

Pharmacogenomics in Pharmacy Education



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Where We're Going



- Historical development of pharmacogenetics
- Concepts in the “New Biology”
- A few population genetics concepts
- Focus on warfarin to exemplify pharmacogenomic information useful for dosing
- Briefly – Pathway Genomics controversy
- Briefly - cancer genetics

Pharmacogenomics – then and now



- Defined as the science that examines the inherited variations in genes that determine drug responses.
- For ~ 45 years, the term was applied primarily to genetic variability with regards to pharmacokinetics parameters (pharmacogenetics).
- Over the past 10 – 15 years, the term has been expanded to include genetic variation in molecular targets and other PD parameters.
- Focus for individualizing the dosing of patients to enhance efficacy and reduce toxicities.

Historical Viewpoint



- First research papers detailing a genetic component to drug PK/PD appear in the 1956-1960 time period.
 - Kalow W. Familial incidence of low pseudocholinesterase level. *The Lancet* 268(6942):15 Sept 1956, 576-7.
 - Carson PE, Flanagan CL, Ickes CE, Alving AS. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 124(3220):14 Sept 1956, 484-5.
 - Evans DAP, Manley KA, McKusick VA. Genetic control of isoniazid metabolism in man. *Br Med J* 2(5197):13 Aug 1960, 485-91.

Isoniazid Metabolism



AUG. 13, 1960

CERVICAL DISK LESIONS

BRITISH MEDICAL JOURNAL 485

collars limits their use to some extent" (*Lancet*, 1958). It has also been suggested that conservative treatment should not include the prolonged use of collars, because prolonged complete immobilization allows neck muscles to atrophy or become lax (Allen, 1952).

Both these views are misguided. A patient who has signs of progressive degeneration of the spinal cord with the prospect of quadriplegia should not be deterred, by self-consciousness about his appearance, from timely immobilization in a light Minerva type of collar if this has a good chance of halting or reversing the disease. While such a collar gives more complete immobilization than a short "under the chin" model, it does not allow the muscles to atrophy any more than does a walking-plaster on a limb. The patient remains ambulant, and can in many cases return to work in his collar.

Such a collar has, we believe, allowed striking improvement in a number of our severely disabled cases, and should be used more often. It should be used reasonably early. Good results cannot be expected where symptoms have been present for over a year. It

- Pallis, C., Jones, A. M., and Spillane, J. D. (1954). *Brain*, **77**, 274.
Russell, W. R. (1956). *Proc. roy. Soc. Med.*, **49**, 198.
Scoville, W. B. (1954). *Conn. med. J.*, **18**, 894.
— (1958). *J. Neurosurg.*, **15**, 615.

GENETIC CONTROL OF ISONIAZID METABOLISM IN MAN

BY

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Plasma Isoniazid Concentrations

Figures show blood levels of isoniazid 6 hrs after dosing.

Upper: persons received 9.8-10 mg/kg body weight.

Lower: persons received 0.8 mg/kg body weight.

<2.5 $\mu\text{g}/\text{mL}$ = Rapid Inactivators

>2.5 $\mu\text{g}/\text{mL}$ = Slow Inactivators

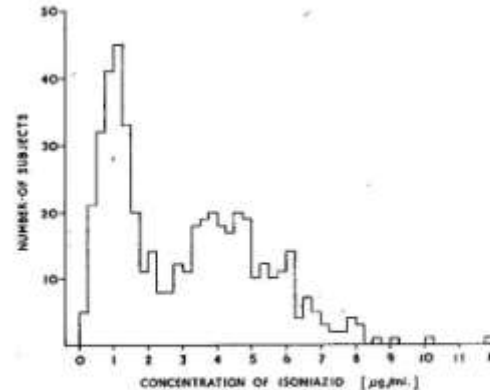


FIG. 1.—Plasma isoniazid concentrations six hours after drug ingestion. 483 persons were investigated. The results for 483 are shown. One result of 14.12 $\mu\text{g}/\text{ml}$ has been omitted. The dose of isoniazid which these subjects received varied between 9.8 and 10 mg. per kg. body weight.

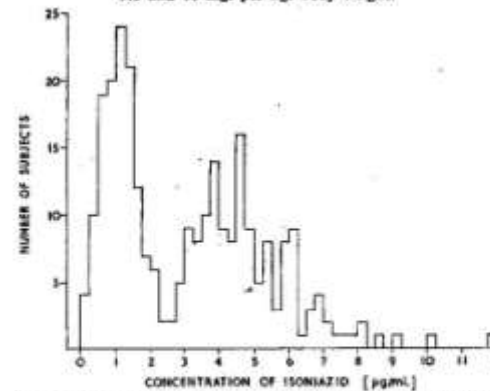


FIG. 2.—Plasma isoniazid concentrations six hours after drug ingestion. Results obtained in 267 members of the 53 complete family units investigated are shown. All these subjects received approximately 9.8 mg. of isoniazid per kg. body weight.

N-acetyl transferase (NAT) based metabolism

Pharmacogenetics (1956-1990)



- Focused on drug metabolism, and the genes that controlled expression of important metabolism and transport proteins.
 - 1977 – debrisoquine elimination due to variability in “debrisoquine hydroxylase” or CYP2D6 [Lancet 1977;17:584]
 - 1980 – thiopurine methyltransferase genetics reported by Weinshilboum [Am J Hum Genet 1980;32:651]
 - 1980s – CYP gene family elucidated and clinically significant variations in activity reported [Trends Pharmacol Sci 1989;10:107]

New Biology



- Rapid advances in molecular biology methods and understanding beginning with the 1980s opened up all proteins for study at the gene level.
- PK related genes/proteins could now be studied directly, and not indirectly by enzyme activities.
- It was possible to analyze PD targets – receptors, transporters, enzymes – as well as PK enzymes.
- Ready to take the next step – use variations in genes to explain differences in individual drug responses.

Human Genome Project



- 1988 – Congress approved the formation of a joint project by the National Institutes of Health and the Department of Energy to sequence of an entire human DNA genome.
- 2003 – Complete sequence released ~ 2 years ahead of schedule.
- This sequence (plus associated DNA markers) allowed for the identification of DNA sequence differences between individuals – the basis for comparative genomics.

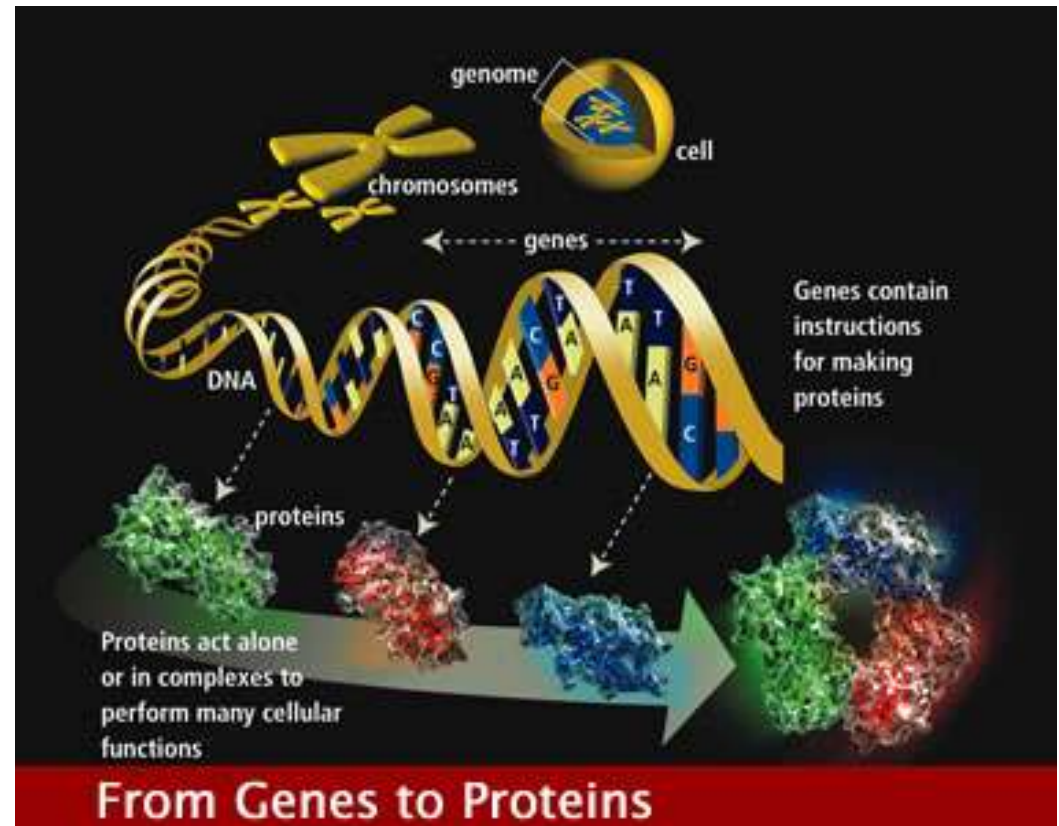
Human Genetic Stability

3.2×10^9 nucleotides per genome

Assembled into 46 chromosomes (2 X 22 autosomes + 2 sex) containing 20-25,000 genes

99.9% of DNA the same between individuals

2% of DNA involved (codes) with protein synthesis



Gene Variability



- Estimated that the DNA for any two humans varies by ~ 1 base/1000 bases.
- Gene sequence may vary due to a rare but stable mutation in nucleotide sequence.
 - These can produce genetic diseases, e.g. sickle cell disease results from a single nucleotide change in the hemoglobin gene.
 - Are stable to inheritance if appropriate selective pressure.
- Gene sequence could be altered by a ***random*** alteration in a single nucleotide base.

Single Nucleotide Polymorphisms (SNPs)



- A single base-pair substitution that arises in >1% of the population is called a single nucleotide polymorphism (SNP).
- These are the most common form of genetic variability in humans constituting 90% of this variation.
- 66% of SNPs are C -> T substitutions
- ~ 1.4×10^6 SNPs with 60,000 within protein coding regions of the gene.

Types of SNPs

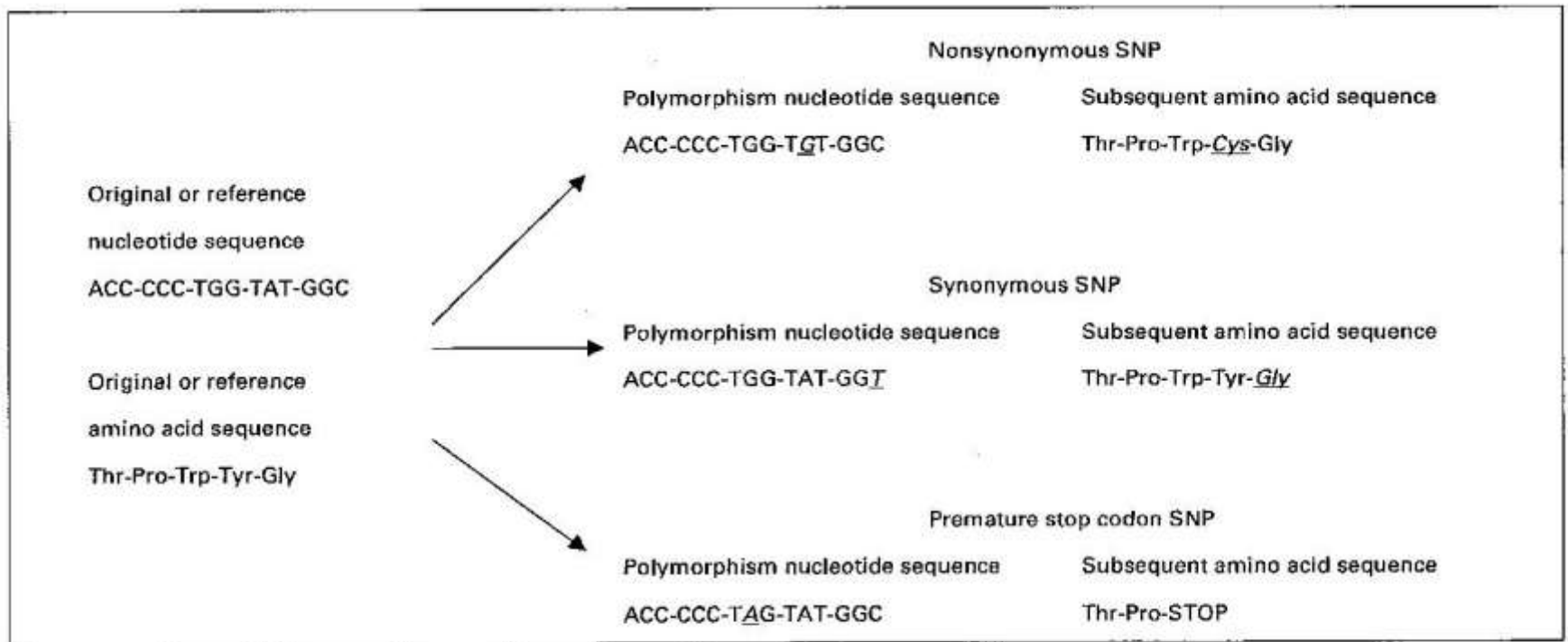


Figure 1. Examples of nonsynonymous, synonymous, and premature stop codon SNPs

Abbreviations used: A, adenosine; C, cytosine; Cys, cysteine; G, guanine; Gly, glycine; Pro, proline; SNP, single nucleotide polymorphism; T, thymine; Thr, threonine; Trp, tryptophan, Tyr, tyrosine.

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Nonsynonymous SNP



- Base change -> change in protein amino acid
- Example: thiopurine methyltransferase
 - Enzyme involved with purine metabolism
 - Activity required for azathioprine [Imuran] and 6-mercaptopurine [Purinethol] metabolism
 - TPMT*3A polymorphism involves a A -> G substitution changing an alanine to threonine
 - This reduces TPMT activity, resulting in higher blood levels and enhanced toxicity - myelosuppression

Synonymous SNP



- Aka a silent polymorphism
- Here the substitution does not change the final amino acid in the protein – however can still get alteration in the functional protein
- Example: ABCB1 3435 C>T
 - Gene codes for the P-glycoprotein efflux pump in human cells
 - This substitution doesn't change amino acid (glycine)
 - Although variant protein the same, less protein in cell
 - Due to decreased mRNA stability
 - Clinically importance shown in decreased risk of hepatotoxicity due to efavirenz [Sustiva]

Premature Stop Codon SNP

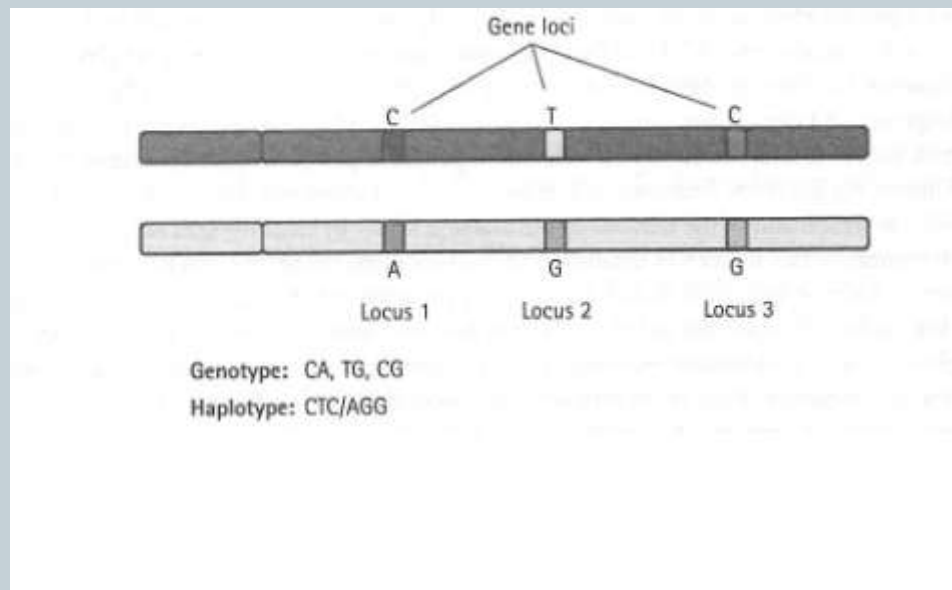


- Here the nucleotide substitution results in a stop codon being introduced.
- Protein chain synthesis is terminated and a shortened protein is produced.
- Example: CYP2C19*3 allele
 - Shortened protein has no enzyme activity
 - Clinically significant for any CYP2C19 substrate drugs.
 - Blood levels of one such drug is omeprazole [Prilosec] was found to be elevated 12X in person with this allele.

Haplotypes



- Haplotype – defined as a group of alleles on a chromosome that are transmitted together.
- These are useful groupings because one can determine information on local linkage between gene loci.



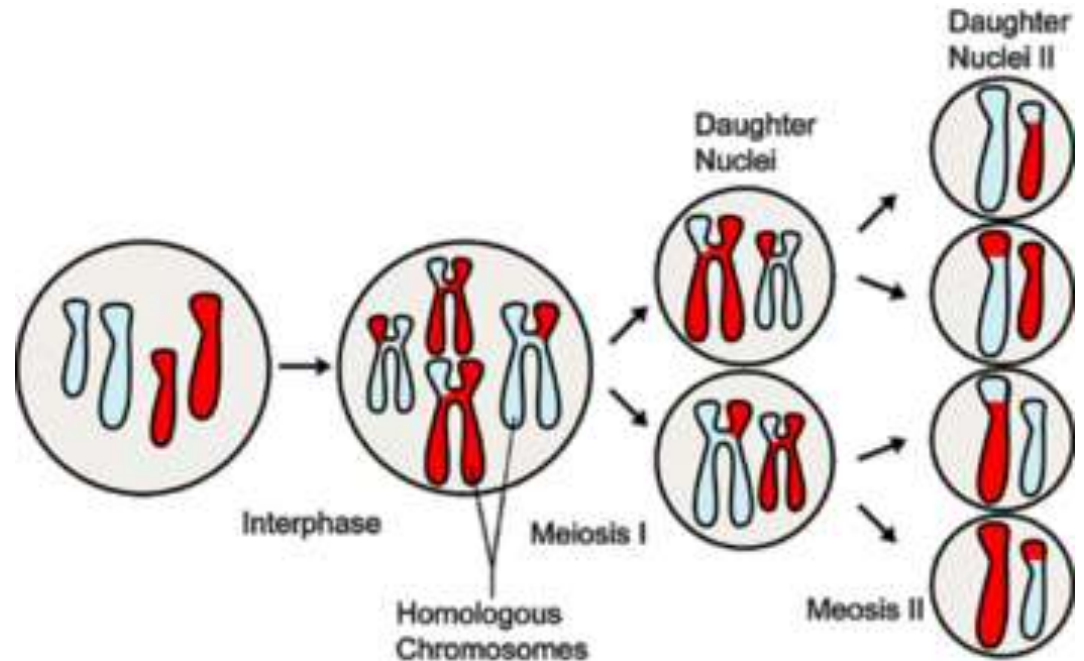
Recombination

During the process of gamete formation, cells undergo meiosis.

Upon pairing up, certain regions of the chromosome cross-over to the other paired chromosome.

This happens 30-40 times/chromosome during each meiosis.

Genes close to each other generally stay together.



Linkage Disequilibrium



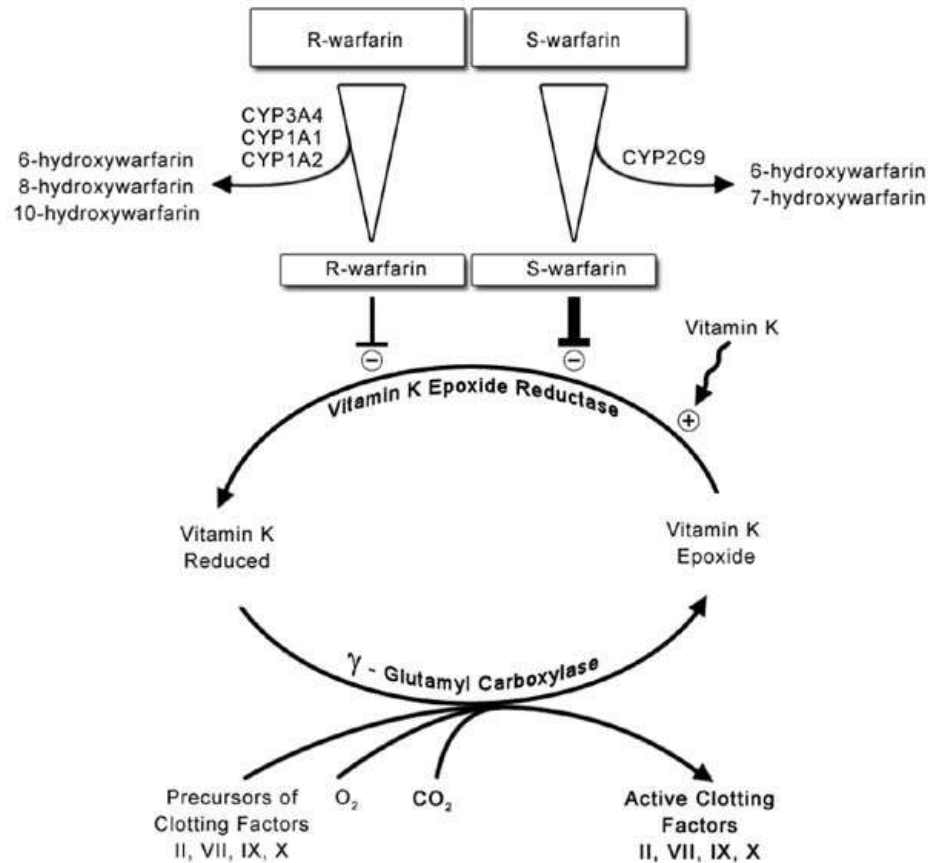
- If two (or more) genes or SNPs are located near each other, then they will tend to stay together through recombination.
- This linkage, is termed disequilibrium because the assortment between paired chromosomes is not random.
- Therefore, one can use the presence of one SNP to infer the presence of other SNPs if they all show strong linkage disequilibrium. This called a tagging SNP.

Warfarin

Warfarin has well defined clinical effects, but substantial potential for bleeding toxicity.

S-form metabolized by CYP2C9

Vitamin K epoxide reductase (complex subunit 1) coded by the VKORC1 gene



Warfarin Dosing Variability



- To understand potential warfarin dosing & toxicity need to differentiate:
 - PK genetic variability – CYP2C9 polymorphisms
 - PD genetic variability – VKORC1 polymorphisms
- Two alleles of CYP2C9 are known with reductions in enzyme activity of 50% and 90%.
- Similarly two SNP haplotypes of the VKORC1 gene have been shown to be associated with modified functional activity of the enzyme complex.

VKORC1 Haplotypes



- Contains 30 SNPs in the non-coding region of the gene.
- 10 of these have been studied for their association with warfarin dosing variability. [NEJM 2005;352:2285]
- 5 of these SNPs were found to exhibit strong linkage disequilibrium forming two haplotypes.

Locus	-4931	-1639	1173	1542	2255
SNPs	C	A	T	C	T
	T	G	C	G	C

Warfarin Genetic Testing - Utility



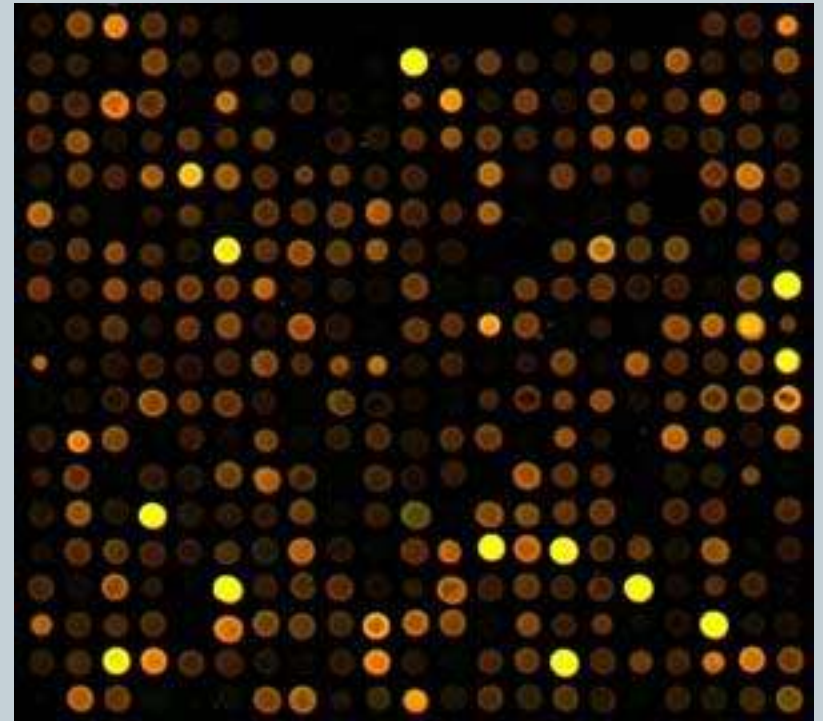
- Multicenter trial of 4043 patients showed that the use of an algorithm including pharmacogenetic information (CYP2C9 & VKORC1) was more accurate in targeting treatment to a defined INR. [NEJM 2009;360:753-64]
- August 2007 FDA updated label for Coumadin to encourage use of pharmacogenomic testing for individualized dosing.
- August 2009 Centers for Medicare & Medicaid Services decided to cover pharmacogenomic testing for Medicare patients.

Pharmacogenomic Testing



- Numerous methodologies can be used to detect DNA alterations depending on whether a limited number of DNA sequences of interest or multiple SNPs need to be examined.
- An efficient method to examine multiple alleles simultaneously is through the use of DNA microarrays.
- Affymetrix provides through Roche Diagnostics a CYP450 Microarray that determines 1936 DNA markers in 225 genes simultaneously.

Amplichip CYP450



Pathway Genomics/ Walgreens

Walgreens was set to sell home DNA test kits beginning May 2010.

FDA issued warning letter that tests had not been validated.

Walgreens removed the test kits, but this leaves the question of the advisability of this approach to pharmacogenomic testing.



Partial List of Markers



- **Pharmacogenomic**

- Abacavir hypersensitivity
- Caffeine metabolism
- Clopidrogel metabolism
- Methotrexate toxicity
- Statin induced myopathy
- Warfarin metabolism
- Tamoxifen response

- **Potential Disease States**

- Lung cancer
- Breast cancer
- Obesity
- Alzheimer's Disease
- Asthma
- Diabetes
- PAD
- Psoriasis

Cancer Genetics



- Identification of molecular targets for cancer chemotherapeutic agents has revolutionized the treatment of selected cancers.
- One can now assay for the presence of a particular gene mutation and predict the effectiveness of drugs.

Cancer Type	Genetic Modification	Drug Therapy
Breast cancer	Her2/neu (ErbB2)	Trastuzumab
Acute myeloid leukemia	FLT3 mutation	Tyr kinase inhib.
Chronic myelogenous leukemia	BCR:ABL fusion	Imatinib

Conclusions



- Pharmacogenomics has evolved from a primary focus on drug metabolism and disposition to include all aspects of persons response to a drug.
- Genetic information is beginning to become incorporated into clinical decision making, particularly in dosing drugs with significant toxicity.
- Individualized medical treatment will require further advances in identification of additional genes involved with drug responses.
- Methodological advances are making detection of individual gene mutations facile.

Resources



- Concepts in Pharmacogenomics, Zdanowicz MM, ed. ASHP, Bethesda, 2010.
- Lee CL, Ma JD, Kuo GM. Pharmacogenomics: bridging the gap between science and practice. J Am Pharm Assoc 2010;50(1):e1-e14.
- Web sites:
 - www.ncbi.nlm.nih.gov/About/primer/index.html
 - www.pharmkb.org
 - www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm