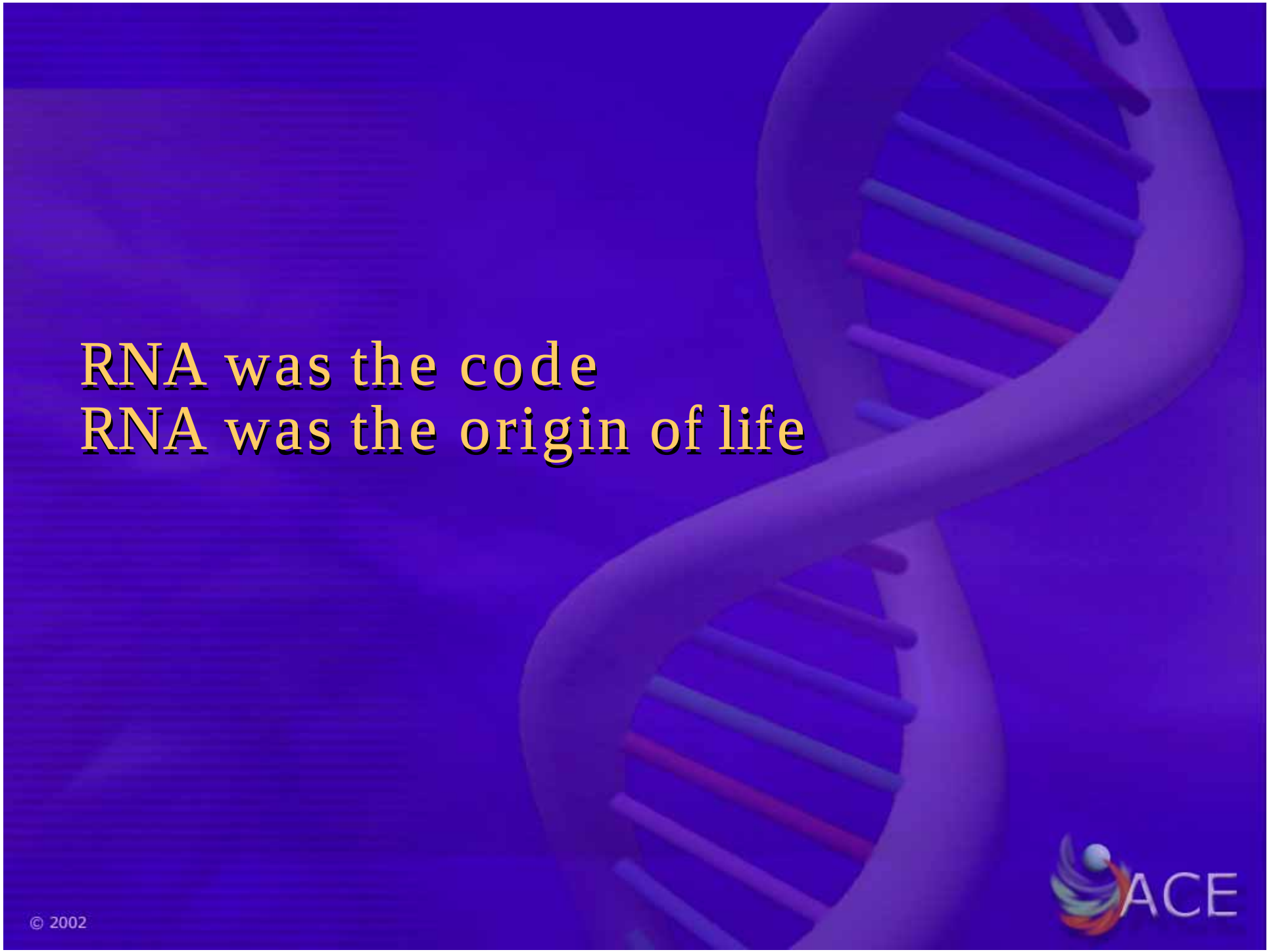


Ribonucleic Acid (RNA)

- RNA is the chemical substance that links the world of DNA with the world of proteins
- RNA, unlike DNA or protein, can replicate itself without assistance
- Further, the most primitive and basic cellular functions require RNA

RNA Functions

- RNA transports the messages of genes
- The ribosome, an RNA-based molecule, builds the proteins
- Transfer RNA transports amino acids to the ribosomes for protein synthesis
- RNA can act as an independent replicator or catalyst



The Ur-Gene

The first gene on this planet was likely an RNA receptor-catalyst, "consuming" chemicals around itself in order to replicate itself

Yet these ribo-organisms were inherently unstable, forming and falling apart quite easily

Gesteland RF and Atkins JF , eds. The RNA World.
Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1993.

ACE

Finding Stability

The first stable life on this planet arose when RNA was able to translate its information into a much more stable form: DNA

The stable information could become even more stable by using proteins to form a "cellular body" to protect itself from a harsh external environment

And the cycle of DNA-RNA-protein was born

ACE


Genes, Sex, and Evolution

Who Controls Whom?

We think of genes as passive recipes awaiting transcription in order to meet the collective need of the whole organism; genes as servants of the body

In reality, it may also be the body that is the servant, the battleground, merely a gravity vehicle for meeting the sundry ambitions of our genes

X and Y

Male and Female likely arose as an antagonistic co-evolution

"The mammalian Y chromosome is thus likely to be engaged in a battle in which it is outgunned by its opponent. A logical consequence is that the Y should run away and hide shedding any transcribed sequences that are not essential to its function."

Amos W and Harwood J. Factors affecting levels of genetic diversity in natural populations. Phil Trans Royal Soc Lon, Series B 1998;353:177-86.

ACE

Competition Between X and Y

- The SRY gene on the Y chromosome determines male sex in mammals
- Two DAX genes determine female sex
- There are rare XY individuals who have two copies of the DAX gene on their X chromosome and they develop into phenotypic females
- SRY can beat one DAX but not two

Swain A, Narvaez V, et al. Dax1 antagonizes Sry action in mammalian sex determination. *Nature* 1998 Feb 19;391(6669):761-7.

ACE

The X Advantage

- 75% of all sex chromosomes are Xs
- Only 25% of sex chromosomes are Ys
- An X chromosome spends 67% of its time in females and only 33% in males
- Clearly the X has a strategic advantage
- Any gene on the Y chromosome is vulnerable to attack and in response, the Y has shed as many genes as possible and shut down the rest in order to “run away and hide”

The SRY Gene

- Yet the SRY gene has become remarkably stable and consistent
- Virtually no point mutations
- Unchanged for 200,000 years
- Yet inter-species variability (e.g., humans vs. chimpanzee) is 10X that of other genes – the SRY gene is the fastest evolving gene in the genome
- Why?

The Balance of Power

- Periodically a driving gene appears on the X chromosome that attacks the Y chromosome by recognizing the SRY coded protein
- Suddenly, there is a huge selective advantage for a mutant SRY gene that is unrecognized by the X attack
- For a time the driving X chromosome distorts the sex ratio in favor of females but eventually, the mutant SRY restores the balance
- The end result is that a new SRY sequence is shared by all members of the species with little variation yet it is distinctly different from the SRY in other closely related species

Amos W and Harwood J. Factors affecting levels of genetic diversity in natural populations. Phil Trans Royal Soc Lon, Series B 1998;353:177-86.

ACE

Sexual Antagonism in Fruit Flies

- Because a Y chromosome will never find itself in a female, it is free to aquire genes that are bad for females and good for males
- In fruit flies, proteins in seminal fluid are absorbed into the female's bloodstream where on migrating to the brain, reduce her sexual appetite and increase her ovulation rate
- Is this good for the species or just for that particular male?

Rice WR. Sexually antagonistic genes: experimental evidence. *Science* 1992 Jun 5;256(5062):1436-9.

- William Rice kept a separate strain of female flies and prevented them from evolving resistance
- Meanwhile he allowed males in another group to breed normally for 29 generations – evolving more and more effective semen against more and more resistant females
- Then he bred the two lines
- The male sperm was now so effective that it killed the females

Juan Carreno de Miranda

La Monstrua vestida

La Monstrua desnuda

Prader-Willi Syndrome

- Morbidly obese, never apparently experiencing satiety – they eat almost until they burst
- Floppy and pale
- Small hands and feet
- Underdeveloped sex organs
- Mild mental retardation
- Bad temper

Angleman's Syndrome

- Taut, thin, hyperactive, insomniac
- Small headed with long jaws and tongues
- Move jerkily, "like puppets"
- Almost perpetually happy and smiling
- Given to paroxysms of laughter
- Often never learn to speak and are severely mentally retarded

Chromosome 15

- In the 1980's geneticists observed that both Prader-Willi syndrome and Angleman's syndrome often occur in the same families
- It soon became clear that both syndromes had the same chunk of chromosome 15 missing
- In Prader-Willi, the father's chromosome section was missing
- In Angelman's, the mother's

Imprinting

- Somehow, some genes "remember" which parent they come from – they are endowed with a paternal or maternal imprint
- Only the maternal or the paternal copy of a particular gene is then expressed

Mice Embryos Formed From Two Mothers or Two Fathers

Two Mothers: embryo properly formed, but it could not make a placenta with which to sustain itself

Two Fathers: healthy placenta but the embryo, though large, was a disorganized blob of cells with no discernable head

McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 1984 May;37(1):179-83.

ACE

Mammalian Imprinting

- Genes inherited from the father are responsible for making the placenta
- Genes inherited from the mother are responsible for organizing the embryo, especially the head and brain

The Placenta and Sexual Antagonism

- Pregnancy has commonly been viewed as a cooperative interaction between a mother and her fetus.
- The placenta is not a maternal organ designed to give sustenance to the fetus, but a fetal organ designed to parasitize the maternal blood supply
- The placenta tries to control, against maternal resistance blood sugar levels and blood pressure to the benefit of the fetus, not the mother

Haig D. Genetic conflicts in human pregnancy. *Q Rev Biol* 1993;68(4):495-532.

ACE

Selfish Gene Hypothesis

- Evolution may not be only about competition between species or between individuals within a single species
- The fundamental competition that leads to “the survival of the fittest” may be only fully manifested at the level of the genes themselves

Dawkins, Richard and Dawkins, Rochard. *The Selfish Gene.* Oxford University Press; Oxford, UK, 1990.

ACE

Imprinting and Promiscuity

- Embryos under the influence of paternal genes might behave differently if they share the womb with full siblings or with embryos that have different fathers
- In the latter case, embryos might have more selfish paternal genes, the better with which to compete

Not All Mice Are Virtuous

Peromyscus maniculatis is a strain of deer mice in which the females are promiscuous, having litters with babies fathered by different males

Peromyscus polionatus is a strain of deer mice that is monogamous each litter containing only full siblings

What happens when we cross the two strains?

Imprinting Demonstrated

P maniculatis (promiscuous) father X
P polionatus (monogamous) mother:
Babies are born giant sized

P maniculatis (promiscuous) mother X
P polionatus (monogamous) father:
Babies are born tiny

Dawson W. Fertility and size inheritance in Peromyscus species cross. *Evolution* 1965;19:44-55.

ACE

Chimera: a mythological monster that was part lion, part she-goat, and part dragon

Chimera: any animal that results from the fused bodies of two genetically distinct individuals– like the opposite of identical twins– two genomes in one body

Mouse Chimeras

Make chimeras by fusing normal mouse embryos with female-female embryos and also with male-male embryos

F-F chimera: really big head and small body; most of the striatum, cortex and hippocampus are made by maternal cells

M-M chimera: really big body and small head: most of the muscle cells, hypothalamus and amygdala (limbic system) are made by the paternal cells

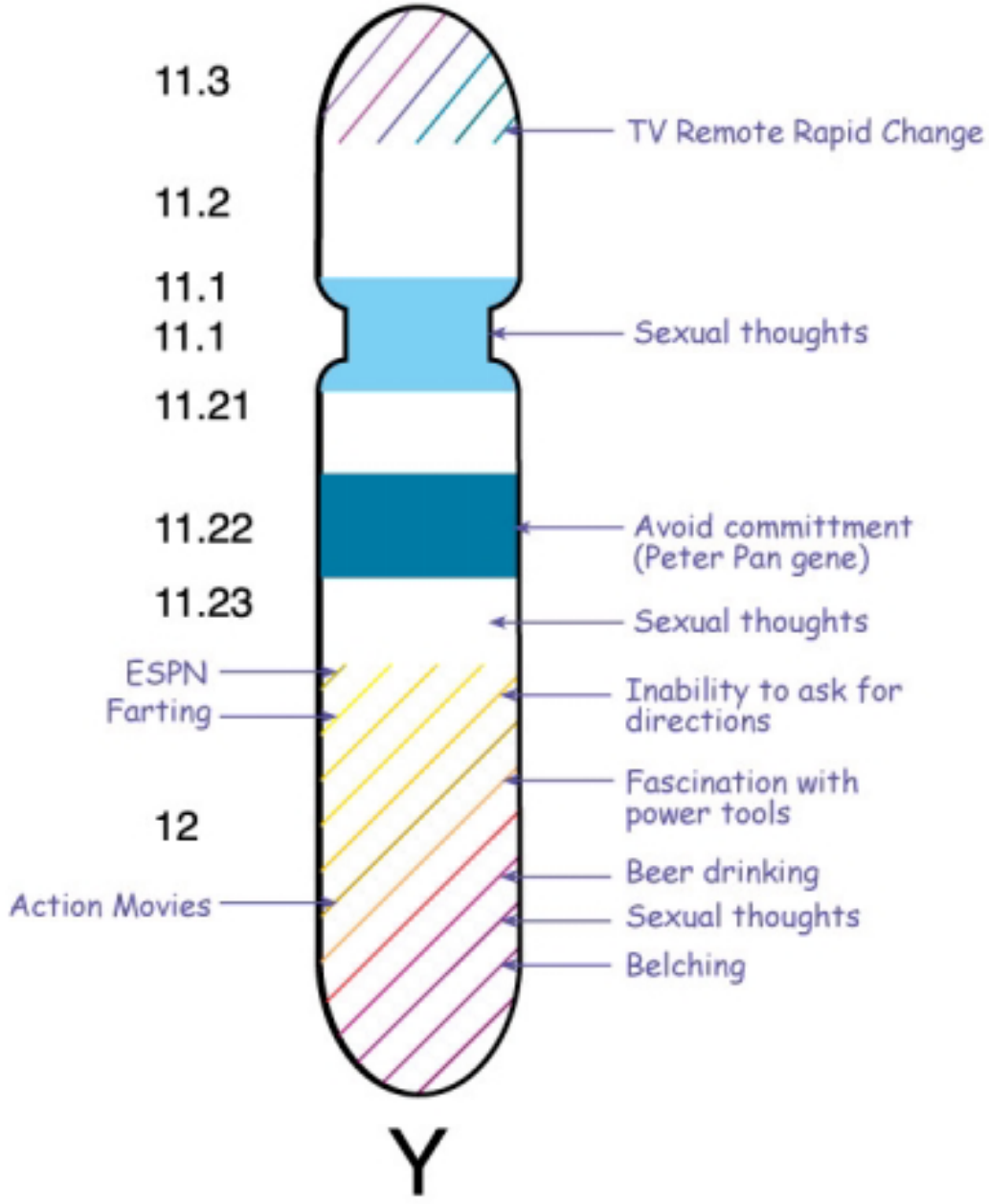
Allen ND, et al. Distribution of parthenogenetic cells in the mouse brain and their influence on brain development and behavior. *Proc Natl Acad Sci U S A* 1995 Nov 7;92(23):10782-6.

Of Mice and Men

If we are like mice, we may be walking around with more of our mother's thinking and our father's moods

Although it is important to remember that crossing over does occur in meiosis so the genes we get from our mother or father bear some resemblance to, but not the same as, the genes they expressed

Mysteries of the Y-Chromosome Revealed

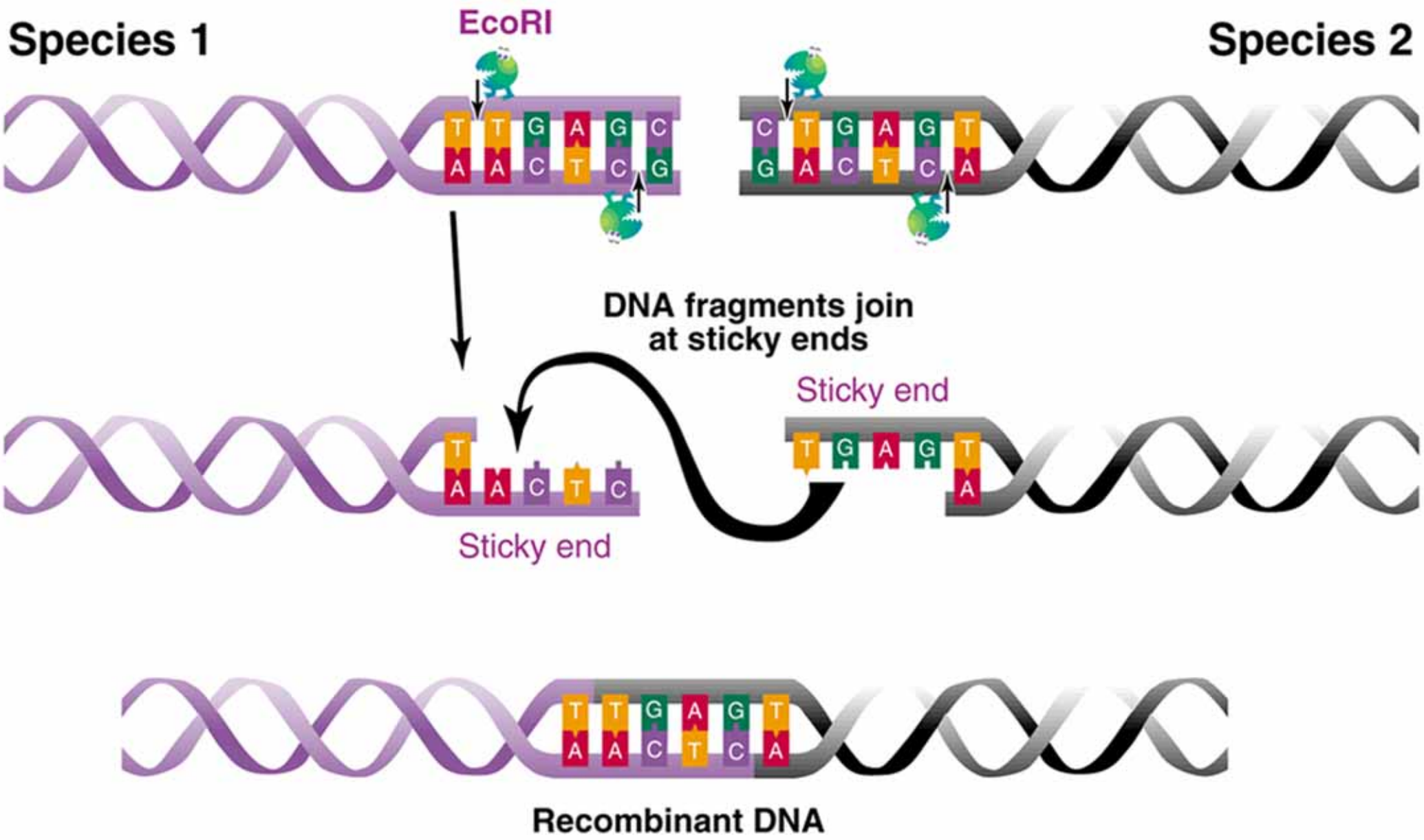



New Methodologies –
New Frontiers

Restriction Enzymes

- Restriction enzymes are bacterial enzymes whose purpose is to protect the bacteria against viral attack by literally shredding the invading viral DNA into bits
- As it turns out, restriction enzymes are absolutely specific in terms of the sequence of DNA they recognize in order to cleave the DNA at precise locations
- More than 1000 restriction enzymes have been isolated from bacteria, each cleaving DNA at its own unique sequence

Restriction Enzyme Action of EcoRI



Herbert Boyer and Stanley Cohen
The Birth of Genetic Engineering - 1973

- Used restriction enzymes to “snip” or “cut down” sections of plasmid DNA from two different bacteria, one resistant to tetracycline, the other resistant to kanamycin
- They mixed the two cut-down plasmid DNAs with a ligase, knitting the DNA fragments back together
- The new hybrid bacteria were able to grow on medium containing both tetracycline and kanamycin

Cohen SN, Chang AC, Boyer HW, Helling RB. Construction of biologically functional bacterial plasmids in vitro. *Biotechnology*. 1973;1992;24:188-92.

ACE

Transgenic Organisms

- Cohen and Boyer asked the next logical question, “Can DNA from different species be as easily exchanged?”
- The answer was, “Yes”
- They cut down a gene from a toad and inserted it into E. coli bacteria
- Each new generation of E. coli bore the toad gene

Morrow JF, Cohen, SN, et al. Replication and transcription of eukaryotic DNA in Escherichia coli. *Proc Natl Acad Sci U S A.* 1974 May;71(5):1743-7.

ACE

Transgenic Organisms "Frankenfoods"

Since 1986, more than 2,000 patents have been filed for transgenic plants and nearly as many for transgenic animals

- Herbicide and insect resistant plants
- Plant-based vaccines
- Nitrogen-fixing plants
- Transgenic pigs producing human hormones like insulin
- Bacteria producing human growth hormone
- Growth hormone genes into farm animals

ACE

The question is no longer whether or not we will allow the creation of transgenic organisms

The only question remaining is how we manage the creation and utilization of transgenic organisms

I Am What I Am… Or Maybe Not

- Only about 3% or your DNA codes for anything that we might call “you” the rest is junk
- Complete viral genomes (Hervs) account for ~1.3% of the human genome
- Reverse transcriptase, a viral enzyme, is repeated so many times in your genome that it accounts for ~15%
- A 280bp viral promoter sequence (Alu repeats) accounts for ~10%

Forensic Uses of Junk DNA

- ~35% of the genome is short sequences repeated over and over and over, known as minisatellite DNA
- The pattern of these repeat sequences is, however unique to individuals
- These unique repeat sequences allow for forensic identification of DNA in paternity testing and DNA “fingerprinting”

Maybe There Really is a Free Lunch in Nature After All

- These viral genes and complete viral genomes are like genetic freeloaders that have hitched a ride on the cellular machinery of mammals
- True genetic parasites that have discovered a way to be replicated generation after generation without ever needing a "body" to do so, adding more fodder to the Selfish Gene Hypothesis

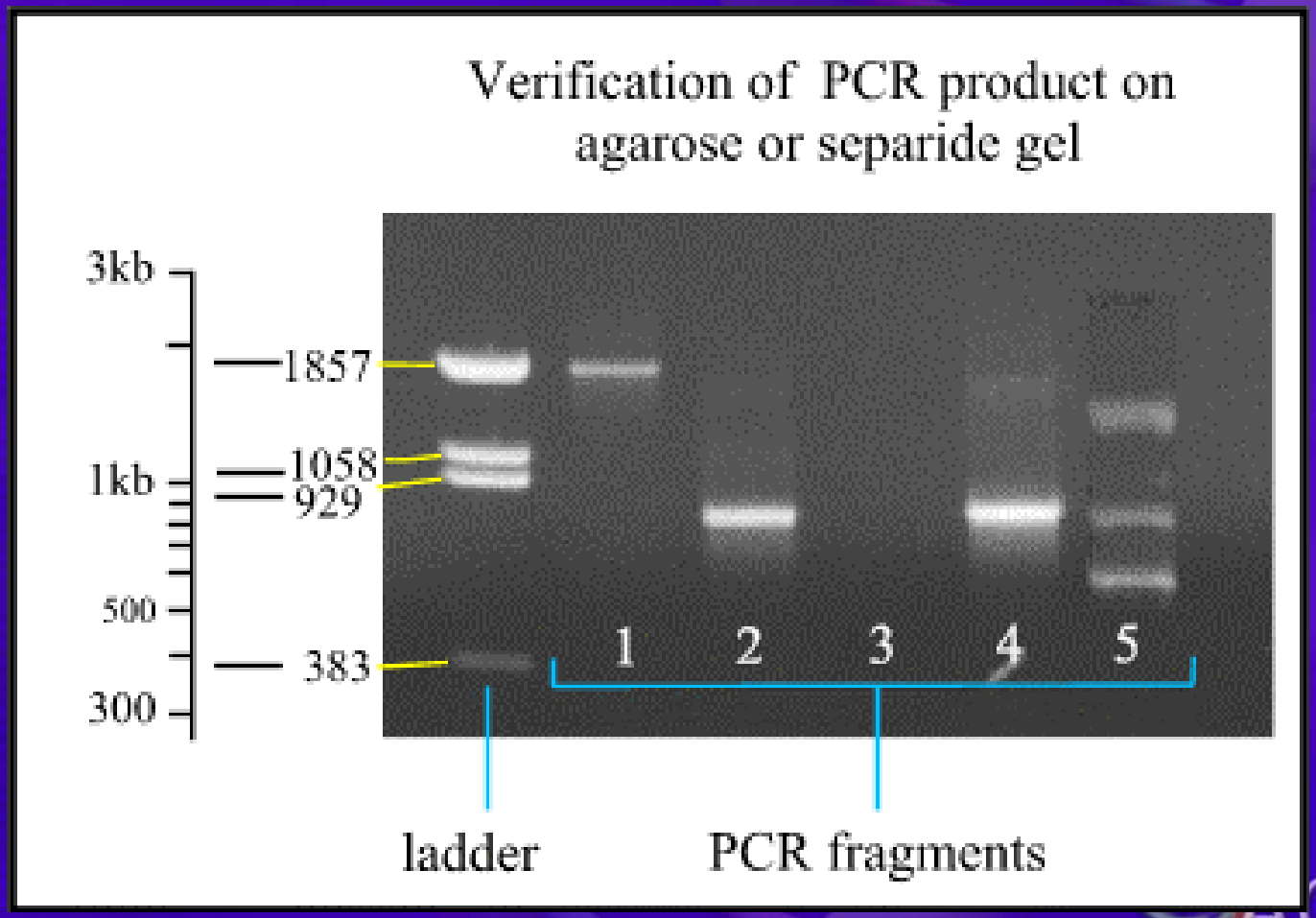
DNA Fingerprinting
Alec Jeffreys – 1984

1. DNA extracted, purified and cut down using restriction enzymes, producing DNA fragnments of varying lengths
2. Placing the fragments on a gel plate and passing an electric current across the plate (gel electrophoresis) causes the smaller fragments to migrate more quickly toward the positive pole than the larger fragments.

DNA Fingerprinting
Alec Jeffreys – 1984

3. The sorted DNA fragments are then subjected to a blotting technique in which they are split into single strands and transferred to a nylon sheet
4. A radioactive DNA probe is added which will anneal (bind) to the single-stranded DNA at specific sequences of minisatellite DNA
5. Finally, a piece of x-ray film is then exposed to the labeled, bound, blotted and separated DNA to reveal unique patterns of minisatellites

Fluorescent DNA "Fingerprints" after Gel Electrophoresis



Restriction Fragment Length Polymorphism (RFLP) Analysis

- Restriction enzymes can be used to analyze specific genes within populations and identify polymorphisms based on DNA fragment lengths
- Known as RFLP analysis, this provides an easy way to screen populations for polymorphisms and look for epidemiological and disease associations

Polymerase Chain Reaction
Kary Mullis – 1983

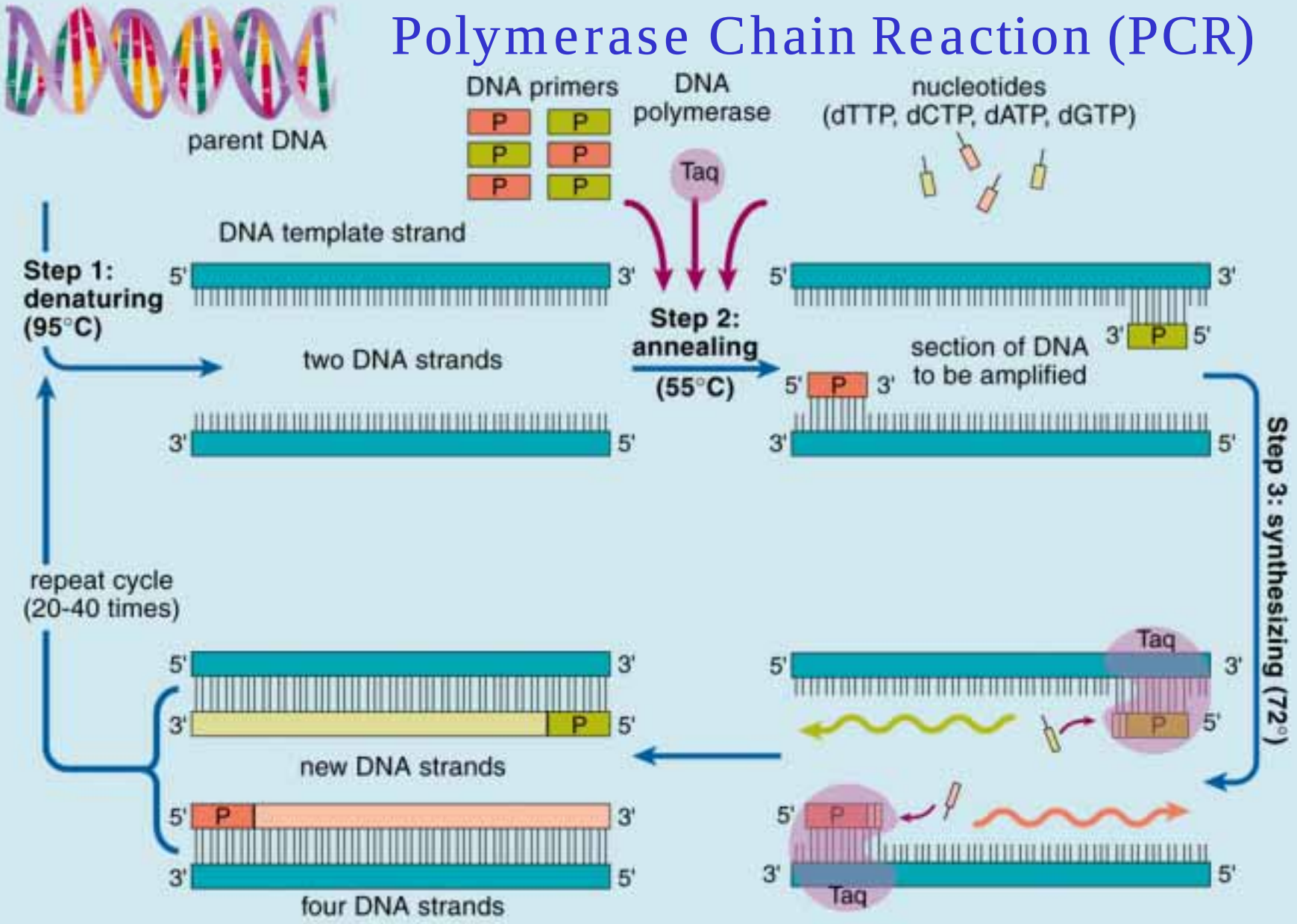
3-step process developed for the exponential amplification of DNA

1. The DNA is denatured, or separated, by heating it to 95°C
2. The temperature is lowered to 55°C so that primers can anneal to the single strands of DNA
3. The temperature is raised to 72°C where DNA polymerase begins adding nucleotides onto the ends of the annealed primer.

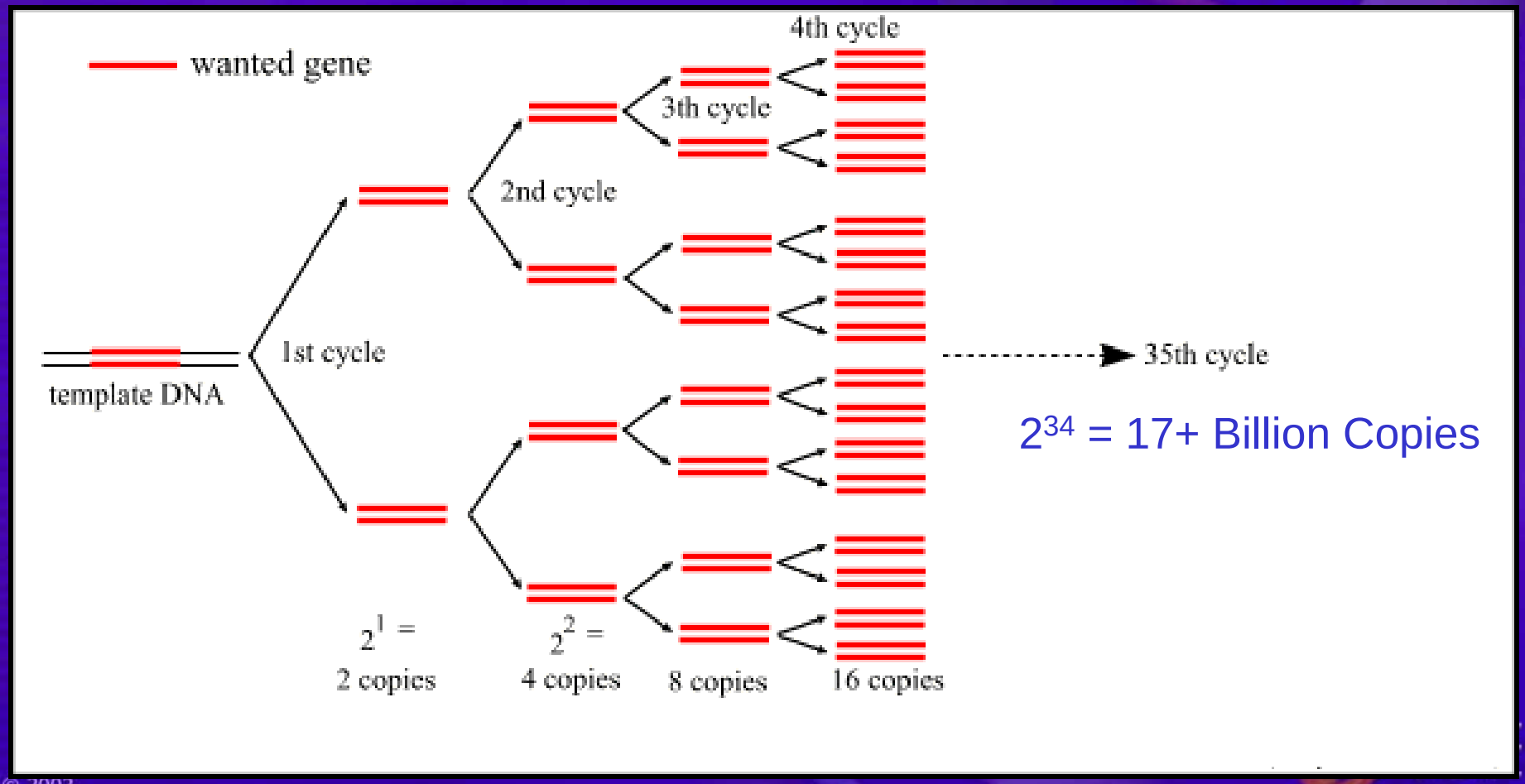
The entire process takes about 5 minutes

ACE

Polymerase Chain Reaction (PCR)



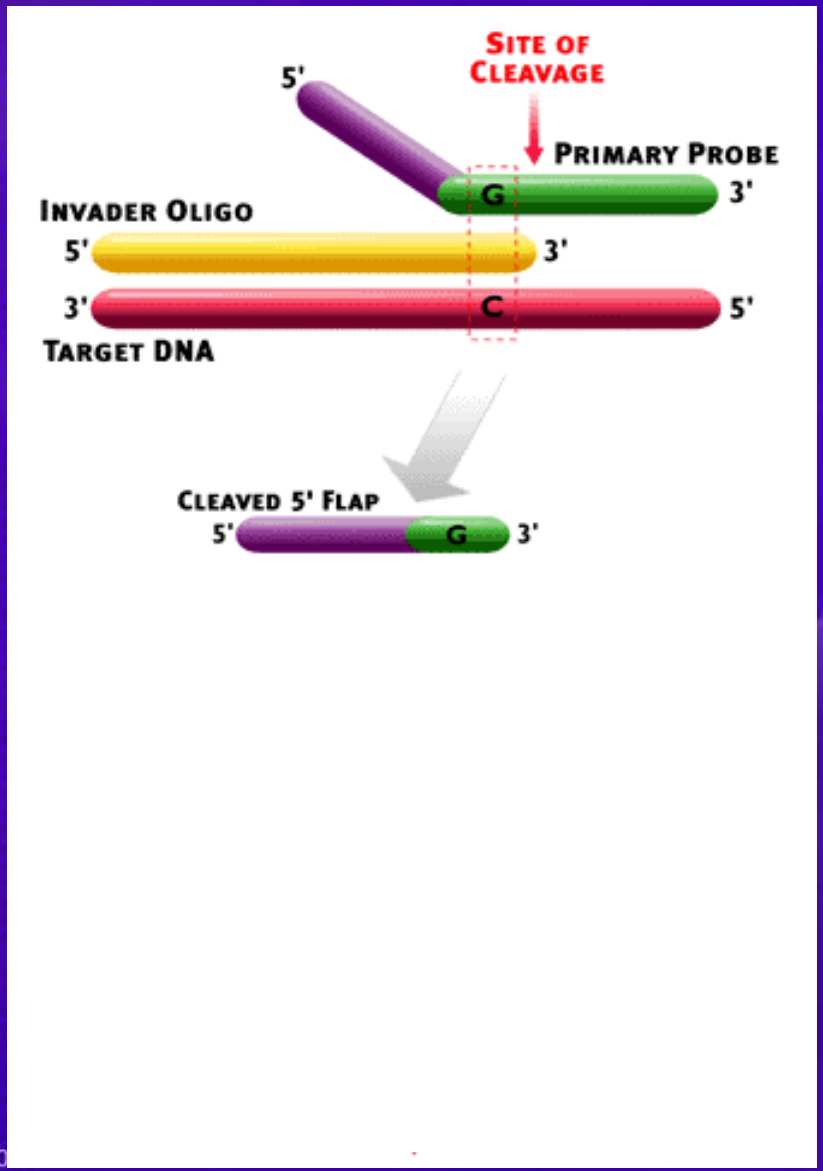
PCR Exponential Amplification



Advances in PCR Technology

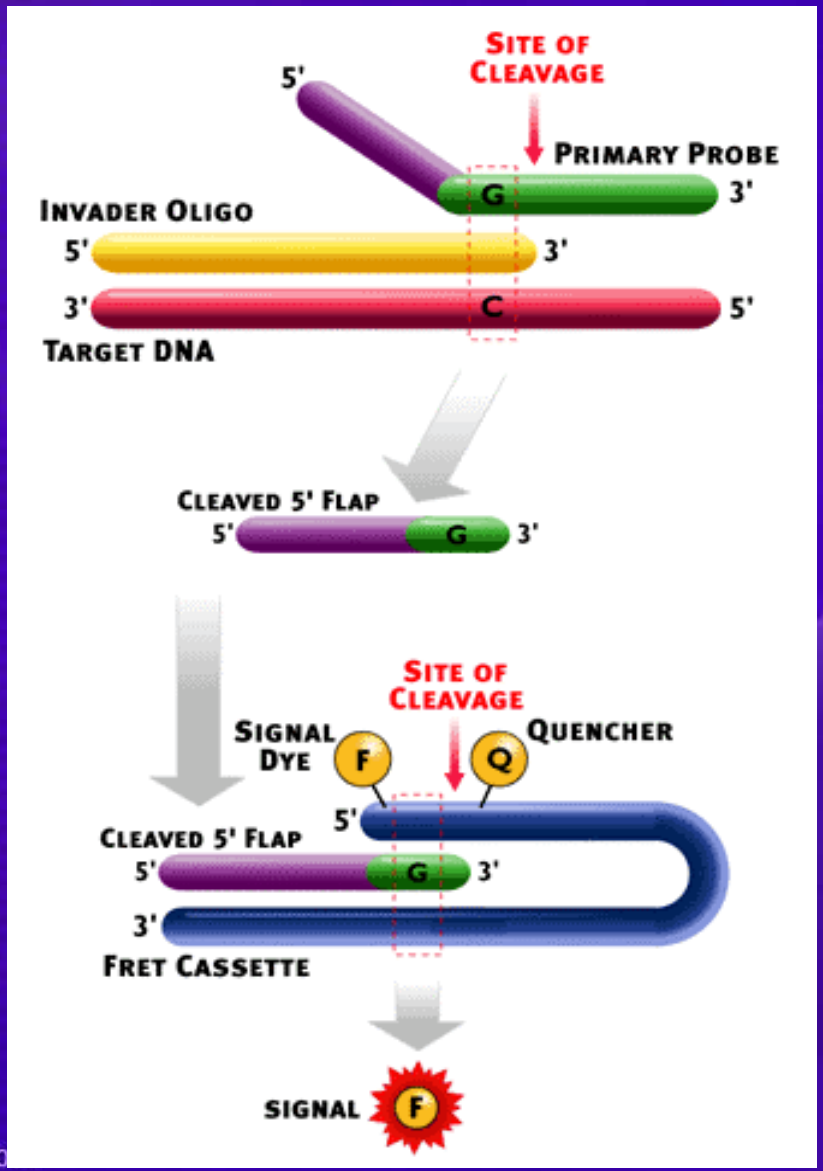
- Originally, new human DNA polymerase had to be added with each replication cycle as it was denatured by the 95°C temperature
- In 1987, polymerase was extracted from a thermophilic bacteria (*Thermus aquaticus*) found naturally in hot springs
- The PCR process is now fully automated, dramatically reducing the cost, time, and skill necessary to carry out PCR

Third Wave Invader® Assay



- Two oligonucleotide probes (100bp) bind to a target sequence, creating a flap
- An enzyme cleaves the flap
- Multiple copies of the primary probe can bind, having their flaps cleaved
- The flaps are amplified linearly

Third Wave Invader® Assay



- The amplified flaps bind to a fluorescence resonance energy transfer (FRET) cassette, creating another overlapping flap, also cleaved
- This cleavage separates the fluorophore (F) from the quencher (Q) producing a fluorescence signal

Comparison of PCR and Invader®

PCR	Invader®
Amplifies Gene Sequence	Amplifies Probe That Binds To Sequence
Exponential Amplification	Linear Amplification
Requires Minute DNA	Requires Substantial DNA
High Risk of Contamination	Low Risk of Contamination

ACE

Advantages of Invader® Technology

1. Cost Effective
2. Ease of use; low tech time
3. Accuracy, precision, sensitivity
4. Flexibility

ACE

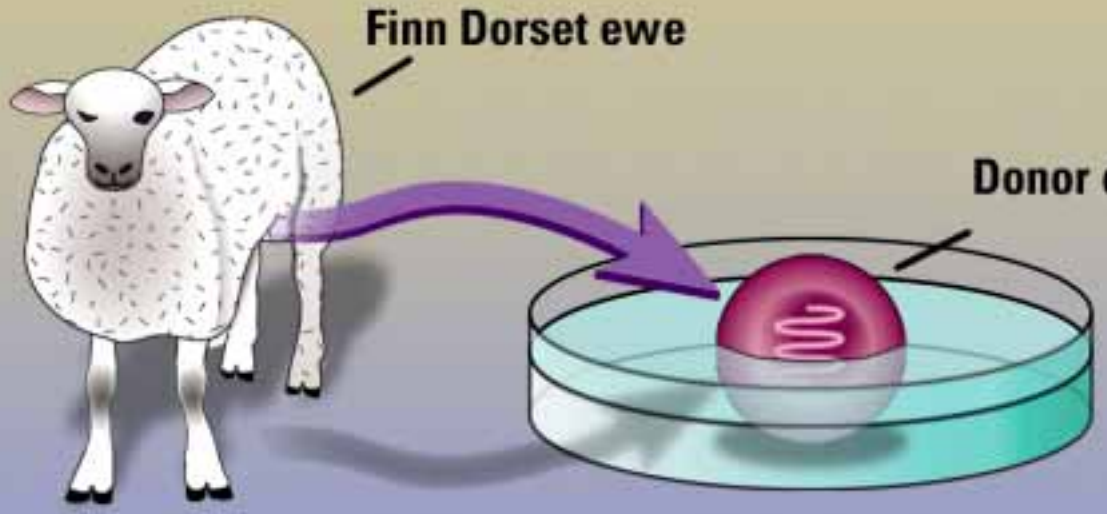
Micro Arrays AKA DNA Chips

- Affymetrix and other companies are developing silicon-coated glass wafers that can be subdivided into ~150,000 distinct locations
- In a matter of years we may be able to look for polymorphisms on >10,000 genes in a matter of minutes, and it will probably cost less than looking at 20 SNPs today

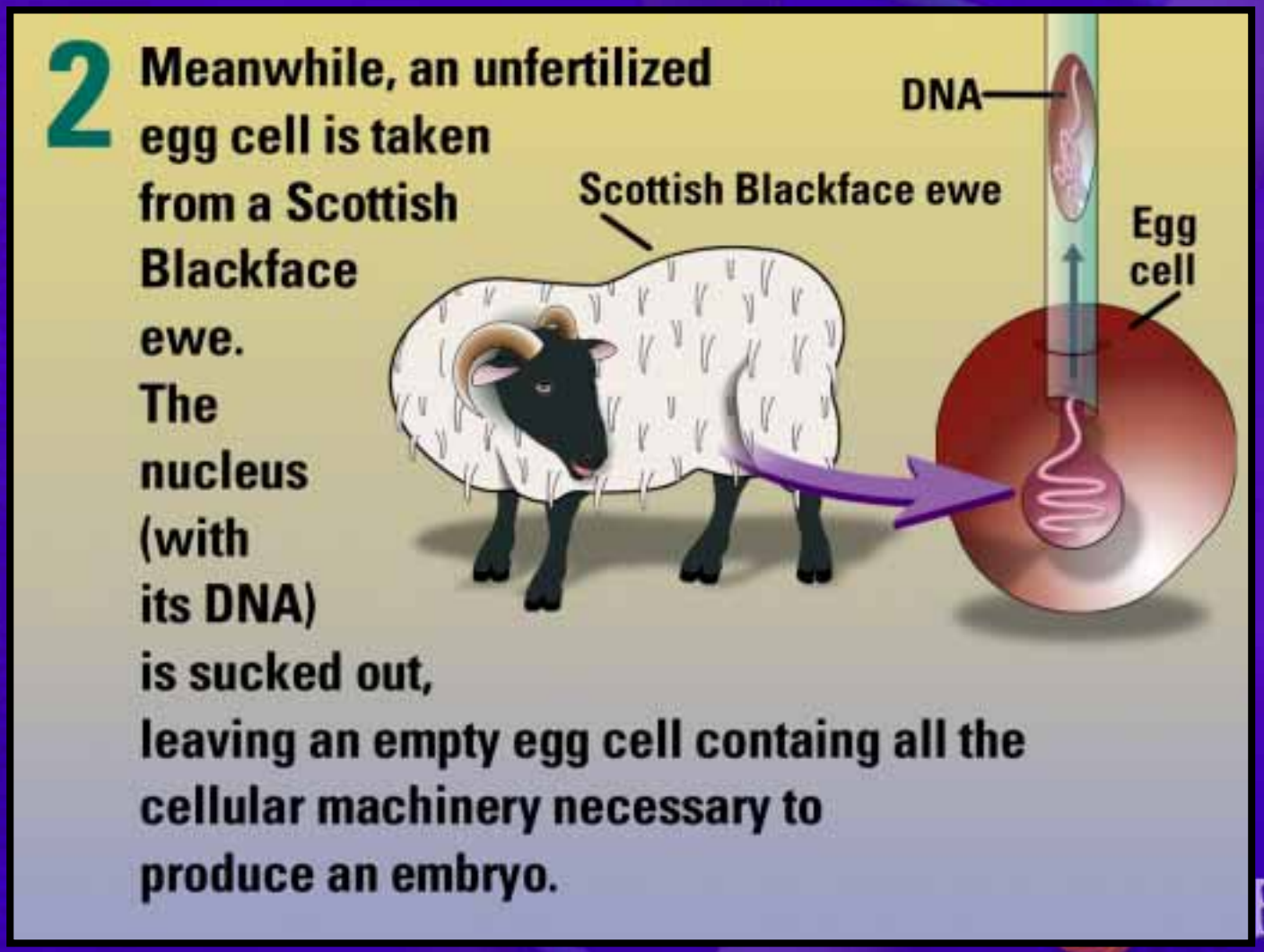
Cloning With Dolly

HOW DOLLY WAS CREATED

1 Cells taken from the udder of a Finn Dorset ewe are placed in a culture with very low concentrations of nutrients. Thus starved, the cells stop dividing and switch off their active genes.

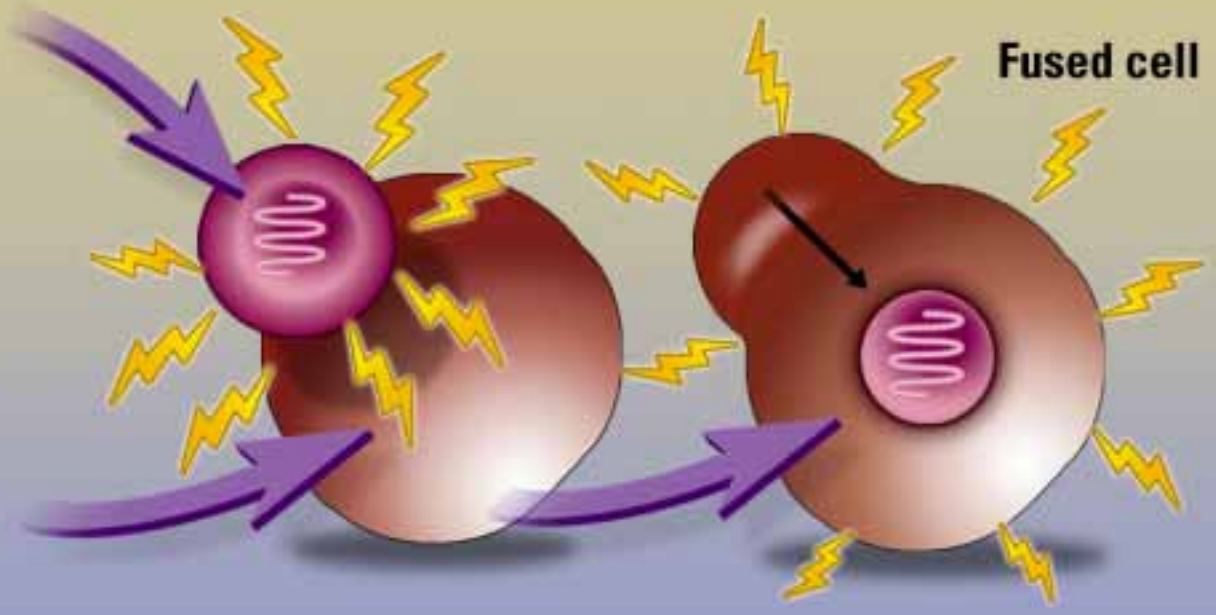


Cloning With Dolly

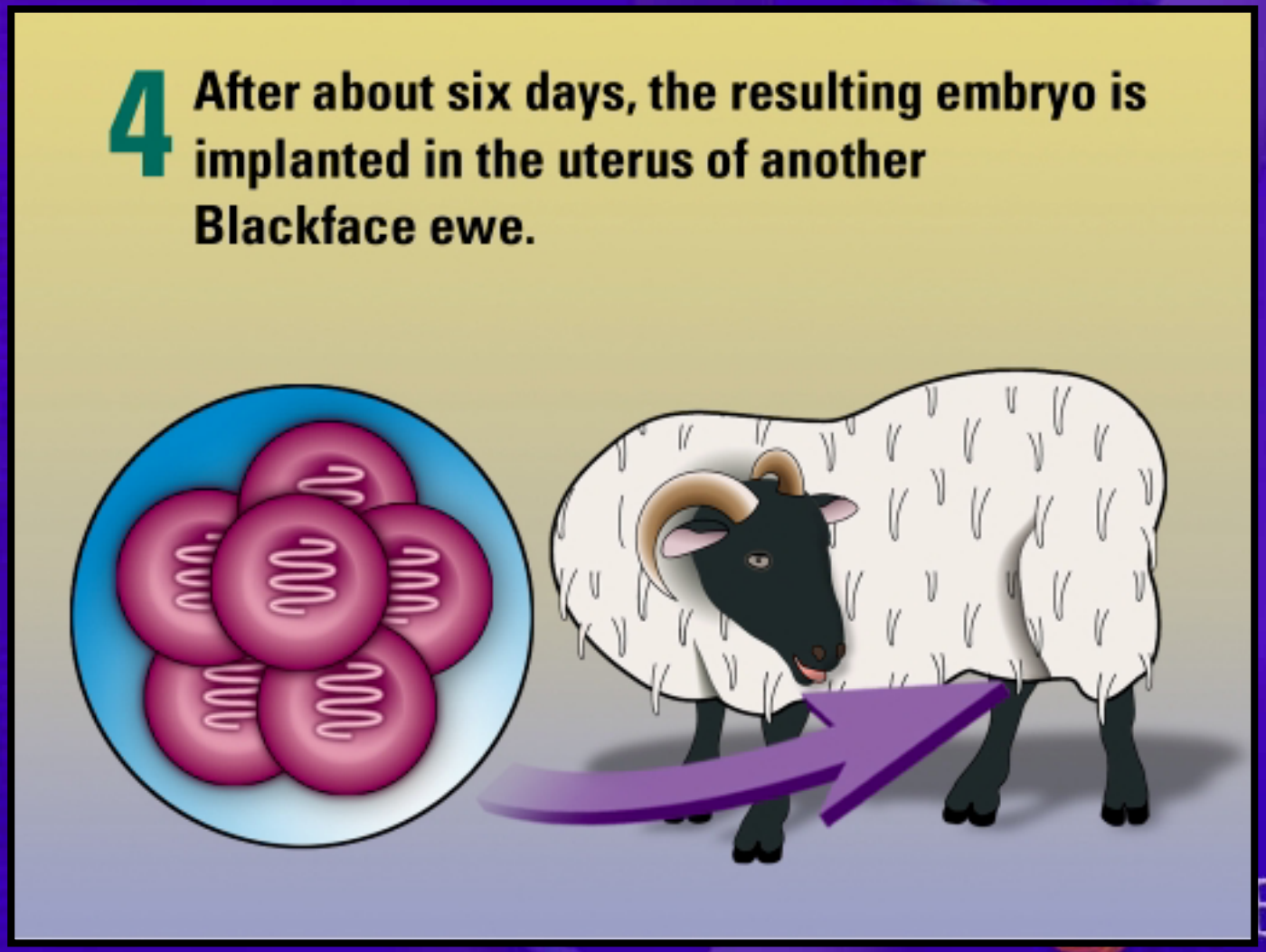


Cloning With Dolly

3 The two cells are placed next to each other and an electric pulse causes them to fuse together like soap bubbles. A second pulse mimics the burst of energy at natural fertilization, jump-starting cell division.



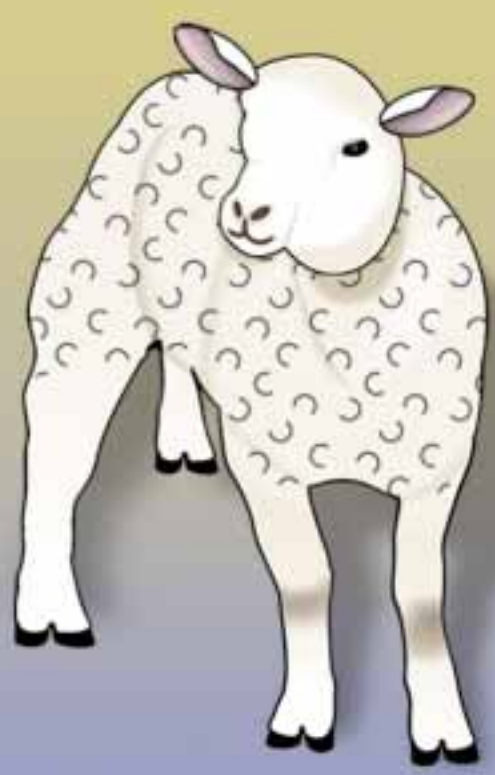
Cloning With Dolly



Cloning With Dolly

5 After a gestation period, the pregnant Blackface ewe gives birth to a baby Finn Dorset lamb, named Dolly, that is - genetically - identical to the original donor.

Finn Dorset lamb



What Dolly Really Means

- Ian Wilmut and Dolly demonstrated once and for all the pluripotentiality of mammalian DNA
- No matter how differentiated a somatic cell may become, no matter how much DNA is shut down, that DNA is still fully viable in every cell in our body


Predictive Genomics and
Functional Medicine

Variety Is the Spice of Life

- Variety is also the driving force of natural selection and of evolution
- All life on this planet “speaks” the same language, viz., DNA
- The same four “letters” write the code for all life on Earth
- In this sense, at least, all life on Earth is one

Evolution and Genetics

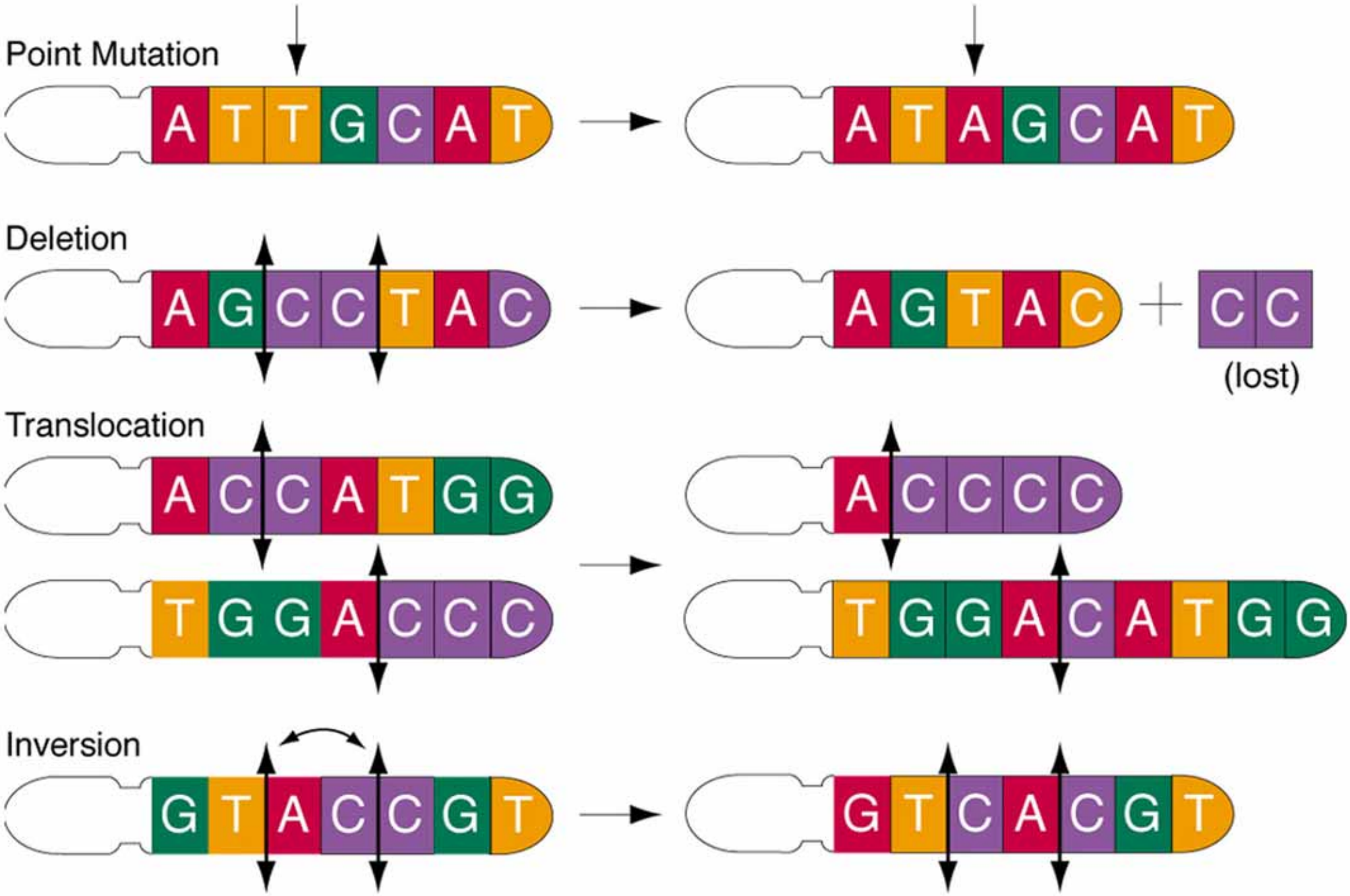
- The more diverse a species' gene pool, the more likely the species will survive and also the more likely one segment of that species will evolve into an entirely new species
- Alterations in the genetic code is the mechanism by which evolution can occur – Nature hedges her bets

Polymorphisms and Evolution

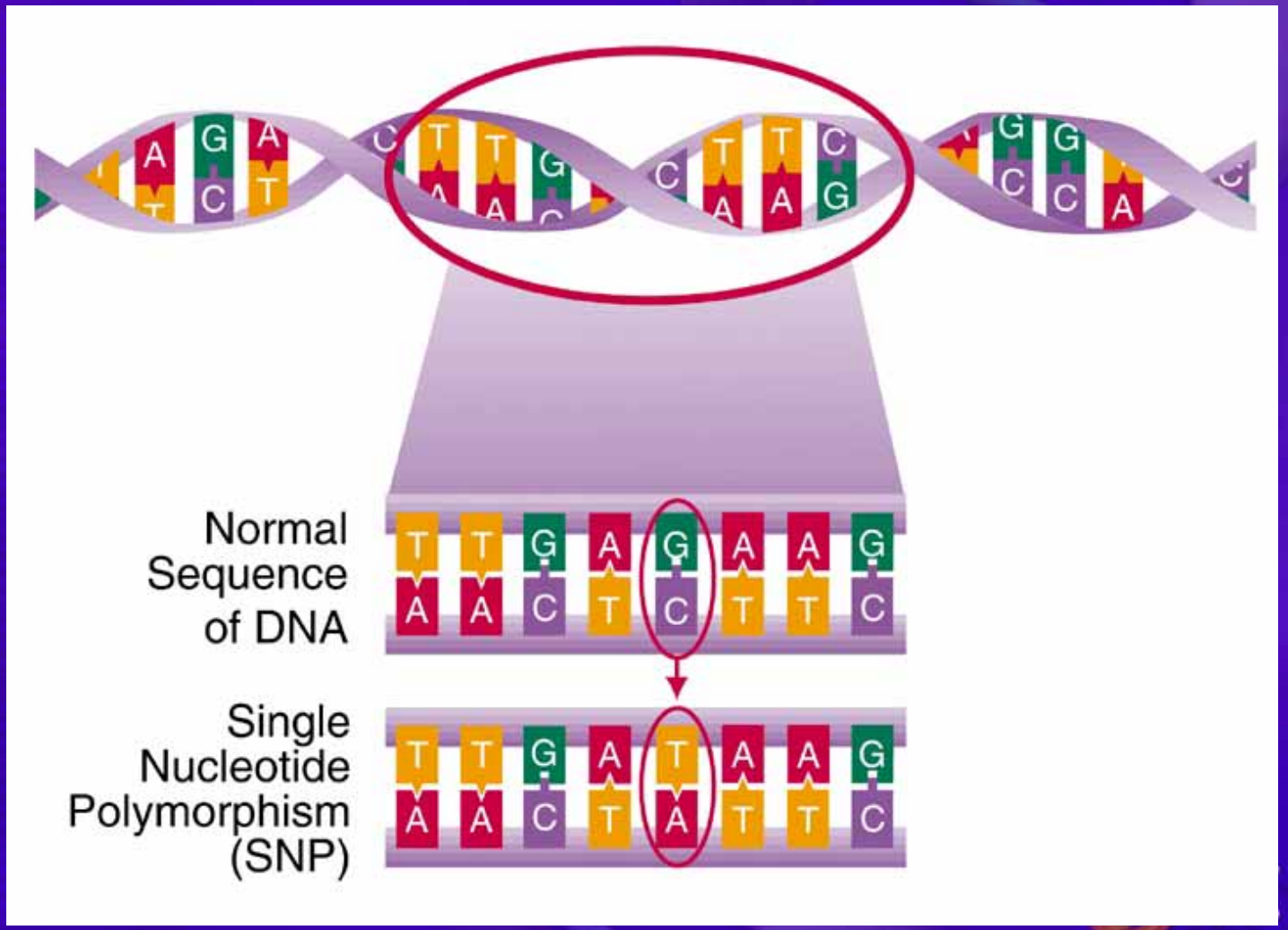
- There is no final "goal" of evolution – no such thing as "evolutionary progress"
- Natural selection merely dictates that organisms better fitted to an environment are more likely to survive and therefore more likely to pass on their genes and their traits
- Genetic variation is the mechanism by which species variation may occur

ACE

Mutations of Chromosomes



Single Nucleotide Polymorphism SNP



Scope of Polymorphic Variation

- 3 billion nucleotides in the human genome
- 40,000+ genes in the human genome
- Each gene is an average of 3,000 nucleotides long
- To date, ~100,000 SNPs have been identified, though there are likely to be 10?, 20?, 100? times as many

Mutation to Polymorphism

- By convention, we move from "mutation" to "polymorphism" when a unique genotype has a 1% prevalence in the population
- Many polymorphisms, however, can affect a significant % of the population

Gene Structure and Function

Altered genes mean altered proteins

Altered proteins mean altered functions

Altered function may be

1. Beneficial
2. Neutral – Harmless
3. Harmful
4. Beneficial in some environments and harmful in others

Thrifty Genotype

- D L Coleman first proposed the idea of a thrifty genotype to explain why Pima Indians were prone to obesity and diabetes
- Survival selected for genes that made this group incredibly efficient at retaining calories from food – a distinct adaptive advantage when food supply was scarce
- However, now with food always abundant, their genes are less well adapted

Coleman DL. Diabetes and obesity: thrifty mutants? *Nutr Rev* 1978 May;36(5):129-32.

Landsteiner Blood Groups
A, B, AB, O

- ABO blood groups result from variation in the enzyme galactosyl transferase coded for by 1,062 base pairs within a gene that is ~18,000 base pairs long
- A and B only differ from one another by 7 nucleotides, three of which are silent, leaving four points of a functional polymorphism

Landsteiner Blood Groups
A, B, AB, O

- O differs from A by a deletion at the 258th nucleotide, but this results in a frame shift and nearly every subsequent amino acid in the resulting protein is different

Adaptive significance of A, B, and O

Are these polymorphisms equally harmless and neutral from the perspective of natural selection

Are there specific adaptive advantages to the various blood groups?

Yamamoto F, Clausen H, et al.. Molecular genetic basis of the histo-blood group ABO system. *Nature* 1990 May 17;345(6272):229-33.

ACE

Blood Type and Cholera

Type O: very susceptible to cholera

Type B: somewhat resistant

Type A: more resistant still

Type AB: virtually immune to cholera

On the Other Hand…

- Type O is more resistant to malaria and syphilis, and is less likely to get various cancers
- Some A, B, and O individuals are non-secretors and are more likely to suffer from meningitis, yeast infections and urinary tract infections but less likely to contract influenza or respiratory sincitial virus

Cooke GS, Hill AV. Genetics of susceptibility to human infectious disease. *Nat Rev Genet* 2001 Dec;2(12):967-77.

ACE

General Principles:

Genetic variability over the course of time often has a lot to do with prevalent infectious diseases

Part of being the fittest, from the perspective of the species, is genomic variety

Opposites Attract

Claus Wederkind and Sandra Furi conducted experiments suggesting that men and women prefer the body odor of members of the opposite sex who are most *unlike* them genetically in terms of major histocompatability genes that are involved in immunological differentiation between 'self' and 'not-self' and in recognizing foreign invaders

Wedekind C and Furi S. Body odor preferences in men and women: do they aim for specific MHC combinations or simple heterogeneity? *Proc Royal Soc Lon Series B* 1997;264:1471-1479.

Opposites Attract, Except When…

Interestingly, women who were taking oral contraception showed no preference for men's smell based on MHC genotypes

Evolution and Medicine

While variety and variation may be good for the species, it may not be beneficial for the individual's health

Medicine is the art and science of restoring sick *individuals* to health, and has nothing to do with populations or natural selection or adaptive advantages

Medicine and Polymorphisms

In medicine, polymorphic variation is likely to convey greater or less susceptibility toward specific diseases by improving or impairing physiological function

Predictive Genomics

Predictive genomics is that branch of medicine which identifies polymorphisms in individuals in order to predict the likelihood of that individual developing a particular chronic disease or functional imbalance given a particular “environment”

Predictive Genomic Profiles

For clinical utility, predictive genomic profiles should gather together as many relevant polymorphisms as possible

The more we know, the more we can do for our patients and tailor our treatment protocols to their unique genomic strengths and weaknesses

ACE

Chronic Disease Manifestation

For most chronic diseases, three elements can play a significant role in their pathophysiology

1. Multiple genes affect the basic physiology
2. Each gene has variable penetrance
3. Environmental modulation of each gene's expression

Four Criteria for Clinical Utility of Polymorphisms in Predictive Genomic Testing

1. Relevant – the only polymorphisms in the genome of interest are those that exert a significant effect on our biochemistry and physiology

Four Criteria for Clinical Utility of Polymorphisms in Predictive Genomic Testing

2. Prevalent – given our current knowledge of the human genome, only polymorphisms that exist in a significant percentage of the population are likely to be identified and evaluated in a cost-effective manner

ACE

Founder Effect

Within sub-populations there is often higher frequencies of certain alleles because that sub-population was derived from a small number of founders

Allele Frequency Variability

- Given population migrations and isolations, there is always something akin to a founder effect
- Racial groups and groups isolated geographically often express very different frequencies of common polymorphisms

Four Criteria for Clinical Utility of Polymorphisms in Predictive Genomic Testing

3. Modifiable – only polymorphisms whose effects are modifiable via reasonable clinical intervention are clinically useful
   - Diet, Nutrition
   - Lifestyle
   - Supplements
   - Pharmaceuticals

Four Criteria for Clinical Utility of Polymorphisms in Predictive Genomic Testing

4. **Measurable** – our genes do not change but our functional physiology and metabolic reserve do change

The progress of our clinical interventions for risk reduction and functional improvement must be measurable

Functional laboratory testing is the primary vehicle by which these changes may be measured.

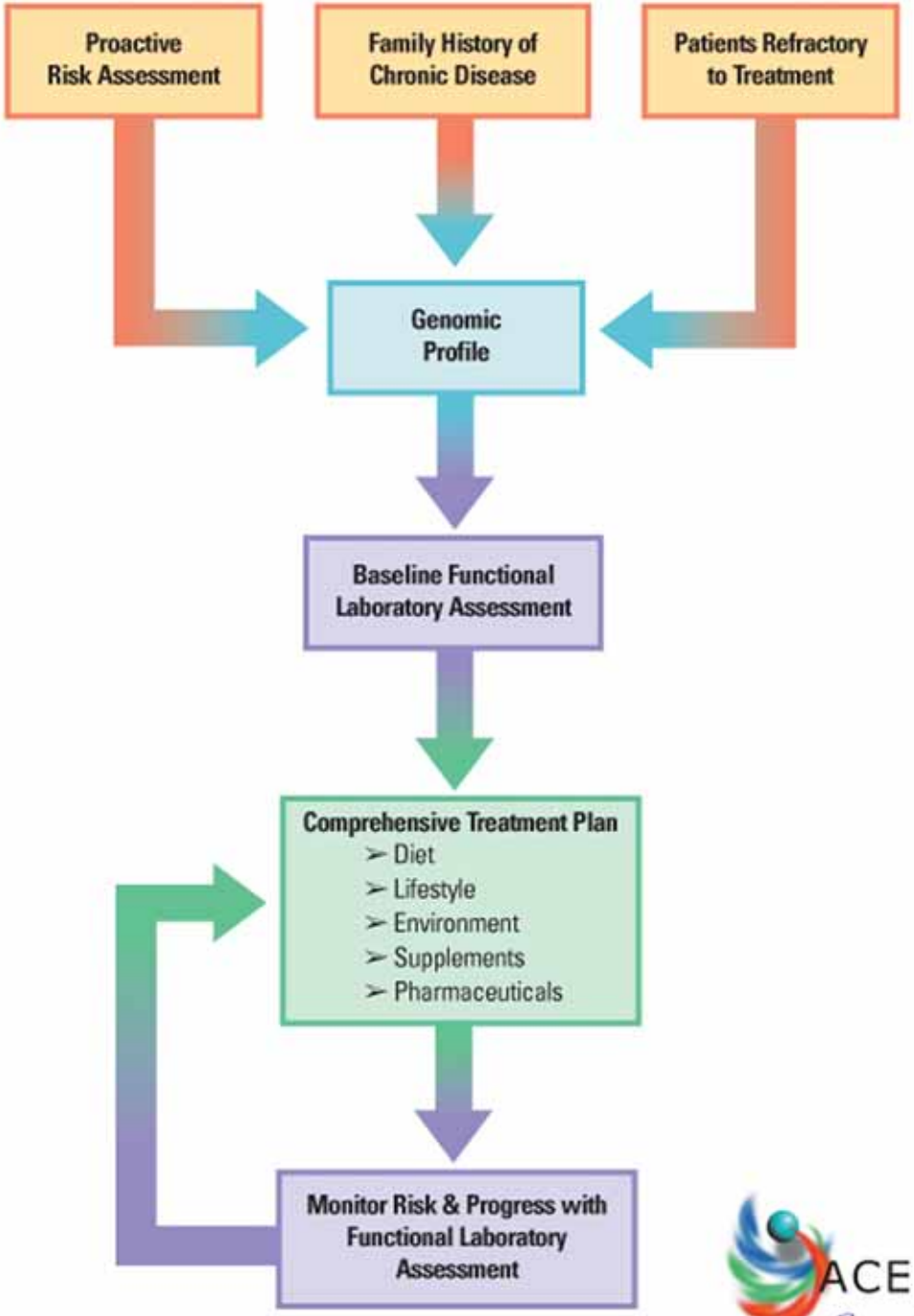
Four Criteria for Clinical Utility of Polymorphisms in Predictive Genomic Testing

1. Relevant
2. Prevalent
3. Modifiable
4. Measurable

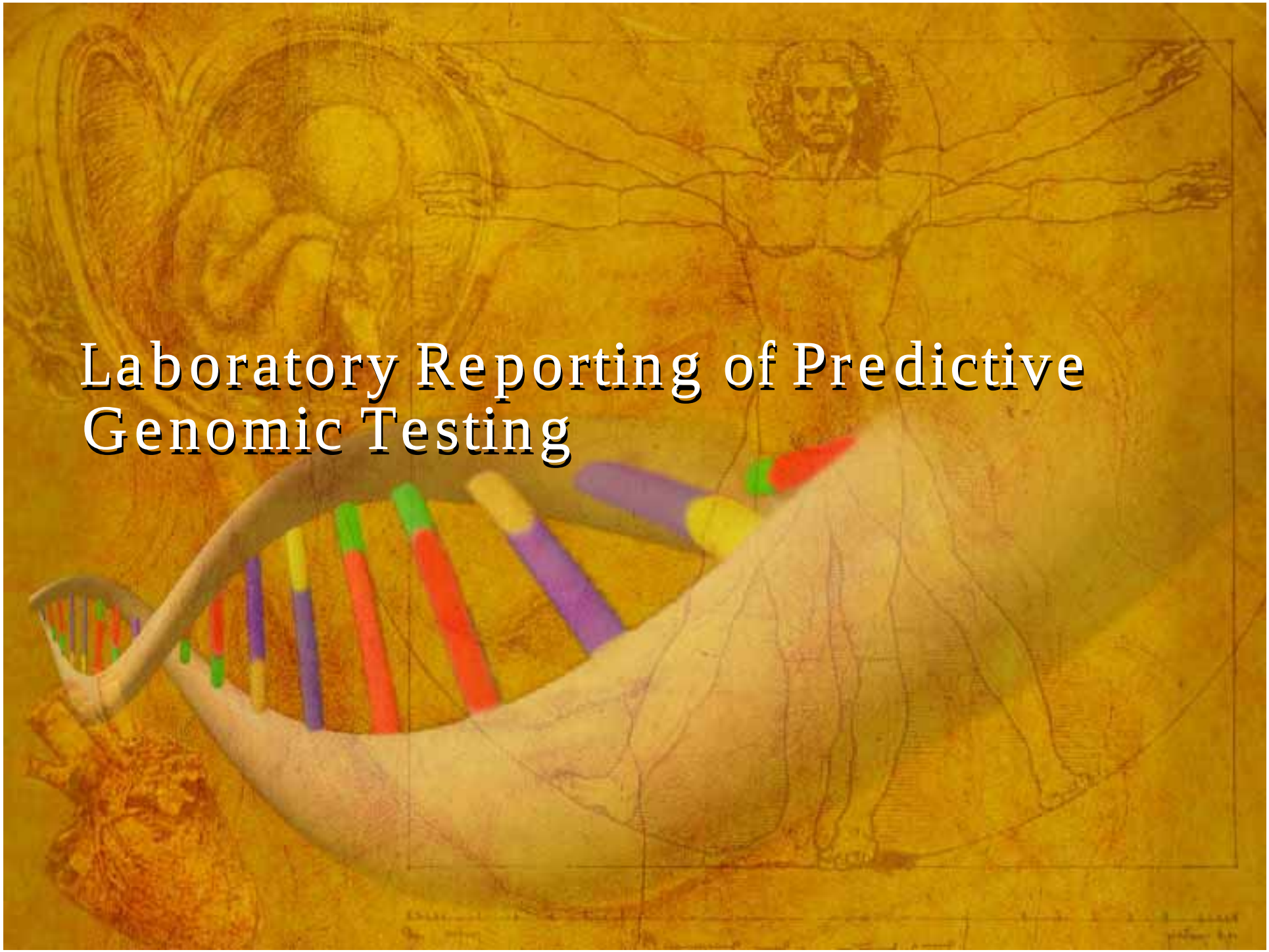
Who Benefits From Predictive Genomic Testing?

1. Proactive patients who want to minimize their risk and optimize their health
2. Patients who have a family history of a disease, like heart disease, colon cancer, osteoporosis, etc.
3. Patients refractory to normal treatment (difficult and challenging cases)

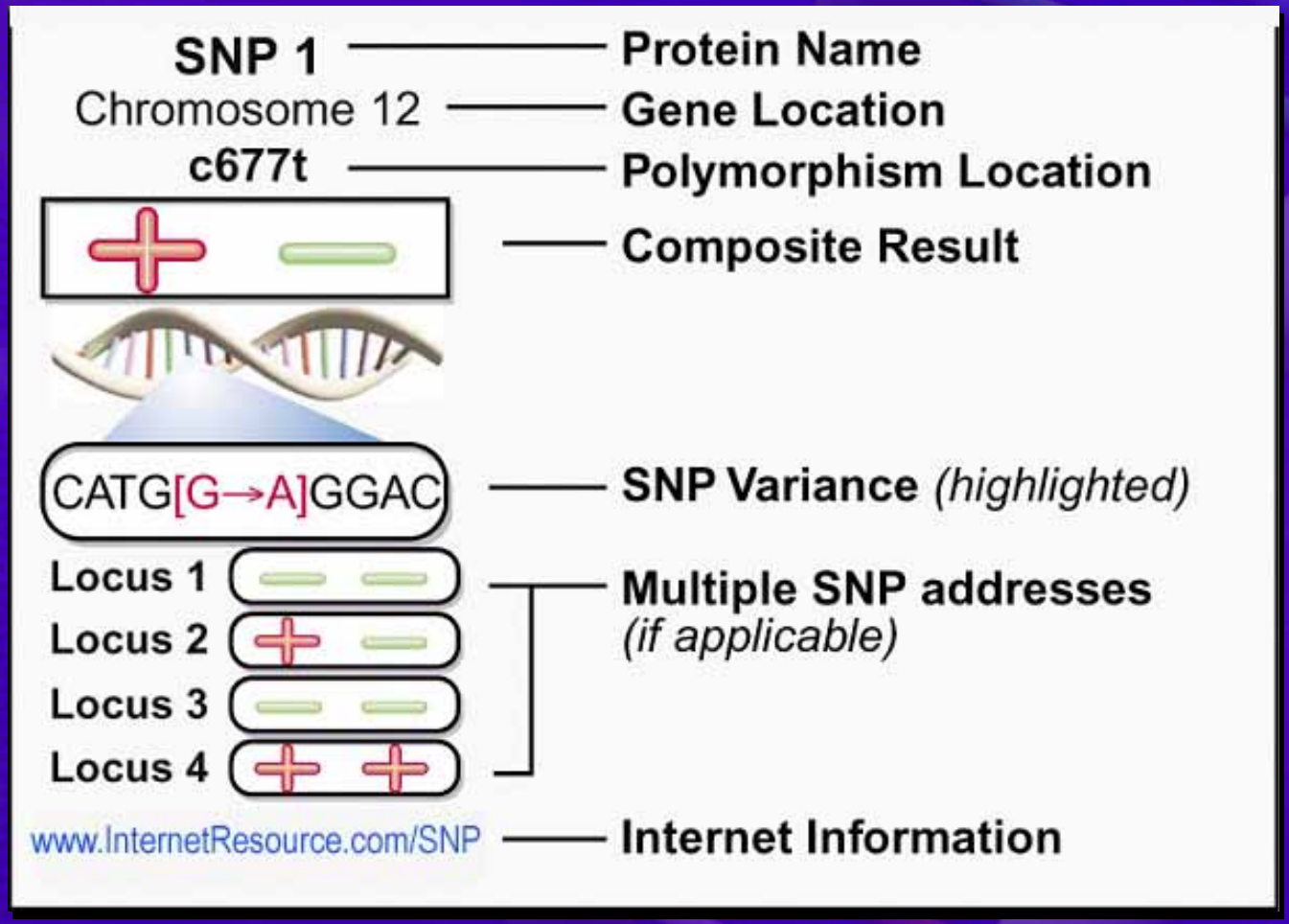
Predictive Genomics and Functional Medicine



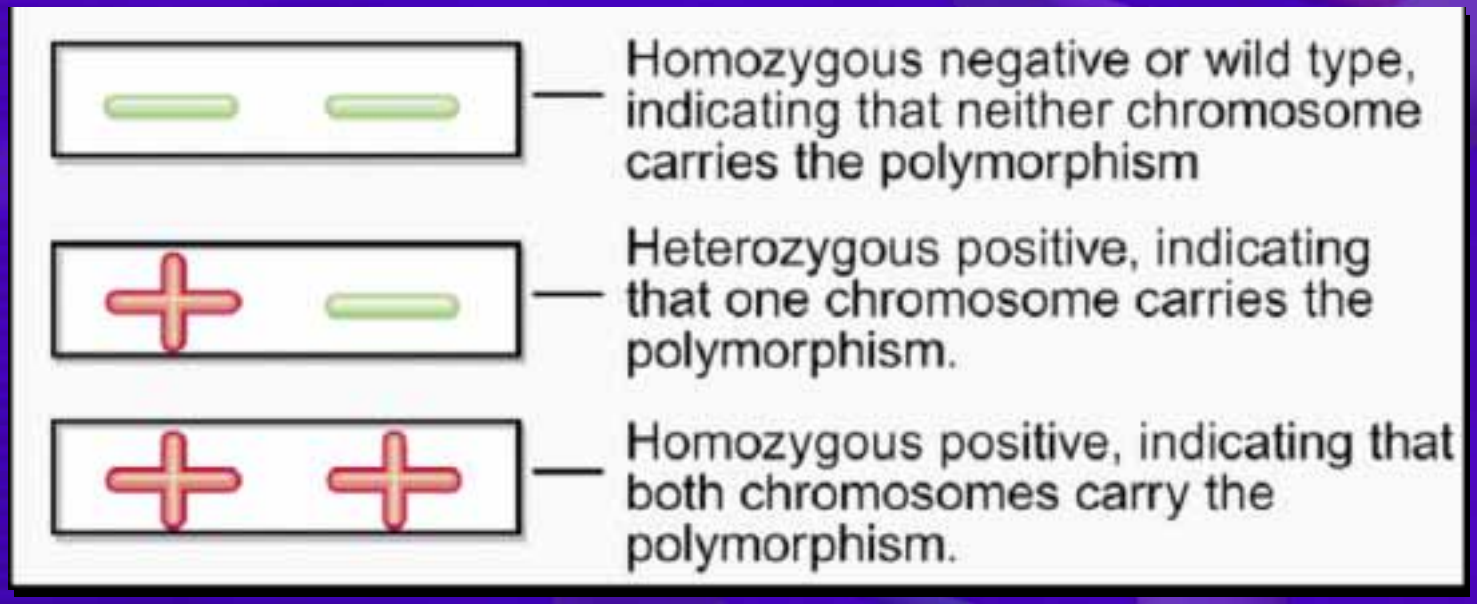
ACE
Genomics
In Primary Care Medicine


Laboratory Reporting of Predictive
Genomic Testing

SNP Identification



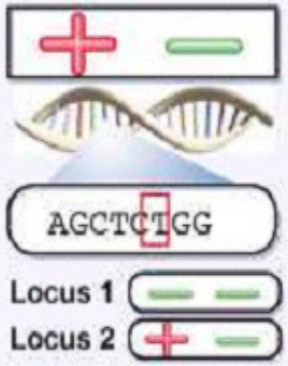
Genotype Identification



Comprehensive Commentary

Inflammation

IL-1RN
Chromosome 12
c677t



www.resource.com/cypla2

HEALTH IMPLICATIONS: Interleukin-1 receptor agonist (IL-1RA) is a naturally occurring competitive inhibitor of IL-1a and IL-1b- a more prolonged and severe inflammatory conditions like atherosclerosis, osteoporosis, P also confers benefit when fighting infections or cancer through amplified immune vigilance.

Health Implications

MINIMIZING RISKS: Eat a diet rich in anti-oxidants (colorful fruits and vegetables). Increase consumption of cold-water fish, like salmon, and reduce intake of vegetable oil and fatty meat. salmon, and reduce intake of vegetable oil and fatty meat. uction. Niacinamide and other anti-inflammatory min (tumeric) may mediate the pro-inflammatory effects press IL-1 levels.
Corticosteroids and cyclosporin A inhibit IL-1 production but with significant immune suppression and numerous other side-effects.

Minimizing Risks

FURTHER EVALUATION: IL-1RA defects lead to increased inflammatory tendencies throughout the body. Consider laboratory sorption (deoxypyridinoline), and for digestive gnizant that there is also an increased risk of

Further Evaluation

ACE

Making Sense of Genetic Gibberish

Rapid Change → Confusion

- The rapid development of genetic technologies means that we're often comparing apples to oranges
- The explosion of genomic information is happening without accepted conventions on notation
- Pure scientific research and applied clinical therapeutics have never been terribly good bedfellows

ACE

Identifying Polymorphisms

- Early research was done using restriction enzymes
- Known as restriction fragment length polymorphism, or RFLP, analysis, it looks for gross dissimilarities between genes in a population
- Results are often reported based on the restriction enzyme used

RFLP Example

- Cutting an individual's DNA with the Bsml restriction enzyme could result in the presence or absence of a fragment identified with gel electrophoresis
- Combining this result with Mendelian notation, we define the possible alleles as
- Fragment present: "B"
- Fragment absent: "b"
- Thus the possible genotypes would be BB, Bb, or bb, but these aren't really genes

ACE

Notation of Single Nucleotide Polymorphisms

The DNA is written in the language of nucleotides: A, C, G, and T

To denote a change found in a SNP, it makes sense to write the nucleotide number and the letters of the variant nucleotides

Possible notations:

A463C

463AC

463A→C

Notation of Amino Acid Substitutions

- An SNP may (or may not) result in a change in the amino acid sequence
- An amino acid change alters the shape and function of the protein
- Instead of looking at nucleotide sequence, we can look at amino acid sequence and substitutions
- Possible notations:
  - A463C
  - 463AC
  - 463A→C

Confusion Reigns – Caveat Emptor

- If we're talking nucleotides, A463C means cytosine substitutes for adenine at the 463d nucleotide
- If we're talking amino acids, A463C means, cysteine substitutes for alanine at the 463d amino acid of a protein
- Of course if one of the amino acids is other than A,C, G, or T, then there's no confusion

ACE Notations

In the interest of clarity, and hopefully to start a world-wide movement

Nucleotide changes:

463A→C or 463AC

Amino Acid changes:

A463C


Osteo Genomics
Polymorphisms Affecting Bone Health

Osteoporosis, What are the risks?

- 1/4 of all post menopausal women have osteoporosis
- 1.5 million fractures/year
- 250,000 hip fractures/year
- 1/3 of all women and 1/6 of all men will sustain a hip fracture in their lifetimes
- morbidity and mortality increase dramatically after hip fracture
  - 20% are dead within two years
  - 50% require long-term nursing home care

Functional Osteoporosis Assessment

Two critical measurements to determine functional risk:

1. Actual bone density

2. The *rate* of bone turnover

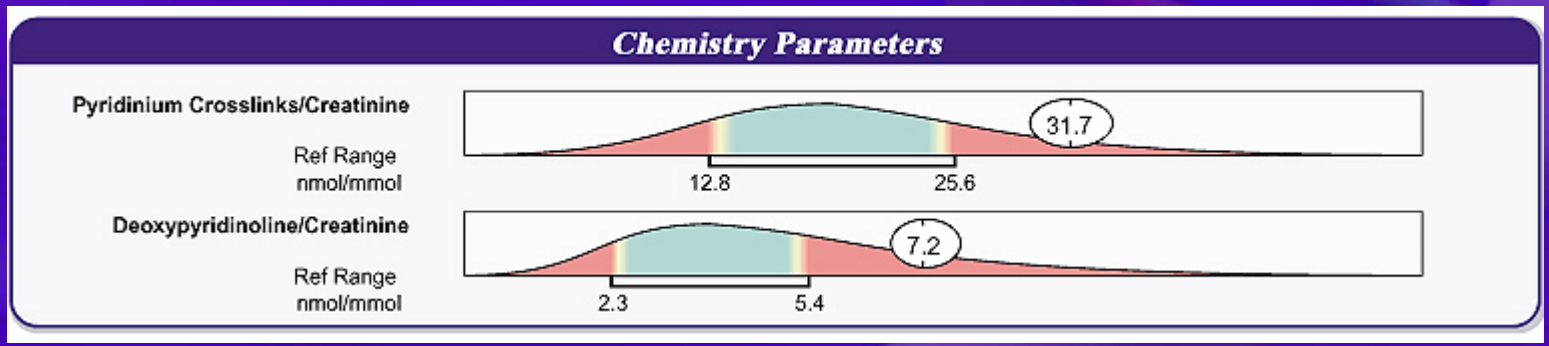
"Changing Perceptions in Osteoporosis."

T J Wilkin British Medical Journal 1999; 318:862-865

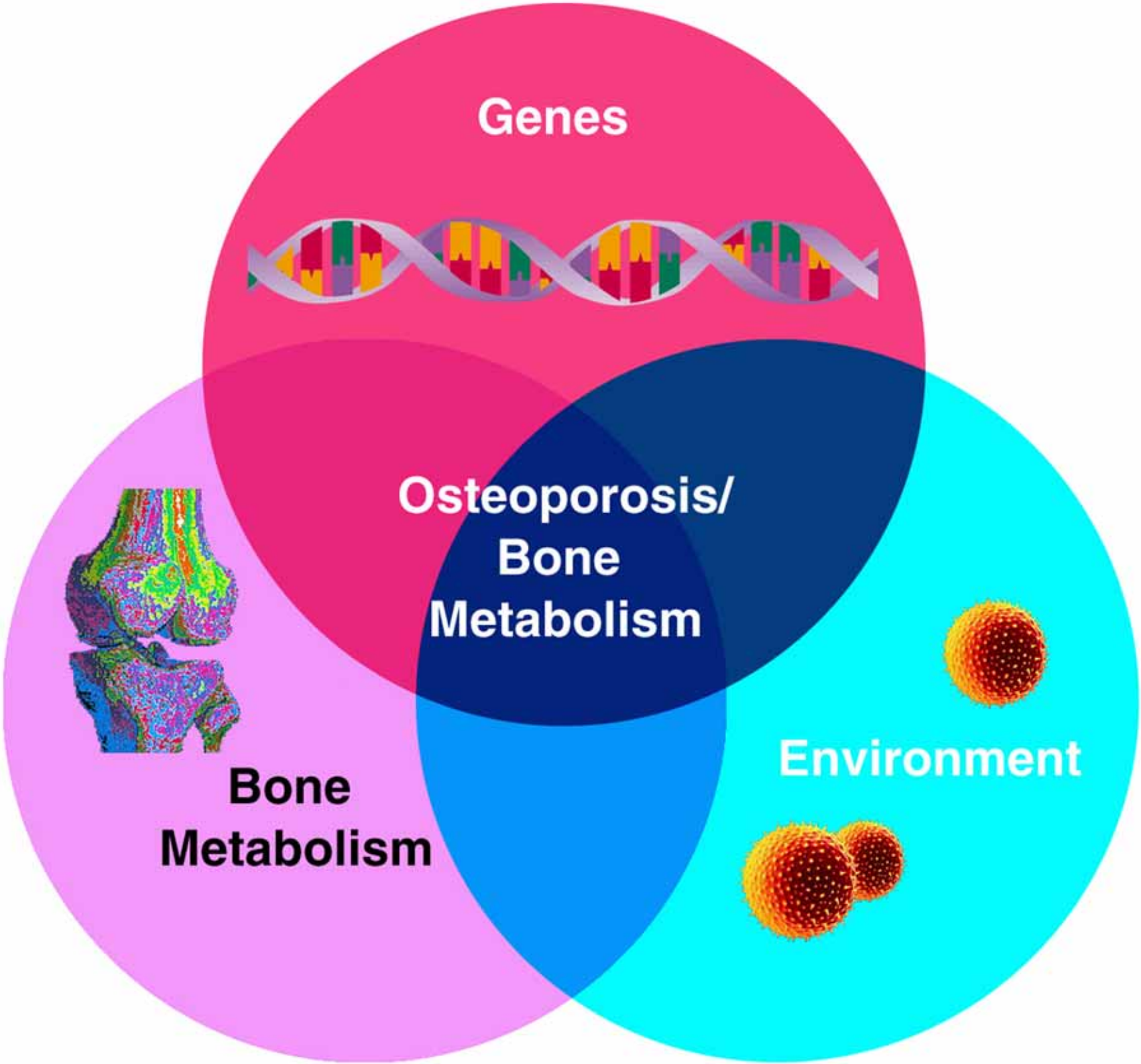
"Bone depends for strength more on its architecture [determined by turnover rate] than on its mass"

"A state of high bone turnover, rather than its prevailing mass, may be the responsive element in fracture prevention, no matter at what age it is encountered."

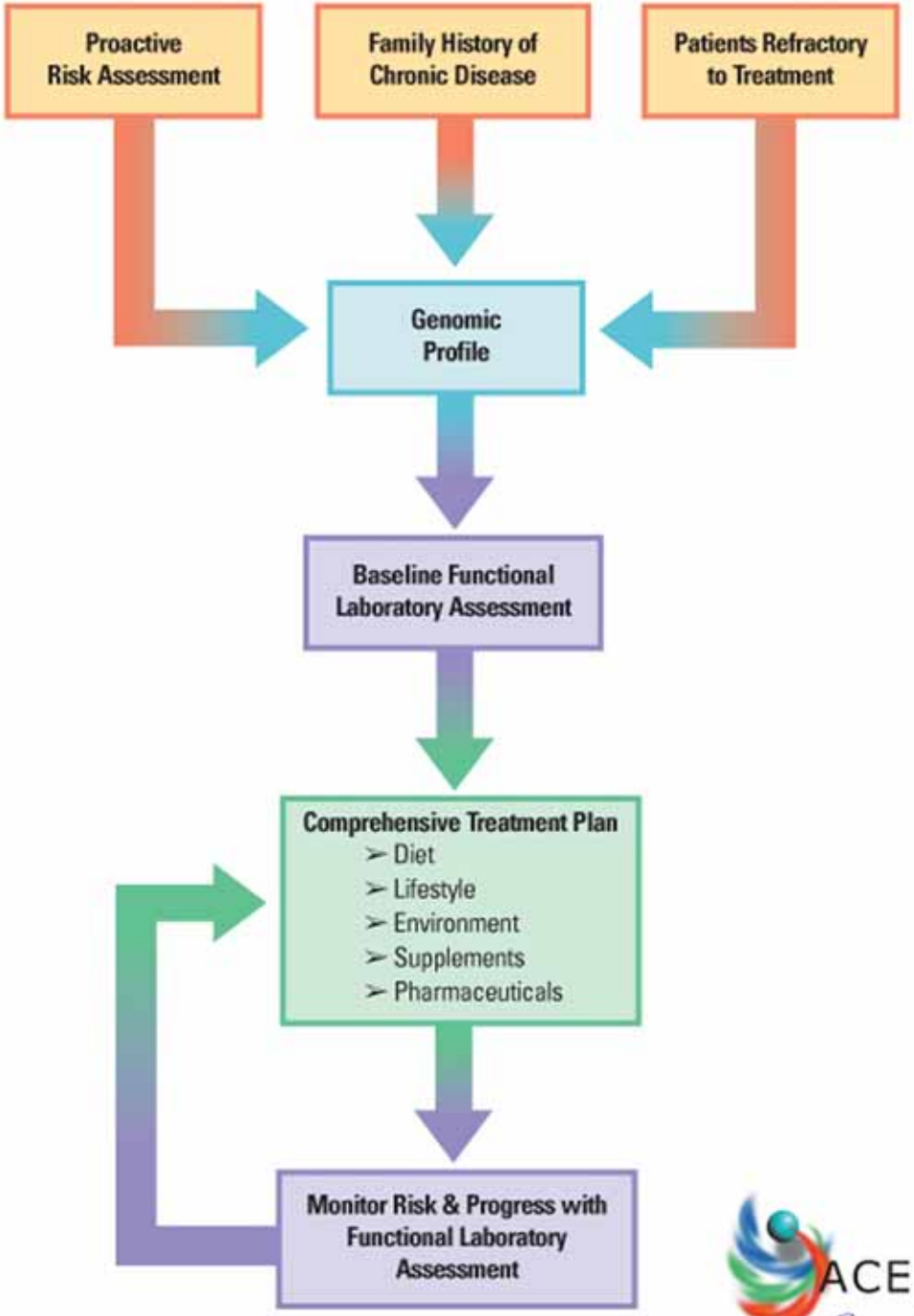
Bone Resorption Profile



Deoxypyridinoline and Pyridinium Collagen Crosslinks in Urine

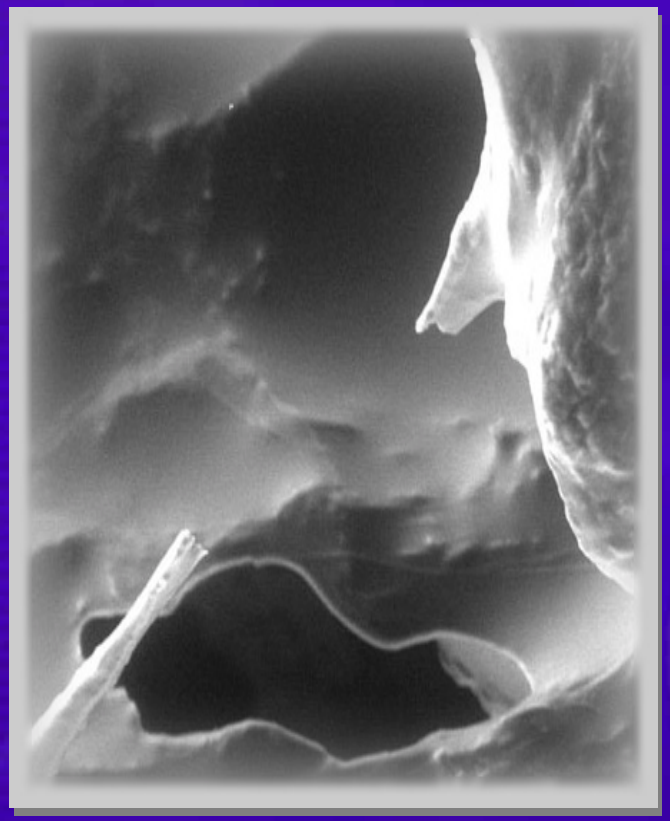

Genes
Osteoporosis/
Bone
Metabolism
Bone
Metabolism
Environment

Predictive Genomics and Functional Medicine



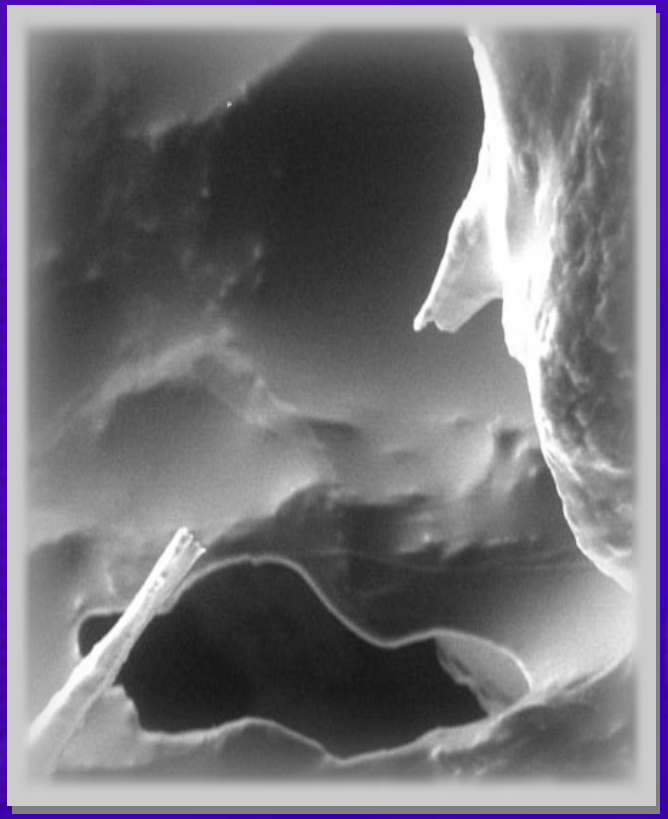
ACE
Genomics
In Primary Care Medicine

Osteo Genomic Polymorphisms



- Identifies polymorphisms associated with increased risk of developing osteopenia and osteoporosis
- Risk factors include impaired collagen synthesis, calcium metabolism, vitamin D3 activity, parathyroid hormone action, osteoclast activity, and chronic inflammation

Osteo Genomic Polymorphisms



Bone Formation Markers

- COL1A1
- CALCR
- VDR

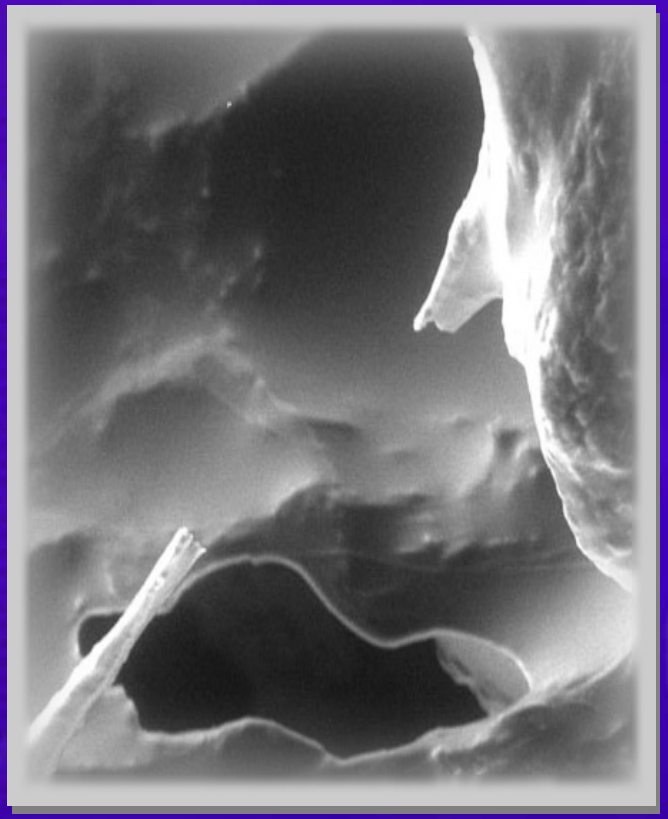
Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

Osteo Genomic Polymorphisms



Bone Formation Markers

- COL1A1
- CALCR
- VDR

Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

Bone Formation Markers
COL1A1 2046G→T

- Collagen 1, alpha-1 is the primary protein matrix used for bone synthesis.
- Polymorphisms in COL1A1 lead to mildly aberrant collagen formation that can lead to reduced bone mineral density and increased risk of osteoporosis.

Therapies to Consider for a COL1A1 2046G→T Polymorphism

- Higher levels of dietary calcium have been shown to reverse the potentially adverse effects of this SNP
- Moderate sunlight exposure or vitamin D supplementation improves calcium absorption
- Post-menopausal women with this SNP respond extremely well to estrogen replacement
- Consider "plant estrogens" like black cohosh, soy isoflavones, etc.

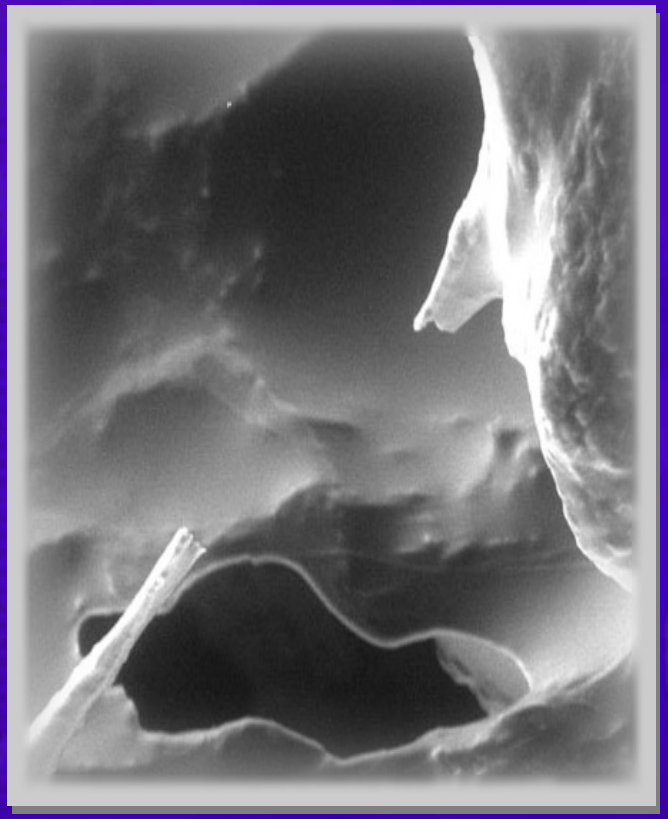
Bone Formation Markers
CALCR P463L

- The calcitonin receptor mediates the cellular action of the hormone calcitonin
- Calcitonin acts to decrease serum calcium levels by decreasing osteoclast activity and preventing bone resorption
- Polymorphisms in CALCR can lead to decreased bone density

Bone Formation Markers
VDR Bsml RFLP

- Vitamin D3 receptors mediate the actions of vitamin D3 including increasing absorption of calcium from the gut and increasing osteoblast activity and mineralization of bone
- Polymorphisms in VDR can inhibit calcium absorption and decrease bone mineralization.

Osteo Genomic Polymorphisms



Bone Formation Markers

- COL1A1
- CALCR
- VDR

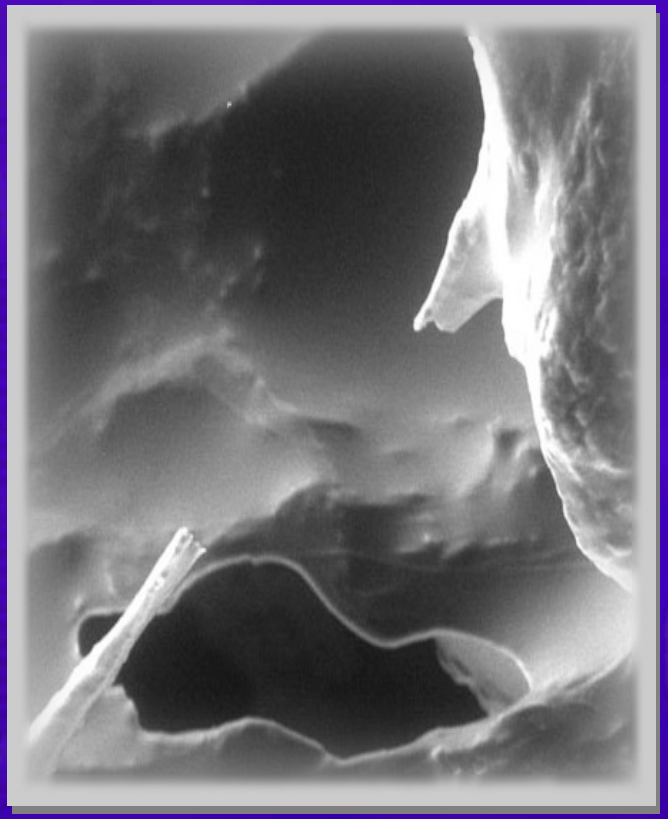
Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

Osteo Genomic Polymorphisms



Bone Formation Markers

- COL1A1
- CALCR
- VDR

Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

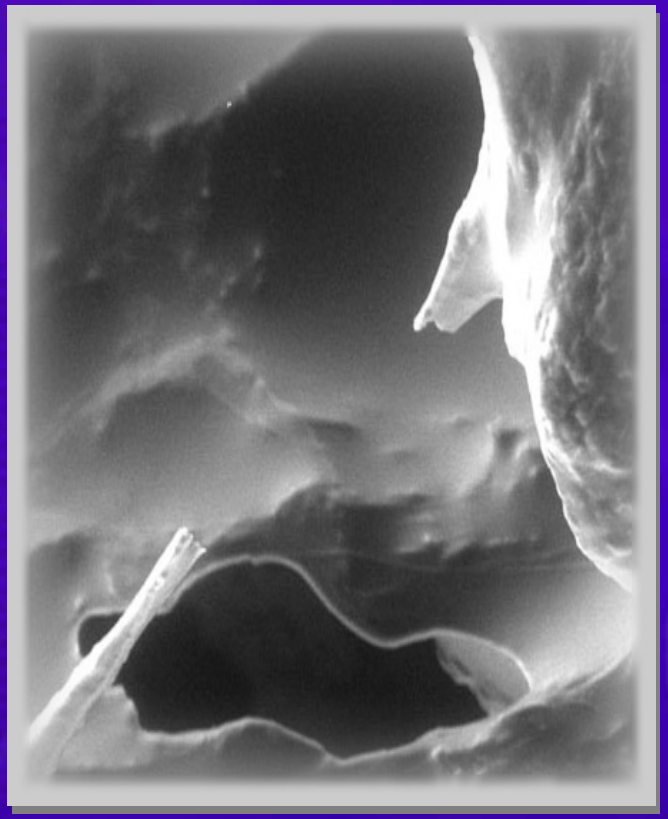
Bone Resorption
PTHR D3S1289

- Parathyroid hormone receptor mediates the actions of parathyroid hormone, including increasing serum calcium by increasing bone resorption, increasing calcium absorption and decreasing calcium excretion
- Polymorphisms in PTHR increase PTH activity and increase bone resorption, contributing to decreased bone density

Therapies to Consider for PTHR D3S1289 Polymorphism

- Vitamin D3 not only increases intestinal absorption of calcium but has a direct inhibitory effect on PTH secretion
- A minimum of 400IU/d of vitamin D from supplement or cod liver oil is recommended
- Insulin resistance exacerbates the effects of PTH on bone resorption
- Evaluation of insulin resistance is recommended, especially if central obesity is present

Osteo Genomic Polymorphisms



Bone Formation Markers

- COL1A1
- CALCR
- VDR

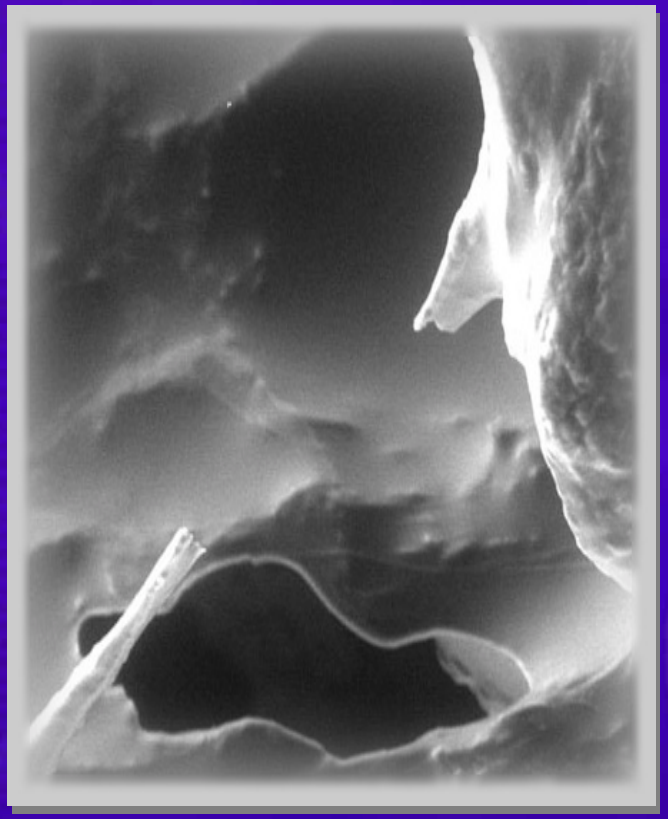
Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

Osteo Genomic Polymorphisms



Bone Formation Markers

- COL1A1
- CALCR
- VDR

Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

Inflammation
IL-1RN*2

- Interleukin-1 receptor antagonist is an anti-inflammatory competitive inhibitor of interleukins 1-α and 1-β
- IL-1RN shuts down inflammatory cascades
- Polymorphisms in IL-1RN pre-dispose an individual to chronic inflammation, including bone inflammation, which can contribute to osteoporosis

Therapies to Consider for IL-1RN Polymorphism

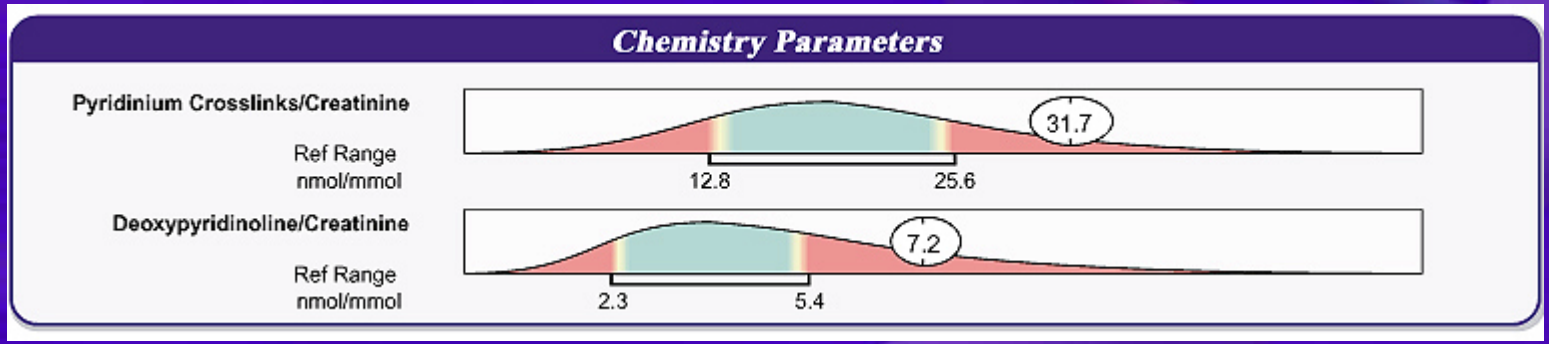
- The IL-1RN SNP prevents the normal negative feedback loop to shut down the inflammatory actions of IL-1α and IL-1β
- Fish oil and milk thistle (silymarin) supplementation have been demonstrated to inhibit IL-1 production directly
- Other anti-inflammatories like boswellia, licorice, and curcumin may also be beneficial

Inflammation
TNF-α -308G→A

- Tissue necrosis factor-alpha is a pro-inflammatory cytokine that can contribute to arthritis, asthma, and osteoporosis
- Polymorphisms of TNF-α increase TNF-α production and inappropriately activate inflammatory response

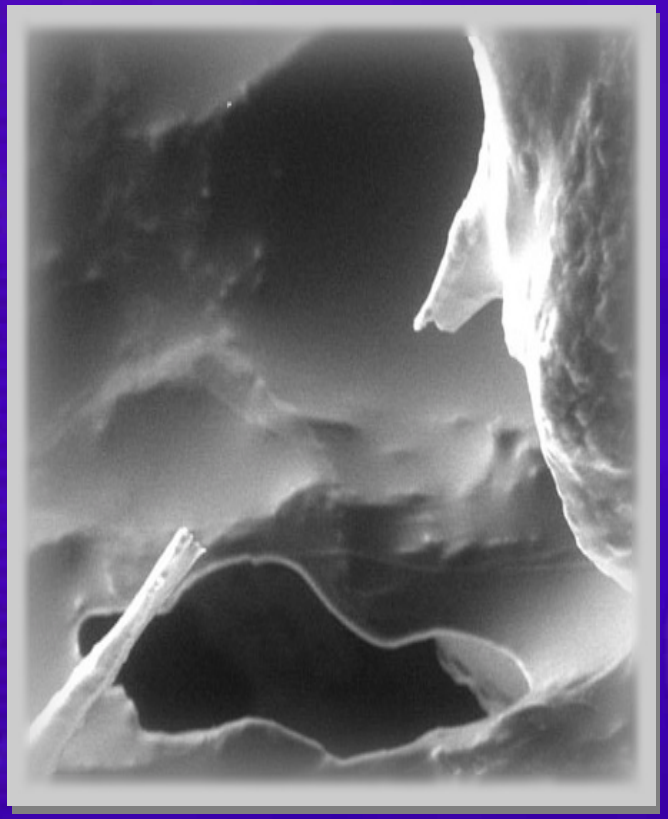
Hypothetical Case

54 y.o. Woman Concerned About Developing Osteoporosis



Doctor puts her on calcium, magnesium, a multi vitamin, isoflavones from soy, and black cohosh, but after three months, there is no change in d-pyd

Osteo Genomic Panel



Bone Formation Markers

- COL1A1
- CALCR
- VDR

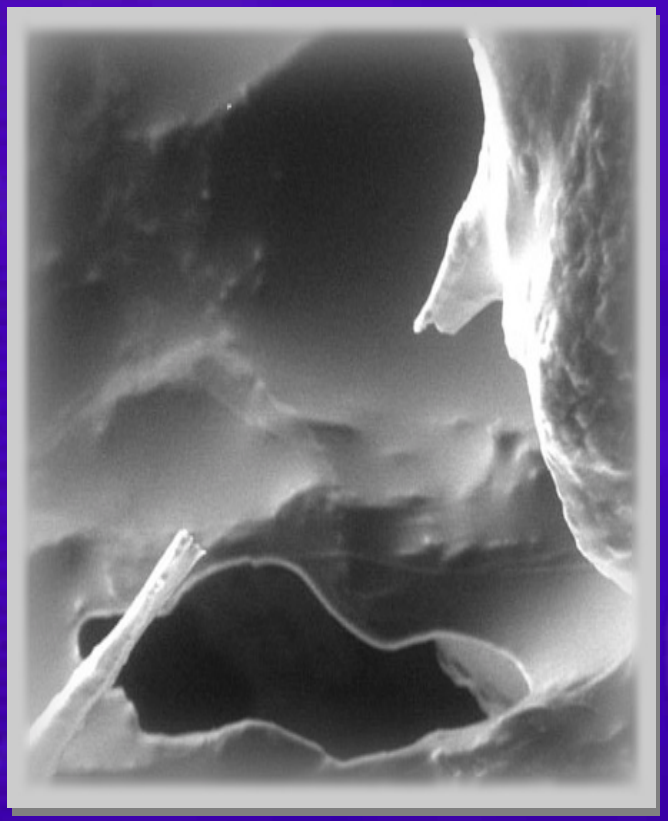
Bone Resorption Markers

- PTHR

Inflammation Markers

- IL-1RN
- TNF-α

Osteo Genomic Panel



Bone Formation Markers

- COL1A1 −/−
- CALCR −/−
- VDR −/−

Bone Resorption Markers

- PTHR +/−

Inflammation Markers

- IL-1RN +/+
- TNF-α −/−

New Treatment Plan

IL-1RN: ++

Fish Oils and Milk Thistle have been shown to inhibit IL-1 production

PTHR: +−

Vitamin D supplementation inhibits the formation of PTH

Insulin resistance exacerbates the effect of PTH on bone – evaluate for metabolic dysglycemia

Osteoporosis and Vegetables

In male rats fed 1g of dry onion per day for 4 weeks,

- bone mineral content increased 18%
- cortical thickness increased 15%
- trabecular bone density increased 13.5%

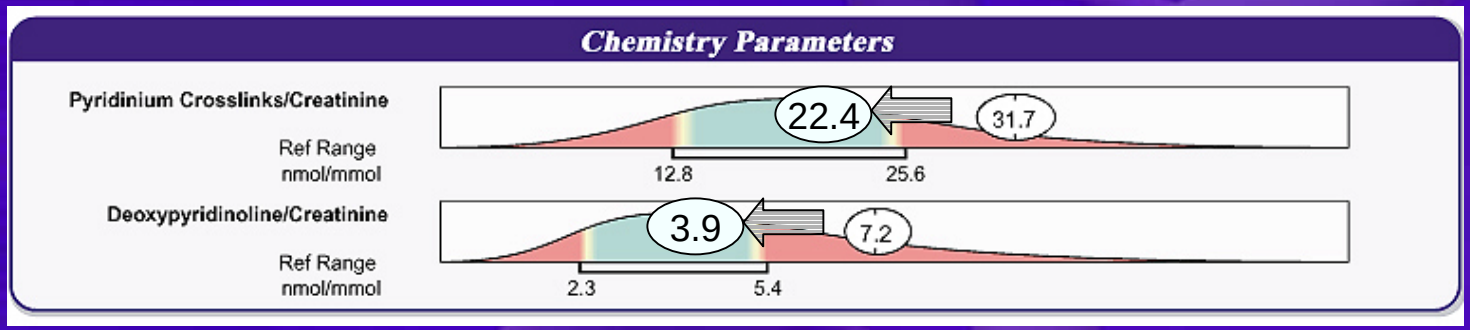
In oophorectomized females, bone resorption was decreased by 25%

Lettuce, tomato, cucumber, arugula, garlic, wild garlic, parsley, and dill showed similar, synergistic results

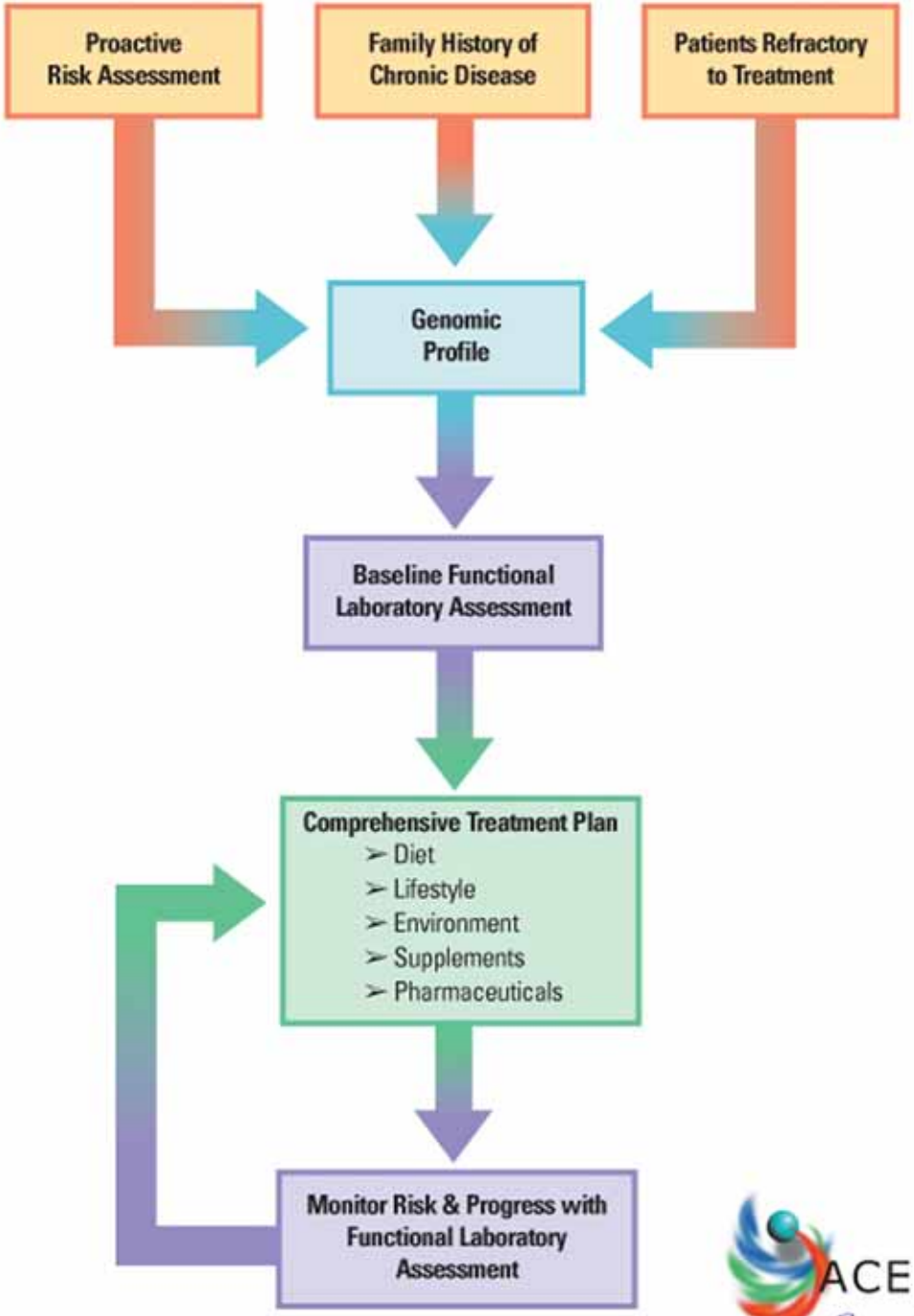
Muhlbauer RC and Li F. Effect of Vegetables on Bone Metabolism. Nature 1999;401:343-344.

ACE

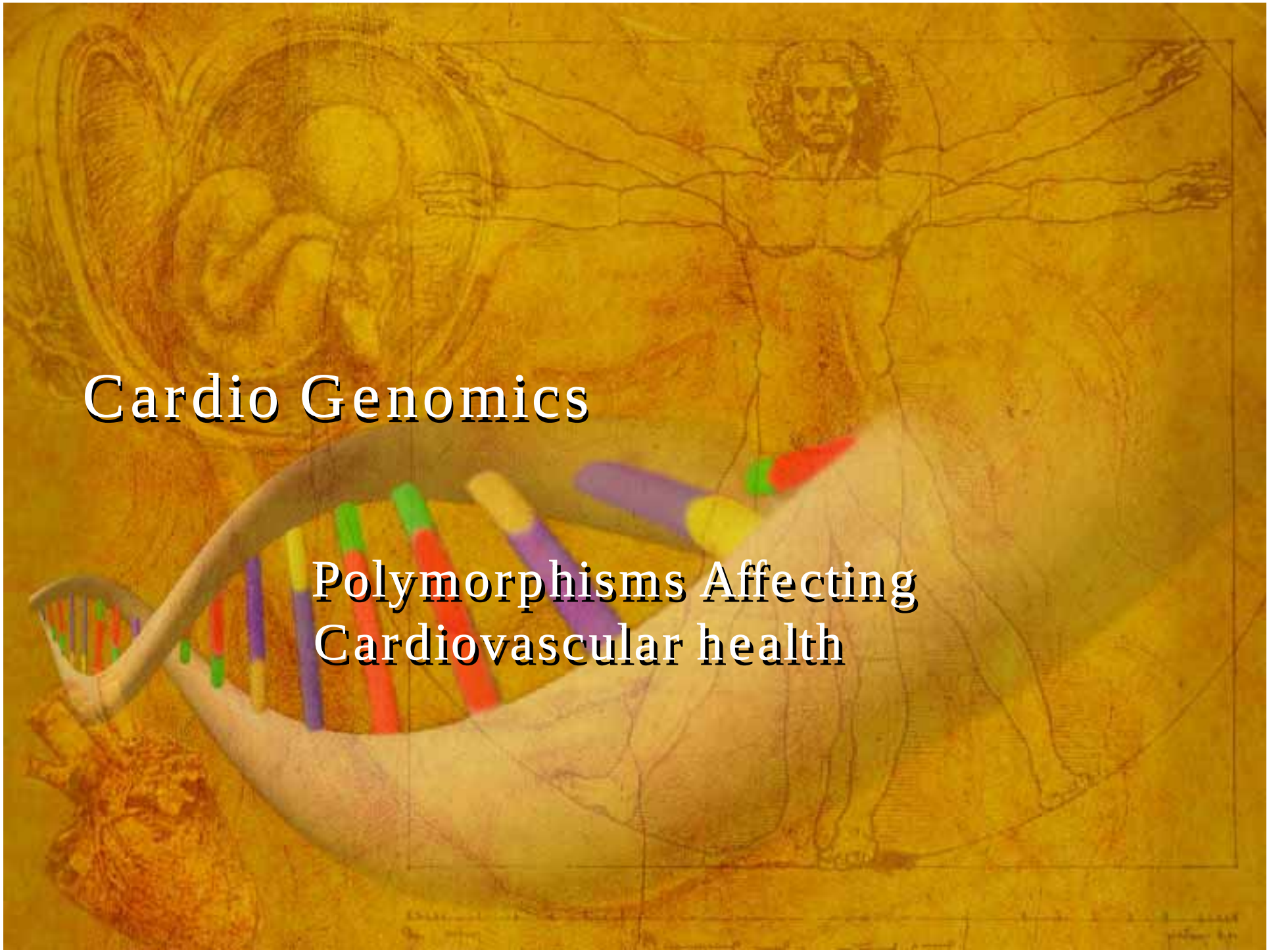
Evaluate Bone Resorption 3 Months After Starting New Treatment Regimen



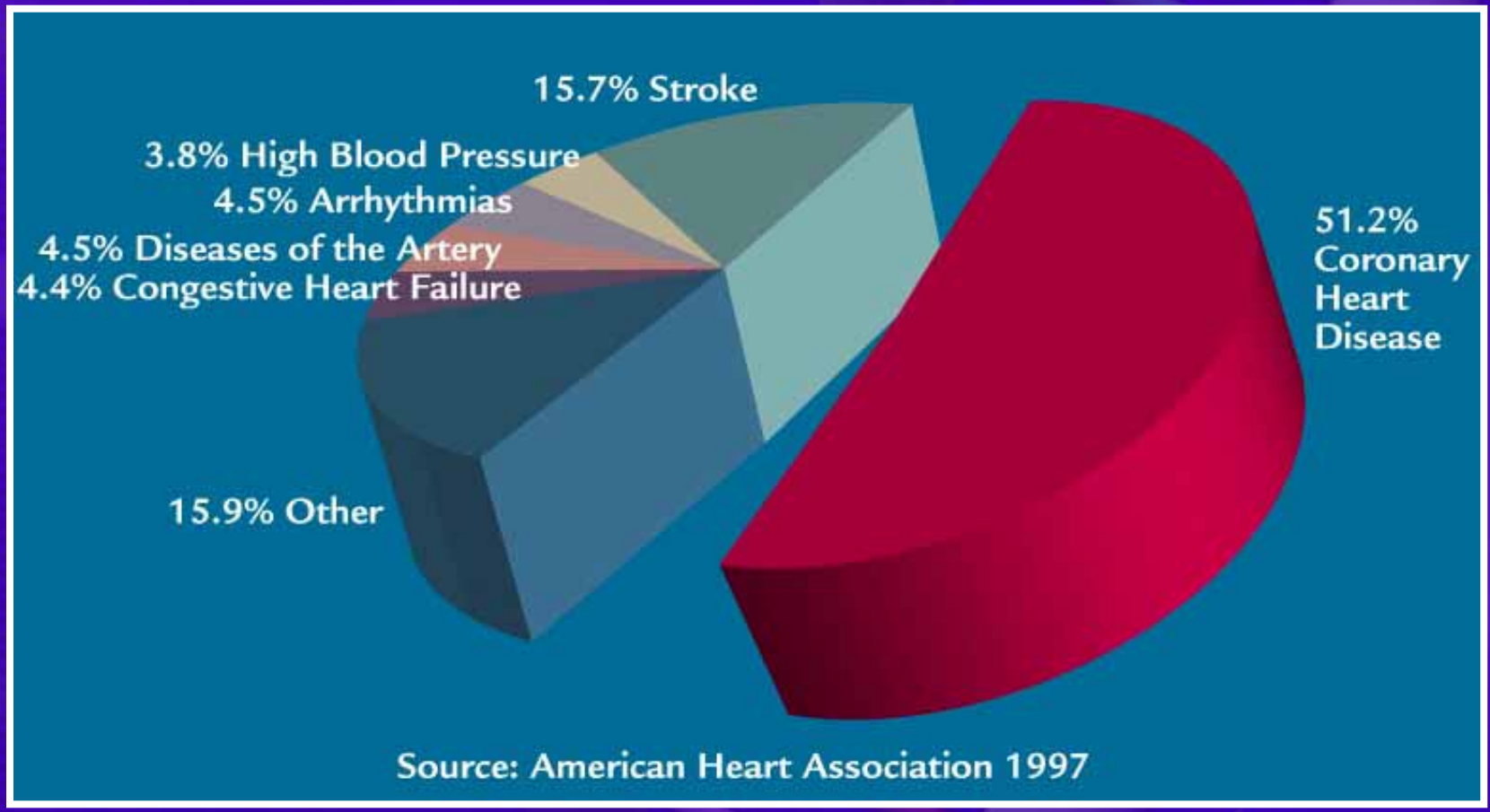
Predictive Genomics and Functional Medicine



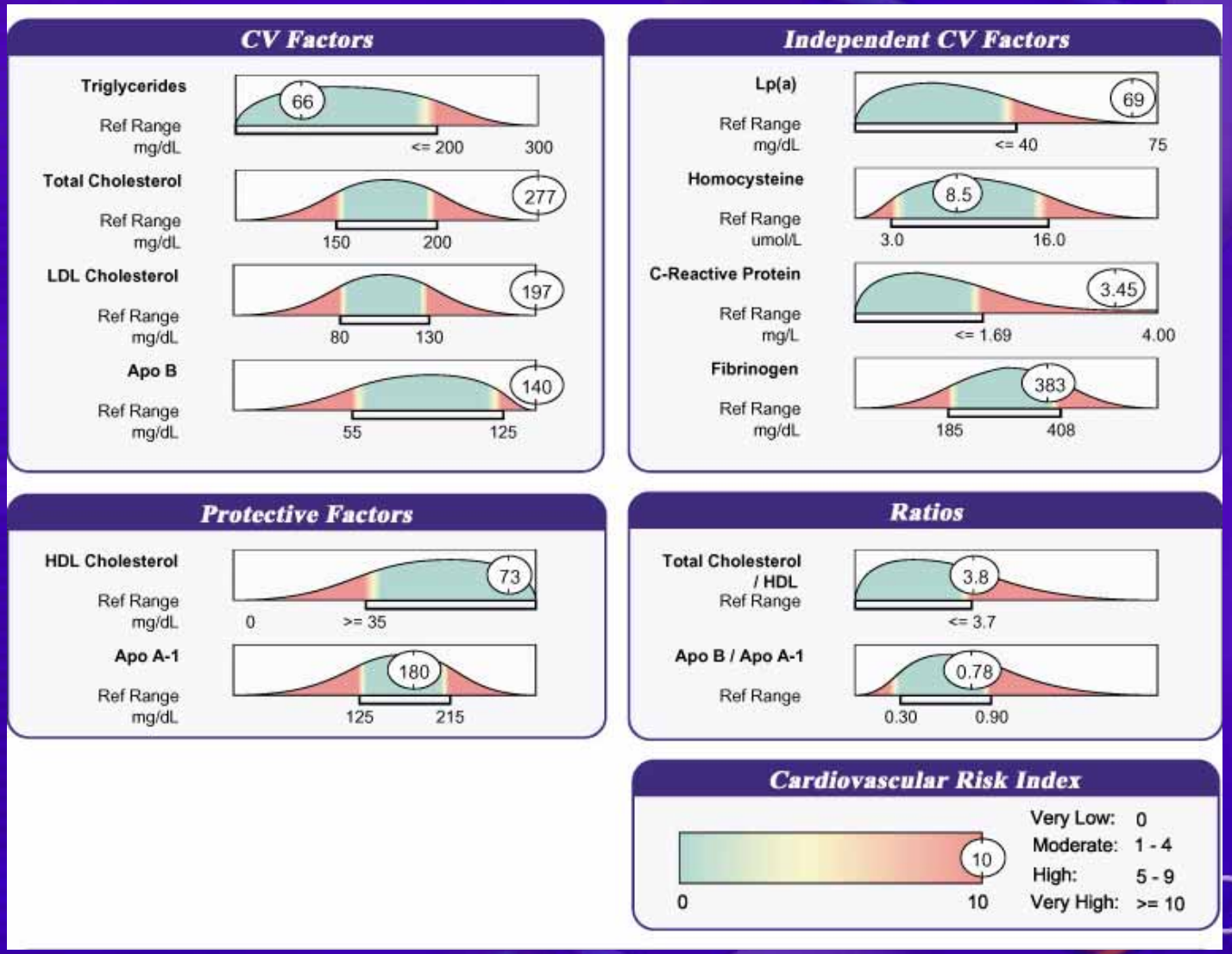
ACE
Genomics
In Primary Care Medicine

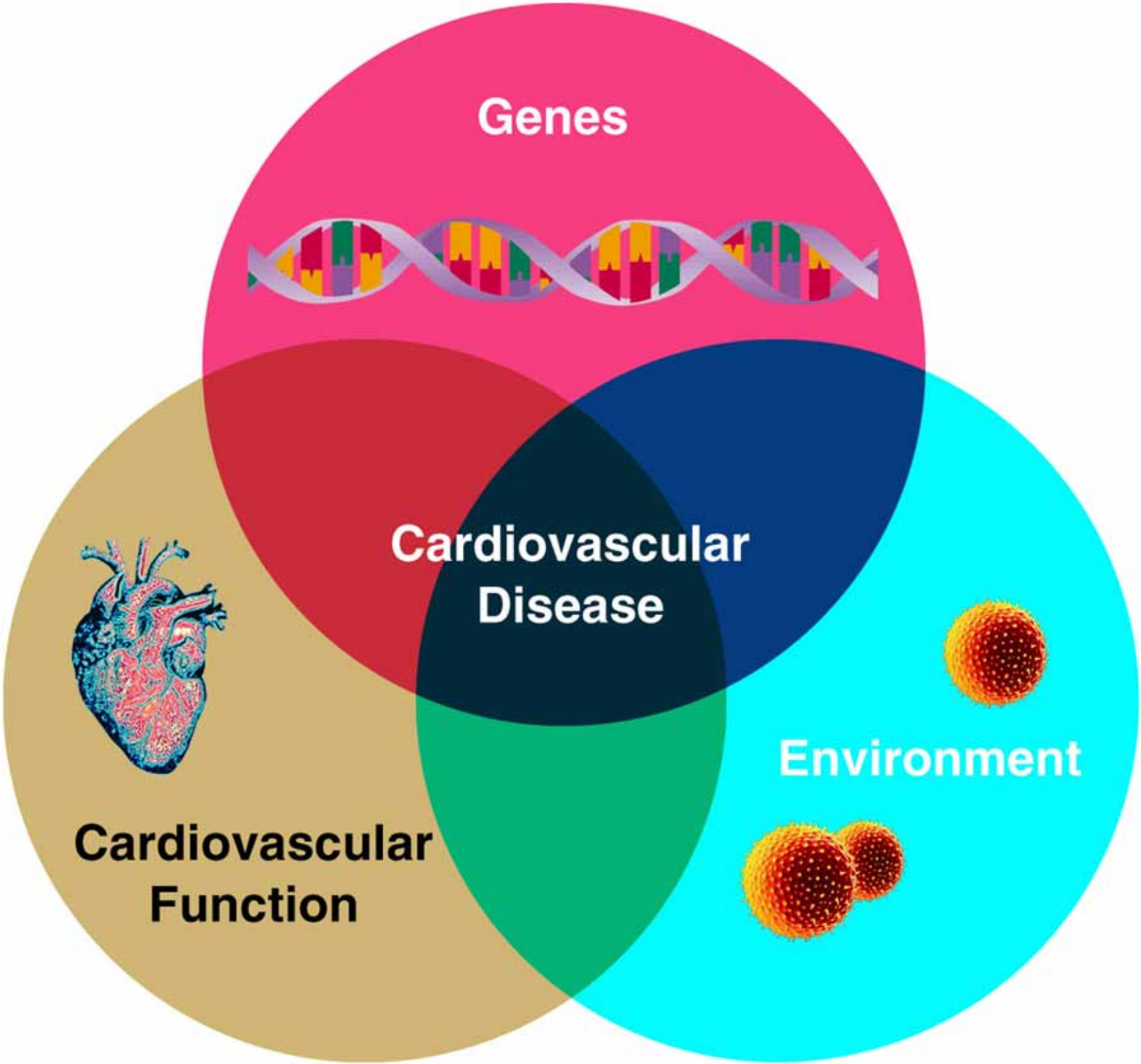

Cardio Genomics
Polymorphisms Affecting
Cardiovascular health

Cardiovascular Disease Accounts for 43% of All Deaths in the United States

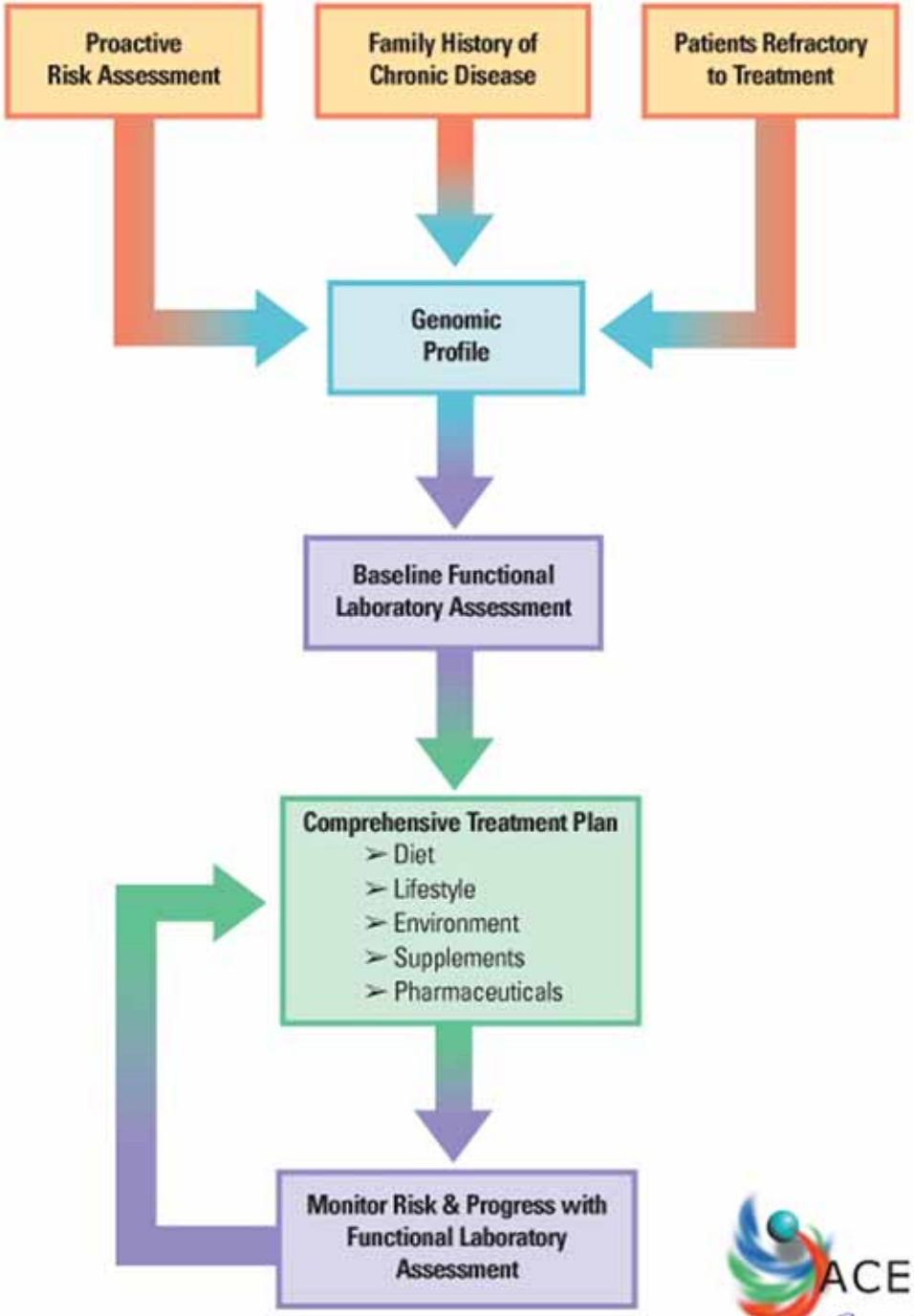


Comprehensive Cardiovascular Assessment




Genes
Cardiovascular Disease
Environment
Cardiovascular Function

Predictive Genomics and Functional Medicine



ACE
Genomics
In Primary Care Medicine

Myocardial Infarction Horizon

- Positive family history is an independent predictor of MI after adjusting for all presently known genetic components
- Risk of MI maps to a single region on chromosome 14 (lod score of 3.9)
- A gene is yet to be identified

Broeckel U, Hengstenberg C, et al. A comprehensive linkage analysis for myocardial infarction and its related risk factors. *Nat Genet* 2002;30(2):210-4.

ACE

Cardio Genomic Polymorphisms

- Identify polymorphisms associated with increased risk of developing atherosclerosis, hypertension, and coronary artery disease
- Risk factors include methylation defects, hypercoagulation, cholesterol regulation defects, chronic inflammation, and cardio-protective markers

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Polymorphisms Affecting Cholesterol Metabolism

Apolipoprotein E (2, 3, 4) – APO E

Cholesteryl Ester Transfer Protein – CETP

Selectin E -- SELE

Apolipoprotein E4

- Apolipoproteins are involved in cholesterol and lipid transport in the blood stream
- The APO E4 allele is associated with higher LDL cholesterol and triglycerides
- The risk of cardiovascular disease and senile plaque is elevated with this polymorphism

Apolipoprotein E

There are three main polymorphisms of APO E, each with distinct alleles occurring at two separate loci in the protein: at amino acid 112 (called site A) and at amino acid 158 (called, surprisingly, site B).

The specifics of each isoform are as follows

APO E2: cysteine / cysteine

APO E3: cysteine / arginine

APO E4: arginine / arginine

APO E4 Confers Risk of Elevated Cholesterol, TG, and IHD

- In patients with elevated cholesterol, 30% carried an APO E4 allele compared to only 15% in patients with normal cholesterol
- In patients with elevated cholesterol, those with one APO E4 allele exhibited a 75% prevalence of IHD compared with only 31% in patients with no APO E4 allele

Eto M, et al. Familial hypercholesterolemia and apolipoprotein E4. *Atherosclerosis* 1988 Aug;72(2-3):123-8.

ACE

Ischemic Heart Disease, Hyperlipoproteinemia and APO E

Variant	No IHD	IHD	HLP Type
E2	3.7%	8.2%	44% III 25% IV
E4	11.7%	17.0%	43% IIb 29% IIa
E3	84.6%	74.8%	

Eto M, et al. Increased frequencies of apolipoprotein epsilon 2 and epsilon 4 alleles in patients with ischemic heart disease. *Clin Genet* 1989 Sep;36(3):183-8.

ACE

Late-Onset Alzheimer's Disease Risk and APO E4

# APO E4 Alleles	Lifetime Risk	Mean Age of Onset
0	20%	84
1	47%	75
2	91%	68

Kamboh MI. Apolipoprotein E polymorphism and susceptibility to Alzheimer's disease. *Hum Biol* 1995 Apr;67(2):195-215.

ACE

Pathophysiology

ApoE appears to contribute directly to the pathogenesis of Alzheimer's disease because it has been immunochemically localized in the three defining lesions of the disease: extracellular amyloid plaques, intracellular neurofibrillary tangles, and vascular amyloid deposits

Kamboh MI. Apolipoprotein E polymorphism and susceptibility to Alzheimer's disease. *Hum Biol* 1995 Apr;67(2):195-215.

ACE

APO E Odds Ratios of Developing Alzheimer's Disease

E3/E3 = 1.0

E4/E3 = 2.7

E2/E3 = 0.5

E4/E4 // E3/E3 = 11.2

E4/E3 // E3/E3 = 2.2

One E4 Allele Lifetime Risk (by age 85):

- 14% for men
- 17% for women

Bickeboller H, et al. Apolipoprotein E and Alzheimer disease: genotype-specific risks by age and sex. *Am J Hum Genet* 1997 Feb;60(2):439-46.

Apolipoprotein E Summary

- APO E4 appears to confer increased risk of heart disease and Alzheimer's disease
- APO E2 appears to confer increased risk of some rarer heart disease but protection for Alzheimer's disease
- APO E3 is the most frequent polymorphism conferring the least risk for heart disease and average risk for Alzheimer's disease

Apolipoprotein E4 and E2 Treatment

Diet: Eat a low fat, very low cholesterol, and high fiber diet; avoid simple carbohydrates; eat salmon 3X/wk; alcohol 1-2/d

Supplements: Fish oil, higher dose vitamin E (~800IU), niacin therapy, red rice yeast

Lifestyle: Exercise boosts HDL; Smoking dramatically increases CAD risk for E4 patients (3X)

Apolipoprotein E4 Treatment

OTC and Pharmaceutical Options:

- Aspirin (83 mg/d) therapy may be advisable
- E4 patients responded better to probucol than to gemfibrozil or cholestryamine in studies

Treatment for Alzheimer's

There are no effective treatments for Alzheimer's dementia to date, however there are promising functional strategies

The earlier such strategies are enjoined the greater their likelihood for success

Kidd PM. A review of nutrients and botanicals in the integrative management of cognitive dysfunction. *Altern Med Rev* 1999 Jun;4(3):144-61.

ACE

Oxidative Stress in Alzheimer's

- Oxidative stress plays a key role in the conversion of soluble to unsoluble beta-amyloid, suggesting that oxidative stress is primary to the beta-amyloid cascade
- Early aggressive anti-oxidant therapy may be warranted, especially for those with an APO E4 allele

Retz W et al. Free radicals in Alzheimer's disease. *J Neural Transm Suppl* 1998;54:221-36.

Strategies for Combating Neurological Oxidative Stress

1. Radical scavengers, agents directly interacting with free radicals – e.g., gingko biloba, vitamins C, E, acetyl-L-carnitine and estrogen.
2. Antioxidants, which are able to prevent or decrease the production of free radicals by use of specific neuropharmacological properties – e.g., MAO-B inhibitors like selegiline and tenilsetam,

Rosler M, et al. Free radicals in Alzheimer's dementia: currently available therapeutic strategies. *J Neural Transm Suppl* 1998;54:211-9.

ACE

Phosphatidylserine and Alzheimer's

- Phosphatidylserine is a phospholipid with a strong affinity for neuron membranes
- The chronic use of phosphatidylserine (~300 mg/d) has been shown to improve cognitive decline in both Alzheimer's and non-Alzheimer's dementias

Engel RR, et al. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. *Eur Neuropsychopharmacol* 1992 Jun;2(2):149-55.

Cholesteryl Ester Transferase Protein CETP

- CETP is a critical step in the transfer of insoluble cholesteryl esters among lipoprotein particles and maintaining normal cholesterol homeostasis
- Polymorphisms in CETP result in impaired ability to remove cholesterol from the system and lower HDL cholesterol levels, with consequent increased risk of developing atherosclerosis and coronary artery disease

TaqI B restriction fragment length polymorphism (RFLP) in the cholesteryl ester transfer protein (CETP) gene

- Wild type homozygotes (B2,B2) had a 45% higher HDL level than did individuals with a polymorphism (B1,B1)
- However, the benefits of the B2 polymorphism were eliminated in individuals who smoked or were obese

Freeman DJ, et al. Regulation of plasma HDL cholesterol and subfraction distribution by genetic and environmental factors: associations between the TaqI B RFLP in the CETP gene and smoking and obesity. *Arterioscler Throm.* 1994;14:336-344.

ACE

Treatment for CETP B1 Polymorphisms

Individuals who had the B1 polymorphism of CETP and who drank alcohol on a daily basis had 30% lower CETP activity and 48% higher HDL cholesterol than non-drinkers

Hannuksela ML, et al. Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. *Atherosclerosis* 1994 Sep 30;110(1):35-44.

ACE

Treatment for CETP B1 Polymorphisms

- Statin drugs (Pravastatin) has been shown to abolish the differences in CETP activity and HDL levels between the B1 wild type and the B2 polymorphism
- Statin mimetics like inositol hexaniacinate and red rice yeast may prove likewise effective in normalizing CETP activity without the common side effects of statin drug use

Kuivenhoven JA, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. *N Engl J Med* 1998 Jan 8;338(2):86-93.

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Polymorphisms Affecting Methylation Capacity

Methylenetetrahydrofolate reductase (MTHFR)

MTHFR is a critical enzyme in folate metabolism and polymorphisms lead to elevated homocysteine levels with defective methylation capacity

The Body's Many Uses for Methylation

1. DNA synthesis (purines and pyrimidines)
2. DNA masking and unmasking
3. Neurotransmitter synthesis
4. Detoxification
5. Heavy Metal Detoxication
6. Nerve myelination
7. Carnitine and CoQ10 synthesis (energy metabolism)

The Methylation Mystery

In 1980, RW Smithells found that women who had previously had a child with a neural tube defect could prevent NTD in future children merely by supplementing with a multi-vitamin containing folic acid

Smithells RW, Sheppard S, et al. Possible prevention of neural tube defects by periconception vitamin supplementation. *Lancet* 1980;??:339-342.

ACE

The Methylation Mystery

- In 1982, CE Butterworth discovered that cervical dysplasia could be improved by supplementing with high oral doses of folic acid (10,000 mg/d —the DRI is 400 µg/d)
- Folic acid also appeared to be protective against papillomavirus infection

Butterworth CE, Hatch KD, et al. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. *Am J Clin Nutr* 1982;35:73-82.

ACE

The Methylation Mystery

- In 1960s, Kilmer McCully noticed an association between atherosclerosis in patients and an elevated serum homocysteine level
- For over 30 years, McCully has been teasing out the relationship between the two and how to reduce serum homocysteine levels using folic acid, B12 and B6

The Methylation Mystery

In 1988, John Lindenbaum was the first to observe that cognitive impairment in the elderly was associated with elevated plasma homocysteine and urinary methylmalonic acid in spite of "normal" folate and B12 levels and an absence of macrocytosis

Lindenbaum J, Healton EB, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 1988;318:1720-28.

ACE

The Methylation Mystery

From conception to old age, methylation defects(indicated by elevated homocyateine) appear to be able to reek havoc on our health

Neural tube defects, heart disease, stroke, cancer, and senile dementia all can be caused from defective methylation capacity

DNA Methylation

- Methylation of cytosine residues in DNA, known as DNA masking, silences transcription
- DNA masking is critical for normal cellular replication and cell differentiation

DNA Methylation

- Methylation of the DNA is critical for genome stability
- Evidence suggests that methylation is also essential for controlling the replication of massive amounts of viral DNA (>35%) within in the human genome
- Methylation-induced processes also play a pivotal role in repairing DNA damaged by oxidative stress or toxin exposure

Ames BN. Endogenous DNA damage as related to cancer and aging. *Mutation Res* 1989;214:41-46.

ACE

Viral Genes and Cancer?

- Methylation capacity may be especially important in the genesis and pathophysiology of cancer since one of the first events associated with cancerous transformation of cells is stripping the DNA of methylation
- This allows not only unfettered cell replication, but also potentially unfettered viral gene replication

Methylation Defects and Colon Cancer

In patients with long standing inflammation as a result of ulcerative colitis, the methylation capacity for masking regions of the DNA in intestinal mucosal cells is reduced

The resulting altered cellular proliferation and differentiation predisposes the patient to develop colorectal neoplasms

Gloria L et al. DNA hypomethylation and proliferative activity are increased in the rectal mucosa of patients with longstanding ulcerative colitis. Cancer 1996;78(11):2300-2306.

ACE

Diseases Associated With Defective Methylation Capacity

- Neural tube defects, spontaneous abortion, placental abruption
- Cervical dysplasia, cervical cancer
- Colon cancer
- Atherosclerosis, coronary artery disease, deep vein thrombosis, stroke
- Cognitive impairment, senility, Alzheimer's disease
- Osteoporosis, rheumatoid arthritis, diabetes

Measuring Methylation Capacity

Serum homocysteine and urinary methylmalonic acid are the most sensitive indicators of defective methylation capacity

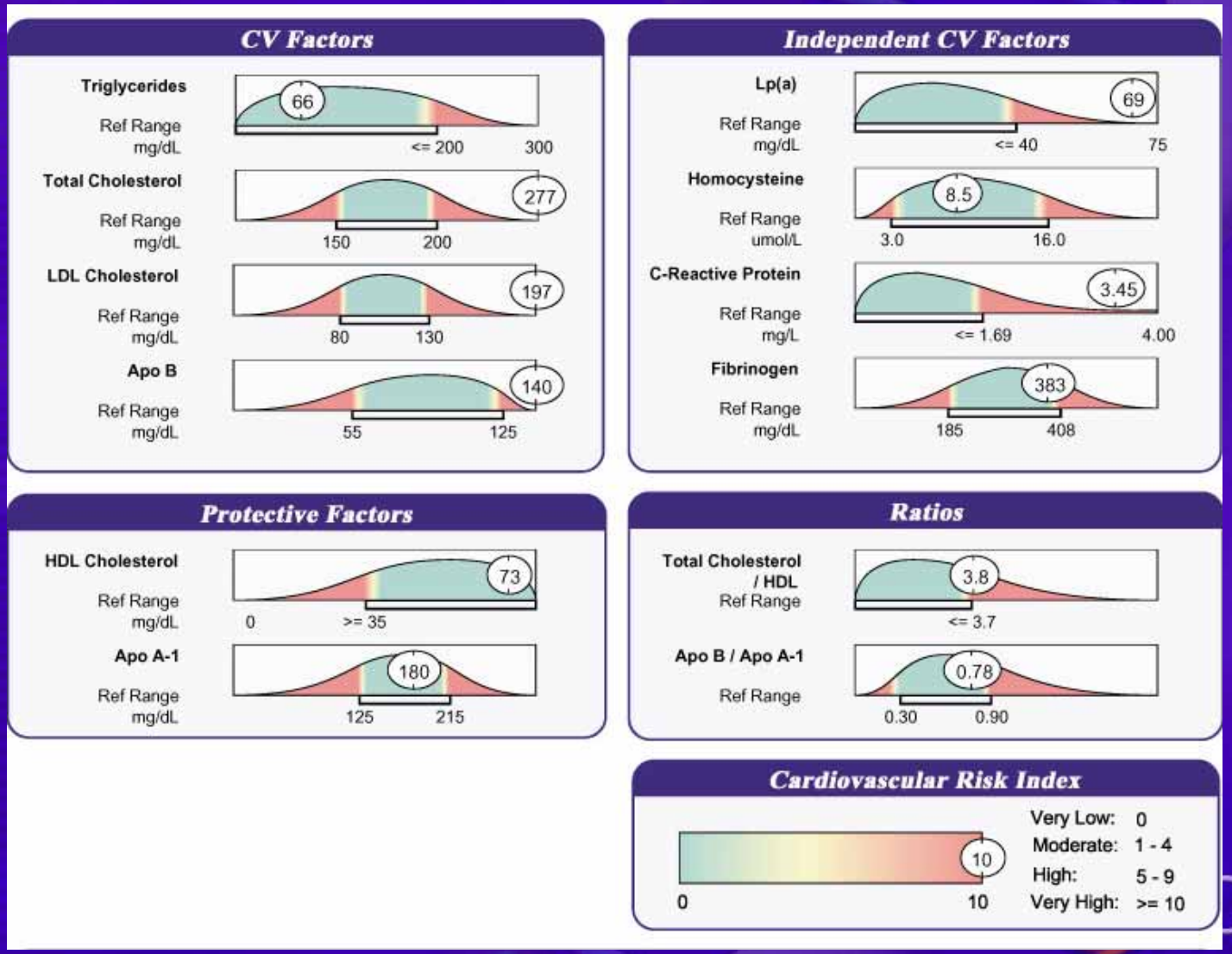
Homocysteine and the Elderly

- Prevalence of homocysteinemia among the elderly is 30-40%
- Grossly elevated homocysteine levels (fasting level > 15 µmol/L) were found in 61% of elderly patients admitted to hospital
- Optimal homocysteine levels may be < 8 µmol/L

Ventura P, Panini R, Verlato C, et al. Hyperhomocysteinemia and related factors in 600 hospitalized elderly subjects. *Metabolism* 2001 Dec;50(12):1466-71.

ACE

Serum Homocysteine Sould Be Part of Any Comprehensive Cardiovascular Risk Profile



Defective Methylation Capacity

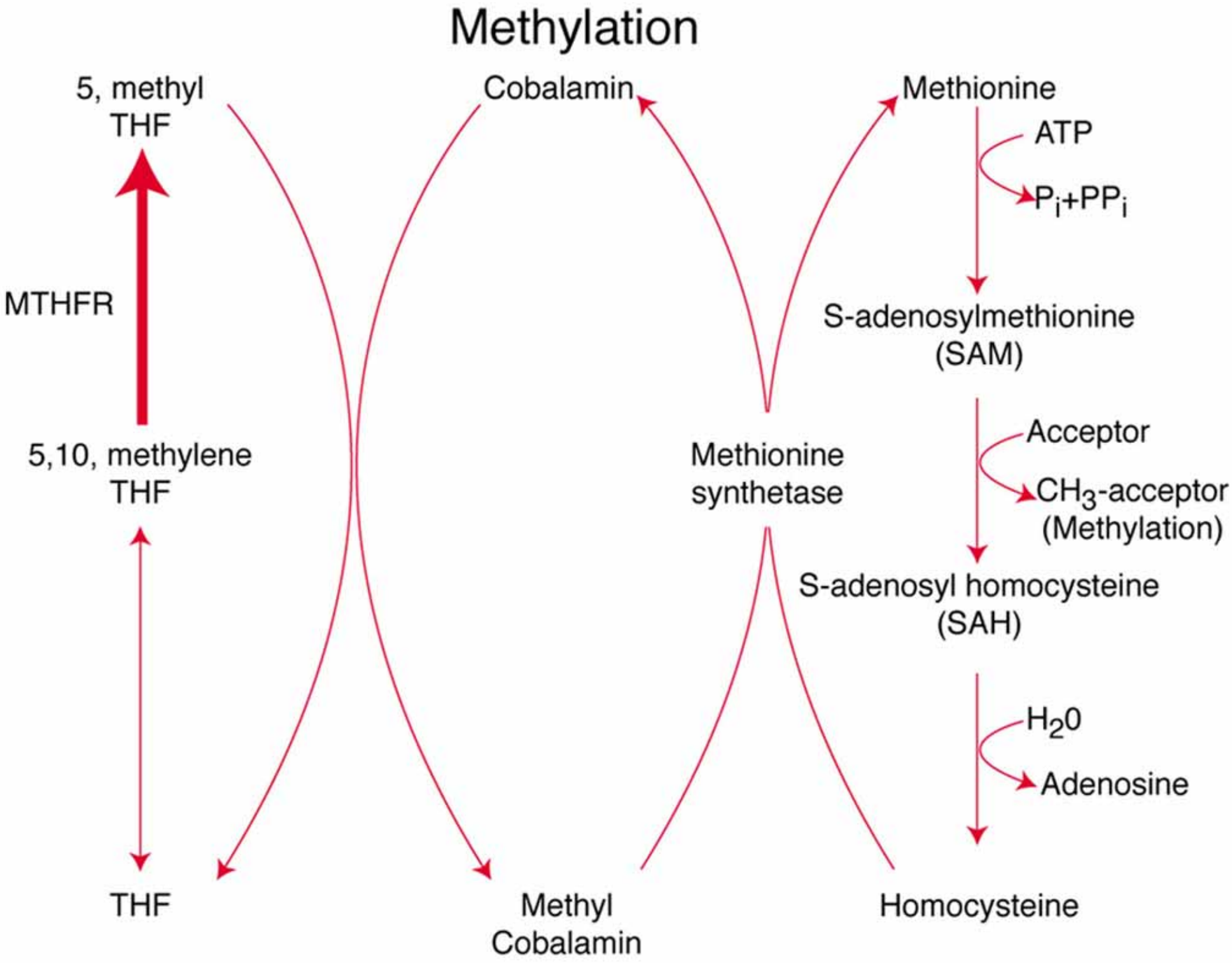
- Not all methylation defects are simply the result of inadequate dietary folic acid, B_{12}, B_6, and/or betaine
- Certain individuals have a single C→T nucleotide polymorphism at the 677th nucleotide in the enzyme methyltetrahydrofolate reductase (MTHFR) which makes them unable to metabolize folate properly for methylation

MTHFR 677C→T SNP Prevalence

- ~50% of Caucasians and Asians are heterozygous for 677C→T with modestly impaired methylation capacity
- ~12% are homozygous with severely impaired methylation capacity
- ~28% of patients with elevated homocysteine did not respond to folic acid, B_{12}, and B_6 because they had the 677C→T SNP

Engbersen AM et al. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995;56:142-150

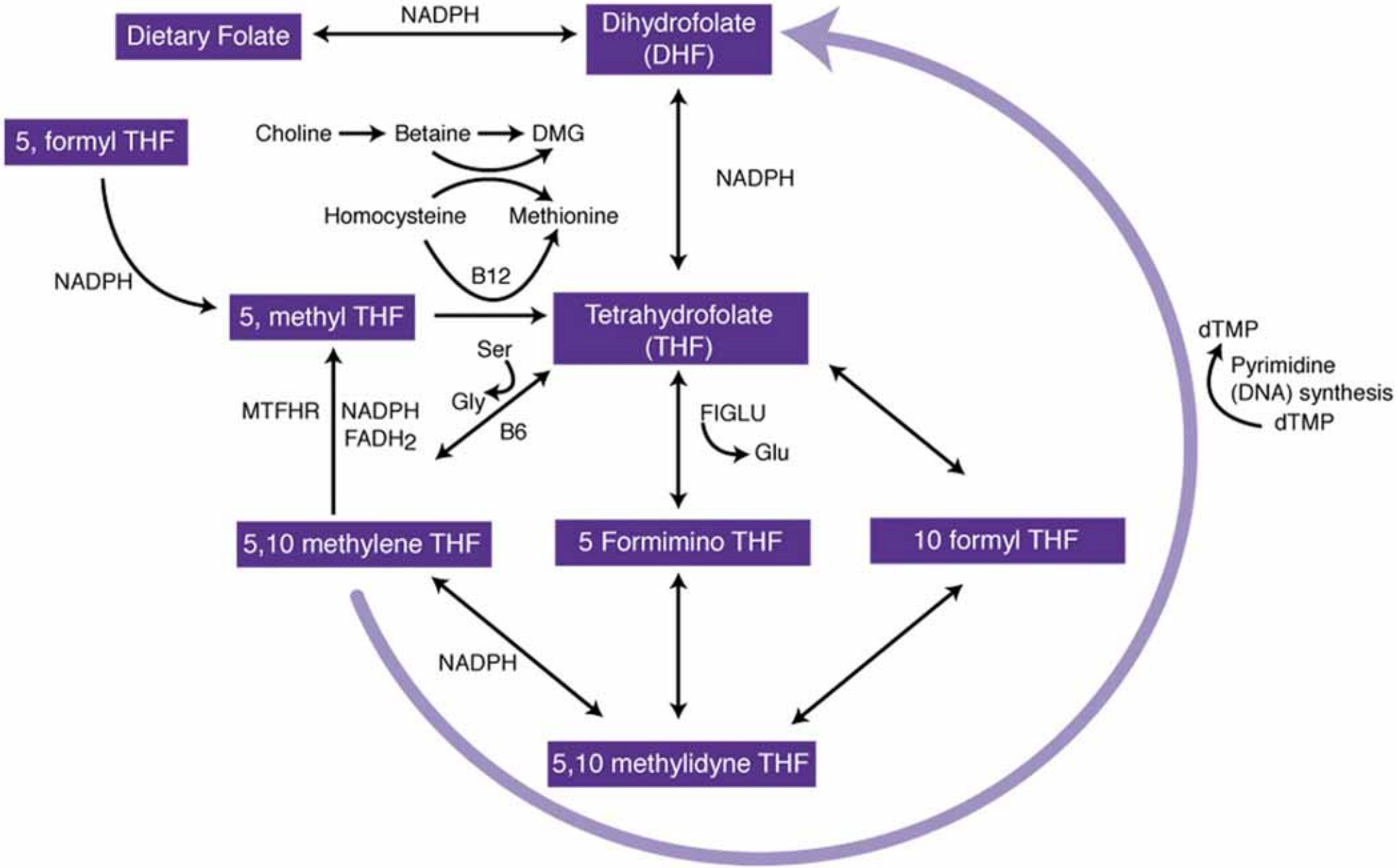
ACE


Methylation
5, methyl
THF
Cobalamin
Methionine
ATP
P_i+PP_i
MTHFR
S-adenosylmethionine
(SAM)
5,10, methylene
THF
Methionine
synthetase
Acceptor
CH_3-acceptor
(Methylation)
S-adenosyl homocysteine
(SAH)
H_20
Adenosine
THF
Methyl
Cobalamin
Homocysteine

Supplement Modifications

- Fortunately, the deleterious effects of a 677C→T SNP can be completely corrected with supplements alone
- 5-methyl tetrahydrofolate (5-MeTHF) is the folate for "downstream" of the SNP
- Betaine is an alternate cofactor in the enzyme methionine synthetase, regenerating methionine from homocysteine

Folate Interconversions



Adapted from Groff J et al. Advanced Nutrition and Human Metabolism. 2nd. West: St. Paul, MN; 1995

Riboflavin (B_2) and Homocysteine

In 423 healthy blood donors, those with the highest homocysteine levels had the lowest riboflavin levels

Riboflavin is the precursor for

- Flavin mononucleotide (FMN) a coenzyme for converting B6 into its active form: pyridoxal-5-phosphate
- Flavin dinucleotide ($FADH_2$) is a coenzyme for MTHFR

Hustad S, Ueland PM, et al. Riboflavin as a determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. *Clin Chem* 2000;46(8):1065-1071.

ACE

Supplement Schedule for Individuals with MTHFR 677C→T Polymorphism

Folic Acid

5-methyl and 5-formyl THF

Betaine

Methylcobalamin (B12)

Pyridoxal-5-phosphate (B6)

Riboflavin (B2)

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Polymorphisms Affecting Blood Pressure

Guanine Nucleotide-binding Protein β-3 (GNB3)

Angiotensin (AGT)

Angiotensin II Receptor-1 (AGTR1)

Guanine Nucleotide-binding Protein β-3 (GNB3)

- GNB3 is pivotal in many cell-to-cell signal transduction pathways, including that by which angiotensin II acts to stimulate vasoconstriction and elevate blood pressure
- The GNB3 825C→T polymorphism is associated with both essential hypertension and obesity

Pharmaceutical Intervention

- GNB3 825C→T appears to increase susceptibility to vasoconstriction
- Homo- and heterozygous 825T allele carriers respond with a stronger decrease in blood pressure to therapy with a thiazide diuretic than homozygous 825C allele carriers

Siffert W. Molecular genetics of G proteins and atherosclerosis risk. *Basic Res Cardiol* 2001 Nov;96(6):606-11.

ACE

Angiotensin (AGT) M235T

- Angiotensin is a polypeptide hormone that stimulates smooth muscle contraction as well as sodium and water retention, resulting in elevated blood pressure
- The M235T polymorphism is associated with increased AGT production and consequently with essential hypertension and coronary artery disease

Salt (Sodium) Restriction

People with hypertension were placed on a sodium restricted diet leading to a 20% reduction in urinary sodium excretion

Blood pressure response to sodium restriction depended on AGT M235T polymorphism genotype

MM Genotype ⇒ -5.3/-1.0 mm Hg

MT Genotype ⇒ -9.0/-5.2 mm Hg

TT Genotype ⇒ -8.6/-3.9 mm Hg

Hunt SC, et al. Enhanced blood pressure response to mild sodium reduction in subjects with the 235T variant of the angiotensinogen gene. *Am J Hypertens* 1999 May;12(5):460-6.

Angiotensin II Receptor-1 (AGTR1) 1166A→T

- The angiotensin II receptor-1 is an important effector controlling blood pressure and volume in the cardiovascular system
- Angiotensin II stimulates vasoconstriction as well as sodium and water retention
- The AGTR1 1166A→T polymorphism is associated with increased binding affinity between angiotensin II and AGTR1and consequently with increased risk for essential hypertension and coronary artery disease

AGTR1(1166A→C)

Losartan, an AGTR1 antagonist, increased the glomerular filtration rate (GFR) and decreased the mean arterial pressure (MAP) in the AC/CC group, but did not influence these parameters in the AA group

Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. *Kidney Int* 1999 Dec;56(6):2173-80.

ACE

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Cardio Genomic Polymorphisms

Cholesterol Metabolism

- ApoE 2, 3, 4
- SELE
- CETP

Methylation

- MTHFR

Hypertension

- GNB3
- AGTR1
- AGT

Coagulation

- Factor 2
- Factor5

Polymorphisms Affecting Coagulation Factors

Coagulation Factor 2, Prothrombin

Coagulation Factor 5 – Leiden

Coagulation Factor 2, Prothrombin 20210G→A

- Coagulation Factor II, prothrombin, is a plasma protein that plays a critical role in blood coagulation and clotting
- Individuals with this polymorphism have elevated plasma prothrombin levels and, therefore, an increased risk of venous thrombosis, myocardial infarction, and stroke

Factor 5 – Leiden 1691A→G

- Factor V Leiden is an important component of the extrinsic pathway for blood clotting combining with Factor X to form prothrombin activator which accelerates the conversion of prothrombin into thrombin
- Individuals with this polymorphism are at increased risk for venous thromboembolism
- Risk of thromboembolism increases dramatically with a concurrent Factor II SNP or with oral contraceptive use

Therapeutic Approaches

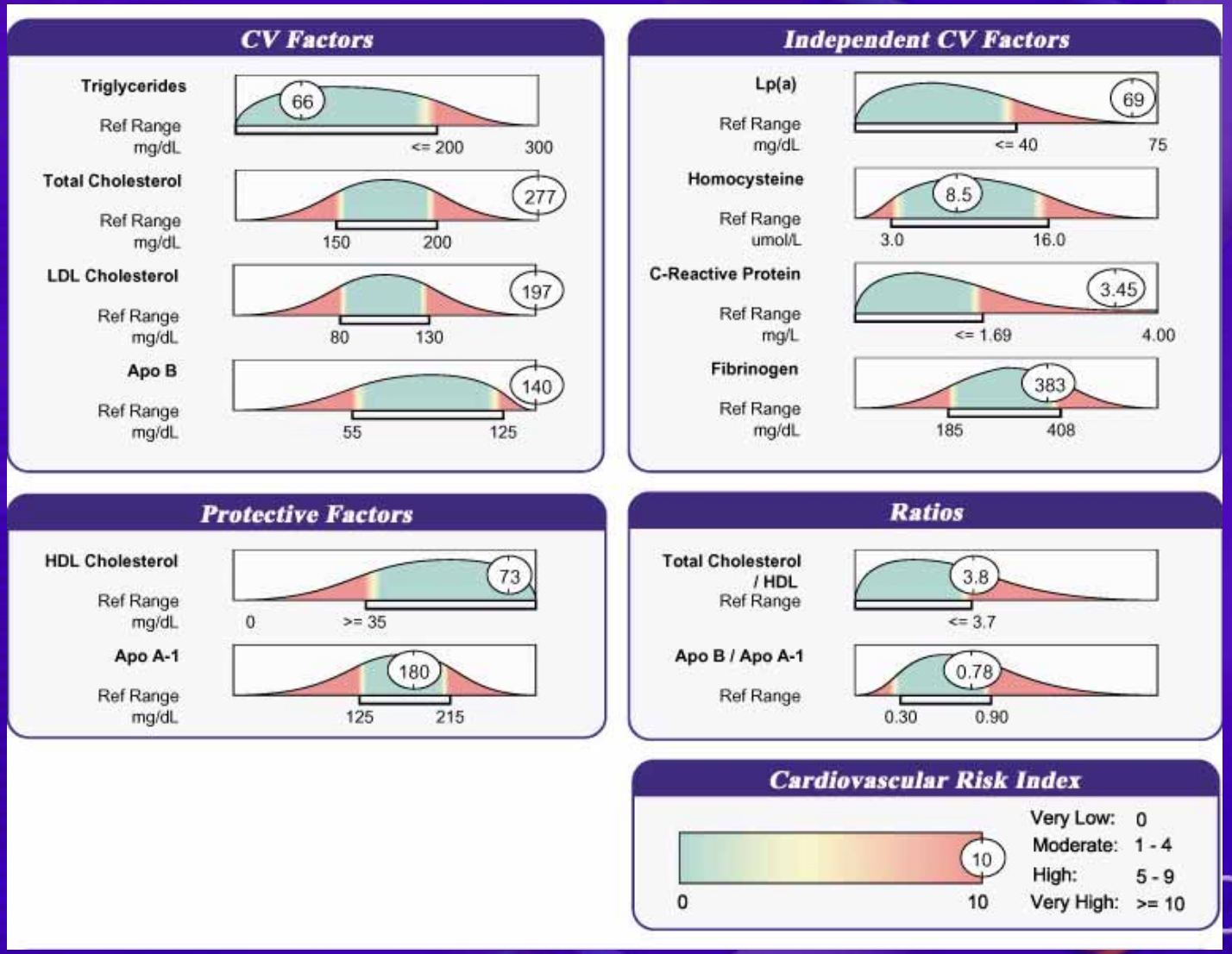
Both Factor 2 and Factor 5 SNPs are associated with dramatic increase of thromboembolism in women on oral contraception and a mild increase in smokers

Monitor Fibrinogen and C-Reactive Protein

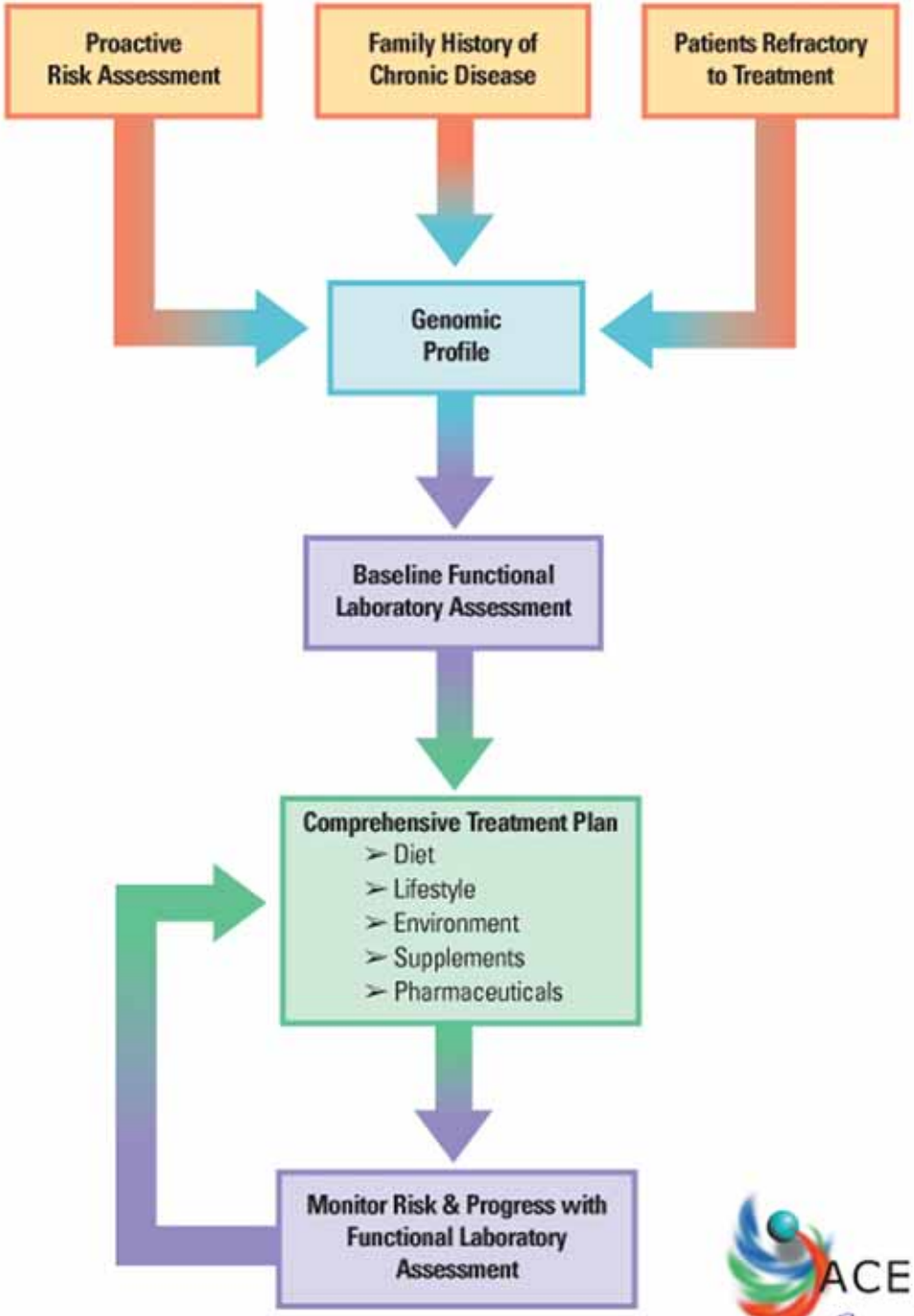
Mild thromboprophylaxis is prudent

- 82 mg aspirin/d
- EPA/DHA/fish oil supplementation
- Licorice inhibits thrombin activation

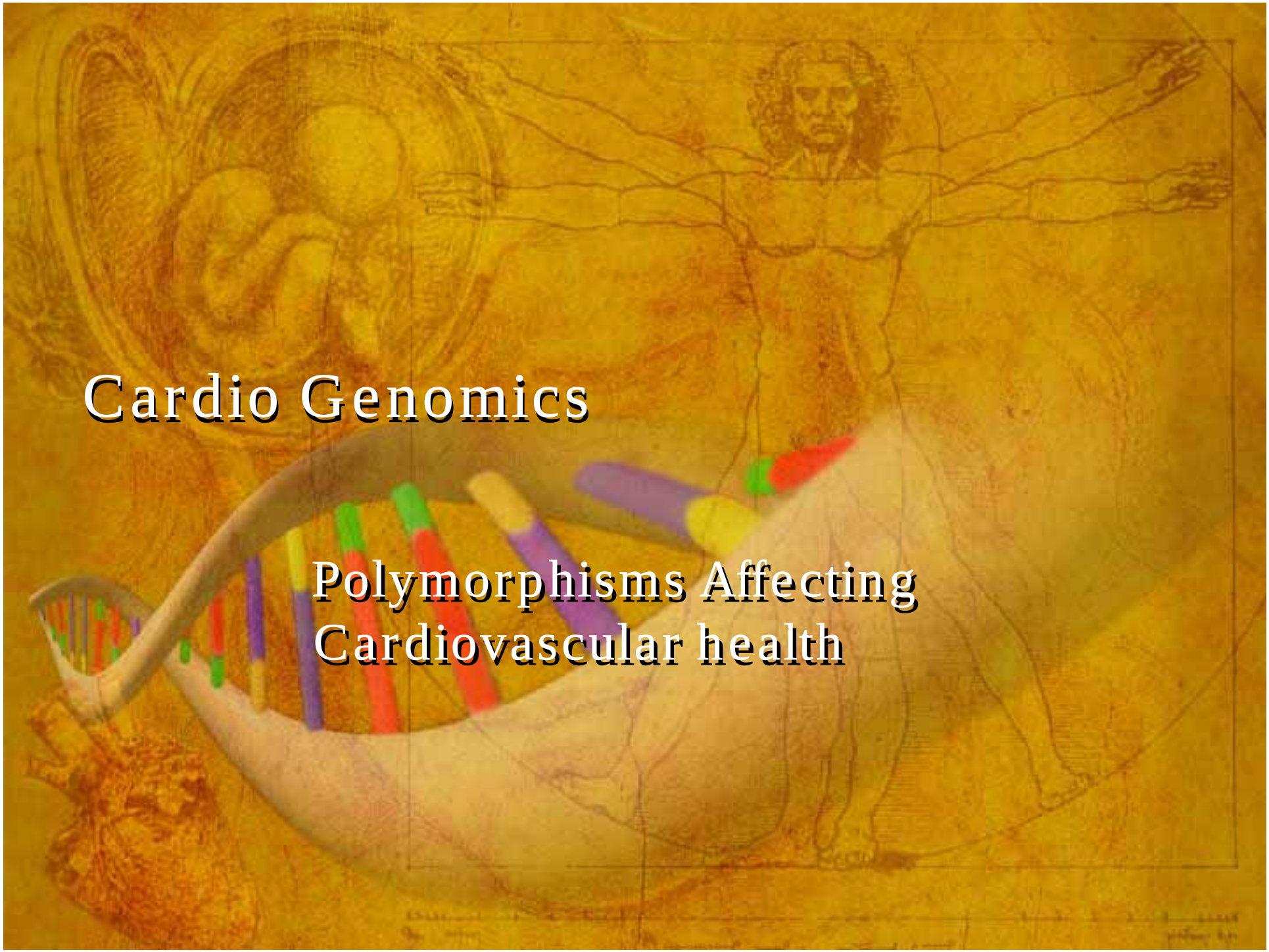
Monitor Progress and Attenuated Risk with Comprehensive Cardiovascular Assessment

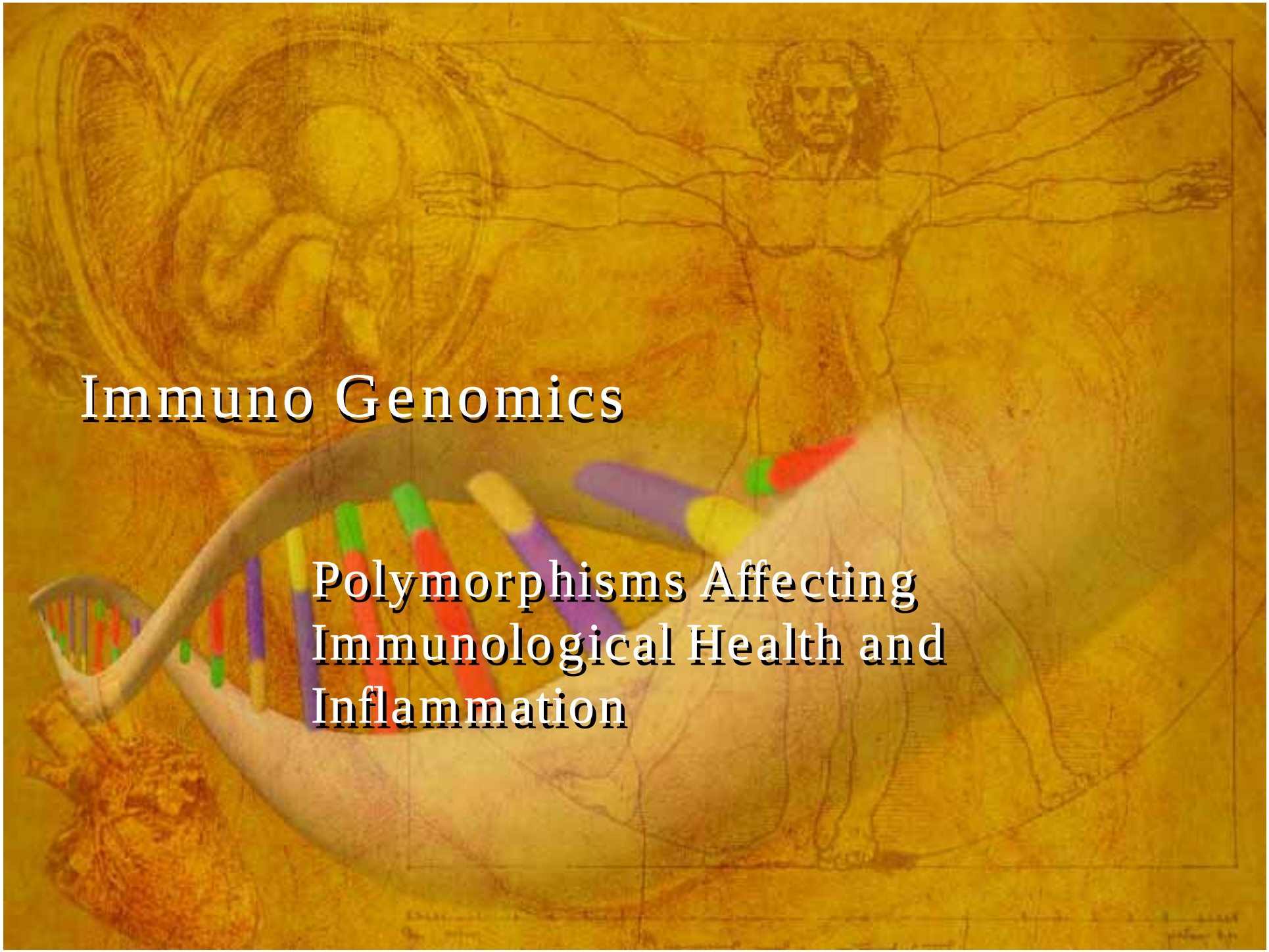


Predictive Genomics and Functional Medicine



ACE
Genomics
In Primary Care Medicine


Cardio Genomics
Polymorphisms Affecting
Cardiovascular health


Immuno Genomics
Polymorphisms Affecting Immunological Health and Inflammation

Cytokines

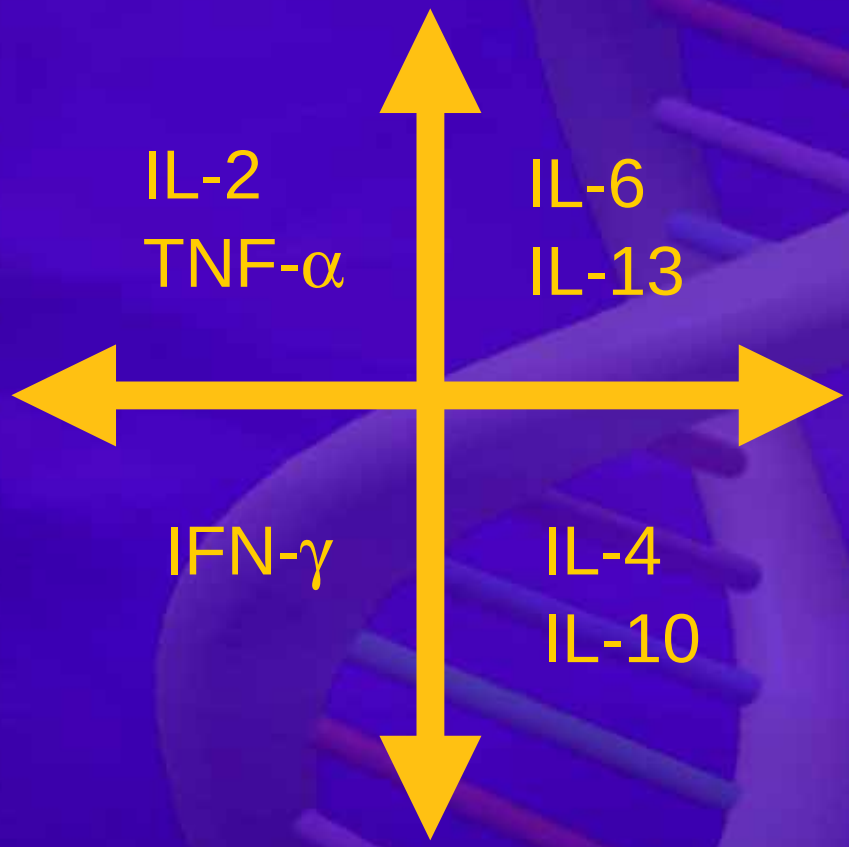
- Polypeptide “immunotransmitters” that initiate and regulate immune and inflammatory responses
- Some constitutive levels of cytokines but production varies based on stimuli and SNPs

Cf.: excellent cytokine website at http://www.copewithcytokines.de/

Cytokine Actions

- Immune regulation
- Inflammation
- Hematopoiesis
- Wound healing
- Chemotaxis
- Mitosis
- Cell differentiation
- Cell death (apoptosis)

Cytokine Classification



Cytokine Characteristics

- **Pleotropy:** the same cytokine has different effects in different circumstances: "contextual"
- **Redundancy:** more than one cytokine may have the same effect
- **Synergy:** cytokines often act in concert amplifying or dampening their physiologic effects

Cytokine Sub-sets

Names of sub-sets came from supposed cell line origins or cytokine functions

Interleukins: from leukocytes

Lymphokines: from lymphocytes

Monokines: from monocytes

Interferons: anti-viral

Colony Stimulating Factors: growth

Chemokines: chemotaxis

T-helper 1 & 2 (TH1 and Th2)

- TH-1 subset of cytokines are more involved with cell-mediated immunity and delayed hypersensitivity response
- TH-2 subset of cytokines are more involved with humoral immunity and allergic response: stimulate cell growth and differentiation, and recruitment of mast cells, basophils, eosinophils, and B-cells

TH-1 and TH-2 Cytokines

IFN-γ suppresses

IL10 suppresses

TH-1	TH-2
IL-2	IL-4
IL-18 (IGIF)	IL-5
IFN- γ	IL-6
TNF- β	IL-10
(TNF-α)*	IL-13

* TNF-α has similar activity to TNF-β but is of MΦ origin (not TH-1)

Cytokines and Inflammation

Inflammation

Pro-	Anti-
IL-1	IL-4
IL-6	IL-10
IL-8	TGF- β
TNF-α	
TNF-β	

Cytokine Sub-Sets

Names of sub-sets came from cell line origin or from cytokine functions

Interleukins: from leukocytes

Lymphokines: from lymphocytes

Monokines: from monocytes

Interferons: anti-viral

Colony Stimulating Factors: growth

Chemokines: chemotaxis

Table Of Important Cytokines

Cytokine	Source	Function
IL-1	MΦ	T & B activation, fever, inflam
IL-2	T-cells	T-cell proliferation
IL-4	T-cells	B-cell growth & proliferation
IL-6	MΦ & T	B-cell stimulation, inflammation
IL-10	T-cells	Inhibits TH-1 cytokines
IL-13	T-cells	B-cell growth & proliferation
IFNα,β	Most cells	Anti-viral
IFNγ	T, NK	Macrophage activation, inflam
TNFα	MΦ	Inflammation, anti-tumor
TNFβ	T-cells	Inflam, anti-tumor, phagocytosis
IL1-RA		Blocks IL-1 action

Interleukin Nomenclature

- Originally interleukins were named for their function: e.g., IL2 was known as T-cell growth factor, but this was replaced by the current system when pleotropy was discovered
- The interleukins are now numbered based on when they were discovered, IL-1 being the first discovered and IL-18 being the most recent

Interleukins

- Polypeptide cytokine mediators between leukocytes
- Modify inflammation and immune response
- Each interleukin functions through a separate receptor system

Immuno Genomic Polymorphisms

- Identifies polymorphisms associated with increased risk of developing defects in immune competence and surveillance
- Risk factors include altered cytokine production and activity that may lead to conditions characterized by chronically up-regulated inflammatory response

Immuno Genomic Polymorphisms

Immune polymorphisms have been associated with increased risk of asthma, atopy, osteopenia, heart disease, auto-immunity and infectious diseases

Immuno Genomic Panel

Chronic Inflammation

- IL-1β
- IL-1RN

TH-1 (Viral Infection & Cancer)

- TNF-α

TH-2 (Allergy, Asthma, & Atopy)

- IL-4
- IL-6
- IL-10
- IL-13

Immuno Genomic Panel

Chronic Inflammation

- IL-1β
- IL-1RN

TH-1 (Viral Infection & Cancer)

- TNF-α

TH-2 (Allergy, Asthma, & Atopy)

- IL-4
- IL-6
- IL-10
- IL-13

Interleukin-1 Family

- IL-1α, IL-1β, Il-1RA (receptor antagonist)
- Regulate inflammatory response to antigen
- IL-1α and IL-1β are pro-inflammatory, while Il-1RA is anti-inflammatory

Interleukin 1-beta: IL-1β -31C→T

- Produced mainly by blood monocytes, mediates the panoply of host inflammatory reactions collectively known as acute phase response
- The -31C→T SNP increases IL-1β production and may predispose individuals to chronic inflammatory conditions by upregulating COX2 activity and prostaglandin production
- Other effects include hypochlorhydria and a predisposition to *H. pylori* infection and gastric cancer

Interleukin 1-beta: IL-1β -31C→T

- The -31C→T SNP increases IL-1β secretion which in turn inhibits gastric acid secretion
- The resulting hypochlorhydria predisposes the individual to *H. pylori* infection and gastric cancer
- Other effects of increased IL-1β include chronic acute phase reaction and increased bone resorption

IL-1β -31C→T Polymorphism and Risk of H Pylori Infection

241 non-cancer outpatients who had participated in a HP eradication program.

Genotype	Infection Rate	Odds Ratio
C/C	45.2%	1.0
C/T	66.7%	2.32
T/T	63.6%	2.46

The OR for the T/T genotype was significantly increased by smoking status = 14.6

Hamajima N, et al. Interleukin 1 polymorphisms, lifestyle factors, and Helicobacter pylori infection. *Jpn J Cancer Res* 2001 Apr;92(4):383-9.

ACE

Eradicating H. pylori

Mastic Gum

Al-Said MS, et al. Evaluation of mastic, a crude drug obtained from Pistacia lentiscus for gastric and duodenal anti-ulcer activity. *J Ethnopharmacol* 1986 Mar;15(3):271-8.

Triple Antibiotic Therapy

Half of all strains of H. pylori are resistant to metronidazole

Ching CK, Chan YK, Ng WC. The combination of omeprazole, amoxycillin, and clarithromycin eradicates Helicobacter pylori in 95% of patients---7 days of therapy is as good as 10 days. *Hong Kong Med J* 1998 Mar;4(1):7-10.

ACE

Direct Inhibition of IL-1β Production

- Fish oil and milk thistle (silymarin) supplementation have been demonstrated to inhibit IL-1β production directly, and can be used for long-term modulation of IL-1β activity
- Other anti-inflammatories like boswellia, licorice, and curcumin may also be beneficial

Immuno Genomic Panel

Chronic Inflammation

- IL-1β
- IL-1RN

TH-1 (Viral Infection & Cancer)

- TNF-α

TH-2 (Allergy, Asthma, & Atopy)

- IL-4
- IL-6
- IL-10
- IL-13

Immuno Genomic Panel

Chronic Inflammation

- IL-1β
- IL-1RN

TH-1 (Viral Infection & Cancer)

- TNF-α

TH-2 (Allergy, Asthma, & Atopy)

- IL-4
- IL-6
- IL-10
- IL-13

Tumor Necrosis Factor (TNF-α, TNF-β)

- TNF is secreted by macrophages, neutrophils, T-cells, and NK cells
- TNF synthesis is induced by interferons, IL-2, substance P, PAF, bradykinins, immune complexes, and cycloxygenase inhibitors
- TNF production is inhibited by IL-6, TGF-β, glucocorticoids, vitamin D3, PGE2, and antagonists of PAF

TNF Actions

- Cytolysis and cytostasis
- Phagocytosis
- Promotes vascular permeability (with IL-1)
- Inhibits anticoagulatory mechanisms
- Chemoattractant for neutrophils
- Promotes synthesis of collagenase, PGE2, and IL-1, CSF, and IFN-γ
- Activates osteoclasts and promotes bone resorption

Tumor necrosis factor-alpha is a pro-inflammatory cytokine that can contribute to arthritis, asthma, and osteoporosis. Polymorphisms of TNF-α inappropriately activate inflammatory response and increase TNF-α production.

Green Tea Polyphenols

- Green tea polyphenols like EGCG, epigallocatechin gallate have been shown to inhibit the genetic expression of TNF-α
- ECGC also inhibits the release of TNF-α, IL-1β, and IL-10
- Inhibition of TNF-α is believed to be the main chemoprotective action of green tea polyphenols

Suganuma M, et al. Mechanisms of cancer prevention by tea polyphenols based on inhibition of TNF-alpha expression. *Biofactors* 2000;13(1-4):67-72.

Fujiki H, et al.. Mechanistic findings of green tea as cancer preventive for humans. *Proc Soc Exp Biol Med* 1999 Apr;220(4):225-8.

ACE

Immuno Genomic Panel

Chronic Inflammation

- IL-1β
- IL-1RN

TH-1 (Viral Infection & Cancer)

- TNF-α

TH-2 (Allergy, Asthma, & Atopy)

- IL-4
- IL-6
- IL-10
- IL-13

Immuno Genomic Panel

Chronic Inflammation

- IL-1β
- IL-1RN

TH-1 (Viral Infection & Cancer)

- TNF-α

TH-2 (Allergy, Asthma, & Atopy)

- IL-4
- IL-6
- IL-10
- IL-13

Interleukin – 6 (IL-6)

- IL6 is a pleiotropic cytokine influencing antigen-specific immune responses and inflammatory reactions contributing to the acute phase reaction as well as to sustained inflammation
- Because of it broad pro-inflammatory action SNPs have been identified contributing to diverse inflammatory disorders

IL-6 SNP: -174G→C

IL6 -174G→C increases hepatic synthesis and release of triglycerides has been associated with elevations in serum triglycerides in response to carbohydrate intake and decreased levels of HDL cholesterol, with expected increased risk of CVD

IL-6 SNP: -174G→C

Since the hypertriglyceride effects of IL6 -174G→C are in response to carbohydrate intake, individuals with this polymorphism and elevated triglycerides are likely improve on a low calorie, low carbohydrate, higher protein diet

IL-6 SNP -634G→C

- IL6 -634G→C has been associated with increased osteoclast activity and osteopenia
- Increased risk of other inflammatory disorders is likely, including inflammatory bowel disease (Crohn's and colitis)
- Sustained anti-inflammatory therapeutics, e.g., fish oils, boswellia, curcumin, etc. may be indicated

Possible Etiological Factors in Asthma

- Atopy (~ 80% co-morbidity)
- Dust mite feces (enzymes → disruption of tight junctions between epithelial cells in lungs)
- Pet dander, esp. cat
- Food Allergy
- Yeast Infections
- Viral infections
- Lead and mercury toxicity

Review article: Miller AL. The etiologies, pathophysiology, and alternative/ complementary treatment of asthma. *Altern Med Rev* 2001 Feb;6(1):20-47.

ACE

Interleukin – 4 (IL-4)

- Produced by antigen-presenting cells (macrophages and dendritic cells)
- Stimulates IgE production in mast cells
- Excess IL-4 appears to be able to "switch" CD8+ cells from their normal production of IFN-γ to produce additional IL-4, augmenting the inflammatory cascade

IL-4 SNP: 590C→T

IL-4 590C→T increases production of IL-4 which decreases the barrier function of lung epithelial cells and gastrointestinal epithelial cells, allowing for increased antigen penetration

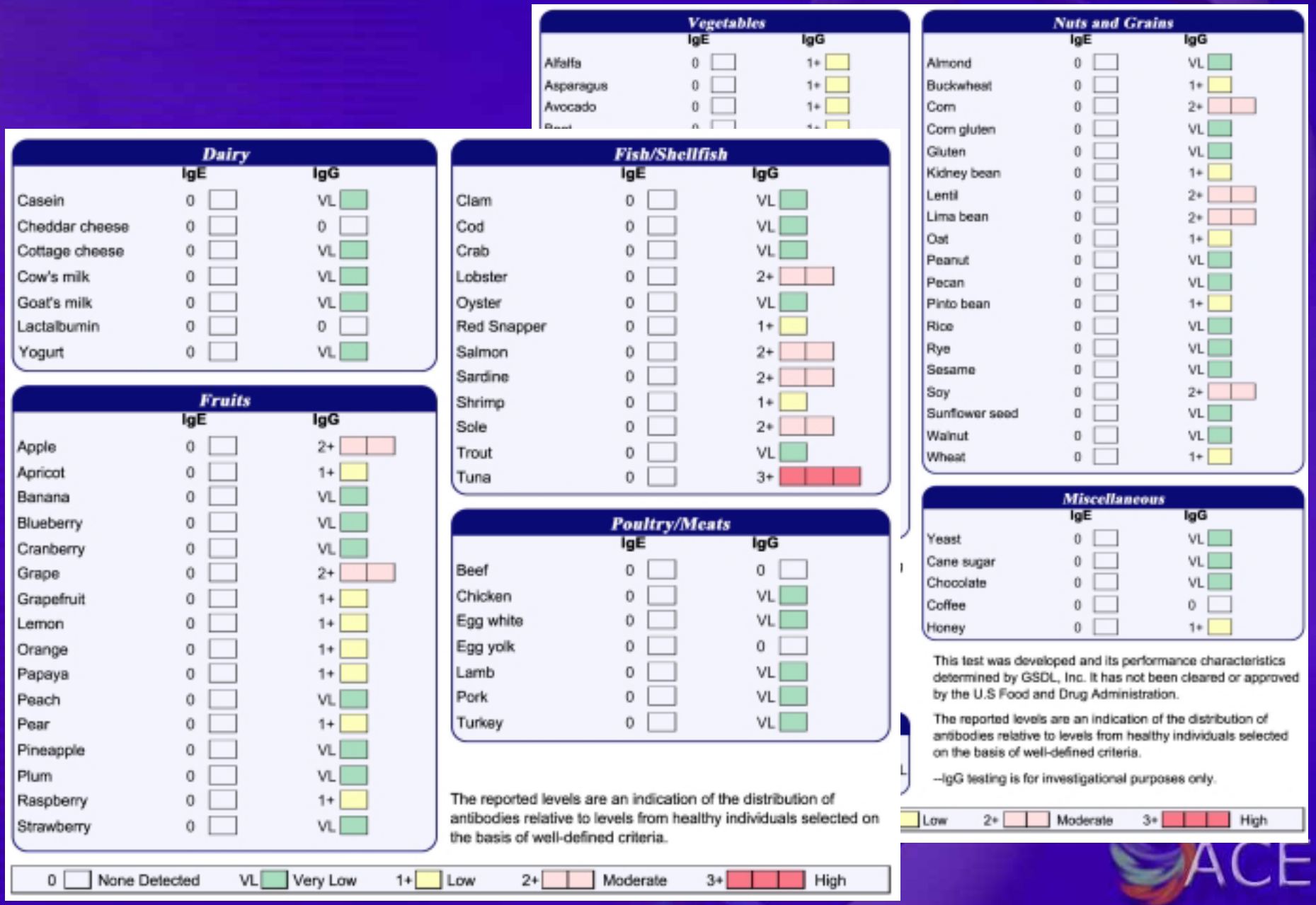
Ahdieh M, et al. Lung epithelial barrier function and wound healing are decreased by IL-4 and IL-13 and enhanced by IFN-gamma. *Am J Physiol Cell Physiol* 2001 Dec;281(6):C2029-38.

ACE

IL-4 SNP: 590C→T

- Functional assessment of intestinal permeability, and for food and inhalant antibodies is warranted
- Based on functional testing results, therapies as for Leaky Gut Syndrome may be appropriate: e.g., quercetin, glutamine, fish oils, vitamin C, etc.

Food Antibody Assessment



Vegetables

	IgE	IgG
Alfalfa	0	1+
Asparagus	0	1+
Avocado	0	1+

Nuts and Grains

	IgE	IgG
Almond	0	VL
Buckwheat	0	1+
Corn	0	2+
Corn gluten	0	VL
Gluten	0	VL
Kidney bean	0	1+
Lentil	0	2+
Lima bean	0	2+
Oat	0	1+
Peanut	0	VL
Pecan	0	VL
Pinto bean	0	1+
Rice	0	VL
Rye	0	VL
Sesame	0	VL
Soy	0	2+
Sunflower seed	0	VL
Walnut	0	VL
Wheat	0	1+

Dairy

	IgE	IgG
Casein	0	VL
Cheddar cheese	0	0
Cottage cheese	0	VL
Cow's milk	0	VL
Goat's milk	0	VL
Lactalbumin	0	0
Yogurt	0	VL

Fruits

	IgE	IgG
Apple	0	2+
Apricot	0	1+
Banana	0	VL
Blueberry	0	VL
Cranberry	0	VL
Grape	0	2+
Grapefruit	0	1+
Lemon	0	1+
Orange	0	1+
Papaya	0	1+
Peach	0	VL
Pear	0	1+
Pineapple	0	VL
Plum	0	VL
Raspberry	0	1+
Strawberry	0	VL

Fish/Shellfish

	IgE	IgG
Clam	0	VL
Cod	0	VL
Crab	0	VL
Lobster	0	2+
Oyster	0	VL
Red Snapper	0	1+
Salmon	0	2+
Sardine	0	2+
Shrimp	0	1+
Sole	0	2+
Trout	0	VL
Tuna	0	3+

Poultry/Meats

	IgE	IgG
Beef	0	0
Chicken	0	VL
Egg white	0	VL
Egg yolk	0	0
Lamb	0	VL
Pork	0	VL
Turkey	0	VL

Miscellaneous

	IgE	IgG
Yeast	0	VL
Cane sugar	0	VL
Chocolate	0	VL
Coffee	0	0
Honey	0	1+

The reported levels are an indication of the distribution of antibodies relative to levels from healthy individuals selected on the basis of well-defined criteria.

This test was developed and its performance characteristics determined by GSDL, Inc. It has not been cleared or approved by the U.S Food and Drug Administration.

The reported levels are an indication of the distribution of antibodies relative to levels from healthy individuals selected on the basis of well-defined criteria.

--IgG testing is for investigational purposes only.

0 None Detected VL Very Low 1+ Low 2+ Moderate 3+ High

1+ Low 2+ Moderate 3+ High

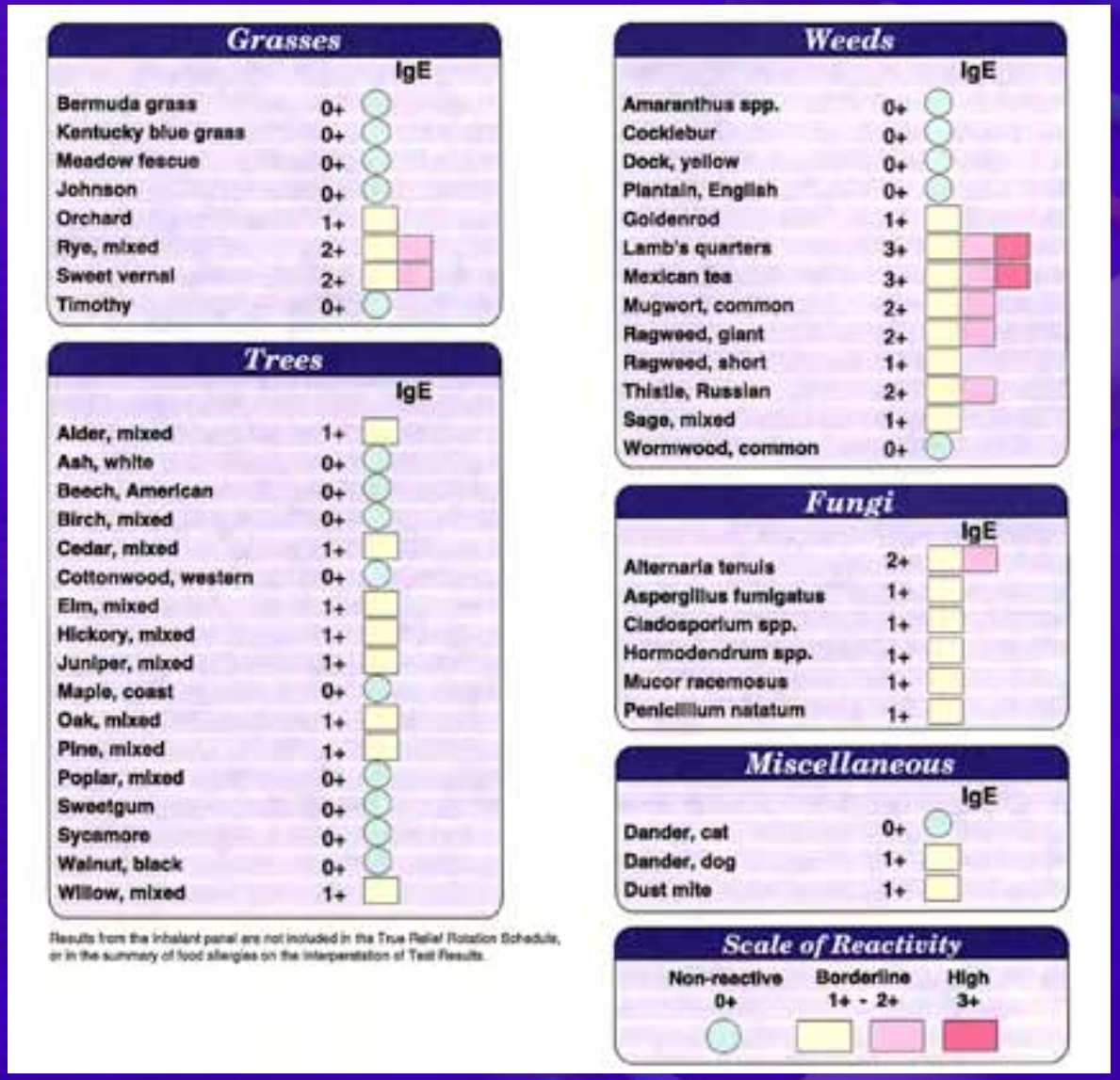
IgG Sensitivity Treatment Protocol

- Eliminate reactive foods
  - 3+ foods should be avoided for ~6 months
  - 2+ foods should be avoided for ~3 months
  - avoid all IgE reactive foods permanently
- Rotate non-reactive foods
  - 4 day rotation for individual foods
  - 2 day rotation for food families
- Re-introduce reactive foods in 3-6 months
  - 1 new food every four days
  - watch for immediate *and* delayed reactions

IgE Inhalant Allergy Treatment Options

- Nutritional support for mast cell stabilization
  - Cod Liver Oil, Quercetin, Vitamin C
- Sub-lingual Neutralizing Dose
- Allergy Desensitization Shots
- Homeopathy by symptom

IgE Inhalants Panel



Grasses

	IgE
Bermuda grass	0+
Kentucky blue grass	0+
Meadow fescue	0+
Johnson	0+
Orchard	1+
Rye, mixed	2+
Sweet vernal	2+
Timothy	0+

Trees

	IgE
Alder, mixed	1+
Ash, white	0+
Beech, American	0+
Birch, mixed	0+
Cedar, mixed	1+
Cottonwood, western	0+
Elm, mixed	1+
Hickory, mixed	1+
Juniper, mixed	1+
Maple, coast	0+
Oak, mixed	1+
Pine, mixed	1+
Poplar, mixed	0+
Sweetgum	0+
Sycamore	0+
Walnut, black	0+
Willow, mixed	1+

Results from the inhalant panel are not included in the True Relief Rotation Schedule, or in the summary of food allergies on the Interpretation of Test Results.

Weeds

	IgE
Amaranthus spp.	0+
Cocklebur	0+
Dock, yellow	0+
Plantain, English	0+
Goldenrod	1+
Lamb's quarters	3+
Mexican tea	3+
Mugwort, common	2+
Ragweed, giant	2+
Ragweed, short	1+
Thistle, Russian	2+
Sage, mixed	1+
Wormwood, common	0+

Fungi

	IgE
Alternaria tenuis	2+
Aspergillus fumigatus	1+
Cladosporium spp.	1+
Hormodendrum spp.	1+
Mucor racemosus	1+
Penicillium natatum	1+

Miscellaneous

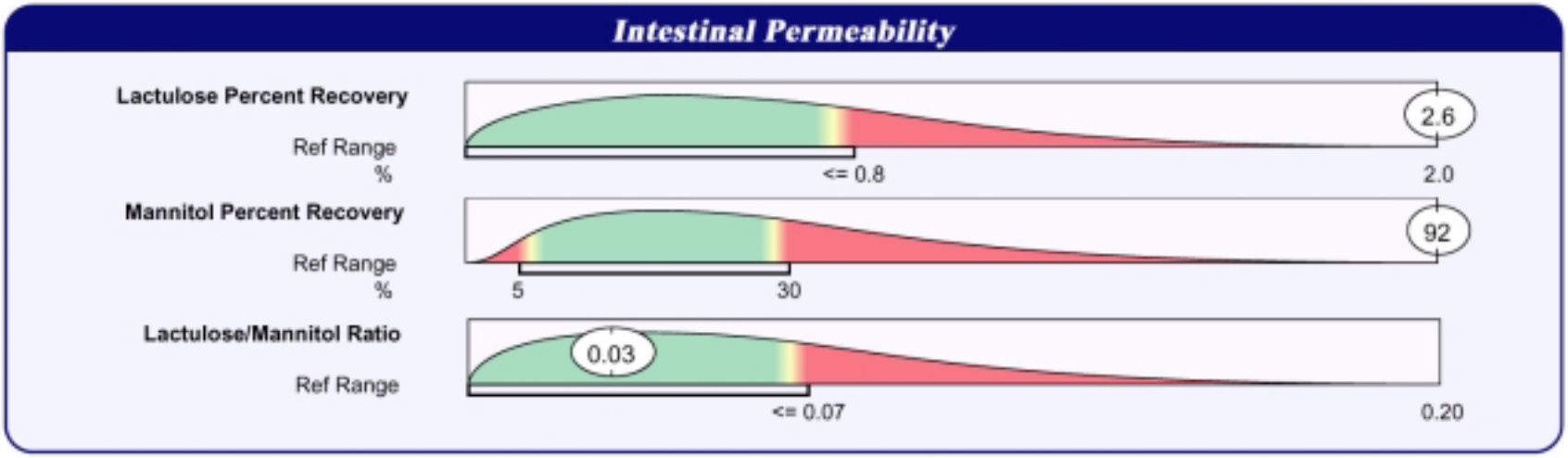
	IgE
Dander, cat	0+
Dander, dog	1+
Dust mite	1+

Scale of Reactivity

Non-reactive	Borderline	High
0+	1+ - 2+	3+

ACE

Intestinal Permeability



This test has been developed and its performance characteristics determined by GSDL, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration.

Interleukin – 10 (IL-10)

- Enhances growth and differentiation of mast cells
- Inhibits TH-1 cytokine production which can adversely affect cell-mediated immune response

IL-10 SNP: -627C→A

- IL-10 -627C→A polymorphism decreases production of IL-10 and accordingly favors a pro-inflammatory reactive state
- Heavy drinkers with at least one A allele are more likely develop advanced liver disease

Grove J, et al. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. *Gut* 2000 Apr;46(4):540-5.

ACE

IL-10 SNP: -627C→A Therapeutic Approach

- Avoid any activity that up-regulates acute inflammatory response
- Minimal alcohol intake appears warranted

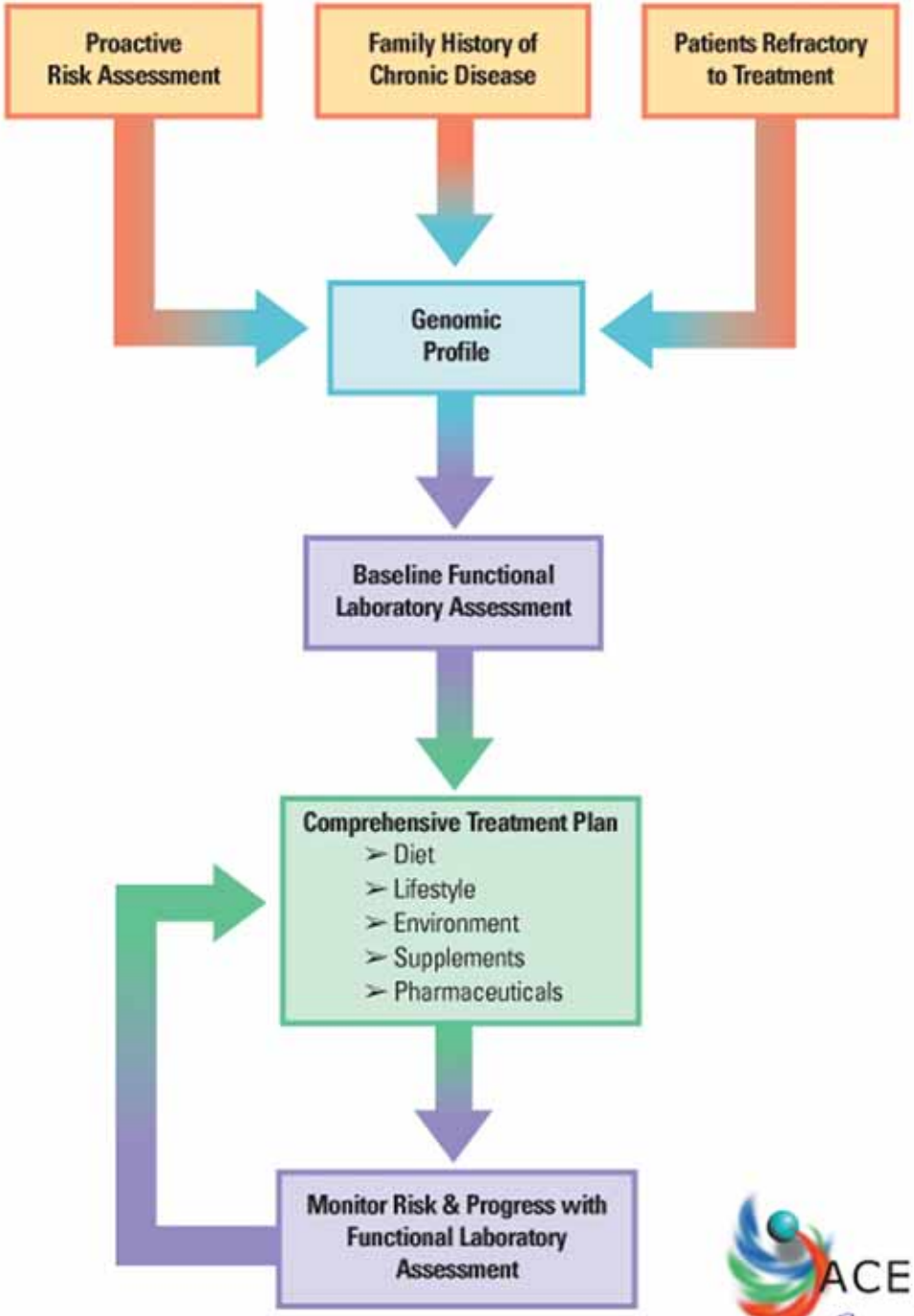
Etiology of Asthma and Atopy?

- Children who are “too clean” are more likely to develop asthma & atopy
- Mycobacteria in dirt stimulate cell-mediated (TH-1) immunity whereas vaccinations stimulate humoral immunity (TH-2)
- A modern, sanitized, vaccinated child is likely to over-develop TH-2 immunity and under-develop TH-1 immunity, rendering him or her susceptible to asthma & atopy

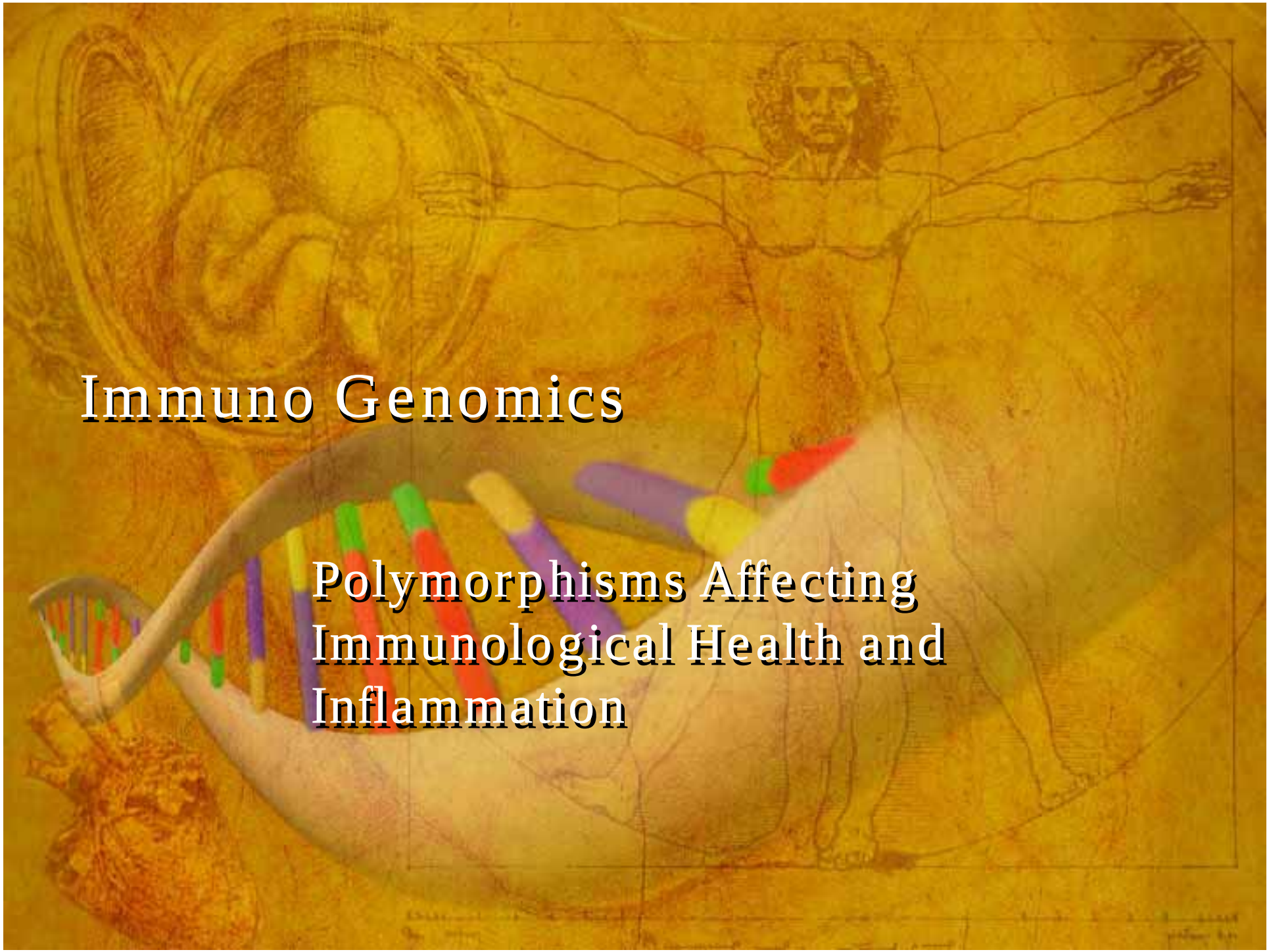
Hamilton G. Let them eat dirt. *New Scientist*, July 18, 1998:26-31. Rook GAW and Stanford JL. Give us this day our daily germs. *Immunology Today* 1998;19:113-116.

ACE

Predictive Genomics and Functional Medicine

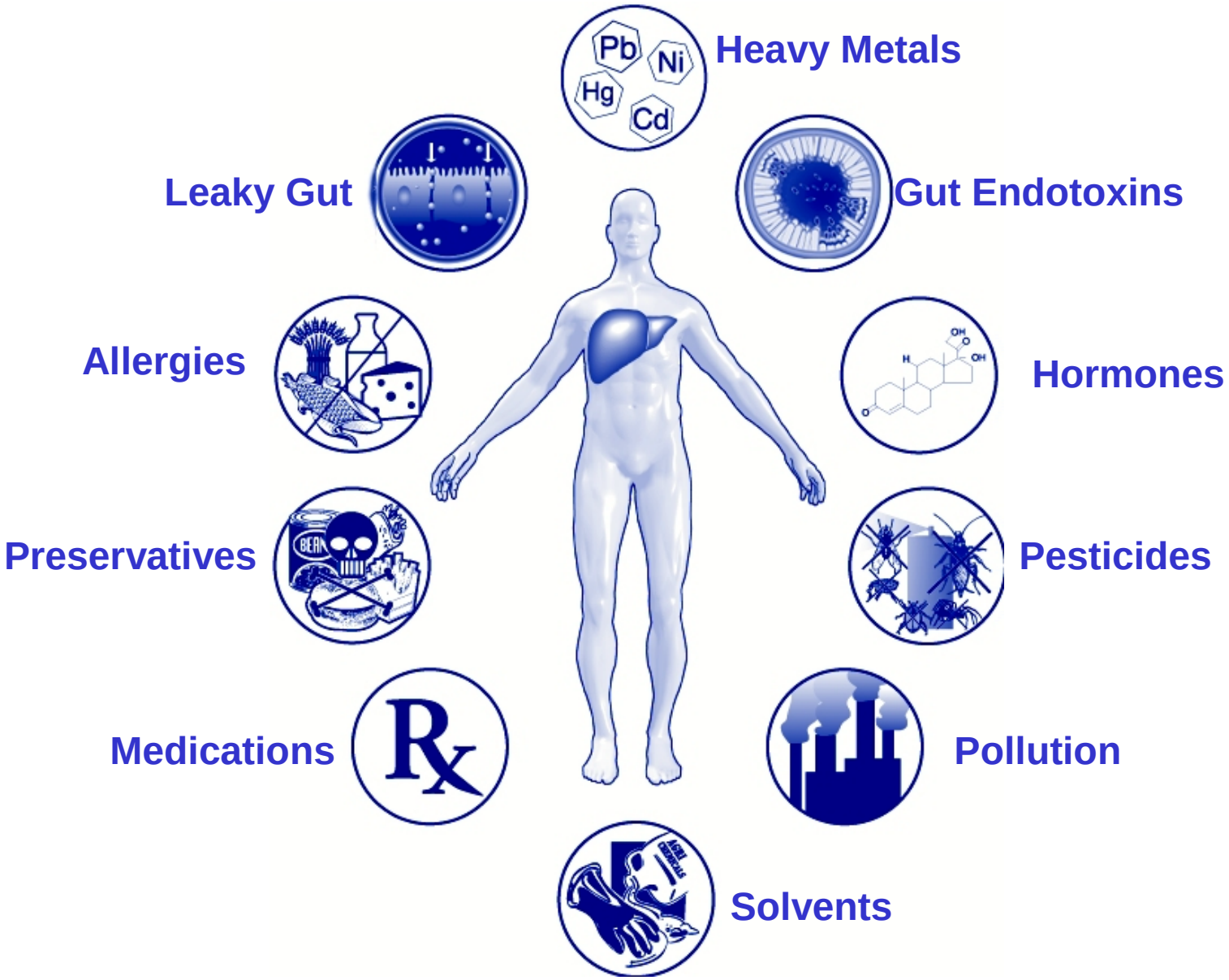


ACE
Genomics
In Primary Care Medicine


Immuno Genomics
Polymorphisms Affecting Immunological Health and Inflammation


Detoxi Genomics
Polymorphisms Affecting
Detoxification Pathways

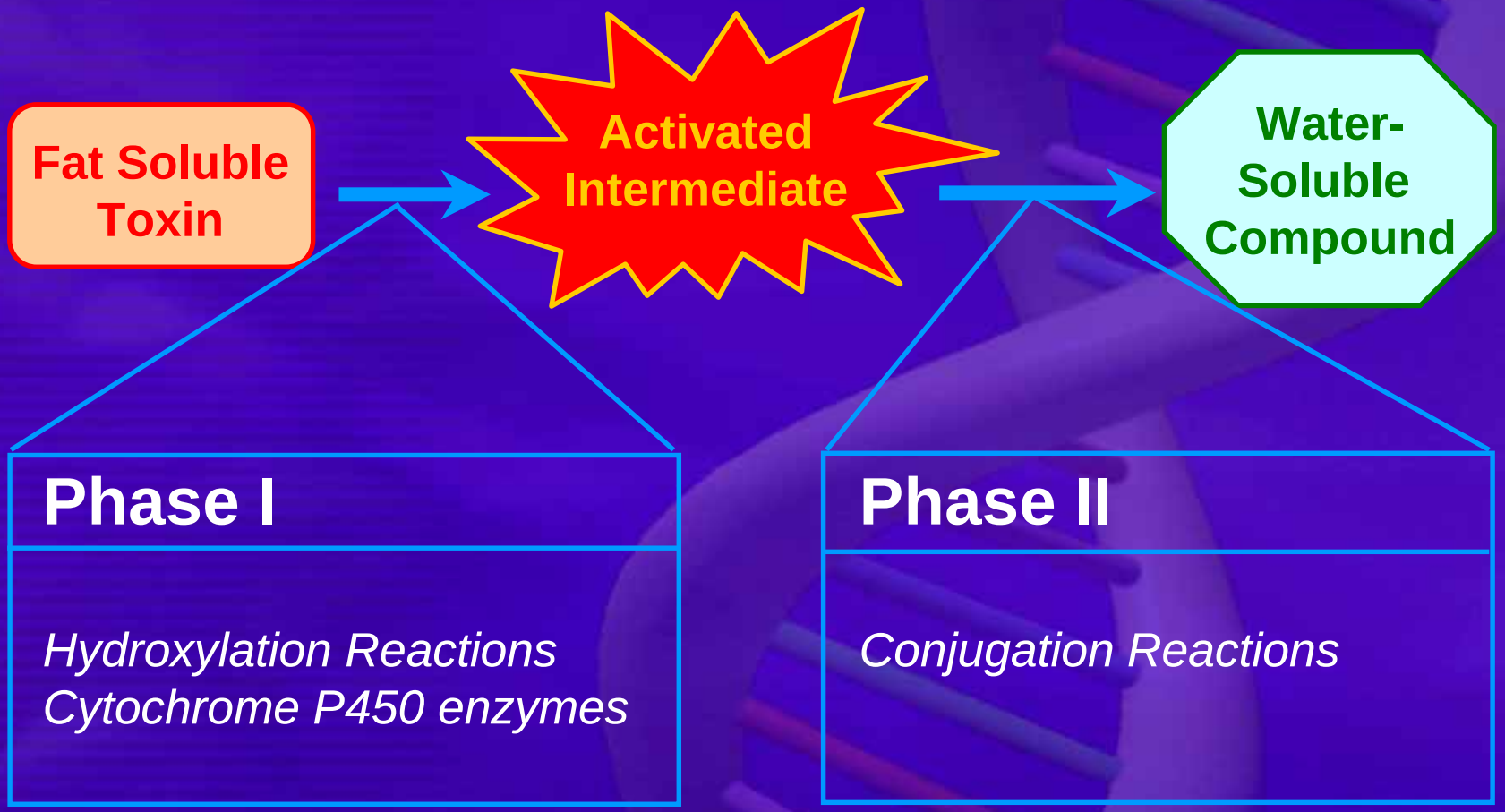
Sources of Toxicity



Hepatic and Cellular Detoxification

- Non-polar toxins are fat-soluble which makes them easy to absorb but difficult to excrete
- When overloaded with exogenous fat-soluble toxins, endogenous wastes like steroid hormones and cholesterol are also difficult to detoxify and excrete

Two Major Pathways of Hepatic Detoxification



Predictive Toxicology
Environmental Exposure and Disease: Before Predictive Genomic Testing

Exposure	Disease
Low	Low Risk
High	High Risk

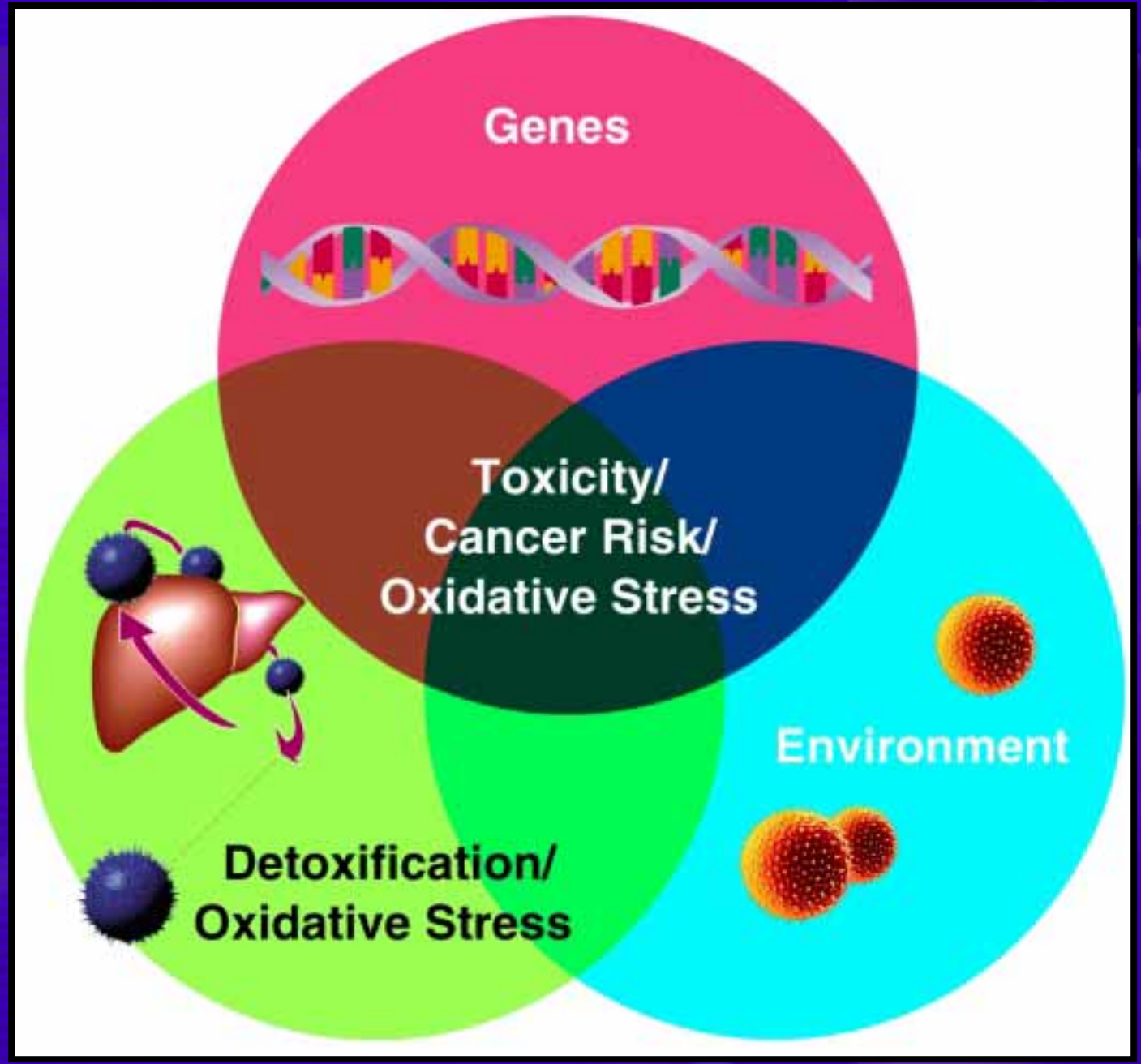
Predictive Toxicology
Environmental Exposure and Disease:
With Predictive Genomics Testing

Environmental Exposure	Genetic Susceptibility	Disease Risk
Low	Low	Low
High	Low	Moderate
Low	High	Moderate
High	High	High

The Straw that Broke the Camel's Back

Classic Signs and Symptoms of Toxic Overload

- Fatigue, malaise, myalgia, headaches
- Symptoms exacerbate with chemical exposure (perfumes, smoke, gasoline)?
- Adverse or paradoxical reactions to common drugs, herbs, vitamins, etc.?
- Adverse reactions to caffeine?
- Physically ill with increased stress or exercise?
- Neuropathies, sleep disturbances


Genes
Toxicity/
Cancer Risk/
Oxidative Stress
Environment
Detoxification/
Oxidative Stress

High and Low Penetrance

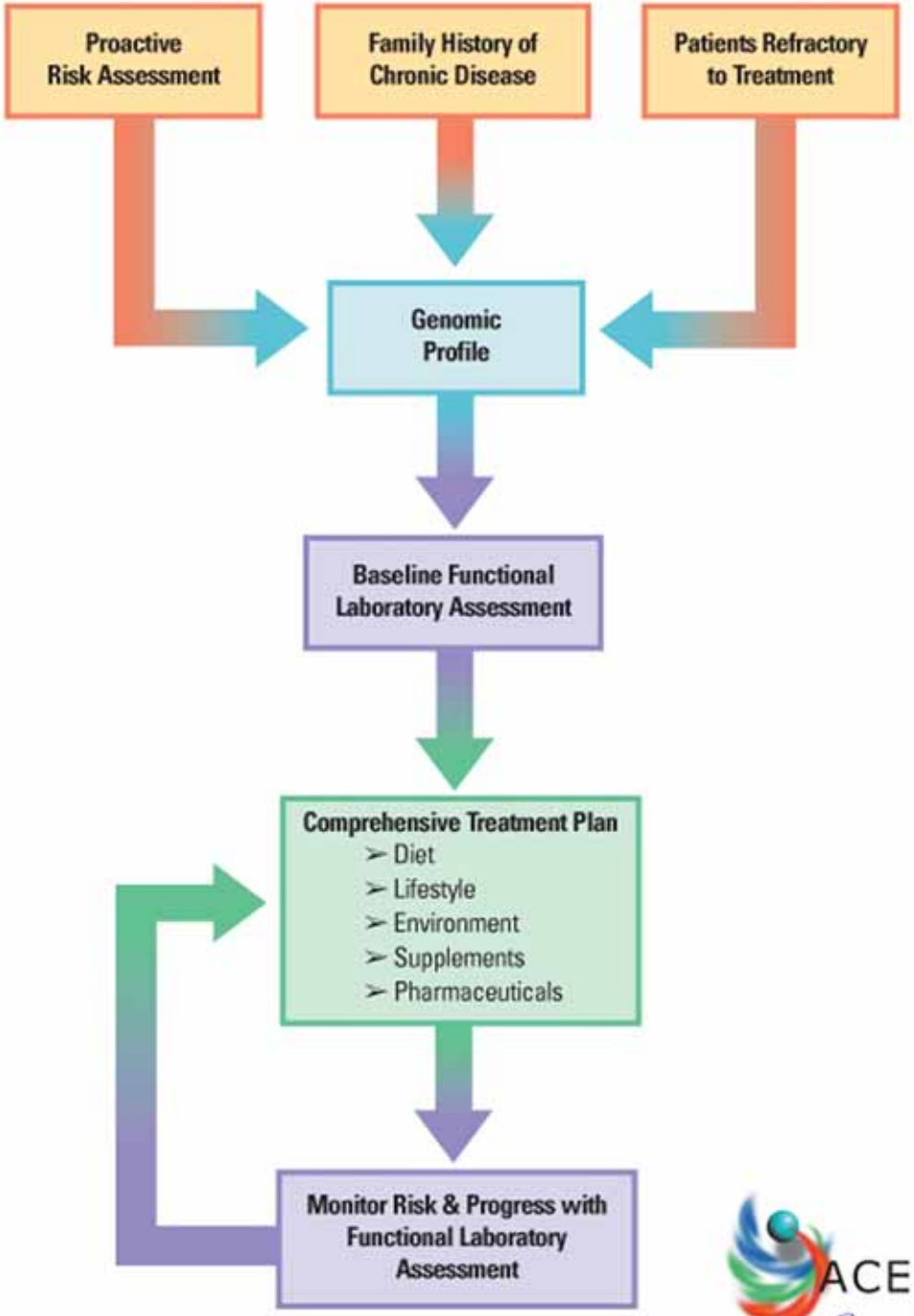
High Penetrance Genes: rare genes causing family syndromes

Low Penetrance Genes: common genes with high gene-environment interaction leading to seemingly sporadic incidence of disease contrary to familial syndromes, AKA, susceptibility genes

Shields PG, Harris CC. Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. J Clin Oncol 2000 ;18(11):2309-15.

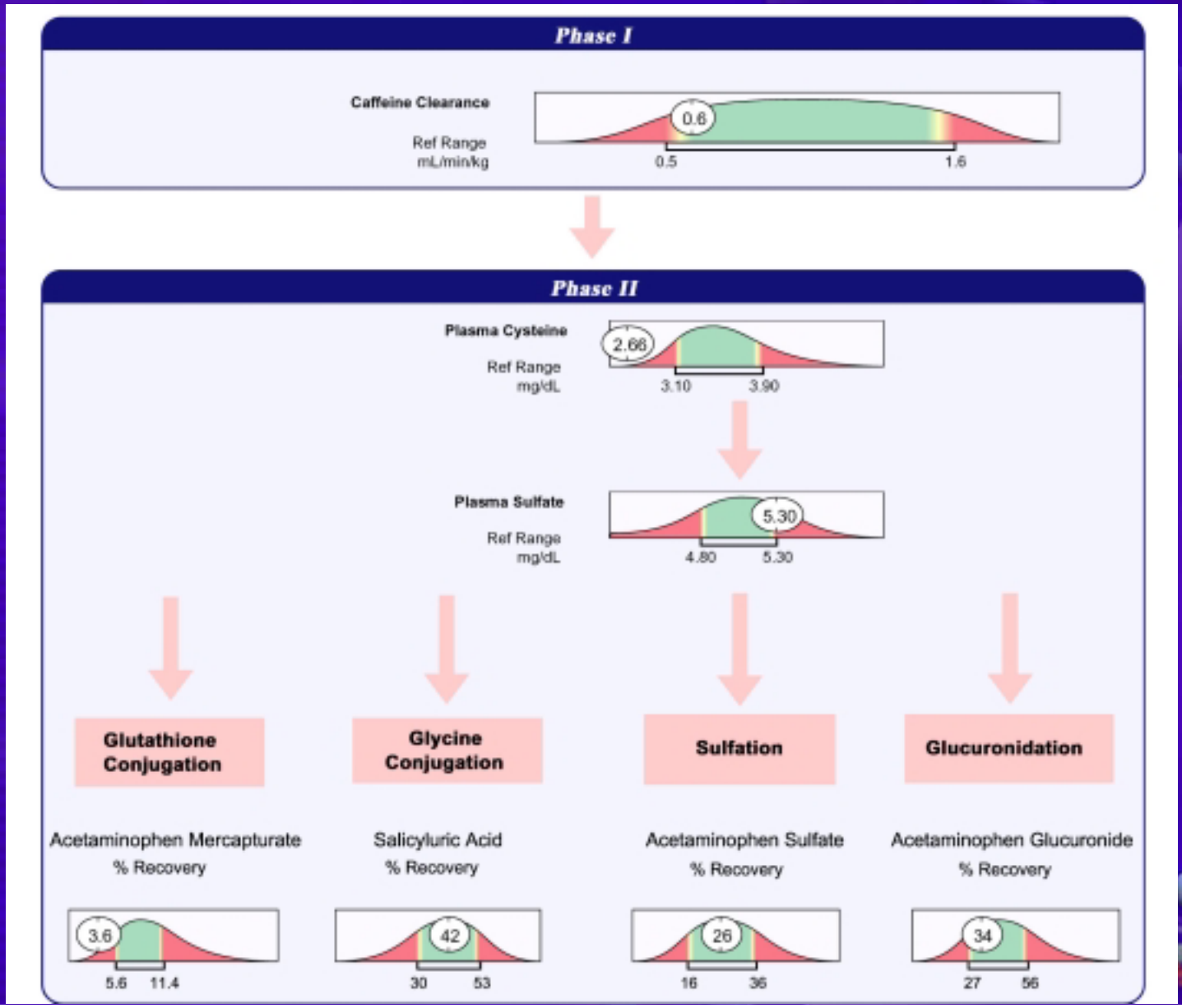
ACE

Predictive Genomics and Functional Medicine



ACE
Genomics
In Primary Care Medicine

Monitoring Detoxification Capacity



Detoxi Genomic Polymorphisms

- Identifies polymorphisms associated with increased risk of developing detoxification defects especially with increased exposure to toxins
- Risk factors include altered phase 1 cytochrome P-450 detoxification, impaired glutathione conjugation and acetylation in phase 2 reactions, altered catecholamine methylation, and increased oxidative stress

ACE

Detoxi Genomic Profile

Detoxification defects have been associated with increased risk for numerous cancers, chronic fatigue, multiple chemical sensitivity, and alcoholism

Detoxi Genomic Panel

Phase I

- CYP1A1
- CYP1B1
- CYP2A6
- CYP2C9
- CYP2D6
- CYP2E1
- CYP3A4

Phase II

- GSTM1
- GSTP1
- GSTT1
- NAT1
- NAT2
- COMT

Ox Stress

- SOD1
- SOD2

Detoxi Genomic Panel

Phase I

- CYP1A1
- CYP1B1
- CYP2A6
- CYP2C9
- CYP2D6
- CYP2E1
- CYP3A4

Phase II

- GSTM1
- GSTP1
- GSTT1
- NAT1
- NAT2
- COMT

Ox Stress

- SOD1
- SOD2

Cytochrome P-450 1A1

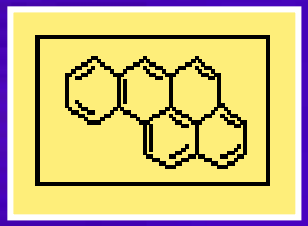
- Detoxifies polycyclic aromatic hydrocarbons (PAHs – fused benzene rings, e.g., benzopyrene) found in cigarette smoke, cooked meats, diesel fumes, and the combustion of any organic material
- Present in lung, intestines, skin, lymphocytes and the placenta
- CYP 1A2 predominates in liver

CYP 1A1 SNPs: I462V and MspI T→C

These polymorphisms are easily induced by moderate exposure to PAHs

Hyperinduction can generate mutagenic metabolites and oxidative stress, increasing risk of developing lung and other cancers.

Detoxifying Cigarette Smoke



Benzopyrene
(cigarette smoke)

Phase 1
CYP 1A1 & 1A2

Benzo-(a)pyren-7,8-diol-9-epoxide

Phase 2
GST

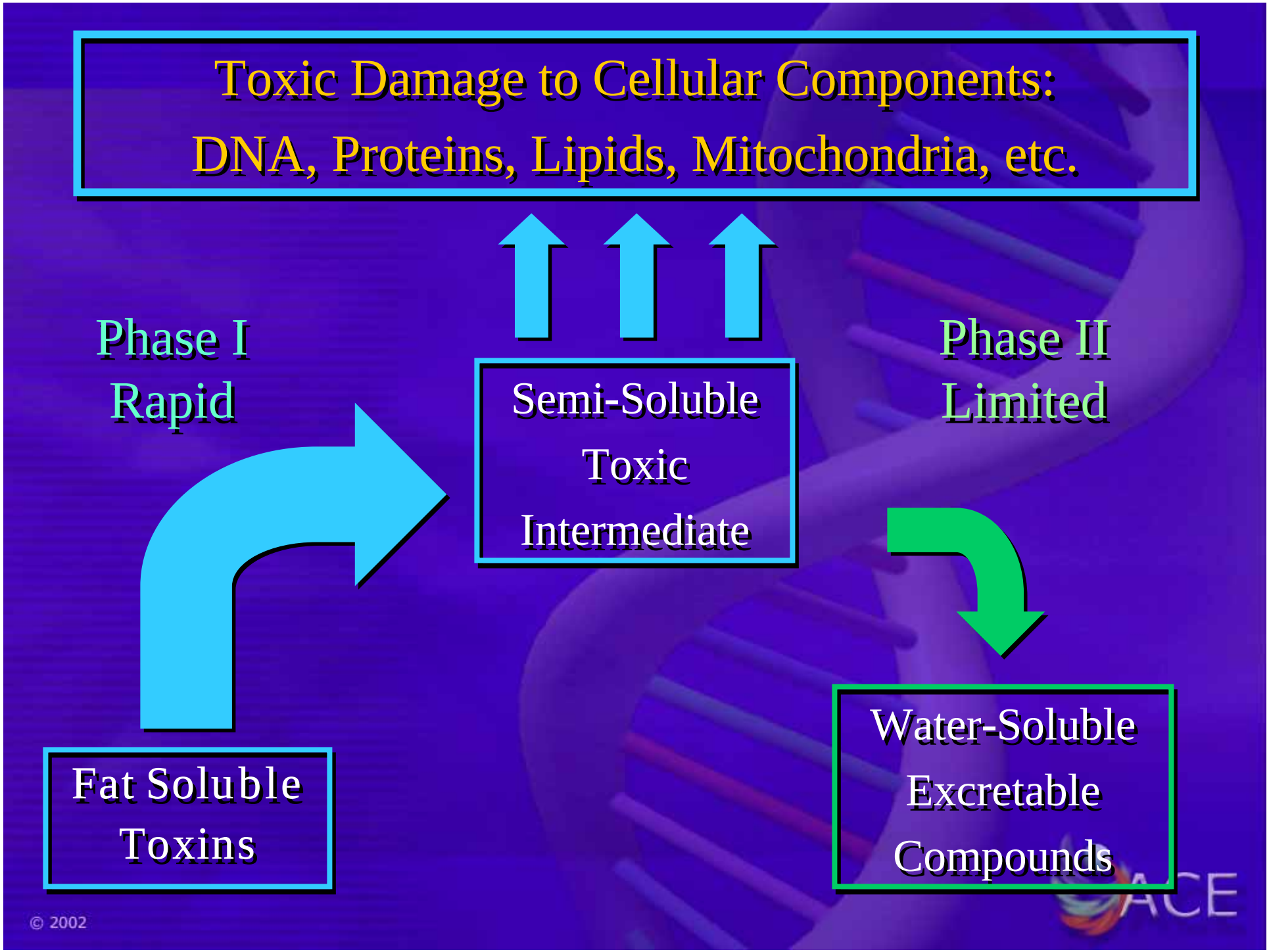
Glutathione Conjugate Excreted

The Special Case of Imbalanced Detoxifiers

Phase I detoxification tends to increase with increased toxic exposure (inducible)

But Phase II detoxification capacity is limited (constitutive)

If Phase I clearance outstrips Phase II, increased toxic intermediates will be produced, causing more damage than the original toxins


Toxic Damage to Cellular Components:
DNA, Proteins, Lipids, Mitochondria, etc.
Phase I
Rapid
Semi-Soluble
Toxic
Intermediate
Phase II
Limited
Fat Soluble
Toxins
Water-Soluble
Excretable
Compounds
© 2002

Symptom Picture of Imbalanced Detoxifiers

Cycle of Constant Pain & Inflammation

- *myofascial & joint pain*
- *recurring headaches*
- *chronic low grade fever*
- *fatigue & malaise*

Chronic Fatigue Syndrome

Fibromyalgia

Long-term Treatment Goals

- Identify and reduce toxin exposure
- Supply conjugating substances for Phase II
  - Glutathione
  - Sulfate
  - Glycine & Taurine
  - Blood sugar balancing diet
- Brassica vegetables to induce conjugation
- Increase anti-oxidant support

CYP 2D6

- Cytochrome P450 2D6 is involved in the detoxification of >20% of all prescribed medications, including tricyclics, MAOIs, SSRIs, opiates, anti-arrhythmics, beta-blockers, etc.
- Polymorphisms help identify slow, moderate and rapid detoxifiers

CYP 2D6 Slow-Metabolizers

- Poor metabolizers have decreased ability to hydroxylate a wide variety of drugs lower dosages may be required to prevent toxicity
- Poor metabolizers have an increased risk of developing Parkinson's disease, probably due to decreased resistance to environmental toxins

CYP 2D6 Fast Metabolizers

- Fast metabolizers may be non-responsive to many drugs including tricyclics, MAOIs, SSRIs, opiates, anti-arrhythmics, beta-blockers
- They have an increased risk of smoking addiction and heavy cigarette use

Cytochrome P-450 2E1

- Detoxifies ethanol, halogenated alkanes (fluorocarbons)
- Acetaminophen, benzene, carbon tetrachloride, chloroform, styrene,polyvinyl chloride
- Present in liver, kidney, lungs, lymphocytes

Cytochrome P-450 2E1

Lung carcinoma

Multiple Chemical Sensitivity

Alcohol damaged liver

CYP 2E1 1019C→T and IVS6, Dra I

- Cytochrome P450 2E1 is involved in the metabolism of nitrosamines and ethanol (acetaldehyde)
- Individuals with polymorphisms may be at greater risk for alcoholism, lymphoma and possibly other cancers

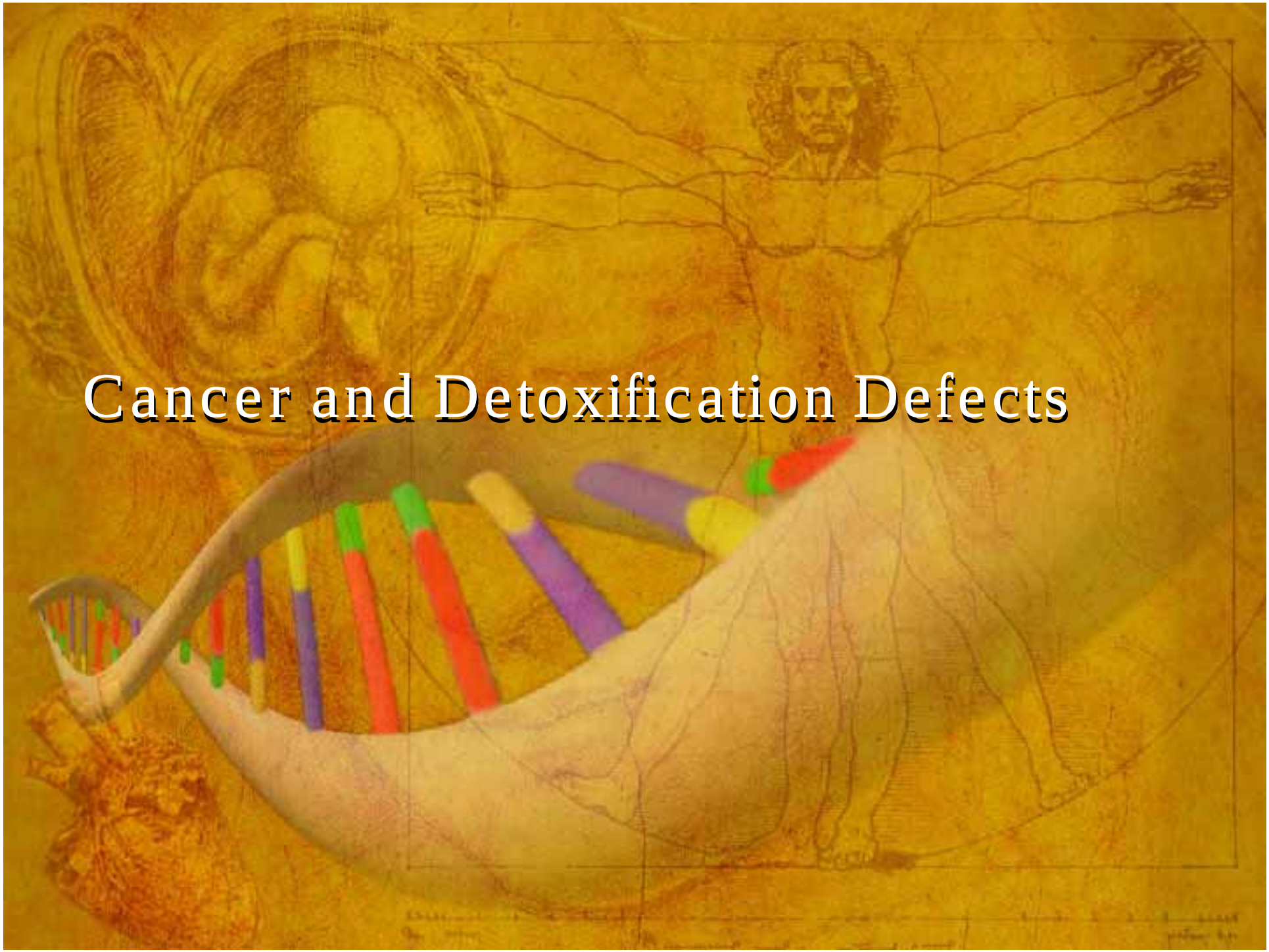
CYP 3A4

- Cytochrome P450 3A4 is used in the metabolism of over 50% of all drugs used by humans as well as steroid hormones
- Individuals with polymorphisms have decreased detoxification capacity and are may be more susceptible to drug toxicity as well as prostate and breast cancer

CYP 3A4 Polymorphisms

4 SNPs with higher prevalence:

1. –290A→G
2. M445T
3. F189S
4. R162Q


Cancer and Detoxification Defects

Heritability of Cancer

Twin studies have allowed researchers to conclude that cancer of the stomach, colon, lung, breast, and prostate have a heritability of between 26 and 42%, suggesting that while environment plays a relatively larger role in these cancers, genetic predisposition nonetheless plays a significant role in cancer etiology

Lichtenstein P, Holm NV, et al. Environmental and heritable factors in the causation of cancer: analyses of cohorts of twins from Sweden, Denmark, Finland. *N Engl J Med* 2000;343(2):78-85.

Breast Cancer and Environment

- Asian-American women born in the USA have a 60% higher risk of breast cancer as Asian-American women born in Asia
- Migrants who had lived in the West for a decade or longer had a risk 80% higher than more recent migrants

Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. *J Natl Cancer Inst* 1993 Nov 17;85(22):1819-27.

ACE

Pathophysiology of Cancer

Most researchers think that cancer results from the accumulation of mutations in genes that regulate growth

- Spontaneous mutations
- Inherited mutations

Bruce Ames 1960s

- Ames realized that many substances that caused cancer had one thing in common: they caused damage to the DNA
- Ames Test: estimates the mutagenicity (or anti-mutagenicity) of a substance by adding it to Salmonella cultures that are unable to synthesize histidine
- Mutagenicity is measured in terms of the number of cultures that can subsequently produce histidine after exposure to the substance

Cancer Suppressors and Promoters

Proto-oncogenes: functional genes actively involved in normal cell proliferation and differentiation

Oncogenes: transformed genes that promote uncontrolled cell growth and proliferation

Tumor Suppressor Genes

- Gatekeeper genes: prevent a cell's entry into the division cycle until conditions are appropriate
- Caretaker genes: maintain the integrity of the cell's genome

Double Hit Theory

For cancer to develop, an oncogene must mutate so as to be jammed "on" and a tumor suppressor gene must mutate so as to be jammed "off"

TP53: "Guardian of the Genome"

- TP53 is a tumor suppressor gene that codes for the protein p53 which acts to begin the process of apoptosis, or cell suicide, in cells whose DNA has been irreparably damaged
- TP53 is the last line of defense against malignancy
- In more than half of all human cancers, TP53 is broken

Radiation and Chemotherapy

- Doctors used to believe that radiation and chemotherapy acted by preferentially killing the most rapidly dividing cells
- But if this is the case why are many rapidly dividing cancers resistant to therapy, and more perplexing, why do tumors become resistant to the effects of radiation and chemotherapy

Radiation and Chemotherapy

- Rather than killing cancer cells directly, perhaps radiation and chemotherapy act by damaging cellular DNA sufficiently to cause TP53 to start producing p53
- When tumors relapse after therapy, the change correlates closely with knockout mutations to TP53
- The most intractable tumors – lung, bladder, melanoma, colorectal, and prostate – are ones in which TP53 is already mutated

Radiation and Chemotherapy

- Instead of looking for agents that kill dividing cells, researchers should have been looking for agents that promote apoptosis
- This is not to say that chemotherapy has been wholly ineffective in treating cancer, only that its effectiveness has been largely accidental

Lowe SW. Cancer therapy and p53. *Curr Opin Oncol* 1995 Nov;7(6):547-53.
Wallace-Brodeur RR, Lowe SW. Clinical implications of p53 mutations. *Cell Mol Life Sci* 1999 Jan;55(1):64-75.

ACE

TP53 and Evolution: The Kamikaze Conundrum

- Genes like TP53 that code for apoptosis by definition cannot be passed on to daughter cells since once engaged, the cell is dead
- Not all evolution, of course, happens at the level of individual genes
- Natural selection is still operating at the level of the whole individual organism

LeGrand EK. An adaptationist view of apoptosis. *Q Rev Biol* 1997 Jun;72(2):135-47.

ACE

DNA Methylation

- Folate deficiency induces DNA strand breaks and hypomethylation in the p53, tumor suppressor gene
- Folate deficiency appears to enhance carcinogenesis
- Even marginal dietary folic acid deficiency (<300µg/d) may alter DNA composition

Kim YI, Pogribny IP, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. *Am J Clin Nutr* 1997;65:46-52.
Jacob RA, Gretz DM, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr* 1998;128:1204-1212.

Health Risks and Exposure

Two critical components to evaluating health risks from exposure to environmental and occupational agents:

1. Hazard identification
2. Dose-response analysis

Low Penetrance (Susceptibility) Genes

- Some genetic polymorphisms have no effect on disease outcome by itself but rather modify the risk associated with exposure to specific environmental toxins
- These polymorphisms are known as effect modifiers

Taioli E, Zocchetti C, Garte S. Models of interaction between metabolic genes and environmental exposure in cancer susceptibility. *Environ Health Perspect* 1998 Feb;106(2):67-70.

ACE

Terminology: Relative Risk

RR estimates the magnitude of an association between a polymorphism and disease and estimates the likelihood of developing the disease in the variant group relative to the wild-type group

Terminology: Odds Ratio

In a case-control study, the relative risk is estimated by calculating the odds of a polymorphism among the affected cases relative to that among controls. The odds ratio represents the magnitude of that association.

Odds Ratio for Bladder Cancer as a Function of Smoking Status and NAT1*10 Genotype

Group	OR (95% CI)
Never Smoked	1.0
Ever Smoked	2.8
+ Normal NAT1	2.5
+ NAT1*10 heterozygote	3.8
+ NAT1*10 homozygote	6.7

Taylor JA, Umbach DM, et al. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. Cancer Res 1998;58(16):3603-10.

ACE

Detoxi Genomic Panel

Phase I

- CYP1A1
- CYP1B1
- CYP2A6
- CYP2C9
- CYP2D6
- CYP2E1
- CYP3A4

Phase II

- GSTM1
- GSTP1
- GSTT1
- NAT1
- NAT2
- COMT

Ox Stress

- SOD1
- SOD2

Detoxi Genomic Panel

Phase I

- CYP1A1
- CYP1B1
- CYP2A6
- CYP2C9
- CYP2D6
- CYP2E1
- CYP3A4

Phase II

- GSTM1
- GSTP1
- GSTT1
- NAT1
- NAT2
- COMT

Ox Stress

- SOD1
- SOD2

Glutathione S-Transferase

There are three isomers of GST:

GST M-1: liver > testis > brain

GST T-1: liver ≈ kidney > GI

GST P-1: brain > heart ≈ lung

Glutathione conjugation is used to detoxify a wide array of electrophilic compounds including, solvents, herbicides, fungicides, polycyclic aromatic hydrocarbons, lipid peroxides, and heavy metals (Hg, Pb, Cd)

Glutathione S-Transferase Polymorphisms

GST M-1: deletion

GST T-1: deletion

GST P-1:

I104V (313A→G)

A113V (341C→)

More than 50% of the population has one or more GST polymorphism

Board, P.; Coggan, M.; et al. Genetic heterogeneity of the human glutathione transferases: a complex of gene families. *Pharm. Therap.* 48: 357-369, 1990.

ACE

Glutathione S-Transferase Polymorphisms and Disease

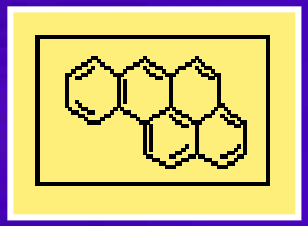
In general GST defects increase the risk of numerous cancers, including colorectal, lung, breast, ovarian, nasopharyngial, head and neck, and basal cell carcinoma,

GST defects have also been to contribute to poor prognosis with cystic fibrosis, multiple sclerosis, and asthma

Hayes JD, Strange RC. Glutathione S-Transferase Polymorphisms and Their Biological Consequences. Prhamacology 2000;61:154-166.

ACE

Detoxifying Cigarette Smoke



Benzopyrene
(cigarette smoke)

Phase 1
CYP 1A1 & 1A2

Benzo-(a)pyren-7,8-diol-9-epoxide

Phase 2
GST

Glutathione Conjugate Excreted

GSTM1 and NAT2 Polymorphisms and Chromosomal Aberrations in Urban Bus Drivers

Genotype	Chromosomal Aberrations Avg.
GSTM+; NAT2 fast	1.53
GSTM+; NAT2 slow	2.67
GSTM-; NAT2 fast	2.63
GSTM-; NAT2 slow	3.20

Knudsen LE, Norppa H, et al. Chromosomal aberrations in humans induced by urban air pollution: influence of DNA repair and polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. *Cancer Epidemiol Biomarkers Prev* 1999;8(4 Pt 1):303-10.

Employment and Lifestyle Concerns?

- Bus driver
- Petroleum refinery
- Industrial factory
- Waiter / bartender
- Smoker

NAT 1 – Slow Acetylators
R64W (190C→T)
R187Q (560G→A)

- N-acetyltransferase 1 is found in many extra-hepatic tissues in the body and is used in the Phase II acetylation of numerous xenobiotics, including caffeine and heterocyclic aromatic amines
- Slow acetylators exposed to xenobiotics, cigarette smoke, or other toxins have increased risk lung, colon, and bladder cancer due to a decreased efficiency with which toxins are conjugated

NAT-2 Slow Acetylators

- N-acetyltransferase 2 is found predominantly in the liver and the gut and is used in the Phase II acetylation of numerous xenobiotics, including heterocyclic aromatic amines
- If exposed to xenobiotics, the risk of lung, colon, and bladder cancer as well as head, neck and oral/pharyngeal cancer increases due to a decreased efficiency with which toxins are conjugated

NAT-2 Slow Acetylators

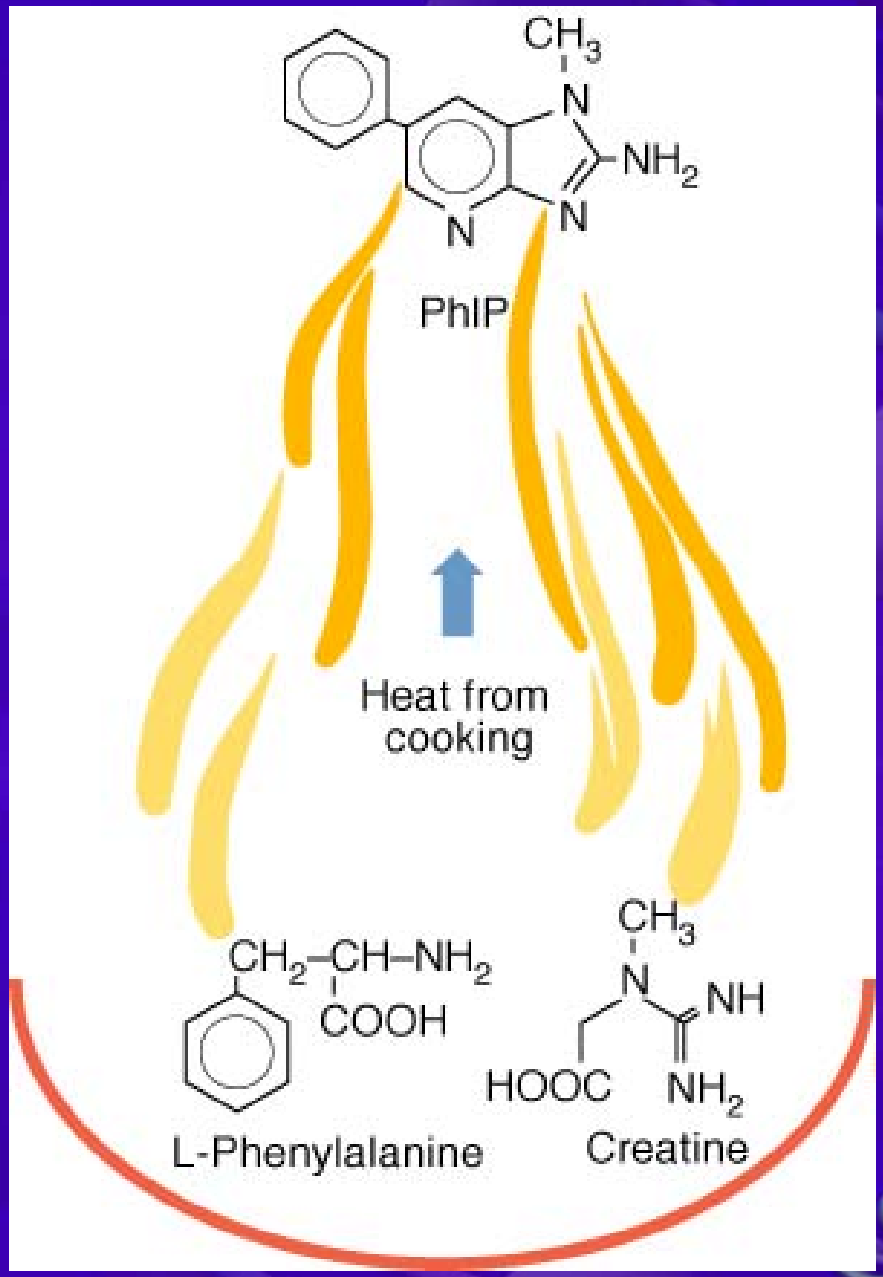
4 SNPs with higher prevalence:

1. I114T (341T→C)
2. A197Q (590G→A)
3. G286E (857G→A)
4. R64Q (191G→A)

NAT-2 Rapid Acetylators

- Rapid acetylation can increase the production of DNA adducts and therefore increase risk of developing adenomas and colorectal cancers, especially if combined with high dietary meat consumption
- Similarly, moderate smokers have a higher risk of lung cancer

Creating heterocyclic amines from cooking



ACE

Acetylation
The Good, The Bad, and The Ugly

Carcinogenic heterocyclic amines can be acetylated in 2 ways

N-acetylation: usually deactivation

O-acetylation: usually activation

The kinetics of acetylation are such that normal acetylation is N-acetylation but if someone is a rapid acetylator, *O*-acetylation increased dramatically

Acetylation
The Good, The Bad, and The Ugly

- Both slow and rapid acetylators are at increased risk for numerous cancers
- Slow Acetylators because they cannot clear toxic heterocyclic amines efficiently
- Rapid Acetylators because they produce more activated *O*-acetylated heterocyclic amine byproducts

Hein DW, et al. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev* 2000 Jan;9(1):29-42.

ACE

NAT-1 and NAT-2 Treatment

- Both slow and rapid acetylators should avoid consuming well done of flame broiled meats
- Both should encourage other phase II pathways by consuming copious quantities of brassica vegetables

Protective Dietary Phenolics

O-methylcatechol derivatives (lipophilic metabolites) of flavonoids from onion, lettuce, apples and red wine have been shown to confer protection against bladder cancer (and possibly other sites) in smokers

Malaveille C, Hautefeuille A, Pignatelli B, et al. Antimutagenic dietary phenolics as antigenotoxic substances in urothelium of smokers. *Mutat Res* 1998 Jun 18;402(1-2):219-24.

ACE

Let Your Food Be Your Medicine

Detoxi Genomic Panel

Phase I

- CYP1A1
- CYP1B1
- CYP2A6
- CYP2C9
- CYP2D6
- CYP2E1
- CYP3A4

Phase II

- GSTM1
- GSTP1
- GSTT1
- NAT1
- NAT2
- COMT

Ox Stress

- SOD1
- SOD2

Detoxi Genomic Panel

Phase I

- CYP1A1
- CYP1B1
- CYP2A6
- CYP2C9
- CYP2D6
- CYP2E1
- CYP3A4

Phase II

- GSTM1
- GSTP1
- GSTT1
- NAT1
- NAT2
- COMT

Ox Stress

- SOD1
- SOD2

Denham Harman

First proposed the idea of “free radicals” in 1956 and later postulated that these compounds play a role in aging through cross-linking reactions, covalently modifying lipids, proteins, cellular and mitochondrial DNA

Harman D. Free radical theory of aging: consequences of mitochondrial aging. *Age* 1983;6:86-94.

ACE

Free Radicals

ROS react with and damage structural or functional components of the body

- Membranes
- Enzymes
- Cellular DNA
- Mitochondrial DNA

Generation of Free Radicals and Reactive Oxygen Species

About 1-2% of oxygen consumed by our mitochondria is converted to superoxide and hydrogen peroxide

A single rat liver mitochondria produces ~$3X10^7$ superoxide radicals per day and each liver cell contains ~1000 mitochondria

Richter C. Oxidative damage to mitochondrial DNA and its relationship to ageing. Int J Biochem Cell Biol 1995;27:647-653.

ACE

Mitochondrial DNA and ROS Damage

- Mitochondrial DNA is 16-20 times as susceptible to oxidative damage than nuclear DNA
- mtDNA is fragmented by ROS and the fragments can accumulate in nDNA
- Over time, the accumulating mtDNA progressively alters the competence and nuclear information content of the nDNA, thereby causing aging, dysfunction, and cell death

Richter C. Reactive oxygen and DNA damage in mitochondria. *Mut Res* 1992;275:249-255.
Richter C. Do mitochondrial DNA fragments cause cancer and aging? *FEBS Lett* 1988;M241:1-5

Mitochondria, ROS, and Aging

Mitochondria of aged insects and mammals produce more ROS than those of young animals, and also contain more lipid peroxides

Maximal lifespan correlates inversely to the rate of oxygen consumption and positively to anti-oxidant capacity

3 Mechanisms To Protect Against Reactive Oxygen Species?

1. Enzymes

- Superoxide dismutase-SOD *(Zn, Cu, Mn)*
- Glutathione peroxidase *(Se)* and glutathione reductase (B_2)
- Catalase *(Fe)*

Groff JL, Gropper SS, Hunt SM. Advanced Nutrition and Human Metabolism. 2nd Ed.West Publishing. Minneapolis, MN; 1995, chapter 10.

ACE

3 Mechanisms To Protect Against Reactive Oxygen Species?

2. Dietary Anti-Oxidants

- Vitamin C for aqueous compartments
- Vitamin E for lipid compartments
- Carotenoids, flavonoids, etc

3 Mechanisms To Protect Against Reactive Oxygen Species?

3. Endogenous Molecules with Secondary Anti-Oxidant Properties

Glutathione,

Coenzyme Q_{10},

Lipoic acid, etc.

Superoxide Radical

Mitochondrial transport of electrons is particularly weak at complex #3 and can result in the release of free electrons that can react with molecular oxygen to produce the superoxide radical

$$e^- + O_2 \rightarrow O_2^-$$

The unpaired electron of O_2^- is highly reactive

ACE

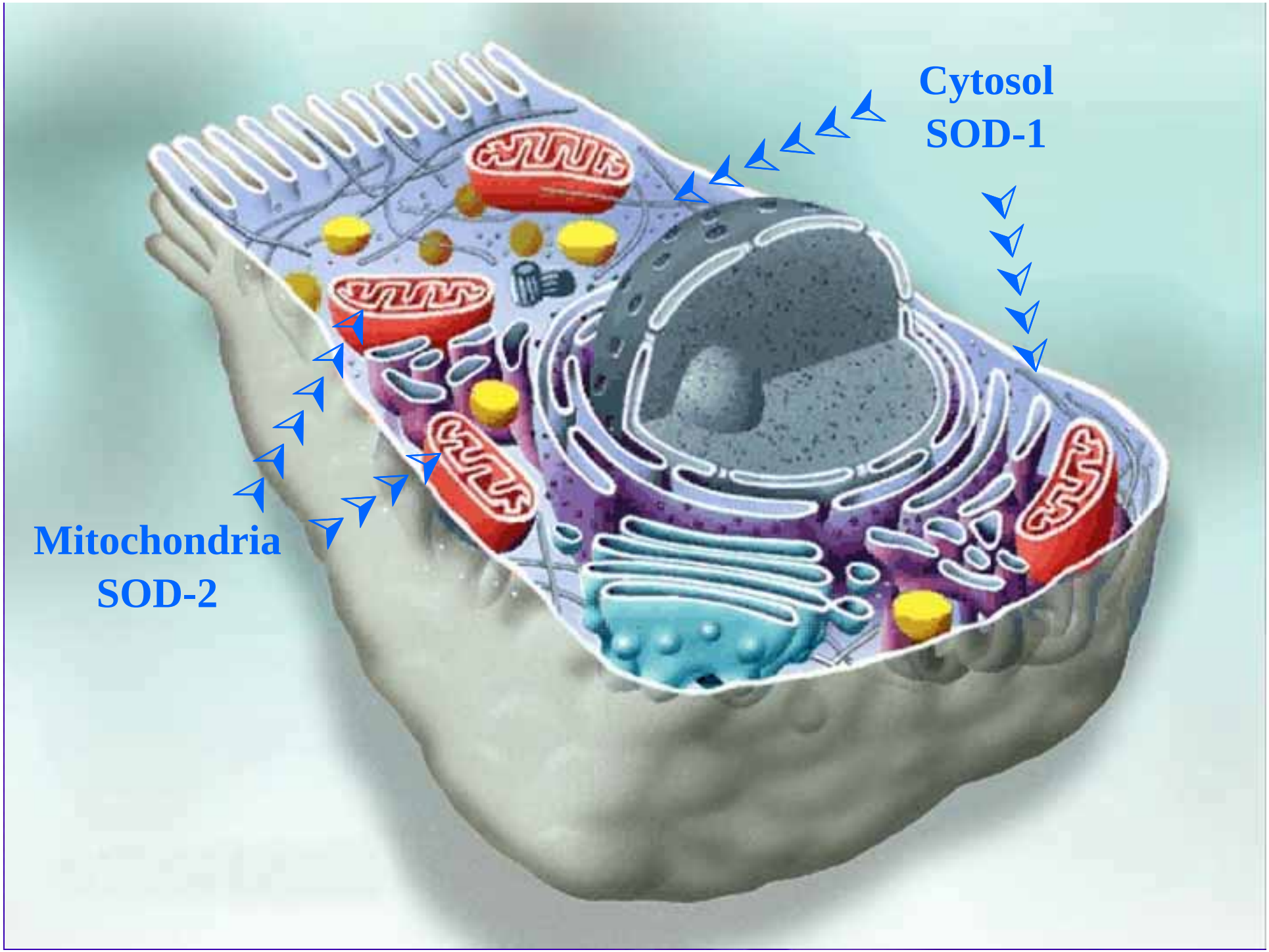
Superoxide Dismutase
SOD

SOD transforms O_2^- into less reactive H_2O_2

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Two isomers exist:

- Cytosolic SOD-1 requires Cu and Zn
- Mitochondrial SOD-2 requires Mn


Cytosol
SOD-1
Mitochondria
SOD-2

SOD-1 Polymorphism

Leads to a higher risk for

- Cardiovascular disease
- Rheumatoid Arthritis
- Neurodegenerative and motor neuron diseases
- Pre-mature aging

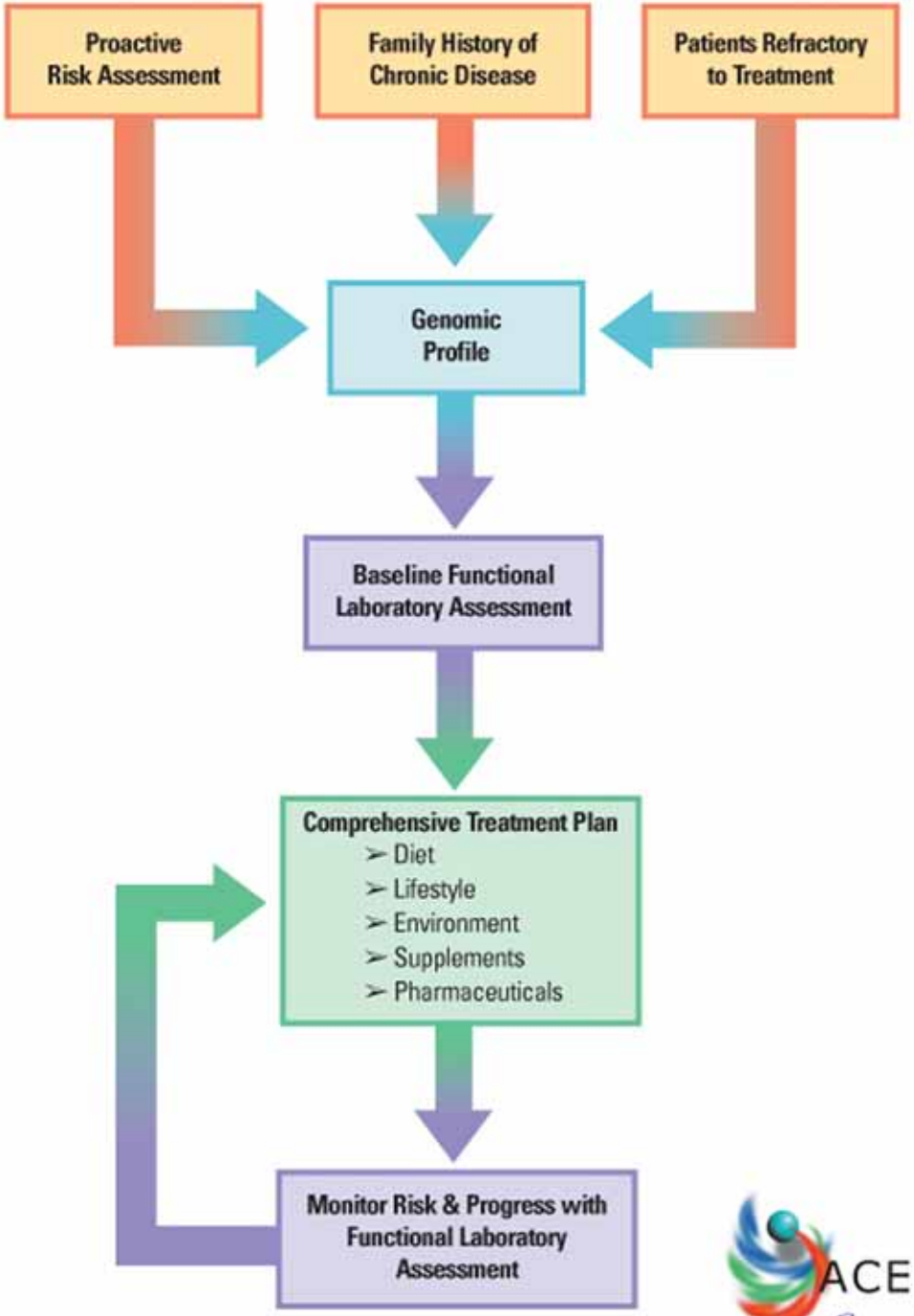
SOD-2 Polymorphism

Excess physical activity leads to excessive oxidative damage

Leads to a higher risk for

- Cardiovascular disease
- Rheumatoid Arthritis
- Neurodegenerative diseases
- Pre-mature aging
- Diabetes
- Breast cancer

Predictive Genomics and Functional Medicine

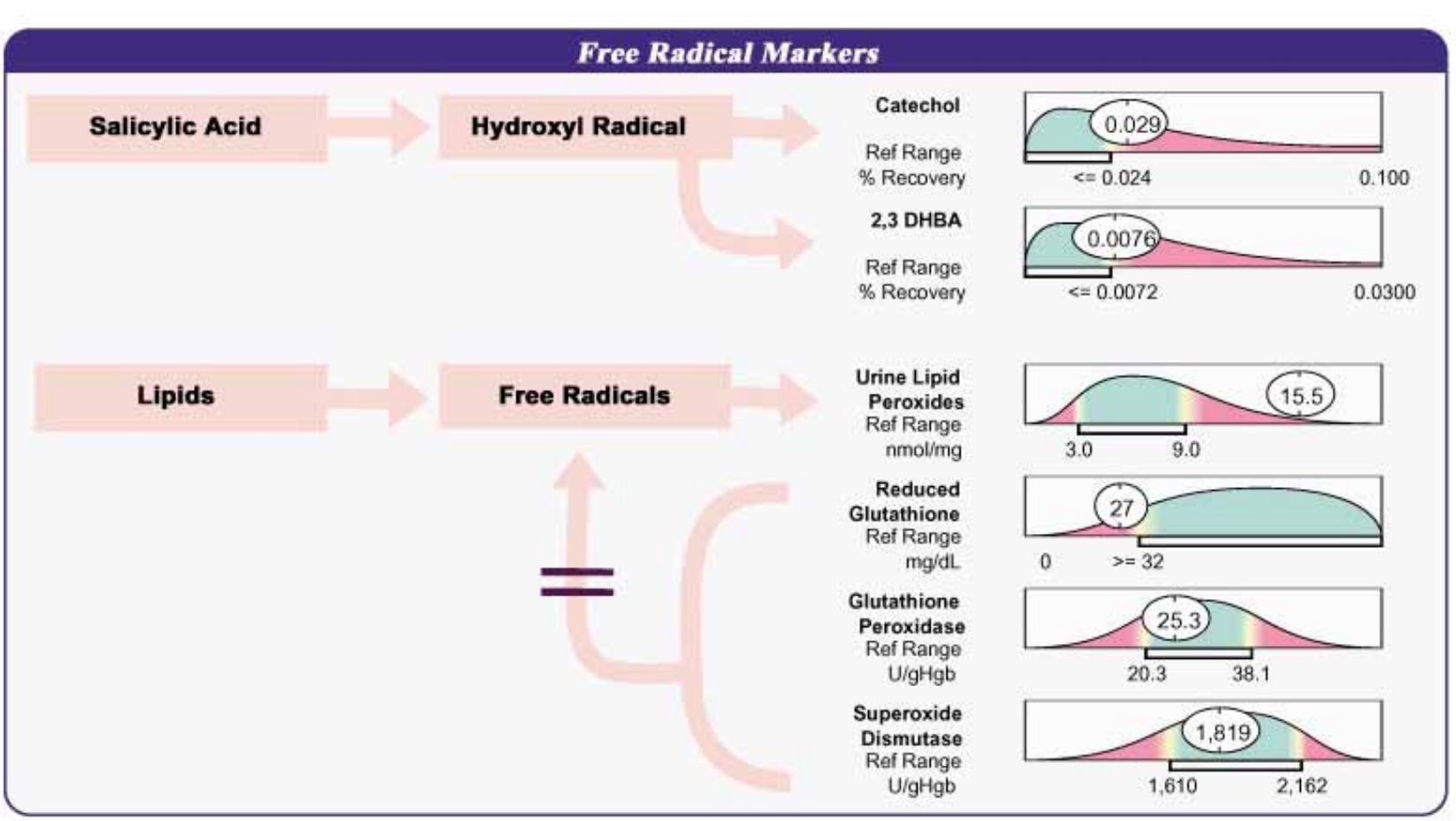


ACE
Genomics
In Primary Care Medicine

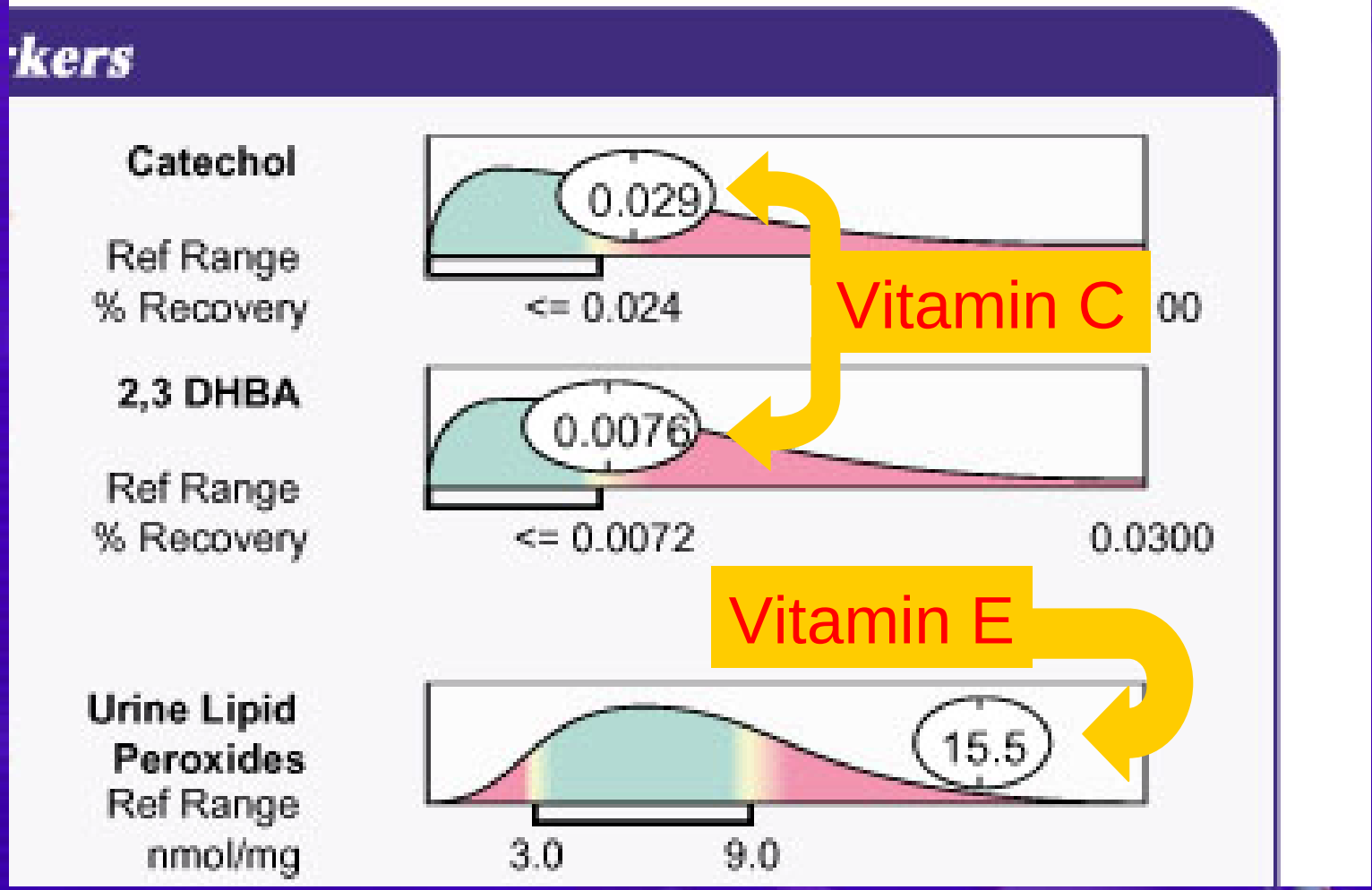
How do we measure individual oxidative stress ?

Oxidative Stress Profile

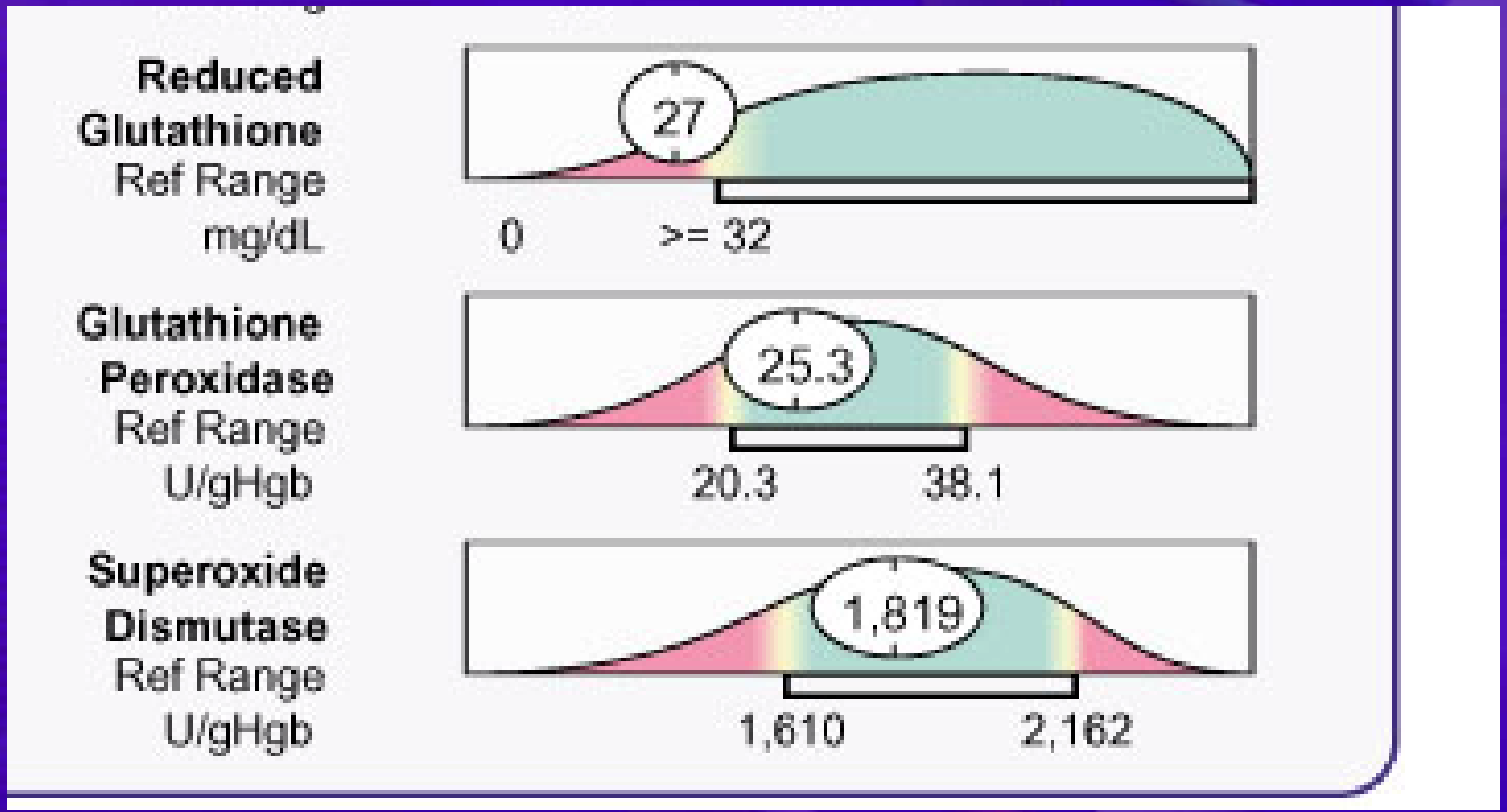
Free Radical Load
Vs.
Anti-Oxidant Reserve



Free Radical Load



Anti-Oxidant Reserve



SOD-1 and SOD-2 Polymorphism Treatment

- Reduce oxidative load
- Sustained increase of ancillary antioxidants and anti-oxidative pathways

Caloric Restriction and Oxidative Stress

- Calorie restriction has been shown to greatly increase the maximal lifespan in mammals and lower animals
- Calorie restriction greatly reduces the formation of free radical and oxidative damage to both mitochondrial and cellular DNA, as well as to membrane lipids and proteins

How can you tell if food has high levels of dietary anti-oxidants in it?

Color

Color	Food Source	Phytochemical
Orange-Red	Carrot, Apricot,	Carotenes
	Squash, Yams,	Lutein
	Tomato, Pepper,	Lycopene
	Apricot, Mango,	Zeaxanthin
Purple-Red	All Berries	Anthocyanins
	Grapes	Reversatrol
	Red Wine	Catechols
		Ellagic Acid

ACE

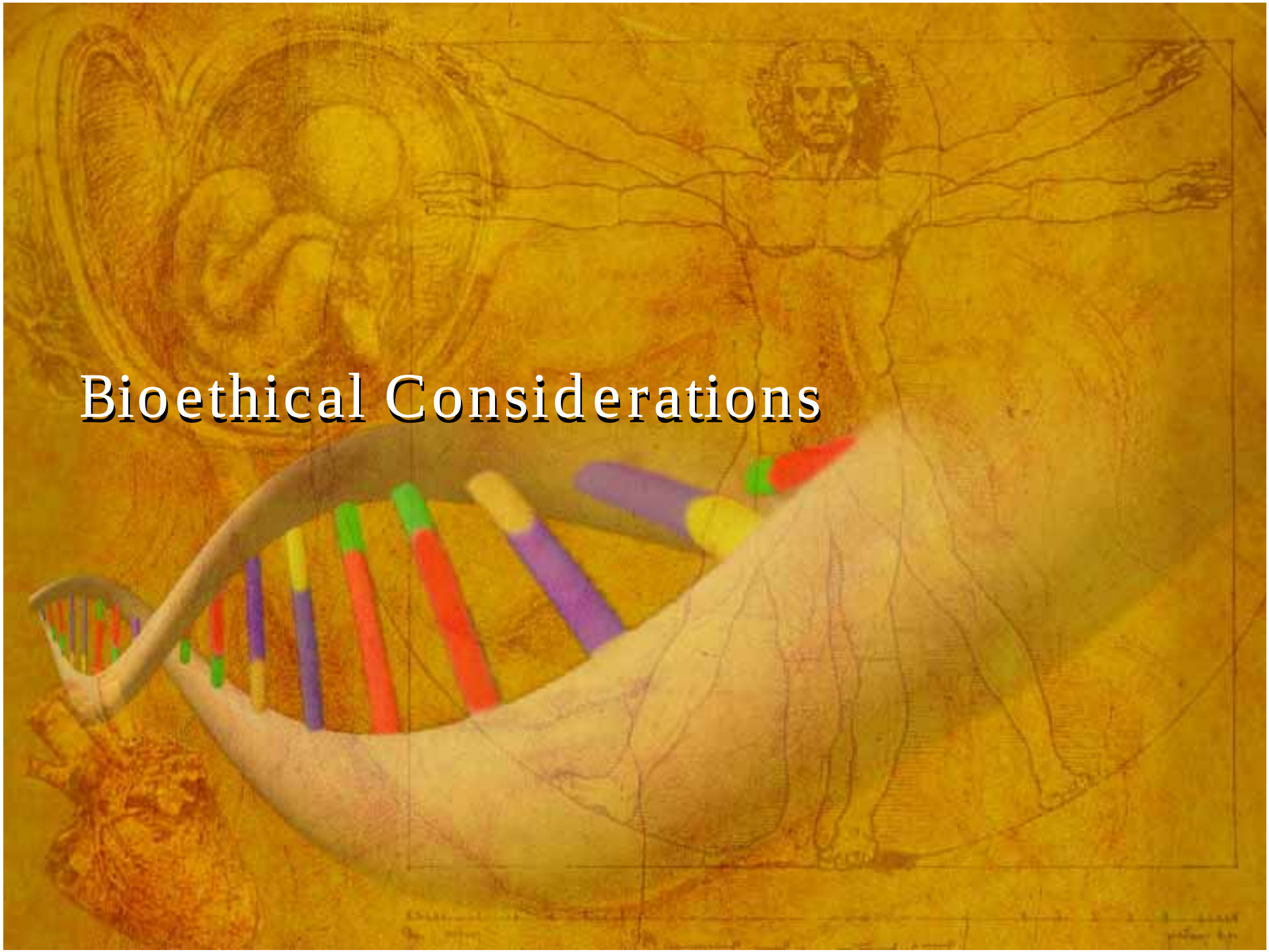
Color	Food Source	Phytochemical
Green	Broccoli, Okra,	Chlorophyll
	Greens, Spinach,	Sulforaphanes
	Cabbage, Beans	Carotenoids
Yellow	Lemons, Citrus	Limonene
Cream	Cauliflower, Potato	Anthoxanthins
White	Garlic, Onions	Allium, Quercetin
Brown	Dried Beans	Isoflavones
	Soy	Saponins
	Peanuts, Lentils	Fiber

ACE

Let Your Food Be Your Medicine


Detoxi Genomics
Polymorphisms Affecting
Detoxification Pathways

Bioethical Considerations



Bioethics

With every new paradigm shift in medicine, ethical issues arise, and genomic testing is no different

A plethora of bioethical and social issues arises in the face of genetic testing, especially if the genetic testing reveals a condition for which no medical treatment is currently available

Questions/Concerns are natural

Where there is ignorance there is potential for misunderstanding

New technology ahead of public policy

DNA="Dolly"

Real issues do arise from genomics

Issues

- How is my genetic information protected?
- Is DNA testing reliable?
- Why test for an “inevitable” disease, if I can’t change it?
- Is “genetic discrimination” a possibility?
- Do I need to share significant genomic information with my family?

How is my genetic information protected?

Genetic information is considered “health-related” and is protected through stringent federal HIPPA legislation.

Genetic information should be held in the strictest of confidence between the laboratory, practitioner and patient.

Laboratories and providers should exercise the highest of security around all genetic information.

How is my genetic information protected?

Model Privacy Policy

We are dedicated to safeguarding patient privacy and the confidentiality of all patient information. For this reason, your genetic test results are protected by a security code that is disclosed only to the health care provider who ordered your test. Your information otherwise will only be utilized internally for company operational purposes and as required by law. Your records, electronic and hard copy, will be maintained under a strict policy of confidentiality.

How is my genetic information protected?

Solution

Laboratories and providers maintain genetic information in the strictest of confidence.

All genomic profile reports should be encoded with a “privacy code.”

Results of genomic profiles should only be shared or discussed with a healthcare practitioner in possession of the patient’s privacy code. Report status requires doc ID#.

How is my genetic information protected?

Solution

Genomic reports should NEVER be faxed

No patient-direct shipments of testing kits

Payment type verified before running sample

Ordering physician and patient signature required before sample will be run.

How is my genetic information protected?

Solution

Genomic reports interpretation and discussion between laboratory medical staff and provider only.

Who should receive genetic information about a patient from a genomics laboratory?

Their physician

Who should NOT receive genetic information about a patient from a genomics laboratory?

Everyone else

Is DNA testing reliable?

SNPs do not indicate the presence of a disease.

SNPs demonstrate the potential for biochemical imbalances that can leave a patient more susceptible to environmental influences (infectious, chemical, physical, nutritional, and behavioral factors).

Is DNA testing reliable?

- SNPs testing should be performed on stable samples, free from contamination and degradation.
- SNP testing should be conducted in a laboratory environment and with a methodology which limits the chance of cross contamination.

Is DNA testing reliable?

Solution

- Utilize technology based upon signal amplification (as opposed to target amplification) to increase accuracy.
- Utilize proprietary collection and environment processing to reduce contamination influences
- Utilize profiles that maintain a specificity of >99.7%

Why test for an "inevitable" disease, if I can't change it?

- SNPs for incurable and life threatening illnesses carry specific ethical challenges.
- The psycho-social implications of this knowledge necessitates specific genetic counseling before and after conducting such testing.

Genes as Fate?

Ingres, oedipus and Sphinx, 1808

Teiresias, the blind seer of Thebes, can see the future but do nothing to change it.

He says to Oedipus,

"It is but sorrow to be wise when wisdom profits not"

Do we want to know our fate, if that fate cannot be changed?

Mendelian Diseases

- In the case of many Mendelian diseases like Huntington's disease, cystic fibrosis, sickle cell anemia, Tay Sachs disease, to name but a few, knowledge of an almost inevitable fate carries with it significant psychological and emotional burdens, not just for the affected individual, but often also for all members of the extended family
- All genetic conditions are, by definition, familial

Predictive Genomic Testing

- Much of the psychological and emotional trauma that may exist in testing for Mendelian disease simply does not exist for predictive genomic testing
- With predictive genomic testing the focus is on relative risk given a specific polymorphism and on modulating that risk using environmental modifications

Why test for an “inevitable” disease, if I can’t change it?

- SNPs for modifiable risk factors offers opportunities for intervention and risk reduction.
- Carefully selected SNPs can enhance a wellness program by offering specificity and direction for prophylactic intervention.

Why test for an "inevitable" disease, if I can't change it?

Solution

Predictive genomic profiles should include SNPs which are relevant, prevalent, modifiable, and measurable

Predictive genomic profiles empower patients by helping them to understand how to match their environment to their genotype.

Is "genetic discrimination" a possibility?

- Patients are concerned about whether genetic information might be used against them.
- Insurance, employment, and social discrimination are all appropriate and natural concerns in today's "open-information" age.

Is "genetic discrimination" a possibility?

- The chance of genetic discrimination occurring is low based upon testing to date.
- Legal protection from genetic discrimination from employers and insurers is covered in some states and by the federal government in the Health Insurance Portability and Accountability Act (HIPAA).
- Few laws have been tested because genetic discrimination cases are so rare.

ACE

Is “genetic discrimination” a possibility?

Genetic discrimination is more likely relative to SNPs associated with non-modifiable, life-threatening.

In most cases, insurers already have access to the family medical history, which already provides much information about the personal risks of common, inherited, complex diseases.

Is “genetic discrimination” a possibility?

As we develop more genetic tests and they become commonly available, we'll find that virtually everybody has risks, and some people believe the problem of discrimination will disappear.

Is "genetic discrimination" a possibility?

Solution

Predictive genomic profiles should include only modifiable SNPs associate with health risks, not with inevitable life-threatening diseases.

Genomic laboratories and providers should utilize a stringent privacy and confidentiality policy to protect patient's health information.

Is "genetic discrimination" a possibility?

Solution

Genomic testing is a new medical tool, and few cases of potential discrimination have been tested.

Federal and state law makers and patient protection groups are evolving a public policy to help protect patient's rights.

Do I need to share significant genomic information with my family?

- Testing “many” by testing “one”
- Patients wonder if they have an obligation to share their genomic testing results with family.
- Inevitable, inherited diseases pose specific family communication challenges.

Do I need to share significant genomic information with my family?

Fortunately, in predictive genomic testing, practical intervention strategies are generally available, and genetic diagnosis will likely do far more to relieve stress rather than to increase it.

Do I need to share significant genomic information with my family?

Solution

Genomic testing is a new medical tool, and offers health risk information about both the patient and their family.

Patients performing Predictive genomic testing should be encouraged to share and discuss the information acquired with other family members, since their risk may also be affected.

Do I need to share significant genomic information with my family?

Solution

Predictive genomic testing should be encouraged for family members desirous of early implementation of risk reduction strategies.

Utilizing predictive genomic testing may be the first step towards an early, comprehensive risk reduction and targeted treatment strategy of family members.

Bioethics Resources

American College of Medical Genetics

Principles of Screening: Report of The Subcommittee on Screening of the American College of Medical Genetics Clinical Practice Committee

Screening for genetic disease or genetic predisposition to disease provides a unique opportunity to prevent the effects of the disease. Retrieval, diagnosis and intervention before irreversible damage represent goals for an effective genetic screening program.

The screening program should have a clearly defined purpose.

- Distinctions must be made between carrier screening, screening for predisposition to disease, screening for presymptomatic disease, and screening for those affected with disease. The terms presymptomatic and symptomatic should be defined based on the nature and severity of specific symptoms. Certain phenotypic signs may have minimal clinical significance. The program should determine whether such signs will, or will not, result in classification of an individual as symptomatic.
- Newborn screening is a special case that, depending on the disorder and the screening method, may identify carriers, presymptomatic individuals, or affected neonates. Newborn screening represents an example of population-based screening as opposed to selective screening where a specified subset of the population is targeted.
- There should be a defined population for screening, e.g., newborns, a specific ethnic group for a disorder with increased frequency in that group, women at risk for breast cancer, etc.
- When a program is established, it must be clear whether the purpose is research or medical care. The decision to move a test from the research to the clinical arena must be carefully considered. The participant must be aware of benefits and risks of the information that will be forthcoming. If the screening is for research purposes, the subjects should be fully informed of the sources of financial support

THE GENETIC PRIVACY ACT AND COMMENTARY (Selected Excerpts)

George J. Annas, JD, MPH
Leonard H. Glantz, JD
Patricia A. Roche, JD

Health Law Department; Boston University School of Public Health; 80 East Concord Street; 02118
Tel. (617) 638-4626; FAX: (617) 638-5299; email: annasgj@bu.edu

The Genetic Privacy Act and Commentary is also the **Final Report** of a project entitled "Gui Protecting Privacy of Information Stored in Genetic Data Banks" which was funded by the Et & Social Implications of the Human Genome Project, Office of Energy Research, U.S. Depar Energy, No. DE-FG02-93ER61626

Additional support was provided by Boston University School of Public Health

---

INTRODUCTION

The Genetic Privacy Act is a proposal for federal legislation. The Act is based on the premis information is different from other types of personal information in ways that require special The DNA molecule holds an extensive amount of currently indecipherable information. The r the Human Genome Project is to decipher this code so that the information it contains is acce privacy question is, accessible to whom?

The highly personal nature of the information contained in DNA can be illustrated by thinkin containing an individual's "future diary."*[1]* A diary is perhaps the most personal and private person can create. It contains a person's innermost thoughts and perceptions, and is usually hi locked to assure its secrecy. Diaries describe the past. The information in one's genetic code c

RL30006: Genetic Information: Legal Issues Relating to Discrimination and Privacy

Nancy Lee Jones

Legislative Attorney
American Law Division
Redistributed as a Service of the National Library for the Environment

Updated January 24, 2001

On June 26, 2000, in a special ceremony at the White House, the completion of the "rough draft" of the human genome was announced. This milestone, which has been compared to the discoveries of Galileo, and other advances in genetics have created novel legal issues relating to genetic information. The Human Genome Project, with its goal of producing detailed maps of the 23 pairs of human chromosomes and sequencing the three billion nucleotide bases that make up the human genome, has been instrumental in the identification of genes responsible for various diseases including glaucoma, colon cancer, and cystic fibrosis. With the identification of these genes comes the hope of genetic therapies to cure disease but this scientific accomplishment is not without potential problems. For instance the presence of a cancer causing gene may indicate a predisposition but does not guarantee that the person will contract the disease: How should an employer or insurer respond? The ethical, social and legal implications of these technological advances have been the subject of significant scrutiny and concern.

The legal implications of such information have been mainly on the state level but there are some relevant Federal statutes. The Health Insurance Portability and Accountability Act of 1996, P.L. 104-191, is the first federal law to specifically address discrimination and insurance issues relating to genetic discrimination. This report discusses current federal and state law as well as legislation which was introduced in the 106th Congress. It will be updated as needed.

Background

On June 26, 2000, in a special ceremony at the White House, the completion of the "rough draft" of sequence of the human genome was announced. More specifically, the scientists involved in the Human Genome Project (HGP) (1)

Bioethics Resources

Human Genome: "Ethical Issues in Pharmacogenetics" by Carol Isaacson Barash, Ph.D.

authorbio

Dr. Carol Isaacson Barash is the founder and principal of Genetics, Ethics & Policy Consulting (GEPC)

human genome: applications of genomic mapping

Ethical Issues in Pharmacogenetics

By Carol Isaacson Barash, Ph.D.

An actionbioscience.org original article

article highlights

Pharmacogenetics promises drugs specific to an individual's condition. However, it poses some ethical concerns:

- *invasion of medical privacy*
- *unequal distribution of benefits*
- *discrimination because it involves genetic tests*
- *research/business conflict-of-interest*

February 2001

Ethical Issues in Pharmacogenetics

By Carol Isaacson Barash, Ph.D.

Drugs can be developed for individuals.

Pharmacogenetics is the study of how genes influence an individual's response to drugs. Though the field would seem to be brand new, it is really half a century old. In the 1950's, scientists first identified deficiencies in enzymes that explained adverse reactions to drugs and that they could be inherited.

Bioethics Resources

authorbio

Philip L. Bereano, Ph.D., J.D., is a professor in the College of Engineering, Department of Technical Communication, University of Washington, Seattle.

human genome: genetic information and privacy

Does Genetic Research Threaten Our Civil Liberties?

By Philip Bereano, Ph.D., J.D.

An actionbioscience.org original article

article highlights

Mapping the human genome may lead to new medical breakthroughs; however, it may also lead to:

- *an individual's loss of privacy*
- *discrimination by class or genetic profile*
- *genetic enhancement of select individuals or populations*

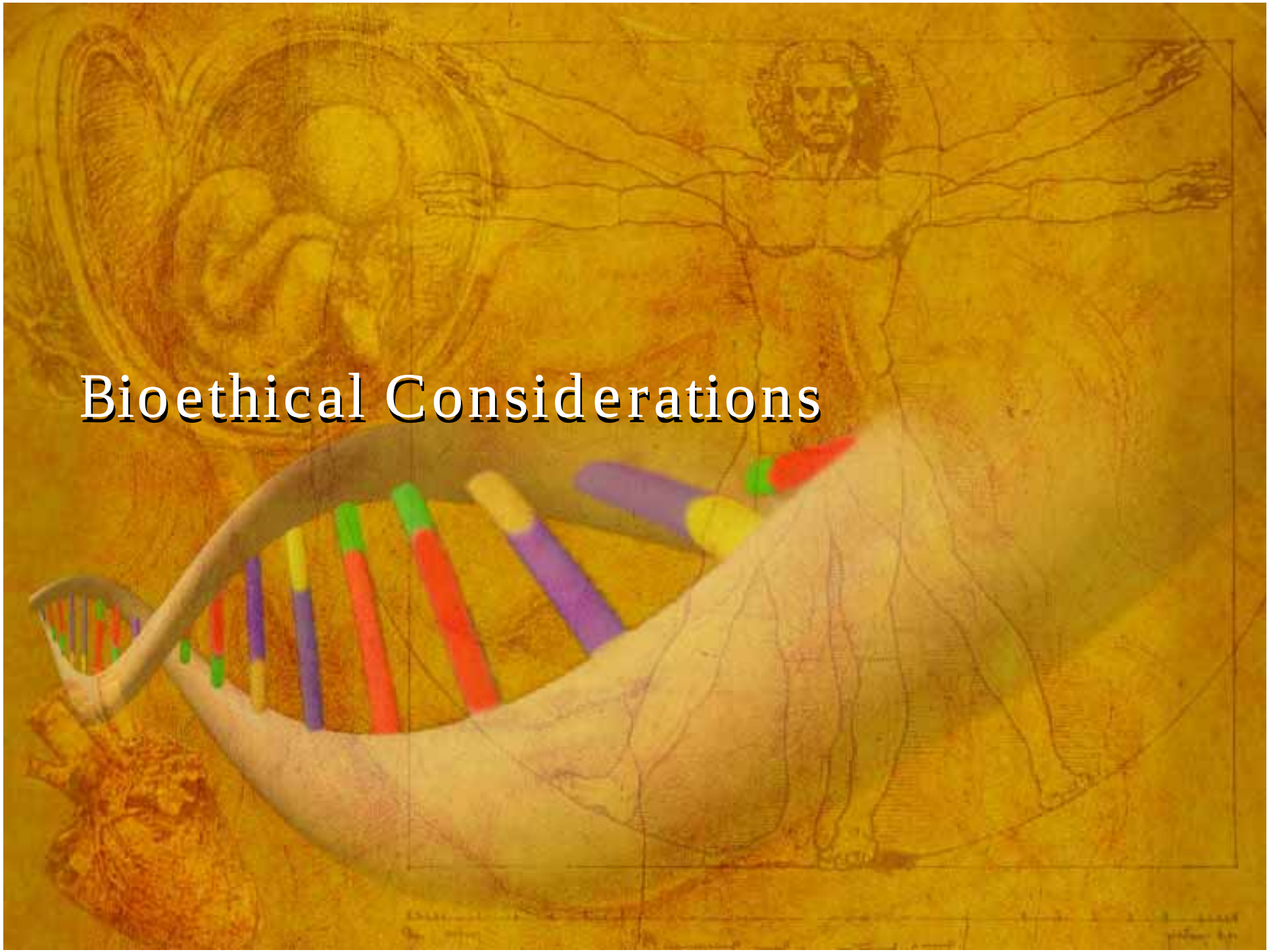
August 2000

Does Genetic Research Threaten Our Civil Liberties?

By Philip Bereano, Ph.D., J.D.

The Human Gene Project at the National Institutes of Health, also being supported in universities all across America, will one day in the not-too-distant future enable every set of parents that has a little baby to get a map of the genetic structure of their child. So if their child has a predisposition to a certain kind of illness or a certain kind of problem, or even to heart disease or stroke in the early 40's, they will be able to plan that child's life, that child's upbringing, to minimize the

Bioethical Considerations




Implementation Strategies

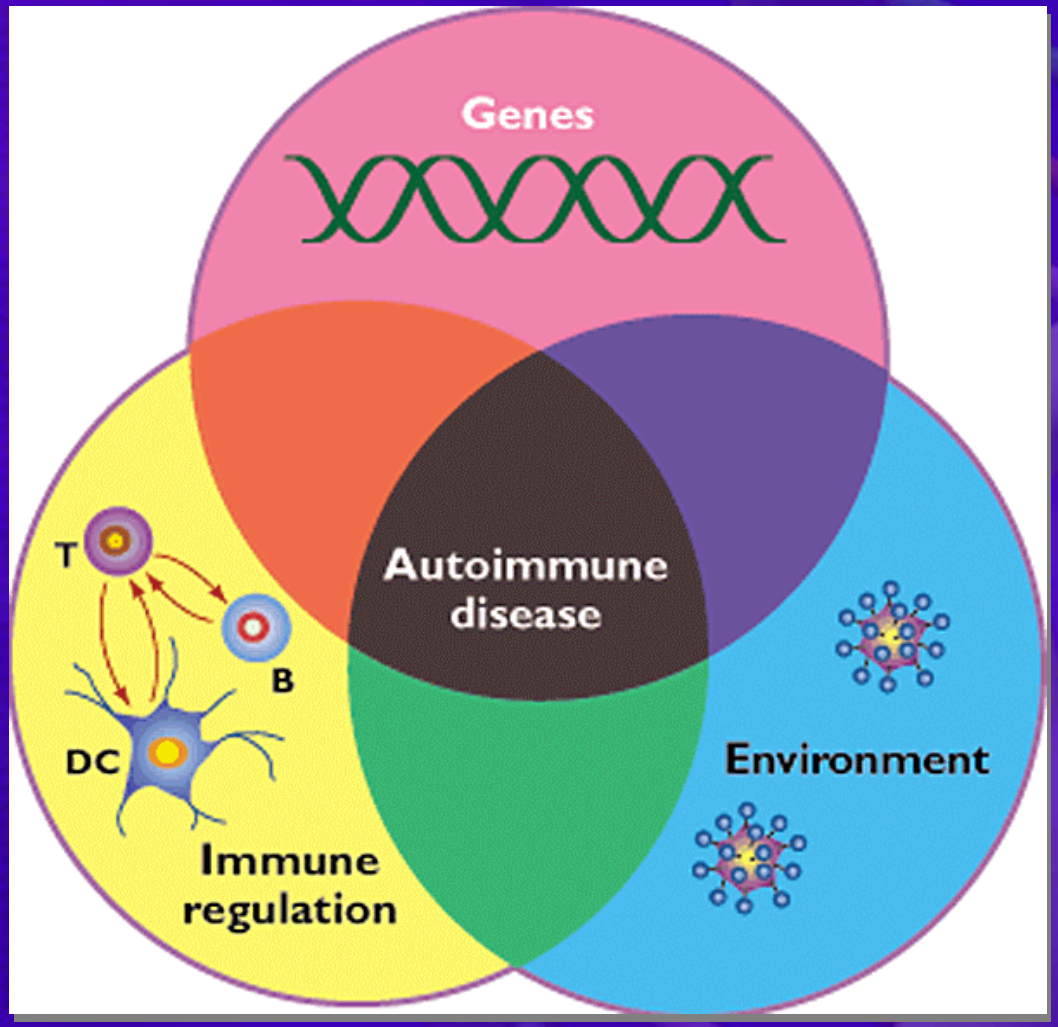
Modifying Gene Expression

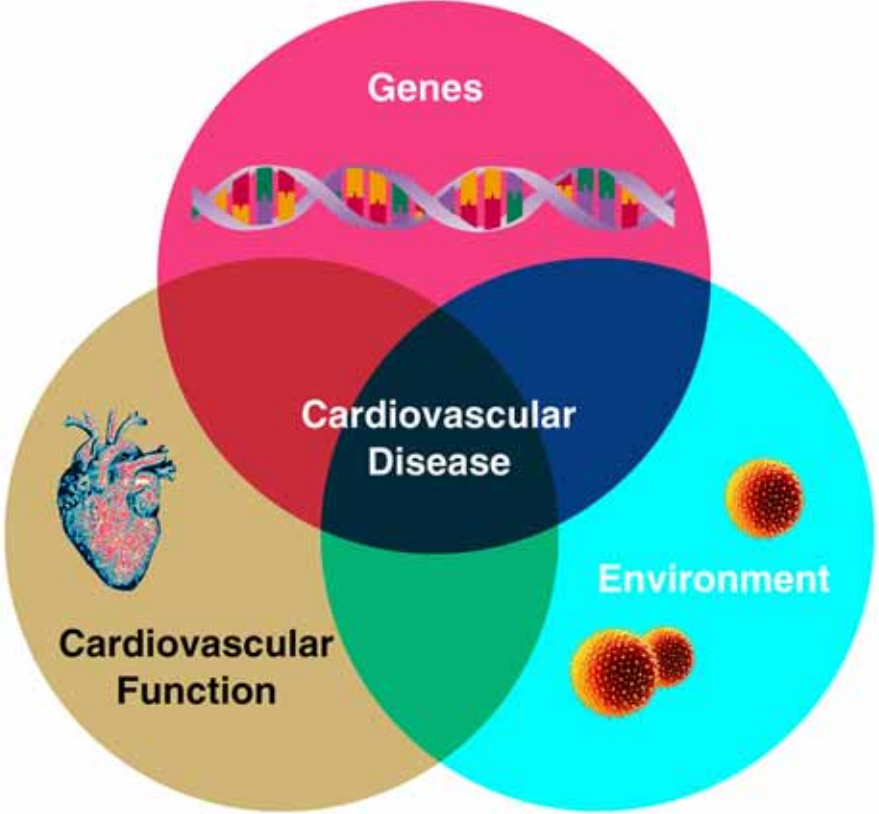
Exposure	Result (Disease)
↓	Low Risk
↑	Low Risk
↓	Mod Risk
↑	High Risk

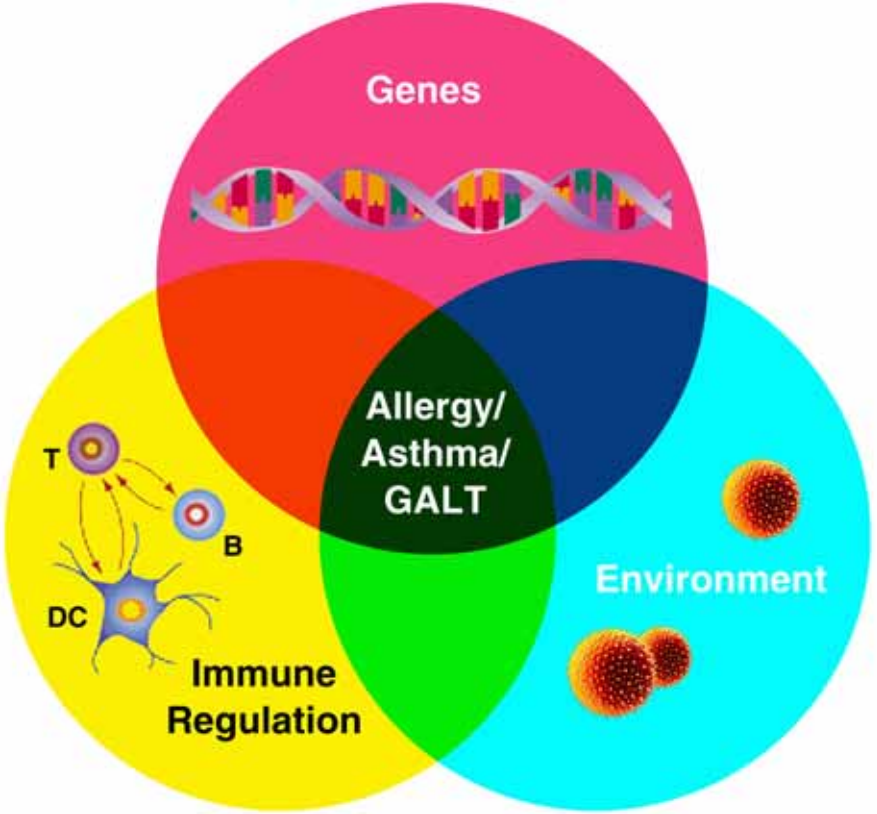
Modifying Gene Expression

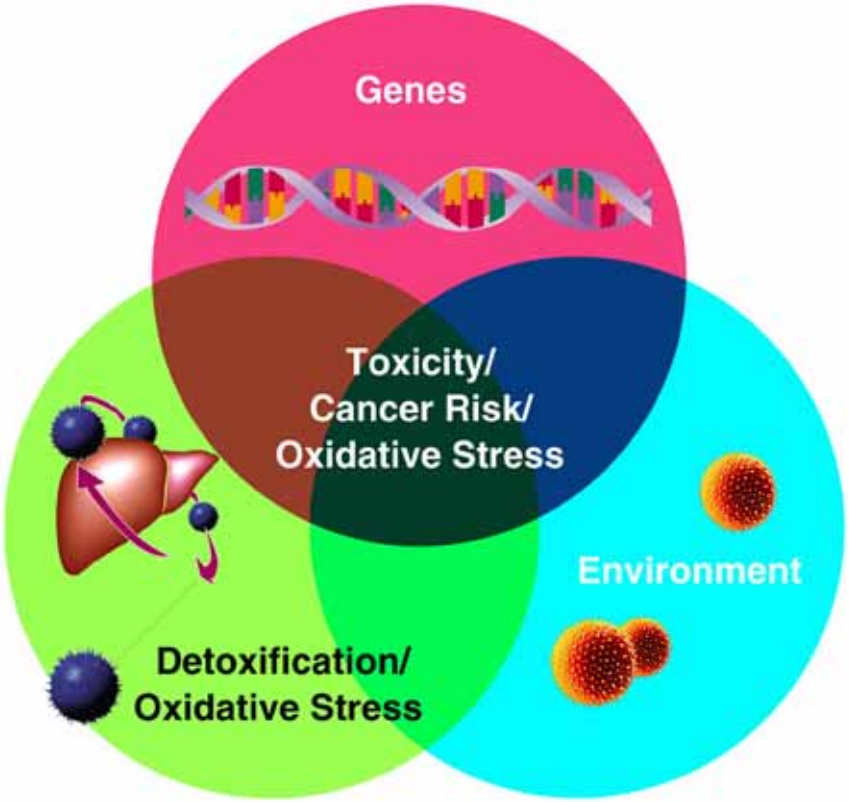
Exposure	Susceptibility (Genotype)	Result (Disease)
↓	↓	Low Risk
↑	↓	Low Risk
↓	↑	Mod Risk
↑	↑	High Risk

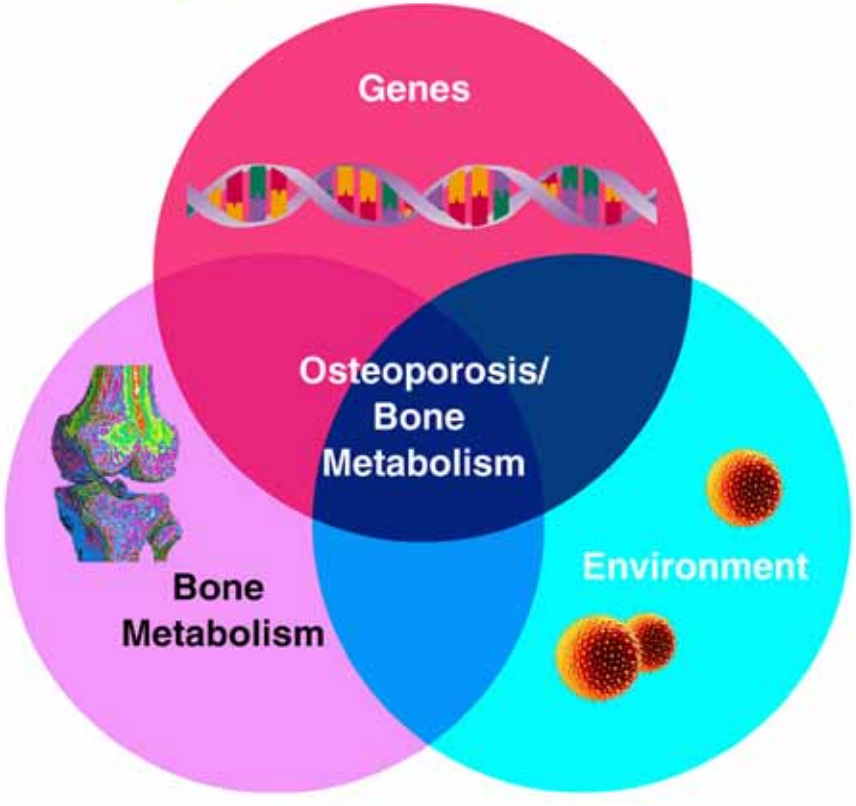
Interactions Cause Disease



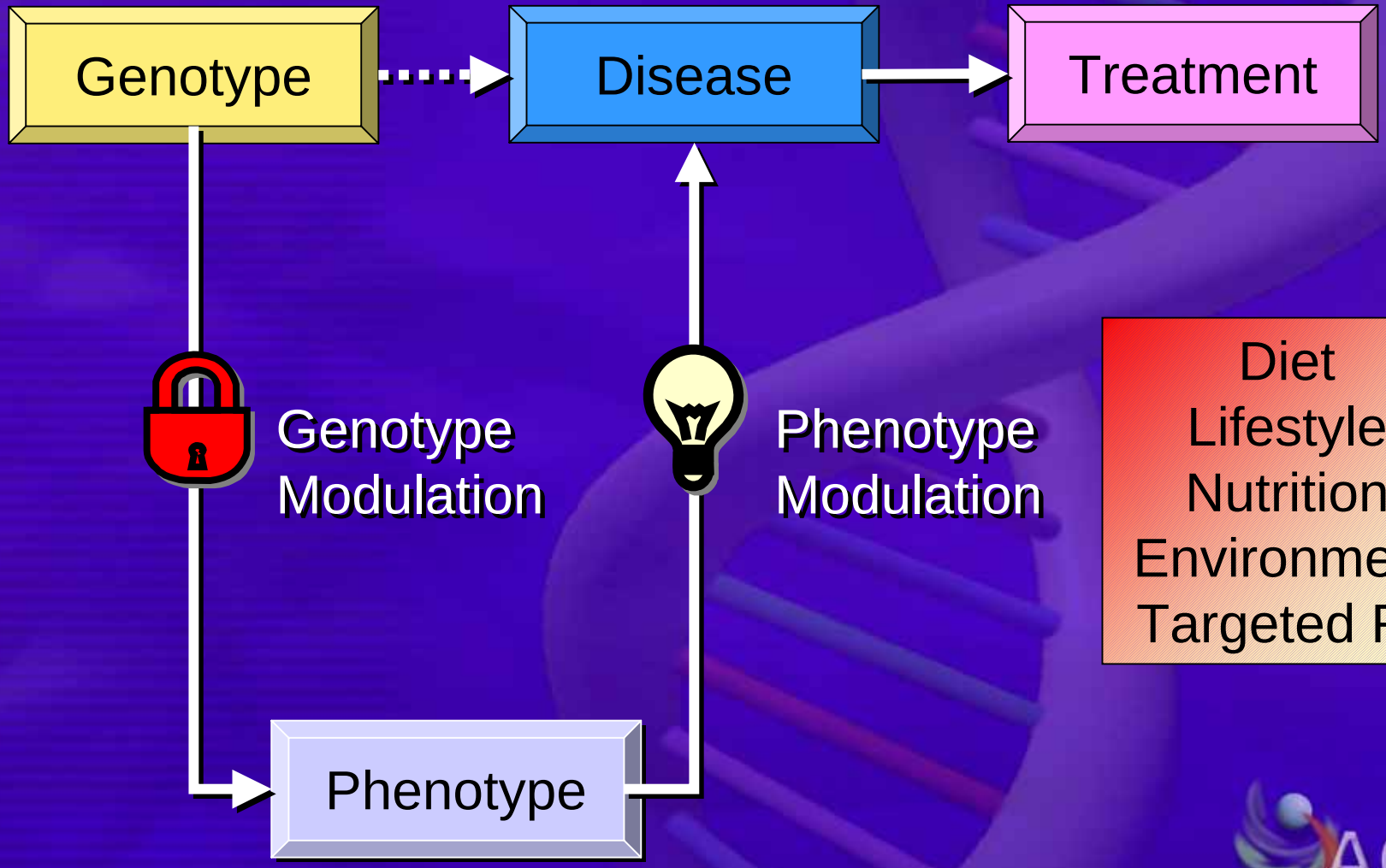

Genes
Cardiovascular Disease
Environment
Cardiovascular Function


Genes
Allergy/ Asthma/ GALT
T
B
DC
Environment
Immune Regulation


Genes
Toxicity/ Cancer Risk/ Oxidative Stress
Environment
Detoxification/ Oxidative Stress


Genes
Osteoporosis/ Bone Metabolism
Environment
Bone Metabolism

Predictive Genomics



Proactive risk screening for all patients

The "River" Analogy

- Most health care is "downstream" medicine.
- Practitioners spend most of their time rescuing drowning patients from the river of "ill-health."

Proactive risk screening for all patients

The "River" Analogy

- Predictive Genomic Diagnostics allow practitioners to move "upstream" and stop their patients from falling into "ill-health."
- All patients can benefit from advanced knowledge of modifiable risk factors

6 Key Concepts

1. All disease come from gene-environment interactions.
2. SNPs underlie almost all diseases.
3. SNPs do not cause disease- rather they increase susceptibility to environment.

6 Key Concepts

4. SNPs should be assessed in condition-related profiles (Osteo Genomic, Cardio Genomic, etc).
5. Predictive genomic profiles contain SNPs which are prevalent, relevant, modifiable, and measurable.
6. Predictive genomic diagnostics should be used for assessing risk in 3 major groups…

ACE

Clinical Implementation

1. Proactive screening for all patients
2. Familial associations
3. Difficult/Challenging Cases

Proactive risk screening for all patients

"River" Analogy

Many practitioners practice "downstream" medicine.

They spend their time rescuing drowning patients from the river of "ill-health."

Predictive Genomic Diagnostics allow practitioners to move "upstream" and stop their patients from falling into "ill-health."

All patients can benefit from advanced knowledge of modifiable risk factors

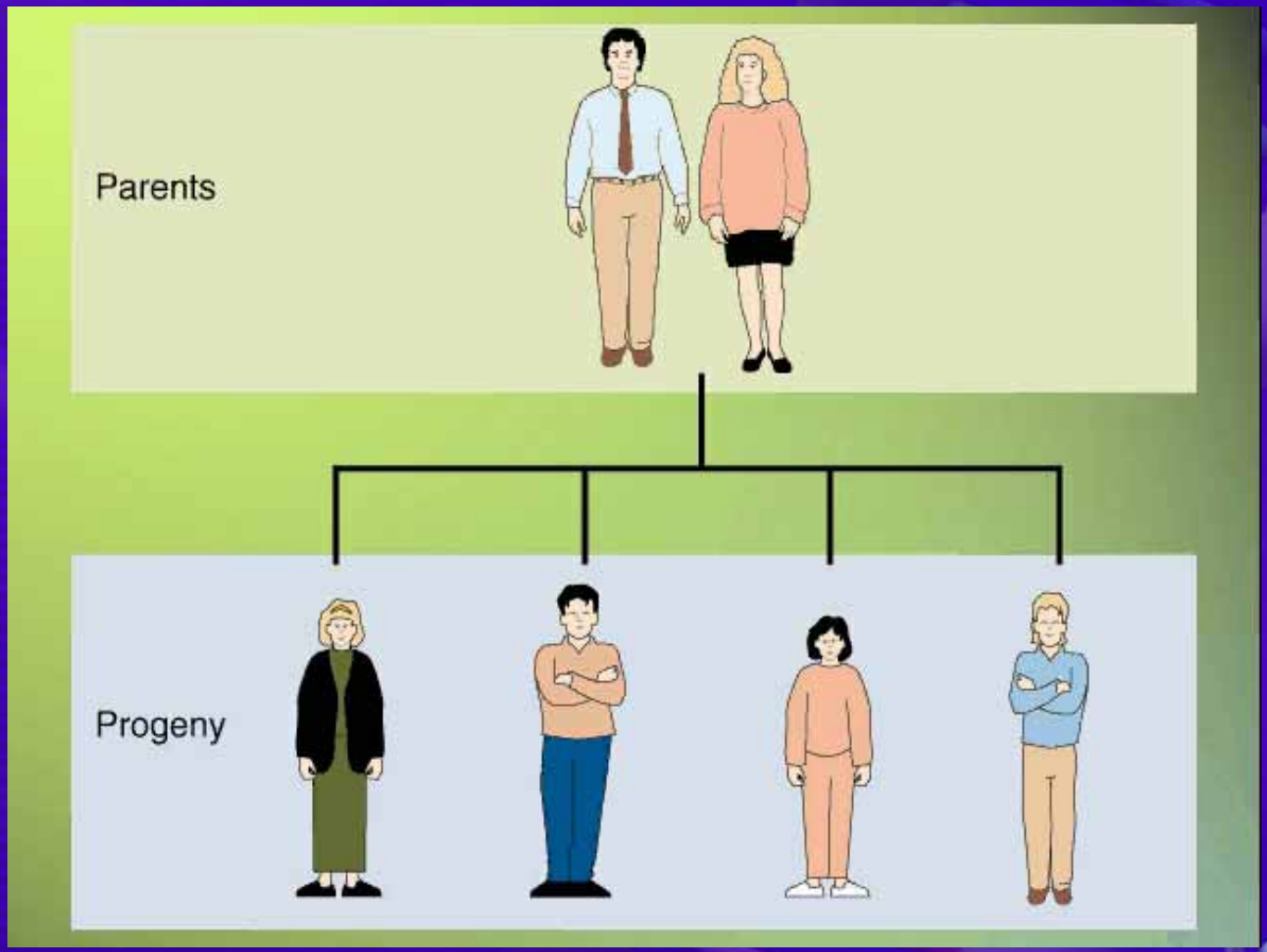
Familial Associations

Family Risk Examples

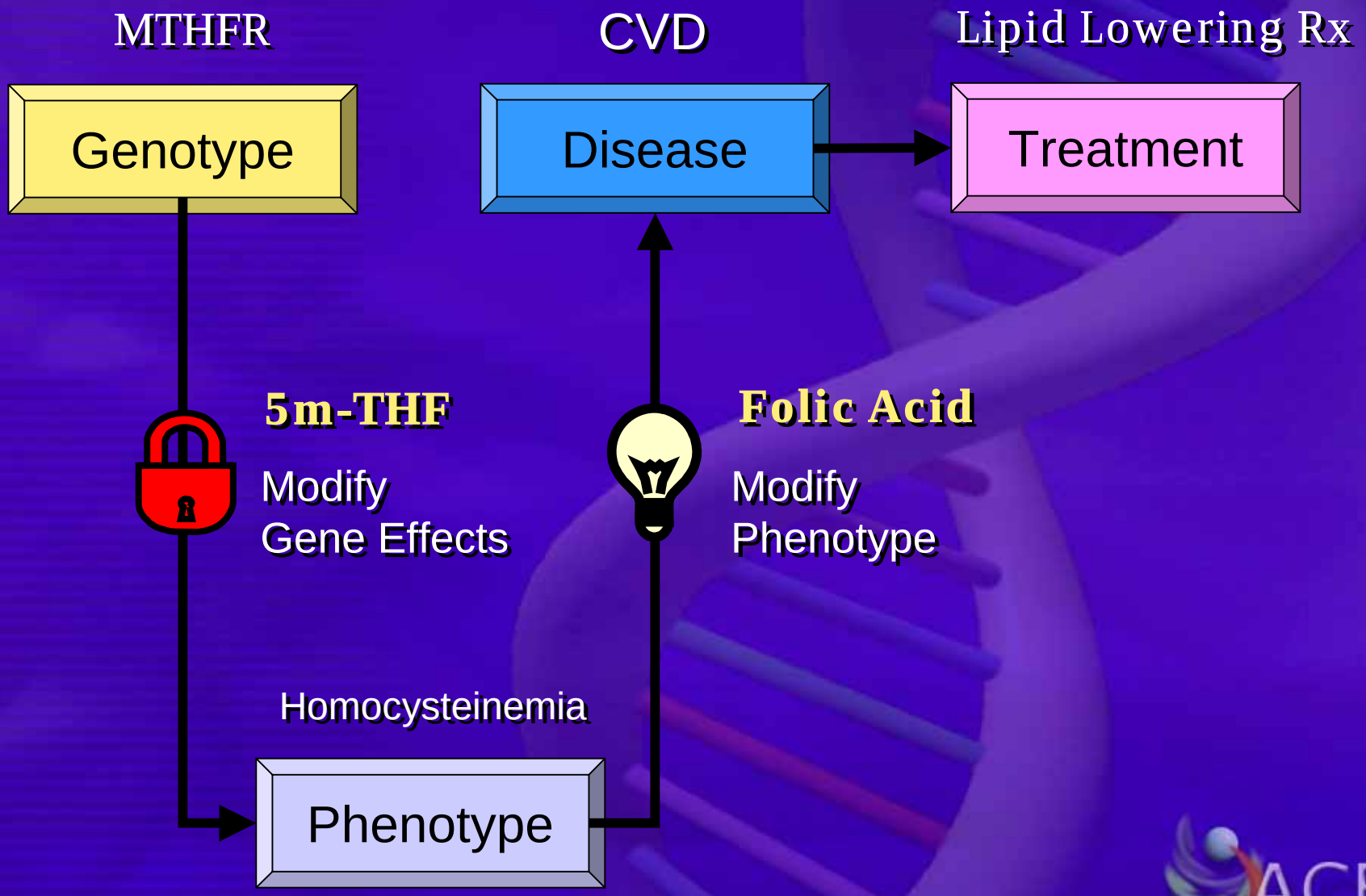
Patients with a family risk of a disease should be tested to see if they carry a modifiable genetic variant.

Patients with a known modifiable genetic variant should have other family members tested to see if they too carry the variant.

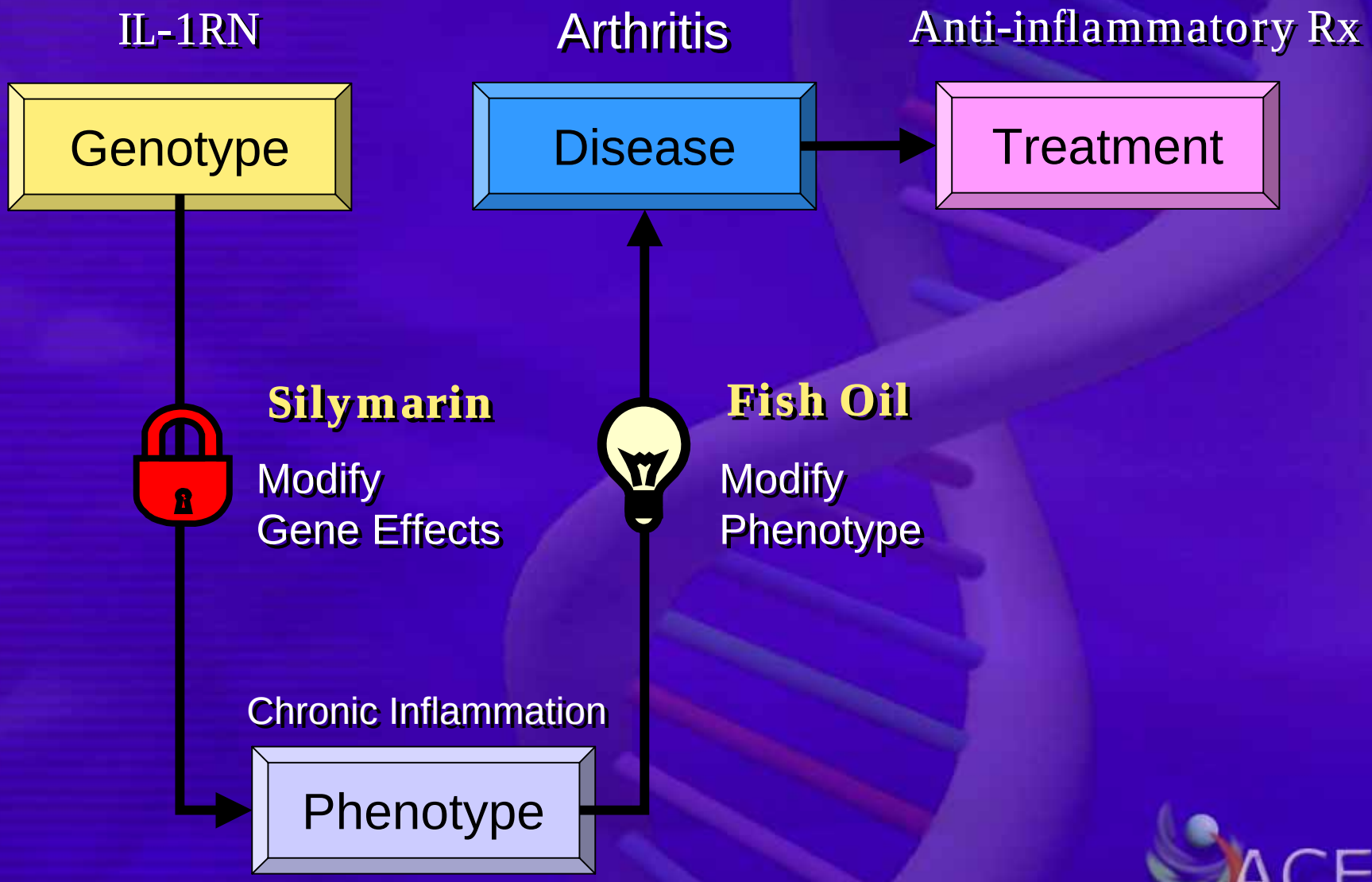
Familial Associations



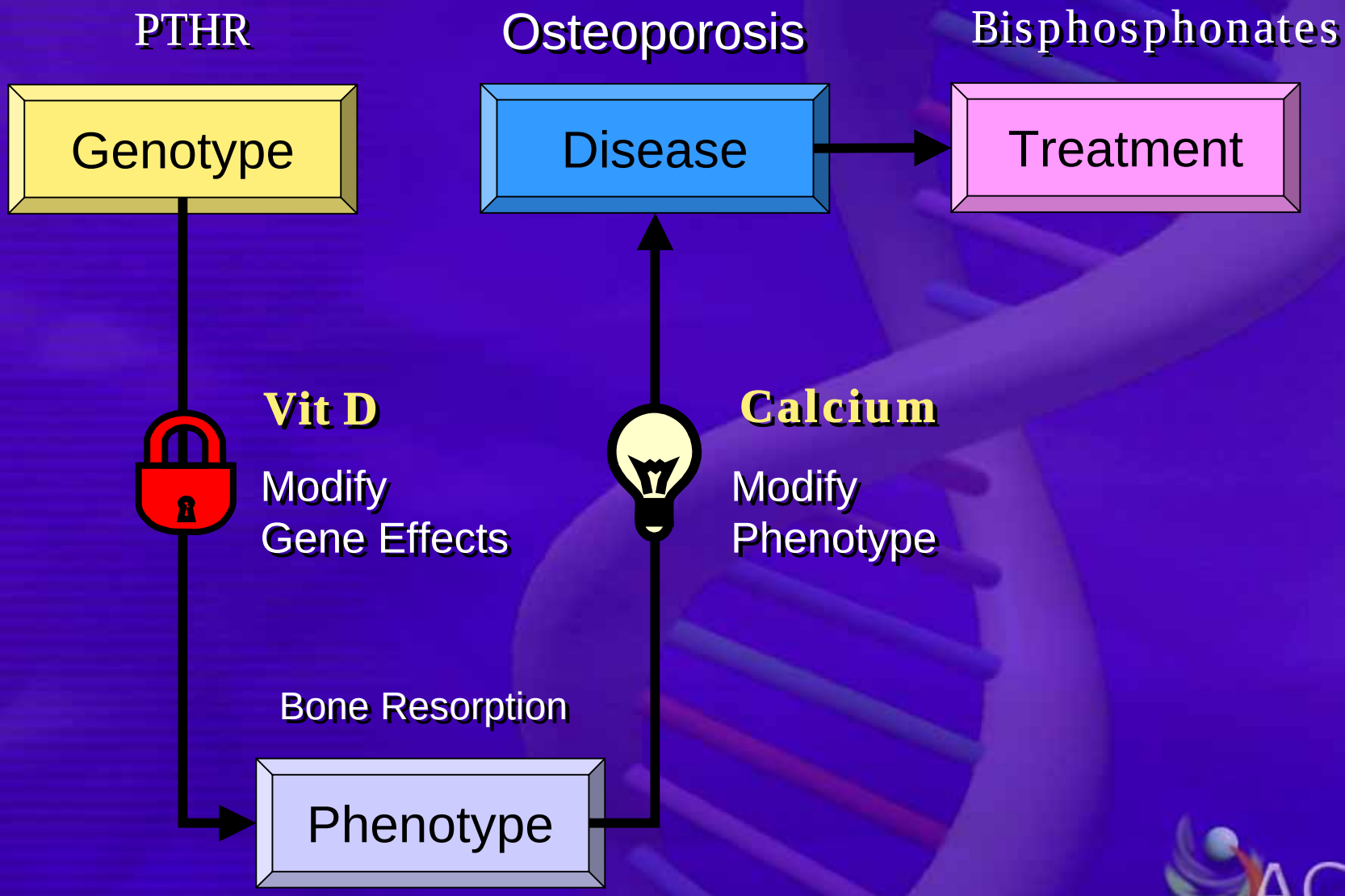
Clinical Cardio Genomics



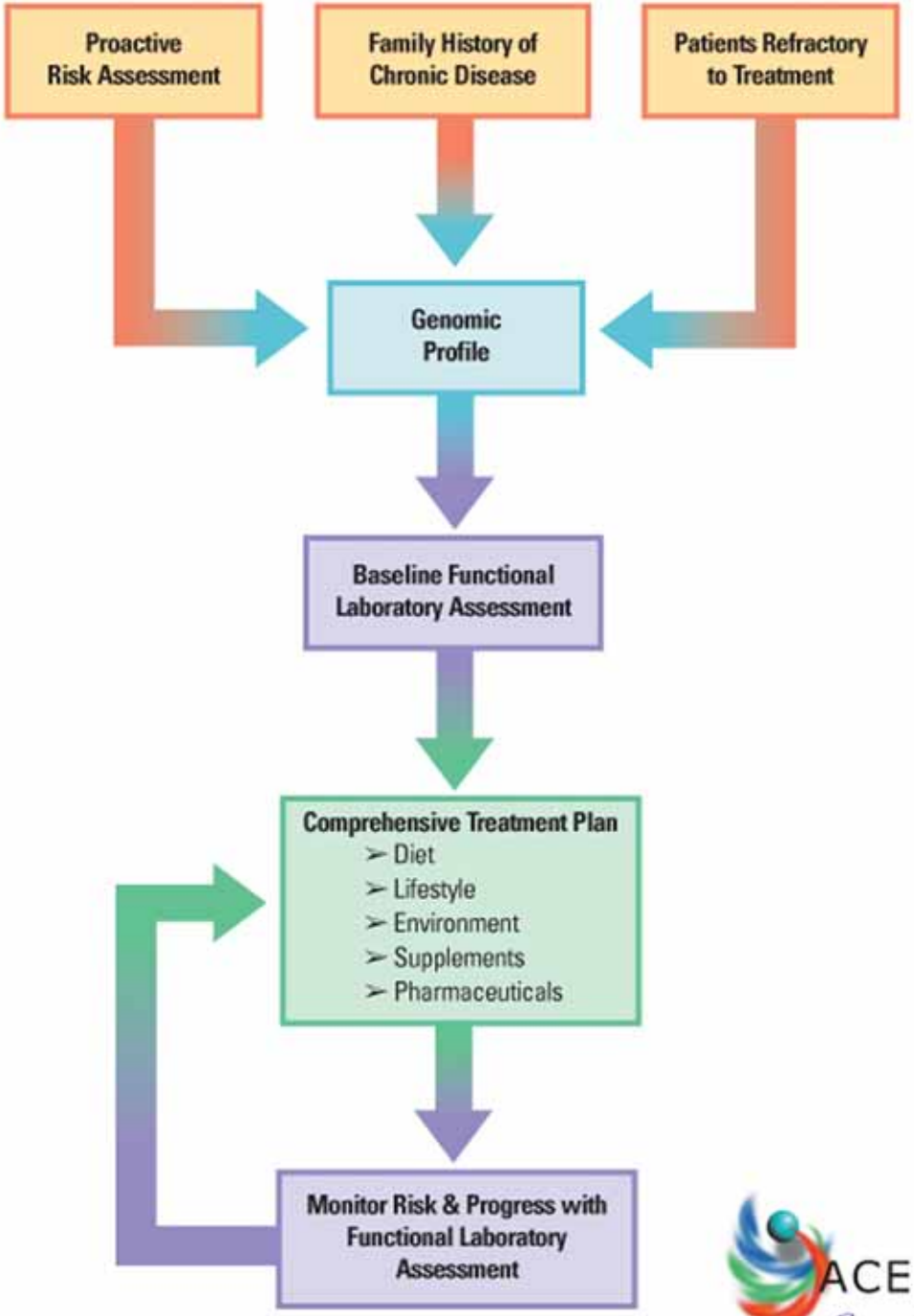
Clinical Immuno Genomics



Clinical Osteo Genomics



Predictive Genomics and Functional Medicine



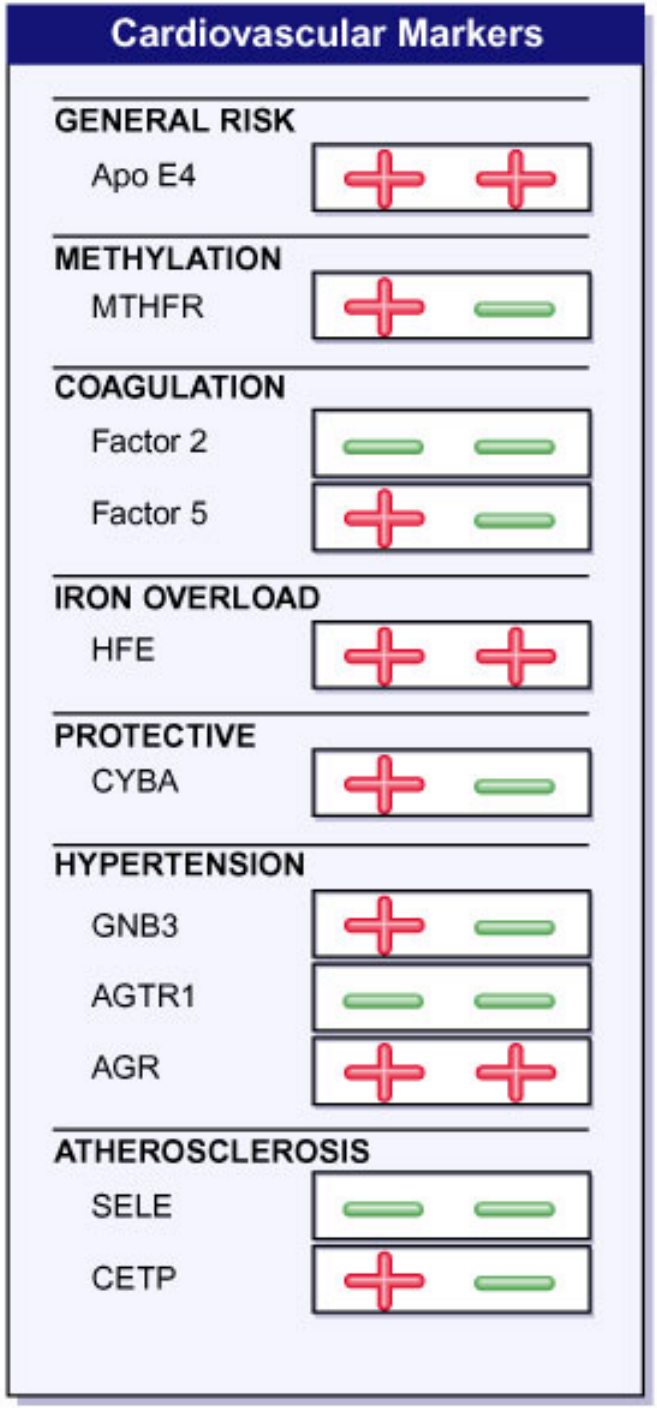
ACE
Genomics
In Primary Care Medicine

Linking Genotype to Phenotype

Genotype	Components	Phenotype
Cardio Genomic	Methylation, Coagulation, Gen Risk, Protection, Hypertension, Atherosclerosis,	Cardiovascular, Amino Acids, Elemental Analysis
Osteo Genomic	Bone Formation, Bone Resorption, Inflammation,	Bone Resorption, Elemental Analysis, Dysglycemia
Immuno Genomic	Inflammation, Immune Dysfunction	Digestive Analysis, Intestinal Permeability, Food Antibody, Inhalants, Cardiovascular, Bone Resorption
Detoxi Genomic	Phase 1, Phase 2, Oxidative Stress	Detox, Ox Stress, Amino Acids, Metabolic Analysis, Cellular Energy, Hormonal Assessment

Notes

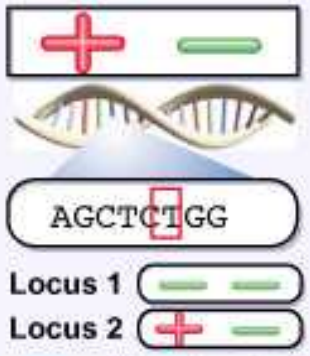
Sample Results



Sample Results

Inflammation

IL-1RN
Chromosome 12
c677t



www.gsdl.com\cyp1a2

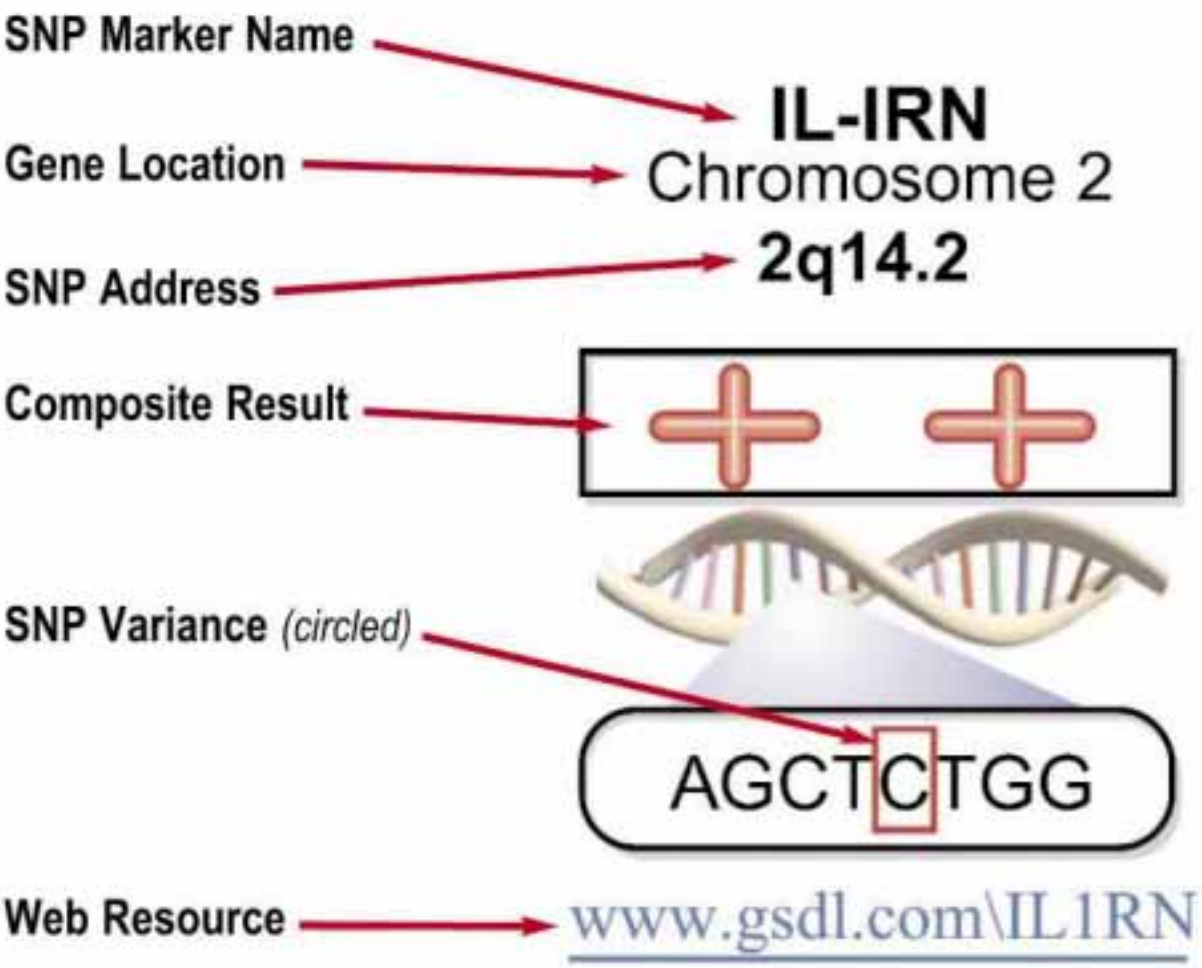
HEALTH IMPLICATIONS: Interleukin-1 receptor agonist (IL-1RA) is a naturally occurring competitive inhibitor of IL-1a and IL-1b-induced pro-inflammatory activity. A defect in the IL-1RA gene can contribute to a more prolonged and severe inflammatory response and has been associated with increased risk for chronic inflammatory conditions like atherosclerosis, osteoporosis, rheumatoid arthritis, lupus, colitis, and Crohn's disease. However, the IL-1RA SNP also confers benefit when fighting infections or cancer through amplified immune vigilance.

MINIMIZING RISKS: Eat a diet rich in anti-oxidants (colorful fruits and vegetables). Increase consumption of cold-water fish, like salmon, and reduce intake of vegetable oil and fatty meat. salmon, and reduce intake of vegetable oil and fatty meat. Fish oil supplementation, silymarin (milk thistle) directly inhibit IL-1 production. Niacinamide and other anti-inflammatory botanicals like boswellia (frankincense), glycyrrhiza (licorice), and curcumin (tumeric) may mediate the pro-inflammatory effects of increased IL-1. Compounds in cannabis have also been shown to suppress IL-1 levels.
Corticosteroids and cyclosporin A inhibit IL-1 production but with significant immune suppression and numerous other side-effects.

FURTHER EVALUATION: IL-1RA defects lead to increased inflammatory tendencies throughout the body. Consider laboratory evaluation of cardiovascular health (lipids, C-reactive protein, etc.), for bone resorption (deoxypyridinoline), and for digestive health (bowel microbiology, food antibodies, etc.). The physician should be cognizant that there is also an increased risk of auto-immunity in this patient. d inflammatory tendencies throughout the body.

ACE

Sample Results



Sample Results

[redacted]

Optimizing your Genomic Potential

The following pages summarize dietary, lifestyle, supplemental, and prescription considerations based on your unique genetic make-up that can help maximize your genetic potential to maintain a healthy cardiovascular system. **Predictive genomic testing only reveals your predisposition to a particular condition. All of the genes identified in predictive genomic testing are modifiable based on your environment and how you choose to live.**

Diet

This section offers dietary considerations based on your unique genetic make-up.

- Eat a diet rich in green vegetables, as these are not only high in mineral content but also the primary source of dietary anti-oxidants. Eat foods that are high in magnesium. Excellent food sources of magnesium include all green foods, nuts, and seeds.

Lifestyle / Environment

This section offers lifestyle and environmental considerations based on your unique genetic make-up.

- Regular, moderate alcohol consumption (1-2 drinks/d) is advised. Red wine may be especially beneficial. A salt restricted diet (DASH diet) and maintaining normal body weight may be therapeutically beneficial. Do not smoke, as this may affect blood pressure.

Nutritional Suplementation

This section offers nutritional supplementation considerations based on your unique genetic make-up.

- Red rice yeast extract (~2,000 mg/d) has statin-like effects with very few side effects. Inositol hexaniacinate (up to 3 g/d) may be beneficial in lowering cholesterol. Additional anti-oxidant support is advised.

Pharmaceutical Considerations

This section offers pharmaceutical considerations based on your unique genetic make-up.

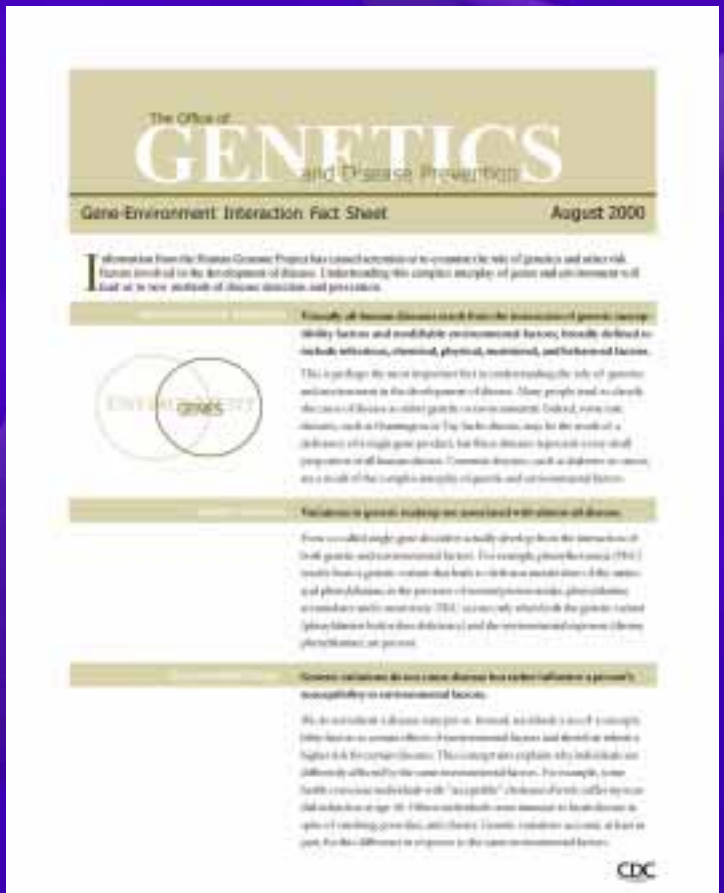
- AGTR antagonists like losartan (Hyzaar™) or ACE inhibitors are likely to be more effective than other anti-hypertensive medications. Statin drugs like Pravastatin™ have been shown to lower total and LDL cholesterol significantly.

Slide Presentation

- MS PowerPoint
  - PowerPoint Player
    - Lecture Version
    - Continuous Play
- Adobe Acrobat
  - Overheads
  - Color
  - B&W
  - Handouts
- HTML Web

Educational Support

CDC “Gene Environment Interaction Fact Sheet



The Office of
GENETICS
and Disease Prevention

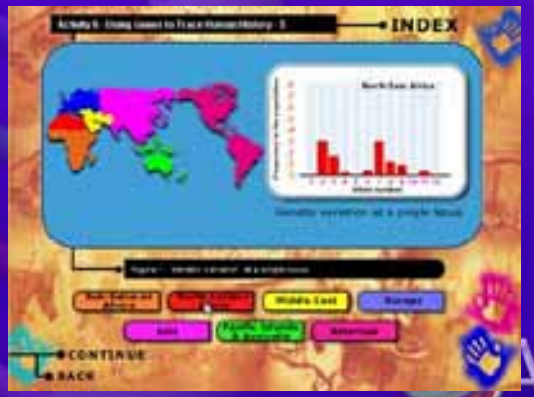
Gene-Environment Interaction Fact Sheet August 2000

CDC

Notes

Educational Support

Human Genome Project
Multimedia Lessons

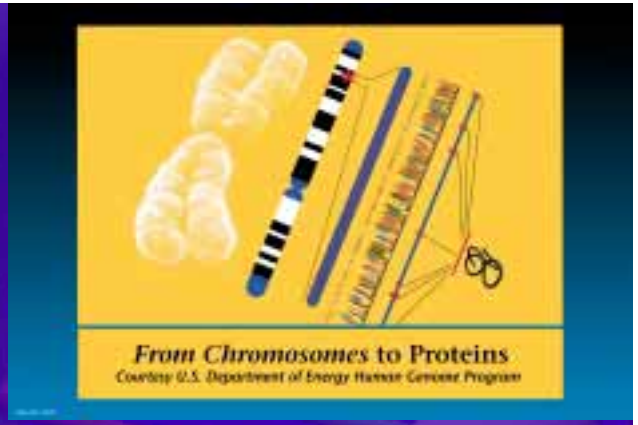


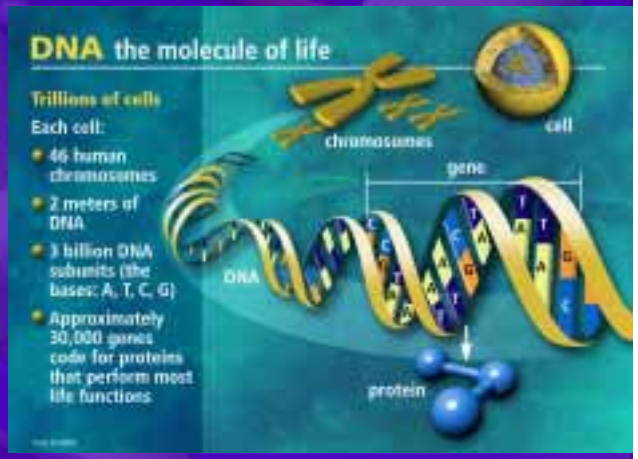
Educational Support

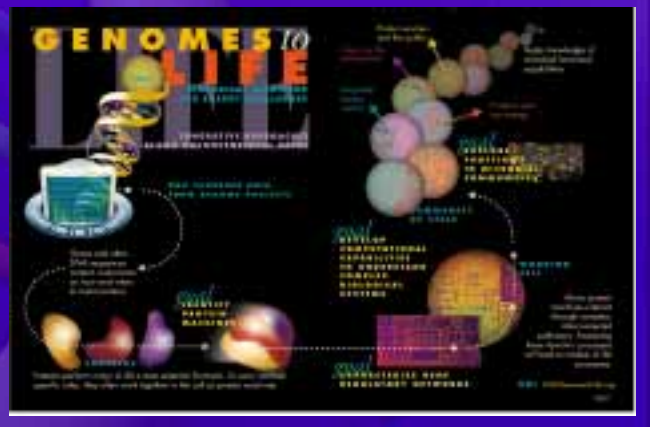
Genetics: The Future of Medicine- NHGRI, NIH

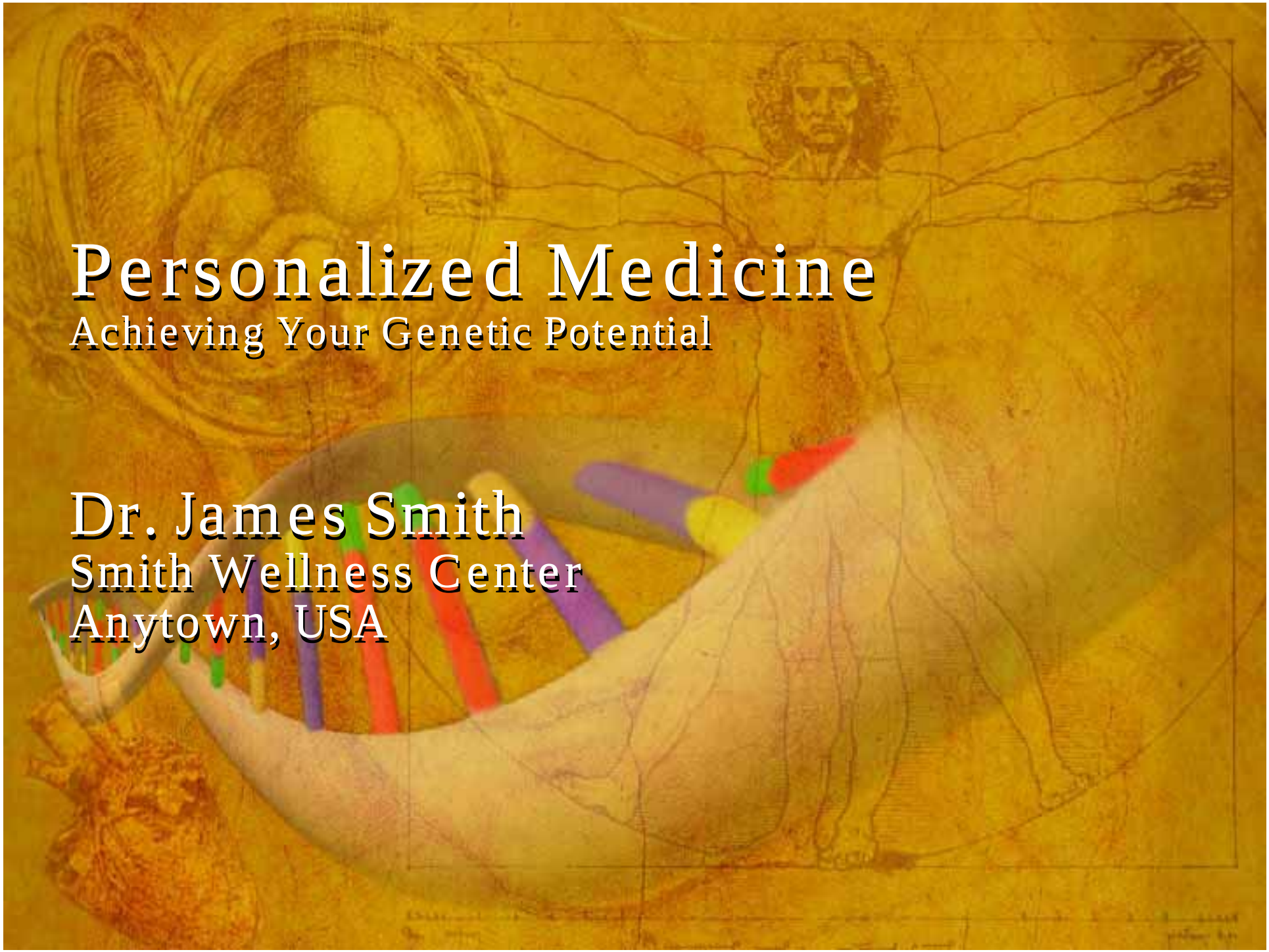
Educational Support

Royalty-free images








Personalized Medicine
Achieving Your Genetic Potential
Dr. James Smith
Smith Wellness Center
Anytown, USA

Expand your reach

Internal Communication

- Staff training
- Reception area
- Routine office visits
- New patient orientation
- Advanced classes/workshops

External Communication

- Community lectures
- Web information

Staff Training

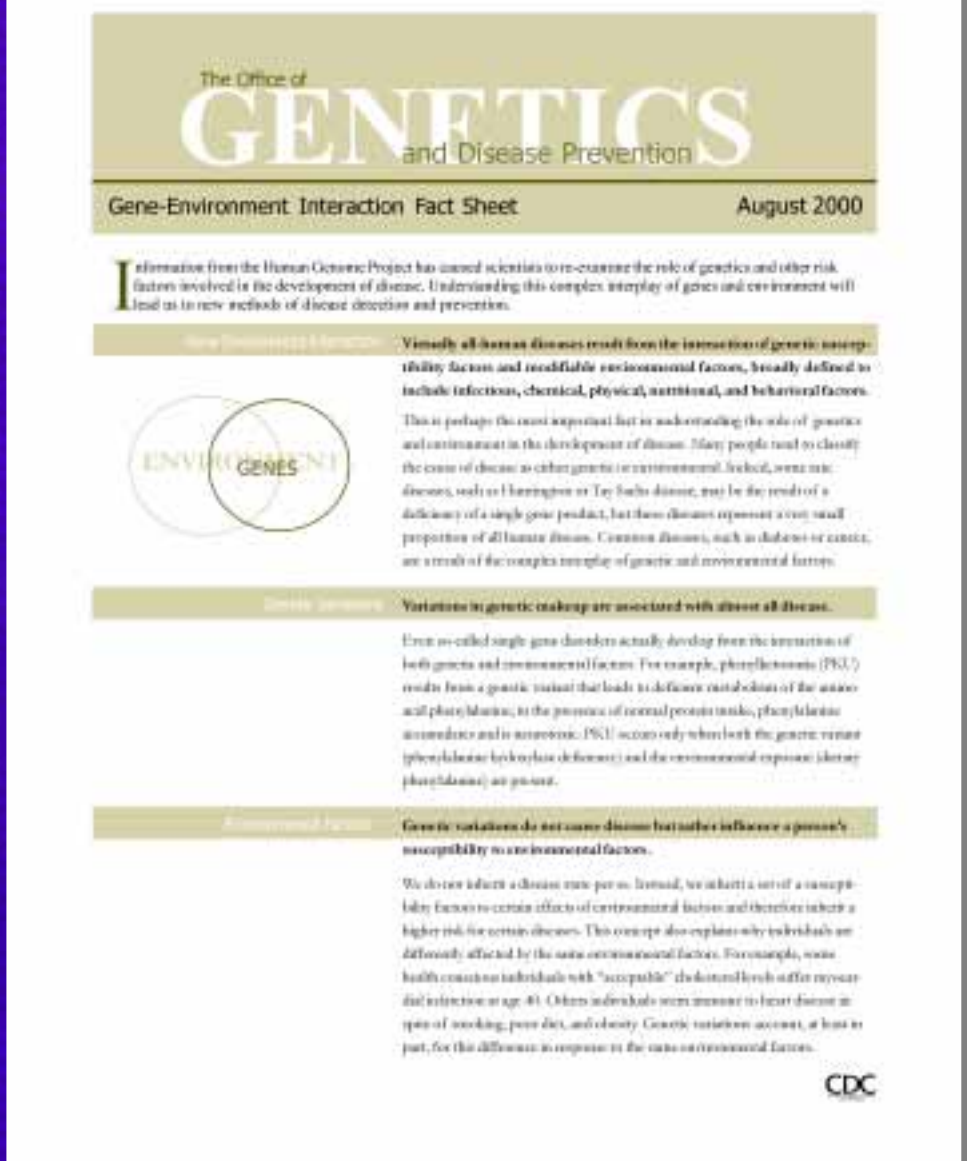
- Missing link to success
  - Implementation assistants
  - Majority of patient interaction
  - Need full support
- Internal Training
  - Can split over several lunch hours
  - Have all assessed & under care for risk factors

Reception Area

- Self-running presentation
- Profile information sheets/Brochures

  Lucite holders
- Video of community lecture

Centers for Disease Control
Gene Environment Interaction Fact Sheet
August 2000



The Office of
GENETICS
and Disease Prevention

Gene-Environment Interaction Fact Sheet August 2000

Information from the Human Genome Project has caused scientists to re-examine the role of genetics and other risk factors involved in the development of disease. Understanding this complex interplay of genes and environment will lead us to new methods of disease detection and prevention.

Virtually all human diseases result from the interaction of genetic susceptibility factors and modifiable environmental factors, broadly defined to include infectious, chemical, physical, nutritional, and behavioral factors.

This is perhaps the most important fact to understanding the role of genetics and environment in the development of disease. Many people tend to classify the cause of disease as either genetic or environmental. Indeed, some rare diseases, such as Huntington or Tay Sachs disease, may be the result of a deficiency of a single gene product, but these diseases represent a very small proportion of all human disease. Common diseases, such as diabetes or cancer, are a result of the complex interplay of genetic and environmental factors.

Variations in genetic makeup are associated with almost all disease.

Even so-called single gene disorders actually develop from the interaction of both genetic and environmental factors. For example, phenylketonuria (PKU) results from a genetic variant that leads to deficient metabolism of the amino acid phenylalanine; in the presence of normal protein intake, phenylalanine accumulates and is neurotoxic. PKU occurs only when both the genetic variant (phenylalanine hydroxylase deficiency) and the environmental exposure (dietary phenylalanine) are present.

Genetic variations do not cause disease but rather influence a person's susceptibility to environmental factors.

We do not inherit a disease state per se. Instead, we inherit a set of a susceptibility factors to certain effects of environmental factors and therefore inherit a higher risk for certain diseases. This concept also explains why individuals are differently affected by the same environmental factors. For example, some health conscious individuals with "acceptable" cholesterol levels suffer myocardial infarction at age 40. Others individuals seem immune to heart disease in spite of smoking, poor diet, and obesity. Genetic variations account, at least in part, for this difference in response to the same environmental factors.

CDC

ACE

Lectures

- New patient orientation
  - Mandatory
  - Bring spouse/significant other
- Advanced classes/workshops
  - Existing patients and guest
- Community lectures
  - Clubs
  - Groups
  - Health Clubs
  - Service organizations
  - Self organized/promoted

Lectures- Tips

- Regular time & place
- Send announcements to patient base
- Slides/overheads
- Keep it simple
- Keep it short: ~45 min
- Handouts
- Close with call to action
  - Courtesy consultation
  - Consultation to discuss questionnaire
  - Focus on advanced assessment tools

Overcoming Resistance to Change

1) Dissatisfaction with the status quo
2) Clear goal or vision of where you want to be
3) Concrete first step


Implementation Strategies

Abstracts

ACE

Abstracts *for* Genomics in Primary Care Medicine

Ahdieh M, Vandenbos T, Youakim A. Lung epithelial barrier function and wound healing are decreased by IL-4 and IL-13 and enhanced by IFN-gamma. *Am J Physiol Cell Physiol* 2001 Dec;281(6):C2029-38.

To understand the effects of cytokines on epithelial cells in asthma, we have investigated the effects of interleukin (IL)-4, IL-13, and interferon (IFN)-gamma on barrier function and wound healing in Calu-3 human lung epithelial cells. IL-4 and IL-13 treatment of Calu-3 cells grown on Transwell filters resulted in a 70-75% decrease in barrier function as assessed by electrophysiological and [(14)C]mannitol flux measurements. In contrast, IFN-gamma enhanced barrier function threefold using these same parameters. Cells treated concurrently with IFN-gamma and IL-4 or IL-13 showed an initial decline in barrier function that was reversed within 2 days, resulting in barrier levels comparable to control cells. Analysis of the tight junction-associated proteins ZO-1 and occludin showed that IL-4 and IL-13 significantly reduced ZO-1 expression and modestly decreased occludin expression compared with controls. IFN-gamma, quite unexpectedly given its enhancing effect on barrier function, reduced expression of ZO-1 and occludin to almost undetectable levels compared with controls. In wound-healing assays of cells grown on collagen I, IL-4 and IL-13 decreased migration, whereas IFN-gamma treatment enhanced migration, compared with control cells. Addition of IFN-gamma, in combination with IL-4 or IL-13, restored migration of cells to control levels. Migration differences observed between the various cytokine treatments was correlated with expression of the collagen I-binding alpha(2)beta(1)-integrin at the leading edge of cells at the wound front; alpha(2)beta(1)-integrin expression was decreased in IFN-gamma-treated cells compared with controls, whereas it was highest in IL-4- and IL-13-treated cells. These results demonstrate that IL-4 and IL-13 diminish the capacity of Calu-3 cells to maintain barrier function and repair wounds, whereas IFN-gamma promotes epithelial restitution by enhancing barrier function and wound healing.

Allen ND, Logan K, Lally G, et al. Distribution of parthenogenetic cells in the mouse brain and their influence on brain development and behavior. *Proc Natl Acad Sci U S A* 1995 Nov 7;92(23):10782-6.

A systematic analysis of parthenogenetic (PG) cell fate within the central nervous system (CNS) was made throughout fetal development and neonatal and adult life. Chimeras were made between PG embryos carrying a

ubiquitously expressed lacZ transgene and normal fertilized embryos. After detailed histological analysis, we find that the developmental potential of PG cells is spatially restricted to certain parts of the brain. PG cells are prevalent in telencephalic structures and are largely excluded from diencephalic structures, especially the hypothalamus. These spatial restrictions are established early in development. Behavioral studies with chimeras identified an increase in male aggression when the proportion of PG cells in the brain was high. These studies demonstrate that imprinted genes play key roles in development of the CNS and may be involved in behavior.

Al-Said MS, Ageel AM, Parmar NS, Tariq M. Evaluation of mastic, a crude drug obtained from Pistacia lentiscus for gastric and duodenal anti-ulcer activity. *J Ethnopharmacol* 1986 Mar;15(3):271-8.

The effect of mastic, a concrete resinous exudate obtained from the stem of the tree Pistacia lentiscus, has been studied on experimentally-induced gastric and duodenal ulcers in rats. Mastic at an oral dose of 500 mg/kg produced a significant reduction in the intensity of gastric mucosal damage induced by pyloric ligation, aspirin, phenylbutazone, reserpine and restraint + cold stress. It produced a significant decrease of free acidity in 6-h pylorus-ligated rats and a marked cytoprotective effect against 50% ethanol in rats which could be reversed by prior treatment with indomethacin. The protective effect was not seen when it was given intraperitoneally in phenylbutazone and restraint + cold stress models. The reduction in the intensity of ulceration in cysteamine-induced duodenal ulcers was not found to be statistically significant in mastic-pretreated rats. The results suggest that mild antisecretory and a localized adaptive cytoprotectant action may be responsible for its anti-ulcer activity. These observations support the results of an earlier study on the clinical effectiveness of mastic in the therapy of duodenal ulcer.

Ames BN. Endogenous DNA damage as related to cancer and aging. *Mutat Res* 1989 Sep;214(1):41-6.

The endogenous background level of oxidant-induced DNA damage in vivo has been assayed by measuring 8-hydroxydeoxyguanosine (oh8dG), thymine glycol and thymidine glycol in urine and oh8dG in DNA. The level of oxidative DNA damage as measured by oh8dG in normal rat liver is shown to be extensive (1/130,000 bases in nuclear DNA and 1/8000 bases in mitochondrial DNA), especially in mtDNA. The methylation adduct 7-methylguanine (m7G) has also been found. m7G is one of about 5 adducts found on methylating DNA, and oh8dG is one of about 20 adducts found on oxidizing DNA, e.g., by radiation. We also discuss 3 hitherto unrecognized antioxidants in man.

Amos W, Harwood J. Factors affecting levels of genetic diversity in natural populations. *Philos Trans R Soc Lond B Biol Sci* 1998 Feb 28;353(1366):177-86.

Genetic variability is the clay of evolution, providing the base material on which adaptation and speciation depend. It is often assumed that most interspecific differences in variability are due primarily to population size effects, with bottlenecked populations carrying less variability than those of stable size. However, we show that population bottlenecks are unlikely to be the only factor, even in classic case studies such as the northern elephant seal and the cheetah, where genetic polymorphism is virtually absent. Instead, we suggest that the low levels of variability observed in endangered populations are more likely to result from a combination of publication biases, which tend to inflate the level of variability which is considered 'normal', and inbreeding effects, which may hasten loss of variability due to drift. To account for species with large population sizes but low variability we advance three hypotheses. First, it is known that certain metapopulation structures can result in effective population sizes far below the census size. Second, there is increasing evidence that heterozygous sites mutate more frequently than equivalent homozygous sites, plausibly because mismatch repair between homologous chromosomes during meiosis provides extra opportunities to mutate. Such a mechanism would undermine the simple relationship between heterozygosity and effective population size. Third, the fact that related species that differ greatly in variability implies that large amounts of variability can be gained or lost rapidly. We argue that such cases are best explained by rapid loss through a genome-wide selective sweep, and suggest a mechanism by which this could come about, based on forced changes to a control gene inducing coevolution in the genes it controls. Our model, based on meiotic drive in mammals, but easily extended to other systems, would tend to facilitate population isolation by generating molecular incompatabilities. Circumstances can even be envisioned in which the process could provide intrinsic impetus to speciation.

Baker JC, Duncanson RC, Tunnicliffe WS, Ayres JG. Development of a standardized methodology for double-blind, placebo-controlled food challenge in patients with brittle asthma and perceived food intolerance. *J Am Diet Assoc* 2000 Nov;100(11):1361-7.

OBJECTIVE: To develop a standardized, double-blind, placebo-controlled, food challenge (DBPCFC) methodology for identifying food intolerance in patients with brittle asthma. SUBJECTS/SETTING: Patients with brittle asthma and perceived food intolerance were studied in hospital. DESIGN: Each of 3 protocols began with 5 days of dietary exclusion. Protocol 1 consisted of open food challenges in 29 patients, protocol 2 consisted of 2 daily DBPCFCs in 22 patients, and protocol 3 involved 1 daily DBPCFC in 18

patients. Total immunoglobulin E level was measured and food-specific radioallergosorbent tests and skin prick tests were conducted. A standard panel of hyperallergenic foods were masked in a soup (developed specially for this study) for every food challenge. Peak expiratory flow, forced expiratory volume, and symptoms were assessed as objective measures of response. Open food challenges at home followed each protocol. Each protocol took approximately 14 days in the hospital and 4 to 6 months at home. RESULTS: For protocols 1, 2 and 3, positive reactions were experienced by 52%, 55%, and 66% of patients, respectively. Radioallergosorbent tests and skin prick tests were shown to have 40% and 71% sensitivity, respectively, and 74% and 77% specificity for predicting a positive food challenge. APPLICATIONS/CONCLUSIONS: The high prevalence of food intolerance in patients with brittle asthma was confirmed, as was the poor positive predictive value of skin prick tests and radioallergosorbent tests. The food challenge method developed enables standardized identification of food intolerances in patients with brittle asthma and may be useful in other groups.

Benard A, Desreumeaux P, Huglo D, et al. Increased intestinal permeability in bronchial asthma. *J Allergy Clin Immunol* 1996 Jun;97(6):1173-8.

T lymphocytes are a major component of bronchial inflammatory processes in asthma. Because lymphocytes have the ability to migrate from one mucosal site to another, we initiated this prospective study to demonstrate mucosal abnormalities of the digestive barrier in asthma. To establish this we studied intestinal permeability in a group of 37 patients with asthma (21 allergic and 16 nonallergic) by measuring chromium 51-labeled ethylenediaminetetraacetatic acid (CrEDTA) urinary recovery. The results were compared with those obtained in a group of 13 nonasthmatic patients with chronic obstructive pulmonary disease and 26 healthy control subjects. Urinary recovery of CrEDTA was significantly higher in patients with asthma (2.5% +/- 1.95%) than in patients with chronic obstructive pulmonary disease (1.16% +/- 0.48%) and healthy control subjects (1.36% +/- 0.14%). There was no significant difference in intestinal permeability between patients with allergic asthma (2.94% +/- 2.4%) and those with nonallergic asthma (1.92% +/- 0.9%). Intestinal permeability was not correlated with the severity of asthma as measured by FEV1. Similarly, intestinal permeability did not significantly vary according to Aas score or steroid treatment. Serum IgE values and eosinophil blood count were not correlated with intestinal permeability. Intestinal permeability was evaluated sequentially in seven patients with asthma (4 allergic and 3 nonallergic) with a mean interval of 7.6 months (range, 2 to 13 months) and did not significantly change. Our results support the hypothesis that a general defect of the whole mucosal system is present as a cause or a consequence of bronchial asthma.

Bickeboller H, Campion D, Brice A, et al. Apolipoprotein E and Alzheimer disease: genotype-specific risks by age and sex. *Am J Hum Genet* 1997 Feb;60(2):439-46.

The distribution of apolipoprotein E (APOE) genotypes as a function of age and sex has been examined in a French population of 417 Alzheimer disease (AD) patients and 1,030 control subjects. When compared to the APOE epsilon3 allele, an increased risk associated with the APOE epsilon4 allele (odds ratio [OR] [epsilon4] = 2.7 with 95% confidence interval [CI] = 2.0-3.6; P < .001) and a protective effect of the APOE epsilon2 allele (OR[epsilon2] = 0.5 with 95% CI = 0.3-0.98; P = .012) were retrieved. An effect of the epsilon4 allele dosage on susceptibility was confirmed (OR[epsilon4/epsilon4] vs. the epsilon3/epsilon3 genotype = 11.2 [95% CI = 4.0-31.6]; OR[epsilon3/epsilon4] vs. the epsilon3/epsilon3 genotype = 2.2 [95% CI = 1.5-3.5]). The frequency of the epsilon4 allele was lower in male cases than in female cases, but, since a similar difference was found in controls, this does not lead to a difference in OR between sex. ORs for the epsilon4 allele versus the epsilon3 allele, OR(epsilon4), were not equal in all age classes: OR(epsilon4) in the extreme groups with onset at < 60 years or > 79 years were significantly lower than those from the age groups 60-79 years. In epsilon3/epsilon4 individuals, sex-specific lifetime risk estimates by age 85 years (i.e., sex-specific penetrances by age 85 years) were 0.14 (95% CI 0.04-0.30) for men and 0.17 (95% CI 0.09-0.28) for women.

Bouic PJ. The role of phytosterols and phytosterolins in immune modulation: a review of the past 10 years. Curr Opin Clin Nutr Metab Care 2001 Nov;4(6):471-5.

Although plant sterols (phytosterols) were chemically described in 1922, their biological role in human and animal health has been underestimated. Their ability to control cholesterol plasma levels in hypercholesterolimic patients was first described in 1983 when the structure of phytosterols implied that they could, by steric hindrance, inhibit the absorption of cholesterol from our diets. This has led to the development of functional foods containing high contents of these plant molecules or their esters as cholesterol controlling foods. Over the last 15 years, however, several reports have appeared in the literature indicating that phytosterols have some immunological activity as highlighted in animal models of inflammation or even in in-vitro and in-vivo models of cancer (colorectal and breast cancer). These findings were paralleled by epidemiological studies correlating the reduced risk of numerous diseases and the dietary intake of phytosterols. It is only in the last 10 years, however, that their direct immune modulatory activity on human lymphocytes has been proven and the mechanism of action in cancer cells has been elucidated. The use of phytosterols as supportive therapies in certain chronic conditions has been tested under clinical trial conditions. This

review presents a summary of the in-vitro and in-vivo studies published to date.

Bouic PJ, Clark A, Lamprecht J, et al. The effects of B-sitosterol (BSS) and B-sitosterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: inhibition of post marathon immune suppression and inflammation. *Int J Sports Med* 1999 May;20(4):258-62.

A pilot study was undertaken to investigate the effects of the intake of capsules containing the plant sterols and sterolins (BSS:BSSG mixture) on selected immune parameters of volunteers participating in an ultra-marathon in Cape Town, South Africa. Those runners having received active capsules (n=9) showed less neutrophilia, lymphopenia and leukocytosis when compared to their counterparts having received placebo capsules (n=8): the placebo treated individuals showed significant increases in their total white blood cell numbers as well as in their neutrophils (p=0.03 and 0.03 respectively). Furthermore, statistically significant increases within lymphocyte subsets were observed in the runners having received the active capsules: CD3+ cells increased (p=0.02) as did CD4+ cells (p=0.03). In parallel, the BSS:BSSG capsules decreased the plasma level of IL6 in the runners using the active capsules (p=0.08) and significantly decreased the cortisol: DHEAs ratio (p=0.03), suggesting that these volunteers had less of an inflammatory response and were less immune suppressed during the post-marathon recovery period. These findings justify further investigations into the use of the phytosterols to prevent the subtle immunosuppression associated with excessive physical stress.

Bouic PJ, Lamprecht JH. Plant sterols and sterolins: a review of their immune-modulating properties. *Altern Med Rev* 1999 Jun;4(3): 170-7.

Beta-sitosterol (BSS) and its glycoside (BSSG) are sterol molecules which are synthesized by plants. When humans eat plant foods phytosterols are ingested, and are found in the serum and tissues of healthy individuals, but at concentrations orders of magnitude lower than endogenous cholesterol. Epidemiological studies have correlated a reduced risk of numerous diseases with a diet high in fruits and vegetables, and have concluded that specific molecules, including b-carotene, tocopherols, vitamin C, and flavonoids, confer some of this protective benefit. However, these epidemiologic studies have not examined the potential effect that phytosterols ingested with fruits and vegetables might have on disease risk reduction. In animals, BSS and BSSG have been shown to exhibit anti-inflammatory, anti-neoplastic, anti-pyretic, and immune-modulating activity. A proprietary BSS:BSSG mixture has demonstrated promising results in a number of studies, including in vitro

studies, animal models, and human clinical trials. This phytosterol complex seems to target specific T-helper lymphocytes, the Th1 and Th2 cells, helping normalize their functioning and resulting in improved T-lymphocyte and natural killer cell activity. A dampening effect on overactive antibody responses has also been seen, as well as normalization of the DHEA:cortisol ratio. The re-establishment of these immune parameters may be of help in numerous disease processes relating to chronic immune-mediated abnormalities, including chronic viral infections, tuberculosis, rheumatoid arthritis, allergies, cancer, and auto-immune diseases.

Broeckel U, Hengstenberg C, et al. A comprehensive linkage analysis for myocardial infarction and its related risk factors. ***Nat Genet*** **2002;30(2):210-4.**

Coronary artery disease and myocardial infarction (MI) are leading causes of death in the western world. Numerous studies have shown that risk factors such as diabetes mellitus, arterial hypertension and hypercholesterolemia contribute to the development of the disease. Although each risk factor by itself is partly under genetic control, a positive family history is an independent predictor, which suggests that there are additional susceptibility genes. We have scanned the whole genome in 513 families to identify chromosomal regions linked to myocardial infarction and related risk factors that are known to be under genetic control. Here we show, by using variance component analysis and incorporating risk factors, that risk of myocardial infarction maps to a single region on chromosome 14 with a significant lod score of 3.9 (pointwise P=0.00015, genome-wide P<0.05), providing evidence of a principal MI locus. To characterize this locus we analyzed each risk factor by itself. Serum concentrations of lipoprotein (a) show linkage to both the apolipoprotein (a) locus (lod score 26.99) and a new locus on chromosome 1 (lod score 3.8). There is suggestive linkage for diabetes mellitus on chromosome 6 (lod score 2.96), for hypertension on chromosomes 1 and 6, for high-density and low-density lipoprotein cholesterol on chromosomes 1 and 17, and for triglyceride concentrations on chromosome 9. Although some of these risk factors overlap with previously identified loci, none overlaps with the newly identified susceptibility locus for myocardial infarction and coronary artery disease.

Butterworth CE Jr, Hatch KD, Gore H, Mueller H, Krumdieck CL. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. *Am J Clin Nutr* 1982 Jan;35(1):73-82.

Forty-seven young women with mild or moderate dysplasia of the uterine cervix (cervical intraepithelial neoplasia) diagnosed by cervical smears, received oral supplements of folic acid, 10 mg, or a placebo (ascorbic acid, 10 mg) daily for 3 months under double-blind conditions. All had used a combination-type oral contraceptive agent for at least 6 months and

continued it while returning monthly for follow-up examinations. All smears and a biopsy obtained at the end of the trial period were classified by a single observer without knowledge of treatment status using an arbitrary scoring system (1 normal, 2 mild, 3 moderate, 4 severe, 5 carcinoma in situe). Mean biopsy scores from folate supplemented subjects were significantly better than in folate-unsupplemented subjects (2.28 versus 2.92, respectively; p less than 0.05). Final versus initial cytology scores were also significantly better in supplemented subjects (1.95 versus 2.32, respectively; p less than 0.05), unchanged in patients receiving the placebo (2.27 versus 2.30, respectively). Before treatment the mean red cell folate concentration was lower among oral contraceptive agent users than nonusers (189 versus 269 ng/ml, respectively; p less than 0.01) and even lower among users with dysplasia (161 versus 269 ng/ml, respectively; p less than 0.001). Morphological features of megaloblastosis were associated with dysplasia and also improved in folate supplemented subjects. These studies indicate that either a reversible, localized derangement in folate metabolism may sometimes be misdiagnosed as cervical dysplasia, or else such a derangement is an integral component of the dysplastic process that may be arrested or in some cases reversed by oral folic acid supplementation.

Ching CK, Chan YK, Ng WC. The combination of omeprazole, amoxycillin, and clarithromycin eradicates Helicobacter pylori in 95% of patients---7 days of therapy is as good as 10 days. *Hong Kong Med J* 1998 Mar;4(1):7-10.

More than half of the known Helicobacter pylori strains are resistant to metronidazole, according to previous Hong Kong studies. The response rates to treatment regimens that comprise metronidazole as one of the antimicrobial agents have usually been disappointing in cases involving metronidazole-resistant strains. The objective of this open cohort evaluation was to assess the efficacy of an alternative regimen that combines omeprazole with amoxycillin and clarithromycin in Helicobacter pylor-positive ulcer and non-ulcer patients in Hong Kong. Furthermore, we aimed to investigate if 7 days were as good as 10 days of therapy. We studied 186 Helicobacter pylor-positive subjects; 149 subjects received 7 days of combination therapy and 37 subjects received 10 days. Our results showed that the overall Helicobacter pylori eradication efficiency was identical (94.6%) for both treatments. The incidences of adverse effects were also very similar (16.8% versus 16.2%) and both treatments were well tolerated. Thus, we propose that omeprazole in combination with amoxycillin and clarithromycin should be considered as one of the first-line therapies for patients with Helicobacter pylor infection in Hong Kong.

Cooke GS, Hill AV. Genetics of susceptibility to human infectious disease. *Nat Rev Genet* 2001 Dec;2(12):967-77.

Before Robert Koch's work in the late nineteenth century, diseases such as tuberculosis and leprosy were widely believed to be inherited disorders. Heritability of susceptibility to several infectious diseases has been confirmed by studies in the twentieth century. Infectious diseases, old and new, continue to be an important cause of mortality worldwide. A greater understanding of disease processes is needed if more effective therapies and more useful vaccines are to be produced. As part of this effort, developments in genetics have allowed a more systematic study of the impact that the human genome and infectious disease have on each other.

Coyle AJ, Bertrand C, Tsuyuki S, et al. IL-4 differentiates naive CD8+ T cells to a "Th2-like" phenotype: a link between viral infections and bronchial asthma. *Ann N Y Acad Sci* 1996 Oct 31;796:97-103.

Viral infections of the lung have been postulated to be a major factor in the etiology of bronchial asthma, a disease characterized by eosinophilic inflammation of the airways. In addition, upper respiratory tract infection in asthmatic individuals results in an exacerbation of the disease. Nevertheless, the mechanisms by which viral infection leads to disease exacerbation are poorly understood. CD8+ T cells play an important role in the host defense responses against viral infection, although to date, there are no reports to suggest that CD8+ T cells play any role in eosinophil recruitment. In the present study, we report that CD8+ T cells activated by either immobilized CD3 mAb or specific antigen can switch to a phenotype that produces Th2 cytokines and secretes less IFN-gamma. Moreover, in vivo, if a lung mucosal Th2 immune response exists, then antigen-specific activation of CD8 cells results in the development of lung eosinophilic inflammation mediated by the secretion of IL-5 from CD8+ T cells. These results may explain the link between viral infections and bronchial asthma, as this IL-4-dependent switch to CD8+ T cells to IL-5 secretion may not only exacerbate asthma by recruiting eosinophils into the lungs, but the impaired IFN-gamma production may also lead to delayed viral clearance.

Engbersen AM, Franken DG, Boers GH, et al. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995 Jan;56(1):142-50.

Thermolability of 5,10-methylenetetrahydrofolate reductase (MTHFR) was examined as a possible cause of mild hyperhomocysteinemia in patients with premature vascular disease. Control subjects and vascular patients with mild hyperhomocysteinemia and with normohomocysteinemia were studied. The

mean (+/- SD) specific MTHFR activity in lymphocytes of 22 control subjects was 15.6 (+/- 4.7) nmol CH2O/mg protein/h (range: 9.1-26.6), and the residual activity (+/- SD) after heat inactivation for 5 min at 46 degrees C was 55.3 (+/- 12.0)% (range: 35.9-78.3). By measurement of MTHFR activity, two distinct subgroups of hyperhomocysteinemic patients became evident. One group (n = 11) had thermolabile MTHFR with a mean (+/- SD) specific activity of 8.7 (+/- 2.1) nmol CH2O/mg protein/h (range: 5.5-12.7) and a residual activity, after heat inactivation, ranging from 0% to 33%. The other group (n = 28) had normal specific activity (+/- SD) of 21.5 (+/- 7.2) nmol CH2O/mg protein/h (range: 10.0-39.0) and a normal residual activity (+/- SD) of 53.8 (+/- 9.2)% (range: 33.1-71.5) after heat inactivation. The mean (+/- SD) specific activity of 29 normohomocysteinemic patients was 20.7 (+/- 6.5) nmol CH2O/mg protein/h (range: 9.4-33.8), and the mean (+/- SD) residual activity after heat inactivation was 58.2 (+/- 10.2)% (range: 43.0-82.0). Thus, in 28% of the hyperhomocysteinemic patients with premature vascular disease, abnormal homocysteine metabolism could be attributed to thermolabile MTHFR.

Engel RR, Satzger W, Gunther W, et al. Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type. *Eur Neuropsychopharmacol* 1992 Jun;2(2):149-55.

Thirty-three patients with mild primary degenerative dementia according to DSM-III (MMS between 15 and 27) took part in a double-blind cross-over study of phosphatidylserine (Fidia, 300 mg/d) versus placebo. Both treatment phases lasted for 8 weeks with an 8 week washout phase in between and a 4 week washout phase before treatment phase one. Clinical global improvement ratings showed significantly more patients improving under BC-PS than under placebo during treatment phase one. The improvement carried over to the following wash-out and treatment phases. There were no significant improvements in GBS dementia rating scale, psychometric tests or P300-latency. 16-channel EEG mapping findings indicated that the patients initially showed higher power values in all frequency bands (except alpha), when compared to a younger, healthy control group. BC-PS reduced the higher power values compared to placebo, shifting EEG power more towards the normal level.

Eto M, Watanabe K, Makino I. Increased frequencies of apolipoprotein epsilon 2 and epsilon 4 alleles in patients with ischemic heart disease. *Clin Genet* 1989 Sep;36(3):183-8.

It has been demonstrated that the genetic polymorphism of apolipoprotein (apo) E is associated with atherosclerosis. Thus, in this study, we have examined the apo E allele frequencies in 109 patients with ischemic heart disease (IHD) and 576 Japanese people as controls, and we have compared these frequencies between patients with IHD and controls. The frequencies of

the epsilon 2 and epsilon 4 alleles were significantly higher in patients with IHD than in the controls (epsilon 2: 8.2% vs 3.7%, epsilon 4: 17.0% vs 11.7%), whereas the frequency of the epsilon 3 allele was significantly lower in patients with IHD than in the controls (74.8% vs 84.6%). The epsilon 2-carrying patients with IHD were characterized by type III (43.8%) and IV (25.0%) hyperlipoproteinemia (HLP), whereas the epsilon 4-carrying patients with IHD were characterized by hypercholesterolemia (type IIb HLP: 42.8%, type IIa HLP: 28.6%). It is concluded that both epsilon 2 and epsilon 4 alleles are more associated with IHD than the epsilon 3 allele.

Eto M, Watanabe K, Chonan N, Ishii K. Familial hypercholesterolemia and apolipoprotein E4. *Atherosclerosis* 1988 Aug;72(2-3):123-8.

The purpose of this study was to elucidate the relationship between two genetic factors associated with raised blood cholesterol, i.e. familial hypercholesterolemia (FH) and apolipoprotein (apo) E4. A group of 50 unrelated heterozygous FH patients aged 33-71 years were studied together with 129 normolipidemic subjects. A significantly higher frequency of apo E4 phenotypes was found in FH patients (30.0%) than in normolipidemic subjects (15.5%). FH patients were divided into two groups with and without apo E4. Plasma total cholesterol (Chol) and triglyceride (TG) levels were significantly higher, and plasma low density lipoprotein-cholesterol (LDL-Chol) level tended to be higher in FH patients with apo E4 than in those without apo E4. In addition, the prevalence of ischemic heart disease (IHD) was significantly higher in FH patients with apo E4 (73.3%) than in those without apo E4 (31.4%). No significant difference was noted in age and in the prevalence of obesity, diabetes, hypertension and smoking between the FH groups with and without apo E4. These results suggest that apo E4 is associated with higher levels of total Chol and TG and, at least in part, contributes to the predisposition to IHD in FH.

Freeman DJ, Griffin BA, Holmes AP, et al. Regulation of plasma HDL cholesterol and subfraction distribution by genetic and environmental factors. Associations between the TaqI B RFLP in the CETP gene and smoking and obesity. Arterioscler Thromb 1994 Mar;14(3):336-44.

This study investigated in a healthy population (n = 220) the association of the TaqI B restriction fragment length polymorphism (RFLP) in the cholesteryl ester transfer protein (CETP) gene with plasma high-density lipoprotein (HDL) cholesterol concentration and subfraction distribution. A raised HDL cholesterol level was found in B2B2 homozygotes (B2 cutting site absent) and was associated specifically with a 45% increase in HDL2 compared with B1B1 homozygotes (B1B1, 77 +/- 39 mg/100 mL, mean +/- SD;

B2B2, 112 +/- 59 mg/100 mL; P < 0.01). Total plasma, very-low-density lipoprotein, and HDL triglyceride levels did not differ among the genotype groups, nor did plasma apolipoprotein AI levels (B1B1, 1.45 +/- 0.35 mg/mL, mean +/- SD; B2B2, 1.56 +/- 0.33 mg/mL). Thus, the genetic variation appeared to be independent of metabolic factors that are known to regulate HDL levels. Plasma CETP exchange activity was unlikely to be the cause of the association, since it did not differ between genotype groups and was not correlated with HDL2 concentration. Multivariate analysis demonstrated that the TaqI B polymorphism had an effect on HDL cholesterol and HDL2 that was independent of age, sex, body mass index, oral contraceptive use, exercise, alcohol consumption, and plasma triglycerides. In smokers, the presence of the B2B2 genotype did not result in increased HDL cholesterol or HDL2, whereas in obese subjects, the difference between B1B1 and B2B2 individuals was diminished. We conclude that the TaqI B RFLP is associated with a quantitatively significant effect on plasma HDL2 levels that is independent of plasma triglycerides and interacts with lifestyle factors.

Fujiki H, Suganuma M, Okabe S, Sueoka E, Suga K, Imai K, Nakachi K, Kimura S. Mechanistic findings of green tea as cancer preventive for humans. *Proc Soc Exp Biol Med* 1999 Apr;220(4):225-8.

Based on our initial work with green tea, in which repeated topical applications of (-)-epigallocatechin gallate (EGCG), the main green tea polyphenol, inhibited tumor promotion in a two-stage carcinogenesis experiment on mouse skin (Phytother Res 1, 44-47, 1987), numerous scientists have since provided so much additional evidence of the benefits of drinking green tea that it is now an acknowledged cancer preventive in Japan, and will possibly soon be recognized as such in other countries. Our work has so far produced several important results with EGCG and green tea: a wide range of target organs in animal experiments for cancer prevention, wide bioavailability of 3H-EGCG in various organs of mice, delayed cancer onset of patients with a history of consuming over 10 cups of green tea per day, and absence of any severe adverse effects among volunteers who took 15 green tea tablets per day (2.25 g green tea extracts, 337.5 mg EGCG, and 135 mg caffeine) for 6 months. This paper introduces three new findings: 1) EGCG interacted with the phospholipid bilayer membrane resulting in confirmation of the sealing effect of EGCG; 2) EGCG inhibited TNF-alpha gene expression in the cells and TNF-alpha release from the cells; 3) high consumption of green tea was closely associated with decreased numbers of axillary lymph node metastases among premenopausal Stage I and II breast cancer patients, and with increased expression of progesterone and estrogen receptors among postmenopausal ones. These results provide new insights into our understanding of the mechanisms of action of tea polyphenols and green tea extract as a cancer preventive.

Furlow FB, Armijo-Prewitt T, Gangestad SW, Thornhill R. Fluctuating asymmetry and psychometric intelligence. Proc R Soc Lond B Biol Sci 1997 Jun 22;264(1383):823-9.

Little is known about the genetic nature of human psychometric intelligence (IQ), but it is widely assumed that IQ's heritability is at loci for intelligence per se. We present evidence consistent with a hypothesis that interindividual IQ differences are partly due to heritable vulnerabilities to environmental sources of developmental stress, an indirect genetic mechanism for the heritability of IQ. Using fluctuating asymmetry (FA) of the body (the asymmetry resulting from errors in the development of normally symmetrical bilateral traits under stressful conditions), we estimated the relative developmental instability of 112 undergraduates and administered to them Cattell's culture fair intelligence test (CFIT). A subsequent replication on 128 students was performed. In both samples, FA correlated negatively and significantly with CFIT scores. We propose two non-mutually exclusive physiological explanations for this correlation. First, external body FA may correlate negatively with the developmental integrity of the brain. Second, individual energy budget allocations and/or low metabolic efficiency in high-FA individuals may lower IQ scores. We review the data on IQ in light of our findings and conclude that improving developmental quality may increase average IQ in future generations.

Grove J, Daly AK, Bassendine MF, Gilvarry E, Day CP. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. *Gut* 2000 Apr;46(4):540-5.

BACKGROUND: The factors determining why less than 10% of heavy drinkers develop advanced alcoholic liver disease (ALD) remain elusive, although genetic factors may be important. Interleukin 10 (IL-10) is an important cytokine with anti-inflammatory, anti-immune, and antifibrotic functions. Several polymorphisms have been identified in the IL-10 promoter and recent evidence suggests that some of these may have functional effects on IL-10 secretion. AIMS: To test the hypothesis that IL-10 promoter region polymorphisms are associated with susceptibility to ALD. METHODS: The allele frequencies for the two single base pair substitutions at positions -627 (C-->A) and -1117 (A-->G) in the IL-10 promoter were determined in 287 heavy drinkers with biopsy proved advanced ALD, 107 heavy drinkers with no evidence of liver disease or steatosis only on biopsy, and 227 local healthy volunteers. RESULTS: At position -627, 50% of patients with advanced ALD had a least one A allele compared with 33% of controls ($p<0.0001$) and 34% of drinkers with no or mild disease ($p=0.017$). At position -1117, the slight excess of the A allele in drinkers with advanced disease was because of linkage disequilibrium between the A alleles at the two sites. CONCLUSIONS: Among heavy drinkers, possession of the A allele at position -627 in the IL-10 promoter is associated with an increased risk of advanced liver disease. This is consistent with recent functional data that the

-627*A allele is associated with low IL-10 expression which will favour inflammatory, immune mediated, and profibrotic mechanisms of alcohol related liver injury.

Haig D. Genetic conflicts in human pregnancy. *Q Rev Biol* 1993 Dec;68(4):495-532.

Pregnancy has commonly been viewed as a cooperative interaction between a mother and her fetus. The effects of natural selection on genes expressed in fetuses, however, may be opposed by the effects of natural selection on genes expressed in mothers. In this sense, a genetic conflict can be said to exist between maternal and fetal genes. Fetal genes will be selected to increase the transfer of nutrients to their fetus, and maternal genes will be selected to limit transfers in excess of some maternal optimum. Thus a process of evolutionary escalation is predicted in which fetal actions are opposed by maternal countermeasures. The phenomenon of genomic imprinting means that a similar conflict exists within fetal cells between genes that are expressed when maternally derived, and genes that are expressed when paternally derived. During implantation, fetally derived cells (trophoblast) invade the maternal endometrium and remodel the endometrial spiral arteries into low-resistance vessels that are unable to constrict. This invasion has three consequences. First, the fetus gains direct access to its mother's arterial blood. Therefore, a mother cannot reduce the nutrient content of blood reaching the placenta without reducing the nutrient supply to her own tissues. Second, the volume of blood reaching the placenta becomes largely independent of control by the local maternal vasculature. Third, the placenta is able to release hormones and other substances directly into the maternal circulation. Placental hormones, including human chorionic gonadotropin (hCG) and human placental lactogen (hPL), are predicted to manipulate maternal physiology for fetal benefit. For example, hPL is proposed to act on maternal prolactin receptors to increase maternal resistance to insulin. If unopposed, the effect of hPL would be to maintain higher blood glucose levels for longer periods after meals. This action, however, is countered by increased maternal production of insulin. Gestational diabetes develops if the mother is unable to mount an adequate response to fetal manipulation. Similarly, fetal genes are predicted to enhance the flow of maternal blood through the placenta by increasing maternal blood pressure. Preeclampsia can be interpreted as an attempt by a poorly nourished fetus to increase its supply of nutrients by increasing the resistance of its mother's peripheral circulation.

Hamajima N, Matsuo K, Saito T, Tajima K, Okuma K, Yamao K, Tominaga S. Interleukin 1 polymorphisms, lifestyle factors, and Helicobacter pylori infection. *Jpn J Cancer Res* 2001 Apr;92(4):383-9.

Associations between Helicobacter pylori (HP) infection and lifestyle factors have been reported by several authors, but little is known about the host factors associated with the infection. This study aims to examine the infection rate of HP according to gene polymorphisms of interleukin (IL)-1A, IL-1B, and IL-1RN, and to investigate the interactions with lifestyle factors. Subjects were 241 non-cancer outpatients who had participated in a HP eradication program. Polymorphisms at - 889 (T to C) of IL-1A, at - 31 (C to T; T allele makes a TATA box) and - 511 (C to T) of IL-1B, and at intron 2 (86-bp VNTR (variable number of tandem repeats)) of IL-1RN were genotyped by PCR (polymerase chain reaction), PCR-RFLP (restriction fragment length polymorphism) and PCR-CTPP (PCR with confronting two-pair primers). It was found that IL-1B polymorphisms at - 31 and - 511 were near-completely linked, but in the opposite way to that in Caucasians; - 31C / - 511T and - 31T / - 511C alleles were dominant in the present subjects. The HP infection rate was substantially different among the genotypes of IL-1B C - 31T; 45.2% (19 / 42) for the C / C, 67.7% (90 / 133) for the C / T, and 63.6% (42 / 66) for the T / T. The age-sex adjusted odds ratio (OR) relative to the C / C genotype was 2.32 (95%CI (confidence interval), 1.10 - 4.92) for the T / C genotype and 2.46 (1.06 - 5.74) for the T / T genotype. The OR for the T / T genotype was significantly modified by smoking status; interaction term = 14.6 (1.12 - 190). The polymorphisms of IL-1A and IL-1RN were not associated with the infection rate. The results suggested that the T allele of IL-1B C - 31T is associated with vulnerability to persistent HP infection, and that the vulnerability is modified by smoking.

Hannuksela ML, Liinamaa MJ, Kesaniemi YA, Savolainen MJ. Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. *Atherosclerosis* 1994 Sep 30;110(1):35-44.

We investigated the interaction between genetic and environmental factors in the regulation of plasma HDL cholesterol concentration by determining TaqI and EcoN I restriction fragment length polymorphisms at the cholesteryl ester transfer protein (CETP) gene locus in 93 male alcohol drinkers and 82 control men. The highest plasma CETP activity and the lowest HDL cholesterol concentration were in the control subjects who were homozygous for the presence of the TaqI B restriction site (genotype 1-1). The lowest CETP activity and the highest HDL cholesterol among the control subjects were in those with genotype 2-2. These associations were, however, evident only in the non-smokers (P = 0.03 for CETP activity and P = 0.05 for HDL cholesterol). The non-smoking control subjects with genotype 1-1 had 19% higher CETP activity and 16% lower HDL cholesterol than those with genotype 2-2 (mean +/- S.D., 113 +/- 25 nmol/h/ml and 1.16 +/- 0.30 mmol/l vs. 95 +/- 16 nmol/h/ml and 1.38 +/- 0.34 mmol/l, respectively), and CETP activity and HDL cholesterol were negatively correlated (r = -0.280, P = 0.03, n = 59). The alcohol drinkers had 30% lower CETP activity (P < 0.001) and 48% higher HDL cholesterol (P < 0.001) than the controls. CETP activity was not

affected by the TaqI B genotype in the alcohol drinkers. The lowest HDL cholesterol was in subjects with genotype 1-1 (1.68 +/- 0.60 mmol/l), but those with genotype 2-2 had lower HDL cholesterol than those with genotype 1-2 (1.78 +/- 0.59 and 1.93 +/- 0.66 mmol/l, respectively). The data of the alcohol drinkers fitted better with the quadratic regression model than with the linear one, suggesting a trend towards a curved relationship between the TaqI B genotype and HDL cholesterol in both the non-smoking and smoking alcohol drinkers. Total, LDL or VLDL cholesterol, total or VLDL triglycerides did not differ between the TaqI B genotypes either in the alcohol drinkers or the controls. Lipid and lipoprotein levels and CETP activities were likewise similar in the TaqI A and EcoN I polymorphisms. Our data indicate that CETP TaqI B polymorphism is related to plasma CETP activity and HDL cholesterol concentration in non-smoking men, but these associations are affected by smoking and alcohol drinking.

Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 2000 Sep;61(3):154-66.

Two supergene families encode proteins with glutathione S-transferase (GST) activity: the family of soluble enzymes comprises at least 16 genes; the separate family of microsomal enzymes comprises at least 6 genes. These two GST families are believed to exert a critical role in cellular protection against oxidative stress and toxic foreign chemicals. They detoxify a variety of electrophilic compounds, including oxidized lipid, DNA and catechol products generated by reactive oxygen species-induced damage to intracellular molecules. An increasing number of GST genes are being recognized as polymorphic. Certain alleles, particularly those that confer impaired catalytic activity (e.g. GSTM1(*)0, GSTT1(*)0), may be associated with increased sensitivity to toxic compounds. GST polymorphisms may be disease modifying; for example, in subgroups of patients with basal cell carcinoma or bronchial hyper-responsiveness, certain GST appear to exert a statistically significant and biologically relevant impact on disease susceptibility.

Hein DW, Doll MA, Fretland AJ, et al. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev* 2000 Jan;9(1):29-42.

The focus of this review is the molecular genetics, including consensus NAT1 and NAT2 nomenclature, and cancer epidemiology of the NAT1 and NAT2 acetylation polymorphisms. Two N-acetyltransferase isozymes, NAT1 and NAT2, are polymorphic and catalyze both N-acetylation (usually deactivation) and O-acetylation (usually activation) of aromatic and heterocyclic amine carcinogens. Epidemiological studies suggest that the NAT1 and NAT2 acetylation polymorphisms modify risk of developing urinary bladder, colorectal, breast, head and neck, lung, and possibly prostate

cancers. Associations between slow NAT2 acetylator genotypes and urinary bladder cancer and between rapid NAT2 acetylator genotypes and colorectal cancer are the most consistently reported. The individual risks associated with NAT1 and/or NAT2 acetylator genotypes are small, but they increase when considered in conjunction with other susceptibility genes and/or aromatic and heterocyclic amine carcinogen exposures. Because of the relatively high frequency of some NAT1 and NAT2 genotypes in the population, the attributable cancer risk may be high. The effect of NAT1 and NAT2 genotype on cancer risk varies with organ site, probably reflecting tissue-specific expression of NAT1 and NAT2. Ethnic differences exist in NAT1 and NAT2 genotype frequencies that may be a factor in cancer incidence. Large-scale molecular epidemiological studies that investigate the role of NAT1 and NAT2 genotypes and/or phenotypes together with other genetic susceptibility gene polymorphisms and biomarkers of carcinogen exposure are necessary to expand our current understanding of the role of NAT1 and NAT2 acetylation polymorphisms in cancer risk.

Hunt SC, Geleijnse JM, Wu LL, et al. Enhanced blood pressure response to mild sodium reduction in subjects with the 235T variant of the angiotensinogen gene. *Am J Hypertens* 1999 May;12(5):460-6.

The relationship of high salt intake to elevated blood pressure levels has been demonstrated in most populations by cross-sectional, longitudinal, physiological, and clinical intervention studies. Variation within the angiotensinogen gene has been implicated in the genetic control of blood pressure levels and has been suggested to contribute to increased salt sensitivity. A total of 86 hypertensive men and women who had never been treated and who had participated in a 6-month randomized, placebo-controlled, clinical trial of low-sodium mineral salt (19% reduction in urinary sodium versus 12% increase in placebo group) were genotyped at the angiotensinogen M235T locus to test the hypothesis that the 235T allele is associated with a significant blood pressure response to a sodium reduction intervention whereas the 235M allele is not. After adjustment for gender and baseline blood pressure, persons with the TT and MT genotypes showed significant systolic blood pressure reductions on mineral salt compared with control subjects (P = .02 and P = .001, respectively) but not persons with the MM genotype (P = .10). Net adjusted diastolic blood pressure reductions also showed greater significance for persons with the TT and MT genotypes than for persons with the MM genotype (P = .08, P = .01, and P = .83, respectively). The net adjusted systolic and diastolic blood pressure reduction was -8.6/-3.9 mm Hg for persons with the TT genotype, -9.0/-5.2 mm Hg for the MT genotype, and -5.3/-1.0 mm Hg for the MM genotype. We conclude that the 235T allele of the angiotensinogen gene is associated with greater blood pressure decreases than the 235M allele after a sodium reduction intervention. The angiotensinogen gene accounts for some of the interindividual variation of the blood pressure response to sodium reduction.

Hustad S, Ueland PM, Vollset SE, et al. Riboflavin as a determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. *Clin Chem* 2000 Aug;46(8 Pt 1):1065-71.

BACKGROUND: Plasma total homocysteine (tHcy) is a risk factor for cardiovascular disease. tHcy concentrations are partly determined by folate, cobalamin, and vitamin B(6) status, and methylenetetrahydrofolate reductase (MTHFR) and other flavoenzymes are important for the biotransformation of these vitamins. This motivates the investigation of the possible relationship between riboflavin status and tHcy. METHODS: The study had a cross-sectional design and included 423 healthy blood donors, ages 19-69 years. We determined plasma tHcy, serum folate, serum cobalamin, serum creatinine, and MTHFR C677T genotype. In addition, we measured riboflavin and its two coenzyme forms, flavin mononucleotide and flavin adenine dinucleotide, in EDTA plasma by capillary electrophoresis and laser-induced fluorescence detection. RESULTS: Riboflavin determined tHcy independently in a multiple linear regression model with adjustment for sex, age, folate, cobalamin, creatinine, and MTHFR genotype (P = 0.008). tHcy was 1.4 micromol/L higher in the lowest compared with the highest riboflavin quartile. The riboflavin-tHcy relationship was modified by genotype (P = 0.004) and was essentially confined to subjects with the C677T transition of the MTHFR gene. CONCLUSIONS: Plasma riboflavin is an independent determinant of plasma tHcy. Studies on deficient populations are needed to evaluate the utility of riboflavin supplementation in hyperhomocysteinemia.

Jacob RA, Gretz DM, Taylor PC, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr* 1998 Jul;128(7):1204-12.

To determine the human folate requirement on the basis of changes in biochemical pathways, we studied the effect of controlled folate intakes on plasma homocysteine and lymphocyte DNA methylation and deoxynucleotide content in healthy postmenopausal women. Eight women (49-63 y of age) were housed in a metabolic unit and fed a low folate diet containing 56 microg/d of folate for 91 d. Folate intake was varied by supplementing 55-460 microg/d of folic acid (pteroylglutamic acid) to the diet to provide total folate intake periods of 5 wk at 56 microg/d, 4 wk at 111 microg/d and 3 wk at 286-516 microg/d. A subclinical folate deficiency with decreased plasma folate was created during the first two periods. This resulted in significantly elevated plasma homocysteine and urinary malondialdehyde, and lymphocyte DNA hypomethylation. The folate depletion also resulted in an increased ratio of

dUTP/dTTP in mitogen-stimulated lymphocyte DNA and decreased lymphocyte NAD, changes suggesting misincorporation of uracil into DNA and increased DNA repair activity. The DNA hypomethylation was reversed with 286-516 microg/d of folate repletion, whereas the elevated homocysteine decreased with 516 but not 286 microg/d of folate. The results indicate that marginal folate deficiency may alter DNA composition and that the current RDA of 180 microg/d may not be sufficient to maintain low plasma homocysteine concentrations of some postmenopausal women.

Kamboh MI. Apolipoprotein E polymorphism and susceptibility to Alzheimer's disease. *Hum Biol* 1995 Apr;67(2):195-215.

Apolipoprotein E (apoE, protein; APOE, gene) plays an important role in lipoprotein metabolism by acting as a ligand for two specific cell receptors to mediate the uptake of apoE-containing lipoproteins by cells. The APOE gene, located on chromosome 19, exhibits a genetic polymorphism with three common alleles, APOE*2, APOE*3, and APOE*4, which show marked variation in their distribution among various ethnic groups. APOE polymorphism has a profound effect on interindividual variation in plasma cholesterol level in the general population, and this effect has made the APOE gene one of the most recognized and appreciated genes today. In addition to its pivotal involvement in lipoprotein metabolism, apoE is thought to participate in seemingly unrelated metabolic pathways, including the normal development of the nervous system and peripheral nerve regeneration after injury. The most dramatic and unexpected finding in this regard was made in early 1993, when it was reported that the presence of the APOE*4 allele is a significant risk factor for the development of late-onset familial Alzheimer's disease, a debilitating brain disorder. Since then, a number of studies have confirmed this provocative association and also have found that the APOE*4 allele is a major risk factor for Alzheimer's disease regardless of age at onset or family history. ApoE appears to contribute directly to the pathogenesis of Alzheimer's disease because it has been immunochemically localized in three defining lesions of the disease (extracellular amyloid plaques, intracellular neurofibrillary tangles, and vascular amyloid deposits). In this article I review current data about the association between APOE polymorphism and Alzheimer's disease and possible physiological mechanisms behind this association.

Kidd PM. A review of nutrients and botanicals in the integrative management of cognitive dysfunction. *Altern Med Rev* 1999 Jun;4(3):144-61.

Dementias and other severe cognitive dysfunction states pose a daunting challenge to existing medical management strategies. An integrative, early intervention approach seems warranted. Whereas, allopathic treatment

options are highly limited, nutritional and botanical therapies are available which have proven degrees of efficacy and generally favorable benefit-to-risk profiles. This review covers five such therapies: phosphatidylserine (PS), acetyl-l-carnitine (ALC), vinpocetine, Ginkgo biloba extract (GbE), and Bacopa monniera (Bacopa). PS is a phospholipid enriched in the brain, validated through double-blind trials for improving memory, learning, concentration, word recall, and mood in middle-aged and elderly subjects with dementia or age-related cognitive decline. PS has an excellent benefit-to-risk profile. ALC is an energizer and metabolic cofactor which also benefits various cognitive functions in the middle-aged and elderly, but with a slightly less favorable benefit-to-risk profile. Vinpocetine, found in the lesser periwinkle Vinca minor, is an excellent vasodilator and cerebral metabolic enhancer with proven benefits for vascular-based cognitive dysfunction. Two meta-analyses of GbE demonstrate the best preparations offer limited benefits for vascular insufficiencies and even more limited benefits for Alzheimer's, while "commodity" GbE products offer little benefit, if any at all. GbE (and probably also vinpocetine) is incompatible with blood-thinning drugs. Bacopa is an Ayurvedic botanical with apparent anti-anxiety, anti-fatigue, and memory-strengthening effects. These five substances offer interesting contributions to a personalized approach for restoring cognitive function, perhaps eventually in conjunction with the judicious application of growth factors.

Kim YI, Pogribny IP, Basnakian AG, et al. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. *Am J Clin Nutr* 1997 Jan;65(1):46-52.

Folate is essential for the de novo biosynthesis of purines and thymidylate, and is an important mediator in the transfer of methyl groups for DNA methylation. Folate deficiency, therefore, could contribute to abnormal DNA integrity and methylation patterns. We investigated the effect of isolated folate deficiency in rats on DNA methylation and DNA strand breaks both at the genomic level and within specific sequences of the p53 tumor suppressor gene. Our data indicate that folate deficiency induces DNA strand breaks and hypomethylation within the p53 gene. Such alterations either did not occur or were chronologically delayed when examined on a genome-wide basis, indicating some selectivity for the exons examined within the p53 gene. Folate insufficiency has been implicated in the development of several human and experimental cancers, and aberrations within these regions of the p53 gene that were examined in this study are thought to play an integral role in carcinogenesis. The aforementioned molecular alterations may therefore be a means by which dietary folate deficiency enhances carcinogenesis.

Knudsen LE, Norppa H, et al. Chromosomal aberrations in humans induced by urban air pollution: influence of DNA repair and

polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. ***Cancer Epidemiol Biomarkers Prev*** **1999;8(4 Pt 1):303-10.**

We have studied the influence of individual susceptibility factors on the genotoxic effects of urban air pollution in 106 nonsmoking bus drivers and 101 postal workers in the Copenhagen metropolitan area. We used the frequency of chromosomal aberrations in peripheral blood lymphocytes as a biomarker of genotoxic damage and dimethylsulfate-induced unscheduled DNA synthesis in mononuclear WBCs, the glutathione S-transferase M1 (GSTM1) genotype, and the N-acetyltransferase 2 (NAT2) genotype as biomarkers of susceptibility. The bus drivers, who had previously been observed to have elevated levels of aromatic DNA adducts in their peripheral mononuclear cells, showed a significantly higher frequency of cells with chromosomal aberrations as compared with the postal workers. In the bus drivers, unscheduled DNA synthesis correlated negatively with the number of cells with gaps, indicating a protective effect of DNA repair toward chromosome damage. Bus drivers with the GSTM1 null and slow acetylator NAT2 genotype had an increased frequency of cells with chromosomal aberrations. NAT2 slow acetylators also showed elevated chromosomal aberration counts among the postal workers. Our results suggest that long-term exposure to urban air pollution (with traffic as the main contributor) induces chromosome damage in human somatic cells. Low DNA repair capacity and GSTM1 and NAT2 variants associated with reduced detoxification ability increase susceptibility to such damage. The effect of the GSTM1 genotype, which was observed only in the bus drivers, appears to be associated with air pollution, whereas the NAT2 genotype effect, which affected all subjects, may influence the individual response to some other common exposure or the baseline level of chromosomal aberrations.

Kuivenhoven JA, Jukema JW, Zwinderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. *N Engl J Med* 1998 Jan 8;338(2):86-93.

BACKGROUND: The high-density lipoprotein (HDL) cholesterol concentration is inversely related to the risk of coronary artery disease. The cholesteryl ester transfer protein (CETP) has a central role in the metabolism of this lipoprotein and may therefore alter the susceptibility to atherosclerosis. METHODS: The DNA of 807 men with angiographically documented coronary atherosclerosis was analyzed for the presence of a polymorphism in the gene coding for CETP. The presence of this DNA variation was referred to as B1, and its absence as B2. All patients participated in a cholesterol-lowering trial designed to induce the regression of coronary atherosclerosis and were randomly assigned to treatment with either pravastatin or placebo for two years. RESULTS: The B1 variant of the CETP gene was associated with both higher plasma CETP concentrations

(mean [+/-SD], 2.29+/-0.62 microg per milliliter for the B1B1 genotype vs. 1.76+/-0.51 microg per milliliter for the B2B2 genotype) and lower HDL cholesterol concentrations (34+/-8 vs. 39+/-10 mg per deciliter). In addition, we observed a significant dose-dependent association between this marker and the progression of coronary atherosclerosis in the placebo group (decrease in mean luminal diameter: 0.14+/-0.21 mm for the B1B1 genotype, 0.10+/-0.20 mm for the B1B2 genotype, and 0.05+/-0.22 mm for the B2B2 genotype). This association was abolished by pravastatin. Pravastatin therapy slowed the progression of coronary atherosclerosis in B1B1 carriers but not in B2B2 carriers (representing 16 percent of the patients taking pravastatin). CONCLUSIONS: There is a significant relation between variation at the CETP gene locus and the progression of coronary atherosclerosis that is independent of plasma HDL cholesterol levels and the activities of lipolytic plasma enzymes. This common DNA variant appears to predict whether men with coronary artery disease will benefit from treatment with pravastatin to delay the progression of coronary atherosclerosis.

LeGrand EK. An adaptationist view of apoptosis. *Q Rev Biol* 1997 Jun;72(2):135-47.

A cell's decision whether to undergo apoptosis (cell suicide) is examined here from an adaptationist perspective, rather than a mechanistic one. External and internal inputs to the cell's protein-based information processing network are used in making this decision, with the cell factoring in its replaceability. A system in which each cell takes primary responsibility for deciding its own fate has great adaptive value because it harnesses each cell's self-knowledge rather than waiting for external cues to be recognized by other cells. Cell self-destruction can be an important selective mechanism, potentially leading to better performance of tissues over time. However, reliance on cells to monitor themselves has a flaw, since cells may incur selfish mutations that impair their apoptotic responsibility. The tight control exerted over somatic cells serves to check selfish genes involved in neoplasia and viral infections. Germ cells appear to be similarly monitored, both by other germ cells and by supporting follicular or Sertoli cells, thus maintaining the advantages offered by an apoptotic system. The adaptationist approach views the limited replacement of neurons and cardiac myocytes as likely to have net survival value. The linkage of these cells into a network with their neighbors throughout a lifetime allows for a precisely functioning team of cells expected to compensate for gradual declines in individual cell functionality. Replacement of apoptotic cells with naive cells might decrease brain functionality and might risk upsetting the conduction of cardiac impulses. The evolutionary viewpoint lends itself to new hypotheses, but only the boldest speculator would have predicted a system in which cells are given primary responsibility for deciding whether to kill themselves when they deem it beneficial to the organism.

Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med* 2000 Jul 13;343(2):78-85.

BACKGROUND: The contribution of hereditary factors to the causation of sporadic cancer is unclear. Studies of twins make it possible to estimate the overall contribution of inherited genes to the development of malignant diseases. METHODS: We combined data on 44,788 pairs of twins listed in the Swedish, Danish, and Finnish twin registries in order to assess the risks of cancer at 28 anatomical sites for the twins of persons with cancer. Statistical modeling was used to estimate the relative importance of heritable and environmental factors in causing cancer at 11 of those sites. RESULTS: At least one cancer occurred in 10,803 persons among 9512 pairs of twins. An increased risk was found among the twins of affected persons for stomach, colorectal, lung, breast, and prostate cancer. Statistically significant effects of heritable factors were observed for prostate cancer (42 percent; 95 percent confidence interval, 29 to 50 percent), colorectal cancer (35 percent; 95 percent confidence interval, 10 to 48 percent), and breast cancer (27 percent; 95 percent confidence interval, 4 to 41 percent). CONCLUSIONS: Inherited genetic factors make a minor contribution to susceptibility to most types of neoplasms. This finding indicates that the environment has the principal role in causing sporadic cancer. The relatively large effect of heritability in cancer at a few sites suggests major gaps in our knowledge of the genetics of cancer.

Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 1988 Jun 30;318(26):1720-8.

Among 141 consecutive patients with neuro-psychiatric abnormalities due to cobalamin deficiency, we found that 40 (28 percent) had no anemia or macrocytosis. The hematocrit was normal in 34, the mean cell volume was normal in 25, and both tests were normal in 19. Characteristic features in such patients included paresthesia, sensory loss, ataxia, dementia, and psychiatric disorders; longstanding neurologic symptoms without anemia; normal white-cell and platelet counts and serum bilirubin and lactate dehydrogenase levels; and markedly elevated serum concentrations of methylmalonic acid and total homocysteine. Serum cobalamin levels were above 150 pmol per liter (200 pg per milliliter) in 2 patients, between 75 and 150 pmol per liter (100 and 200 pg per milliliter) in 16, and below 75 pmol per liter (100 pg per milliliter) in only 22. Except for one patient who died during the first week of treatment, every patient in this group benefited from cobalamin therapy. Responses included improvement in neuropsychiatric

abnormalities (39 of 39), improvement (often within the normal range) in one or more hematologic findings (36 of 39), and a decrease of more than 50 percent in levels of serum methylmalonic acid, total homocysteine, or both (31 of 31). We conclude that neuropsychiatric disorders due to cobalamin deficiency occur commonly in the absence of anemia or an elevated mean cell volume and that measurements of serum methylmalonic acid and total homocysteine both before and after treatment are useful in the diagnosis of these patients.

Lowe SW. Cancer therapy and p53. *Curr Opin Oncol* 1995 Nov;7(6):547-53.

Apoptosis is now recognized as an important process in tissue homeostasis. In malignancy, mutations in apoptotic programs may promote tumor progression as well as reduce the efficacy of cancer therapy. Recent studies identify the product of the p53 tumor-suppressor gene as an important regulator of apoptosis in tumor cells. At the same time, clinical studies implicate p53 mutations in pleiotropic resistance to cytotoxic cancer therapy. Together, these observations suggest that inactivation of p53 promotes resistance to anticancer agents by attenuating apoptosis. This view identifies p53 as a potential drug target and suggests several strategies for therapeutic intervention.

Malaveille C, Hautefeuille A, Pignatelli B, et al. Antimutagenic dietary phenolics as antigenotoxic substances in urothelium of smokers. *Mutat Res* 1998 Jun 18;402(1-2):219-24.

Human urine is known to contain substances that strongly inhibit bacterial mutagenicity of aromatic and heterocyclic amines in vitro. The biological relevance of these anti-mutagens was examined by comparing levels of tobacco-related DNA adducts in exfoliated urothelial cells from smokers with the anti-mutagenic activity in corresponding 24-h urine samples. An inverse relationship was found between the inhibition of PhIP-mutagenicity by urine extracts in vitro and two DNA adduct measurements: the level of the putatively identified ABP-dG adduct and the total level of all tobacco-smoke-related carcinogen adducts including those probably derived from PhIP. These substances appear to be dietary phenolics and/or their metabolites because (i) the anti-mutagenic activity of urine extracts (n=18) was linearly related to their content in phenolics; (ii) the concentration ranges of these substances in urine extracts were similar to those of various plant phenols (e.g., quercetin, isorhamnetin) for which an inhibitory effect on the liver S9-mediated mutagenicity of PhIP was obtained; (iii) treatment of urines with beta-glucuronidase and arylsulfatase enhanced both anti-mutagenicity and the levels of phenolics in urinary extracts; (iv) urinary extracts inhibited non-competitively the liver S9-mediated mutagenicity of PhIP as did quercetin, used as a model phenolics. Onion, lettuce, apples and red wine are important sources of dietary flavonoids which are probably responsible for the anti-mutagenicity associated with foods and beverages. After HPLC fractionation

of urinary extracts, the distribution profile of anti-mutagenic activity corresponded roughly to that of onion and wine extract combined. Overall, our study strongly suggests that smokers ingesting dietary phenolics, probably flavonoids, are partially protected against the harmful effects by tobacco carcinogens within their bladder mucosal cells.

Malaveille C, Hautefeuille A, Pignatelli B, et al. Dietary phenolics as anti-mutagens and inhibitors of tobacco-related DNA adduction in the urothelium of smokers. *Carcinogenesis* 1996 Oct;17(10):2193-2200.

Human urine is known to contain substances that strongly inhibit bacterial mutagenicity of aromatic and heterocyclic amines in vitro. The biological relevance of these anti-mutagens was examined by comparing levels of tobacco-related DNA adducts in exfoliated urothelial cells from smokers with the anti-mutagenic activity in corresponding 24-h urine samples. An inverse relationship was found between the inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-mutagenicity by urine extracts in vitro and two DNA adduct measurements: the level of the putatively identified N-(deoxyguanosine-8-yl)-4-aminobiphenyl adduct and the total level of all tobacco-smoke-related carcinogen adducts including those probably derived from PhIP. Urinary anti-mutagenicity in vitro appears thus to be a good indicator of the anti-genotoxicity exerted by substances excreted in urine, that protect the bladder mucosal cells (and possibly other cells) against DNA damage. These substances appear to be dietary phenolics and/or their metabolites because (i) the anti-mutagenic activity of urine extracts (n = 18) was linearly related to their content in phenolics; (ii) the concentration ranges of these substances in urine extracts were similar to those of various plant phenols (quercetin, isorhamnetin and naringenin) for which an inhibitory effect on the liver S9-mediated mutagenicity of PhIP was obtained; (iii) treatment of urines with beta-glucuronidase and arylsulfatase enhanced both anti-mutagenicity and the levels of phenolics in urinary extracts; (iv) urinary extracts inhibited noncompetitively the liver S9-mediated mutagenicity of PhIP as did quercetin, used as a model phenolics. Several structural features of the flavonoids were identified as necessary for the inhibition of PhIP and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxiline mutagenicity. Fractionation by reverse-phase HPLC and subsequent analysis of two urinary extracts, showed the presence of several anti-mutagenic substances and phenolics; more lipophilic phenolics displayed the highest specific inhibitory activity. This suggests that enzymatic conversion of dietary flavonoids into their more lipophilic and anti-mutagenic O-methylcatechol derivatives, as noted for quercetin, may occur in vivo in man. Onion, lettuce, apples and red wine are important sources of dietary flavonoids which are

probably responsible for the anti-mutagenicity associated with foods and beverages. After HPLC fractionation of urinary extracts, the distribution profile of anti-mutagenic activity corresponded roughly to that of onion and wine extract combined. Our study strongly suggests that smokers ingesting dietary phenolics, probably flavonoids, are partially protected against the harmful effects by tobacco carcinogens within their bladder mucosal cells. This protective effect of dietary phenolics against the cancer of the bladder (and possibly other sites) should be verified and explored as a part of a chemoprevention strategy.

McCully KS. Chemical pathology of homocysteine. I. Atherogenesis. *Ann Clin Lab Sci* 1993 Nov-Dec;23(6):477-93.

The atherogenic properties of homocysteine were discovered by observation of arteriosclerosis in children with homocystinuria caused by inherited deficiency of three different enzymes. Hyperhomocysteinemia is generally recognized as an independent risk factor for coronary, cerebral, and peripheral atherosclerosis. Hyperhomocysteinemia is caused by heterozygosity for homocystinuria, micronutrient deficiency from dietary imbalance, toxins, drugs, hormones, and other factors, explaining many key observations concerning the epidemiology of atherosclerosis. The etiological factors for atherosclerosis are believed to increase conversion of methionine to homocysteine thiolactone, the reactive cyclic internal lactone of homocysteine. The free amino groups of low density lipoprotein (LDL) are thiolated by homocysteine thiolactone, causing aggregation and increased uptake of LDL by macrophages, explaining lipid deposition in atheromas. Homocysteine thiolactone, released from homocysteinylated LDL within vascular wall, promotes intimal injury, oxidation of cholesterol and unsaturated lipids, platelet aggregation, thrombogenic factors, myointimal hyperplasia, deposition of sulfated glycosaminoglycans, fibrosis and calcification of atherosclerotic plaques.

McCully KS. Chemical pathology of homocysteine. II. Carcinogenesis and homocysteine thiolactone metabolism. *Ann Clin Lab Sci* 1994 Jan-Feb;24(1):27-59.

Abnormalities of methionine metabolism in malignancy include carcinogenicity of methionine deficiency, methionine auxotrophy of cultured malignant cells, deficient methylation of DNA, and aerobic glycolysis that is reversed by methionine. Cells from children with homocystinuria form an aggregated sulfated extracellular matrix and grow in a pattern similar to cultured malignant cells. Normal cells metabolize homocysteine thiolactone to sulfate, but malignant cells accumulate homocysteine thiolactone, which thiolates proteins and other cellular macromolecules. Thioretinamide, the amide of retinoic acid homocysteine thiolactone, and its cobalamin complex,

thioretinaco, are antineoplastic and chemopreventive against carcinogenesis. Deficiency of these compounds in malignant cells is believed to increase conversion of methionine to homocysteine thiolactone and thioco, its cobalamin complex. These compounds are believed to participate in oxidative phosphorylation by formation of thioretinaco ozonide disulfonium complexes that are the active sites of adenosine triphosphate (ATP) binding in mitochondrial membranes. Hypothetical deficiency of thioretinaco may explain important metabolic abnormalities of malignant cells.

McCully KS. Chemical pathology of homocysteine. III. Cellular function and aging. *Ann Clin Lab Sci* 1994 Mar-Apr;24(2):134-52.

The homocysteine thiolactonyl derivative, thioretinaco ozonide, is believed to function as an electron acceptor in oxygen metabolism and as the binding site for adenosine triphosphate (ATP) synthesis by mitochondria, preventing damage by free radical oxidants in resting cells. During cell division, methionine is converted to homocysteine thiolactone, converting thioretinaco to thioco, increasing free radical oxidants, and oxidizing cellular glutathione and ascorbate. Homocysteic acid has growth hormone activity and releases insulin-like growth factor in hypophysectomized rats, promoting oxidation of homocysteine thiolactone to sulfated glycosaminoglycans of cartilage. The free base of homocysteine thiolactone produces keratinization, squamous metaplasia, dysplasia, and carcinogenesis in normal mouse tissues. The efficiency of homocysteine thiolactone metabolism declines with aging, explaining decreased formation of adenosyl methionine in aging and suggesting loss of thioretinaco ozonide from membranes of aging cells. The effects of aging on enzyme activity, connective tissues, lipid synthesis, auto-immune diseases, atherogenesis and carcinogenesis are related to these changes in homocysteine metabolism.

McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 1984 May;37(1):179-83.

Transplantation of pronuclei between one-cell-stage embryos was used to construct diploid mouse embryos with two female pronuclei (biparental gynogenones) or two male pronuclei (biparental androgenones). The ability of these embryos to develop to term was compared with control nuclear-transplant embryos in which the male or the female pronucleus was replaced with an isoparental pronucleus from another embryo. The results show that diploid biparental gynogenetic and androgenetic embryos do not complete normal embryogenesis, whereas control nuclear transplant embryos do. We conclude that the maternal and paternal contributions to the embryonic genome in mammals are not equivalent and that a diploid genome derived from only one of the two parental sexes is incapable of supporting complete embryogenesis.

Miller AL. The etiologies, pathophysiology, and alternative/complementary treatment of asthma. *Altern Med Rev* 2001 Feb;6(1):20-47.

Technical Advisor, Thorne Research, Inc; Senior Editor, Alternative Medicine Review. Correspondence address: PO Box 25, Dover, ID 83825.
E-mail: alan@thorne.com

A chronic inflammatory disorder of the respiratory airways, asthma is characterized by bronchial airway inflammation resulting in increased mucus production and airway hyper-responsiveness. The resultant symptomatology includes episodes of wheezing, coughing, and shortness of breath. Asthma is a multifactorial disease process with genetic, allergic, environmental, infectious, emotional, and nutritional components. The underlying pathophysiology of asthma is airway inflammation. The underlying process driving and maintaining the asthmatic inflammatory process appears to be an abnormal or inadequately regulated CD4+ T-cell immune response. The T-helper 2 (Th2) subset produces cytokines including interleukin-4 (IL-4), IL-5, IL-6, IL-9, IL-10, and IL-13, which stimulate the growth, differentiation, and recruitment of mast cells, basophils, eosinophils, and B-cells, all of which are involved in humoral immunity, inflammation, and the allergic response. In asthma, this arm of the immune response is overactive, while Th1 activity, generally corresponding more to cell-mediated immunity, is dampened. It is not yet known why asthmatics have this out-of-balance immune activity, but genetics, viruses, fungi, heavy metals, nutrition, and pollution all can be contributors. A plant lipid preparation containing sterols and sterolins has been shown to dampen Th2 activity. Antioxidant nutrients, especially vitamins C and E, selenium, and zinc appear to be necessary in asthma treatment. Vitamins B6 and B12 also may be helpful. Omega-3 fatty acids from fish, the flavonoid quercetin, and botanicals Tylophora asthmatica, Boswellia serrata and Petasites hybridus address the inflammatory component. Physical modalities, including yoga, massage, biofeedback, acupuncture, and chiropractic can also be of help.

Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. *Kidney Int* 1999 Dec;56(6):2173-80.

Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. BACKGROUND: Most of the known actions of angiotensin II (Ang II) are mediated by the Ang II type 1 receptor (AGT1R). A noncoding polymorphism of the AGT1R gene has been described in which there is either an adenine (A) or cytosine (C) base at position 1166. The functional significance of this polymorphism is unknown, prompting us to

examine the relationship between this polymorphism and the systemic and renal responses to AGT1R blockade and subpressor Ang II infusion. METHODS: Sixty-six healthy Caucasian men and women, genotyped for the AGT1R polymorphism by polymerase chain reaction, were chosen to form two homogeneous groups: AA and AC/CC. Renal hemodynamic function was assessed with inulin and para-aminohippurate clearance before and after AGT1R receptor blockade with losartan and Ang II infusion. RESULTS: The mean values at baseline for glomerular filtration rate (GFR), renal plasma flow (ERPF), and renal blood flow (RBF) were significantly lower in the AC/CC group compared with the AA group. Losartan increased the GFR and decreased the mean arterial pressure (MAP) in the AC/CC group, but did not influence these parameters in the AA group. The aldosterone responses to losartan were blunted in the AA subgroup. During Ang II infusion, AC/CC subjects maintained GFR despite equivalent declines in RBF, suggesting an enhanced efferent arteriolar constrictive response. CONCLUSIONS: Taken together, these results suggest that there is a relationship between the AGT1R A1166-->C polymorphism and the humoral and renal hemodynamic responses to AGT1R blockade and to Ang II infusion in the sodium-replete state, and that the C allele is associated with enhanced intrarenal and peripheral Ang II activity. Further studies are required to determine the genetic locus for this effect.

Muhlbauer RC and Li F. Effect of Vegetables on Bone Metabolism. *Nature* 1999;401:343-344.

Onion consumption by rats has been shown to increase bone mass. In male rats fed 1 g of dry onion per day for 4 weeks, bone mineral content increased by 17.7%, mean cortical thickness increased by 14.8%, and the mineral density of trabecular bone increased by 13.5% relative to controls. In a rat model, it was shown that 14 vegetables eaten by humans can significantly inhibit bone resorption in the rat. A mixture of 500 mg each of onion and Italian parsley, and a mixture of lettuce, tomato, cucumber, arugula, onion, garlic, wild garlic, common parsley, Italian parsley and dill at 100 mg of each daily significantly inhibited bone resorption, which suggested an additive effect. There was no inhibition by soybeans at the same dose, or by foodstuffs of animal origin, and even milk powder had no significant effect, despite its 1.29% calcium content. The mean 20% inhibition by 1 g onion per day is slightly higher than the effect of calcitonin at doses used to treat postmenopausal osteoporosis. In ovariectomized rats, bone resorption was increased by 32% compared with sham-operated animals, but this was inhibited by onion at 30-1,500 mg/day in a dose-dependent fashion. The highest dose decreased resorption by 25%. Onions not only prevent bone resorption in male rats, but also in female rats in which bone resorption has been stimulated by estrogen withdrawal.

Onorato J, Merland N, Terral C, et al. Placebo-controlled double-blind food challenge in asthma. J *Allergy Clin Immunol* 1986 Dec;78(6):1139-46.

To determine the prevalence of food allergy as a cause of exacerbation of asthma, we studied 300 consecutive patients with asthma (7 months to 80 years of age) who attended a respiratory clinic. Each patient was screened for possible food allergy by means of a questionnaire and by skin prick tests with the six food allergens most common in our area. Patients with either a suggestive history and/or a positive prick test and/or RAST underwent double-blind food challenge with lyophilized food in capsules or food mixed in a broth to disguise its taste. Pulmonary function tests and symptoms were followed for 8 hours after each challenge. Of the 300 patients screened, only 25 had either a history or skin prick tests or RAST responses suggestive of food allergy. Twenty patients had interpretable food challenges. In these 20 patients, food challenge caused asthma in six and caused other symptoms (atopic dermatitis and gastrointestinal symptoms) in five. On rechallenge after pretreatment with disodium cromoglycate (300 mg 30 minutes before the food challenge), the asthmatic response was blocked in four of five subjects. The patients with asthma with food allergy were generally young, had a current or past history of atopic dermatitis, and high total serum IgE levels. Our findings confirm that food allergy can elicit asthma, but its incidence is low, even in the population attending a specialty clinic. Food elimination diets should not be prescribed for all patients reporting an adverse reaction to foods or having a positive skin prick test and/or RAST with food allergens. In patients with asthma caused by food allergy, disodium cromoglycate may be used to complement elimination diets.

Retz W, Gsell W, Munch G, et al. Free radicals in Alzheimer's disease. *J Neural Transm Suppl* 1998;54:221-36.

Alzheimer's disease is a neurodegenerative disorder comprising multisystem atrophies probably caused by multifactorial processes. The disease is characterized by typical neuropathology, impaired synaptic function and massive cell loss. The pathobiochemistry of this disorder involves oxidative stress, which accumulates free radicals leading to excessive lipid peroxidation and neuronal degeneration in certain brain regions. Moreover, radical induced disturbances of DNA, proteins and lipid membranes have been measured. The hypothesis has been proposed that cellular events involving oxidative stress may be one basic pathway leading to neurodegeneration in Alzheimer's disease. In this work we report evidence for increased oxidative stress and disturbed defense mechanisms in Alzheimer's disease, which may result in a self-propagating cascade of neurodegenerative events. Furthermore it is evident from experimental data, that aggregation of beta-amyloid and beta-amyloid toxicity is favourably caused by oxidative stress. Therefore, oxidative stress plays a key role in the conversion of soluble to

unsoluble beta-amyloid, suggesting that oxidative stress is primary to the beta-amyloid cascade.

Rice WR. Sexually antagonistic genes: experimental evidence. *Science* 1992 Jun 5;256(5062):1436-9.

When selection differs between the sexes, a mutation beneficial to one sex may be harmful to the other (sexually antagonistic). Because the sexes share a common gene pool, selection in one sex can interfere with the other's adaptive evolution. Theory predicts that sexually antagonistic mutations should accumulate in tight linkage with a new sex-determining gene, even when the harm to benefit ratio is high. Genetic markers and artificial selection were used to make a pair of autosomal genes segregate like a new pair of sex-determining genes in a Drosophila melanogaster model system. A 29-generation study provides experimental evidence that sexually antagonistic genes may be common in nature and will accumulate in response to a new sex-determining gene.

Richter C. Oxidative damage to mitochondrial DNA and its relationship to ageing. *Int J Biochem Cell Biol* 1995 Jul;27(7):647-53.

Mitochondria are the most important intracellular source of reactive oxygen species and are protected against them by enzymatic and nonenzymatic antioxidants. Nevertheless, mitochondrial DNA (mtDNA) is subject to severe oxidative damage, and much more so than nuclear DNA (nDNA). Damage is indicated by the detection of various base modifications, particularly 8-hydroxydeoxyguanosine (8OHdG), which can lead to point mutations because of mispairing. MtDNA is also fragmented to some extent. Conceivably, such fragmentation relates to the deletions found in mtDNA. Several hypotheses suggest that defective mitochondria contribute to, or are responsible for, ageing. Recent observations indicate that mitochondria in an old organism differ in many respects from those in a young organism. Thus, with ageing there is an increased production of reactive oxygen species, a decrease in certain antioxidants, a decreased transcription, translation, and cytochrome oxidase content, and an increase in the extent of DNA modifications. Major unresolved questions concerning the role of mtDNA changes in ageing are addressed: is there a causal relationship; what is the true extent of DNA damage; what are significance and functional consequences of mtDNA oxidation; are reactive oxygen species the cause of the DNA modifications found in vivo; what is the relationship between DNA damage and alterations of RNAs and proteins? Future studies promise to clarify the possible causal relationship between mitochondrial dysfunction, reactive oxygen species production, mtDNA modifications, and ageing.

Richter C. Reactive oxygen and DNA damage in mitochondria. *Mutat Res* 1992 Sep;275(3-6):249-55.

During the last decade the importance of reactive oxygen species as major contributors to various types of cancer, heart diseases, cataracts, Parkinson's and other degenerative diseases that come with age, and to natural aging has become apparent. Mitochondria are the most important intracellular source of reactive oxygen. Mitochondrial DNA is heavily damaged by reactive oxygen at the bases, as indicated by the high steady-state level of 8-hydroxydeoxyguanosine, the presence of which causes mispairing and point mutations. Mitochondrial DNA is also oxidatively fragmented to a certain extent. Conceivably, such fragmentation relates to deletions found in mitochondrial DNA. Point mutations and deletions have recently been shown to be etiologically linked to several human diseases and natural aging. Future studies should address the causal relationship between mitochondrial dysfunction, production of reactive oxygen species, and aging.

Richter C. Do mitochondrial DNA fragments promote cancer and aging? *FEBS Lett* 1988 Dec 5;241(1-2):1-5.

Reactive oxygen species are important in carcinogenesis, diseases, and aging, probably through oxidative damage of DNA. Our understanding of this relationship at the molecular level is very sketchy. It has recently been found that in mitochondria oxidative DNA damage is particularly high and may not be repaired efficiently. I propose that oxidatively generated DNA fragments escape from mitochondria and become integrated into the nuclear genome. This may transform cells to a cancerous state. Time-dependent nuclear accumulation of mitochondrial DNA fragments may progressively change the nuclear information content and thereby cause aging. This proposal can be tested experimentally.

Rosler M, Retz W, Thome J, Riederer P. Free radicals in Alzheimer's dementia: currently available therapeutic strategies. *J Neural Transm Suppl* 1998;54:211-9.

Substantial evidence now exists that oxidative stress may play an important role in the etiopathogenesis of DAT. The different sources of oxidative stress in DAT are suggesting several pharmacological opportunities for influencing the disease. It is possible to distinguish 2 major types of possible therapeutic agents according to their pharmacological point of attack. 1. Radical scavengers, agents directly interacting with free radicals. Candidates of this type are gingko biloba, vitamins A, C, E and estrogen. 2. Antioxidants, which are able to prevent or decrease the production of free radicals by use of specific neuropharmacological properties. Candidates are selegiline, a MAO-B inhibitor well established in the therapy of Parkinson's disease, and

tenilsetam, which is believed to be an AGE-inhibitor. Recent in vitro studies have demonstrated the efficacy of both types of therapeutic agents by preventing or delaying oxidative neural damage. Some clinical data exist regarding the antidementive properties particularly in terms of gingko biloba, selegiline and vitamin E. The efficacy studies about these compounds seem to indicate a promising future strategy in the therapy of DAT. But it is too early to draw definite conclusions since it is well known that all of our candidate substances do not act specifically as radical scavengers or antioxidants.

Scarr S. Developmental theories for the 1990s: development and individual differences. *Child Dev* 1992 Feb;63(1):1-19.

Understanding both typical human development and indivdual differences within the same theoretical framework has been difficult because the 2 orientations arise from different philosophical traditions. It is argued that an evolutionary perspective can unite the study of both species-typical development and individual variation. Research on determininants of development from many perspectives can be understood within an evolutionary framework in which organism and environment combine to produce development. Species-normal genes and environments and indidividual variations in genes and environments both affect personality, social, and intellectual development. These domains are used as examples to integrate theories of normal development and individual differences. Within the usual samples of European, North American, and developed Asian countries, the results of family and twin studies show that environments within the normal species range are crucial to normal development. Given a wide range of environmental opportunities and emotional supports, however, most children in these societies grow up to be individually different based on their individual genotypes. Understanding the ways in which genes and environments work together helps developmentalists to identify children in need of intervention and to tailor interventions to their particular needs.

Shields PG, Harris CC. Cancer risk and low-penetrance susceptibility genes in gene-environment interactions. J Clin Oncol 2000;18(11):2309-15.

PURPOSE: To provide a concise review for human cancer risk related to low-penetrance genes and their effects on environmental carcinogen exposure. METHODS: Citation of relevant and recent references for molecular epidemiology, focusing on lung cancer, ethical issues, and some clinical implications of recent molecular epidemiology studies. RESULTS: Low-penetrance genes contribute to cancer risk by augmenting the effects of carcinogen exposures. These exposures can be measured in the body through molecular dosimetry (ie, the amount of DNA damage), which reflects a

biologically effective dose. The examination of tumors and the mutations within tumor suppressor genes, such as p53, can provide etiologic clues for both exposure and susceptibility. Although many studies have focused on carcinogen metabolism and cancer risk, more recent studies are considering DNA repair. Also, we are learning that behavior, such as tobacco addiction, also may be genetically controlled. CONCLUSION: Sporadic cancers are caused by gene(n)-environment(n) interactions rather than a dominant effect by a specific gene, environmental exposure, or gene-environment interaction. New paradigms, where we categorize genes as caretaker or gatekeeper genes, will allow for new hypotheses to be tested and will require advanced methods of analysis. The goal of molecular epidemiology is to develop risk assessment models for individuals, but currently the most achievable goal will be population risk assessment and a better understanding of carcinogenesis.

Siffert W. Molecular genetics of G proteins and atherosclerosis risk. *Basic Res Cardiol* 2001 Nov;96(6):606-11.

Using a classical candidate gene approach, we have described a common C825T polymorphism in the gene GNB3 which encodes the ubiquitously expressed beta3 subunit of heterotrimeric G proteins. The 825T allele is associated with alternative splicing of the gene and the formation of a truncated but functionally active beta3 subunit which is referred to as Gbeta3s. Expression of the splice variant results in an enhanced G protein activation on stimulation of G protein-coupled receptors. Carriers of the 825T allele show an increased risk for hypertension and left ventricular hypertrophy. Homo- and heterozygous 825T allele carriers respond with a stronger decrease in blood pressure to therapy with a thiazide diuretic than homozygous 825C allele carriers. Moreover, 825T allele carriers appear to have an increased risk for obesity which appears sensible given the established role of G protein signaling in adipogenesis. The highest frequencies of the 825T allele are found in ethnicities with the highest lifestyle-dependent risk for obesity, e.g., black Africans and East Asians. This suggests that the 825T allele fulfills the criteria of a thrifty genotype.

Smithells RW, Sheppard S, Schorah CJ, et al. Possible prevention of neural-tube defects by periconceptional vitamin supplementation. *Lancet* 1980 Feb 16;1(8164):339-40.

Women who had previously given birth to one or more infants with a neural-tube defect (NTD) were recruited into a trial of periconceptional multivitamin supplementation. 1 of 178 infants/fetuses of fully supplemented mothers (0.6%) had an NTD, compared with 13 of 260 infants/fetuses of unsupplemented mothers (5.0%).

Suganuma M, Sueoka E, Sueoka N, Okabe S, Fujiki H. Mechanisms of cancer prevention by tea polyphenols based on inhibition of TNF-alpha expression. *Biofactors* 2000;13(1-4):67-72.

Among various biochemical and biological activities of tea polyphenols, we believe inhibition of the expression and release of tumor necrosis factor-alpha (TNF-alpha) is crucial, since our study with TNF-alpha-deficient mice has revealed that TNF-alpha is an essential factor in tumor promotion. We found that EGCG dose-dependently inhibited AP-1 and NF-kappaB activation in BALB/3T3 cells treated with okadaic acid, resulting in inhibition of TNF-alpha gene expression. Furthermore, treatment with 0.1% green tea extract in drinking water reduced TNF-alpha gene expression as well as TNF-alpha protein level in the lung of TNF-alpha transgenic mice; and IL-1beta and IL-10 gene expression in the lung was also inhibited by treatment with green tea extract, indicating that green tea inhibits both TNF-alpha and the cytokines induced by TNF-alpha in organs. We recently found synergistic effects of EGCG and cancer preventive agents such as tamoxifen and sulindac, on cancer preventive activity. Taken together, the results show that green tea is efficacious as a non-toxic cancer preventive for humans.

Swain A, Narvaez V, Burgoyne P, Camerino G, Lovell-Badge R. Dax1 antagonizes Sry action in mammalian sex determination. *Nature* 1998 Feb 19;391(6669):761-7.

DAX1, which encodes an unusual member of the nuclear hormone-receptor superfamily, is a gene that may be responsible for a sex-reversal syndrome in humans, referred to as dosage-sensitive sex reversal, in which XY individuals carrying duplications of Xp21, part of the small arm of the X chromosome, develop as females. XY mice carrying extra copies of mouse Dax1 as a transgene show delayed testis development when the gene is expressed at high levels, but do not normally show sex reversal. Complete sex reversal occurs, however, when the transgene is tested against weak alleles of the sex-determining Y-chromosome gene Sry. These results show that DAX1 is largely, if not solely, responsible for dosage-sensitive sex reversal and provide a model for early events in mammalian sex determination, when precise levels and timing of gene expression are critical.

Taioli E, Zocchetti C, Garte S. Models of interaction between metabolic genes and environmental exposure in cancer susceptibility. *Environ Health Perspect* 1998 Feb;106(2):67-70.

Polymorphic metabolic genes that confer enhanced genetic susceptibility to the carcinogenic effects of certain environmental carcinogens act according to a type 2 interaction between genetic and environmental risk factors. This type of interaction, for which the gene has no effect on disease outcome by

itself but only modifies the risk associated with exposure, must be treated differently from other types of gene-environment interaction. We present a method to analyze different dose effects often seen in studies involving these genes. We define a low exposure-gene effect, when a greater degree of gene environment interaction appears at lower doses of exposure (the interaction follows an inverse dose function), and a converse high exposure-gene effect, when the interaction increases as a function of dose. Using a standard logistic regression model, we define a new term, alpha, that can be determined as a function of exposure dose in order to analyze epidemiological studies for the type of exposure-gene effect. These models are illustrated by the use of hypothetical case-control data as well as examples from the literature.

Taylor JA, Umbach DM, et al. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res* 1998;58(16):3603-10.

Arylamines are known bladder carcinogens and are an important constituent of tobacco smoke. The handling of arylamines in the body is complex and includes metabolism by NAT1 and NAT2, enzymes that play a role in both activation and detoxification of arylamines and their congeners. Both NAT1 and NAT2 are polymorphic, with alleles that have been shown to correlate with higher or lower enzyme activity. To explore the combined role of these genes and exposure on bladder cancer risk, we examined the NAT1 and NAT2 genotype in a case-control study of bladder cancer in which detailed exposure histories were available on all 230 cases and 203 frequency-matched controls. Using PCR-RFLP genotyping, we determined NAT2 genotype for the five most common alleles, NAT2*4, NAT2*5, NAT2*6, NAT2*7, NAT2*14 (frequently referred to as WT, M1, M2, M3, and M4, respectively). Similarly, the NAT1 genotype was determined for the four most common alleles NAT1*3, NAT1*4, and NAT1*11, and the putative high-activity allele, NAT1*10. No association between NAT2 genotype and bladder cancer risk was found whether genotype was considered alone or in combination with smoking, in either stratified or logistic regression analysis that adjusted for age, sex, and race. Stratified and logistic regression analysis both demonstrated an increased risk for individuals carrying the NAT1*10 allele among smokers. There was evidence of a gene-dosage effect, such that those who were homozygous for the NAT1*10 allele had the highest risks. There was also evidence of a statistically significant gene-environment interaction, such that bladder cancer risk depends on both NAT1 genotype and smoking exposure. Interestingly, although NAT2 genotype did not influence risk either alone or in combination with smoking exposure, there was evidence of a statistically significant gene-gene-environment three-way interaction. Bladder cancer risk from smoking exposure is particularly high in those who inherit NAT2 slow alleles in combination with one or two copies of the NAT1*10 allele. A biological mechanism for this finding is suggested.

Ventura P, Panini R, Verlato C, et al. Hyperhomocysteinemia and related factors in 600 hospitalized elderly subjects. *Metabolism* 2001 Dec;50(12):1466-71.

Hyperhomocysteinemia (HHcy) is a metabolic disorder frequently occurring in the elderly population. Recently several reports have suggested abnormalities in homocysteine (tHcy) metabolism implicating HHcy as a metabolic link in the multifactorial processes characterizing many geriatric illnesses-with special emphasis on atherosclerotic vascular diseases and cognitive impairment. The present study was undertaken in a large sample of elderly hospitalized subjects to determine (1) the prevalence of HHcy, (2) the association of HHcy with vascular and cognitive disorders, and (3) the factors independently predicting Hhcy. Six hundred elderly subjects (264 men and 336 women; mean age, 79 +/- 9 years) were randomly chosen from those admitted as inpatients over a period of 3 years. In all patients, body mass index (BMI), mid-upper arm muscle area (MUAMA), plasma cholesterol, triglycerides, total proteins, albumin, lymphocyte count, creatinine, homocysteine (fasting and 4 hours after methionine oral load), serum vitamin B(6), vitamin B(12), and folate concentrations were measured. The presence of disease or use of medications known to affect homocysteine plasma levels were also recorded. The mean fasting tHcy level was 16.8 +/- 12 micromol/L in the whole sample, 18.18 +/- 13.25 micromol/L in men, and 15.86 +/- 12.14 micromol/L in women (P =.005 men v women). The mean Hcy level 4 hours after methionine load was 37.95 +/- 20.9 in the whole sample. Prevalence of hyperhomocysteinemia (fasting Hcy > or = 15 micromol/L or 4 hours after methionine load > or = 35 micromol/L) was 61% (365/600) (67% in men and 56% in women, P <.05). HHcy was rarely (8%) an isolated disorder; in addition to diabetes (20%), renal failure (48.2%), and malnutrition (20.2%), it was often associated with heart failure (30%), malignancies (20.5%), and the use of diuretics (56%) and anticonvulsant drugs (13%). Plasma homocysteine progressively increases across subjects from those with no diabetes, malnutrition, renal failure, obesity, inflammatory bowel disease, heart failure to those with 1, 2, or more concurrent diseases. Multiple stepwise regression analysis showed that 72% of plasma total fasting tHcy variability was explained by age, serum folate, plasma albumin, use of diuretics, and renal function (measured as plasma creatinine clearance). In conclusion, the present study documents that hyperhomocysteinemia, in elderly hospitalized patients is (1) a common finding, (2) frequently associated with vascular and cognitive disorders, and (3) probably a secondary phenomenon in most cases. The major predictor of high plasma homocysteine levels were age, serum folate, plasma albumin, plasma creatinine clearance, and use of diuretic drugs. These variables explain a large proportion of plasma Hcy variability.

Wallace-Brodeur RR, Lowe SW. Clinical implications of p53 mutations. *Cell Mol Life Sci* 1999 Jan;55(1):64-75.

The ultimate goal of basic cancer research is to provide a theoretical foundation for rational approaches to improve cancer therapy. Our extensive insight into the biology of the p53 tumour suppressor and the clinical behaviour of tumours harbouring p53 mutations indicates that information concerning p53 will be useful in diagnosis and prognosis, and may ultimately produce new therapeutic strategies. At the same time, efforts to understand the clinical implications of p53 mutations have revealed conceptual and technical limitations in translating basic biology to the clinic. The lessons learned from p53 may lay the groundwork for future efforts to synthesize cancer gene function, cancer genetics and cancer therapy.

Wan H, Winton HL, Soeller C, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999 Jul;104(1):123-33.

House dust mite (HDM) allergens are important factors in the increasing prevalence of asthma. The lung epithelium forms a barrier that allergens must cross before they can cause sensitization. However, the mechanisms involved are unknown. Here we show that the cysteine proteinase allergen Der p 1 from fecal pellets of the HDM Dermatophagoides pteronyssinus causes disruption of intercellular tight junctions (TJs), which are the principal components of the epithelial paracellular permeability barrier. In confluent airway epithelial cells, Der p 1 led to cleavage of the TJ adhesion protein occludin. Cleavage was attenuated by antipain, but not by inhibitors of serine, aspartic, or matrix metalloproteinases. Putative Der p 1 cleavage sites were found in peptides from an extracellular domain of occludin and in the TJ adhesion protein claudin-1. TJ breakdown nonspecifically increased epithelial permeability, allowing Der p 1 to cross the epithelial barrier. Thus, transepithelial movement of Der p 1 to dendritic antigen-presenting cells via the paracellular pathway may be promoted by the allergen's own proteolytic activity. These results suggest that opening of TJs by environmental proteinases may be the initial step in the development of asthma to a variety of allergens.

Wedekind C, Furi S. Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proc R Soc Lond B Biol Sci* 1997 Oct 22;264(1387):1471-9.

The major histocompatibility complex (MHC) is an immunologically important group of genes that appears to be under natural as well as sexual selection. Several hypotheses suggest that certain MHC-allele combinations (usually heterozygous ones) are superior under selective pressure by

pathogens. This could influence mate choice in a way that preferences function to create MHC-heterozygous offspring, or that they function to create specific allele combinations that are beneficial under the current environmental conditions through their complementary or epistatic effects. To test these hypotheses, we asked 121 men and women to score the odours of six T-shirts, worn by two women and four men. Their scorings of pleasantness correlated negatively with the degree of MHC similarity between smeller and T-shirt-wearer in men and women who were not using the contraceptive pill (but not in Pill-users). Depending on the T-shirt-wearer, the amount of variance in the scorings of odour pleasantness that was explained by the degree of MHC similarity (r2) varied between nearly 0 and 23%. There was no apparent effect of gender in this correlation: the highest r2 was actually reached with one of the male odours sniffed by male smellers. Men and women who were reminded of their own mate/ex-mate when sniffing a T-shirt had significantly fewer MHC-alleles in common with this T-shirt-wearer than expected by chance. This suggests that the MHC or linked genes influence human mate choice. We found no significant effect when we tested for an influence of the MHC on odour preferences after the degree of similarity between T-shirt-wearer and smeller was statistically controlled for. This suggests that in our study populations the MHC influences body odour preferences mainly, if not exclusively, by the degree of similarity or dissimilarity. The observed preferences would increase heterozygosity in the progeny. They do not seem to aim for more specific MHC combinations

Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. *Nature* 1990 May 17;345(6272):229-33.

The histo-blood group ABO, the major human alloantigen system, involves three carbohydrate antigens (ABH). A, B and AB individuals express glycosyltransferase activities converting the H antigen into A or B antigens, whereas O(H) individuals lack such activity. Here we present a molecular basis for the ABO genotypes. The A and B genes differ in a few single-base substitutions, changing four amino-acid residues that may cause differences in A and B transferase specificity. A critical single-base deletion was found in the O gene, which results in an entirely different, inactive protein incapable of modifying the H antigen.

Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. *J Natl Cancer Inst* 1993 Nov 17;85(22):1819-27.

BACKGROUND: Breast cancer incidence rates have historically been 4-7 times higher in the United States than in China or Japan, although the reasons remain elusive. When Chinese, Japanese, or Filipino women migrate to the United States, breast cancer risk rises over several generations and approaches that among U.S. Whites. PURPOSE: Our objective was to quantify breast cancer risks associated with the various migration patterns of Asian-American women. METHODS: A population-based, case-control study of breast cancer among women of Chinese, Japanese, and Filipino ethnicities, aged 20-55 years, was conducted during 1983-1987 in San Francisco-Oakland, California, Los Angeles, California, and Oahu, Hawaii. We successfully interviewed 597 case subjects (70% of those eligible) and 966 control subjects (75%). RESULTS: A sixfold gradient in breast cancer risk by migration patterns was observed. Asian-American women born in the West had a breast cancer risk 60% higher than Asian-American women born in the East. Among those born in the West, risk was determined by whether their grandparents, especially grandmothers, were born in the East or the West. Asian-American women with three or four grandparents born in the West had a risk 50% higher than those with all grandparents born in the East. Among the Asian-American women born in the East, breast cancer risk was determined by whether their communities prior to migration were rural or urban and by the number of years subsequently lived in the West. Migrants from urban areas had a risk 30% higher than migrants from rural areas. Migrants who had lived in the West for a decade or longer had a risk 80% higher than more recent migrants. Risk was unrelated to age at migration for women migrating at ages less than 36 years. Ethnic-specific incidence rates of breast cancer in the migrating generation were clearly elevated above those in the countries of origin, while rates in Asian-Americans born in the West approximated the U.S. White rate. CONCLUSIONS: Exposure to Western lifestyles had a substantial impact on breast cancer risk in Asian migrants to the United States during their lifetime. There was no direct evidence of an especially susceptible period, during either menarche or early reproductive life. IMPLICATIONS: Because heterogeneity in breast cancer risk in these ethnic populations is similar to that in international comparisons and because analytic epidemiologic studies offer the opportunity to disentangle correlated exposures, this study should provide new insights into the etiology of breast cancer.

Glossary

ACE

Glossary

Allele: one of several forms of the same gene; e.g. smooth peas and wrinkled peas. If the two alleles are identical, the organism is said to be homozygous. If the alleles are different, the organism is said to be heterozygous.

Antisense: the strand of DNA that does NOT code for messenger RNA.

Antisense Therapy: gene therapy that stops a faulty gene from producing its defective protein.

Base: a subunit of DNA or RNA. There are four bases in DNA: adenine (A) which will only pair with thymine (T), and cytosine (C) which will only pair with guanine (G). RNA also has four bases but uracil (U) replaces thymine.

Chimera: a transgenic organism.

Chromatin: the DNA-histone protein complex that makes up chromosomes.

Chromosome: *(Gr: "colored body")* the rod-like structures containing DNA found in the cell nucleus. Occur in pairs in somatic cells, one coming from each parent.

Clone: two or more organisms with exactly the same DNA. Identical twins are naturally occurring clones.

Codon: three letter "words" that code for specific amino acids. There are also start and stop codons that begin and end translation of proteins.

Cytokine: polypeptide signal molecules , or "immunotransmitters", that initiate and regulate immune and inflammatory responses.

DNA: deoxyribonucleic acid.

DNA Probes: short sequences of nucleotides with radioactive tags. They will bind to complimentary sections of DNA. Used in DNA testing.

Dominant: a trait is dominant of it appears in the next generation *(phenotype)* regardless of the genotype of the other, paired gene. Compare recessive.

Exon: the portion of a gene that codes for proteins. Compare intron.

Gene: the basic units of heredity; discrete sequences of DNA that code for specific proteins.

Genetic Engineering: the process and science of splicing one organism's genes into another organism's genome.

Genetics: the scientific study of heredity.

Genome: the totality of the DNA in an organism.

Genomics: the study of genomes, including genome mapping, gene sequencing, determination of gene function, and the identification of polymorphisms and their physiologic effects.

Genotype: the actual genetic composition of an individual organism or its genes. Compare phenotype.

Ghost Gene: a DNA sequence that used to be a gene and code for a protein but whose sequence has changed enough that it is no longer a coding gene.

Heredity: the way traits or characters are passed from one generation to the next.

Heterozygous: having two different alleles at a single gene locus.

Homozygous: having two identical alleles at a single gene locus.

Imprinting: determination of the expression of a gene depending on parental origin of that gene. E.g., the frontal cortex develops from the mother's genes while the placenta and the hippocampus develop from the father's genes. Praeder-Willi syndrome and Angelman's syndrome are examples of imprinted genetic diseases. In the former, a section of the paternal chromosome 15 is missing; in the latter the same segment is missing from the maternal chromosome.

Invader® Technology: a proprietary methodology using linear signal amplification and a fluorescent signal to identify polymorphisms in genes. It is distinct from PCR that uses exponential amplification of the gene to be identified, rather than a linear amplification of a signal.

Intron: the portion of a gene that does not code for proteins and is removed post-transcriptionally to form messenger RNA; a type of junk DNA. Compare exon.

Junk DNA: any DNA that does not code for proteins. More than 97% of the human genome is junk DNA.

Ligase: an enzyme that stitches together two sections of DNA or RNA, acting like molecular "glue".

Meiosis: the process of duplicating DNA and subsequent division so that the resulting germ cells contain the haploid number of chromosomes *(half the number found in a somatic cell).*

Mendelian Trait or Heredity: any trait determined by a single gene, independently assorted, and either recessive or dominant.

Messenger RNA: mRNA codes off the sense strand of DNA and transports the genetic message from the cell nucleus to the ribosome, where it codes for protein synthesis. mRNA is made in the process known as transcription. The synthesis of a protein from mRNA is known as translation.

Mitosis: the process of duplicating DNA during somatic cell division. When divided, each daughter cell has an exact copy of the original cell's DNA.

Mutation: any change in the DNA code.

Natural Selection: Theory first proposed by Charles Darwin: living creatures that are better fitted to a specific environment are more likely to survive and therefore to pass their genes on to subsequent generations. Similarly, those not well suited to an environment are less likely to survive and pass on their genetic information. Over time, this leads to polymorphisms, species-wide change, and eventually to the emergence of new species *(evolution).*

Nucleoside: a glycoside composed of a base (A, C, G, T, or U) and a ribose sugar (deoxyribose or ribose).

Nucleotide: the structural "building blocks" of DNA and RNA; one of five possible bases: adenine, cytosine, guanine, thymine, and uracil.

Oncogene: a transformed gene that promotes uncontrolled cell growth and proliferation. Involved in the pathophysiology of cancer. Compare tumor-suppressor gene.

PCR: see Polymerase Chain Reaction.

Penetrance: the frequency with which a genotype expresses itself as a particular phenotype, or, the degree to which a person having a gene will express that gene *(e.g., disease).*

Phenotype: the physical manifestation or observable appearance of an organism or trait; usually the result of genetic and environmental influences in combination.

Point Mutation: any mutation in which a single nucleotide of the DNA sequence is changed.

Polygenic Disease: resulting from the interaction of more than one gene.

Polymerase: an enzyme that copies DNA.

Polymerase Chain Reaction: a method of exponential gene amplification used in forensic medicine and other areas involving 3 steps basic steps that are repeated until adequate quantities of the gene have been copied: **1)** denaturing, **2)** annealing of primers, and **3)** DNA synthesis.

Polymorphism: two or more variants (alleles, phenotypes, genotypes, etc.) at significant frequencies in the population. By convention, a polymorphism must be present with a frequency >1% of the population.

Population Genetics: the study of the hereditary makeup of different groups within the population.

Primer: a short sequence of nucleotides that starts the process of copying DNA. The primer is necessary to start the action of polymerase.

Promoter: a region of DNA in front of a gene that promotes the expression of that gene. Promoters function something like an on/off switch.

Recessive: a trait or character is recessive if its expression is masked by the presence of a dominant allele. Two recessives genotypes must be present for the trait to express itself as phenotype.

Restriction Enzyme: bacterial enzymes that cut DNA at specific nucleotide sequences. Bacteria use restriction enzymes to protect against viral infection. Scientists use restriction enzymes for RFLP analysis of genomes, and with a ligase for genetic engineering and DNA splicing.

Ribosome: cell organelle made of RNA and protein that translates messenger RNA into proteins: protein synthesis.

RFLP: restriction fragment length polymorphism analysis: a method of DNA analysis using restriction enzymes to cleave the DNA into different fragment lengths and then using Southern Blot analysis or PCR analysis to identify polymorphisms.

RNA: ribonucleic acid.

Sense: the sense strand of DNA is the one that codes for messenger RNA; the other DNA strand is known as antisense.

Silent Mutation: a mutation that occurs in a gene but that has no affect on the outcome of the protein coded for by that gene. Silent mutations can occur in an intron fragment, or a codon change may still code for the same amino acid.

Single Nucleotide Polymorphism: the mutation of a single nucleotide in a gene. Acronym: SNP *(pronounced "snip").*

SNP: Single Nucleotide Polymorphism.

Spacer DNA: non-coding DNA that occurs between separate genes on a chromosome.

SRY: Sex-determining Y gene plays a crucial role in sexual differentiation and determination of males

Tandem Repeat: repeated sequences of non-coding DNA, like a genetic stutter. The repeat patterns for individuals are unique, allowing for forensic identification of individual DNA samples.

Transcription: the process whereby a copy of mRNA is made from the sense strand of DNA in the cell nucleus.

Transgenic: an organism *(plant., animal, bacteria, yeast, etc.)* possessing one or more genes from another organism spliced into its genome through genetic engineering.

Translation: the process whereby proteins are synthesized in the ribosome.

Transport RNA: tRNA are small segments of RNA that attach to amino acids in the cytosol ad then present the amino acid for use in protein synthesis in the ribosome.

Transposon: a gene that can "jump" from locus to locus on a chromosome, or even between chromosomes. Discovered by Barbara McClintock and was a critical component for the selfish-gene theory. These mobile genetic elements, or "jumping genes", account for ~10% of spontaneous mutations in mice but only 1 in 700 in humans.

Tumor-Suppressor Gene: genes which promote normal cellular differentiation and proliferation. Includes gate-keeper genes which prevent a cell from entering into the division cycle until conditions are appropriate and caretaker genes which maintain the integrity of the cellular DNA.

Wild Type: homozygous "normal" *(non-mutant)* for a particular trait or gene, or, the most prevalent variant of a polymorphism.

BioEthics

ACE

authorbio

Philip L. Bereano, Ph.D., J.D., is a professor in the College of Engineering, Department of Technical Communication, University of Washington, Seattle.

human genome: genetic information and privacy

Does Genetic Research Threaten Our Civil Liberties?

By Philip Bereano, Ph.D., J.D.

An actionbioscience.org original article

article highlights

Mapping the human genome may lead to new medical breakthroughs; however, it may also lead to:

- *an individual's loss of privacy*
- *discrimination by class or genetic profile*
- *genetic enhancement of select individuals or populations*

August 2000

Does Genetic Research Threaten Our Civil Liberties?

By Philip Bereano, Ph.D., J.D.

> *The Human Gene Project at the National Institutes of Health, also being supported in universities all across America, will one day in the not-too-distant future enable every set of parents that has a little baby to get a map of the genetic structure of their child. So if their child has a predisposition to a certain kind of illness or a certain kind of problem, or even to heart disease or stroke in the early 40's, they will be able to plan that child's life, that child's upbringing, to minimize the possibility of the child developing that illness or that predisposition, to organize the diet plan, the exercise plan, the medical treatment that would enable untold numbers of people to have far more full lives than would have been the case before ...*[1]
>
> *President Bill Clinton*

However, the confluence of a number of technical and social trends has greatly enhanced the capacity for using genetic techniques for surveillance and tracking:

The goal of The Human Genome Project is to identify every human gene.

- The science of genetics is a flourishing new industry, nourished in large part by the federally funded Human Genome Project. The goal of this ambitious research endeavor is to identify every gene found in the human body, perhaps 100,000 in all. Several months ago, the US government and a private corporation announced that they had "completed" the "map" of the genome, although actually there are still many gaps. Much related research focuses on genetic diagnostics -- tests designed to identify genes thought to be associated with various medical conditions. More than 50 new genetic tests have been identified in the past five years alone.

- The increasing speed, sophistication, affordability, and interconnectivity of computer systems allows the rapid monitoring and matching of many millions of records. A 1994 benchmark study by the ACLU found that "concerns about personal privacy run deep among the American people."[2]
- The promotion of an ideology of geneticization fosters the belief that genes are determinative of an individual's behavior, character, and future.
- Capitalist economic relations have created a scramble for venture capital, the altering of patent laws, and calls for mass genetic testing by researchers who trade on the old image of the altruistic scientist to mask their conflicts of interest in testing labs, patents, consulting contracts, etc.

Genes have become a business commodity.

Our technological society

New technology does not benefit everyone equally.

Technologies are not value-neutral; they usually embody the perspectives, purposes, and political objectives of powerful social groups. The dominant ideology in Western society proclaims that science and technology are value-neutral, and the only problems caused by technologies are either "externalities" (unintended side effects) or abuses. However, because technologies are the result of human interventions into the otherwise natural progression of activities (and not acts of God or of nature), they are themselves actually imbued with human intentions and purposes. Current technologies do not equally benefit all segments of society (and indeed are not intended to do so), although to maximize public support for these developments and to minimize potential opposition, their proponents rarely acknowledge these distributional ramifications.

Because it needs large capital, new technology is influenced by the rich and powerful.

The United States is a society in which the differential access to wealth and power has been exacerbated during recent years. Thus, those people with more power can determine the kinds of technological developments that are researched and implemented. Because of their size, scale, and requirements for capital investments and for knowledge, modern technologies are powerful interventions into the natural order. They tend to be the mechanisms by which already powerful groups extend, manifest, and further consolidate their powers. Thus, technologies themselves are not neutral; they are social and political phenomena. Genetic technologies and computerization exhibit these characteristics, and reflect power differentials in our society.

Genetic enhancement of individuals or races (eugenics) is possible.

The resulting milieu of technological triumphalism appears to offer omniscience -- capabilities of enhanced surveillance and control over people and events, as well as promises of perfectionism (thus leading to both a loss of privacy and increased opportunities for discrimination by powerful entities). Predictability will replace a tolerance for natural variation and diversity. Powerful scientists have already called for programs of eugenics, labeled as "genetic enhancement" to create a less distasteful package.[3]

Loss of privacy

Genetic privacy is as real a concern as

Genetic privacy, like medical privacy in general, involves notions of the dignity and integrity of the individual. Is data accurate; can individuals access their own files; can the donor correct inaccurate data; are the

medical privacy.

custodians faithful and are technical security systems protecting the data where possible; does the individual have control over which third parties are allowed access, and under what conditions?

The US Department of Defense will not bar third-party use of employee DNA samples.

- The US Department of Defense insists on taking DNA samples from all its personnel, ostensibly for identification of those killed in action and body parts from military accidents -- despite the fact that the samples are to be kept for 50 years (long after people have left active duty), the program includes civilian employees, the agency refuses to issue regulations barring all third party use, and the Department will not accept waivers from the next of kin of subjects not wanting to donate tissues.

FBI criminal data includes DNA collected from the convicted as well as the accused.

- The FBI has been promoting the genetic screening of criminals to establish state DNA identification data banks to be used in criminal investigations; indeed, Federal legislation penalizes states fiscally if they don't participate, and now all do. Yet the data includes samples from those whose crimes have low recidivism rates or don't leave tissue samples; in some states people merely accused are forced into the program, and in others there are politicians calling for an expansion along these lines, despite the Constitutional presumption of innocence.

Infant blood tests are stored in databases.

- Infant blood samples, from the heel-sticks used to determine blood type and test for PKU, are stored as "Guthrie blots." California alone has more than seven million in its repository.

An individual's rights should include informed consent to genetic screening.

The American Civil Liberties Union advocates that "the decision to undergo genetic screening is purely personal" and it should not be "subject to control or compulsion by third parties" or the government. And "where a person has intentionally undergone genetic screening procedures there must be no disclosure of findings to third parties without the express and informed consent of the subject given after the results of the screening are made known to the subject and upon such times and conditions as the subject may require..."[4]

Yet patients' records "are commodities for sale," in the words of the New York Times a few years ago,[5] and a panel of the US National Research Council has warned that the computerized medical records of millions of citizens are open to misuse and abuse.[6]

Genetic discrimination

People have been denied health insurance because of genetic screening.

Genetic discrimination is the other major civil liberty threatened by genetics research. Scientists working with the Council for Responsible Genetics have documented hundreds of cases where healthy people have been denied insurance or employment based on genetic "predictions."[7] Of course, relatively few genetic diseases are deterministic; most tests (which have inherent limits themselves) cannot tell us if a genetic mutation will become manifest; if it does do so, it cannot tell us when in life this will occur; and if it happens, how severe the condition will be. In addition, many genetic conditions can be controlled or treated by interventions and environmental changes; that is why governments mandate testing newborns for PKU.

The growth of the mania for testing in the US is a manifestation of class relationships, through new technological possibilities: employers test employees, insurance companies and health organizations test patients, college officials test students, legislators pass bills to test a variety of

disempowered groups (welfare recipients, prisoners, immigrants and the like). Such indignities are never foisted upon the ruling class by the masses.

Examples of such discrimination include:

An HMO stated it would pay for an abortion of a fetus carrying a genetic disorder, but not provide coverage if born.

- A pregnant woman, whose fetus tested positive for cystic fibrosis, was told by her health maintenance organization (HMO) that it would be willing to cover the cost of an abortion but would not cover the infant under the family's medical policy if she elected to carry the pregnancy to term.
- A healthy woman, who casually mentioned to her family doctor that her father had been diagnosed with Huntington's disease, and that she herself was at risk for inheriting this genetic disorder, was later denied disability insurance. The insurance company rejected her because they found a note about her father's diagnosis written in the margin of her medical records.

Healthy people were denied coverage despite their disease prevention measures.

- A healthy boy, who carried a gene predisposing him to a heart disorder, was denied health coverage by his parents' insurance company, even though the boy took medication that eliminated his risk of heart disease.
- One healthy man in his 20s with a gene for the degenerative brain condition Huntington's disease was refused life insurance. His older brother, on the other hand, tested negative and was able to reduce his premium which had been previously set on a family history of the disease.
- Another case involved a well woman in her 30s whose genetic test indicated a 70 to 90 per cent risk of developing cancer. Despite having regular screening for cancer, her superannuation was reduced and the life cover component refused.

Insurance companies in many states can charge higher health premiums.

Federal legislation, the Health Insurance Portability and Accountability Act (HIPPA, 1996), limits genetic discrimination as a basis for denying certain insurance medical insurance policies, but it does not prohibit charging higher premiums, nor does it cover life, disability, or automobile insurance or to employment -- all areas of documented discrimination. Slowly, state by state, the CRG, ACLU, and patients' rights groups are trying to get legislation passed to reduce or eliminate genetic discrimination; about 40 states have enacted some type of protections, but many are very weak and/or partial.

Federal rules for medical privacy (including genetic information) under HIPPA were announced in August 2000, after weaker proposals by the Clinton Administration received a great deal of criticism. While providing standards for the disclosure of bio-information, the rules require that the patient only receive notice, not give consent; thus, there still would not be full patient control over sensitive information.

The President has also announced his support of a Federal bill which would prohibit health insurance providers from using any type of genetic information for making decisions about whether to cover a person or what premium to charge. This legislation would address some of the discrimination problems which have been occurring. And he has issued an Executive Order barring genetic discrimination in Federal employment.

Conclusion

Our fate is not determined solely by genes; environmental factors play a role, too.

Beyond the risks of discrimination and loss of privacy, however, society's fascination with genetic determinism has other social and political consequences. An overemphasis on the role of genes in human health neglects environmental and social factors, thus contributing to the image of people with "defective" genes as "damaged goods." This, in effect, encourages a "blame the victim" mindset, directly contrary to the public policy embodied in the Americans with Disabilities Act, now 10 years old. Economic and social resources end up being diverted into finding biomedical "solutions" while societal measures get short-changed.

Conclusion: The politics of genetic technologies must be monitored.

Although new technologies claim to offer us more "freedom," they really can threaten our civic values. This is certainly true of the new biology. As Jefferson warned, "the price of liberty is eternal vigilance" -- it isn't genetically hard-wired to happen automatically.

About the author: Philip L. Bereano, Ph.D., J.D., is a professor in the College of Engineering, Department of Technical Communication, University of Washington, Seattle. For over 30 years he has worked on issues regarding technologies and public policies. He chairs the national Committee on Databases and Civil Liberties of the American Civil Liberties Union, and is a co-founder of the Council for Responsible Genetics. He participated in the development of the UN Cartagena Biosafety protocol.
http://www.uwtc.washington.edu/getting_to_know_us/faculty/bereano/default.htm

author bio
Dr. Carol Isaacson Barash is the founder and principal of Genetics, Ethics & Policy Consulting (GEPC)

human genome: applications of genomic mapping

Ethical Issues in Pharmacogenetics

By Carol Isaacson Barash, Ph.D.

An actionbioscience.org original article

article highlights

Pharmacogenetics promises drugs specific to an individual's condition. However, it poses some ethical concerns:

- *invasion of medical privacy*
- *unequal distribution of benefits*
- *discrimination because it involves genetic tests*
- *research/business conflict-of-interest*

February 2001

Ethical Issues in Pharmacogenetics

By Carol Isaacson Barash, Ph.D.

Drugs can be developed for individuals.

Pharmacogenetics is the study of how genes influence an individual's response to drugs. Though the field would seem to be brand new, it is really half a century old. In the 1950's, scientists first identified deficiencies in enzymes that explained adverse reactions to drugs and that they could be inherited.

For example, early research showed that 10% of African American men serving in the Korean war became anemic after ingesting an anti-malarial drug, which rarely, if ever caused problems for Caucasian soldiers. To pinpoint the cause, it took years of study:

Trial and error used to determine drug development.

- The anemic reaction was determined to be caused by a variation of the G6PD gene, and the variation was found to be common among people of African descent but not so among Caucasians.
- It was later discovered that the normal form of the gene makes an enzyme that helps protect red blood cells against certain chemicals. Lacking that protective effect, those with the variant form are vulnerable to deleterious effects.
- Since that time, numerous other enzyme variants have been identified and found to cause adverse reactions. Such adverse effects were identified, until recently, by trial and error methods. Specifically, drugs were administered, and an individual's metabolism of that drug was tracked by recording the amount of by-product in their urine.

Genomic mapping eliminates trial

The Human Genome Project has enabled us to identify the molecular composition of the enzymes in question so that we can study correlations between genotypic (gene trait) and phenotypic (physical trait) variability. These advances will increasingly enable us to detect

and error drug trial methods.

individuals who are likely to experience adverse reactions to medicines without having to use potentially dangerous methods of trial and error.

In coming years, we are likely to learn that particular single nucleotide polymorphisms (SNPs) are associated with sensitivities or resistances to chemical compounds in the environment. Scientists are now rushing to not only identify common SNPs, but to determine what drug effects can be correlated to them.

Pharmacogenomics is a recent offshoot of pharmacogenetics. Its scope is broader; for example, it attempts to understand not only the molecular composition of genetic variants associated with drug response but also the behavior of those variants, including how those genes affect drug receptor sites.

Ethical Issue #1: "Good" or "Bad" Allocation of Scarce Resources?

Critics say that genomic mapping is a waste of money and time.

Many believe that pharmacogenomics, like other new fields spawned by the Human Genome Project, represent a misallocation of resources. Rather than embark on learning how genes indicate a predisposition to disease and developing cures and enhancements, or experimenting with ways to change the human germ cell, global efforts should be spent on solving more urgent problems facing humanity, such as global famine or accessibility to potable water.

Others contend that pharmacogenomics, in particular, offers enormous potential for clinical benefits to patients as well as economic benefits for health care delivery. The arguments in favor include:

Every year, adverse reactions to drugs possibly kill 100,000 American patients.

- In the U.S. alone, adverse drug reactions are thought to KILL about 100,000 hospitalized patients annually. It is believed that many of these reactions are due to genetic variants and thus many of these deaths can be avoided by testing people for adverse drug response before giving them drugs. The science and technology for such tests, however, are in their infancy.

Over 2 million people have serious reactions to medication.

- Another 2.2 million incur serious, but non-fatal, reactions. Physicians, in view of their Hippocratic oath, are obligated to do no harm. Can this obligation be fulfilled when the information available to physicians about how particular medicines will fare in their patients is so meager? At present, physicians, generally have no way of knowing in advance whether the drug they prescribe will or will not cause an adverse effect in their patients.

'One-size- fits-all' medication can be dangerous.

- This situation is further compounded by the fact that most adverse drug reactions result from the fact that medicines are "a one-size-fits-all." In other words, although medicines are taken in different dosages depending on symptoms, patient age, weight and other clinical factors, these criteria may not be adequate to ensure that a particular medicine will be safe and effective for a particular individual. Until recently, there has been no alternative to either developing or prescribing medicines. Pharmacogenomics promises to take the guesswork out of developing and prescribing safe and effective drugs.

Ethical Issue #2: What is a fair distribution of burdens and benefits in developing the field of pharmacogenomics?

Monies and people (as research subjects and as researchers) will develop the field to the point that customized medicine will be possible. Who will benefit?

Designer drugs may be too costly at first for all to benefit.

- The availability of this new technology may be costly initially, and thus accessible only to those wealthy enough to pay for both the test and the designer drug best suited to them. Yet, the cost will likely diminish so as to become affordable to most. However, will lower costs influence a person to submit to the required genetic testing, thus creating threats, if not violations, to one's autonomy (the basic tenet of bioethics)?

Commercialization of research results may lead to conflict of interest.

- Researchers who have investments in companies competing in their field may be in a conflict of interest if they are conducting research for such a company. Substantial concerns about conflicts of interest as both a threat to quality research as well as to the well being of research subjects have abounded for decades.[1]
- A recent study found that policies governing conflicts of interests at major medical institutions varied considerably in both disclosure requirements and the nature of permitted academic-industry relationship, thereby opening a door to the possibility that an interest in financial gain could overpower an interest in either achieving valid research or protecting the well-being of subjects.[2]
- Further, there are several examples in the history of medical research where the patient population standing to benefit from advances (i.e., people who have donated their time, bodies, and hearts to research, though compensated per standard National Institute of Health [NIH] terms), did not receive the anticipated medical benefits because new therapies were unaffordable, when they became commercially available, or not covered by insurers, as these two examples illustrate:

Participants do not always benefit from successful outcomes of drug trials.

  - Numerous sufferers of Gauchier Disease, who helped companies developed safe and effective treatment (clinical research), were denied access to treatments by insurance companies by refusing to cover the high cost therapies. The patients couldn't afford to pay costs out of their own pocket.
  - A Cannavan's Disease Support Group has been instrumental in helping a company develop treatment by raising research funds as well as supplying researchers with willing research participants. The group is suing research facilities not for financial return on investment but for the opportunity to play an active role in furthering research/treatment goals.[3]

Ethical Issue #3: Will individualized medicine be used ethically?

Knowing if a person will respond to a drug in ways that are safe and effective for that individual will enable patients to avoid medications that are dangerous or ineffective for them.

Gene profiling is not the only factor

This is not to say that genes are the only key to cures. Environment plays a role, too. Dietary and lifestyle behaviors are likely to still affect the safety and efficacy of medicines for particular individuals. As well,

in creating designer drugs.

variation in drug response is not limited to micro polymorphisms. Environmental factors also play a role (such as sun exposure, drug/drug interaction, drug/food interaction). However, scientists are poised to uncover why the metabolism of particular individuals absorbs and dispels pharmaceuticals in a particular manner.

Consider the following *hypothetical* clinical scenario as illustrating some of the ethical issues that can arise in clinic:

Not all physicians will take advantage of new testing methods.

- A 42 year old man of Scandinavian descent presents to his physician with a general feeling of malaise.
- Five years previously he was diagnosed with high serum cholesterol, which he attempted to control with a regimen of exercise and dietary regulation, with no success. His physician then prescribed for him a drug therapy.
- Before agreeing to take the prescribed medication, the patient retrieved volumes of information from the Web, including but not limited to peer-reviewed journal articles about his condition and his physician's first choice drug.
- After six months of therapy there was only a modest lowering of cholesterol levels, so the medication was changed. After nine months on the second medication, there was still no marked effect.
- By the time the patient was able to see his physician again, a newer therapy had become available. This new drug had become the physician's favorite. The physician advised the patient to switch to this new drug, and the patient was eager to try it. Three weeks later, the patient came to see the physician to complain of continued malaise.

The patient may have been better served if he had undergone the following genetic tests, the results of which could have provided valuable management information:

Genetic testing can provide an array of diagnostic results.

- Test 1: a pre-dispositional test to determine whether the patient has a polymorphism associated with plaque development leading to coronary heart disease.
- Test 2: a test to see whether the patient has a polymorphism associated with a non-response to the medication (the newest medicine). A positive test 2 indicates that the patient lacks an enzyme needed to metabolize the drug. The absence of the enzyme means that the drug is dispelled from the body without absorption.
- Test 3: a test to see whether the patient has a polymorphism which indicates the presence of an enzyme responsible for metabolizing the dosage too slowly, making the drug in that dosage toxic to the patient.
- The rationale sequence of testing is 1-3.

If the patient tests negative, meaning that he does not have the polymorphism associated with plaque development, then his high cholesterol poses no health risk and medication to lower cholesterol levels are not indicated. If the patient tests positive, meaning that he does have the polymorphism, then he is predisposed to coronary heart disease (CAD) by virtue of being a plaque maker. In this case,

cholesterol-lowering medication is indicated.

Ethical Issue #4: Whose right predominates?

The father of a research subject opened a letter addressed to his child and learned that his child had enrolled in a genetic research study.

Whose privacy was invaded -- the father's or the child's?

- The letter indicated that for the purpose of research the research facility had obtained some of the father's medical records. The father objected to what apparently was non-consensual disclosure of his medical information, even for the purpose of obtaining an informative family history to be used to provide optimal care for the son/daughter.
- Outraged, the father phoned the Office for the Protection of Human Research Subjects (OPHR) of NIH and protested that the researchers' obtaining his family history without his explicit consent constituted a violation of his privacy rights. OPHR, apparently siding with the father, blocked the offspring from using the father's information and forbade any further attempts to obtain more information on grounds that an individual's (specifically *his*) right to privacy and autonomy is paramount.

Among the interesting and difficult issues in this case is the fact that it challenges us to think deeply about the weighted values we assign to first principles, namely the right to privacy. Whose right predominates in this case - the father's or the child's?

Laws exist to ensure medical privacy.

New federal medical privacy rules under HIPPA spell out the requirements to ensure privacy of all individuals. These rules, though scheduled to become practice February 2001, are facing strict opposition from several different sectors of the health care industry, primarily because of the cost and impracticalities involved in implementation. For numerous reasons, it is far from clear if these rules would support the father's claim, and if so how.[4]

Conclusion

In spite of our best efforts to anticipate and resolve ethical quandaries arising from the application of new genetic technologies, it is likely that unexpected conflicts will arise. Those discussed in this article are not intended as an exhaustive list.

Pharmacogenetics has fewer ethical issues than other medical biotechnologies.

The ethical issues here are remarkably similar to those standardly invoked in pre-dispositional testing discussions. Yet, arguably the stakes are lower here. The risk of psychological harm is, for the most part, far less substantial than testing for a late onset disorder like Huntington Disease, for which, effective treatment does not exist. Still, in the absence of guidance about what constitutes high and low stakes, ethically defensible decision making requires acknowledgement of the competing interests and a broad enough scope of concern to analyze how an apparent low risk can become a real high risk and vice versa.

Pharmacogenetics will permit gene profiling to answer questions about medicine responses, as well as enable researchers to design better and

Pharmacogenetics will ensure safer drugs.

safer medicines. The science and its applications are real today and will be increasingly common in coming years. While the likelihood that individuals will be shut out from health insurance because they do not respond to a single drug or because a particular drug formula is toxic to them is extremely low, as would be employment exclusions (in hiring, promoting or job responsibilities), the issues underscore the importance of debating more widely the ethical use of pharmacogenetics.

Conclusion: The end product of this technology -- individualized drugs -- must be made easily available to all.

In the U.S., 45 million people lack any health insurance, and thus are at the mercy of hospitals' budgets for unrecoverable expenditures. Further, these individuals, and the many more millions of people with health insurance, have no access to sophisticated medical care due to limits imposed by insurers, especially for-profit managed care organizations and self-insured employers. Whether customized medicine will be available to all remains a large unknown. If history is a hint to how this new field will be used, we ought to act now to ensure that the benefits are available to ALL.

About the author: Dr. Carol Isaacson Barash is the founder and principal of Genetics, Ethics & Policy Consulting (GEPC), which specializes in optimizing the integration of genomics into 21st-century health care delivery. GEPC offers solutions to a broad range of challenges facing health care in the post genomic era. Prior to establishing GEPC, Dr. Barash was director of a study on genetic discrimination funded by the Department of Energy. She is a trained philosopher with over twenty years experience in health care.
http://www-unix.oit.umass.edu/~fholmes/directory2.html

American College of Medical Genetics

Principles of Screening: Report of The Subcommittee on Screening of the American College of Medical Genetics Clinical Practice Committee

Screening for genetic disease or genetic predisposition to disease provides a unique opportunity to prevent the effects of the disease. Retrieval, diagnosis and intervention before irreversible damage represent goals for an effective genetic screening program.

The screening program should have a clearly defined purpose.

- Distinctions must be made between carrier screening, screening for predisposition to disease, screening for presymptomatic disease, and screening for those affected with disease. The terms presymptomatic and symptomatic should be defined based on the nature and severity of specific symptoms. Certain phenotypic signs may have minimal clinical significance. The program should determine whether such signs will, or will not, result in classification of an individual as symptomatic.

- Newborn screening is a special case that, depending on the disorder and the screening method, may identify carriers, presymptomatic individuals, or affected neonates. Newborn screening represents an example of population-based screening as opposed to selective screening where a specified subset of the population is targeted.

- There should be a defined population for screening, e.g., newborns, a specific ethnic group for a disorder with increased frequency in that group, women at risk for breast cancer, etc.

- When a program is established, it must be clear whether the purpose is research or medical care. The decision to move a test from the research to the clinical arena must be carefully considered. The participant must be aware of benefits and risks of the information that will be forthcoming. If the screening is for research purposes, the subjects should be fully informed of the sources of financial support to minimize any perception of conflict of interest.

- Screening for clinical purposes preferably should be tied to the availability of intervention, including prenatal diagnosis, counseling, reproductive decision making, lifestyle changes, enhanced phenotype screening, etc.

A screening program is more than a laboratory test.

- The introduction of the screening test is best accompanied by a public and professional education program.

- An essential component of a screening program is follow up evaluation and counseling by genetic professionals for participants with positive results in order to assure appropriate understanding and treatment, and to reduce anxiety and stigmatization. Counseling of individuals with negative results may, in some cases, be appropriate.

- All screening results are confidential. The results may only be revealed to the participant and/or the participant's personal physician. Research programs should apply for a certificate of confidentiality (Earley and Strong, 1995).

- In screening programs where there is less than 100% sensitivity, participants should be informed that a negative result does not necessarily rule out the possibility that they are carriers of, or affected with, the disorder tested. When there are a priori risks for the participants, the statistics should be accurately individualized for the particular patient.

- Due to potential problems of insurability and employability for carriers and/or affected individuals, participants may not want their insurance company or employer to know they are having the screening test performed. This may place the burden of payment for the screening test on the participant.

A screening program should be reviewed by the appropriate board.

- New screening programs should be considered by an appropriate review board, which will determine if consent is necessary.

- Review boards should determine, within the bounds of current federal regulations, if it is appropriate to involve fetuses, minor children, or incompetent adults in specific screening programs because of special risks and considerations, such as the implications for later onset diseases, self esteem, stigmatization, and altered family dynamics.

- Because of the sensitive nature of screening programs involving fetuses, minor children, and incompetent adults, review boards should weigh the risks and benefits to these groups separately from those of competent adults.

The screening program should be evaluated periodically to determine if it is meeting its goals.

- Tests should be simple, accurate, and relatively inexpensive. They should identify most of the carriers and affected persons (high sensitivity) with few false positives (high specificity). Sensitivity, specificity and predictive value should be appropriate for the screening venue. Acceptable sensitivity and specificity will depend on the a priori risk of the screened population, and may vary for individuals within the population.

- The disorder for which screening is carried out should be of significant clinical severity. Severity will be viewed differently by individual patients.

- The correlation between phenotype and genotype should be understood for the targeted disease.

- Results of screening programs and the screening specimens themselves need to be maintained in a safe and secure environment.

REFERENCE

C.L. Earley and L.C. Strong; Certificates of confidentiality: A valuable tool for protection of genetic data. American Journal of Human Genetics. 57:727-731, 1995.

Members of the Subcommittee

Edward R.B. McCabe, M.D., Ph.D., Subcommittee Chair
UCLA School of Medicine
Corinne D. Boehm, M.S.
Johns Hopkins Hospital
George P. Henry, M.D.
Reproductive Genetics Center
Michelle M. LeBeau, Ph.D.
University of Chicago
Vicki M. Park, Ph.D.
University of Tennessee
Jerome I. Rotter, M.D.
Cedars-Sinai Medical Center
Margretta R. Seashore, M.D.
Yale University School of Medicine

This guideline is designed primarily as an educational resource for medical geneticists and other health care providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily assure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

Revised and approved by the ACMG Executive Committee on February 28, 1997.

Genetics Privacy and Legislation

Human Genome Project Information Website
U.S. Department of Energy Office of Science, Office of Biological and Environmental Research, Human Genome Program

I. FEDERAL POLICY HISTORY

No federal legislation has been passed relating to *genetic discrimination in individual insurance coverage* or to *genetic discrimination in the workplace*. Several bills were introduced during the last decade. Some of these bills attempted to amend existing civil rights and labor laws, while others stood alone. The primary public concerns are that (1) insurers will use genetic information to deny, limit, or cancel insurance policies or (2) employers will use genetic information against existing workers or to screen potential employees. Because DNA samples can be held indefinitely, there is the added threat that samples will be used for purposes other than those for which they were gathered.

Executive Order Protecting Federal Employees
On February 8, 2000, U.S. President Clinton signed an *executive order* prohibiting every federal department and agency from using genetic information in any hiring or promotion action. This executive order, endorsed by the American Medical Association, the American College of Medical Genetics, the National Society of Genetic Counselors, and the Genetic Alliance

- *Prohibits* federal employers from requiring or requesting genetic tests as a condition of being hired or receiving benefits. Employers cannot request or require employees to undergo genetic tests in order to evaluate an employee's ability to perform his or her job.
- *Prohibits* federal employers from using protected genetic information to classify employees in a manner that deprives them of advancement opportunities. Employers cannot deny employees promotions or overseas posts because of a genetic predisposition for certain illnesses.
- *Provides* strong privacy protections to any genetic information used for medical treatment and research. Under the EO, obtaining or disclosing genetic information about employees or potential employees is prohibited, except when it is necessary to provide medical treatment to employees, ensure workplace health and safety, or provide occupational and health esearchers access to data. In every case where genetic information about employees is obtained, it will be subject to all Federal and state privacy protections.

U.S. House of Representatives Committee on Energy and Commerce
Hearing on Potential for Discrimination in Health Insurance Based on Predictive Genetic Tests, July 11, 2001

Senate Committee on Health, Education, Labor, and Pensions
Hearing on Genetic Information in the Workplace, July 20, 2000

Genetic Nondiscrimination Bills from the 106th Congress 1999-2000
[See the PHRMA Genomics Legislation website for the latest details on all genomics bills.]

- H.R.293, Genetic Information Health Insurance Nondiscrimination Act of 1999, SPONSOR: Rep John E. Sweeney (introduced 01/06/99). A bill to amend the Public Health Service Act and the Employee Retirement Income Security Act of 1974 to prohibit health insurers and group health plans from discriminating against individuals on the basis of genetic information.

- H.R.306, Genetic Information Nondiscrimination in Health Insurance Act of 1999, SPONSOR: Rep Louise McIntosh Slaughter (introduced 01/06/99). A bill to prohibit discrimination against individuals and their family members on the basis of genetic information or of a request for genetic services.

- S.300, Patients' Bill of Rights Plus Act, SPONSOR: Sen Trent Lott (introduced 01/22/99). A bill to improve access and choice of patients to quality, affordable health care. Includes section on genetic information nondiscrimination in health insurance.

- S.326, Patients' Bill of Rights Act, SPONSOR: Sen James M. Jeffords (introduced 01/28/99). A bill to improve the access and choice of patients to quality, affordable health care. Includes section on genetic-information nondiscrimination in health insurance.

II. STATE POLICY HISTORY

States have a patchwork of genetic-information nondiscrimination laws, none of them comprehensive. Existing state laws differ in coverage, protections afforded, and enforcement schemes. Some of the first state laws enacted to address this issue prohibited discrimination against individuals with specific genetic traits or disorders. Other state laws regulate both the use of genetic testing in employment decisions and the disclosure of genetic test results. These state laws generally prohibit employers from requiring workers and applicants to undergo genetic testing as a condition of employment. Some states permit genetic testing when it is requested by the worker or applicant for the purpose of investigating a compensation claim or determining the worker's susceptibility to potentially toxic chemicals in the workplace. These statutes often require the worker to provide informed written consent for such testing, contain specific restrictions governing disclosure, and prevent the employer from taking adverse action against the employee.

[See the NIH NHGRI chart of all genetics insurance discrimination legislation and the NIH NHGRI chart of all genetics workplace discrimination legislation that has been enacted at the state level.]

State Genetics Reports

- IL: The Challenges of Human Cloning for Public Policy in Illinois (February 2001)

- OR: Assuring Genetic Privacy in Oregon (November 2000)

- KY: Genetic Testing in Health, Life, and Disability Insurance in Kentucky (January 2000)
- MI: Report of the Michigan Commission on Genetic Privacy and Progress (February 1999)
- NE: Report of the Nebraska Commission on Human Genetic Technologies Commission (December 1998)

III. EXISTING FEDERAL ANTI-DISCRIMINATION LAWS AND HOW THEY APPLY TO GENETICS

Although no specific federal genetic nondiscrimination legislation has been enacted, some believe that parts of existing nondiscrimination laws could be interpreted to include genetic discrimination. Here is a brief overview of these laws and how they apply to genetics.

Americans with Disabilities Act of 1990 (ADA)

The most likely current source of protection against genetic discrimination in the workplace is provided by laws prohibiting discrimination based on disability. Title I of the Americans with Disabilities Act (ADA), enforced by the Equal Employment Opportunity Commission (EEOC), and similar disability-based antidiscrimination laws such as the Rehabilitation Act of 1973 do not explicitly address genetic information, but they provide some protections against disability-related genetic discrimination in the workplace.

- Prohibits discrimination against a person who is regarded as having a disability.
- Protects individuals with symptomatic genetic disabilities the same as individuals with other disabilities.
- Does not protect against discrimination based on unexpressed genetic conditions.
- Does not protect potential workers from requirements or requests to provide genetic information to their employers after a conditional offer of employment has been extended but before they begin work. (Note: this is a heightened concern because genetic samples can be stored.)
- Does not protect workers from requirements to provide medical information that is job related and consistent with business necessity.

In March 1995, the EEOC issued an interpretation of the ADA. The guidance, however, is limited in scope and legal effect. It is policy guidance that does not have the same legal binding effect on a court as a statute or regulation and has not been tested in court. According to the interpretation,

- Entities that discriminate on the basis of genetic predisposition are regarding the individuals as having impairments, and such individuals are covered by the ADA.
- Unaffected carriers of recessive and X-linked disorders, individuals with late-onset genetic disorders who may be identified through genetic testing or family history as being at high risk of developing the disease are not covered by the ADA

Health Insurance Portability and Accountability Act of 1996 (HIPAA)
The Health Insurance Portability and Accountability Act (HIPAA) applies to *employer-based and commercially issued group health insurance only*. HIPAA is the only federal law that directly addresses the issue of genetic discrimination. There is no similar law applying to private individuals seeking health insurance in the individual market. HIPAA

- Prohibits group health plans from using any health status-related factor, including genetic information, as a basis for denying or limiting eligibility for coverage or for charging an individual more for coverage.
- Limits exclusions for preexisting conditions in group health plans to 12 months and prohibits such exclusions if the individual has been covered previously for that condition for 12 months or more.
- States explicitly that genetic information in the absence of a current diagnosis of illness shall not be considered a preexisting condition.
- Doesn't prohibit employers from refusing to offer health coverage as part of their benefits packages.

HIPPA National Standards to Protect Patients' Personal Medical Records, Dec. 2000
This new regulation will protect medical records and other personal health information maintained by health care providers, hospitals, health plans and health insurers, and health care clearinghouses. The regulation was mandated by Congress when it failed to pass comprehensive privacy legislation (as required by HIPPA) by 1999.. The new standards: limit the non-consensual use and release of private health information; give patients new rights to access their medical records and to know who else has accessed them; restrict most disclosure of health information to the minimum needed for the intended purpose; establish new criminal and civil sanctions for improper use or disclosure; and establish new requirements for access to records by researchers and others. They are not specific to genetics, rather they are sweeping regulations governing all personal health information.

For more on the standards, see

- U.S. Department of Health and Human Services (DHHS) Announces Final Regulation Establishing First-ever National Standards to Protect Patients' Personal Medical Records: DHHS Press Release
- Summary of the Final Regulation: DHHS Fact Sheet

Title VII of the Civil Rights Act of 1964
An argument could be made that genetic discrimination based on racially or ethnically linked genetic disorders constitutes unlawful race or ethnicity discrimination.

- Protection is available only where an employer engages in discrimination based on a genetic trait that is substantially related to a particular race or ethnic group.
- A strong relationship between race or national origin has been established for only a few diseases.

IV. RECOMMENDATIONS FOR FUTURE LEGISLATION

Workplace Discrimination
Based on previous recommendations from the National Action Plan on Breast Cancer (NAPBC) and the NIH-DOE Working Group on the Ethical, Legal, and Social Implications (ELSI) of human genome research, in a 1998 report the Clinton Administration announced recommendations for future legislation to ensure that discoveries made possible by the Human Genome Project are used to improve health and not to discriminate against workers or their families. These recommendations are

- Employers should not require or request that employees or potential employees take a genetic test or provide genetic information as a condition of employment or benefits.
- Employers should not use genetic information to discriminate against, limit, segregate, or classify employees in a way that would deprive them of employment opportunities.
- Employers should not obtain or disclose genetic information about employees or potential employees under most circumstances.

Genetic testing and the use of genetic information by employers should be permitted in the following situations to ensure workplace safety and health and to preserve research opportunities. However, in all cases where genetic information about employees is obtained, the information should be maintained in medical files that are kept separate from personnel files, treated as confidential medical records, and protected by applicable state and federal laws.

- An employer should be permitted to monitor employees for the effects of a particular substance found in the workplace to which continued exposure could cause genetic damage under certain circumstances. Informed consent and assurance of confidentiality should be required. In addition, employers may use the results only to identify and control adverse conditions in the workplace and to take action necessary to prevent significant risk of substantial harm to the employee or others.

- The statutory authority of a federal agency or contractor to promulgate regulations, enforce workplace safety and health laws, or conduct occupational or other health research should not be limited.

- An employer should be able to disclose genetic information for research and other purposes with the written, informed consent of the individual.

These recommendations should apply to public and private-sector employers, unions, and labor-management groups that conduct joint apprenticeship and other training programs. Employment agencies and licensing agencies that issue licenses, certificates, and other credentials required to engage in various professions and occupations also should be covered.

Individuals who believe they have been subjected to workplace discrimination based on genetic information should be able to file a charge with the Equal Employment Opportunity Commission, Department of Labor, or other appropriate federal agency for investigation and resolution. The designated agency should be authorized to bring lawsuits in the federal courts to resolve issues that would not settle amicably. The courts should have the authority to halt the violations and order relief, such as hiring, promotion, back pay, and compensatory and punitive damages to the individual. Alternatively, an individual should be able to elect to bring a private lawsuit in federal or state court to obtain the same type of relief plus reasonable costs and attorney's fees. To enforce these protections, the designated enforcement agency must be given sufficient additional resources to investigate and prosecute allegations of discrimination.

Insurance Discrimination

In 1995, the NIH-DOE Joint Working Group on Ethical, Legal, and Social Implications of Human Genome Research (ELSI Working Group) and the National Action Plan on Breast Cancer (NAPBC) developed and published the following recommendations for state and federal policymakers to protect against genetic discrimination (*Science*, vol. 270, Oct. 20, 1995):

Definitions:

- "Genetic information" is information about genes, gene products, or inherited characteristics that may derive from the individual or a family member.

- "Insurance provider" means an insurance company, employer, or any other entity providing a plan of health insurance or health benefits, including group and individual health plans whether fully insured or self-funded.

Recommendations:

- Insurance providers should be prohibited from using genetic information or an individual's request for genetic services to deny or limit any coverage or establish eligibility, continuation, enrollment, or contribution requirements.

- Insurance providers should be prohibited from establishing differential rates or premium payments based on genetic information or an individual's request for genetic services.
  Insurance providers should be prohibited from requesting or requiring collection or disclosure of genetic information. Insurance providers and other holders of genetic information should be prohibited from releasing genetic information without the individual's prior written authorization. Written authorization should be required for each disclosure and include to whom the disclosure would be made.

Sample Genetic Privacy Act and Commentary
A draft bill (Genetic Privacy Act) was written in 1995 by George Annas of the Boston University School of Public Health to assist legislators. This bill proposed that access to information in genetic data banks should be regulated during sample collection, storage, disclosure, and use. Several state lawmakers adapted language and concepts from the draft bill to write proposals for legislation in their own states.

V. WHY LEGISLATION IS NEEDED NOW

(1) Based on genetic information, employers may try to avoid hiring workers they believe are likely to take sick leave, resign, or retire early for health reasons (creating extra costs in recruiting and training new staff), file for workers' compensation, or use healthcare benefits excessively.

(2) Some employers may seek to use genetic tests to discriminate against workers--even those who do not and may never show signs of disease--because the employers fear the cost consequences.

(3) The economic incentive to discriminate based on genetic information is likely to increase as genetic research advances and the costs of genetic testing decrease.

(4) Genetic predisposition or conditions can lead to workplace discrimination, even in cases where workers are healthy and unlikely to develop disease or where the genetic condition has no effect on the ability to perform work

(5) Given the substantial gaps in state and federal protections against employment discrimination based on genetic information, comprehensive federal legislation is needed to ensure that advances in genetic technology and research are used to address the health needs of the nation--and not to deny individuals employment opportunities and benefits. Federal legislation would establish minimum protections that could be supplemented by state laws.

(6) Insurers can still use genetic information in the individual market in decisions about coverage, enrollment, and premiums.

(7) Insurers can still require individuals to take genetic tests.

(8) Individuals are not protected from the disclosure of genetic information to insurers, plan sponsors (employers), and medical information bureaus, without their consent.

(9) Penalties in HIPPA for discrimination and disclosure violations should be strengthened in order to ensure individuals of the protections afforded by the legislation.

THE GENETIC PRIVACY ACT AND COMMENTARY (Selected Excerpts)

George J. Annas, JD, MPH
Leonard H. Glantz, JD
Patricia A. Roche, JD

Health Law Department; Boston University School of Public Health; 80 East Concord Street; Boston, MA 02118
Tel. (617) 638-4626; FAX: (617) 638-5299; email: annasgj@bu.edu

The Genetic Privacy Act and Commentary is also the **Final Report** of a project entitled "Guidelines for Protecting Privacy of Information Stored in Genetic Data Banks" which was funded by the Ethical, Legal & Social Implications of the Human Genome Project, Office of Energy Research, U.S. Department of Energy, No. DE-FG02-93ER61626

Additional support was provided by Boston University School of Public Health

INTRODUCTION

The Genetic Privacy Act is a proposal for federal legislation. The Act is based on the premise that genetic information is different from other types of personal information in ways that require special protection. The DNA molecule holds an extensive amount of currently indecipherable information. The major goal of the Human Genome Project is to decipher this code so that the information it contains is accessible. The privacy question is, accessible to whom?

The highly personal nature of the information contained in DNA can be illustrated by thinking of DNA as containing an individual's "future diary."*[1]* A diary is perhaps the most personal and private document a person can create. It contains a person's innermost thoughts and perceptions, and is usually hidden and locked to assure its secrecy. Diaries describe the past. The information in one's genetic code can be thought of as a coded probabilistic future diary because it describes an important part of a unique and personal future.

Genetic information is powerful and personal. As the genetic code is deciphered, genetic analysis of DNA will tell us more and more about a person's likely future, particularly in terms of physical and mental well-being. The search for genetic information often involves locating predictors of undesirable and stigmatizing conditions - such as cancers, and conditions that lead to mental illness and dementia. This information is uniquely sensitive for a number of reasons. First, unlike ordinary diaries that are created by the writer, the information contained in the genetic code is largely unknown to the person in whose genetic material it is found. Therefore, if this information is obtained by someone else without the individual's permission, another person would learn intimate details of the individual's likely future life. A stranger could, in effect, read the future diary of an individual without the individual even knowing that the diary exists. There are many people, including insurers and employers, to whom information about an individual's likely health future would be useful.*[2]*

Second, deciphering an individual's genetic code also provides the reader of that code with probabilistic health information about that individual's family, especially parents, siblings and children. Third, since the DNA molecule is stable, once removed from a person's body and stored, it can become the source of an increasing amount of information as more is learned about how to read the genetic code. Finally, genetic information (and misinformation) has been used by governments to viciously discriminate against those perceived as genetically unfit.

Collection, Analysis and Storage of DNA and Genetic Information

Focusing solely on any or all of these types of DNA databanks assumes that the DNA samples have been legitimately obtained and analyzed, and the only issues are the proper storage of genetic information, and rules governing the disclosure of the genetic information by DNA databanks. But meaningful privacy

protection must regulate the collection, analysis and storage of DNA **samples**, as well as the storage and disclosure of the genetic **information** derived from the analysis of these samples, no matter who performs that analysis. It is, after all, the DNA samples that contain the individual's private genetic information. Control of these samples enables the custodian to analyze and reanalyze them to derive increasing amounts of genetic information as new tests are developed. It is also possible to obtain biological material for the purpose of DNA analysis without the person knowing that such material was obtained or analyzed. For example, DNA can even be obtained from hair samples left on a barber's floor or from saliva found on a licked stamp.

Therefore, to effectively protect genetic privacy unauthorized collection and analysis of individually identifiable DNA must be prohibited. As a result, the overarching premise of the Act is that no stranger should have or control identifiable DNA samples or genetic information about an individual unless that individual specifically authorizes the collection of DNA samples for the purpose of genetic analysis, authorizes the creation of that private information, and has access to and control over the dissemination of that information.

The rules protecting genetic privacy must be clear and known to the medical, scientific, business and law enforcement communities and the public. The purpose of the Genetic Privacy Act is to codify these rules. It has been drafted as a federal statute to provide uniformity across state lines. However, the Act could be adopted by individual states and used as guidelines by professional societies, at least until such time as Congress acts.*[7]*

Under the Act, each person who collects a DNA sample (e.g., blood, saliva, hair or other tissue) for the purpose of performing genetic analysis is required to:

- provide specific information verbally prior to collection of the DNA sample;
- provide a notice of rights and assurances prior to the collection of the DNA sample;
- obtain written authorization which contains required information;
- restrict access to DNA samples to persons authorized by the sample source;
- abide by a sample source's instructions regarding the maintenance and destruction of DNA samples.

Special rules regarding the collection of DNA samples for genetic analysis are set forth for minors, incompetent persons, pregnant women, and embryos. DNA samples may be collected and analyzed for identification for law enforcement purposes if authorized by state law, and for identifying dead bodies, without complying with the authorization provisions of the Act. Research on individually identifiable DNA samples is prohibited unless the sample source has authorized such research use, and research on nonidentifiable samples is permitted if this has not been prohibited by the sample source. Pedigree research and research involving DNA from minors are also governed by specific provisions of the Act.

Individuals are prohibited from analyzing DNA samples unless they have verified that written authorization for the analysis has been given by the sample source or the sample source's representative. The sample source has the right to:

- determine who may collect and analyze DNA;
- determine the purposes for which a DNA sample can be analyzed;
- know what information can reasonably be expected to be derived from the genetic analysis;
- order the destruction of DNA samples;
- delegate authority to another individual to order the destruction of the DNA sample after death;

- refuse to permit the use of the DNA sample for research or commercial activities; and
- inspect and obtain copies of records containing information derived from genetic analysis of the DNA sample.

A written summary of these principles and other requirements under the Act must be supplied to the sample source by the person who collects the DNA sample. The Act requires that the person who holds private genetic information in the ordinary course of business keep such information confidential and prohibits the disclosure of private genetic information unless the sample source has authorized the disclosure in writing or the disclosure is limited to access by specified researchers for compiling data.

The Genetic Privacy Act protects individual privacy while permitting medical uses of genetic analysis, legitimate research in genetics, and genetic analysis for identification purposes.

II

THE GENETIC PRIVACY ACT

A BILL

To protect the genetic privacy of individuals.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

Sec. 1. SHORT TITLE; TABLE OF CONTENTS
(a) SHORT TITLE. -- This act may be cited as the "Genetic Privacy Act."
(b) TABLE OF CONTENTS. -- The table of contents for this Act is as follows:

Sec. 2. FINDINGS AND PURPOSES

(a) FINDINGS. -- The Congress finds as follows:

(1) The DNA molecule contains information about one's probable medical future, and this information is written in a code that is currently being broken at a rapid pace.

(2) Genetic information has a history of being used by governments to harm individuals.

(3) Genetic information is uniquely private and personal information that should not be collected or disclosed without the individual's authorization.

(4) The improper use and disclosure of genetic information can lead to significant harm to the individual, including stigmatization and discrimination in areas such as employment, education, health care, and insurance.

(5) An analysis of an individual's DNA provides information not only about an individual, but also about that individual's parents, siblings and children, thus implicating family privacy.

(6) Genetic information is uniquely tied to reproductive decisions which are among the most private and intimate decisions that an individual can make.

(7) Current legal protections for medical information, tissue samples, and DNA samples are inadequate to protect genetic privacy.

(8) Uniform rules for the collection, storage and use of identifiable DNA samples and private genetic information obtained from them are needed both to protect individual privacy and to permit legitimate genetic research.

(b) PURPOSES. -- The purposes of this Act are as follows:

(1) To define the circumstances under which DNA samples may be collected, stored and analyzed.

(2) To define the circumstance under which private genetic information may be created, stored and disclosed.

(3) To define the rights of individuals whose DNA samples are collected, stored, and analyzed.

(4) To define the rights of individuals whose genetic information is stored and disclosed.

(5) To define the responsibilities of persons who collect, analyze and use DNA samples and the genetic information derived from them.

(6) To establish effective mechanisms to enforce the rights and responsibilities defined in this Act.

Sec. 3. DEFINITIONS

For purposes of this Act:

(a) COMPULSORY DISCLOSURE. -- The term "compulsory disclosure" means any disclosure of private genetic information mandated or required by federal or state law in connection with a judicial, legislative, or administrative proceeding, including but not limited to, disclosure required by subpoena, subpoena duces tecum, request or notice to produce, court order, or any other method of requiring a person maintaining private genetic information to produce private genetic information under the criminal or civil discovery laws of any state or the federal law.

(b) DISCLOSE. -- The term "disclose", when used with respect to private genetic information, means to provide access to the information, or the verification of the information, but only if such access or verification is provided to a person other than the sample source or the sample source's representative.

(c) DISCLOSURE. -- The term "disclosure" means the act or an instance of disclosing.

(d) DNA. -- The term "DNA" means deoxyribonucleic acid.

(e) DNA SAMPLE. -- The term "DNA sample" means any human biological specimen from which DNA can be extracted, or DNA extracted from such specimen.

(f) DNA TYPING. -- The term "DNA typing" means a scientifically reliable method for characterizing and comparing sequences of DNA, and applying a statistical analysis of population frequency to determine that if the DNA sequences match, the probability that the match occurs by chance.

(g) IDENTIFIABLE INDIVIDUAL. -- The term "identifiable individual" means any individual whose name, address, Social Security number, health insurance identification number, or similar identifying information is known, available, or can be determined with reasonable accuracy either directly or by reference to other available information.

(h) INDIVIDUAL IDENTIFIER. -- The term "individual identifier" means a name, address, Social Security number, health insurance identification number, or similar information by which the identity of a sample source can be determined with reasonable accuracy, either directly or by reference to other available information. The term does not include characters, numbers, or codes assigned to an individual or a DNA sample which cannot be used to determine the identity of a sample source.

(i) INDIVIDUALLY IDENTIFIABLE DNA SAMPLE. -- The term "individually identifiable DNA sample" means any DNA sample linked to an individual identifier.

(j) INDIVIDUALLY IDENTIFIABLE RECORD. -- The term "individually identifiable record" means any record that contains private genetic information linked to an individual identifier.

(k) INSTITUTIONAL REVIEW BOARD. -- The term "Institutional Review Board" means a board established in accordance with 45 CFR 46.102(g)(1992) as such regulation may be amended.

(l) PERSON. -- The term "person" shall include an individual, a corporation, partnership, association, joint venture, government, governmental subdivision or agency, and other legal or commercial entity.

(m) PRIVATE GENETIC INFORMATION. -- The term "private genetic information" means any information about an identifiable individual that is derived from the presence, absence, alteration, or mutation of a gene or genes, or the presence or absence of a specific DNA marker or markers, and which has been obtained:

(1) from an analysis of the individual's DNA; or

(2) from an analysis of the DNA of a person to whom the individual is related.

(n) SAMPLE SOURCE. -- The term "sample source" means the individual from whose body the DNA sample originated.

(o) SAMPLE SOURCE'S REPRESENTATIVE. -- The term "sample source's representative" means any person who has the legal authority to make health care decisions concerning a minor or an incompetent

person, or the administrator or executor of a deceased person's estate, if any, otherwise the next of kin of a deceased person.

PART A - - COLLECTION AND ANALYSIS OF DNA SAMPLES

Sec. 101. COLLECTION OF DNA SAMPLES

(a) REQUIREMENT OF WRITTEN AUTHORIZATION. -- Except as otherwise provided in sections 121, 122, and 123, no person may collect or cause to be collected an individually identifiable DNA sample for genetic analysis without the written authorization of the sample source or the sample source's representative.

(b) REQUIRED INFORMATION. -- Prior to the collection of a DNA sample from a sample source for genetic analysis, the person collecting the sample or causing the sample to be collected shall verbally inform the sample source or the sample source's representative:

(1) that consent to the collection or taking of the DNA sample is voluntary;
(2) that consent to the genetic analysis is voluntary;
(3) of the information that can reasonably be expected to be derived from the genetic analysis;
(4) of the use, if any, that the sample source or the sample source's representative will be able to make of the information derived from the genetic analysis;
(5) of the right to inspect records that contain information derived from the genetic analysis;
(6) of the right to have the DNA sample destroyed;
(7) of the right to revoke consent to the genetic analysis at any time prior to the completion of the analysis;
(8) that the genetic analysis may result in information about the sample source's genetic relatives which may not be known to such relatives but could be important, and if so the sample source will have to decide whether or not to share that information with relatives;
(9) that in the future someone else may ask if the sample source has obtained genetic testing or analysis and condition a benefit on the disclosure of information regarding such testing or analysis;
(10) that the collection and analysis of the DNA sample, and the private genetic information derived from the analysis is protected by this Act; and
(11) of the availability of genetic counseling.

Sec. 102. ANALYSIS OF DNA SAMPLES

(a) ANALYSIS PROHIBITED WITHOUT AUTHORIZATION. -- Except as otherwise provided in sections 121, 122, and 123, genetic analysis of an individually identifiable DNA sample is prohibited unless specifically authorized in writing by the sample source or the sample source's representative.

(b) ASCERTAINMENT OF AUTHORIZATION. -- No person may analyze an individually identifiable DNA sample without ascertaining that written authorization for the analysis has been obtained.

Sec. 103. AUTHORIZATION FOR COLLECTION AND STORAGE OF INDIVIDUALLY IDENTIFIABLE DNA SAMPLES FOR GENETIC ANALYSIS

(a) WRITTEN AUTHORIZATION. -- To be valid, the authorization required by sections 101 and 102 must satisfy each of the following requirements:

(1) WRITING. -- The authorization must be in writing, signed by the sample source or the sample source's representative, and dated on the date of such signature;
(2) COLLECTOR IDENTIFIED. -- The authorization must identify the person who collects the DNA sample or causes the DNA sample to be collected;
(3) ANALYZER IDENTIFIED. -- The authorization must identify the facility in which the analysis will be performed;
(4) STORAGE FACILITY IDENTIFIED. -- The authorization must identify the facility in which the DNA sample will be stored;
(5) COLLECTION DESCRIBED. -- The authorization must state the manner in which the sample is to be collected;

(6) AUTHORIZED USE. -- The authorization must include a description of all authorized uses of the DNA sample;
(7) STATEMENT REGARDING STORAGE AFTER COMPLETION OF ANALYSIS. -- The authorization must indicate whether or not the sample source permits the sample to be maintained or stored in an identifiable form after the analysis is completed;
(8) STATEMENT REGARDING USE OF UNIDENTIFIABLE DNA SAMPLES FOR RESEARCH OR COMMERCIAL PURPOSES. -- The authorization form must include a provision that enables the sample source or the sample source's representative to prohibit the use of the DNA sample for research or commercial purposes even if the sample is not in an individually identifiable form.

(b) RETENTION OF AUTHORIZATION. -- The authorization for the collection and analysis of an individually identifiable DNA sample shall be retained at least as long as the DNA sample is retained.

(c) COPY. -- A copy of the authorization shall be provided to the sample source or the sample source's representative.

Sec. 104. OWNERSHIP AND DESTRUCTION OF DNA SAMPLES

(a) OWNERSHIP OF THE DNA SAMPLE. -- An individually identifiable DNA sample is the property of the sample source.

(b) RIGHT TO ORDER DESTRUCTION OF THE DNA SAMPLE. -- Except when a DNA sample has been collected pursuant to section 122 or 123 of this Act, the sample source or the sample source's representative shall have the right to order the destruction of the DNA sample.

(c) ROUTINE DESTRUCTION OF SAMPLES OR IDENTIFIERS. -- An individually identifiable DNA sample must be destroyed on completion of genetic analysis unless:
(1) the sample source or the sample source's representative, has directed otherwise in writing, or
(2) all individual identifiers linking the sample to the sample source are destroyed.

Sec. 105. NOTICE OF RIGHTS AND ASSURANCES

A person who collects or stores DNA samples for genetic analysis shall provide a sample source or a sample source's representative prior to the collection, storage, or analysis of a DNA sample, and any other person upon request, with a notice of rights and assurances that contains the following information and assurances that:

(a) a DNA sample will only be used as authorized in the written authorization;

(b) an individually identifiable DNA sample is the property of the sample source;

(c) unless specifically prohibited by the sample source or sample source's representative, researchers may be granted access to DNA samples that cannot be linked to individual identifiers;

(d) the sample source or the sample source's representative has the right to order the destruction of the individually identifiable DNA sample at any time;

(e) the individually identifiable DNA sample will be destroyed on the completion of the analysis unless the sample source or the sample source's representative has previously directed otherwise in writing;

(f) the sample source can designate another individual as the person authorized to make decisions regarding the individually identifiable DNA sample after the death of the sample source; and if any person is so designated, the sample source should notify the facility in which the DNA sample is stored;

(g) the sample source or the sample source's representative has the right to examine the records containing private genetic information, to obtain copies of such records and to request correction or amendment of them;

(h) private genetic information may be disclosed to researchers who qualify for such access under this Act;

(i) the collection and analysis of the DNA sample and the private genetic information derived from the analysis is protected by this Act, and anyone whose rights under this Act have been violated can seek civil remedies, including damages, as provided in this Act; and

(j) genetic counseling is available.

PART B - - DISCLOSURE OF PRIVATE GENETIC INFORMATION

Sec. 111. DISCLOSURE OF PRIVATE GENETIC INFORMATION

(a) REQUIREMENT OF WRITTEN AUTHORIZATION. -- Except as provided in section 115 and section 132(b) no person who, in the ordinary course of business, practice of a profession, or rendering of a service, creates, stores, receives or furnishes private genetic information may by any means of communication disclose private genetic information except in accordance with a written authorization as provided for in section 112.

(b) REDISCLOSURE PROHIBITED. -- Redisclosure of private genetic information which has been disclosed to any person pursuant to a valid written authorization is prohibited.

Sec. 112. AUTHORIZATION FOR DISCLOSURE OF PRIVATE GENETIC INFORMATION

(a) WRITTEN AUTHORIZATIONS. -- To be valid, an authorization for disclosure of private genetic information must satisfy each of the following requirements:

(1) WRITING. -- The authorization must be in writing, signed by the sample source or the sample source's representative and dated on the date of such signature;
(2) SAMPLE SOURCE OR REPRESENTATIVE IDENTIFIED. -- The authorization must identify the individual granting authorization and the individual's relationship to the sample source;
(3) PERSON MAKING DISCLOSURE IDENTIFIED. -- The authorization must identify the person permitted to make the disclosure;
(4) INFORMATION DESCRIBED. -- The authorization must describe the specific genetic information to be disclosed;
(5) RECIPIENT IDENTIFIED. -- The authorization must identify the person to whom the information is to be disclosed;
(6) PURPOSE DESCRIBED. -- The authorization must describe the purpose for which the disclosure is being made;
(7) EXPIRATION DATE. -- The authorization must state the date upon which the authorization will expire, which in no event shall be longer than 30 days after the date of the authorization; and
(8) REVOCATION STATEMENT. -- The authorization must include a statement that the authorization is subject to revocation at any time before the disclosure is actually made.

(b) COPY. -- A copy of the authorization shall be provided to the person making the authorization.

(c) REVOCATION OR AMENDMENT OF AUTHORIZATION. -- A sample source or the sample source's representative may revoke or amend the authorization, in whole or in part, at any time.

(d) NOTICE OF REVOCATION. -- A sample source may not maintain an action against a person for disclosure of private genetic information made in good faith reliance on a valid authorization if the person had no notice of the revocation of the authorization at the time the disclosure was made.

(e) IDENTIFICATION OF INFORMATION AS PROTECTED BY LAW. -- Each disclosure made with the written authorization described in subsection (a) must be accompanied by the following written statement:

"This information has been disclosed to you from confidential records protected under the Genetic Privacy Act and any further disclosure of the information without specific authorization is prohibited."

(f) EFFECT OF GENERAL AUTHORIZATION FOR RELEASE OF MEDICAL RECORDS. -- A general authorization for the release of medical records or medical information shall not be construed as an authorization for disclosure of private genetic information.

Sec. 113. INSPECTION AND COPYING OF RECORDS CONTAINING PRIVATE GENETIC INFORMATION

(a) INSPECTION OF RECORDS. -- Except as otherwise provided in section 131(c)(2) and 131(f), a person who maintains private genetic information shall upon written request permit the sample source or the sample source's representative to inspect records containing private genetic information and shall provide a copy of any such records upon request by the sample source or the sample source's representative.

(b) RESPONSE TO REQUEST EXAMINATION AND COPYING OF INFORMATION. -- Upon receipt of a written request from a sample source or the sample source's representative to inspect or copy all or part of records containing private genetic information, a person as promptly as required under the circumstances but no later than 30 business days after receiving the request, shall make the information available to the sample source or the sample source's representative for inspection during regular business hours or provide a copy, if requested, to the individual.

(c) EXPLANATION OF TERMS AND CODES. -- A person shall provide an explanation of terms and any code or abbreviations used in records containing the private genetic information upon request of the sample source or the sample source's representative.

(d) FEE. -- A person may charge a reasonable fee, not to exceed the person's actual duplication cost, for copies of records which are provided.

Sec. 114. AMENDMENT OF RECORDS

(a) IN GENERAL. -- Within 45 days of receipt of a written request by a sample source or a sample source's representative to correct or amend in whole or in part any record containing private genetic information, a person who maintains records containing private genetic information shall:

(1) make the correction or amendment requested;
(2) inform the individual that the correction or amendment has been made;
(3) make reasonable efforts to inform any person to whom the uncorrected or unamended portion of the information was previously disclosed of the correction or amendment that has been made; and
(4) at the request of the individual, make reasonable efforts to inform any known source of the uncorrected or unamended portion of the information about the correction or amendment that has been made.

(b) REASONS FOR REFUSAL AND REVIEW PROCEDURES. -- If correction or amendment is refused, the person maintaining the records shall inform the sample source or the sample source's representative of:

(1) the reasons for the refusal of the person to make corrections or amendment;
(2) any procedures for further review of such refusal; and

(3) the individual's right to file with the person a concise statement setting forth the requested correction or amendment and the individual's reasons for disagreeing with the refusal of the person to make the correction or amendment.

(c) STANDARDS FOR CORRECTION OR AMENDMENT. -- A person maintaining records containing private genetic information shall correct or amend information in accordance with a request made under subsection (a) if the information is not accurate or complete for the purposes for which the information may be used or disclosed by the person.

(d) STATEMENT OF DISAGREEMENT. -- After a sample source or a sample source's representative has filed a statement of disagreement under subsection (b)(3), the person, in any subsequent disclosure of the disputed portion of the information, shall include a copy of the individual's statement and may include a statement of the reasons for not making the requested correction or amendment.

Sec. 115. DISCLOSURES PURSUANT TO COMPULSORY PROCESS

(a) PROCEEDINGS IN WHICH AVAILABLE. -- No person who maintains private genetic information may be compelled to disclose such information pursuant to a request for compulsory disclosure in any judicial, legislative, or administrative proceeding, unless:

(1) The person maintaining the genetic information has received the authorization of the sample source or the sample source's representative to release the information in response to such request for compulsory disclosure;

(2) The sample source or the sample source's representative is a party to the proceeding and the private genetic information is at issue; or

(3) The genetic information is for use in a law enforcement proceeding or investigation in which the person maintaining the information is the subject or party;

(b) NOTICE. -- If genetic information is sought under subparagraph (2) of subsection (a), or in a proceeding or investigation pursuant to subparagraph (3) of subsection (a), the person requesting compulsory disclosure shall serve upon the person maintaining the genetic information, and upon the sample source, the sample source's representative, or on the sample source's attorney, the original or a copy of the compulsory disclosure request at least thirty days in advance of the date on which compulsory disclosure is requested, and a statement of the right of the sample source or sample source's representative, and of the person maintaining the genetic information, to have any objections to such compulsory disclosure heard by such court or governmental agency prior to the issuance of an order for such compulsory disclosure, and the procedure to be followed to have any such objections heard. Such service shall be made by certified mail, return receipt requested, or by hand delivery, in addition to any form of service required by applicable state or federal law.

(c) CERTIFICATION. -- Service of compulsory process or discovery requests upon a person maintaining private genetic information must be accompanied by a written certification, signed by the person seeking to obtain the private genetic information or his or her authorized representative, identifying at least one subparagraph of subsection (a) under which compulsory process or discovery is being sought. The certification must also state, in the case of information sought under subparagraphs (2) or (3) of subsection (a), that the requirements under subsection (b) for notice have been met. A person may sign the certification only if the person reasonably believes that the subparagraph of subsection (a) identified in the certification provides an appropriate basis for the use of discovery or compulsory process. A copy of the written certification shall be maintained as a permanent part of the records of private genetic information.

(d) STANDARD FOR ISSUANCE OF ORDER. -- An order under this section may only be entered by a court of competent jurisdiction after a hearing and determination that good cause exists. To make this determination the court must find that:

(1) other ways of obtaining the private genetic information are not available or would not be effective; and

(2) there is a compelling need for the private genetic information which outweighs the potential harm to the privacy interest of the subject of the information.

(e) CONTENT OF ORDER. -- An order under this section which authorizes disclosure of private genetic information must:

(1) limit disclosure to those parts of records containing such information which are essential to fulfill the objective of the order;

(2) limit disclosure to those persons whose need for the information is the basis of the order;

(3) require the deletion of individual identifiers from any documents made available to the public; and

(4) include such other measures as are necessary to limit disclosure for the protection of the subject of the information including, but not be limited to, sealing from public scrutiny the record or any portion of the record of any proceeding for which disclosure of the information has been ordered.

THE GENETIC PRIVACY ACT

PART E - - MINORS AND INCOMPETENT PERSONS

Sec. 141. AUTHORIZATION FOR COLLECTION AND ANALYSIS OF DNA FROM MINORS

(a) INDIVIDUALS UNDER 16. -- Except as provided in sections 131(c) and 151, the individually identifiable DNA sample of a sample source who is under 16 years of age shall not be collected or analyzed to determine the existence of a gene that does not in reasonable medical judgment produce signs or symptoms of disease before the age of 16, unless:

(1) there is an effective intervention that will prevent or delay the onset or ameliorate the severity of the disease; and

(2) the intervention must be initiated before the age of 16 to be effective; and

(3) the sample source's representative has received the disclosures required by section 101 of this Act and has executed a written authorization which meets the requirements of section 103 of this Act and which also limits the uses of such analysis to those permitted by this section.

(b) INDIVIDUALS AGE 16 OR 17. -- Except as otherwise provided in sections 131(c) and 143, the individually identifiable DNA sample of a sample source who is 16 or 17 years of age may be collected and analyzed provided that--

(1) the sample source receives the information required by section 101 of this Act while accompanied by a parent or other adult family member; and

(2) the sample source executes a written authorization which meets the requirements of section 103 of this Act.

(c) DESTRUCTION OF DNA SAMPLES OF INDIVIDUALS UNDER 16. -- A sample source's representative may, on behalf of a sample source who is under 16 years of age, order the destruction of a DNA sample collected pursuant to subsection (a) of this section.

Sec. 142. AUTHORIZATION FOR DISCLOSURE OF PRIVATE GENETIC INFORMATION ABOUT INDIVIDUALS AGE 16 OR 17

(a) AUTHORIZATION REGARDING INDIVIDUALS. -- Except as provided by section 144, private genetic information about an individual who is age 16 or 17 shall not be disclosed unless the sample source has executed a written authorization which meets the requirements of section 112.

(b) AUTHORIZATION REGARDING INDIVIDUALS UNDER 16. -- Except as provided in section 152, private genetic information about a minor who is under 16 years of age shall not be disclosed unless a parent or other sample source's representative has executed a written authorization that meets the requirements of section 112.

PART F - - PREGNANT WOMEN, FETUSES, AND EXTRACORPOREAL EMBRYOS

Sec. 151. AUTHORIZATION FOR COLLECTION AND ANALYSIS OF DNA FROM PREGNANT WOMEN AND FETUSES

Regardless of her age, a pregnant woman shall have all the rights and authority of an adult sample source in regard to her DNA sample and the DNA sample of her fetus unless she is otherwise incompetent under the provisions of section 143.

Sec. 152. AUTHORIZATION FOR DISCLOSURE OF PRIVATE GENETIC INFORMATION ABOUT PREGNANT WOMEN AND FETUSES

Regardless of her age, a pregnant woman shall have all the rights of an adult sample source in regard to records containing private genetic information as provided in section 113, 114, and 115 of this Act, and in regard to disclosure of genetic information resulting from an analysis of her DNA sample or the DNA sample of her fetus, unless she lacks the ability to understand the information contained in an authorization under section 112.

III

COMMENTARY

This commentary explains why and how decisions were made about provisions of the Genetic Privacy Act to help readers understand both its scope and the intent of the drafters. Those parts of the Act that are self-explanatory are not referenced in this section.

Sec. 3. DEFINITIONS

(m) PRIVATE GENETIC INFORMATION. - The term "private genetic information" means any information about an identifiable individual that is derived from the presence, absence, alteration, or mutation of a gene or genes, or the presence or absence of a specific DNA marker or markers, and which has been obtained:

(1) from an analysis of the individual's DNA; or

(2) from an analysis of the DNA of a person to whom the individual is related.

The term "Private Genetic Information" is the key to the Act because it defines the information that is protected by it. This definition recognizes that not all genetic information needs or warrants legal protection, and limits the Act's protection to information derived from DNA analysis. The Act, accordingly, does not protect genetic information derived from medical examinations, family histories, or pedigrees.

Like other kinds of personal information, some genetic information is more sensitive than other genetic information. Control of some genetic information is more critical for the exercise of personal autonomy, and publication or disclosure of some genetic information can be more damaging or stigmatizing than disclosure of other genetic information. For instance, although height, eye and skin color, and other physical characteristics are inherited and therefore genetic information, such externally-expressed genetic information is not private. On the other hand, knowledge about the presence of a gene that makes it probable that the individual will suffer a debilitating disease later in life is private information, at least until a point in time when symptoms become manifest or the individual intentionally discloses the information.

We wanted to draft a definition that is based on a principled distinction between "private" and other genetic information, and at the same time susceptible to practical application. The manner in which genetic information is created contributes to its private nature. Genetic analysis of an individual's DNA, such as testing for a specific disease gene, particularly if signs and symptoms of the disease are not manifested, is an obvious source of such private information. Similarly, if an analysis reveals that an individual is the carrier of a recessive disease gene which could be passed on to offspring, this carrier status is private information if derived from a DNA analysis. Carrier status could also be inferred from a genetic condition in an individual's child. Therefore, another source of private genetic information about an individual is the analysis of the DNA of a close relative of the individual.

Private genetic information can also be obtained from a family history of a genetic disease. Physicians who inquire about the incidence of a particular condition in a patient's family acquire private genetic information on a regular basis. This source of private genetic information is the least susceptible to regulation and control because it is virtually impossible to distinguish such private genetic information from other family medical history in any principled way.

Development of a genetic medical history can be a complex process involving review of medical records of several family members, or it can result simply from asking the patient a few questions about specific relatives. Regardless of the nature of the inquiry, the purpose is the same: to determine an individual's risk of having inherited a gene. For example, developing a family pedigree or history can be used to determine whether or not a woman is likely to have inherited a breast cancer gene. The prediction that an individual family member has inherited the gene may be based solely on the patient's report of the age and relationship of other women in the family who have developed cancer. *[8]*

Although one process uses DNA analysis and the other does not, both lead to the creation of the same private genetic information: the prediction of a predisposition to disease. Nonetheless, distinguishing between "private genetic information" derived from a family history and other medical information derived from a family history is problematic. For example, it is difficult, if not impossible, to distinguish between the prediction of having inherited the breast cancer gene, based on disease occurrence in the family, and establishing a person's risk for other diseases, such as heart disease or diabetes, based on the prevalence of these diseases in a family.

Inclusion of family history-based risk information in the definition of "Private Genetic Information" would protect information that has historically been collected and disclosed as ordinary medical information, and virtually all medical records would be subject to the provisions of the Act. Extending the umbrella of protection through such an expansive definition would necessitate the overhaul of well established medical information practices and policies.

A similar analysis leads to the same conclusion regarding biochemical tests that detect the presence or absence of a protein that indicates the presence or absence of a particular gene. By not including genetic information derived from family histories, biochemical tests, or methods other than DNA analysis, we recognize that some genetic information will escape the protection of the Act. We have opted to exclude this type of genetic information to avoid the enormous practical problems presented by including it. Despite this underinclusiveness, we believe our definition is consistent with the goal of protecting information developed within the context of the Human Genome Project as a result of mapping the human genome: information derived from DNA analysis is subject to uniform and comprehensive privacy protection.

PART A

COLLECTION AND ANALYSIS OF DNA SAMPLES

Sec. 101. COLLECTION OF DNA SAMPLES

(a) REQUIREMENT OF WRITTEN AUTHORIZATION. -- Except as otherwise provided in sections 121, 122, and 123, no person may collect or cause to be collected an individually identifiable DNA sample for genetic analysis without the written authorization of the sample source or the sample source's representative.

(b) REQUIRED INFORMATION. -- Prior to the collection of a DNA sample from a sample source for genetic analysis, the person collecting the sample or causing the sample to be collected shall verbally inform the sample source or the sample source's representative:

(1) that consent to the collection or taking of the DNA sample is voluntary;
(2) that consent to the genetic analysis is voluntary;
(3) of the information that can reasonably be expected to be derived from the genetic analysis;
(4) of the use, if any, that the sample source or the sample source's representative will be able to make of the information derived from the genetic analysis;
(5) of the right to inspect records that contain information derived from the genetic analysis;
(6) of the right to have the DNA sample destroyed;
(7) of the right to revoke consent to the genetic analysis at any time prior to the completion of the analysis;
(8) that the genetic analysis may result in information about the sample source's genetic relatives which may not be known to such relatives but could be important, and if so the sample source will have to decide whether or not to share that information with relatives;
(9) that in the future someone else may ask if the sample source has obtained genetic testing or analysis and condition a benefit on the disclosure of information regarding such testing or analysis;
(10) that the collection and analysis of the DNA sample, and the private genetic information derived from the analysis is protected by this Act; and
(11) of the availability of genetic counseling.

This section sets forth the general prohibition against collection of identifiable DNA samples without the written authorization of the sample source or that person's representative. In addition, this section requires that particular information be verbally communicated before an authorization is obtained. These requirements are designed to foster a knowledgeable and voluntary decision to proceed with the collection and analysis of a DNA sample. A perfunctory recitation should be discouraged, despite the fact that mere delivery of the information would technically satisfy the requirement of this section. Those who collect DNA samples should be encouraged to expand upon the minimum information required by providing additional information they believe to be beneficial to individuals who plan to have their DNA analyzed.

The information that must be provided under this Act is similar to the kind of information that must be disclosed before obtaining consent for diagnostic tests that reveal highly private and sensitive information. For example, several state laws require that anyone undergoing an HIV test must first be told about the information that the test can yield, the reliability of the test, and how the information can be used by the individual that is tested, in addition to how the information may be used by others who become aware of it.*[11]* Such requirements are warranted because, if disclosed, information on HIV status could result in economic, social or psychological harm. Similarly, genetic information may be used to preclude the sample source from obtaining an economic or social service benefit.

Disclosure of genetic information can also have a harmful effect because it can also indicate the presence or absence of a stigmatizing condition or disease. The sample source should therefore be told that others may ask if the sample source has had a DNA analysis, and the results obtained.

An additional disclosure, required by section 101(b)(8), is intended to address the fact that the results of genetic analysis can reveal that others are likely to be affected by the same genetic condition or disease as the individual whose DNA is to be analyzed. This section, therefore, also requires that the person be informed:

that the genetic analysis may result in information about the sample source's genetic relatives which may not be known to such relatives but could be important and if so the sample source will have to decide whether or not to share the information with relatives.

DNA analysis may reveal that other relatives are likely to be gene carriers, to have a gene that codes for disease, or to be predisposed to developing a particular disease or condition by reason of their genetic relationship to the sample source. In effect, the uncollected DNA of family members is indirectly analyzed. This aspect of genetic analysis raises questions about whether such family members should be told about their possible risks and if so, by whom and how? One suggestion is that access to genetic testing in some circumstances be made conditional on a prior agreement to disclose information to other family members who become identified as at risk.*[12]* This suggestion, however, has not been widely supported for several reasons, including the fact that it would deter individuals from seeking information about themselves.*[13]*

Creating either a contractual or statutory obligation for individuals to share such information with their family members would be not only unprecedented, but inadvisable. The creation of new substantive rights or duties of family members is not our intention and is beyond the scope of this Act. However, because the Act creates rules that govern the use and disclosure of information, it is imperative that individuals be informed of the fact that by seeking genetic information about themselves through genetic analysis, they may also become privy to information about other family members who would also want and/or need such information. A person seeking genetic analysis will not always be able to anticipate the nature of the information that can result and must therefore be informed of this possibility before the analysis is authorized. While it will be an individual choice as to whether or not to share that information with others, this disclosure should instigate discussion between the sample source and the collector of the sample.

For example, if as a result of the analysis of the DNA of the sample source it could be determined that the person's sibling is also the carrier of a genetic condition, and could pass the condition to offspring, or could suffer in the future from a genetic condition that can be ameliorated or treated, the sample source must be informed that he or she will have to decide whether or not to share that information with the sibling once the results are known. Despite the absence of a legal obligation to do so, the sample source should be encouraged out of moral obligation to share as much of the information as would provide the sibling, or other relatives, with the opportunity to obtain information about their own condition or risk. Since this is a foreseeable and a relatively common burden resulting from DNA analysis, its disclosure is necessary. This issue is discussed in more detail in the Appendix.

Availability of genetic counseling can also provide the sample source with help in deciding how and when to initiate discussion with relatives, and in determining how much information about their own status they are comfortable sharing with others. Consequently, in addition to disclosing the nature and scope of the information that the analysis will produce, section 101(b)(11) requires that the person who collects the sample must provide information on the **availability** of such counseling. This requirement can be fulfilled

by telling the individual about the existence of genetic counselors whose expertise is to help individuals understand what genetic information that can be derived from DNA analysis means, and plan in light of such information. The person could suggest how a genetic counselor could be located by those who decide a consultation would be desirable. The person collecting the sample is not, however, required to provide such counseling, nor would they be obligated to take any steps to ensure that the individual is referred to a specific counselor.

This limited requirement will not be burdensome, since it would be rare for anyone who regularly collects and analyzes DNA samples not to have information about genetic counseling services. Research and clinical programs that conduct DNA analysis often utilize such services, receive references from such services or at least recommend that subjects or patients take advantage of the assistance counselors can give. Anyone collecting and analyzing DNA samples as a regular part of their business or practice should have some awareness of this emerging field, and requiring some discussion about the availability of genetic counseling is consistent with present practices of many programs.

This requirement is supported by the recommendations of other experts who have studied the effects of genetic information.*[14]* Research and experience with Huntington Disease linkage studies and other genetic testing has demonstrated that pre-test counseling as well as post-test counseling is needed for those who face the choice of having DNA analyzed and the possibility of sharing such information with others.*[15]* Test results can have an impact, not only on the self perception of the individual who has been tested, but on family relationships as well. Particular attention has been focused on the effect of information about the inheritance of this disease on family relationships and personal identity.*[16]* Although Huntington Disease is an extreme example because the disease itself is devastating, it presents issues that are typical in genetic testing and analysis.*[17]*

Sec. 104. OWNERSHIP AND DESTRUCTION OF DNA SAMPLES

(a)OWNERSHIP OF THE DNA SAMPLE. - An individually identifiable DNA sample is the property of the sample source.

(b)RIGHT TO ORDER DESTRUCTION OF THE DNA SAMPLE. - Except when a DNA sample has been collected pursuant to section 122 or 123 of this Act, the sample source or the sample source's representative shall have the right to order the destruction of the DNA sample.

(c)ROUTINE DESTRUCTION OR REMOVAL OF IDENTIFIERS. - An individually identifiable DNA sample must be destroyed on completion of genetic analysis unless:

(1)the sample source or the sample source's representative has directed otherwise in writing, or

(2)all individual identifers linking the sample to the sample source are destroyed.

Some individuals will want to take maximum advantage of the evolving nature of knowledge about the human genome, and will welcome the opportunity to have their DNA collected, stored or analyzed. Others are wary of the potential harm that can result from information derived from genetic analysis, and will want reassurance that they alone control when their DNA is analyzed and who has access to their samples and information. The provisions of this section are intended to preserve the autonomy of all individuals regardless of their varying views on the benefits and dangers of genetic information.

Giving individuals control over their DNA is accomplished first by establishing that an individually identifiable DNA sample is the property of the sample source. Since the sample source has this property right, control of a sample can be transferred to another individual through a will or other legal instrument. Consequently, individuals who do not want their DNA analyzed during their own lifetime may nevertheless have a sample collected and stored for the benefit of others. Descendants to whom control over DNA samples is transferred could thus benefit from future developments in genetics which require analysis of DNA from multiple generations. Until the complete genome is mapped, locating genes through linkage analysis will be dependent upon the availability of such samples. This provision can promote this availability.

In addition to being able to transfer ownership of a sample, the sample source also has the right, except in limited circumstances, to order the destruction of a sample that has been collected. [section 104(b)] This gives those who want to limit the availability of such samples reassurance that once authorized analysis has been completed, the sample itself can be destroyed, preventing any additional unauthorized analysis. In some circumstances, a sample source's representative, such as the parent of a minor, can exercise this right on behalf of the individual from whom the sample has been collected. However, this right is not exercisable by either the sample source or a sample source's representative when samples have been collected for identification use in law enforcement (section 122), or when the sample has been collected pursuant to a court-ordered analysis (section 123). Requiring that the person analyzing such samples destroy them at the direction of a sample source would directly conflict with the compulsory nature of collection and analysis in these situations.

Finally, section 104 provides for routine destruction of DNA samples or removal of identifiers, after the completion of the authorized analysis. This routine destruction can be overridden by the explicit directions of the sample source or the sample source's representative. [section 104(c)] Routine destruction would not result in an irreplaceable loss, since each individual is the source of an abundant supply of DNA samples. If an individual anticipates having a series of analyses conducted, and wants to avoid what is perceived as the inconvenience of collecting multiple samples, the authorization for collection of a specimen containing DNA can include specific directions for storage of the sample for analysis in the future, provided, of course, that storage services are offered by the collector or analyzer.

Sec. 105. NOTICE OF RIGHTS AND ASSURANCES. -- A person who collects or stores DNA samples for genetic analysis shall provide a sample source or a sample source's representative prior to the collection, storage, or analysis of a DNA sample, and any other person upon request, with a notice of rights and assurances that contains the following information and assurances that:

(a)a DNA sample will only be used as authorized in the written authorization;

(b)an individually identifiable DNA sample is the property of the sample source;

(c)unless specifically prohibited by the sample source or sample source's representative, researchers may be granted access to DNA samples that cannot be linked to individual identifiers;

(d)the sample source or the sample source's representative has the right to order the destruction of the individually identifiable DNA sample at any time;

(e)the individually identifiable DNA sample will be destroyed on the completion of the analysis unless the sample source or the sample source's representative has previously directed otherwise in writing;

(f)the sample source can designate another individual as the person authorized to make decisions regarding the individually identifiable DNA sample after the death of the sample source; and if any person is so designated, the sample source should notify the facility in which the DNA sample is stored;

(g)the sample source or the sample source's representative has the right to examine the records containing private genetic information, to obtain copies of such records and to request correction or amendment of them;

(h)private genetic information may be disclosed to researchers who qualify for such access under this Act;

(i)the collection and analysis of the DNA sample and the private genetic information derived from the analysis is protected by this Act, and anyone whose rights under this Act have been violated can seek civil remedies, including damages, as provided in this Act; and

(j)genetic counseling is available.

Individuals who authorize the collection and analysis of their DNA may not be aware of their rights under this Act and therefore be unable to exercise them. To enhance the knowledge of one's rights, this section requires that persons who collect DNA samples provide written notice to the individual when authorization for collection, storage and analysis of the DNA sample is obtained. This notice is similar in function and content to notices of fair information practices required by other informational privacy statutes.*[19]* However, since the Act has provisions relating to the collection and analysis of samples, in addition to provisions that govern the information that results from such activities, the notice required by section 105 is more inclusive than other information practices.

A notice prepared under this section does not contain contractual assurances, but will consist of a series of statements regarding the legal responsibilities of those who collect, store and analyze samples, and the legal rights of the sample source.

PART B

DISCLOSURES OF PRIVATE GENETIC INFORMATION

Sec. 111. DISCLOSURE OF PRIVATE GENETIC INFORMATION

(a) REQUIREMENT OF WRITTEN AUTHORIZATION. - Except as provided in section 115 and section 132(b) no person who, in the ordinary course of business, practice of a profession, or rendering of a service, creates, stores, receives or furnishes private genetic information may by any means of communication disclose private genetic information except in accordance with a written authorization as provided in section 112.

(b) REDISCLOSURE PROHIBITED. - Redisclosure of private genetic information which has been disclosed to any person pursuant to a valid written authorization is prohibited.

This section states the general rule that any person who creates, maintains or furnishes private genetic information as part of their ordinary business or professional activities may disclose such information only in accordance with written authorization. (Exceptions to this general rule are presented in sections 115 and 132(b) and are discussed below.) These provisions apply to health care providers, lab technicians, genetic counselors, researchers, insurers and anyone else whose activities fall within the description in this section, regardless of the number of individuals on whom they have information. Section 111 also prohibits redisclosure of information received pursuant to a valid authorization.

Not all disclosures of private genetic information are prohibited by this or any other section of the Act. For example, nothing in the language of this statute prohibits a friend, neighbor, relative or any other person not engaged in such business activities from repeating genetic information that is learned directly or indirectly from a sample source or someone knowledgeable about the sample source. Consequently, anyone who wants to recover for unauthorized disclosures of information by such individuals will have to look to common law torts or other statutes for a cause of action and a remedy.

The Act does not carve out an exception for disclosures of genetic information without the individual's authorization, as do some other statutes that deal with medical information. Where some statutes governing medical information permit breaches of confidentiality by professionals in emergency circumstances to prevent harm to another individual,*[20]* the Act does not permit disclosure of private genetic information without authorization, regardless of how well-intentioned the purpose of the contact with another individual. A full discussion of the common law on this issue appears in the Appendix.

Therefore, when it is anticipated that the analysis of one person's DNA will reveal that a second individual (usually a close relative)is or may be at risk, the individual who has authorized an analysis should be encouraged to share the information with other family members who might benefit from it.

Sec. 112. AUTHORIZATION FOR DISCLOSURE OF PRIVATE GENETIC INFORMATION

(a)WRITTEN AUTHORIZATIONS. -- To be valid, an authorization for disclosure of private genetic information must satisfy each of the following requirements:

(1)WRITING. -- The authorization must be in writing, signed by the sample source or the sample source's representative and dated on the date of such signature;

(2)SAMPLE SOURCE OR REPRESENTATIVE IDENTIFIED. -- The authorization must identify the individual granting authorization and the individual's relationship to the sample source;

(3)PERSON MAKING DISCLOSURE IDENTIFIED. -- The authorization must identify the person permitted to make the disclosure;

(4)INFORMATION DESCRIBED. -- The authorization must describe the specific genetic information to be disclosed;

(5)RECIPIENT IDENTIFIED. -- The authorization must identify the person to whom the information is to be disclosed;

(6)PURPOSE DESCRIBED. -- The authorization must describe the purpose for which the disclosure is being made;

(7)EXPIRATION DATE. -- The authorization must state the date upon which the authorization will expire, which in no event shall be longer than 30 days after the date of the authorization; and

(8)REVOCATION STATEMENT. -- The authorization must include a statement that the authorization is subject to revocation at any time before the disclosure is actually made.

(b)COPY. -- A copy of the authorization shall be provided to the person making the authorization.

(c)REVOCATION OR AMENDMENT OF AUTHORIZATION. -- A sample source or the sample source's representative may revoke or amend the authorization, in whole or in part, at any time.

(d)NOTICE OF REVOCATION. -- A sample source may not maintain an action against a person for disclosure of private genetic information made in good faith reliance on a valid authorization if the person had no notice of the revocation of the authorization at the time the disclosure was made.

(e)IDENTIFICATION OF INFORMATION AS PROTECTED BY LAW. -- Each disclosure made with the written authorization described in subsection (a) must be accompanied by the following written statement:

"This information has been disclosed to you from confidential records protected under the Genetic Privacy Act and any further disclosure of the information without specific authorization is prohibited."

(f)EFFECT OF GENERAL AUTHORIZATION FOR RELEASE OF MEDICAL RECORDS. -- A general authorization for the release of medical records or medical information shall not be construed as an authorization for disclosure of private genetic information.

This section sets forth the requirement for a valid authorization which must be specific and in writing. The purpose is to prevent disclosures of genetic information under blanket releases of information and overly broad and unnecessary access to highly personal information.

The individual who authorizes the disclosure may revoke it at any time. However, anyone who does not receive notice of a revocation, and who makes a disclosure in good faith reliance on the authorization, will not be liable for violating this Act [section 112(d)]. The individual may only be able to express a revocation orally, so a written revocation is not required. However, when possible and to prevent the holder of the authorization from denying awareness of revocation, it would make sense for any individual who intends to revoke authorization, or to amend the provisions of an authorization, to do so in writing.

Those governed by the provisions of sections 111 and 112 would, at a minimum, include researchers, independent databanks, clinical laboratories, medical care providers and insurers. Although few insurers at the present time routinely request or require DNA analysis in the course of processing applications, some insurers are interested in obtaining access to private genetic information that already exists.*[21]* They can

do so by directly asking applicants if they have had genetic analysis and by obtaining information contained in medical records. While most applicants are not likely to have had any DNA analysis done prior to an application for insurance, this may change in the future. This change could be precipitated by several factors, including the identification of genes that predispose individuals to common diseases such as cancer and the development of readily available and cost effective predictive testing for such disorders.*[22]*

When an individual has had a DNA analysis and the resultant private genetic information is entered into medical records, an authorization for disclosure that meets the requirements of this Act is required before such information can be disclosed. The Act specifically provides that a general authorization for disclosure of medical information does not fulfill this requirement [section 112(f)]. Consequently, a provider disclosing medical information to an insurer, an employer, or any other person, must be careful that private genetic information is not disclosed along with other information unless it has been specifically authorized. Those who maintain medical records that include private genetic information as defined by the Act, must develop record keeping policies and procedures that adequately guard against wrongful disclosures of such information under general releases of medical information.

A rule that would require complete segregation of private genetic information from medical records would facilitate compliance with these provisions. Nonetheless, we believe such a statutory requirement is neither practical nor advisable. At least some private genetic information may be necessary for the provision of adequate and appropriate medical treatment. Inclusion of such information in medical records is, therefore, left to the discretion of providers and the developing standards of care. Disclosure of such information, on the other hand, is not discretionary and can only be made when the individual specifically authorizes it, and when the purpose of the disclosure has been explicitly documented. Nothing in these provisions, however, would require that providers disclose private genetic information, if to do so would conflict with any other law or professional ethics.

Accommodating the provisions of these sections should not be burdensome on those who maintain such information whether or not it is incorporated in medical records. Developing authorization forms that meet these requirements should not be any more difficult than development of forms and procedures so as to comply with federal regulations governing the confidentiality of alcohol and substance abuse treatment, as well as other laws governing medical records.*[23]* Since most medical records in the future are likely to be maintained in electronic format, it should be feasible to program record keeping so that private genetic information can be deleted from records prior to release under a general authorization.

PART E

MINORS AND INCOMPETENT PERSONS

Sec. 141. AUTHORIZATION FOR COLLECTION AND ANALYSIS OF DNA FROM MINORS

(a) INDIVIDUALS UNDER 16. -- Except as provided in sections 131(c) and 151, the individually identifiable DNA sample of a sample source who is under 16 years of age shall not be collected or analyzed to determine the existence of a gene that does not in reasonable medical judgment produce signs or symptoms of disease before the age of 16, unless:

(1) there is an effective intervention that will prevent or delay the onset or ameliorate the severity of the disease; and

(2) the intervention must be initiated before the age of 16 to be effective, and

(3) the sample source's representative has received the disclosures required by section 101 of this Act and has executed a written authorization which meets the requirements of section 103 of this Act and which also limits the uses of such analysis to those permitted by this section.

(b) INDIVIDUALS AGE 16 OR 17. -- Except as otherwise provided in sections 131(c) and 143, the individually identifiable DNA sample of a sample source who is 16 or 17 years of age may be collected and analyzed provided that--

(1) the sample source receives the information required by section 101 of this Act while accompanied by a parent or other adult family member; and

(2) the sample source executes a written authorization which meets the requirements of section 103 of this Act.

(c) DESTRUCTION OF DNA SAMPLES OF INDIVIDUALS UNDER 16. -- A sample source's representative may, on behalf of a sample source who is under 16 years of age, order the destruction of a DNA sample collected pursuant to subsection (a) of this section.

Sec. 142. AUTHORIZATION FOR DISCLOSURE OF PRIVATE GENETIC INFORMATION ABOUT INDIVIDUALS AGE 16 OR 17

(a) AUTHORIZATION REGARDING INDIVIDUALS. -- Except as provided by section 144, private genetic information about an individual who is age 16 or 17 shall not be disclosed unless the sample source has executed a written authorization which meets the requirements of section 112.

(b) AUTHORIZATION REGARDING INDIVIDUALS UNDER 16. -- Except as provided in section 152, private genetic information about a minor who is under 16 years of age shall not be disclosed unless a parent or other sample source's representative has executed a written authorization that meets the requirements of section 112.

The collection and genetic analysis of DNA from minors is governed by different standards depending on the circumstances, which fall into these general categories and are summarized in Table 1.

1. Rules that govern genetic analysis in the context of research and which apply to all minors (previously discussed and set forth in section 131);

2. Rules that govern genetic analysis in a non-research context and which apply to minors under the age of 16 [section 141(a)];

3. Rules that govern genetic analysis in a non-research context and which apply to minors age 16 and 17 [section 141(b)]; and

4. Rules that govern genetic analysis of DNA of pregnant minors (sections 151, 152).

The Act forbids the genetic testing of children for conditions that will not be manifested until after the child becomes an adult. This accords with the positions of others who have commented upon this topic. For example, the Institute of Medicine's report on **Assessing Genetic Risks** states:

> Children should generally be tested only for genetic disorders for which there exists an effective curative or preventive treatment that must be instituted early in life to achieve maximum benefit. Childhood testing is not appropriate for carrier status, untreatable childhood diseases, and late onset diseases that cannot be prevented or forestalled by early treatment.*[43]*

Similarly, other commentators have said, "The only justification for doing predictive testing in childhood is if an advantage can clearly be demonstrated for the child.*[44]* These statements and the prohibition of such childhood testing are controversial because they remove authority from parents who may wish to have their offspring tested.

There are two reasons for this prohibition on the exercise of parental discretion. First, if someone learns that the child is a carrier of a gene that disposes the child to some condition later in life, this finding may subject the child to discrimination and stigmatization by both the parents and others who may learn of this fact. Second, a child's genetic status is the **child's** private genetic information and should not be determined or disclosed unless there is some compelling reason to do so.

Parents have an enormous amount of discretion and authority when it comes to making child rearing decisions. Indeed, such authority has constitutional dimensions.*[45]* Parents are given this authority because it is assumed that they will act in the best interests of their children. However, there are social policies that deprive parents of discretion in a number of areas. For example, child labor laws and mandatory education laws forbid parents from sending their young children to work or from withholding basic educational opportunities from their children. Even in circumstances in which parents have a religious

objection to mandatory education, the state may require that children receive enough instruction so that children learn basic reading, writing and math skills.*[46]*

Parents have broad discretion, but not absolute discretion, in making health care decisions for their children. For example, the state may require that children receive certain services, such as vaccination, even over parental objections. When a child is ill parents can choose between alternative suggested remedies and can choose to use no remedies in most cases. However, parents may not refuse to provide children with care that is necessary to sustain the child's life, because in such an instance there can be no argument that the parent is acting in the child's best interest.

Parents also have access to their children's medical records and other medical information as a general rule. This is because parents need to have such access to make informed medical decisions about their children's care. But when parents are not in the position to make health care decisions for their children there is no justification for parents to have access to these records. Thus, when minor children are authorized to make treatment decisions for themselves as a result of emancipation or maturity, their medical records are confidential and their parents are not authorized to obtain access to this private medical information.*[47]*

It is increasingly recognized that children have rights independent of parent's rights. Thus minor women have a constitutional right to obtain abortions without their parents' consent or knowledge because minors have a constitutional right to privacy.*[48]* Likewise, minors have a constitutional right to obtain contraceptives without parental involvement.*[49]* The exercise of these rights by minors is dependent upon their maturity to make the decisions necessary to use these services.

The Act's limitation of parental authorization for genetic testing does not provide minors with decisional rights, but rather provides them with protection from potential harm. In this regard it is similar to the prohibition on parents from consenting to research for their children in which the research presents a risk of harm to the child with no benefit. Not only is such research strictly regulated, there are those who argue that it should be absolutely banned.*[50]* The further purpose of the limitation of parental authority to authorize collection and analysis is to protect the child's privacy interest in his or her own genetic information. This information will not only exist during the child's minority but will continue to exist when the child becomes an adult. As a result, a parent's curiosity about a child's genetic information should not be sufficient to breach the child's (and later the adult's) privacy interest in this genetic information. If, however, there is sufficient justification, a parent may authorize the collection and analysis of DNA samples. It is for this reason that the Act makes an exception for the collection and analysis of genetic material where it is necessary in order to ameliorate, prevent or treat a condition that will manifest itself prior to the time when the child is authorized to consent to such DNA collection and analysis. This exception enables parents to play their traditional protective role, and provides them with the authority to obtain necessary information when needed for them to act in their child's best interest.*[51]*

Under the Act 16 and 17 year olds have the same rights as adults in nonresearch settings to authorize genetic analysis [section 141 (b)]. This accords with the increasing recognition that mature minors are entitled to make medical decisions for themselves. Consequently, if a 16 or 17 year old wanted information about carrier status, such screening could be conducted under his or her sole authorization. This information would mostly be relevant to decisions relating to reproduction. Although unlikely, a 16 or 17 year old could seek genetic analysis either prior to becoming pregnant, or in relation to the decision to continue with a pregnancy. Where the young woman is already pregnant, under the Act, no restrictions are placed on her pursuit of such analysis and genetic information regarding herself or her fetus. (sections 151, 152)

In all other nonresearch circumstances, however, the Act requires that the 16 or 17 year old be accompanied by an adult family member at the time that the information required by section 101 (b) is given to him or her. [section 141 (b)(1)] The decision to include an adult family member in this process is not up to the young person, as some state statutes provide regarding abortion counseling.*[52]* The Act requires an adult's involvement. Although the decision to undergo genetic analysis is a highly personal and private one, it is unlike the decision to continue a pregnancy in that requiring the involvement of a family member does not expose the minor to the same familial repercussions. The goal of this requirement is to provide family support for the minor who is faced with a novel situation which involves obtaining and

processing complex information. Since the collector of the sample is likely a stranger, regardless of how skilled this person is in communicating information, he or she may not be aware of, or sensitive to, the burden that such information can place on even a mature minor. A family member will also have a shared interest in protecting family privacy and will be aligned with the minor if issues of disclosure to other family members arise or are anticipated.

The Act does not require the authorization of the adult family member prior to the collection of a sample for analysis. The role of the adult family member in the authorization process is limited to providing support and guidance. The decision not to require dual consent of parent and minor when the minor is 16 or 17 years old is intentional. We want to avoid giving greater deference to the interests of a parent or family member than to the autonomy of the mature minor who seeks genetic analysis. Actual exercise of this authority by a 16 or 17 year old will undoubtedly be rare. In general, those likely to seek such genetic analysis will do so out of a need to know if they are at risk for a specific genetic disease that is known to be present in the family. Unlike adults, 16 and 17 year olds do not generally seek genetic analysis and information in the context of reproductive planning, since few teenage pregnancies are the result of conscious and careful planning.

[1] Annas GJ & Elias S, eds, *Gene Mapping: Using Law and Ethics as Guides*, New York: Oxford University Press, 1992, p.9.

[2] Council of State Governments, *Advances in Genetic Information: A Guide for State Policy Makers,* Lexington, Ky: Council of State Governments, 1992; Privacy Commissioner of Canada, *Genetic Testing and Privacy*, Ottawa, Ontario: Privacy Commissioner of Canada, 1992.

[3] Annas GJ, "Privacy Rules for DNA Databanks", 270 JAMA 2346 (1993).

[4] Domestic and International Data Protection Issues, Hearings before the Subcommittee on Government Information, Justice, and Agriculture of the Committee on Government Operations, U.S. House of Representatives, 102d Cong., 2d Sess. (1991).

[5] Current confidentiality protections are inadequate even to protect individual medical records, a circumstance widely recognized during the recent health care reform debate. *See*, *e.g.*, the medical records section of *The Health Security Act* as amended in the U.S. House of Representatives, *Report on the Health Security Act*, Committee on Government Operations, Aug. 12, 1994; and *Hearings on Health Reform, Health Records, Computers and Confidentiality*, Committee on Government Operations, U.S. House of Representatives, 103d Cong. 1st Sess., Nov. 4, 1993; and *Hearings on Fair Health Information Practices Act of 1994,* April 20, May 4-5, 1994. *See also*, Wilker NL, Stawski S, Lewontin R, Billings PR, "DNA Data Banking and the Public Interest", in Billings PR, ed, DNA *on Trial: Genetic Information and Criminal Justice*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1992, pp. 141-149; Yates JRW, Malcom S, Read AP, "Guidelines for DNA Banking: Report of the Clinical Genetics Society Working Party on DNA Banking", 16 *J. Med. Genet*. 245 (1989); Ad Hoc Committee on DNA Technology, American Society of Human Genetics, DNA Banking and DNA Analysis: Points to Consider. 42 *Am. J. Hum. Genet*. 781 (1986); and Andrews LB, DNA Testing, Banking and Individual Rights, in Knoppers BM & Laberge GM, eds, *Genetic Screening: From Newborns to DNA Typing*, Amsterdam: Excerpta Medica, 1990, pp. 217-242.

[6] Designing Genetic Information Policy: The Need for an Independent Policy Review of the Ethical, Legal and Social Implications of the Human Genome Project, Committee on Government Operations, U.S. House of Representatives, 102d Cong, 2d Sess., Rep. No.16, April 2, 1992, p.25.

[7] Congress has recently acted to protect genetic information derived from DNA samples held by law enforcement agencies for identification purposes. *See*, Violent Crime Control and Law Enforcement Act of 1994, P.L. 103-322 210305. This law would not be affected by the Genetic Privacy Act.

[8] See discussion of the use of family history by physician to conclude that an individual suffered from breast-ovarian carcinoma syndrome despite no manifested symptoms of disease in Katskee v. Blue Cross/Blue Shield of Nebraska, 245 Neb. 808, 515 N.W.2d 645 (1994), discussed in Annas, GJ, "When Should Preventive Treatment be Paid for by Health Insurance?" 331 New Eng. J. Med. 1027 (1994).

[9] Ad Hoc Committee on DNA Technology, American Society of Human Genetics, "DNA Banking and DNA Analysis: Points to Consider," 42 Am. J. Hum. Genetics 781 (1988).

*[10]*Office of Technology Assessment, U.S. Congress, *Genetic Witness: Forensic Uses of DNA Tests*, Washington, D.C.: U.S. Government Printing Office, 1990, p.132.

For detailed descriptions of the process and procedures used in DNA typing see Thompson, WC, "Evaluating the Admissibility of New Genetic Identification Tests: Lessons form the 'DNA War'", 84 J. Crim. L. 22 (1993); Committee on DNA Technology in Forensic Science, "DNA Typing Technical Considerations" in *DNA Technology in Forensic Science*, National Academy Press, Washington, D.C., 1992, pp.51-73.

[11] See, *e.g.*, Cal. Ins. Code 799.03(Deering 1994) (requiring that prior to execution of consent for HIV related test, insurers must provide printed materials on HIV, information on what the subject can do with the results, a list of available counseling services and sources of additional help); N.Y. Pub. Health Law 2781 (Consol 1993) (requiring that person ordering an HIV related test explain the nature of the illness, and provide information about discrimination problems that might result); Pa. Cons. Stat. 7605 (1993) (requiring that explanation of HIV related test and information on the availability of information about exposure and transmission and suggestion that subject may desire pre-test counseling be communicated prior to test).

[12] President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, *Screening and Counseling for Genetic Conditions* Washington, D.C.: U.S. Government Printing Office, 1983, p.43.

[13] Chapman M, "Invited Editorial: Predictive Testing for Adult Onset Genetic Disease: Ethical and Legal Implications of the Use of Linkage Analysis for Huntington Disease", 47 Am. J. Hum. Genet. 1, 2 (1990).

[14] Chapman M, "Canadian Experience with Predictive Testing for Huntington Disease: Lessons for Genetic Testing Centers and Policy Makers", 42 Am. J. Hum. Genet. 491, 493 (1992); The Institute of Medicine's Committee on Assessing Genetic Risks recommends more stringent requirements in regard to genetic counseling and believes that "genetic counseling and education must be an integral part of genetic testing; anyone who is offering, or referring for, genetic testing must provide--or refer for --appropriate genetic counseling and education prior to testing and follow-up after testing." *Assessing Genetic Risks*, Andrews LB, Fullarton JE, Holtzman NA & Motulsky G, eds., Washington, D.C. National Academy Press, 1994, p.170. As more primary care physicians provide and use genetic tests, they are the likely candidates to perform such counseling. However, before they will be adequately prepared to do so effectively, research and education on appropriate counseling methods must be undertaken. *Id*. at 173.

[15] Chapman, *supra* note 7, at 492; For discussion of issues that arise in the different contexts in which genetic counseling takes place, including pre-natal testing and screening for late onset disorders *see* IOM Report on *Assessing Genetic Risks*, *supra* note 7, Chap. 4, "Issues in Genetic Counseling."

[16] Huggins M, *et al*, "Ethical and Legal Dilemmas Arising During Predictive Testing for Adult-Onset Disease: The Experience of Huntington Disease", 47 Am. J. Human Genet. 4 (1990); Chapman, *supra* note 7 at 493; *Assessing Genetic Risks, supra* note 7, at 88-89.

[17] See Chapman, *supra* note 7, at 492; Huggins M. *et al*, "Predictive Testing for Huntington Disease in Canada: Adverse Effects and Unexpected Results in Those Receiving a Decreased Risk", 42 Am. J. Med. Genet. 508, 514-515 (1992). These commentators view the role of genetic counseling as particularly warranted in predictive testing since results will be an expression of altered risk and the individual who is tested may not appreciate the significance, for example, between being at 11% as opposed to 50% increased risk of having a particular gene or disease and act on such misunderstanding with harmful results.

[18] If discussion and forms are to be understandable by the average individual, considerable effort should go into their development. As a recent study at Johns Hopkins Oncology Center in Baltimore revealed, the average form for experimental therapies required at least an 11th grade reading level, despite the fact that

most specialists recommend that important documents should be written at or below an 8th grade level. McFarling UL, Medical Notebook, Cancer Consent Forms Found Difficult to Read, *Boston Globe*, October 13, 1994, p.3.

[19] See, *e.g.*, 42 CFR 2.22 (1993)(requiring that at the time of admission for alcohol or drug abuse treatment, patients receive notice of the federal law and regulations that protect the confidentiality of such treatment records); Uniform Health Care Information Act 5-101, 9 U. L. A. 509 (1988)(requiring that a health care provider post a notice of information practices); Health Information Model Legislation 106 (Am. H. Info. Manag. Assoc.) (requiring those who receive health care information from patients to provide patients with a statement of the recipient's fair information practices); Insurance Information and Privacy Model Act 4 (N.A.I.C. 1989)(requiring insurance institutions and agents to provide a notice of information practices to applicants).

[20] See, *e.g.*, The Health Security Act, H.R. 3600, 103d Cong. 2d Sess. 5137 (permitting disclosures without patient's authorization if it is believed that the disclosure will avoid or minimize imminent danger to the health or safety of any individual), Uniform Health-Care Act 2-104 (additionally permitting disclosures to immediate family members, unless prohibited by the patient); and 42 U.S.C. 290dd-2(b)(2)(permitting disclosures of substance abuse treatment information to medical personnel in bona fide emergencies). It should be noted that the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research also recognizes that a genetic counselor's ethical duty of confidentiality can be overridden if several conditions are met, including a determination of a high probability of harm from withholding of the information, that the information will actually be used to avert the harm, and that only genetic information necessary for diagnosis or treatment of disease is disclosed. *Screening and Counseling for Genetic Conditions, supra* note 5 at 44. This issue is discussed in detail in the Appendix.

[21] McEwen J, McCarty K & Reilly P, "A Survey of Medical Directors of Life Insurance Companies Concerning Use of Genetic Information", 53 Am. J. Hum. Genet. 33 (1993).

[22] Marshall E, "Genetic Testing Set for Takeoff", 265 Science 464 (1994).

[23] See, 42 CFR 2.31 (1993) for contents of written consent to disclosure of substance abuse treatment information under the regulations and a sample form.

[24] See, *e.g.*, Uniform Health Care Information Act 3-101, 3-102, 9 U. L. A. 499, 501 (1988)(requiring that health care providers permit patients examine and copy records except in particular circumstances); Health Information Model Legislation 106(b)(6) (Am. H. Info. Manag . Assoc.) (providing that patients may have access to health care information); Mass. Gen. Laws Ann. ch. 112 12CC (West 1993); Fair

[25] See, *e.g.*, Mass. Gen. Laws Ann. ch. 112 12CC (permitting provider to withhold inspection of psychotherapy records when in the exercise of reasonable professional judgment, seeing these records would adversely affect the patient's well being; on request of the patient, however, the total record is to be made available to an attorney or another therapist.) In its Report on the Health Security Act, H.R. 3600, the Committee on Government Operations recommended amendment to include provisions of H.R. 4077 which allow withholding of seven categories of information from patients who request record inspection, H.R. Rptr. No. 601, 103d Cong., 2d Sess. Pt. 5, pp.25-26 (1994).Health Information Practices Act 111,112, H. R. 4077 103Rd Cong. 2d Sess. (1994)(requiring health information trustees to permit individuals to inspect and copy most health information).

[26] H.R. 4077. After introduction by Rep. Gary Condit in March, 1994, hearings were held by the Subcommittee on Information, Justice, Transportation and Agriculture, Committee on Government Operations, U.S. House of Representatives. The provisions of this bill form the basis for the Fair Health Information Practices Part of amendments to the Health Security Act (H.R. 3600) as reported in H.R. Rep. No. 601, 103d Cong., 2d Sess., Pt. 5, p.101 (1994).

[27] See, *e.g.*, Terre Haute Regional Hospital v. Trueblood, 600 N.E.2d 1358 (Ind. 1992) (permitting discovery of medical records of non-party patients but nonetheless requiring that identity of patients be redacted from them).

[28] Commonwealth v. Kobrin, 479 N.E.2d 674 (Mass. 1985).

[29] Khajezadeh D, "Patient Confidentiality Statutes in Medicare and Medicaid Fraud Investigations", 13 Am. J. L. & Med. 105, 120-121 (1987) In some cases the court has simply noted that disclosures are limited to the purposes connected to the plan's administration without any specific guidance as to which informational components of patient records meet particular purposes of the plan.

[30] For description of the range of law enforcement activities in which collection and analysis is allowed under various state laws *see* McEwen J & Reilly P, "A Review of State Legislation on DNA Forensic Databanking," 54 Am. J. Hum Genet. 941 (1994).

[31] See generally, Thompson, *supra* note 3.

[32] Shapiro ED & Weinberg ML, "DNA Data Banking: The Dangerous Erosion of Privacy," 38 Cleve. St. L. Rev. 455, 477 (1990).

[33] U.S. v. Laub Baking Company, 283 F.Supp. 217 (1968) (rejecting claims that fingerprinting violates rights against self-incrimination, unreasonable search and seizure, and right of privacy).

[34] Private genetic information could be developed under such laws because state statutes that authorize the establishment of forensic DNA databanks differ in defining the scope of authority to conduct DNA analysis in connection with such banking. For example, while Michigan identifies the process that is authorized as "DNA identification profiling" which is a "validated scientific method of analyzing components of deoxyribonucleic acid molecules for the purpose of identifying the pattern of the components' chemical structure that is unique to an individual" Mich. Comp. Law 28.172 (1992), not all states are as specific. Alabama's statute, which authorizes the establishment of that state's forensic databank, states that "the Alabama Department of Forensic Sciences should be authorized and empowered to analyze, type and record any and all genetic markers contained in or derived from DNA and to create a statewide DNA database system for collection, storage and maintenance of genetic identification information as the same may pertain to the identification of criminal suspects." Code of Ala. 36-18-20 (1994).

[35] The only circumstance in which paternity tests involving genetic analysis would fall outside of this provision, and this Act could be construed as prohibiting them, would be if an order for such tests was not issued under Rule 35 or what was considered a "comparable rule". Paternity actions are routinely brought as civil actions in most states. Even when paternity is one element to be proven in a criminal action for failure to pay support by an enforcement agency, the civil rules of procedure are often applied. Some states, such as Ohio, have specific statutes regarding authority to issue orders for paternity testing on motion to the court. For example, ORCA 2317.47 (refering to blood tests in paternity actions) and ORCA 3111.09 (containing similar provisions for genetic tests in paternity actions). The consequences of willful failure to obey a court order under these circumstances includes having the refusal disclosed in trial or permitting the court to issue an order determining paternity without genetic testing. ORCA 3111.09 Most DNA identification tests are currently done to determine paternity; more than 100,000 a year for paternity and less than 10,000 for use in a criminal proceeding. Bishop JE, How DNA Scientists Help Track Criminals and Clear the Innocent, *Wall St. J.*, Jan. 6, 1995, p.1.

[36] Lewis, Under a Genetic Cloud, *Boston Globe*, August 14, 1994, p.A1. The named plaintiff, Le Ann Sevenson, filed suit in Santa Clara against KTI Chemicals, Inc.

[37] The Common Rule, based on the regulations of the Department of Health and Human Services (*see* 45 CFR Part 46) has been adopted in whole or as modified by individual departments and agencies. For a particular agency's version, *see* 56 *Fed. Reg.* 28019-28031 (1991).

[38] This requirement coincides with the recommendations of the NIH Office for Protection from Research Risks that IRB make sure adequate counseling is provided to pedigree research participants on the meaning of the genetic information they receive so as to minimize the psychological risks of participation. National Institute of Health, *Protecting Human Research Subjects, Institutional Review Board Guidebook*, Washington, D.C.: U.S. Gov. Printing Office, 1993, pp. 5-54.

[39] Revelation during the authorization process that such information may result from participation is also recommended by OPRR. *Id*.

[40] Examples include the Uniform Health Care Information Act 52-104(a)(7), and H.R. 4077, 128 *supra* note 17. These acts contain similar provisions for access for research use to patient information without the authorization of patients if an IRB has determined that the project's importance outweighs the intrusion into the patient's privacy, and that it would be impracticable to conduct the project without such information. Although 42 U.S.C. 290dd-2(b)(2) is less restrictive and permits disclosures of information regarding substance abuse treatment without patient authorization to "qualified personnel for purposes of conducting research", regulations under the same statute require that an independent panel of three persons determine the welfare of the patients will be adequately protected and that the risks in disclosure of alcohol and substance abuse treatment information are outweighed by the benefits of the research. 42 CFR 2.51 (1993). None of these models is more specific in regard to what factors must be considered by the reviewer in conducting such balancing tests.

[41] H.R. Rep. No. 601, *supra* note 19, 124-125.

[42] Courts have held that an administrator of an estate can waive the attorney client priviledge on behalf of the deceased in some instances. In the Matter of John Doe Grand Jury Investigation, 562 N.E.2d (Mass. 1990) Statutes can also vest authority in a decendent's personal representative to exercise the decisional right to waive psychiatrist-patient privilege. *See*, for example, Mont. Code Ann. 50-16-222 (1993) granting such authority in the personal representative, or if none available, in a surviving spouse, parent or adult child.

[43] Assessing Genetic Risks, *supra* note 7, at 10.

[44] Bloch M & Hayden MR, "Opinion: Predictive Testing for Huntington Disease in Childhood: Challenges and Implications," 46 Am. J. Hum. Genet. 1-4 (1990).

[45] Pierce v. Society of Sisters, 268 U.S. 510 (1925).

[46] Wisconsin v. Yoder, 406 U.S. 205 (1972).

[47] See, *e.g.*, Mass. Gen. Laws Ch. 112 12F.

[48] Planned Parenthood v. Danforth, 428 U.S. 52 (1976); Bellotti v. Baird, 443 U.S. 622 (1979).

[49] Carey v. Population Services International, 431 U.S. 678 (1977).

[50] Ramsey P, "Children in Medical Investigation" in *The Patient as Person*, New Haven, Conn., Yale University Press, 1970, pp.11-17; and Ramsey P, "Children as Research Subjects: A Reply," 7(2) Hastings Center Report 40 (April, 1977).

[51] Bloch & Hayden, *supra* note 37 at 1-3. The authors acknowledge that most parents who request such tests are seeking a way to allay their own anxieties about the child's future. However, they caution that the results could have negative impact on the child's upbringing and relationship to siblings with a different risk. They recommend that predictive testing in childhood only be done when an advantage can be clearly demonstrated for the child.

[52] See, *e.g.*, Me. Rev. Stat., tit.22, 1597-A4, requiring that the physician or counselor who provides information to the pregnant minor explore with her whether or not involvement of a parent, guardian or adult family member in the decision making process would be in her best interests.

[53] See, *e.g.*, Strunk v. Strunk, 445 S.W.2d 145 (Ky. 1969) in which the court exercised its equitable power to permit transplantation of a kidney from an incompetent man to his brother who was dying.

[54] Id. *See also,* Curran v. Bosze, 566 N.E.2d 1319 (Ill. 1990). Although the principal issue in this case was whether or not it was in a child's best interests for a parent to withhold consent for a donation to a half-sibling, the court specifically held that a parent could consent to such donation only when it would be in the minor's best interests. *Id.* at 1331.

[55] In In re Richardson, 284 So. 2d 185, 188 (La. App. 1973) the court concluded that before application of the best interests standard was considered to determine if consent could be given for transplantation of a kidney from a brother to a sister, it must first be established that the surgical intrusion was urgent, there were no reasonable alternatives, and that the contingencies were minimal.

[56] S. 1735, 103d Cong., 1st Sess. (1993), The Privacy Protection Act of 1993 was introduced by Sen. Paul Simon to establish a Privacy Protection Commission. Like previous proposals, although granting investigative powers to the Commission, this bill contained no similar grant of enforcement powers to the Commission itself but charged it with reporting violations of the Privacy Act for which criminal but not civil penalties are available, to the president, Attorney General and Congress. It also leaves activities of the private sector outside the Commission's jurisdiction.

[57] Rotenberg M, "In Support of a Data Protection Board in the United States," 8 Gov. Info. Q. 79, 88 (1991).

[58] See, *e.g.*, Iowa Code Ann. 729.6 (1992) (prohibiting employers from requiring or administering genetic tests as a condition of employment); Wis. Stat. Ann. 631.89 (1994) (restricting the use of genetic tests and results of such tests by insurers), Cal. Ins. Code 10146 (1994) (establishing standards for underwriting life and disability insurance on the basis of tests for genetic characteristics) and Cal. Health & Safety Code 1374.7 (1994) (prohibiting health insurance plans from rejecting applicants, or setting higher rates for applicants, on the basis of genetic characteristics).

[59] For instance, in regard to genetic testing that is permissible by life and disability insurers, California requires that written informed consent for such test include, in addition to information that would also be required by this Act, the limitations of the test and procedures for notifying the applicant of the results. Cal. Ins. Code 10148 (1994).

RL30006: Genetic Information: Legal Issues Relating to Discrimination and Privacy

Nancy Lee Jones

Legislative Attorney
American Law Division

Updated January 24, 2001

On June 26, 2000, in a special ceremony at the White House, the completion of the "rough draft" of the human genome was announced. This milestone, which has been compared to the discoveries of Galileo, and other advances in genetics have created novel legal issues relating to genetic information. The Human Genome Project, with its goal of producing detailed maps of the 23 pairs of human chromosomes and sequencing the three billion nucleotide bases that make up the human genome, has been instrumental in the identification of genes responsible for various diseases including glaucoma, colon cancer, and cystic fibrosis. With the identification of these genes comes the hope of genetic therapies to cure disease but this scientific accomplishment is not without potential problems. For instance the presence of a cancer causing gene may indicate a predisposition but does not guarantee that the person will contract the disease: How should an employer or insurer respond? The ethical, social and legal implications of these technological advances have been the subject of significant scrutiny and concern.

The legal implications of such information have been mainly on the state level but there are some relevant Federal statutes. The Health Insurance Portability and Accountability Act of 1996, P.L. 104-191, is the first federal law to specifically address discrimination and insurance issues relating to genetic discrimination. This report discusses current federal and state law as well as legislation which was introduced in the 106th Congress. It will be updated as needed.

Background

On June 26, 2000, in a special ceremony at the White House, the completion of the "rough draft" of sequence of the human genome was announced. More specifically, the scientists involved in the Human Genome Project (HGP) (1) reported that this rough draft consists of overlapping fragments covering 97% of the human genome, and a sequencing of 85%of the human genome. (2) Within the next two or three years, the entire sequence should be complete. (3) These rapid advances provide powerful tools for information about the causes, and potentially the cures, for diseases such as diabetes, heart disease, Parkinson's disease, bipolar illness, and asthma.

In recent testimony Dr. Francis Collins, the Director of the National Human Genome Research Institute, described the dramatic progress of the Human Genome Project and stated that "these revelations hold within them the promise of a true transformation of medical practice. Quite possibly before the end of the first decade of this new millennium, each of us may be able to learn our individual susceptibilities to common disorders, in some cases allowing the design of a program of effective individualized preventive medicine focused on lifestyle changes, diet and medical surveillance to keep us healthy....These same discoveries about genetics likely will lead us to predict who will respond most effectively to a particular drug therapy, and who may suffer a side effect and out to avoid that particular drug." (4)

The first apparently successful genetic therapy has been recently reported (5) and in the not too distant future, the benefits of genetic therapy have been seen by some as little short of miraculous with potential cures of major diseases such as heart disease, and cancer, and Alzheimer's. However, there have been numerous ineffective efforts at gene therapy and at Senate hearings, held following the death of a patient involved in gene therapy, Senator Frist stated that gene therapy holds "great promise, but because gene therapy is an experimental, high risk procedure, there is a need for vigilant oversight to ensure patient safety." (6)

These scientific advances in genetics are not without other potential problems. The ethical, social and legal implications of genetic research have been the subject of significant scrutiny and a portion of the funds for the Human Genome Project are set aside for use in analyzing these issues. (7) As scientific knowledge about genetics becomes increasingly widespread, numerous researchers and commentators, including Dr. Francis Collins, have expressed concerns about how this information is to be used. (8) In recent congressional testimony, Dr. Collins stated: "while genetic information and genetic technology hold great promise for improving human heath, they can also be used in ways that are fundamentally unjust. Genetic information can be used as the basis for insidious discrimination....The misuse of genetic information has the potential to be a very serious problem, both in terms of people's access to employment and health insurance and the continued ability to undertake important genetic research." (9)

This concern has encompassed fears of discrimination in many aspects of life, including employment, and health and life insurance. A study on discrimination found that a number of institutions, including health and life insurance companies, health care providers, blood banks, adoption agencies, the military and schools, were reported to have engaged in genetic discrimination against asymptomatic individuals. (10) The discriminatory practices alleged included treating a genetic diagnosis as a preexisting condition for insurance purposes, refusal by an adoption agency to allow a woman at risk for Huntington's disease to adopt based on the woman's genetic risk, and termination from employment after disclosure of a risk of Huntington's disease. (11) Similarly, another study reported that twenty-two percent of the respondents indicated that they or a family member were refused health insurance as a result of a genetic condition. (12) This study was strongly criticized by the Health Insurance Association of America (HIAA) which has argued that there is no evidence showing that insurers engage in genetic discrimination and that federal legislation to prohibit discrimination based on genetic information is unnecessary. (13)

A joint report by the Department of Labor, the Department of Health and Human Services, the Equal Employment Opportunity Commission and the Department of Justice summarized the various studies on discrimination based on genetic information and argued for the enactment of federal legislation. The report stated that "genetic predisposition or conditions can lead to workplace discrimination, even in cases where workers are healthy and unlikely to develop disease or where the genetic condition has no effect on the ability to perform work" and that "because an individual's genetic information has implications for his or her family members and future generations, misuse of genetic information could have intergenerational effects that are far broader than any individual incident of misuse." (14) Concluding that existing protections are minimal, the report went on to call for the enactment of legislation which states that (1) employers should not require or request that employees or potential employees take a genetic test or provide genetic information as a condition of employment or benefits, (2) employers should not use genetic information to discriminate against, limit, segregate, or classify employees, and (3) employers should not obtain or disclose genetic information about employees or potential employees under most circumstances. (15) According to the Labor Department report, employers should be able to (1) use genetic information for monitoring for the effects of a particular substance in the workplace under certain circumstances, and (2) disclose genetic information for research and other purposes with the written, informed consent of the individuals. In addition, the report states that the statutory authority of federal agencies or contractors to promulgate regulations, enforce workplace safety and health laws, or conduct occupational or other health research should not be limited. (16)

It should be emphasized that legal issues relating to genetics may vary depending on whether insurance, employment or other types of discrimination, or medical research are involved. Approaches to addressing the issues raised in these contexts vary from taking no legislative action, addressing certain specific concerns (as was done in the Health Insurance Portability and Accountability Act), or more far reaching approaches such as comprehensive legislation on genetics or legislation focused on all medical records, including genetics.

Generally legal issues raised regarding genetics have been based on two main concepts: privacy and discrimination. The privacy interests of an individual in his or her genetic information have been seen as significant and protecting these interests is seen as making discriminatory actions based on this information less likely. However, another approach would be to prohibit this potential misuse of the information by prohibiting discrimination. Some statutes, like the Americans with Disabilities Act (ADA), 42 U.S.C. §§ 12101 et seq., take a two-pronged approach to similar issues regarding medical information about disabilities by both protecting the confidentiality of the information and by prohibiting discriminatory acts.

Currently there are no federal laws that directly and comprehensively address the issues raised by the use of genetic information. There are, however, a few laws that address parts of these issues but the only federal law that directly addresses the issue of discrimination based on genetic information is the Health Insurance Portability and Accountability Act. A provision relating to genetic discrimination was included in H.R. 2990, 106th Congress but the bill was not enacted prior to the adjournment of the 106th Congress. On February 8, 2000, President Clinton issued an executive order prohibiting discrimination against federal employees based on protected genetic information. On December 20, 2000, the Department of Health and Human Services issued final regulations on medical privacy which are not specific to genetics but cover all personal health information. (17) In addition, many states have enacted laws which vary widely in their approaches to genetic information.

The Health Insurance Portability and Accountability Act of 1996

P.L. 104-191, the Health Insurance Portability and Accountability Act of 1996 (18), has been hailed as taking "important steps toward banning genetic discrimination in health insurance" but has also been criticized as not going far enough. (19) The Act prohibits a group health plan or issuer of a group health plan from using genetic information to establish rules for eligibility or continued eligibility and provides that genetic information shall not be treated as a preexisting condition in the absence of the diagnosis of the condition related to such information. It also prohibits a group health plan or issuer of a group health plan from using genetic information in setting a premium contribution. However, the Act would not prohibit group health plans or issuers of plans (i.e., insurers) from requiring or requesting genetic testing, does not require them to obtain authorization before disclosing genetic information, and does not prevent them from excluding all coverage for a particular condition or imposing lifetime caps on all benefits or on specific benefits. In addition, this Act does not address the issues of the use of genetic information in contexts other than health insurance such as employment. (20)

Executive Order

On February 9, 2000, President Clinton signed Executive Order 13145 prohibiting genetic discrimination against employees in federal executive departments and agencies. In announcing the executive order at a meeting of the American Association for the Advancement of Science, the President stated that "This extraordinary march of human understanding imposes on us a profound responsibility to make sure that the age of discovery can continue to reflect our most cherished values." (21) Many commentators lauded the executive order, and quoted with approval its description as "preventive policy making- to put in place the kind of protections that the public needs and deserves before we find ourselves in a needless crisis situation." (22) However, it has also been criticized both on a philosophical level (23) and in the details of its coverage. (24)

The executive order defines "protected genetic information" as "(A) information about an individual's genetic tests; (B) information about the genetic tests of an individual's family members; or (C) information about the occurrence of a disease; or medical condition or disorder in family members of the individual." Current health status information would not be protected under this executive order unless it was derived from the information described above.

The executive order requires executive departments and agencies to implement the following nondiscrimination requirements:

the employing entity shall not discharge, fail or refuse to hire, or otherwise discriminate against any employee because of protected genetic information or because of information about a request for or receipt of genetic services;
the employing entity shall not limit, segregate or classify employees in any way that would deprive or tend to deprive any employee of employment opportunities or otherwise adversely affect that employee's status because of protected genetic information or because of information about a request for or receipt of genetic services;
the employing entity shall not request, require, collect, or purchase protected genetic information with respect to an employee or information about a request for or receipt of genetic services;
the employing entity shall not disclose protected genetic information with respect to an employee or information about a request for or receipt of genetic services with certain exceptions;
the employing entity shall not maintain protected genetic information or information about a request for or receipt of genetic services in general personnel files. Such materials shall be treated as confidential medical records and kept separate from personnel files.

There are certain exceptions to these prohibitions. For example, the employing entity may request or require information if such current condition could prevent the applicant or employee from performing the essential functions of the job, or where it is to be used exclusively to determine whether further medical evaluation is needed to diagnose a current disease. Genetic monitoring of biological effects of toxic substances in the workplaces are permitted in certain circumstances.

The Americans with Disabilities Act

The Americans with Disabilities Act (ADA), 42 U.S.C. § 12101 et seq., prohibits discrimination against an individual with a disability in employment, public services, public accommodations, and communications. The threshold issue in any ADA case is whether the individual alleging discrimination is an individual with a disability. The act defines the term disability with respect to an individual as having "(A) a physical or mental impairment that substantially limits one or more of the major life activities of such individual, (B) a record of such an impairment; or (C) being regarded as having such an impairment." (25) Although the statutory language of the ADA does not reference genetic traits, there was a discussion of the issue during congressional debate. (26) So far there have been no reported cases addressing this issue although one case has been filed with the Equal Employment Opportunities Commission.

EEOC Interpretation of the ADA Regarding Genetic Discrimination

The ADA has been interpreted by the Equal Employment Opportunity Commission (EEOC) as including genetic information relating to illness, disease, or other disorders. (27) The legislative history was cited by the EEOC in its guidance to the definition of disability for its compliance manual. In this guidance, the EEOC examined the definition of disability under the ADA, noting that the definition was composed of three prongs: disability means (1) a physical or mental impairment that substantially limits one or more of the major life activities of an individual, (2) a record of such an impairment, or (3) being regarded as having such an impairment. (28) It was under the third prong that the EEOC determined that discrimination based on genetic information relating to illness, disease, or other disorders was prohibited. (29)

Although this EEOC interpretation was widely heralded as a significant step for the protection of rights for individuals whose genes indicate an increased susceptibility to illness, disease or other disorders, it is limited in its application and may be even more limited after the recent Supreme Court decisions on the definition of disability. (30) However, the EEOC has not withdrawn this guidance and at recent Senate hearings, EEOC Commissioner Paul Miller stated that the ADA "can be interpreted to prohibit employment discrimination based on genetic information. However, the ADA does not explicitly address the issue and its protections are limited and uncertain." In addition, Commissioner Miller observed that even if the ADA were found to cover genetic discrimination, the requirements of the ADA may not protect workers from all types of genetic discrimination. He stated, "for example, the ADA does not protect workers from requirements or requests to provide genetic information to their employers....In addition, once the applicant is hired, the employer may request that the employee take a medical exam, such as a genetic test, if the employer can demonstrate that the information from that test is job related and consistent with business necessity." (31)

The first ADA case alleging genetic discrimination was filed with the EEOC by Terri Seargent. Ms. Seargent, whose situation was extensively discussed during Senate debate on genetic discrimination, had had a promising career as a manager for a small insurance broker in North Carolina. She had positive performance evaluations but after medical tests determined that she had Alpha 1 Antitrypson Deficiency, a condition that affects the lungs and liver, and she began taking expensive medication, she was terminated from her employment. (32)

Supreme Court ADA Decisions

Although the combination of the ADA's legislative history and the EEOC's guidance has led commentators to argue that the ADA would cover genetic discrimination, the merit of these arguments has been uncertain since there have been no reported cases holding that the ADA prohibits genetic discrimination. This uncertainty has increased in light of recent Supreme Court decisions on the ADA.

The first Supreme Court ADA case to address the definition of disability was Bragdon v. Abbott, a 1998 case involving a dentist who refused to treat an HIV infected individual outside of a hospital. (33) In Bragdon, the Court found that the plaintiff's asymptomatic HIV infection was a physical impairment impacting on the major life activity of reproduction thus rending HIV infection a disability under the ADA. In two 1999 cases the Court examined the definitional issue whether the effects of medication or assistive devices should be taken into consideration in determining whether or not an individual has a disability. The Court in the landmark decisions of Sutton v. United Airlines (34) and Murphy v. United Parcel Service, Inc, (35) held, contrary to the interpretation given by the EEOC, that the "determination of whether an individual is disabled should be made with reference to measures that mitigate the individual's impairment...." (36) In reaching this holding, the Court looked to the first prong of the definition of disability (having a physical or mental impairment that substantially limits one or more of the major life activities of an individual) and emphasized that the phrase "substantially limits" appears in the present indicative verb form "requiring that a person be presently -- not potentially or hypothetically -- substantially limited in order to demonstrate a disability." In Albertsons Inc. v. Kirkingburg (37) the Court held unanimously that the ADA requires proof that the limitation on a major life activity by the impairment is substantial. The Court in Sutton also looked at the findings enacted as part of the ADA which stated that "some 43,000,000 Americans have one or more physical or mental disabilities" and found that this figure was inconsistent with the argument that the statute covered individuals without looking at the mitigating effects of medications or devices. The individualized nature of the inquiry into whether an individual was an individual with a disability was emphasized.

Although the Court's decision in Sutton did not turn on the third prong of the definition of disability (being "regarded as having such an impairment") the Court did address the interpretation of this part of the definition. There are two ways, the Court stated, that an individual can fall within the "regarded as" prong: (1) a covered entity mistakenly believes that a person has a physical impairment that substantially limits one or more major life activities, or (2) a covered entity mistakenly believes that an actual impairment substantially limits one or more major life activities. The Court found that, on its own, the allegation that an entity has a vision requirement in place does not establish a claim that the entity regards an individual as substantially impaired in the major life activity of working. The term "substantially limits" was regarded as significant. It requires "at a minimum, that plaintiffs allege they are unable to work in a broad class of jobs." The Court emphasized that it was "assuming without deciding" that working is a major life activity and that the EEOC regulations interpreting "substantially limits" are reasonable and found that even using the EEOC interpretation, the plaintiffs in Sutton failed to allege adequately that their vision is regarded as an impairment that substantially limits them in a major life activity. Being precluded from being a global airline pilot was not sufficient since they could obtain other, although less lucrative jobs, as regional pilots or pilot instructors.

The "regarded as" prong was directly at issue in Murphy. In Murphy the Court held that the fact that an individual with high blood pressure was unable to meet the Department of Transportation (DOT) safety standards was not sufficient to create an issue of fact regarding whether an individual is regarded as unable to utilize a class of jobs. Like Sutton, the holding in Murphy emphasized the numerous other jobs available to the plaintiff.

The Supreme Court's decisions on the ADA did not directly address genetic discrimination and it is possible that the ADA could be interpreted to cover a particular genetic defect. However, the reasoning used in the Court's recent decisions appears to make it unlikely that an ADA claim based on genetic discrimination would be successful. There are several factors that lead to this conclusion.

First, the Supreme Court in Sutton specifically struck down an interpretation by the EEOC regarding the use of mitigating factors and raised questions concerning the validity of the EEOC's interpretation. The Court also found no statutory authority for agency interpretation of the definition of disability. The EEOC had taken the position that whether or not an individual has a disability should be determined by what his or her condition would be without medication or an assistive device. Rejecting this EEOC interpretation, in Sutton the Supreme Court noted that no agency was given the authority to interpret the term "disability" but that because both parties accepted the regulations as valid "we have no occasion to consider what deference they are due, if any." Similarly, in Murphy the Court clearly stated that its use of the EEOC regulations did not indicate that the regulations were valid. However, in its earlier decision in Bragdon v. Abbott, the Court had found its conclusion that HIV infection was covered by the ADA to be "reinforced by administrative guidance issued by the Justice Department...." The cases subsequent to Bragdon did not examine this seeming contradiction so exactly how a future decision would view EEOC regulations and guidance is uncertain. This issue is especially important regarding potential cases of genetic discrimination since

the EEOC has published guidance indicating that the ADA covers genetic discrimination, (38) and there are no reported cases.

Similarly, the Supreme Court showed little indication to examine the legislative history of the ADA. The Court in Sutton held that it was not necessary to consider the legislative history of the ADA regarding the issue of whether individuals should be examined in their uncorrected state or with the use of mitigating medications or devices. It found that the statutory language was sufficient to support its holding on this issue. Although the issue regarding genetic discrimination is distinct from that of the use of mitigating medications and devices, the Court's general reluctance to examine legislative history in Sutton may indicate that the language on genetic discrimination quoted above from the congressional debates also would not be examined.

The Court's reliance in Sutton upon the findings in the ADA that 43,000,000 Americans have one or more physical disabilities also indicates that the Court may not find genetic defects to be covered. The number of individuals cited in the findings as having a disability was seen by the Court as inconsistent with the argument that the statute covered individuals whose disabilities were mitigated by medications or devices. Since the prevalence of genetic defects is believed to be widespread, coverage of genetic defects could arguably include almost every individual. Thus, it is possible that the Court could use the same rationale as in Sutton to find genetic defects not included.

In Bragdon v. Abbott, where the Court found that HIV infection was covered under the ADA, the majority opinion spent considerable time discussing the immediate physiological effects of the infection. This would appear to be consistent with the holding in Sutton that the "substantially limits" definitional language requires that the substantial limitation not be potential or hypothetical. This reasoning could be contrasted to the situation presented by genetic defects which in many cases do not ever manifest. Interestingly, in his dissenting opinion in Bragdon v. Abbott, Chief Justice Rehnquist, who was in the majority in Sutton, stated that the argument regarding coverage of HIV infection "taken to its logical extreme, would render every individual with a genetic marker for some debilitating disease 'disabled' here and now because of some possible future effects." Whether the Court would now share the Chief Justice's view that such coverage of genetic discrimination is an invalid interpretation of the definition is uncertain, especially since the Court in Bragdon was discussing the first prong of the definition, not the "regarded as" prong which is the most likely basis for coverage of genetic defects.

In its recent cases the Court provided considerable guidance concerning the "regarded as" prong of the definition of disability, the most likely aspect of the definition to be used to find coverage of genetic defects. Including the requirement that the individual be regarded as "substantially limited" in a major life activity, the Court found that this language meant that being precluded from a particular job was not sufficient to be substantially limited in the major life activity of working if other jobs in the same class could be obtained. And when this specific issue was raised in Murphy, the plaintiff was not found to be regarded as substantially limited in the major life activity of working. The main point of this rather complicated discussion is that making the case that one is regarded as substantially limited in a major life activity, particularly the major life activity of working, is likely to be difficult.

The Supreme Court's recent decisions do not directly address ADA coverage of genetic discrimination. They emphasize an individualized approach to the determination of whether an individual has a disability under the ADA. Although an argument could be made that the ADA would cover individuals with genetic defects in certain cases, the Court's most recent decisions, particularly Sutton and Murphy, use reasoning that would make it unlikely that most ADA claims based on genetic discrimination would be successful.

In addition, even assuming the ADA was found to apply, it may not protect employees from having their employers have access to their genetic information. Although the ADA prohibits an employer from making medical inquiries prior to a job offer, the employer may obtain medical information in certain cases after the offer of employment has been made. Assuming that the prohibitions against discrimination in the ADA would apply, it is difficult to prove that genetic information was the reason for discrimination. This raises issues relating to the privacy of genetic information.

Privacy

Although the Constitution does not expressly provide for a right to privacy, the Supreme Court has found some right to informational privacy. (39) However, these rights are limited by judicial deference to government's need to

acquire the information and by the fact that such a constitutional right would be limited to state action. As a practical matter, this would mean that federal or state collections of information may receive some constitutional protection but the collection and use of information by private health plans or organizations would not be covered. (40) Certain federal statutes may provide some privacy protection for medical records. The Privacy Act of 1974, 5 U.S.C. § 552a, prohibits the disclosure of records maintained on individuals by federal government agencies except under certain conditions. Subsection 552a(f)(3) allows agencies to establish special procedures for individuals who wish to access their medical records. The intent of this provision as described in the House report was to ensure rules so that an individual who would be adversely affected by the receipt of such data may be apprized of it in a manner which would not cause such adverse effects. (41)

The Freedom of Information Act (FOIA), 5 U.S.C. §§ 552 et seq., establishes a right of access to records maintained by agencies within the executive branch of the federal government. It contains several exemptions, including one for "personnel and medical files and similar files the disclosure of which would constitute a clearly unwarranted invasion of personal privacy." (42) Both the Privacy Act and FOIA may, then, provide some privacy protections for genetic information but they are limited in their scope and would not encompass information held by a private entity. (43)

The Health Insurance Portability and Accountability Act (HIPAA) contains requirements for the standardization of electronically transmitted health insurance financial claims and administrative transactions, such as the submission of claims, processing of enrollments, verification of insurance eligibility, and payment and remittance advice. HIPAA required the Secretary of Health and Human Services (HHS) to make recommendations to Congress by August 1997 concerning the protection of privacy of individually identifiable health information and Congress had until August 1999 to enact legislation on this issue. If Congress did not enact legislation, HIPAA requires the Secretary of HHS to promulgate regulations on privacy protections. The Secretary of HHS issued final regulations on December 20, 2000. (44)

The final privacy regulations apply to health insurers, providers, and health care clearinghouses and require patient consent for most sharing of personal health information. The final regulations cover all personal health information in paper, oral or electronic form and limit disclosure of personal health information to the minimum amount necessary for the purposes of the disclosure except where medical records are transferred for the purposes of treatment. Companies that sponsor health plans are prohibited from accessing personal health information for employment purposes unless the patient consents. Civil money penalties are provided and egregious violations carry federal criminal penalties of up to $250,000 and ten years in prison. Although these regulations are general and not specific to genetics, they will have an effect on genetic information. In the comments to the regulations, the Department noted that many commentators requested additional protections for sensitive information, including genetic information. In response, the Department noted that generally the regulations do not differentiate among types of protected health information. (45)

State Statutes

Although there is limited federal law relating to the use of genetic information, many states have enacted statutes dealing with various aspects of these issues. Early state statutes focused on particular genetic conditions. The first statute to prohibit discrimination based on a genetic trait was enacted in North Carolina and prohibited employment discrimination based on the sickle cell trait. In 1991 Wisconsin became the first state to enact a comprehensive law to prohibit discrimination based on genetic test results. Currently, the states vary in their provisions with some prohibiting discrimination in employment while others deal solely with discrimination in insurance. A recent survey of state law found that thirty-four states have passed laws governing genetic testing and information. (46) One of the most contentious aspects of the state legislation has been the definition of genetic information. Some states, like Michigan, limit nondiscrimination protections to the results of genetic tests. On the other hand, New Jersey prohibits the use of information about genes, gene products or inherited characteristics that may derive from an individual or family member. This would include information such as family history which is often used in insurance underwriting.

Although these state statutes do provide some types of coverage, they do not cover employer self-funded plans providing private health insurance for employees and their dependents. These plans are exempt from state insurance laws due to the preemption provision in the federal Employee Retirement Income Security Act (ERISA). (47) Since

it has been estimated that over one-third of the nonelderly insured population obtains its coverage through self-funded plans and these types of plans are increasing, the ERISA exemption limits the application of state laws significantly. (48)

Legislation in the 106th Congress

Although legislation specifically relating to genetic discrimination and privacy was not enacted during the 106th Congress, a provision relating to health insurance was considered in the conference on H.R. 2990. The Senate amended H.R. 2990 as passed by the House, striking all the language after the enacting clause and substituting the language in S. 1344. This Senate bill would have amended ERISA, the Public Health Services Act and the Internal Revenue Code to prohibit health plans or health insurance issuers, in both group and individual markets, from using predictive genetic information to set premiums. It also contained confidentiality provisions. (49)

Senator Daschle had offered a more comprehensive amendment to the FY 2001 Labor-HHS Appropriations bill, S. 2553. It would have prohibited insurance companies from raising premiums or denying coverage on the basis of genetic tests and would have also barred employers from using predictive genetic information to make employment-related decisions. The amendment was defeated by a vote of 54-44.

A number of other bills were introduced on genetic issues but did not receive congressional action. They varied in their approach with some bills prohibiting discrimination on the basis of genetic information in insurance and employment while others were tailored specifically to health insurance. (50) In addition, legislation relating to medical privacy generally could have impacted on genetic issues.

When President Clinton announced his Executive Order on genetic discrimination, he also indicated support for the federal legislation introduced by Representative Slaughter and its companion bills. Representative Slaughter's bill, H.R. 2457, which would have prohibited health insurance and employment discrimination against individuals and their family members on the basis of predictive genetic information or genetic services. The Senate held hearings on July 20, 2000 concerning genetic information in the workplace.

Footnotes

1. The Human Genome Project, begun in 1990, is a 13 year effort coordinated by the U.S. Department of Energy and the National Institutes of Health to identify all the estimated 80,000 genes in human DNA and to determine the sequences of the 3 billion chemical bases that make up human DNA, store this information in data bases, develop tools for data analysis, and address the ethical, legal, and social issues (ELSI) that may arise from the project. The Human Genome Project is funded through the Department of Energy and the National Institutes of Health. For more detailed information see "The National Human Genome Research Institute," http://www.nhgri.nih.gov and "Human Genome Research," http://www.er.doe.gov/production/ober/hug_top.html

2. "International Human Genome Sequencing Consortium Announces 'Working Draft' of Human Genome," http://www/nhgri.nih.gov/NEWS/sequencing_consortium.html

3. Testimony of Francis S. Collins, Director, National Human Genome Research Institute, National Institutes of Health, Before the Senate Health, Education, Labor and Pensions Committee (July 20, 2000).

4. Id.

5. Rick Weiss, "Genetic Therapy Apparently Cures 2," The Washington Post A-1 (April 28, 2000).

6. Testimony of Bill Frist United States Senator Tennessee Before the Senate Committee on Health, Education, Labor and Pensions Subcommittee on Public Health, Gene Therapy: Is there Oversight for Patient Safety?" (February 2, 2000).

7. The group working on these issues is referred to as the Ethical, Legal and Social Implications (ELSI) program. See http://www.nhgri.nih.gov/ELSI.

8. Testimony by Dr. Francis Collins, Director, National Center for Human Genome Research, Before the Senate Committee on Labor and Human Resources (March 6, 1996). See also, Hudson, Rothenberg, Andrews, Kahn, and Collins, "Genetic Discrimination and Health Insurance: An Urgent Need for Reform," 270 Science 391 (Oct. 20, 1995); Annas, Glantz and Roche, "Drafting the Genetic Privacy Act: Science, Policy and Practical Considerations," 23 J. of Law, Medicine and Ethics 360 (1995); Gostin, "Genetic Discrimination: The Use of Genetically Based Diagnostic and Prognostic Tests by Employers and Insurers," 17 Am. J. of Law & Med. 109 (1991); Rothstein, Mark, Genetic Secrets: Protecting Privacy and Confidentiality in the Genetic Era (1997).

9. Testimony of Francis S. Collins, Director, National Human Genome Research Institute, National Institutes of Health, Before the Senate Health, Education, Labor and Pensions Committee (July 20, 2000).

10. Geller, Alper, Billings, Barash, Beckwith, and Natowicz, "Individual, Family, and Societal Dimensions of Genetic Discrimination: A Case Study Analysis," 2 Science and Engineering Ethics 71 (1996). The study has been criticized by the insurance industry as relying on anecdotal information. See American Council of Life Insurance, "Statement Regarding the Council for Responsible Genetics 'Study' on Genetic Discrimination" (April 11, 1996).

11. Id.

12. Lapham, E. Virginia, Kozma, Chahira, Weiss, Joan O., "Genetic Discrimination: Perspectives of Consumers," 274 Science 621 (October 25, 1996).

13. "Testimony of the HIAA on Genetic Testing," Before the Senate Committee on Labor and Human Resources, 105th Cong., 2d Sess. (May 21, 1998), reproduced at http://www.hiaa.org/news/news-state/genetictesting.html

14. Department of Labor, Department of Health and Human Services, Equal Employment Opportunity Commission, Department of Justice, "Genetic Information and the Workplace," http://www.dol.gov/dol/_sec/public/media/reports/genetics.htm 2-3 (January 20, 1998).

15. Department of Labor, Department of Health and Human Services, Equal Employment Opportunity Commission, Department of Justice, "Genetic Information and the Workplace," http://www.dol.gov/dol/_sec/public/media/reports/genetics.htm at 7 (January 20, 1998).

16. Id.

17. 65 Fed. Reg. 82460 (Dec. 20, 2000).

18. For a general discussion of this Act see Beth Fuchs, Bob Lyke, Richard Price, and Madeleine Smith , "The Health Insurance Portability and Accountability Act (HIPAA) of 1996: Guidance on Frequently Asked Questions," CRS Report 96-805 (pdf).

19. Statement of Rep. Louise Slaughter Before the House Science Subcommittee on Technology, Hearing on Technological Advance in Genetic Testing: Implications for the Future, September 17, 1996.

20. It should also be noted the HIPAA contains certain requirements regarding the standardization of claims that raise potential privacy issues. HIPAA addressed these issues by requiring either congressional action or regulatory action to protect privacy. A more detailed discussion of this issue is contained in the section on privacy.

21. "President's Order Bars Discrimination Based on Genetics," Investor's Business Daily A9 (February 9, 2000).

22. Wendy R. Uhlmann, "When Genes are Decoded, Who Should See the Results?; Every one of us at Risk," The New York Times F7 (February 29, 2000).

23. Michael Kinsley, editor of Slate, an online magazine, observed that "genetic discrimination is universal, inevitable and, in some ways, essential....Practice, practice will get you to Carnegie Hall, but only if you've been born on the right bus....The world would be a poorer place if it did not distinguish between me and Yo-Yo Ma in

doling out opportunities to be a concert cellist." Michael Kinsley, "Genetic Correctness," The Washington Post A29 (April 18, 2000).

24. Mark A. Hall, a law professor at Wake Forest University, argues that the order's prohibition of considering predictive genetic information would not allow for the screening of susceptibility to toxic exposure prior to working in such an environment and would not allow for the use of genetic predispositions to future conditions that could effect job performance. Mark A. Hall, "When Genes are Decoded, Who Should See the Results?; Many 'Greatly Overestimate the Risk'," The Washington Post F7 (February 29, 2000).

25. 42 U.S.C. §12102.

26. Rep. Owens stated that "[t]hese protections of the ADA will also benefit individuals who are identified through genetic tests as being carriers of a disease-associated gene. There is a record of genetic discrimination against such individuals, most recently during sickle cell screening programs in the 1970's. With the advent of new forms of genetic testing, it is even more critical that the protections of the ADA be in place. Under the ADA, such individuals may not be discriminated against simply because they may not be qualified for a job sometime in the future. The determination as to whether an individual is qualified must take place at the time of the employment decision, and may not be based on speculation regarding the future. Moreover, such individuals may not be discriminated against because they or their children might incur increased health care costs for the employer." 136 Cong. Rec. H 4623 (daily ed. July 12, 1990) (remarks of Rep. Owens). Similarly, Rep. Edwards and Rep. Waxman also stated that individuals who are carriers of a disease-associated gene may not be discriminated against under the ADA. 136 Cong. Rec. H 4625 (daily ed. July 12, 1990) (Statement of Rep. Edwards); Id. at H 4627 (Statement of Rep. Waxman).

27. Equal Employment Opportunity Commission, Compliance Manual, vol. 2, section 902, order 915.002,902-45 (1995). It is also possible that title VII of the Civil Rights Act of 1964, 42 U.S.C. § 2000e et seq.,may provide some protection against certain kinds of genetic discrimination since an argument could be made that discrimination based on genetic disorders that are racially or ethnically based, such as sickle cell disease, is prohibited under title VII. However, there are relatively few genetic conditions that have a strong connection with a racial or ethnic group, thus limiting the scope of potential coverage.

28. 42 U.S.C. §12102(2).

29. The EEOC gave the following example of its application of the third prong of the definition to genetic discrimination.

CP's genetic profile reveals an increased susceptibility to colon cancer. CP is currently asymptomatic and may never in fact develop colon cancer. After making CP a conditional offer of employment, R learns about CP's increased susceptibility to colon cancer. R then withdraws the job offer because of concerns about matters such as CP's productivity, insurance costs and attendance. R is treating CP as having an impairment that substantially limits a major life activity. Accordingly CP is covered by the third part of the definition of disability.

30. Prior to the Supreme Court's decisions there were three major limitations on the EEOC interpretation. First, the ADA specifically excludes insurance from its coverage except that this exclusion "shall not be used as a subterfuge to evade the purposes of title I and III." The exact parameters of this provision, especially as it relates to genetic information, are unclear although it would appear fair to say the nondiscrimination protections for individuals with certain genes would be considerably stronger in the employment context than when such individuals are being considered for insurance coverage. Second, the EEOC interpretation is part of guidance issued in its compliance manual. Specific prohibitions of discrimination in this area were not included in the statute and were also not part of the EEOC's regulations. Even if a court gives deference to the guidance as indicative of the agency's view of the statute, a court would not likely give such guidance the deference it would accord to statutory or regulatory language. In addition, even assuming the ADA was found to apply, it may not protect employees from having their employers have access to their genetic information. Although the ADA prohibits an employer from making medical inquiries prior to a job offer, the employer may obtain medical information in certain cases after the offer of employment has been made. Even if the prohibitions against discrimination in the ADA would apply, it would be

difficult to prove that genetic information was the reason for discrimination. This raises issues relating to the privacy of genetic information which are beyond the scope of this memorandum.

31. Statement of Commissioner Paul Steven Miller, U.S. Equal Employment Opportunity Commission, Before the Senate Committee on Health, Education, Labor and Pensions (July 20, 2000.

32. 146 Cong. Rec. S6050 (daily ed. June 29, 2000)(remarks of Sen. Kennedy).

33. 524 U.S. 624 (1998). For a more detailed discussion of this decision see Jones, "The Americans with Disabilities Act: HIV Infection is Covered Under the Act," CRS Report 98-599 (July 10, 1998).

34. 527 U.S. 471 (1999).

35. 527 U.S. 516 (1999).

36. Sutton v. United Airlines. See also Murphy v. United Parcel Service, where the Court held that the determination of whether the petitioner's high blood pressure substantially limits one or more major life activities must be made considering the mitigating measures he employs.

37. 527 U.S. 555 (1999).

38. EEOC Compliance Manual, Vol. 2, section 902, order 915.002,902-45 (1995).

39. See e.g., Whalen v. Roe, 429 U.S. 589 (1977).

40. For a more discussion of this issue see Gostin, "Genetic Privacy," 23 J. of Law, Medicine & Ethics 320 (1995).

41. H.Rept. 93-1416. 93d Cong., 2d Sess. 16-17 (1974).

42. 5 U.S.C. §552(b)(6).

43. For a discussion of recent developments in medical records privacy see Stephen Redhead, "Medical Records Privacy: Questions and Answers on the December 2000 Federal Regulation," CRS Rept. RS20500.

44. 65 Fed. Reg. 82461 (Dec. 20, 2000). For a more detailed discussion see Medical Records Privacy: Questions and Answers on the December 2000 Federal Regulation," CRS Rept. RS20500.

45. 65 Fed. Reg. 82731 (Dec. 20, 2000).

46. Claire Hackney, "Genetic Testing," Health Policy Tracking Service (April 5, 2000). The states listed in this survey are: Alabama, Arizona, California, Colorado, Connecticut, Delaware, Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Louisiana, Maine, Maryland, Minnesota, Missouri, Montana, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, Ohio, Oklahoma, Oregon, Rhode Island, South Carolina, Tennessee, Texas, Vermont, Virginia, and Wisconsin.

47. 29 U.S.C. §§ 1001-1145.

48. Hudson, "Genetic Discrimination and Health Insurance: an Urgent Need for Reform," 270 Science 391 (1995); Rothenberg, "Genetic Information and Health Insurance: State Legislative Approaches," 23 J. of Law, Med. & Ethics 312 (1995).

49. For a more detailed discussion see Judy Hearne and Hinda Chaikind, "Side-by-Side Comparison of H.R. 2990 and the Senate Amendment for Patient Protection," CRS Rep. RL30144.

50. See e.g., S. 1322, a bill to prohibit health insurance and employment discrimination against individuals and their family members on the basis of predictive genetic information or services (Sen. Daschle); H.R. 293, a bill to amend

the Public Health Services Act and the Employee Retirement Income Security Act to prohibit health issuers and groups health plans from discriminating against individuals on the basis of genetic information (Rep. Sweeney); S. 543, a bill to prohibit discrimination on the basis of genetic information with respect to health insurance (Sen. Snowe); H.R. 2457, a bill to prohibit health insurance and employment discrimination against individuals and their family members on the basis of predictive genetic information or genetic services (Rep. Slaughter); H.R. 2555, a bill to establish limitations with respect to the disclosure and use of genetic information in connection with group health plans and health insurance coverage, and to prohibit employment discrimination on the basis of genetic information and genetic testing (Rep. Stearns); H.R. 306, a bill to prohibit discrimination against individuals and their family members on the basis of genetic information or a request for genetic services (Rep. Slaughter); S. 479, a bill to amend title XXVII of the Public Health Service Act and other laws to assure the rights of enrollees under managed care plans (Sen. Schumer); H.R. 2878, a bill to protect the privacy of health information in the age of genetics and other new technologies (Rep. McDermott); H.R. 743, a bill to provide for certain military retirees and dependents a special Medicare part B enrollment period and a special Medigap open enrollment period and which prohibits discrimination in the issuance of Medicare supplemental insurance on the basis of genetic information (Rep. Scarborough).

National Council for Science and the Environment
1725 K Street, Suite 212 - Washington, DC 20006
202-530-5810 - info@NCSEonline.org

Monograph

A Physician's Primer *of* Clinical Genomics

Table *of* Contents

Preface

As practitioners, most of us had limited training in genetics, and a lot has changed in recent years. The field of clinical genomics now promises to revolutionize the way we practice not only primary care medicine but also every individual sub-specialty of medical practice. Being able to read the genetic code of individuals will prove to be every bit as great an advance in clinical diagnostics as the pioneering work of Robert Hooke and Anton van Leeuwenhoek with the microscope.

Although many of the advances in the past five years have been methodological, rather than clinical, these new methods of investigating the genome are now being applied to common clinical conditions, revealing novel therapeutic approaches with the promise of highly favorable outcomes. The new information is coming fast and furious, and will continue to do so for years to come, but the time to begin educating ourselves is now.

This guide places the new genomic information into a clinical context for practitioners and provides background information and defines key concepts so that the health care provider can understand the practical clinical applications of current genetic research.

1. Introduction to Clinical Genomics

The concept of "biochemical individuality" was first proposed by Roger Williams in 1956 to explain variability in disease susceptibility, nutrient needs, and drug responsiveness among otherwise seemingly healthy people. It is only in the wake of the ongoing genomic revolution, however, that predictive genetic testing has become available to allow us to assess true biochemical individuality. For the first time, physicians can predict with increasing precision who is more likely to develop specific diseases, who will respond favorably to a particular drug or supplement therapy, or react adversely, and finally, which nutrients are optimal for a particular individual's treatment, health, and well-being.

Genetics is the scientific study of heredity, one gene at a time. Genomics is the study of genomes, or the totality of the DNA of a single species. Genomics attempts to look at the totality of all our genes as a dynamic system, interacting with and influencing our biochemical pathways and physiology. The Human Genome Project is the mapping and sequencing of the entire human genome. The first draft of the entire human genome was published in April 2001, almost exactly one hundred years after the rediscovery of Mendel's "Laws of Heredity." The human genome consists of slightly more than 3,000,000,000 nucleotides (give or take a few hundred million) and it codes for every protein and every enzyme made by the human body. Some 40,000 or so genes are thought to exist in the human genome, yet we know the function of less than half of those genes.

As primary care practitioners, we stand at a critical crossroads where increases in availability of DNA-based testing and demand by patients for genetic information and advice necessitate our need to become genetically literate. This primer will provide broad-based and novel instruction in the advances of genomics with emphasis on clinical implementation of genomic information.

Clinical Genomics

Knowledge of the human genome will revolutionize the practice of medicine. Currently, three broad areas of clinically relevant genomics are rapidly advancing:

1. Mendelian inheritance
2. Pharmacogenetics
3. Predictive genomic testing for chronic diseases with multifactorial etiologies

Mendelian Inheritance

Mendelian genetics, or the inheritance of traits or characters in pedigree patterns, encompasses the history of genetics prior to genomic analysis. Animal husbandry and plant hybridization are early, pre-scientific examples of using Mendelian inheritance, albeit without an awareness of the scientific principles involved, for specific purposes, such as improving wheat yield, breeding sheep with denser wool, hybridizing tomato plants with bigger fruits, domesticating dogs, and so on.

In humans, once patterns of inheritance became clearly recognizable through family pedigrees, doctors were able to offer limited genetic counseling for conditions like hemophilia, muscular dystrophies, cystic fibrosis, Huntington's disease, Down's syndrome, and the like. However, all too often, little or no treatment was available for those affected.

Advances in genetic testing have facilitated the identification of point mutations and chromosomal rearrangements, making the diagnosis of inherited disorders faster, easier, cheaper, and more accurate. It is now relatively simple to determine who will develop a Mendelian genetic disorder, who is a carrier, and who is unaffected within a family. This has greatly enhanced the effectiveness of genetic counseling. Further, pre-implantation genetic diagnosis is also rapidly becoming available as an alternative to intra-uterine genetic diagnosis (e.g., amniocentesis) to rule out single gene defects or chromosomal rearrangements. With such technology, we are able to prevent the need for termination of pregnancy by screening for genetic aberrations before implantation takes place. These and other strategies for prevention, early diagnosis, and treatment are becoming increasingly commonplace as primary care practitioners interface with specialized genetic counselors.

Pharmacogenetics

Pharmacogenetics, simply put, is the field of pharmacology that uses genomic information to find the *right* drug for the *right* person at the *right* time. All drugs in use today act on fewer than 500 known molecular targets. If only 10% of the genes in the genome represent molecular targets for drug therapy – a very conservative estimate – then the possibility exists to develop some 3-4,000 new molecular entities to combat disease – that's 8 times more drugs than are on the market today![1]

Pharmacogenetics begins with functional genomics: determining the functions of genes in order to find those that make good targets for drug discovery. In most cases, genes code for proteins, and the proteins exert a physiological effect and thus are excellent candidates for development as drugs. Indeed, the first wave of

genomic drugs to come on the market will belong to the conventional classes of protein, antibody, and small molecule drugs that are in wide use today. Yet the new drugs are likely to exhibit increased specificity and efficacy. Epogen™, an analogue of the natural protein that stimulates red blood cell manufacture in the marrow, and monoclonal antibodies are examples of these new types of "smart drugs" emerging on today's pharmaceutical markets.

Pharmacogenetics will also focus on tailoring drug treatment to specific individuals. Why do some people have adverse drug reactions or some show no response, while others receive substantial therapeutic benefit – all from the same drug at the same dosage? The answers are increasingly believed to lie in genetic variability from one person to the next.

How we metabolize specific drugs has a lot to do with our genetic capacity for detoxifying and eliminating drugs from the body. If a particular enzyme is missing or is overly abundant, a given drug may not be ideal for that patient. It is hoped that with thorough genetic analysis many adverse drug reactions can be avoided altogether.

Moreover, this same general principle of impaired detoxification enzymes may underlie the pathophysiology of numerous troubling modern chronic diseases like chronic fatigue syndrome or fibromyalgia or autism. Some individuals may be genetically ill equipped to handle the added toxic burden of an increasingly industrialized world.

The ultimate endgame of pharmacogenetics, however, is likely to be therapies such as anti-sense RNA for shutting off specific gene expression and gene therapy for replacing damaged genes. The development of these therapies is only in its infancy with mixed clinical results and will probably not be clinically viable for some time to come.

Predictive Genomic Testing

Probably the largest potential area of medical intervention using clinical genomics may be categorized under the rubric of predictive genomic testing. In nature, changing just one nucleotide out of the 3 billion in the human genome allows for physiologic variation, for natural selection, and for evolution. Yet such changes are frequently disadvantageous for the health of the individual; only rarely is such a change physiologically beneficial. These single nucleotide polymorphisms (SNPs – pronounced "snips") are also believed to account for the vast amount of variation between individuals. Humans share in excess of >99.9% homology; it is the <0.1% of our genes that make us individuals.

Tremendous efforts are underway to identify polymorphisms, as they may hold

the ultimate keys to truly individualized medicine. The future impact of SNP research on primary care medicine cannot be overemphasized: most fundamental physiological processes like detoxification, immune surveillance, hormone signaling, and metabolic pathways are all dependent in large part on individual genetic variation.

SNPs are also known to play a significant role in the development of many chronic diseases. By examining conditions like heart disease, neurological degeneration, and osteoporosis, we can demonstrate how genetic testing for SNPs can play an enormous adjunctive role in treating these common clinical conditions. While SNPs have been shown to increase risk of developing chronic diseases dramatically, there are also intervention strategies involving diet, lifestyle, and specific nutrient and drug intervention that are available to minimize or even eliminate the effects of the increased genetic risk. As SNP testing becomes widely available, the public demand for accurate risk assessment and therapeutic prevention strategies will grow exponentially.

One potential problem is the number of SNPs in the genome. To date, roughly 100,000 SNPs have been identified, and literally millions exist in the DNA, but the vast majority of these have no impact whatsoever on human physiology. Finding the polymorphisms that make a real difference in our physiology is the first task of predictive genomics.

To be of clinical utility, the polymorphisms identified in predictive genomic testing must meet four criteria: they must be,

1. **Relevant** – the only polymorphisms in the genome of interest are those that exert a significant effect on our biochemistry and physiology

2. **Prevalent** – given our current knowledge of the human genome, only polymorphisms that exist in a significant percentage of the population are likely to be identified in a cost-effective manner

3. **Modifiable** – only polymorphisms whose effects are modifiable via clinical intervention (diet, lifestyle, supplements, pharmaceuticals, and toxin exposure reduction) are clinically useful

4. **Measurable** – our genes do not change but our functional physiology and metabolic reserve do change. The progress of our clinical interventions for risk reduction and functional improvement must be measurable. Functional laboratory testing is the primary vehicle by which these changes may be measured.

Over 5% of colon, breast, ovary and prostate cancers are estimated to be due exclusively to mutant genes. Carriers can have a 60-80% chance of developing the disease.[2] But by the same token, 20-40% of carriers of particular mutations will

NOT develop disease. Why? Because, in large part, diet, nutrition, and lifestyle factors can exert an enormous influence on how, or even if, a gene will express itself. The common misconception of genetic testing is that it foretells our fate. While that may be true of certain Mendelian traits, it certainly is not true of most predictive genomic testing. Rather, knowing about increased risk is the first step towards a committed and effective prevention strategy.

Similarly, SNPs in important biochemical pathways can alter the body's detoxification capacity and its ability to maintain proper immune surveillance. For example, multiple variations of cytochrome p-450 enzymes as well as glutathione-s-transferase and N-acetyl transferase have been identified and are known to play important roles in adverse drug reactions, drug resistance, as well as the development of complex syndromes like multiple chemical sensitivity and cancer, perhaps through an increased level of oxidative stress. Alterations in immune parameters can be identified through SNPs that affect the production of various cytokines like IL-1 and TNF-α. Genetic up-regulation of the production of these cytokines can lead to a TH-2 dominant state with increased incidence and severity of chronic inflammatory disorders.

The phenotypic expression of SNPs can frequently be modified through dietary and lifestyle choices, clinical nutrition, and judicious pharmacological intervention. Or at the very least alternative biochemical pathways can be supported to minimize the phenotypic effect of defective enzyme systems. Furthermore, laboratory testing is available to monitor modifications in physiologic capacity and function brought about through such interventions. This is what is commonly referred to as functional medicine or functional laboratory testing, which allows us the means to monitor the effectiveness of our treatment protocols, since, it should go without saying, a person's genetic propensities will never change. All we can do is affect the expression of those genes in the individual's biochemistry and physiology.

The specter of genetic determinism looms large in the public consciousness – most people are convinced that our genes are our fate. Nothing could be further from the truth. In fact, phenotypic expression of genomic determinants is largely modifiable. It is becoming increasingly evident that who we are as individuals is a function of both our genetic make up and the environment we subject our genes and our bodies to.

The goal of predictive genomic testing is to reveal underlying genetic susceptibility to a wide variety of clinical conditions and diseases. Every person wanting to take a proactive role in his or her health would benefit from an understanding of his or her genetic susceptibility and risk. For a few groups of patients, however, the benefits of predictive genomic testing may be especially great:

- Patients with a family history of chronic disease like heart disease, osteoporosis, cancer, allergy, or chronic inflammation, or patients who may have been adopted
- Patients with chronic conditions that have been refractory to standard treatment
- Patients with a history of prolonged toxic exposure

Health and disease lie at the intersection of our genes and our environment. Until now all we have been able to measure is pathology, function, and environment. Now, with the advent of predictive genomic testing we can measure genetic predisposition to many illnesses as well. For the first time in the history of medicine, we can begin to measure the true state of an *individual's* health. Medicine can assuredly never be the same. As a health care practitioner committed to using the latest clinical diagnostics for your patients, the time to develop a working knowledge of genomic testing and responsive intervention strategies is now. Otherwise, the future of medicine will pass by both you and your patients.

2. Piecing the Genetics Puzzle Together

The Mystery of Life

Variety is not only the spice of life it is also the driving force of natural selection and of evolution. Scientists now believe that all life on this planet evolved from a single common ancestor. Why? Because all life "speaks" the same language of heredity: we call that language DNA. The same four nucleotides (or "letters," if you will) are used to write the DNA code in every creature that is now alive or has ever lived on Earth. Variation in the genetic code is the primary means of biological change and it is biological change that has meant fruitful abundance. Using the common language of DNA, Nature was able to write the code that has allowed this abundance of creatures to evolve. In this sense at least, all life on this planet is one.

The 20th century may well be remembered in medicine as the century of Genetics. In 1900, the scientific community knew literally nothing about the mechanisms of heredity, yet, one hundred years later, we had a nearly complete map of all 3 billion nucleotides that make up the human genome and a dozen or so other species' genomes mapped as well. Some fifteen Nobel Prizes in medicine and physiology were awarded to researchers in genetics, far more than to any other field of medical inquiry. And with good reason, for here, we are at the very gates of life itself, reading the epic saga of how life came to be on this planet and how it continues to be passed from generation to generation in an unending chain to infinity. Life, as

difficult as it may be to define, appears minimally to involve two basic phenomena: the ability to replicate and the ability to create order. In Erwin Schroedinger's pregnant phrase, living creatures "drink orderliness" from their environment. In both cases, the key to order and to replication is information. That information is held within and passed on through our DNA.

Matt Ridley, in his riveting book, *Genome: an Autobiography of a Species in 23 Chapters*, draws a striking analogy for the human genome:

"Imagine the genome is a book.
There are twenty-three chapters, called CHROMOSOMES.
Each chapter contains several thousand stories, called GENES.
Each story is made up of paragraphs, called EXONS, which are interrupted by advertisements called INTRONS.
Each paragraph is made up of words, called CODONS.
Each word is written in letters called BASES.
There are 1 billion words in the book which makes it longer than… 800 Bibles."3

Claude Shannon, in the early 1940s, had the idea that information and entropy are opposing forces, each having an intimate connection with energy. In fact the more information a system contains, the less entropy it has, and vice-versa. Living systems defy entropy only in as much as they possess a high degree of information. That information comes from their DNA. It is the information found in DNA that allows living systems to "drink orderliness" from the rest of the universe and to produce offspring.[4]

Of course, the idea that "information" is the key to living beings is not particularly new. Aristotle believed that inherent in an egg was the "idea" of a chicken; so too, within an acorn was the "plan" for a future oak tree. Aristotle's ideas fostered the notion that there was a "vital force" integral to living beings. There is sweet seduction in the idea that the information implicit in living systems is a manifestation of some greater force that animates all life. That vital force is considered to be but one aspect of the Law. It is Law that informs, regulates and even intends the interrelationships of our physical reality. The very orderliness of the universe; our ability to express the world of matter in mathematical equations is evidence that there truly are Laws of Nature.[5] In such metaphors like "vital force" and "Law," we hear echoes of the opening passage of gospel of John, "In the beginning was the Word and the Word was with God and the word was God. The same was in the beginning with God…And the Word was made flesh…." As difficult as it may be to prove scientifically, many cannot help but believe that there are qualitative and not merely quantitative differences between living creatures and inanimate objects. Or in simpler terms, life is, and perhaps always will be, a mystery.

Yet the Word, the Law, the primordial information that intended life, cannot be DNA. It takes proteins to make DNA, and it takes DNA to encode proteins. Thus

we find ourselves stumped with another chicken-and-egg conundrum. Neither DNA nor proteins can exist without the other. Protein is phenotype: metabolism, biochemistry, body, the chicken. DNA is genotype: self-replication, information, code, the egg.

Before proteins, before DNA, there was the chemical substance that links the two together even to this day. That link, the primordial source of information was, in all likelihood, RNA, ribonucleic acid. RNA, unlike DNA and unlike proteins, can replicate itself. Looking at cellular physiology, it is an RNA-dependent enzyme that takes the RNA message from the DNA to a RNA-containing protein complex (ribosomes) that then translates the message from the DNA into a protein using RNA-transported amino acids. Thomas Cech and Sidney Altman postulate that the first gene, the "ur-gene," was a combined "replicator-catalyst": it consumed chemicals around it in order to replicate itself. Not exactly life as we know it, yet these ribo-organisms could create order and replicate themselves.

Still, these ribo-organisms had an inherent problem: in an adverse environment, or even if they got too large, they would fall apart. Over some stretch of evolutionary time, it is thought that these ribo-organisms were able to translate their information to a more stable molecule, i.e., DNA, and from thence to a more stable substance in proteins. RNA maintained its existence as the critical go-between for DNA and proteins, indispensable to the replication process.[6] Thus, life developed into its more stable forms: DNA is transcribed into RNA that is translated into proteins that, in turn, synthesizes and regulates DNA. And the cycle of life as we know it, oscillating between genotype and phenotype via the messenger RNA was born. So pivotal is this relationship to our understanding of cellular life that it is referred to as "The Central Dogma" of molecular biology.

How Genes Work

DNA has three known functions in living organisms:

1. DNA replicates itself
2. DNA codes for RNA which in turn codes for proteins, the primary building blocks of the cell, the tissues, and the body
3. DNA regulates gene expression, allowing for
    a. Cell growth
    b. Cell differentiation
    c. Cell replication
    d. Programmed cell death

The structure of DNA is complementary. It is built from deoxyribose (a sugar), phosphate groups, and four nucleotides or bases: adenine, cytosine, guanine, and thymine (mercifully abbreviated to A, C, G, and T). Adenine can only bind with thymine and cytosine can only bind with guanine, producing the complementary

structure. The 3-dimensional structure of DNA is like a ladder that has been twisted around its vertical axis: the deoxyribose and phosphate form the "rails" of the ladder, while pairs of A & T and C & G form the "rungs." The advantage of the complementary structure is simply that the DNA "ladder" can split with each half binding to complementary nucleotides in order to make two perfect copies of the original DNA.

This is no small project. If all the DNA in a single human cell were unraveled and stretched out into a straight line, it would measure about 2 meters (6 feet). Given the 100 trillion or so cells in your body, if all the DNA in all your cells were stretched out in a straight line, it would reach to the sun and back… a thousand times. That's rather a lot of information, even if, as we shall see, over 97% of it is junk.

The complementary binding of nucleotides to one another also allows DNA to code for RNA faithfully. RNA is structurally similar to DNA except,

1. RNA is single stranded,
2. RNA uses the nucleotide uracil (U) in the place of thymine, and
3. RNA's 3-nucleotide codons (think of them as "3-letter words") code directly for specific amino acids, allowing for the synthesis of proteins in ribosomes.

Only one strand of our DNA codes for RNA. This strand is called the "sense strand" while the other unused strand is referred to as the "anti-sense strand." (As a side note, one area of pharmacogenetics research involves the creation of anti-sense drugs that could bind to sections of DNA, preventing their transcription – effectively silencing a "bad" gene.)

Heredity is dependent on the genes found within the entire genome. Genes are those sections of the DNA that code for RNA (and subsequently protein synthesis). Only about 3% of the human genome is actually used by and for human physiology. The average gene is about 3,000 nucleotides long, but this can vary considerably – the gene for dystrophin, the longest known in the human body, is an enormous 2 million base pairs long. The final messenger RNA made from such genes is much shorter, however.

Genes are composed of exons, portions that actually code for proteins, and long introns interspersed between the exons, that do not code for anything. Introns have been likened to advertisements in a magazine: contributing nothing to the actual storyline and constantly interrupting it. And, like some magazines, the "advertisements" found within a gene are far longer than the actual "story" itself. Indeed, most genes have far more introns than exons in them. The gene is transcribed into RNA containing a complementary copy of the entire gene but before leaving the nucleus, the RNA is shortened. The introns are excised and the exons are joined together to produce the final messenger RNA.

Messenger RNA is composed of triplet codons, or 3-letter "words," that code for specific amino acids. Mathematically, since there are 4 nucleotides in RNA, there are 4^3, or 64, possible three-letter words. 61 triplet codons are specific for one of the 20 amino acids used to make proteins. The remaining 3 codons are stop codons, telling the ribosome to stop translation and release the protein. Obviously more than one codon can code for the same amino acid, but each codon is specific for only one amino acid. This allows for a precise translation of the genetic information in DNA into proteins. After release, the protein may still be modified post-translationally: e.g., a glycoside or a lipid group may be added or the protein may be folded into its final tertiary shape to finish the specific protein being manufactured.

Junk DNA

A close look at the human genome is rather surprising. You might think that all 3 billion nucleotides serve some clearly useful purpose for our survival and health. Surely we humans are such complex creatures that 3 billion nucleotides would be necessary to explain our individuality. Nothing could be further from the truth. In fact, 97% or so of the human genome doesn't consist of genes at all: it's genetic gibberish and is known as junk DNA since it codes for nothing and has no known function. A significant minority of the junk DNA codes for complete viral genomes, although the viruses themselves are never expressed phenotypically as actual viruses. There are several thousand complete viral genomes within our human genome. Known as human endogenous retroviruses, or Hervs, these viral genomes constitute about 1.3% of the entire genome, a staggering figure when you consider human genes only constitute roughly 3% of the genome.

Most of the remaining junk DNA is classified as minisatellite DNA, characterized by the presence of short arrays of tandem repeat units – base pairs that repeat a short to medium sequence over and over and over. Minisatellite DNA is remnant viral DNA that has "hitched a ride" on the cellular machinery of mammals: literal genetic parasites that have discovered a way to be replicated generation after generation without ever needing a "body" to do so.

The most abundant gene in the entire *human* genome is reverse transcriptase, a *viral* gene used to make a DNA copy of viral RNA for insertion into the host DNA. It's not the copy of an entire viral genome (Herv) but only one gene of viral origin. It is estimated that over 100,000 copies of reverse transcriptase exist in the human genome, accounting for a staggering 14.6% of the entire genome – 5 times more genetic material than what is clearly human. Presumably it exists in such staggeringly high quantities because reverse transcriptase has the ability to get itself transcribed and then make a DNA copy of its RNA only to get reinserted into the genome at another location.

Almost as common are shorter sequences of a viral promoter sequence known as Alu repeats. The 280-basepair Alu text may repeat over a million times in the human genome, constituting another 10% of the entire genome. All tolled, more than 35% of human DNA is minisatellite DNA.

The presence of these "genetic freeloaders" has caused many Evolutionary Biologists to rethink evolution itself. Perhaps natural selection has less to do with competition between species or individual organisms within a particular group and is actually far more about competition between genes using individuals and species as mere vehicles for their propagation. Genes may be thus be thought of as "selfish replicators" or "selfish genes," to use Richard Dawkins' terminology.[7] From one perspective, evolution's greatest successes may be those genes that have figured out a way to replicate themselves even though they have dispensed with their bodies altogether. Selfish genes may prove that for some, there really is a "free lunch" in nature.

Unfortunately for their hosts, these genetic parasites are not passive passengers but can pose credible threats to the integrity of the host genome. Replicating in the "wrong place" of the genome could be disastrous, especially given the tendency of selfish DNA to jump from one locus to another seemingly at random. Barbara McClintock discovered this phenomenon of "jumping genes," or transposons, in the 1940s while working with Indian corn, but the importance of her discoveries would not be recognized for many years. It is now estimated that 1 out of 700 spontaneous human mutations are due to the action of "jumping genes" (the number may be as high as 1 out of 10 spontaneous mutations in other mammals).

Mounting evidence suggests a scenario in which viral genes replicate freely within mammalian genomes until the mammalian genome "learns" to suppress the spread of viral genes. Yet the mammalian genome, while able to stop rampant replication of viral genes within its own genome is powerless to excise the genes already incorporated. Thus, we see cumulative evidence of past infections.

The primary means of controlling viral gene replication within the human genome appears to be methylation. A methyl group may be added to a cytosine nucleotide to "shut off" that segment of DNA. Researchers have long thought that methylation of DNA allows for tissue differentiation in an individual cell (nerve cells versus heart or skin cells, etc.), but new evidence suggests that an equally important function of DNA methylation may be to suppress replication of transposons – viral parasitic elements – within the mammalian genome. This may be especially important in the genesis and pathophysiology of cancer since one of the first events associated with cancerous transformation of cells is stripping the DNA of methylation. This allows not only unfettered cell replication, but also unfettered viral transposon replication. This is yet another area where competent methylation capacity is critical for normal physiology.

Ironically, the transposons and minisatellite regions of the DNA were the first to have clinical applications, especially in forensic medicine. These highly repetitive and variable regions of DNA are the source of "DNA fingerprinting" (see discussion below in "diagnostic genetic methodologies"). The minisatellite arrays with the genome can serve as unique identifiers for individuals.

A Brief Timeline of the Discoveries and Concepts of Heredity and Molecular Genetics

If I have seen further, it is because I have stood on the shoulders of giants
– Sir Isaac Newton

1655 Robert Hooke discovers that living matter is made up of "cells."

1759 CF Wolff proposes the general cell theory.

1838 Matthias Schleiden concludes that all plants are made up of cells.

1839 Theodor Schwann concludes that all animals are made up of cells.

1859 Charles Darwin publishes *The Origin of Species* and discovers the Law of Natural Selection.

1865 After breeding ~28,000 pea plants for seven characteristics, Gregor Mendel discovers the Laws of Heredity and publishes *Experiments in Plant Hybridization* in the Brunn Society for the Study of Natural Science's journal. No one noticed; not even Darwin.

1869 Johann Miescher isolates DNA from the nuclei of white blood cells as pus in soldier's bandages, but didn't have a clue as to what it was or did.

1869 Francis Galton publishes *Hereditary Genius*, claiming that heredity alone is responsible for character traits – his work would evolve into the eugenics movement.

1875 Francis Galton demonstrates the usefulness of twin studies in elucidating the relative importance of genes and environment in determining characteristics.

1882 Walther Fleming, using dyes to stain cells, discovers and names "chromosomes."

1889 Francis Galton publishes *Natural Inheritance* and describes the quantitative measurement of metric traits in populations. This is the first statistical study of variation, a field now known as biometry.

1892 August Weismann proposes that heredity is transmitted by a substance with a "chemical and molecular constitution."

1885-1901 Albrecht Kossel discovers that DNA contains the bases adenine, cytosine, guanine, and thymine (ACGT). Nobel Prize 1910.

1900 Karl Erich Correns, Hugo de Vries, and Erich Tschermak independently re-discover Mendel's Laws.

1900 Karl Landsteiner discovers the blood-agglutination phenomenon in humans, later classified as A, B, O, and AB blood types.

1900 Pearson develops the chi-square test in statistics.

1902 Archibald Garrod first reports that a human disease (alkoptonuria) behaves as a Mendelian recessive.

1902 William Bateson coins the terms, "genetics," "allele," "heterozygote," and "homozygote."

1902-3 Walter Sutton formulates the chromosome theory and discovers that they come in pairs. Later he discovered that sperm and egg cells had but one copy of each chromosome.

1903 Wilhelm Ludwig Johannsen introduces the ideas of genotype, phenotype, and selection.

1908 Godfrey Harold Hardy suggests that Mendelian mechanisms acting alone have no effect on allele frequencies in populations. This observation forms the mathematical basis for population genetics.

1909 Wilhelm Johannsen coins the term "gene" for the sections of chromosomes responsible for passing on a particular trait. He also coins the terms, "genotype" and "phenotype."

1909 Archibald Garrod publishes *Inborn Errors of Metabolism*, the earliest discussion of the biochemical genetics.

1915 *The Mechanism of Mendelian Heredity* by Morgan, Sturtevant, Bridges, and Muller is published.

1920s Thomas Hunt Morgan conducts thousands of experiments with *Drosophila* and shows that genes lie in a row on chromosomes, "like beads on a string." He is awarded the first Nobel Prize for work in Genetics in 1933.

1920s Frederick Griffith found that he could kill mice by injecting them with live benign bacteria combined with dead pathogenic bacteria. He postulated a "transforming principle," later shown by Avery in 1944 to be DNA.

1927 Hermann Muller discovers that X-rays can induce mutations in *Drosophila*. Nobel Prize 1946.

1930s Phoebus Levene discovers that DNA also contains deoxyribose, a sugar.

1941 George Beadle and Edward Tatum formulate the one gene/one enzyme theory: each individual gene causes the production of only one protein. Nobel Prize 1958.

1944 Oswald Avery proposes that DNA is the stuff of heredity and of genes. He concludes that DNA was the "transforming principle" that killed the mice in Griffith's experiments. Not confirmed until 1952. Until this time, the biochemical mechanisms of heredity were unknown and hotly disputed.

1940s Barbara McClintock discovers transposons, a.k.a. jumping genes. Ignored at first, her research became instrumental in explaining "selfish genes." Nobel Prize 1983.

1940s Ochoa and Kornberg discover the biosynthetic pathways for RNA and DNA. Nobel Prize 1959.

1950 Erwin Chargaff discovers that DNA contains equal numbers of adenine as thymine and cytosine as guanine; known as Chargaff's ratios.

1951 Rosalind Franklin uses X-ray diffraction to help elucidate the structure of DNA.

1952 Alfred Hershey and Martha Chase prove that DNA is the hereditary material.

1952 Frederick Sanger's team works out the complete amino acid sequence for insulin. Nobel Prize in Chemistry 1958.

1953 James Watson and Francis Crick discover the double-helix structure of DNA. Noble Prize 1962.

1957 Arthur Kornberg synthesizes DNA in a test tube.

1959 J. Lejeune, M. Gautier, and R. Turpin show that Down syndrome is trisomy 21, a genetic disorder.

1960s Werner Arber, Daniel Nathans and Hamilton Smith discover restriction enzymes in bacteria that cleave viral DNA at specific places. Nobel Prize 1978.

1966 Nirenberg and Ochoa "crack the genetic code" by identifying the 3 letter "words" coding for the 20 amino acids.

1973 Stanley Cohen and Herbert Boyer create the first transgenic organism by puting a toad gene into a bacteria using restriction enzymes.

1978 Louise Brown, the first in vitro fertilized embryo, or "test tube baby," was born.

1980 US Patent and Trademark office grants a patent on a genetically engineered bacteria.

1982 The first recombinant DNA drug approved for use in humans: insulin from transgenic pigs.

1983 Kary Mullis develops polymerase chain reaction (PCR) technology. Noble Prize 1993.

1984 Alec Jeffries develops DNA typing (DNA "fingerprinting") after discovering the unique patterning of minisatellite DNA in individuals.

1988 US Patent and Trademark office grants a patent for the first genetically altered mammal: the oncomouse.

1988 The Human Genome Project begins.

1989 Bishop and Varmus awarded Nobel Prize for discovery of the cellular origin of retroviral oncogenes.

1990 W. French Anderson performs the first approved gene therapy on a human; Ashanti DeSilva.

1994 Calgene Corporation introduces the first genetically engineered food on the market: the FLAVR SAVR‰ tomato.

1996 Ian Wilmut clones "Dolly," the sheep from an adult utter cell, demonstrating the pluripotency of the somatic cell genome.

2000 First "draft" of the human genome completed simultaneously by the Human Genome Project and by Celera Corporation.

2002 First commercially available laboratory profiles measuring polymorphic variants for predictive genomics for common clinical conditions by Great Smokies Diagnostic Laboratory.

Diagnostic Genetic Methodologies

Genetic Engineering

The genomics revolution has of course been predicated on the development of new laboratory methodologies that enable us to read the sequence of specific DNA nucleotides, thereby mapping genes themselves. A chance meeting between two scientists at a conference in Hawaii in 1973 set in motion the development of the biotechnology necessary for reading the code of life. Herbert Boyer was a researcher at UCSF working with restriction enzymes. Restriction enzymes are bacterial enzymes whose purpose is to protect the bacteria against viral attack by literally shredding the invading viral DNA into bits. As it turns out, restriction enzymes are absolutely specific in terms of the sequence of DNA they recognize in order to cleave the DNA at precise locations. Moreover, different bacteria express different restriction enzymes recognizing different nucleotide sequences for cutting DNA. Today, more than 1000 different restriction enzymes have been identified. Meanwhile, Stanley Cohen of Stanford was working on bacterial exchange of plasmid DNA and was intensely interested in the potential of using restriction enzymes to facilitate exchange of DNA between bacterial plasmids. Cohen and Boyer began working together to this end.

Their research was simple, elegant, and effective. First, restriction enzymes were used to "snip" or "cut down" sections of plasmid DNA from two different bacteria (one strain resistant to tetracycline, the other resistant to kanamycin) at exactly the same sequence location. Mixing the identically cut down segments of DNA in the presence of a ligase enzyme allowed them to be knit back together and resulted in the formation of new hybrid plasmid DNA. They demonstrated the successful creation of the new hybrids by the transformed bacteria's ability to grow on medium containing both tetracycline and kanamycin. For the first time DNA was transferred directly and *in vitro* from one organism to another by humans. The field of recombinant DNA was born.

Cohen and Boyer asked the next logical question, "Can DNA from different species be as easily exchanged?" Accordingly, they cut down a gene from a toad and mixed it with bacterial DNA. With every new generation of bacteria, the toad gene was present. Species boundaries could be crossed, they demonstrated, and the first transgenic organism was created.

The terms genetic engineering, gene cloning, gene splicing, and recombinant DNA are all used inter-changeably to indicate the essential process created by Boyer and Cohen. Today it is common to buy cheese, for instance, that has listed in its ingredients, "microbial enzymes," indicating that the enzyme to make the cheese, originally from cows, has been grown from transgenic bacteria bioengineered with the bovine enzyme gene. Other areas of transgenic experimentation

abound, including novel plant breeding: herbicide and insect resistant plants; nitrogen fixing plants; plants producing vaccines; etc. The list of possibilities is endless because the capacity to mix DNA from different species is endless.

The first commercially marketed, genetically altered food offered to consumers came on the market in 1994. Calgene, a biotechnology company in California, created the FLAVR SAVR‰ tomato: a tomato plant that would not easily bruise or rot once picked off the vine and so could be picked later in the ripening cycle to improve flavor and still make to long journey to the supermarket without being prematurely damaged.

Research into transgenic animals (known also as chimeras) is also proceeding rapidly. Animals can be genetically engineered to produce biological products that humans need. Transgenic pigs, for instance, now produce human insulin for diabetic patients. This type of process - putting human genes into animal carriers - is known colloquially as molecular farming, or "pharming." Further, transgenic genes have been spliced into animals and plants, inducing an adaptive advantage for specifically designed ends. For instance, human growth hormone genes have been spliced into salmon, chickens, and pigs in an effort to produce faster growing and bigger animals for human consumption. Humans' needs for organ transplants may one day be met by growing and harvesting pigs genetically altered to reduce the likelihood of organ rejection.

Critics of bioengineering have coined the term "frankenfoods" to describe transgenic plants and animals. To be sure, the future of how we use transgenic organisms is yet to be fully determined, but, like it or not, Pandora has opened her box. Since 1986, over 2,000 transgenic plants and nearly as many transgenic animals have been developed and, of course, patented.

DNA Fingerprinting

In 1984, Alec Jeffreys noticed that certain segments of DNA had short sequences that repeated over and over, sometimes as introns (non-coding segments of DNA that sit in-between the coding segments, known as exons) within a specific gene and sometimes within the larger, non-coding sections of the genome. He called theses segments "minisatellites." He also noticed that the pattern of minisatellites was unique to each individual – no two were alike (except, of course, in identical twins). From this he reasoned that minisatellites in DNA could be used as unique identifiers in forensic medicine and elsewhere, much as fingerprints are currently used, only with a higher degree of accuracy.

The procedure for DNA typing is fairly simple: a sample of DNA is extracted and purified and is then cut at specific locations using restriction enzymes, producing

DNA fragments of varying lengths. Placing the fragments on a gel plate and passing an electric current across the plate (gel electrophoresis) causes the smaller fragments to migrate more quickly toward the positive pole than the larger fragments. The sorted DNA fragments are then subjected to a blotting technique in which they are split into single strands and transferred to a nylon sheet. A radioactive DNA probe is added which will anneal (bind) to the single-stranded DNA at specific sequences of minisatellite DNA. Finally, a piece of x-ray film is then exposed to the labeled, bound, blotted, and separated DNA to reveal unique patterns of minisatellites.

Polymerase Chain Reaction (PCR)

DNA fingerprinting only works if you have a fairly large amount of DNA to use in the process. Polymerase Chain Reaction or PCR was developed in 1983 by Kary Mullis in order to generate multiple copies of DNA from a small sample. PCR can take even a single strand of DNA and within several hours generate billions of copies of that DNA. Simply put, PCR allows for exponential amplification of a specific genetic sequence.

PCR is a three-step process carried out in repeated cycles. Ingredients required for the process include, a sample of the DNA or DNA segment to be copied, two short primer sequences that can bind (or anneal) to the template and form a starting point for copying a specific DNA segment, free nucleotides, and DNA polymerase, the enzyme that promotes DNA replication.

First, the DNA is denatured, or separated, by heating it to 95ºC (203ºF). Second, the temperature is lowered to 55ººC (131ºF) so that the primers can anneal to the template. Third, the temperature is raised to 72ºC (162ºF) where DNA polymerase begins adding nucleotides onto the ends of the annealed primer. The entire process takes only about 5 minutes and then can begin again. Usually 20-40 cycles is more than enough to produce an adequate sample of DNA for "fingerprinting" analysis; 35 PCR cycles can produce a little over 17 billion copies of the DNA.

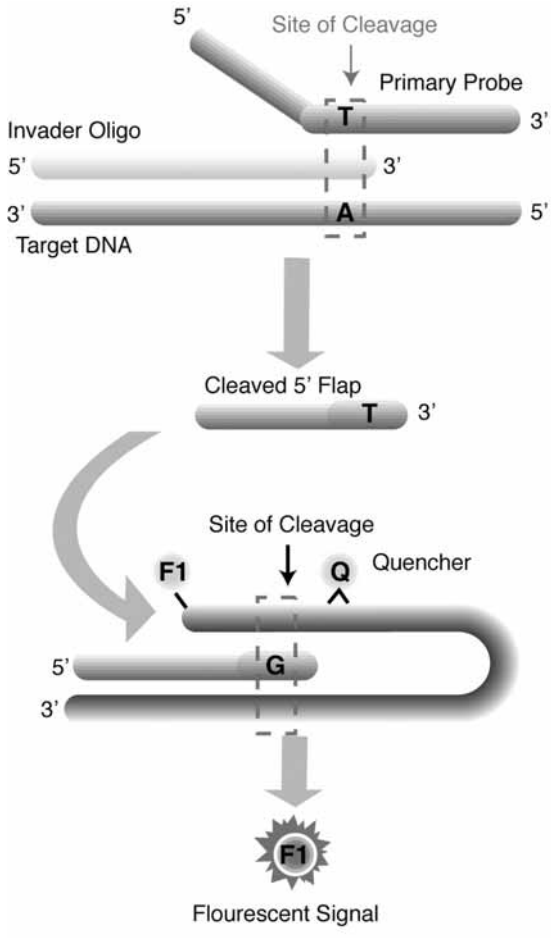
When PCR was first developed it had one serious limitation: new DNA polymerase had to be added after each cycle since the 95ºC temperature would destroy human DNA polymerase (it is, after all, a protein). The problem was solved in 1987 with the discovery that a thermophilic bacteria (*Thermus aquaticus*) found naturally in hot springs made DNA polymerase that could withstand the exceedingly high temperatures of their own natural environment (~100ºC). By using heat-stable, bacterial DNA polymerase, the cycles can continue uninterrupted and without needing to add new reactants with each cycle. This allowed researchers to develop automated PCR machines, dramatically reducing the cost, time, and skill necessary to carry out PCR.

PCR has many applications since it requires only a very small sample of DNA to be able to work. It is used in forensic medicine when only a small amount of DNA is culled from a crime scene, even a single hair; it is used to detect low-level viral infections like HIV; it is used to amplify DNA fragments found in a 40,000 year old woolly mammoth; and it has been an integral tool in the human genome project, allowing us to map all 3 billion nucleotides.

Invader®, Assay

New, inexpensive DNA assays are coming on the market, making DNA analysis possible for many conditions, including routine single nucleotide polymorphism, or SNP, analysis. A unique diagnostic picture of a person's genetic potential is now commercially available. The Invader®, DNA assay is one such method.

Invader®, technology is a novel, homogenous platform that can analyze DNA without prior PCR amplification of the target sequence. Instead of amplifying the target itself, the Invader®, assay amplifies a target specific signal, detected by a standard fluorescence microtiter plate. So, rather than exponentially amplifying the DNA itself, as in PCR, the Invader® platform uses a larger sample of DNA and then linearly amplifies the signal which detects a particular DNA sequence. Invader®, technology uses oligonucleotides that bind to the target in a sequence-specific manner and an enzyme that cleaves in a structure-specific manner, enabling it to detect single base changes.[8,9]



The Invader®, DNA reaction involves two steps:

In the primary reaction, two synthetic oligonucleotides hybridize in tandem to the target DNA, forming an overlapping structure, or flap. An enzyme recognizes and cleaves off this flap, releasing it as the target specific product. Multiple copies of the primary probe can bind, having its flap clipped off, resulting in the amplification of flaps in the reaction well (see diagram).

In the secondary reaction, the flaps are free to bind to a fluorescence resonance energy transfer (FRET) cassette, creating another overlapping flap this time in the FRET cassette, also cleaved by the enzyme. When the FRET cassette is cleaved, the fluorophore (F) and quencher (Q) are separated, producing a fluorescence signal that can be read.

If the oligonucleotides do not bind, no flap is released, no secondary binding and cleavage takes place and no fluorescence signal is produced or detected. The procedure is sensitive, accurate, precise, and flexible.[10,11]

3. Mendelian Inheritance

(a.k.a. the genetics you probably remember)

The Laws of Heredity are few; their implications for life are vast. The simplest genetic characteristics are those whose presence depends on the genotype at a single locus: one gene controls the expression of one characteristic. Such characters are known as Mendelian. Over 10,000 Mendelian characters have been identified in humans.

After breeding some 28,000 pea plants, Gregor Mendel proposed a number of laws regulating inheritance in plants. He studied seven simple traits (alleles) in pea plants. For instance, some plants were tall, others short; some had wrinkled peas, some smooth, etc. Each of these traits was determined by an allelic variant at an individual gene locus. What we refer to as genes today, he simply called "factors" of inheritance.

In sum, the Laws of Heredity Mendel discovered were,

I. Each physical characteristic corresponds to a single gene
II. Genes come in pairs
III. Only one gene of the pair is passed on to the next generation from the parent
IV. It is equally probable that either gene will be passed on
V. Some characteristics are "dominant" while others are "recessive"

In spite of a lukewarm reception at the Brunn Society for the Study of Natural Science, Mendel published his findings in the Society's journal.[12] Mendel sent copies of his work to botanists around the world but no one recognized the importance of his findings. Thirty-five years later, and sixteen years after Mendel's death, Correns, de Vries, and von Tschermak each independently arrived at similar conclusions as Mendel had and each was stunned to realize that his own "revolutionary discoveries" were merely repetitions of Mendel's previous work.

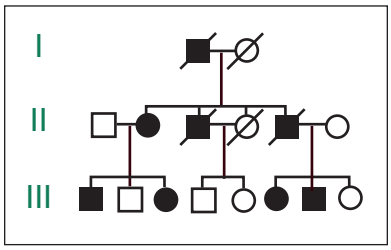
A trait (character) is dominant if it is expressed in the heterozygote (only one of the chromosome pair carries the gene) and recessive (both chromosomes carry the

gene) if it is only expressed in a homozygote. Dominant and recessive are properties of traits, not of genes themselves. Mendelian pedigree patterns are not always as evident in humans as they are in pea plants due to a number of complicating factors. Chief among these is incomplete penetrance. The penetrance of a character is the probability that a person with the genotype will manifest the dominant character. Other confounders include delayed onset of late-age genetic disorders, multigene effects, and variable expression of genes (different features of a single genetic syndrome will appear in similarly affected individuals). In addition, spontaneous mutations can occur where no pedigree association exists.

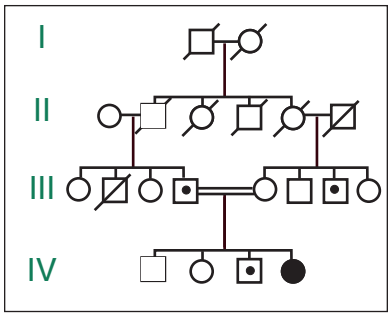
There are six basic Mendelian pedigree patterns:
1. Autosomal dominant
2. Autosomal recessive
3. X-linked recessive
4. X-linked dominant
5. Y-linked
6. Mitochondrial (matrilineal)

Complications aside, the ideal characteristics of these patterns of inheritance are as follows:

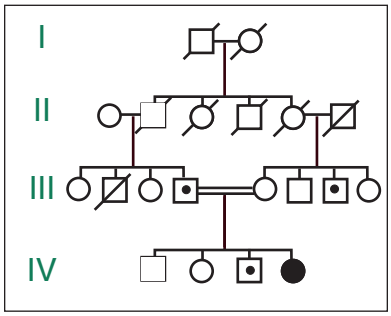
Autosomal dominant inheritance
- The affected person has at least one affected parent
- Affects either sex equally
- Transmitted by either sex
- Offspring of an affected person have a 50% chance of inheriting the trait
- E.g., Huntington's disease



Autosomal recessive inheritance
- Affected people are usually born to unaffected parents, unless one or both parents is homozygous for the trait
- Heterozygous "carriers" are usually asymptomatic
- Incidence increases with increased consanguinity
- Affects either sex
- The offspring or two heterozygous carriers have a 25% chance of being affected

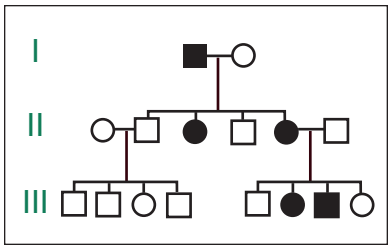


X-linked recessive
- Affects males predominately
- Parents are usually unaffected (mother is usually an asymptomatic carrier)
- Females can be affected if the father is affected and the mother is a carrier (probability = 50%)
- There is no male to male transmission in the pedigree
- E.g., hemophilia



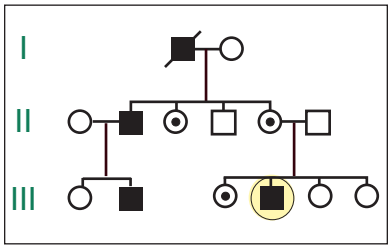
X-linked dominant inheritance

- Affects either sex but more females than males, since an affected male will always transmit the trait to his daughters
- Females are often affected more mildly than males, and they usually have an unaffected X-chromosome
- The child of an affected female has a 50% chance of being affected regardless of sex
- For an affected male, all of his daughters but none of his sons will be affected



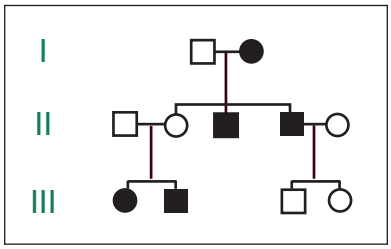
Y-linked inheritance

- Affects only males
- All sons of an affected father are affected
- Traits are usually not severe
- E.g., hairy ears



Mitochondrial (matrilineal) inheritance

- Mitochondria are inherited from the maternal egg, not the paternal sperm
- Heteroplasty (multiple mitochondrial genomes in one individual) is possible but fairly uncommon



Mendelian inheritance patterns were the first evidence to unlock to mysteries of heredity. While 10,000 traits are known to be Mendelian (e.g., eye color), at least as many traits are non-Mendelian. Height, intelligence, personality, and a thousand more characteristics of creatures are multi-factorial, controlled by the interaction of numerous genes, each independently assorted. Furthermore, the same confounders for simple Mendelian inheritance - incomplete penetrance, environmental influences on gene expression, spontaneous mutations - also occur in multi-genic characteristics, but their effects are exponentially multiplied. Still, the Laws of Heredity have taught us much and form the basis from which we learn to know what we do not know about dynamic interactions between genes within the genome and between the genome and the environment.

4. Predictive Genomics

Polymorphic Variation and Single Nucleotide Polymorphisms

Polymorphisms, literally, "many shapes," include any variation in the genome between individuals. What makes you different from your neighbor in genetic

terms? As it turns out, it's about 0.0003% of the genome! Polymorphisms can be harmless. If they occur randomly in the DNA, which appears likely, then >97% would be harmless to the individual since they would occur in the non-coding portion of the DNA (recall that only about 3% of the human genome actually codes for genes). But polymorphisms can also be disastrous – cystic fibrosis, muscular dystrophy, sickle cell anemia, and the like are examples of very serious diseases that result from changes in only one letter of the genetic code. Finally, some polymorphisms are somewhere between harmless and full-blown disease – they affect function but only moderately, sometimes improving that function, but more often, impairing it.

In thinking about polymorphisms, it is helpful to remember that there is no final "goal" of evolution; no such thing as evolutionary "progress." Natural selection is simply the process whereby organisms change to fit the limitations and opportunities afforded by a changing physical environment. To the extent that organisms can change to meet their challenges and opportunities, they are successful, i.e., they survive and thrive. Variation within a species improves the likelihood that at least some individuals in that species will be better able to meet those environmental challenges and carry the future of the species within their loins. Species variation at its most basic level occurs in the genome first. Polymorphisms are another name we give to genomic variability within a species.

Landsteiner blood groups (A, B, AB, and O) are an excellent example of polymorphisms and illustrate the evolutionary implications for change. ABO blood groups result from variation in the enzyme galactosyl transferase coded for by 1,062 base pairs within a gene that is ~18,000 base pairs long. There are only seven nucleotides that are different between type A and type B individuals. Three of the mutations are silent, i.e., they code for the same amino acids in the final protein, leaving only four points of functional genetic polymorphism.[13] Further, type O differs from type A by a single deletion: the 258th nucleotide is missing in type O, but this produces enormous changes since there is a frame-shift and every three-letter codon after that is different than for type A.

Is it the case that all blood groups are equally harmless and innocuous and that we are merely observing variation that is neutral from the perspective of natural selection? Was it merely by chance that the Americas were originally populated only with individuals with type O blood? Or is it possible that other blood groups came to the Americas but subsequently died out. Simply put, are there specific adaptive advantages to the various blood groups?

The latter idea appears to have considerable merit when considering the epidemiological evidence. In the 1980s it became apparent that children with certain blood types were more or less susceptible to various types of infections. For instance, type O individuals appear to be more susceptible to cholera; type B are somewhat more resistant; type A more resistant still; and Type AB individuals are virtually

immune to cholera. This illustrates a common principle in genetics, known as frequency-dependent selection: adaptive advantage always lies with the rare version of a gene so that neither version can become extinct (recall that if two AB individuals mate, only half of their offspring will be AB; 25% will be BB and 25% will be AA; i.e., if AB individuals survive, so to will BB and AA individuals).

On the other hand, type O individuals are more resistant to malaria and to syphilis, and are less likely to get various types of cancer. Similarly, one in five individuals are unable to secrete ABO blood group proteins into their saliva and other body fluids. These non-secretors are more likely to suffer conditions like meningitis, yeast infections, and urinary tract infections, but less likely to catch influenza or respiratory syncitial virus.[14] This illustrates the general principle that genetic variability often has a lot to do with prevalent infectious diseases. This should not surprise us given our understanding of competition between species as a central element in natural selection: "survival of the fittest."

Other examples abound. Pima Indians have a genetic constitution that would allow them to survive longer than most humans without food, often referred to as the "thrifty genotype." In an age when food supply was anything but certain, this was a distinct adaptive advantage. However, in the current world of ample food, it is a distinct disadvantage. Almost every adult Pima Indian is obese and diabetic. Their "thrifty genotype," once an advantage, is now clearly a liability.

Part of being fittest, from the perspective of the species, is genomic variety. Which might lead us to speculate that the old wives tale of opposites attracting may have some foundation in biology. Indeed, evolutionary behaviorists have supplied us with some intriguing confirmatory evidence. Claus Wederkind and Sandra Furi conducted experiments suggesting that men and women prefer the body odor of members of the opposite sex who are most *unlike* them genetically in terms of major histocompatability genes that are involved in immunological differentiation between "self" and "not-self." MHC proteins are especially important in recognizing foreign invaders.

Similar findings have been found in mice where females appear to choose mates based on the smell of their urine, choosing mates that are the most dissimilar to them genetically. As an interesting side note, in the human study, only women taking oral contraceptives failed to show a preference for different MHC genotypes. The researchers concluded, "No one smells good to everybody; it depends on who is sniffing whom." And so it appears, "opposites really do attract."[15]

The general principle here appears to be that different versions of genes become more or less frequent in response to the threat of specific diseases, and variation is good for the species. Yet what is good for the species may not be so good for the individual. Indeed, here we have the crux of the difference between natural history and medicine. Medicine is the art and science of restoring sick *individuals* to

health, and has nothing to do with populations or natural selection or adaptive advantages.

From the perspective of medicine, we must view polymorphisms in an entirely different light. In medicine, polymorphic variation is likely to convey greater or less susceptibility toward specific diseases by improving or impairing physiological function. The most common type of polymorphism is known as single nucleotide polymorphisms or SNPs in which, as we have said, a single nucleotide in a gene is changed.[16] Currently, a consortium of private companies and governmental agencies has set for itself the task of identifying and cataloguing as many SNPs as possible and as quickly as possible in order to keep this intellectual property within in the public domain (since genetic variations are patentable under US law). Their goal is to identify 100,000 SNPs in the human genome by the end of 2002.

SNPs analysis may be critical for the complete understanding of complex human diseases since certain genotypes will be consistently associated with those individuals who develop particular diseases – both acute and chronic. Aberrant genes produce aberrant proteins and enzymes. By identifying the genetic aberrations, we may come to a more complete understanding of the molecular basis of diseases, from which novel therapeutics may arise. Thus, population genetics and epidemiological genetics may lead to advances in molecular genetics and to more effective therapeutics for individuals. This research may prove especially fruitful for common chronic diseases like heart disease, diabetes, depression, senile dementia and many cancers, where a combination of multi-factorial genetic and environmental influences are all but certain.[17,18] It is estimated that by the year 2020, 73% of all deaths in industrialized countries will be from non-communicable diseases.[19] The importance of fully understanding chronic multifactorial diseases will become increasingly important for more effective clinical intervention.

To this end, it will also become increasingly important to identify SNPs in individuals that confer greater risk or protection in developing chronic diseases. Those SNPs that will be most important clinically are the SNPs that are relevant to the development of common chronic diseases, have a reasonably high prevalence in the general population, and whose physiological effects are modifiable using diet, nutritional intervention, lifestyle changes, and specific pharmacological intervention. In other words, clinically important SNPs must be relevant, prevalent, and modifiable.

At this stage of our medical knowledge, we do not have a plethora of outcome studies of intervention trials to support specific therapeutic regimens for specific single nucleotide polymorphisms. Some use this limitation as justification for not testing altogether – if we have no proven therapies to ameliorate the adverse physiologic effects of a particular SNP, is it ethical or wise to inform a patient that s/he has a particular genetic limitation?

Even in the absence of clear clinical outcome trials, we can still make sound medical inferences based on our knowledge of biochemistry and physiology. While many therapies have not been validated as specifically effective, many therapies are known to be generally effective for chronic diseases, and we can be absolutely certain that no intervention will definitely be ineffective. Furthermore, it is only by identifying individuals who are at increased risk based on their genetic profiles that we will be able to construct and conduct thorough therapeutic efficacy studies.

Consider the following hypothetical situation: it's difficult to win at poker if you don't know what cards you have been dealt. You don't know which cards to hold, which to discard; you don't know how much to bet or whether to fold and wait for the next hand. By analogy, without some understanding of your genetic strengths and weaknesses, you don't know how to play the genetic "hand" life has dealt you.

Predictive genomic testing is currently available for numerous chronic diseases, including cardiovascular disease, osteoporosis, detoxification defects, and immunological defects associated with the gut associated lymphoid tissue and chronic inflammatory conditions. In each of these areas, functional laboratory testing also exists which allows the practitioner to measure the functional integrity of physiological systems as well as the system's metabolic reserve. The combination of genomic SNP analysis and functional laboratory testing provides a novel, effective, and truly comprehensive assessment of both risk and function.

Nature vs. Nurture

Our health and, indeed, who we are at any given moment in time is the result of a combination of two powerful influences in our lives: our genes and our environment. Virtually all human characteristics, not to mention all human diseases, result from the complex interplay of genetic susceptibility and modifiable environmental factors. Environmental factors should be thought of broadly to include infectious, chemical, physical, nutritional, psychological, and social influences. Literally, "we" are the intersection of our genes and our environment.

Estimating the relative impact of genetic and environmental influences for any particular condition or character is difficult. Many genes express varying degrees of penetrance in the population – you may have the gene that predisposes you to some condition, but the gene never manifests and you never get the disease. Often this variable penetrance is due to environmental influences that affect gene expression. For example, a person may have a defect in n-acetyl transferase but never develop bladder cancer because she did not smoke cigarettes, whereas another person who had the same defect and did smoke would have 7 times the relative risk for developing bladder cancer.[20] In addition, many complex diseases like heart disease or diabetes are multifactorial both in terms of genetic influences and envi-

ronmental influences. Dozens of genes and even more environmental "exposures" may play a significant role in pathophysiology.

How do researchers determine the relative contribution of environment and specific genes in determining the physical manifestation of a condition or character (phenotype)? Twin studies have long been considered the gold standard since the larger the genetic component of a condition, the higher the expected congruence between identical twins. By contrast, the greater the environmental influence the less the expected difference between congruence between identical twins and congruence between fraternal twins. Indeed if fraternal twins have the same frequency of congruence of a condition as identical twins do, it is assumed that the condition is largely environmental (but not entirely environmental, for fraternal twins still share half their genes in common as do any pair of siblings). Twin studies have allowed researchers to conclude that cancer of the stomach, colon, lung, breast, and prostate have a heritability of between 26 and 42%, suggesting that while environment plays a larger role in these cancers, genetic predisposition plays a nonetheless significant role.[21]

In recent years, with the advent of feasible genetic testing for single nucleotide and other polymorphisms, large epidemiological studies have begun to replace twin studies as the preferred method for estimating genetic and environmental influences in diseases. Starting with diseased individuals, scientists begin looking for SNPs within that population that appear in higher frequency than in healthy populations. Candidate genes, once identified, may then be followed in prospective clinical trials to observe if carriers manifest a disease more frequently than non-carriers. Multiple regression analysis can also allow an estimation of the relative influence of numerous genes in the development of a single complex chronic disease.

From the environmental side of the equation, other epidemiological studies can provide useful information. For instance, rates of breast cancer among Asian-American women in the United States vary widely depending on how long they have been in America. First generation immigrants have a breast cancer risk that is similar to their native homelands and is 80% lower than the cancer rates for third-generation Asian-American women. Since both populations have similar genes, the huge difference in breast cancer rates is likely largely due to environmental differences of their new homeland: altered diets, toxin exposure, lifestyle, etc.[22]

The task for any given chronic disease is Herculean, and while new evidence is being published every day, it will likely be some years before we get precise estimates of the relative contribution of specific genes and specific environmental factors in every chronic disease. Yet with each new polymorphism identified and with each new epidemiological study and intervention trial we come closer to a full appreciation of the complex pathophysiology of chronic disease processes. One

thing seems clear already, however: in virtually every disease, both genetic susceptibility and environmental influences play pivotal roles in disease and character development. It is almost never a case of Nature or Nurture, and almost always a case of Nature *and* Nurture. This is comforting, for while we may not be able to alter our genes, we can alter our environment.

5. Case Study Examples

Cardiovascular Disease Refractory to Treatment

Take a hypothetical patient at increased risk for developing cardiovascular disease, for instance. Physical exam and a comprehensive laboratory cardiovascular risk profile reveals that the patient has hypertension, low HDL cholesterol, and elevated homocysteine levels, each of which would increase the risk of developing cardiovascular disease. You prescribe additional magnesium, folic acid, B12, B6, and aerobic exercise. Three months later you reevaluate only to find that none of the parameters has changed.

You decide to investigate further and order a cardiovascular predictive genomics profile that identifies polymorphisms in three critical enzymes in your patient:

1) methylenetetrahydrofolate reductase (MTHFR)
2) cholesteryl ester transfer protein (CETP)
3) angiotensin receptor (AGTR1)

Elevated serum homocysteine indicates a defect in methylation capacity and is independently associated with a 3-fold increase in the risk of a cardiovascular event. In most patients, homocysteine levels fall when the patient is supplemented with folic acid, B12, and B6, but in ~28% of patients, this therapy is ineffective because they have a single nucleotide polymorphism (677C‡T) in a critical enzyme that regenerates methionine from homocysteine: methylenetetrahydrofolate reductase.[23] With this polymorphism, an individual cannot efficiently convert 5,10-methylenetetrahydrofolate into 5-methyl tetrahydrofolate, the active methyl donor, and methylation slows. Folic acid is ineffective as a treatment. However, 5-methyl tetrahydrofolate, the "downstream" product of the impaired biochemical pathway, can be supplemented directly. Giving this special, albeit more expensive, form of folate, improves methylation capacity, lowers the homocysteine levels, and reduces CVD risk. In addition, supplementing with betaine engages a secondary "back-up" pathway that also promotes efficient remethylation of homocysteine.

High-density lipoprotein (HDL) cholesterol concentration is inversely related to the risk of coronary artery disease. The cholesteryl ester transfer protein (CETP) has a central role in the metabolism of this lipoprotein and may therefore alter the susceptibility to atherosclerosis. Your patient has B1B1 genotype for CETP (Taq1B; intron 1) that is associated with higher CETP levels and 30% lower HDL cholesterol levels.[24]

As for treatment options, subsequent studies have shown that moderate alcohol consumers with the B1B1 genotype had 30% lower CETP activity and 48% higher HDL cholesterol levels than those with the same genotype who consumed no alcohol.[25] Furthermore, B1B1 genotypes for CETP have been shown to have dramatic reductions in cholesterol levels from taking Pravastatin, one of the statin drugs.[26] Natural statin mimetics like red rice yeast might reasonably be considered as an alternative since the side effects are considerably less than pharmaceutical statin drugs.

Angiotensin II is an important effector controlling blood pressure and volume in the cardiovascular system. Its importance is reflected by the efficacy of angiotensin-converting enzyme inhibitors in the treatment of hypertension and congestive heart failure. Type 1 receptors mediate the major cardiovascular effects of angiotensin II. One SNP of the AGTR1 gene (1166A‡C) has been strongly associated with hypertension. AGTR1 antagonists like losartan (Hyzaar‰) are likely to produce the best clinical response in patients with receptor polymorphisms.[27,28] Likewise ACE-inhibitors would likely be less effective in this individual, since the dysfunction is at the level of the receptor and not with angiotension conversion itself. This illustrates one of the potentially clinically useful aspects of pharmacogenetics – finding the right drug for the right individual based on his or her genetic polymorphic limitations.

In this hypothetical example, we see how genotyping of an individual's SNPs can give enormous direction in terms of therapeutic intervention: isolating the right therapies for the right individuals. Through the judicious use of diet, nutrition, lifestyle changes and pharmacological therapies it is possible to modify the expression of the genes and to overcome genetic limitations of biochemical pathways. Predictive genomic testing allows us to be smarter clinicians with more effective therapeutics and fewer side-effects. Furthermore, the therapeutic gains are measurable through continued functional laboratory testing.

The genomic revolution is happening now. Medicine will never be the same. Truly individualized medicine is rapidly becoming a reality for us as practitioners and for our patients, who only stand to gain from our increased diagnostic genomic capabilities.

6. A Few Bioethical Considerations

With every new paradigm shift in medicine, ethical issues arise, and genomic testing is no different. A plethora of bioethical and social issues arises in the face of genetic testing, especially if the genetic testing reveals a condition or conditions for which no medical treatment is currently available. In Sophocles' tragedy "Oedipus Rex," Teiresias, the blind seer of Thebes, has been given the power to see

the future but cannot change it. Perceiving Fate is a heavy burden, indeed. He says to Oedipus, "It is but sorrow to be wise when wisdom profits not."

In the case of many Mendelian diseases like Huntington's disease, cystic fibrosis, sickle cell anemia, and Tay Sachs disease, to name but a few, knowledge of an almost inevitable fate carries with it significant psychological and emotional burdens, not just for the affected individual, but often also for all members of the extended family. All genetic conditions are, by definition, familial. Diagnosis of a genetic disease in one individual raises the specter at least of diagnosis in other members of the family, or confirmation that other family members are carriers and will put any future offspring at risk as well.

Not surprisingly, the penetrance of genetic conditions runs the gamut from nearly 100% likelihood of developing the condition as in Huntington's disease and a 40-80% chance of developing Alzheimer's disease given an apoE4 allele to a 10-30% chance of developing heart disease with a defective MTHFR allele. But in reality, there are extremely few conditions for which penetrance nears 100%. If penetrance is anything less than 100%, phenotypic expression is mediated by two important factors. First, most complex conditions have multiple gene interactions. There is not one gene locus that determines heart disease or cancer. Dozens of genes are likely to modify risk and ultimately play a role in phenotype expression. Second, the expression of any given gene is modified by environmental factors including diet, lifestyle, toxic exposure, etc. Invariably our health is a combination of nature AND nurture, or what is sometimes referred to as gene-gene-environment interaction.

This is especially true of predictive genomic testing where we are attempting to estimate and reduce the risk of developing complex chronic diseases. The focus is on relative risk given a specific polymorphism and on modulating that risk using environmental modifications. It is useful to recall the four criteria for clinically useful predictive genomic testing.

Relevant- The polymorphisms identified should exert significant influence on the development of disease. Polymorphisms should be carefully selected based on their direct influence over specific biochemical pathways that create known symptom clusters or diseases.

Prevalent- Polymorphisms should have a significant prevalence in the population so that testing is practical and economically feasible, and so that outcomes trials are likely to occur and novel therapeutics developed and validated.

Modifiable- Polymorphisms for which specific risk reduction strategies are known, including dietary, nutritional, lifestyle, and pharmaceutical interventions, are the most clinically useful and pose few bioethical issues.

Measurable- Functional laboratory testing should be available to measure risk reduction as well as to monitor therapeutic progress, by evaluating phenotypic expression of genetic tendency, functional integrity, and metabolic reserve.

Fortunately, in predictive genomic testing, since practical intervention strategies are generally available, genetic diagnosis will likely do far more to relieve stress rather than to increase it. Furthermore, since phenotypic or physiologic progress may be monitored using functional laboratory testing, we can monitor patients closely, changing therapeutic regimens as appropriate. In predictive clinical genomics, rather than being a harbinger of a fate to come, SNP testing may be the first step towards comprehensive risk reduction or comprehensive treatment strategy.

7. Resources

There are a plethora of websites springing up on the Internet that deal with genomics – even the entire human genome, all 3 billion letters, is available online (talk about your somnolent reading). Simply searching with the word "genome" will elicit hundreds of sites. Obviously, some are more user-friendly than others.

One of the best websites, especially focused on single nucleotide polymorphisms is a site run by the National Center for Biotechnology Information: Online Mendelian Inheritance in Man (OMIM) at http://www.ncbi.nlm.nih.gov/Omim/. It is extraordinarily useful in researching individual SNPs and the enzymes and other proteins for which they code. References are cross-linked to PubMed so that abstracts of relevant studies may be reviewed instantly.

Another very useful site is the Office of Genomics and Disease Prevention, run by the Centers for Disease Control, with numerous Powerpoint slide shows available on a wide variety of topics. The site is best located by entering "Office of Genomics and Disease" in a commercial search engine.

An excellent website for vetting bioethical concerns may be accessed at the Genetics and Ethics page, http://www.ethics.ubc.ca/brynw/

Footnotes

1. Bloom BR. Genetics and developing countries. *BMJ* 2001;322:1006-7.
2. Donnai D, Elles R. Integrated regional genetic services: current and future provision. BMJ 2001;322:1048-52.
3. Ridley M. *Genome: an Autobiography of a Species in 23 Chapters*. New York: Perrenial, 1999.
4. Campbell J. *Grammatical man: information, entropy, language and life*. London: Allen Lane, 1983.
5. Mitchell W. "Food and the Naturopathic Physician." In MJ Cronin, ed., *The Best of Naturopathic Medicine*. Tempe, AZ: Southwest College Press, 1996.
6. Gesteland RF, Atkins JF , eds. *The RNA World*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1993.
7. Dawkins, R. *The Selfish Gene*. Oxford, UK: Oxford University Press, 1990.
8. Lyamichev V, Mast AL, Hall JG, et al. Polymorphism identification and quantitative detection of genomic DNA by invasive cleavage of oligonucleotide probes. *Nat Biotechnol* 1999;17:292-296.
9. Lyamichev VI, Kaiser MW, Lyamicheva NE, et al. Experimental and theoretical analysis of the invasive signal amplification reaction. *Biochemistry* 2000;39:9523-9532.
10. Kwiatkowski RW, Lyamichev V, de Arruda M, Neri B. Clinical, genetic, and pharmacogenetics applications of the Invader assay. *Mol Diagn* 1999;4:353-364.
11. Eis PS, Olson MC, Takova T, et al. An invasive cleavage assay for direct quantitation of specific RNAs. *Nat Biotechnol* 2001;19:673-676.
12. Mendel G. Versuche über Pflanzen-Hybriden. *Vorgelegt in den Sitzungen vom 8. Februar und 8. März 1865*
13. Yamamoto F, Clausen H, White T, et al. Molecular genetic basis of the histo-blood group ABO system. *Nature* 1990 May 17;345(6272):229-33.
14. Hill AVS. Genetics of infectious disease resistance. *Curr Opin in Genetics and Develop* 1996;6:348-353.
15. Wedekind C, Furi S. Body odor preferences in men and women: do they aim for specific MHC combinations or simple heterogeneity? *Proc R Soc Lond B Biol Sci* 1997;264:1471-1479.
16. Kwok PY, Deng Q, Zakeri H, et al. Increasing the information content of STS-based genome maps: identifying polymorphisms in mapped STSs. *Genomics* 1996;31:123-126.
17. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996;273:1516-1517.
18. Kwok P, Gu Z. Single nucleotide polymorphism libraries: why and how are we building them? *Mol Med Today* 1999;5:538-543.
19. Lopez AD, Murray C. The Global Burden of Disease. Report for the World Health Organization at http://www.hsph.harvard.edu/organizations/bdu/summary.html
20. Taylor JA, Umbach DM, Stephens E, et al. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res* 1998 Aug 15;58(16):3603-10.
21. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med* 2000 Jul 13;343(2):78-85.
22. Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. *J Natl Cancer Inst* 1993 Nov 17;85(22):1819-27.
23. Engbersen AM, Franken DG, Boers GH, et al. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 1995 Jan;56(1):142-50.
24. Freeman DJ, Griffin BA, Holmes AP, et al. Regulation of plasma HDL cholesterol and subfraction distribution by genetic and environmental factors: associations between the TaqI B RFLP in the CETP gene and smoking and obesity. *Arterioscler Throm*. 1994;14:336-344.

25. Hannuksela ML, Liinamaa MJ, Kesaniemi YA, Savolainen MJ. Relation of polymorphisms in the cholesteryl ester transfer protein gene to transfer protein activity and plasma lipoprotein levels in alcohol drinkers. *Atherosclerosis* 1994 Sep 30;110(1):35-44.

26. Kuivenhoven JA. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. *New Eng J Med* 1998;338: 86-93.

27. Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. *Kidney Int* 1999 Dec;56(6):2173-80.

28. Azuma H, Niimi Y, Hamasaki H. Prevention of intimal thickening after endothelial removal by a nonpeptide angiotensin II receptor antagonist, losartan. *Br J Pharmacol* 1992 Jul;106(3):665-71.

z,ac,PhysPrimer,021402

Achieving Clinical Excellence

Date: March 16, 2002 Location: Asheville

SEMINAR EVALUATION

In order to assess today's seminar, we would appreciate your taking a few minutes to help us understand what you enjoyed and where we might improve. We encourage any notes you feel are appropriate.

Please circle the appropriate score beside each topic:
(1) Poor, (2) Fair, (3) Good, (4) Excellent, (5) Outstanding

Speaker: Dr. Brad Rachman

Evaluation

1) The information presented was useful and practical. 1 2 3 4 5

2) The information was presented clearly. 1 2 3 4 5

3) The information was presented in an interesting and lively manner. 1 2 3 4 5

4) The speaker had a thorough grasp of the subject matter. 1 2 3 4 5

5) The presentation held my interest. 1 2 3 4 5

6) The subject matter was clinically relevant. 1 2 3 4 5

7) I will be able to apply what I learned in my practice. 1 2 3 4 5

8) The amount of material presented was appropriate. 1 2 3 4 5

ACE

Achieving Clinical Excellence

Date: March 16, 2002 Location: Asheville

SEMINAR EVALUATION

In order to assess today's seminar, we would appreciate your taking a few minutes to help us understand what you enjoyed and where we might improve. We encourage any notes you feel are appropriate.

Please circle the appropriate score beside each topic:
(1) Poor, (2) Fair, (3) Good, (4) Excellent, (5) Outstanding

Speaker: Dr. Patrick Hanaway

Evaluation

1) The information presented was useful and practical. 1 2 3 4 5

2) The information was presented clearly. 1 2 3 4 5

3) The information was presented in an interesting and lively manner. 1 2 3 4 5

4) The speaker had a thorough grasp of the subject matter. 1 2 3 4 5

5) The presentation held my interest. 1 2 3 4 5

6) The subject matter was clinically relevant. 1 2 3 4 5

7) I will be able to apply what I learned in my practice. 1 2 3 4 5

8) The amount of material presented was appropriate. 1 2 3 4 5

ACE

Achieving Clinical Excellence

Date: March 16, 2002 Location: Asheville

SEMINAR EVALUATION

In order to assess today's seminar, we would appreciate your taking a few minutes to help us understand what you enjoyed and where we might improve. We encourage any notes you feel are appropriate.

Please circle the appropriate score beside each topic:
(1) Poor, (2) Fair, (3) Good, (4) Excellent, (5) Outstanding

Speaker: Dr. R. W. Watkins

Evaluation

1) The information presented was useful and practical. 1 2 3 4 5

2) The information was presented clearly. 1 2 3 4 5

3) The information was presented in an interesting and lively manner. 1 2 3 4 5

4) The speaker had a thorough grasp of the subject matter. 1 2 3 4 5

5) The presentation held my interest. 1 2 3 4 5

6) The subject matter was clinically relevant. 1 2 3 4 5

7) I will be able to apply what I learned in my practice. 1 2 3 4 5

8) The amount of material presented was appropriate. 1 2 3 4 5

ACE

IN GENERAL...

1) The course materials were valuable. 1 2 3 4 5

2) The facility was comfortable and convenient. 1 2 3 4 5

3) This seminar met personal objectives. 1 2 3 4 5

4) I would recommend today's seminar to others. 1 2 3 4 5

5) The course met stated objectives. 1 2 3 4 5

6) The topics were thoroughly discussed. 1 2 3 4 5

7) Sufficient time was allowed for each session. 1 2 3 4 5

8) Have you been to other ACE Educational Seminars? No. One. More than one.

9) The best aspects of today's seminar were:

_____

_____

_____

_____

_____

10) This program could be improved by:

_____

_____

_____

_____

_____

11) Any additional comments?

_____

_____

_____

_____

_____

_____

_____