Outline

Hardy - Weinberg Law
- Example

Approaches to Genetic Diagnosis
- Newborn Screening
- Adult Screening

Hardy - Weinberg Law

Hardy-Weinberg equilibrium was derived to explain why dominant traits do not eventually replace detrimental recessive traits.

Also, it is used to determine genotype frequencies from the relative frequencies of the alleles at a specific locus.

Hardy - Weinberg Law

General

For a gene locus, in a population with random mating and reproduction, the genotype frequencies will be determined by the relative frequencies of the alleles at the locus.

Assumptions

1. Random mating
2. No inbreeding
3. No migration
4. No mutation
5. Large population
6. No selection

Genotype equilibrium based on stable allele frequencies

Hardy - Weinberg Law (or Equilibrium)

Assume that a gene locus has two alleles (A + a)

Let the gene frequencies of the two alleles be:

Frequency of A = p

Frequency of a = q

p + q = 1 (Only two choices)

The genotype (phenotype) frequencies will be determined by the frequency of the alleles at the locus.

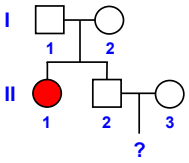
Genotype	Frequencies
AA	$p \cdot p = p^2$
Aa aA	$p \cdot q$ $q \cdot p$ } $= 2pq$
aa	$q \cdot q = q^2$

$AA + Aa + aA + aa = p^2 + pq + qp + q^2$

$= p^2 + 2pq + q^2 = 1$

Genotype	AA	Aa	aa
Frequency	p^2 +	2pq +	q^2 = 1

Example



- II-2 and II-3 are planning to have children
- II-1 has cystic fibrosis (CF)
- What is the chance that II-2 and II-3 will have a child with CF?
- CF occurs in 1/2,500 in Caucasians

How to use the Hardy - Weinberg Law

Cystic Fibrosis (CF) = 1/2,500

Gene	C = Normal allele
	c = Cystic fibrosis allele
Genotype	CC = Normal
	Cc = Normal Carrier
	cc = Cystic fibrosis

We know that the frequency of affected individuals (c/c) is 1/2,500.

We want to determine the frequency of carriers (C/c).

The frequency of the heterozygote (C/c) is 2pq.

$cc = 1/2{,}500 = q^2$

$q = 1/50$

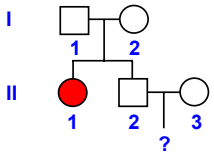
$p + q = 1$

$p = 1 - 1/50 = 49/50$

$2pq = 2 \times 49/50 \times 1/50$

$= 98/2{,}500 \cong 1/25$

This is the frequency of carriers (Cc)



I-1 and I-2 are Cc

Chance that II-2 = Cc = 2/3

Chance that II-3 = Cc = 1/25

Chance that their offspring will have albinism

$$\frac{2}{3} \times \frac{1}{25} \times \frac{1}{4} = \frac{2}{300} = \frac{1}{150}$$

Autosomal Recessive Mating
Aa x Aa

Gametes	A	a
A	A A	A a
a	a A	a a

cc = 1/2,500 = Affected

Cc = 1/25 = Carriers

Hardy - Weinberg Equilibrium explains why rare deleterious alleles remain in the population.

→ **Prenatal Screening**

Newborn Screening
- **Screen for metabolites**

Adult Screening
- **Diagnosis**
- **Predisposition mutations**
- **Carrier Status**

Why do prenatal Screening?

Pursue potential interventions that may exist

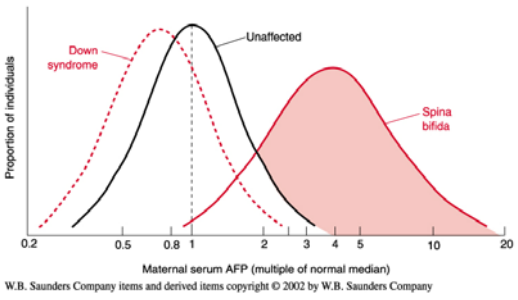
Begin planning for a child with special needs

Start addressing anticipated lifestyle changes

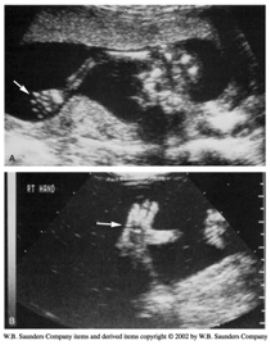
Identify support groups and resources

Make a decision about carrying the child to term

http://www.americanpregnancy.org/prenataltesting/cvs.html



Ultrasonography



Normal

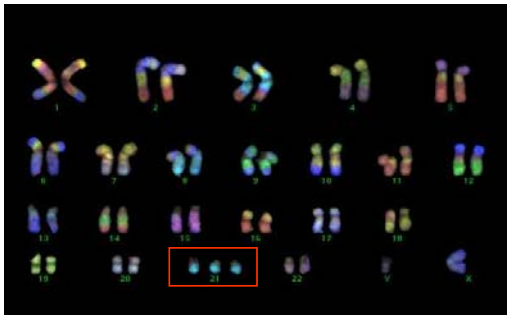
Abnormal

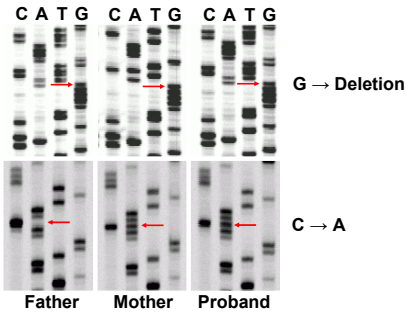
Several types of DNA alterations that need to be detected:

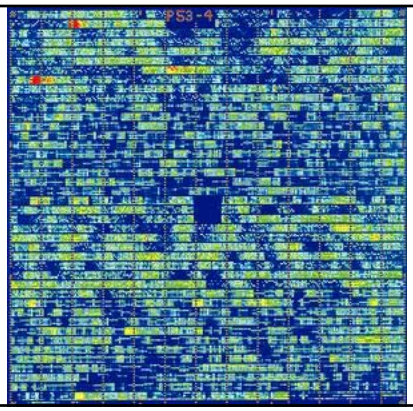
Chromosomal abnormalities:
- **Rearrangements**
- **Deletions/Duplications**

Mutations at level of the gene(s)
- **Deletions/Duplications**

Mutations at the nucleotide level:
- **Missense**
- **Nonsense**
- **Frameshift**
- **Microdeletions**

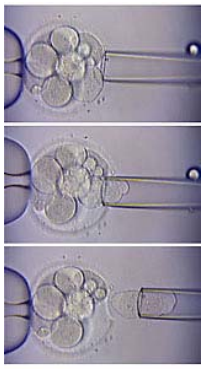




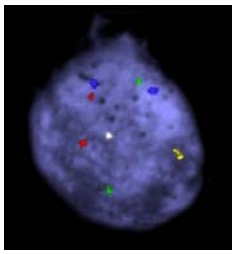


Pre-implementation Genetic Diagnosis (PGD)

This is the testing of a fertilized egg for genetic disorders after in vitro fertilization (IVF), before placing the embryo in the uterus for development. It is now possible to remove a single cell from an eight cell embryo (embryo biopsy), and analyze the chromosomal composition.



yalefertilitycenter.org/patient/pgd.html

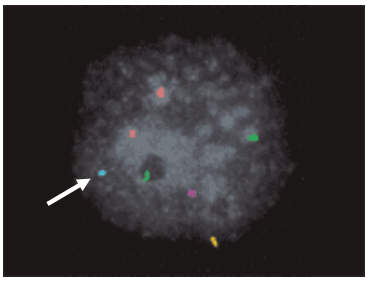


PGD on normal male blastomera.

Red: LSI 21,
Green: LS I 13,
Blue: CEP 18,
White: CEP Y,
Yellow: CEP X.

O. Scheinost, Dept. of Medical Genetics, Hospital České Budějovice.

http://www.lucia-cytogenetics.com/index.php?lang=en&&inc=pgd



13 - Red
18 – Cyan (1)
21 - Green
X – Purple
Y - Yellow

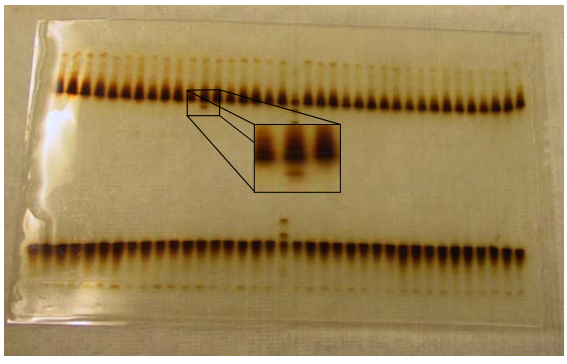
Prenatal Screening

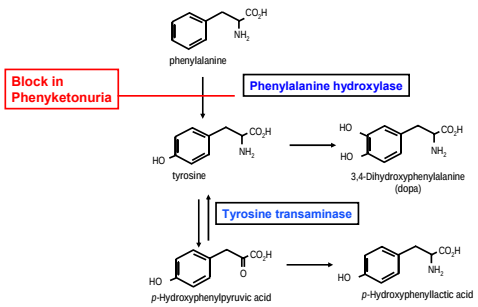
→ **Newborn Screening**
Screen for metabolites

Adult Screening
Diagnosis
Predisposition mutations
Carrier Status

Why Do Newborn Screening?

- **Disease is deleterious**
- **Disease is treatable**
- **Has a reliable test**
- **Early treatment makes a difference**
- **Cost effective**





Presentation of untreated PKU

1. Unusual “musty” order
2. Lighter pigmentation
3. Mental retardation
4. Behavioral changes
5. Seizures
6. Skin changes (eczema)

Symptoms of 51 never treated PKU patients

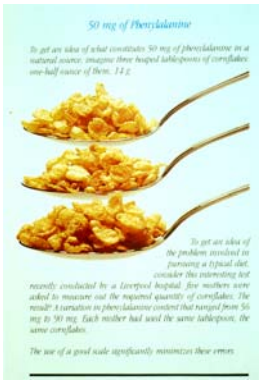
Epilepsy		25%
Profoundly retarded	(IQ, < 35)	~50%
Moderately retarded	(IQ, 36 - 67)	~50%
Slightly retarded	(IQ, >68)	~5%

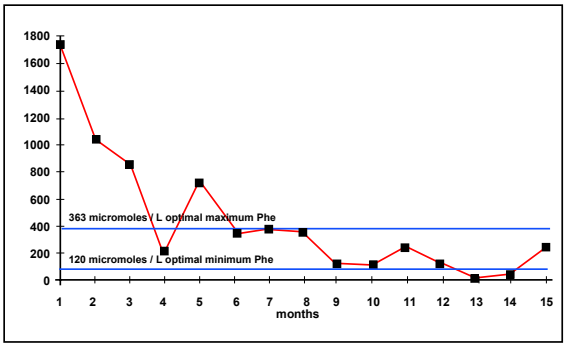
Treatment

Treatment of PKU requires a lifelong diet of reduced phenylalanine.

Estimates of Phenylalanine Requirements in Humans

200-500 mg



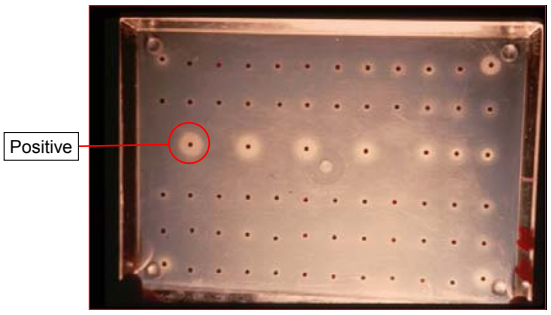


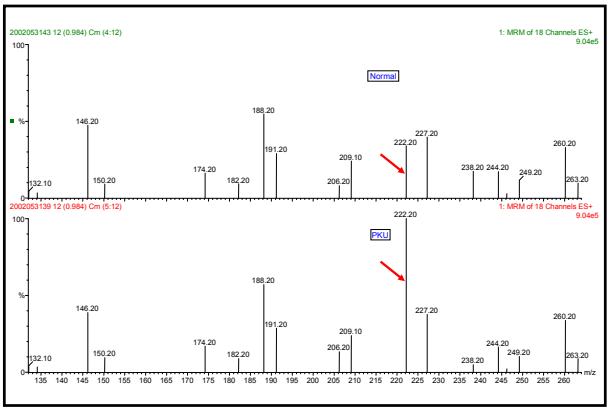
The Montreal Children's Hospital
Hyperphenylalaninemia (PKU) Resource Booklet for Families

Genetic Diseases Screened in Newborns

Disorder Screened	Number of States	Reliability at <24 Hours	
Phenylketonuria	51	Yes	Phenylalanine (Guthrie)
Congenital hypothyroidism	51	Possibly	thyroxine (T_4) (RIA)
Sickle cell disease	44	Yes	Electrophoresis (IEF)
Glactosemia	44	Yes	Galactose (Bacterial)
Congenital adrenal hyperplasia	14	Yes	21 hydroxylase (RIA 17-OHP)
Maple syrup urine disease	24	Yes	Leucine (Guthrie)
Homocystinuria	21	No	Methionine (Guthrie)
Biotinidase deficiency	17	Yes	Enzyme assay
Tyrosinemia	7	No	Tyrosine
Cystic fibrosis	3	Possibly	Immunoreactive trypsin

Newborn Screening by Guthrie test





Minnesota Department of Health Newborn Metabolic Screening Program

Amino Acid Disorders

Phenylketonuria	Phenylalanine hydroxylase (PAH)
Maple Syrup Urine Disease	Branched-Chain α-keto acid Dehydrogenase (BCKDH)
Citrullinemia	Argininosuccinate synthetase (ASS)
Arginosuccinic Aciduria	Argininosuccinate lyase (ASL)
Homocystinuria	Cystathionine β-synthase (CBS)
Argininemia	Arginase (ARG1)
Tyrosinemia Type I	Fumarylacetoacetase (FAH)

Disorders of Organic Acid Metabolism

Organic Acidemia Type I	Glutaryl-CoA dehydrogenase (GCDH)
Propionic Acidemia	Propionyl-CoA carboxylase (PCCA)
	Propionyl-CoA carboxylase (PCCB)
Methylmalonic Acidemia	Methylmalonyl-CoA isomerase (MUT)
Isovaleric Acidemia	Isovaleryl-CoA dehydrogenase (IVD)
3-Metyl-Crotonyl-CoA Carboxylase Deficiency	
	3-methylcrotonyl-CoA carboxylase-α (MCCA)
	3-methylcrotonyl-CoA carboxylase-β (MCCB)
3-Hydroxy-Methyl-Glytaryl-CoA Lyase Deficiency	
	3-hydroxy-3-methylglutaryl CoA lyase (HMGCL)

Fatty Acid Oxidation Disorders

Very Long Chain Acyl-CoA Dehydrogenase Deficiency (ACADVL)
Medium Chain Acyl-CoA Dehydrogenase Deficiency (ACADM)
Short Chain Acyl-CoA Dehydrogenase Deficiency (ACADS)
Long Chain Hydroxy Acyl-CoA Dehydrogenase Deficiency (HADHA) and (HADHB)
Carnitine/Acylcarnitine Translocase Deficiency (CACT)
Carnitine/Acylcarnitine TransferaseII Deficiency (CPT2)
Hydroxy Acyl-CoA Dehydrogenase/ 3-Ketoacyl-CoA Thiolase/Enoyl-CoA Hydratase Deficiency

Prenatal Screening

Newborn Screening
- **Screen for metabolites**

→ **Adult Screening**
- **Diagnosis**
- **Predisposition mutations**
- **Carrier Status**

Medical Genetics

It's not just for pediatricians anymore.

Adult onset diseases

Pharmacogenetics

Toxicogenetics

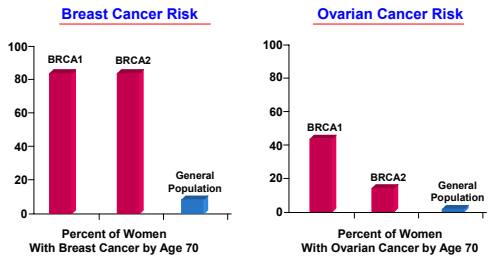
"Everyone carries anywhere from five to fifty significant genetic flaws, and that virtually all diseases - even AIDS - have a genetic component"

Francis Collins

Director of the National Human Genome Research Institute

NIH

BRCA1 and BRCA2 Mutations Increase the Risk of Breast and Ovarian Cancer



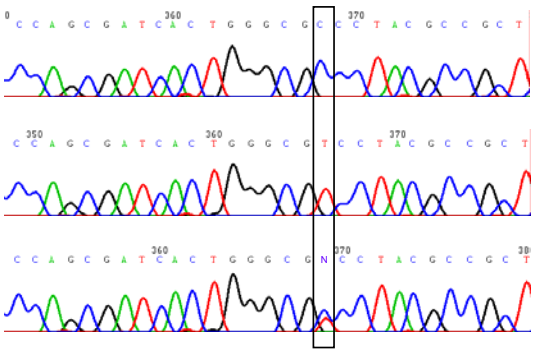
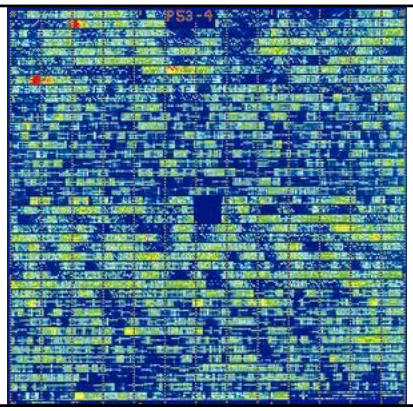
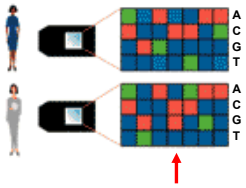



Figure 2. High-density oligonucleotide probe array package.

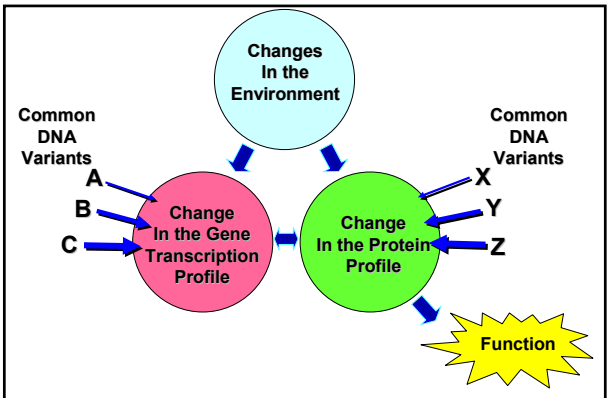




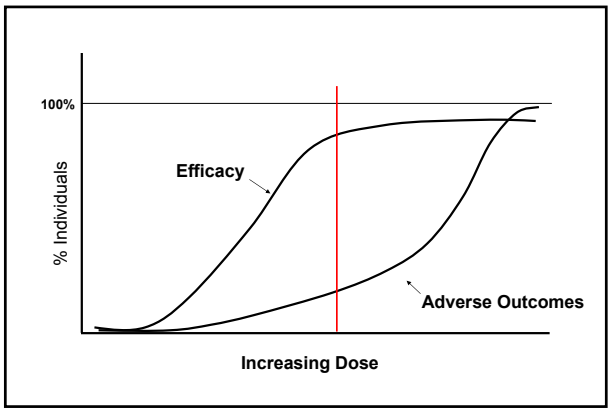
Genetic Variation

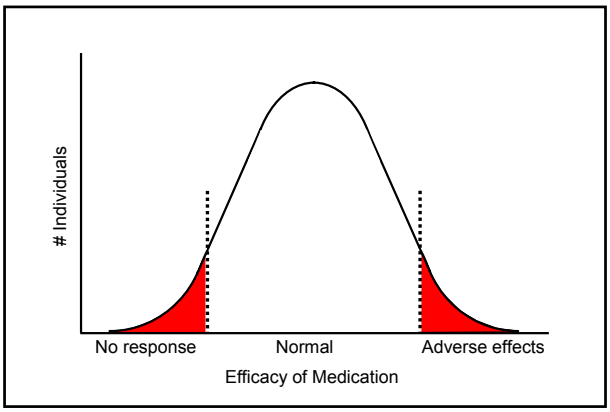
Genetic Disease	Genetic Susceptibility
Classic Medical Genetics	
~20%	~80%
Single gene Chromosome	Common Gene Variation Gene + Environment
Early onset (usually pediatric)	Delayed onset (usually adult)
Marfan Syndrome PKU Cystic Fibrosis Neurofibromatosis Down syndrome	Coronary Heart Disease Hypertension Diabetes Cancer Vascular Disease

Sir Archibald E. Garrod



Pharmacogenetics





Japan jumps towards personalized medicine

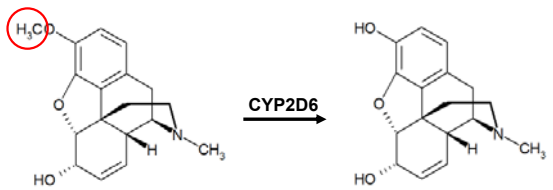
NATURE 437, October 6, 2005

Codeine is a prodrug with no analgesic activity. About 10% of a dose of codeine is converted in the body by CYP2D6 to morphine, an active metabolite.

The morphine is glucuronidated to both active and inactive metabolites that are eliminated by the kidneys. The rest of the codeine is metabolized by glucuronidation and CYP3A4 to inactive metabolites. Thus, CYP2D6 is necessary for much of the analgesic action of codeine.

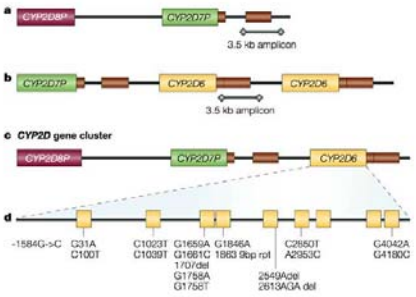
Most Caucasians rapidly convert codeine to morphine via CYP2D6. Approximately 7% to 10% of Caucasians, however, have a genetic variant that produces limited CYP2D6 activity and slow metabolism of CYP2D6 substrates. In these patients, conversion of codeine to morphine is reduced, as is the analgesic efficacy. Administration of codeine to these patients will not provide the expected degree of analgesia.

Codeine Metabolism

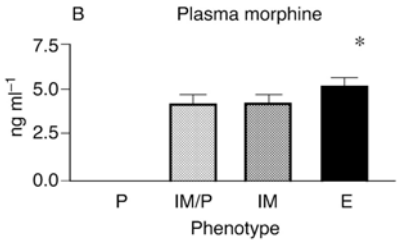


Codeine

Morphine



CYP2D6 Polymorphisms and Pain Relief from Codeine



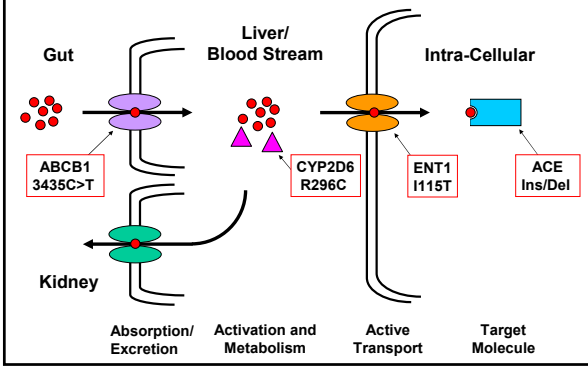
Williams, British Journal of Anaesthesia, 2002, 89(6); 839-45

Population Frequency of Cytochrome p450 (CYP) genotypes

Gene	PM	IM	EM	UM
CYP2D6	10%	35%	48%	7%
CYP2C9	4%	38%	58%	N/A
CYP2C19	3-21%	N/A	79-97%	N/A

http://www.healthanddna.com/professional/pharmacogenetics.html

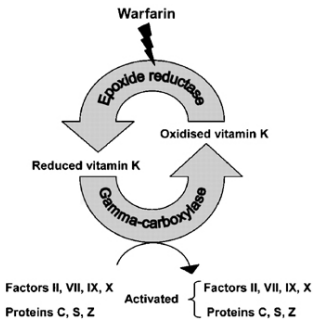
ADME (Absorption, Distribution, Metabolism and Excretion)



Warfarin is widely prescribed for the prevention and control of thrombo-embolism. (Used for anticoagulation)

Warfarin inhibits the vitamin K cycle, specifically vitamin K epoxide reductase (VKORC1). Reduced vitamin K is a required cofactor for gamma-glutamyl carboxylase (GGCX) that converts precursor forms of blood clotting factors; VII, IX, X and prothrombin to active forms.

Steady-state concentrations of Warfarin are maintained through the balance of the dose administered, level of cytochrome P4502C9 metabolism and renal elimination of the inactive hydroxy-metabolites and the active form.



Wadelius M et al., Pharmacogenomics J. 2005;5:262-70.

Warfarin is a narrow therapeutic index agent; a small change in systemic concentration of the drug may lead to significant changes in pharmacodynamic response. Careful clinical management is required to balance the risks of bleeding (over-anticoagulation) with those of thrombosis (under-anticoagulation).

The international normalized ratio (INR) measures blood coagulation relative to a standardized coagulation time. The INR target for Warfarin therapy is between 2.0 and 3.0.

The distribution of Warfarin, to obtain an INR target between 2.0 and 3.0, can range from <15 mg Warfarin / week (7%) to dosages
>60 mg / week (4%).

Polymorphisms of the P4502C9 cytochrome

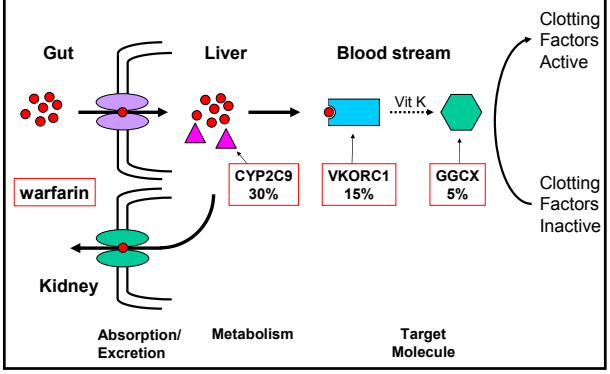
Allele	Functional Nucleotide Change	Amino-Acid Change	Enzyme Activity	Allele Frequency
CYP2C9*1	None	None	Normal	0.819
CYP2C9*2	C→T exon 3	Arg144Cys	12%	0.107
CYP2C9*3	A→C exon 7	Ile359Leu	5%	0.074

Mean required daily warfarin dose in 185 patients receiving long-term therapy in relation to genotype.

Genotype	Number of patients	Mean daily warfarin maintenance dose (mg)
CYP2C9*1/*1	127	5.63
CYP2C9*1/*2	28	4.88
CYP2C9*1/*3	18	3.32 ←
CYP2C9*2/*2	4	4.07
CYP2C9*2/*3	3	2.34 ←
CYP2C9*3/*3	5	1.6 ←

Approximately 30% of the population are carriers and 7% homozygous for reduced activity alleles.

JAMA 287: 1690-1698, 2002



Do not be surprised if in the next year or two, this kind of DNA testing will be considered as a necessary step before writing a prescription.

- Dr. Francis Collins, the director of the National Human Genome Research Institute

Wall Street Journal, June 26, 2002

HEALTH

At-Home DNA Tests Are Here

Doctors Warn Amateurs of Undue Alarm, Risky False Assurance

By Helen Pearson

London

Gene Science by Direct Mail

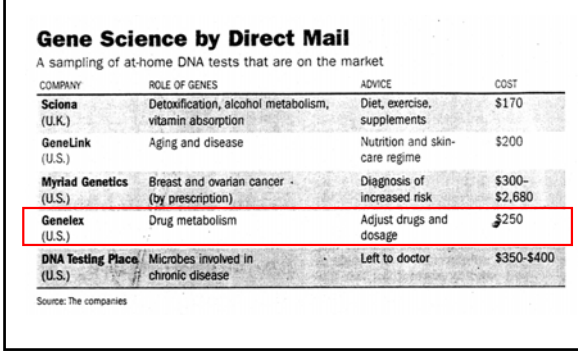
A sampling of at-home DNA tests that are on the market

Company	Role of genes	Advice	Cost
Sciona (U.K.)	Detoxification, alcohol metabolism, vitamin absorption	Diet, exercise, supplements	$170
GeneLink (U.S.)	Aging and disease	Nutrition and skin-care regime	$200
Myriad Genetics (U.S.)	Breast and ovarian cancer (by prescription)	Diagnosis of increased risk	$300-$2,680
Genelex (U.S.)	Drug metabolism	Adjust drugs and dosage	$250
DNA Testing Place (U.S.)	Microbes involved in chronic disease	Left to doctor	$350-$400

Source: The companies

Risk assessments for complex diseases can be misleading, because many genes and lifestyle factors are involved, so do-it-yourself tests are 'more harmful than useful,' a researcher says.

Wall Street Journal, June 26, 2002



Gene Science by Direct Mail

A sampling of at-home DNA tests that are on the market

COMPANY	ROLE OF GENES	ADVICE	COST
Sciona (U.K.)	Detoxification, alcohol metabolism, vitamin absorption	Diet, exercise, supplements	$170
GeneLink (U.S.)	Aging and disease	Nutrition and skin-care regime	$200
Myriad Genetics (U.S.)	Breast and ovarian cancer (by prescription)	Diagnosis of increased risk	$300-$2,680
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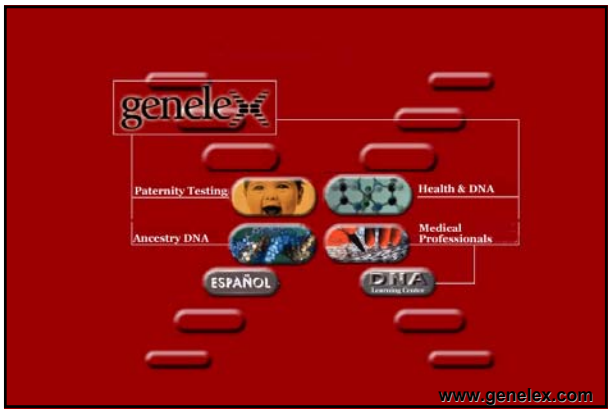
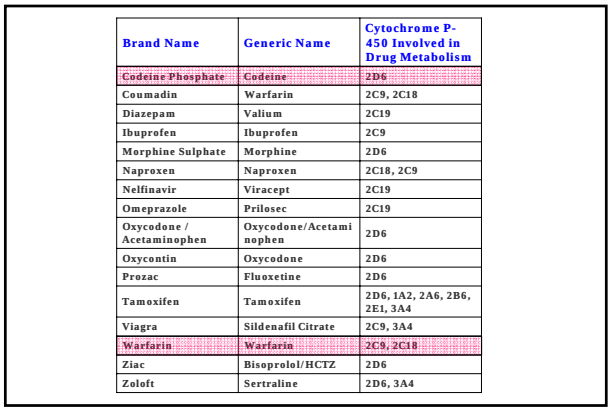
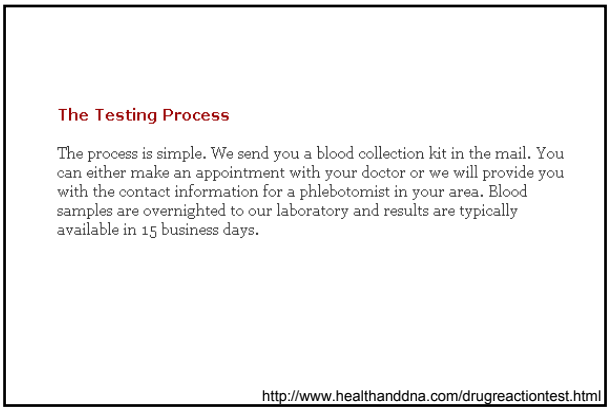
DNA Testing Place (U.S.)	Microbes involved in chronic disease	Left to doctor	$350-$400

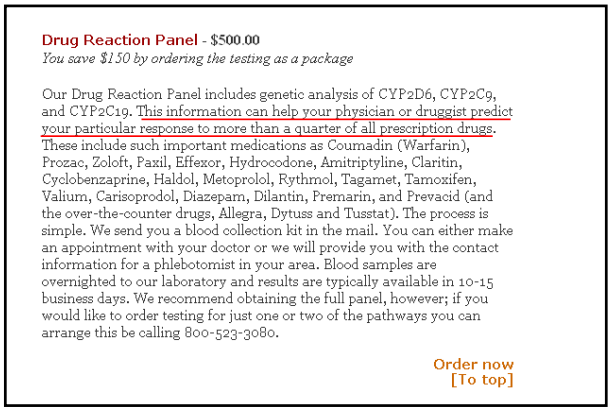
Source: The companies

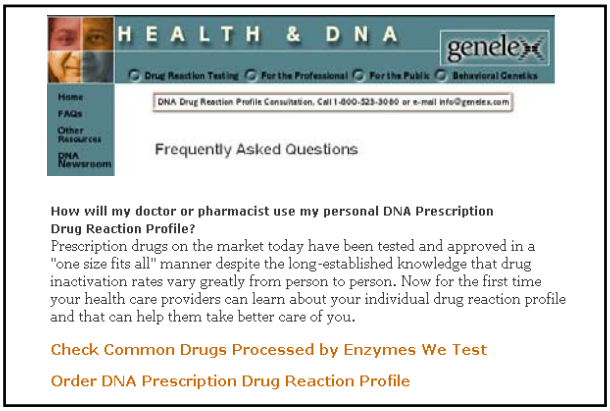




Brand Name	Generic Name	Cytochrome P-450 Involved in Drug Metabolism
Codeine Phosphate	Codeine	2D6
Coumadin	Warfarin	2C9, 2C18
Diazepam	Valium	2C19
Ibuprofen	Ibuprofen	2C9
Morphine Sulphate	Morphine	2D6
Naproxen	Naproxen	2C18, 2C9
Nelfinavir	Viracept	2C19
Omeprazole	Prilosec	2C19
Oxycodone / Acetaminophen	Oxycodone/Acetaminophen	2D6
Oxycontin	Oxycodone	2D6
Prozac	Fluoxetine	2D6
Tamoxifen	Tamoxifen	2D6, 1A2, 2A6, 2B6, 2E1, 3A4
Viagra	Sildenafil Citrate	2C9, 3A4
Warfarin	Warfarin	2C9, 2C18
Ziac	Bisoprolol/HCTZ	2D6
Zoloft	Sertraline	2D6, 3A4







Warfarin dosage reductions based on CYP2C9 genotype

CYP 2C9 Genotype	Dose (% of normal)
CYP *1/*1 (WT)	100%
CYP *1/*2	81
CYP *1/*3	70
CYP *2/*2	62
CYP *2/*3	51
CYP *3/*3	40

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Cytochrome P-450 2D6

Phenotype prevalence is 10 % PM, 7% UM, and 35% IM.

Therapy Modification

PM (Poor metabolizers)
Avoid medications that are altered to their active form through 2D6, such as opioids. (For instance, 10% of a codeine dose is transformed to morphine through demethylation in the liver.) If you are uncertain, contact the drug manufacturer or look up the pharmacology data.

Reduce dosage 6-10 fold for medications that are administered in their active form and deactivated through 2D6 as are many antidepressants. (Desipramine, for example, is absorbed from the gastrointestinal tract following oral administration and is extensively bound to tissue and plasma proteins in the order of 90-95%. It is inactivated by hydroxylation and by further demethylation in the liver.) If you are uncertain, contact the drug manufacturer or look up the pharmacology data. Therapeutic drug monitoring is recommended for PMs to confirm that steady-state drug concentrations are within the therapeutic target interval.

UM (Ultra-extensive metabolizers)
Increase dosage 2-5 fold depending on the number of duplications noted in the report. Success has also been achieved by concurrently administering another substrate or an inhibitor of CYP2D6.

IM (Intermediate metabolizers)
Start IMs at lowest efficacious dose and avoid multiple drug therapy that inhibits or activates through the same pathway.

Genelex plans to offer personal screens for behavior markers as a research project. If you wish to be notified when we offer personal screens in this category, please **inform us**. In the meantime, start to compile your **DNA Prescription Drug Reaction Profile** and **Nutritional Genetic Profile** and share your results with your physician and pharmacist.