Clinical Pharmacogenetics

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Pharmacogenetics

The study of heredity as it relates to the absorption, distribution, elimination and action of medicines:

A tool to limit variability and individualize therapy

Pharmacogenomics

The study of the human genome, and its structure as relates to genes involved in drug absorption, action and elimination:

A tool for drug discovery

Mechanisms of Inherited Genetic Variability

(All are in germ line DNA or mitochondrial DNA)

Single nucleotide polymorphisms (SNPs)

Deletions

Duplications

Early History of Pharmacogenetics

- 1932: First Inherited Difference in an Inherited Response to a Chemical: Inability to Taste Phenylthiourea.
- Motulsky: 1957 “inheritance might explain many individual differences in the efficacy of drugs and in the occurrence of adverse drug reactions”
- 1959: Friedrich Vogel :“Pharmacogenetics: the Role of Genetics in Drug Response”
- 1959: Genetic influence on isoniazid blood concentrations
- 1964: Genetic variation in ethanol metabolism
- 1977: CYP2D6 polymorphism

Methods in Pharmacogenetics

- Family and twin studies to confirm genetic characteristics (dominant, recessive, Mendelian, maternal etc.)
- Population distribution analysis with Normit plots using a valid probe to detect phenotypic polymorphism (> 1% of population)
- Identification of gene and mutations
- Development of a genetic test for mutations in DNA
- Correlation between genotype and phenotype

Phenylthiourea Nontaster Trait

(Snyder *et al*: The inheritance of taste deficiency in man. *Ohio J Sci* 1932: **32**, 436–468.

- 800 families including 2043 children
- Serial Dilution Testing
- Mendelian Inheritance
- US prevalence of the nontaster trait = 30 %.

Genetically Polymorphic Trimethylaminuria

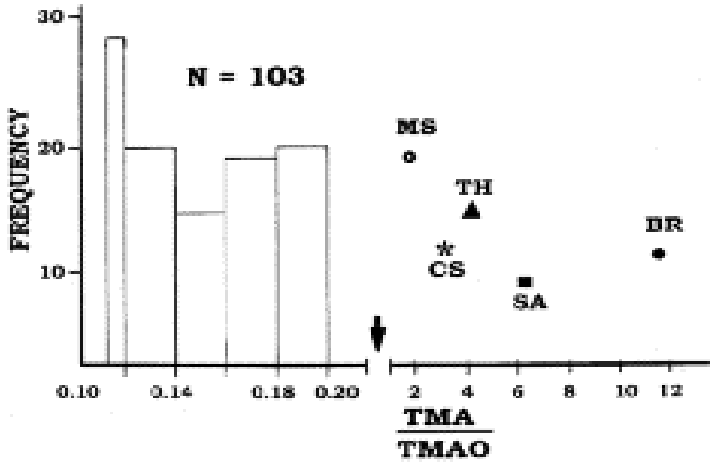


Fig. 1. Population frequency of the urinary TMA/TMAO ratios (μmol per 24 h) for 103 normal healthy volunteers. The ratio of each affected proband is also shown for comparison, with the position on the *y*-axis arbitrarily assigned.

From: **Thithapandha, A.**
A pharmacogenetic study of trimethylaminuria in orientals
Pharmacogenetics (1997) **7**, 497–501

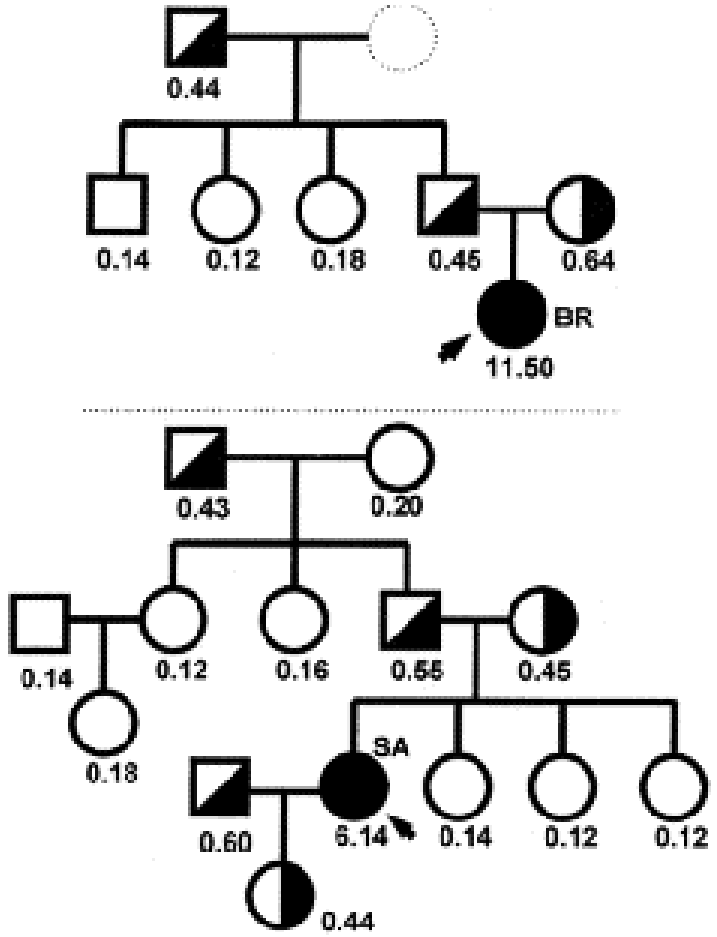


Fig. 2. Family studies on the urinary excretion (μmol per 24 h) of TMA and TMAO under normal dietary conditions. All values shown are the TMA/TMAO ratios. Each affected proband is indicated by an arrowhead.

N-Acetylation Polymorphism NAT-2

- Late 1940's : Peripheral Neuropathy noted in patients treated for tuberculosis.
- 1959 : Genetic factors influencing isoniazid blood levels in humans. *Trans Conf Chemother Tuberc* 1959: **8**, 52–56.

Incidence of the Slow Acetylator NAT-2 phenotype

- 50% among Caucasians
- 50% among Africans
- 90 % among Japanese
- 20% among Egyptians

Properties of an ideal pharmacogenetic probe for phenotype

- Specific for the trait in question
- Sensitive
- Easy to assay
- Clinically benign

NAT-2 substrates
(All have been used as probes)

- Caffeine
- Dapsone
- Hydralazine
- Isoniazid
- Procainamide

Clinical relevance of the NAT-2 polymorphism

- Higher isoniazid levels, greater neuropathy in slow acetylators
- Faster ANA appearance with procainamide in slow acetylators
- Lack of N-Acetyl Procainamide in slow acetylators, and therefore lack ofK-channel blockade

Examples of Genetic Effects on Human Drug Absorption, Action and Elimination

- Absorption:
  - Alcohol Dehydrogenase
  - Cytochrome P450 3A5
  - Cytochrome P450 2C19
- Action
  - Angiotensin II receptor
  - $\beta_2$receptor
  - Dopamine D4 receptor
  - Endothelial NO synthase
  - $5HT_4$receptor
  - Glucose 6 phosphate dehydrogenase

Examples of Genetic Effects on Human Drug Absorption, Action and Elimination (continued)

- Cytochrome P450 2A6
- Cytochrome P450 2C9
- Cytochrome P450 2C19
- Cytochrome P450 2D6
- Regulation of cytochrome P450 3A4
- Dihydropyridine Dehydrogenase (DPD)
- UDP-Glucuronyl Transferase 1A1 (UGT 1A1)
- Glutathione - S - Transferase (GST)
- Thiopurine methyl transferase (TPMT)
- Flavin Mono-Oxygenase 3 (FMO-3)
- Multidrug Resistance Transporter (MRP)

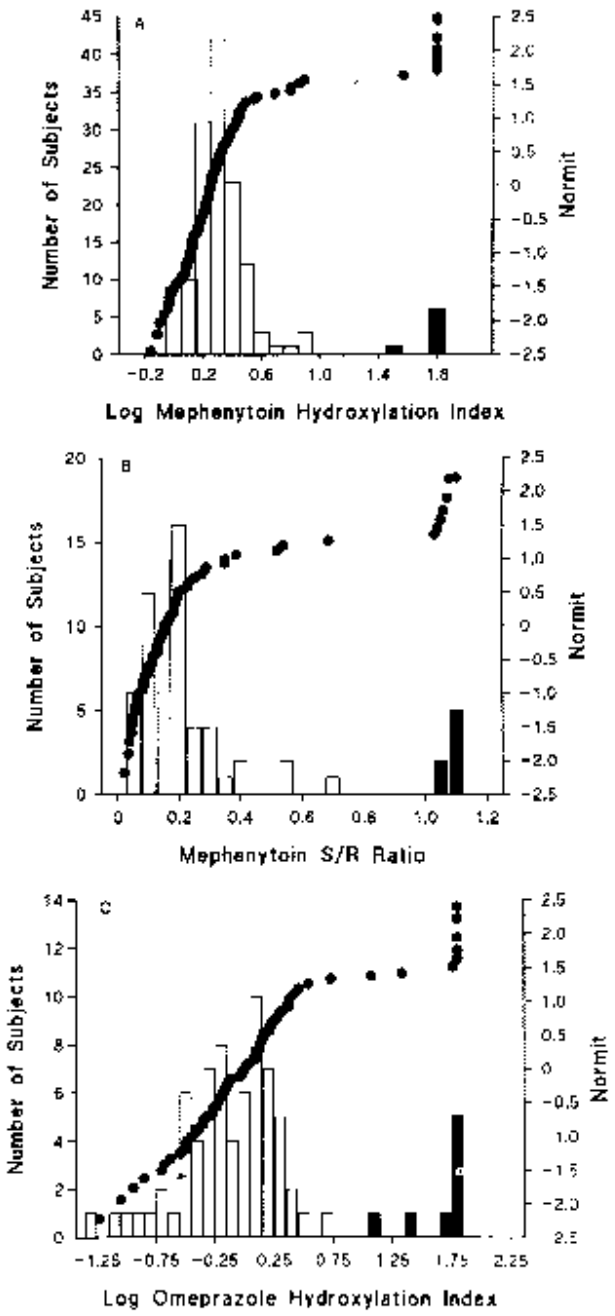
Examples of genetic effects on Drug Absorption in Humans

(Phenotype ahead of the genotype)

Aldehyde Dehydrogenase Genetics

- 10 human ALDH genes
- 13 different alleles
- autosomal dominant trait because of lack of catalytic activity if one subunit of the tetramer is inactive
- ALDH2 deficiency results in build up of toxic acetaldehyde
- Absent in up to 45% of Chinese, not at all in Caucasians or Africans

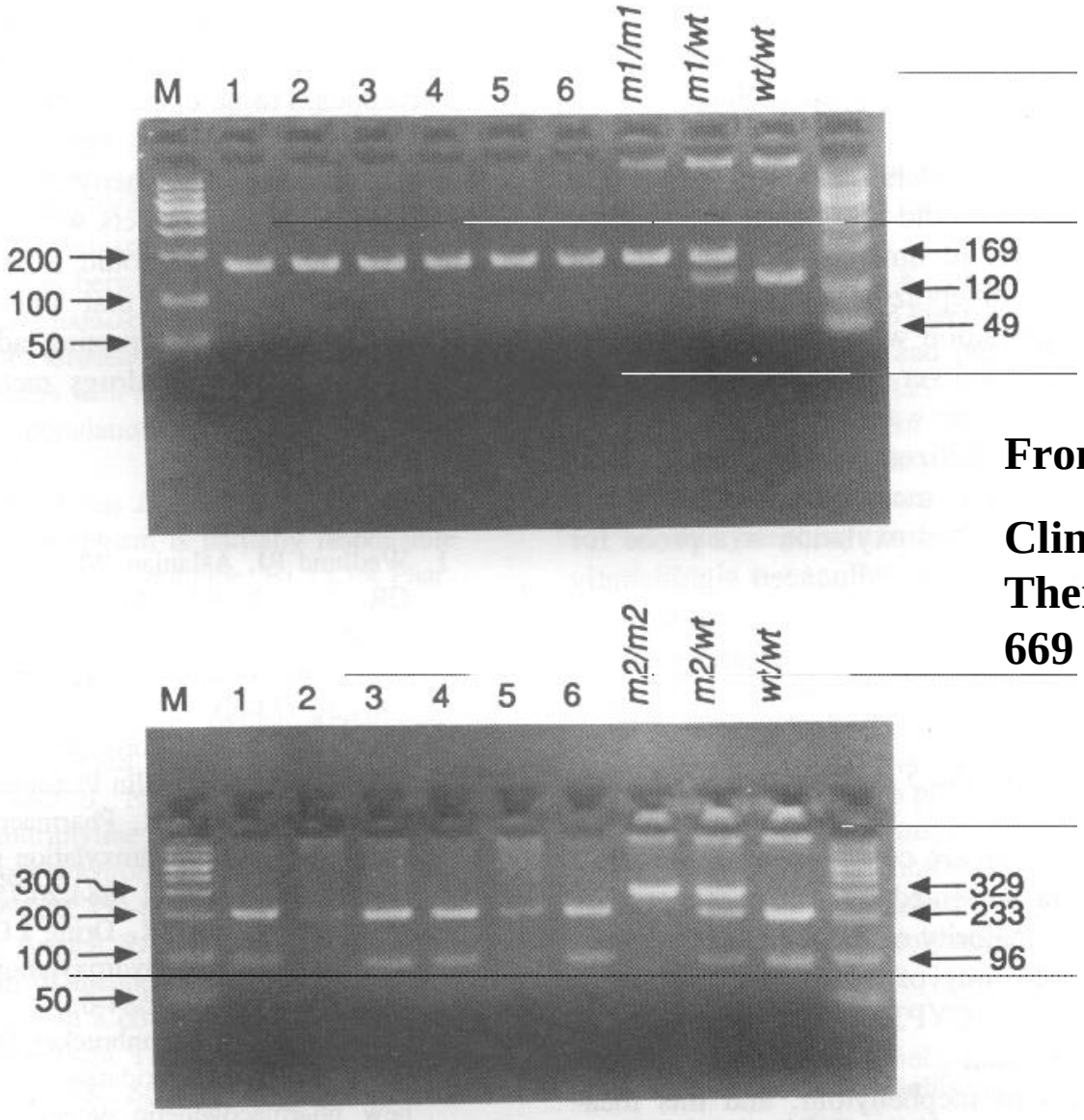
Population Distribution of CYP2C19 phenotype



Balian et al:

Clin Pharmacol Ther 1995;57:662-669

Genotyping of CYP2C19



From : Balian et al:

Clin Pharmacol Ther 1995;57:662-669

Distribution of CYP2C19 genotypes

Population	n	wt *Allele*	m1 *Allele*	m2 *Allele*	*Calculated*[a] *(observed) PM genotypes*
European-Americans	105	0.87	0.13	0	2% (2%)
Saudi Arabians	97	0.85	0.15	0	2% (2%)
Japanese	53	0.67[bcd]	0.23[de]	0.104	11% (15%)
Filipinos	52	0.54[bcd]	0.39[def]	0.077	21% (23%)
Chinese-Taiwanese	118	0.63[bcd]	0.32[de]	0.055	14% (15%)
African-Americans (NC)	108	0.75[bcd]	0.25[de]	0	6% (7%)

From: Goldstein et al: Pharmacogenetics 1997;7:59-64.

Examples of CYP2C19 substrates

- Amitryptyline, Imipramine
- Citalopram
- Diazepam
- Nelfinavir
- Omeprazole, Lansoprazole
- Mephenytoin
- Phenytoin
- Proguanil

Clinical relevance of the CYP2C19 polymorphism

- Increased toxicity with citalopram in PMs
- Prolonged sedation by diazepam in PMs
- Increased sedation by mephenytoin in PMs
- Altered metabolic saturation threshold for phenytoin in PMs
- Decreased activity of proguanil in PMs

Examples of genetic effects on drug action in humans

(Genotype ahead of the phenotype)

Glucose 6 Phosphate Dehydrogenase:

Carson PE *et al*: Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 1956: **124**, 484–485.

- Deficient in 1 in 10 Africans
- X-linked recessive
- Primaquine induces hemolysis, as does:
- Quinine, quinidine
- Sulfonamides
- Dapsone, methylene blue

Examples of Human Receptors shown to be genetically polymorphic with *possible* alterations in clinical phenotype

- Angiotensin II receptor
- Angiotensin converting enzyme
- $\beta_2$receptor
- Dopamine D_4 receptor
- Endothelial NO synthase
- $5HT_4$receptor

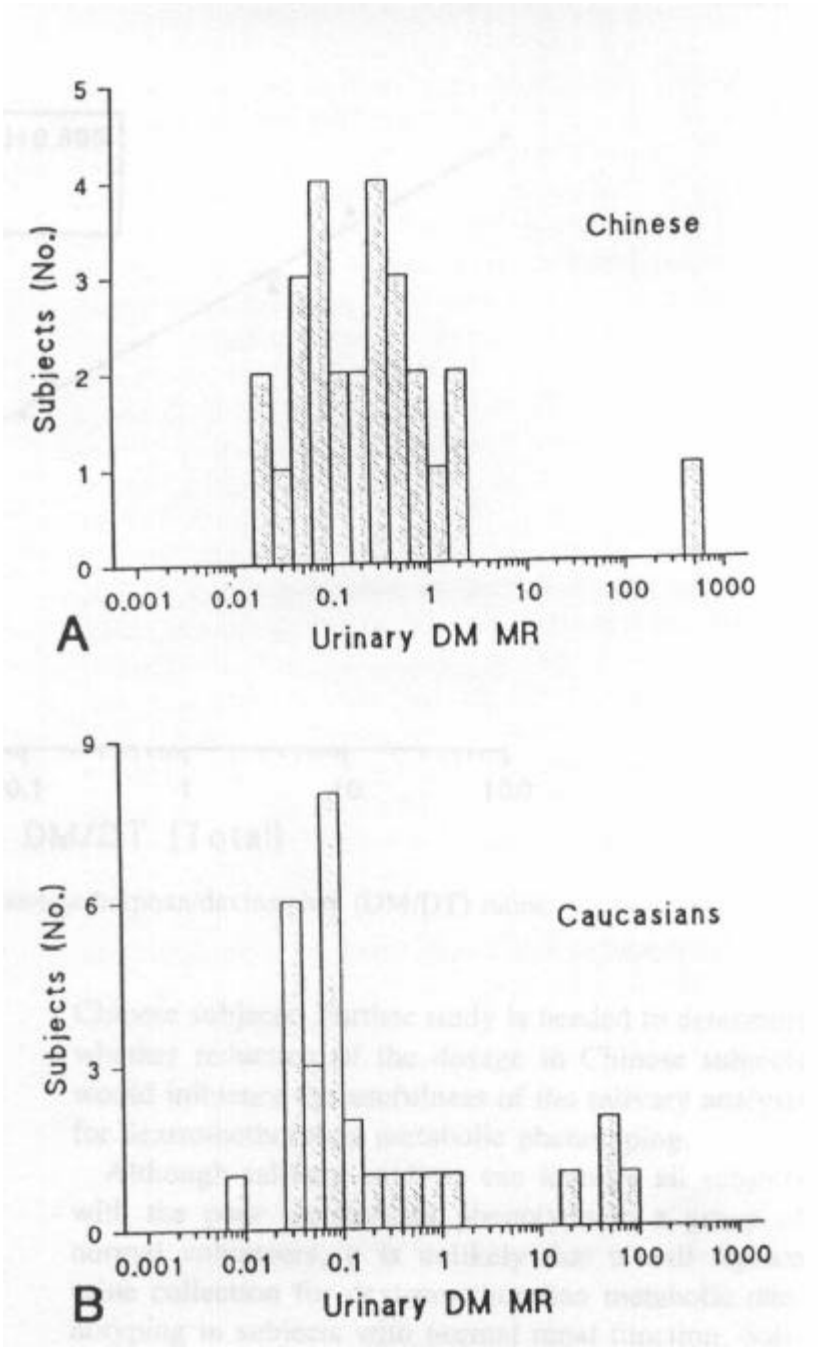
Not all mutations in DNA have a phenotypic consequence
(most do not)
Not all genetic polymorphisms have a clinical consequence

Examples of genetic effects on drug elimination in humans

Population Distribution of CYP2D6 phenotype using Dextromethorphan

From Woosley *et al*:

Clin Pharmacol Ther 1991;49:410-419



CYP2D6 Substrate Probes used to Determine Phenotype

- Dextromethorphan
- Sparteine
- Debrisoquin
- S- Metoprolol
- Bufarol (*in vitro*)

Cytochrome P450 2D6

- Absent in 7% of Caucasians
- Hyperactive in up to 30% of East Africans
- Catalyzes primary metabolism of:
  - codeine
  - β-blockers
  - tricyclic antidepressants
- Inhibited by:
  - fluoxetine
  - haloperidol
  - paroxetine
  - quinidine

Effect of CYP2D6 on timolol pharmacokinetics and pharmacodynamics

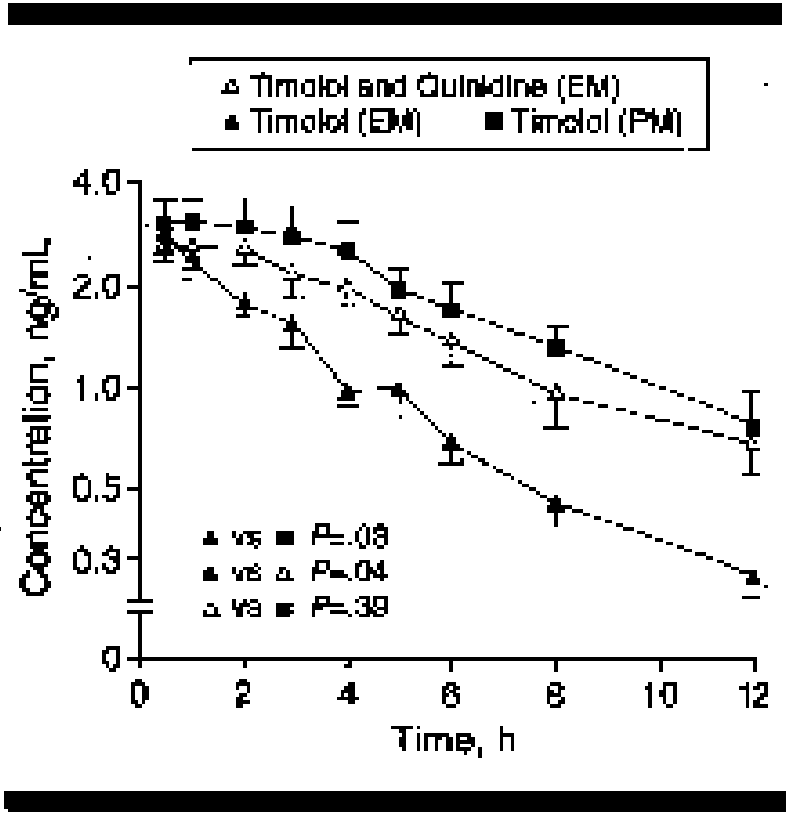


Figure 3.—Plasma concentrations (±SEM) of timolol in eight extensive metabolizers (EMs) with and without quinidine and five poor metabolizers (PMs) of debrisoquin following nasal administration of two drops of ophthalmic solution into each nostril.

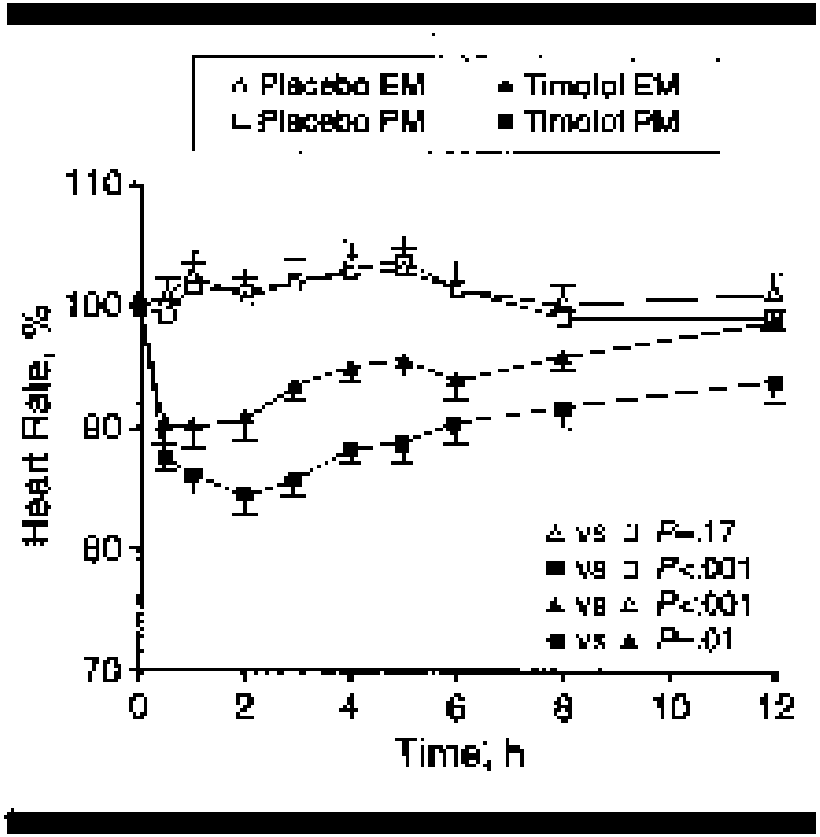


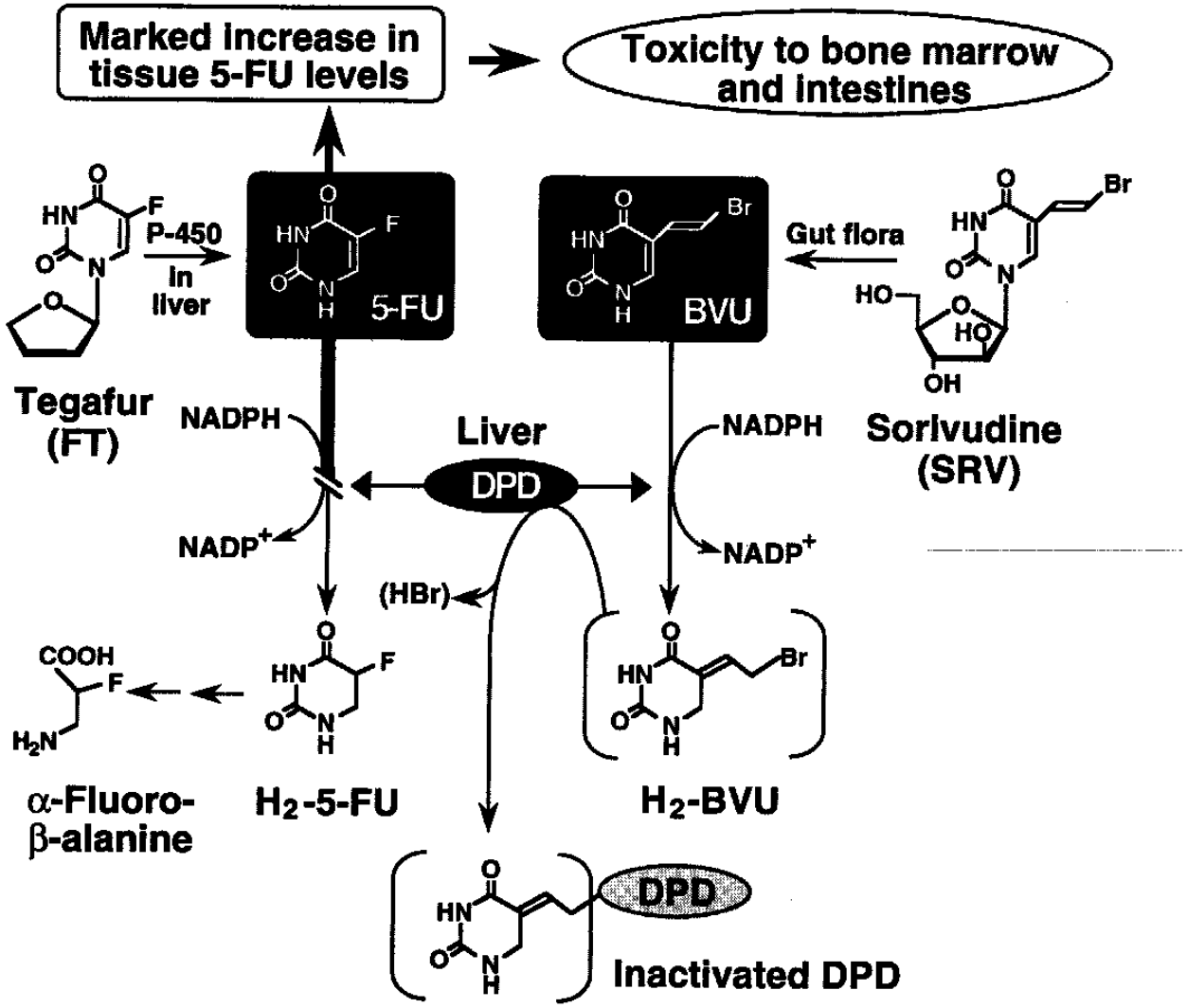
Figure 1.—Percentage of baseline heart rate (±SEM) in eight extensive metabolizers (EMs) and five poor metabolizers (PMs) of debrisoquin on two different occasions following administration of timolol eye drops and placebo (artificial tears).

From: Wood AJJ et al. JAMA 1995;274:1611-1613

Dihydropyridine Dehydrogenase

- Absent in ~ 3% of Caucasians
- Responsible for metabolism of 5-fluorouracil
- 80-90% of 5-FU is metabolized, 10 - 20% is renal
- Deficient patients treated with conventional doses of 5-FU experience diarrhea, stomatitis, mucositis, myelosuppression and neurotoxicity.

Dihydropyridine Dehydrogenase



Okuda et al. Eighteen deaths due to an interaction with DPD.JPET 1998;287:791-809

UGT 1A1

- Responsible for Gilbert's
- absent in ~15% of Caucasians
- < 5% Asians
- > 50% of Africans
- > 50% of Hispanics

Sulfonamide Hypersensitivity Reactions

- 50 % Prevalence HIV patients
- 1 % Prevalence in healthy normals

Glutathione-S-Transferase

- 55 % of Caucasians have no GSTM1
- 15 % of Caucasians have no GSTT1

Glutathione *S*-transferase (*GSTM1*) null genotype and sulphonamide intolerance in acquired immunodeficiency syndrome

Claudine Deloménie[1], Pascale Mathelier-Fusade[2], Sandrine Longuemaux[1], Willy Rozenbaum[3], Francisque Leynadier[2], Rajagopal Krishnamoorthy[1] and Jean-Marie Dupret[1]*

Pharmacogenetics (1997) **7**, 519–520

	Number of subjects	*GSTM1*0/*0 Number (%)*	*GSTT1*0/*0 Number (%)*
Control individuals	205	115 (56)	39 (19)
AIDS patients			
Sulphonamide intolerants[a]	36	26 (72)	5 (14)
Sulphonamide tolerants	44	21 (48)	6 (14)
Total of AIDS patients	80	47 (59)	11 (14)

[a] Odds ratio and 95% confidence interval for sulphonamide hypersensitivity reactions among AIDS patients with the *GSTM1* null genotype is 2.8 [95% CI, 1.1–7.3]. No association was observed with the *GSTT1* null genotype (OR = 1.0; [95% CI, 0.3–3.7]).

Thiopurinemethyltransferase (TPMT)

Current Methods for genetic testing

- By phenotype: metabolic probe drug or Western blot
- By PCR with mutation-specific endonuclease
- By PCR and allele-specific hybrization
- By oligonucleotide chip hybridization
- By laser lithography - guided oligonucleotide chip hybridization.

Reality Check

- No genetic test, based on DNA testing is currently approved by the United States Food and Drug Administration, for the treatment of patients.
- The CYP2D6 polymorphism was first described in 1977, genetic tests have been available since 1986

Clinical Pharmacogenetics Summary Pearls

- A good phenotyping probe is critical
- Genetic tests need validation just as any other tests
- A potent inhibitor can mimick a genetic polymorphism
- Not all genetic polymorphisms have a phenotypic correlate, or clinical effect
- The clinical relevance of genetic polymorphisms is greatest with drugs of narrow therapeutic range, but not confined to them