

# PHARMACOGENETICS LECTURE SUPPLEMENT

9/13/02

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## Objectives:

Understand some of the genetic factors involved in inter-patient variability in drug response.

Learn some of the common pharmacogenetic polymorphisms.

Appreciate the important role of drug metabolism in determining drug response.

## CASE STUDIES:

*Patient 1.* A 69 year old woman was hospitalized for moderate to severe depression and treated with nortriptyline in a modest dose of 25 mg 3 times daily. Two days after the start of treatment she complained of dizziness. After a further 6 days of treatment, she complained of increasing tiredness and vertigo and appeared slightly confused. Low clearance of nortriptyline was suspected, blood was taken for nortriptyline analysis and the dosage was decreased to 25 mg once daily. The plasma concentration of nortriptyline after 8 days of treatment with the 75 mg daily dose was 1300 nM (recommended plasma concentration range 200-600 nM). The concentration on 25 mg daily for 12 days was 742 nM. When the dosage was further reduced to 20 mg at night, the patient had no side effects, and made an excellent recovery.

*Patient 2.* A 41 year old woman had been treated for long periods with high doses of nortriptyline (300-500 mg /day) to achieve therapeutic plasma levels (200-600 nM). The mean (+s.d.) plasma level in seven samples drawn at a dose of 300 mg/day was 291 +/- 56 nm. The plasma concentration of unconjugated 10-hydroxynortriptyline was about 10 times higher than that of the parent drug, which is much higher than usual.

**Pharmacogenetics:** originally defined as “..clinically important hereditary variation in response to drugs” (F. Vogel, 1959)

2 Sources of inpatient variability in response to drugs:

### 1) Environmental

- disease
- other drugs or chemicals
  - inhibition at receptors
  - inhibition of drug metabolism and clearance
  - induction of drug metabolism
  - induction or “up-regulation” of receptors
- usually results in a unimodal or “normal” distribution

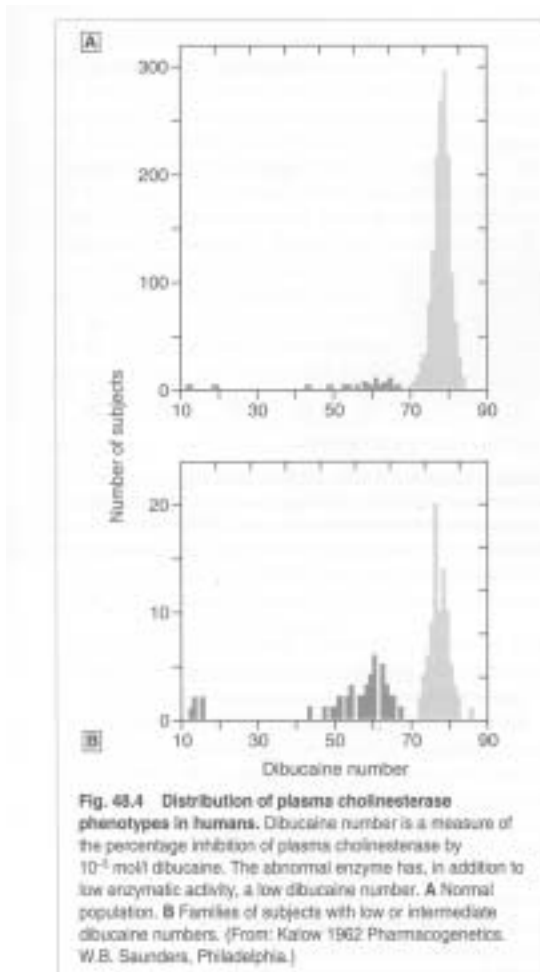
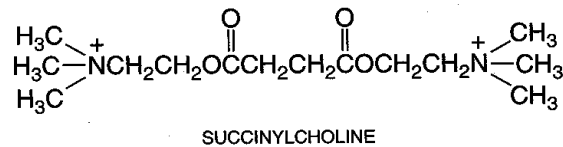
### 2) Hereditary (or Pharmacogenetic)

- receptors
- “disposition” factors, primarily metabolism
- initially observed as bi- or tri- modal distribution (classic Mendelian model)

## Historical examples

### 1) Serum esterases

Prolonged apnea following succinylcholine administration



### 2) G6P Dehydrogenase

High incidence of hemolytic anemia among Afro-Caribbean males treated for malaria with primaquine.

Red cell lysis due to toxic oxygen species generated by primaquine metabolites.

Susceptibility to anemia due to lack of protection by G6P dehydrogenase:

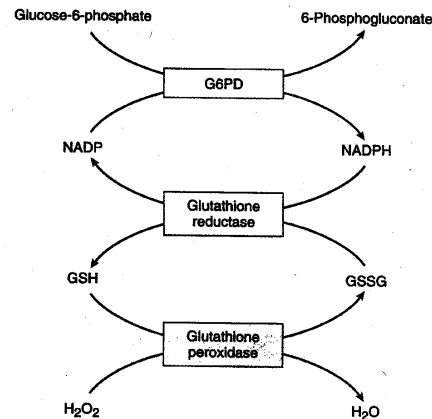
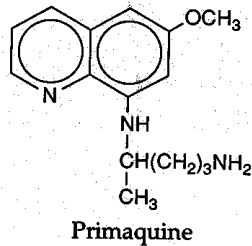


Figure 14-7

The role of glucose-6-phosphate dehydrogenase (G6PD) in the defense against oxidant injury. The disposal of  $H_2O_2$ , a potential oxidant, is dependent on the adequacy of reduced glutathione (GSH), which in turn is generated by the action of NADPH. The synthesis of NADPH is dependent on the activity of G6PD. GSSG, oxidized glutathione.

X-linked recessive defect(s) in G6Pdh enzyme; especially prevalent among African and Mediterranean ethnic groups.

### 3) N-Acetyltransferase

Peripheral neuropathy as side-effect of isoniazid treatment for tuberculosis.

Due to defect(s) in N-acetyltransferase enzyme ("Slow acetylator" phenotype)  
Autosomal recessive trait whose prevalence varies widely among ethnic groups

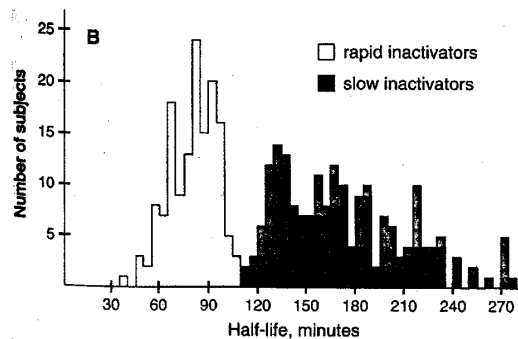
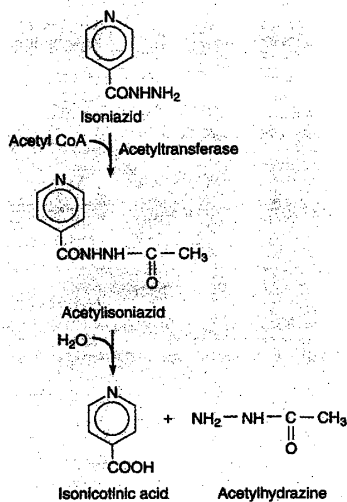


Figure 48-1. Bimodal distribution of serum isoniazid concentrations and half-lives in a large group of Finnish patients

Prevalence of "slow acetylator" phenotype in various gene pools:

Ethiopian 90%

European Caucasian 50%

East/Central Asian 10%-20%

Canadian Eskimo 5%

## More Recent Examples:

### 1) Thiopurine N-Methyl Transferase (TPNMT)

Approximately 1 in 300 pediatric cancer patients treated with thiopurine drugs is at risk for life-threatening severe myelosuppression, due to complete lack of ability to methylate thiopurines

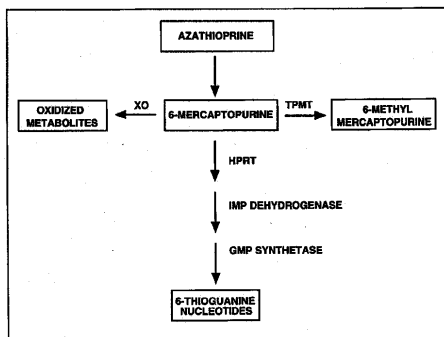


FIG. 2. Thiopurine biotransformation.

A simplified outline of the biotransformation of azathioprine and 6-mercaptopurine to yield 6-TGN. XO, xanthine oxidase; HPRT, hypoxanthine phosphoribosyl-transferase; IMP, inosine monophosphate; and GMP, guanosine monophosphate.

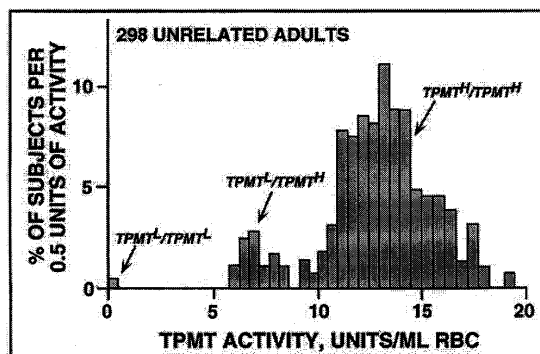


FIG. 1. RBC TPMT frequency distribution histogram for 298 randomly selected Caucasian subjects.

Metabolic deficiency due to 2 Single Nucleotide Polymorphisms (SNPs) in open reading frame of TPMT gene, resulting in inactive enzyme

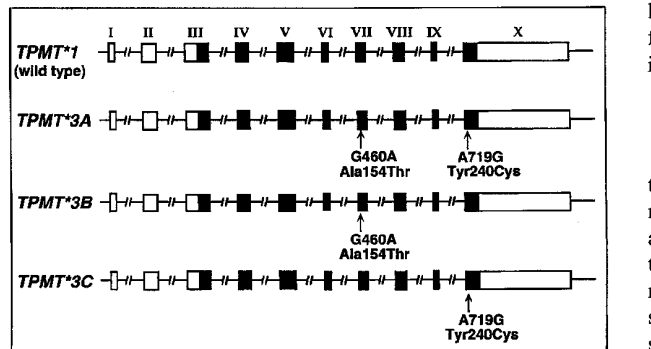


FIG. 4. Selected human TPMT alleles.

The wild-type human TPMT allele (*TPMT\*1*) and variant alleles *TPMT\*3A*, *TPMT\*3B*, and *TPMT\*3C*. Rectangles represent exons, with black coding areas and white untranslated regions.

Additional variations in 5' flanking region (17 bp repeat element repeated 4 - 8 times); probably affects level of enzyme expression.

Phenotype screening routinely done on rbc TPNMT activity; genotyping done as confirmatory followup.

### 2) CYP2D6

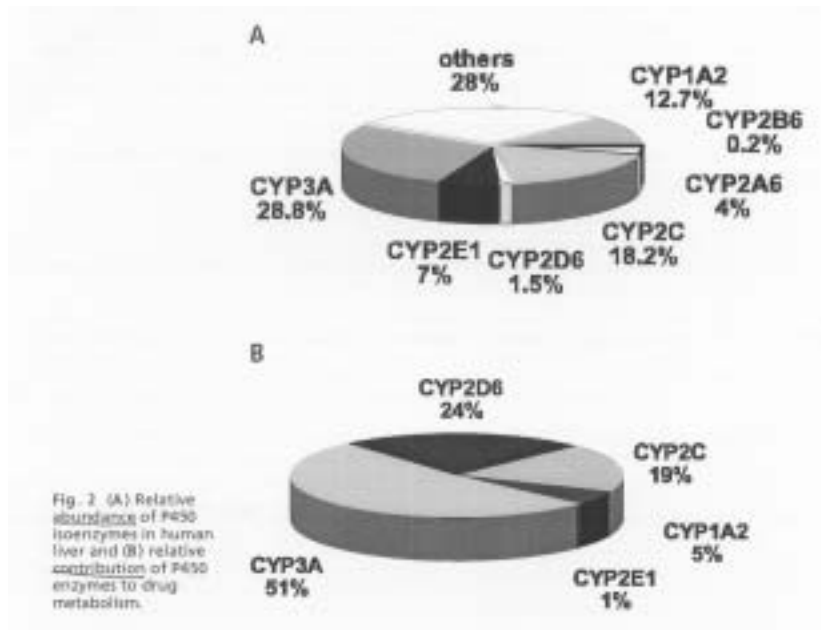
First described as idiosyncratic adverse responses to debrisoquine, an antihypertensive drug not used in US; adverse responses included postural hypotension and dizziness.

Variability reflected in variations in rates of debrisoquine hydroxylation, the major metabolic pathway for this drug.

Distribution of “Poor Metabolizer” (PM) phenotype varies in different ethnic groups:

- 7%-10% among Caucasian Europeans
- <1% among East Asians

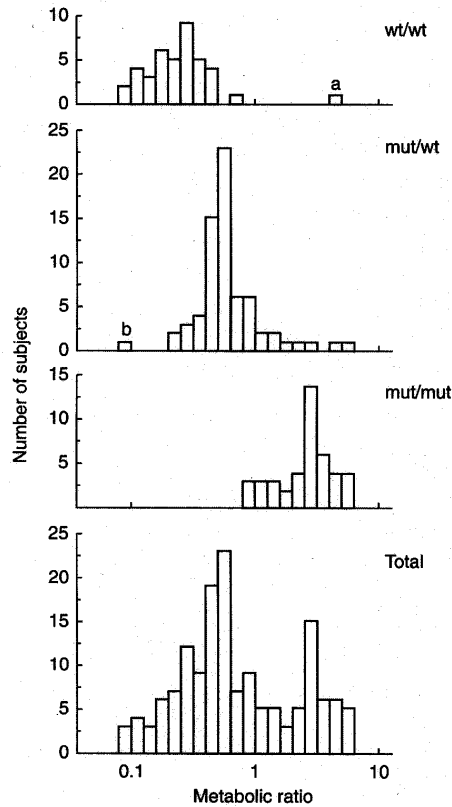
CYP2D6 is the primary enzyme that hydroxylates debrisoquine; it also metabolizes many other prescription drugs (~ 1/4 of all known drugs)



**Table 2** Some drugs whose metabolism is catalysed by CYP2D6.

<i>β-Adrenoceptor blockers</i>	<i>Antidepressants</i>	<i>Neuroleptics</i>
Metoprolol	Amitriptyline	Haloperidol
Propranolol	Clomipramine	Perphenazine
Timolol	Desipramine	Risperidone
	Fluoxetine	Thioridazine
<i>Antiarrhythmic drugs</i>	Fluvoxamine	Zuclopenthixol
Encainide	Imipramine	
Flecainide	Mianserin	<i>Miscellaneous</i>
Perhexilene	Nortriptyline	Codeine
Propafenone	Paroxetine	Debrisoquine
Sparteine	Venlafaxine	Dextromethorphan
		Phenformin
		Tolterodine
		Tramadol

Debrisoquine hydroxylase activity correlates with presence of variant alleles of CYP2D6



**Figure 1** Distribution of the debrisoquine MR (parent drug/4-hydroxy metabolite) in three genotype groups related to the *CYP2D6\*10* allele in 152 Korean subjects. wt = *CYP2D6\*1*(or\*2) and mut = *CYP2D6\*10*. Reproduced with permission from Roh *et al.* [26].

At least 20 variant *CYP2D6* alleles exist; different variants predominate in different ethnic groups. Variants include SNPs in open reading frame causing frame shifts or aa substitutions, SNPs at exon junctions causing splice variants, gene deletion and multiple gene copies.

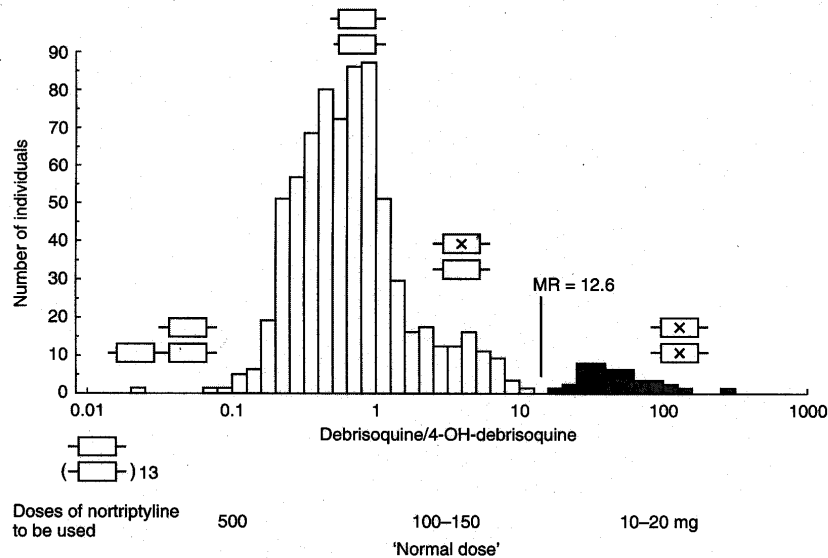
**Table 1** Frequency of *CYP2D6\*1* or \*2 alleles (causing 'normal' enzyme activity) and some alleles causing no or deficient *CYP2D6* activity in three different ethnic populations.

<i>CYP2D6</i> alleles	Functional mutation	Consequence	Swedish	Allele frequency (%)		
				Chinese	Zimbabwean	
*1 or *2			69	43	54	
*3	A2637 del	Frame shift	2	0	0	
*4	G1934A	Splicing defect	22	0-1	2	
*5	Gene deletion	No enzyme	4	6	4	
*10	C188T	Unstable enzyme	n.d.	51	6	
*17	C1111T	Reduced affinity	n.d.	n.d.	34	

n.d. = not determined.

Data are from original publications [23, 25, 27] and reviews [4, 5].

“Extensive metabolizer” (EM) phenotype also exists; these people have duplicate or multiple copies of the CYP2D6 gene (2-12 copies), producing rapid metabolism (low MR - ratio of parent drug/metabolite)



**Figure 2** Distribution of the urinary debrisoquine MR in 757 healthy Swedish subjects with schematic presentation of *CYP2D6* genotypes, where a cross in an allele indicates a detrimental mutation. Also tentative doses of nortriptyline to be used in different genotypes are indicated. From [64].

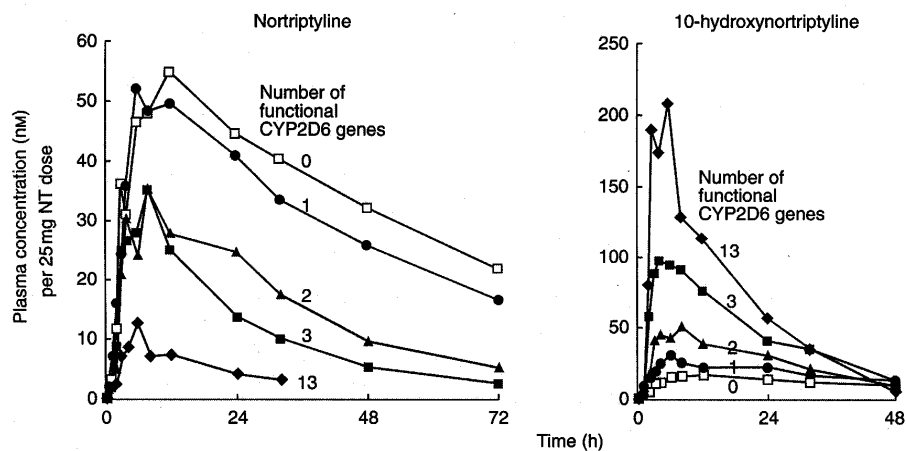
Percentage of EM phenotype also varies among ethnic groups:

Northern Europeans (Sweden) 1%-2%

Southern Europe (Spain, Sicily) 7% - 10%

Northern Africa, Middle East (Ethiopia, Saudi Arabia) 20%-30%

Variation in CYP2D6-dependent metabolic capacity can have major clinical implications. Rapid genotyping screens are being developed.



**Figure 3** Mean plasma concentrations of nortriptyline (left) and 10-hydroxynortriptyline (right) in different *CYP2D6* genotype groups after a single oral dose of nortriptyline. The numerals close to the curves represent the number of functional *CYP2D6* genes in each genotype group. In groups with 0-3 functional genes, there were five subjects in each group while there was only one subject with 13 functional genes. Reproduced with permission from Dalén *et al.* [41].

**Table 4 CYP2D6 – implications for poor metabolisers**

Decreased elimination - accumulation of parent compound resulting in toxicity	
<b>β-Blockers</b>	Metoprolol Timolol Bifuralol
<b>Anti-arrhythmics</b>	Sparteine Mexiletine Flecainide
<b>Tricyclic Antidepressants</b>	Nortryptiline Clomipramine
<b>Neuroleptics</b>	Perphenazine Zuclopenthixol
Decreased prodrug activation	
Encaidine	(active metabolite: O-desmethyl encaidine)
Codeine	(active metabolite: morphine)
Decreased elimination of active metabolite	
Imipramine	(active metabolite: desipramine)
Decreased elimination of parent compound and active metabolite	
Amitryptiline	(active metabolite: nortryptiline)

Polymorphisms for other CYP forms exist as well; Example - CYP2C19, responsible for metabolism of the commonly prescribed anticoagulant Warfarin. PM phenotype present in 3%-5% of Caucasians, 20% of ethnic Chinese. PM pts at risk for hemorrhage.

Some pharmacogenetic variations are known for specific drug receptors. The number of therapeutically significant “receptor polymorphisms” appear to be much more limited than the number of therapeutically significant “drug metabolism polymorphisms”

Panel 2: Examples of genetically determined variations in drug targets which influence therapeutic outcome			
Drug	Polymorphic gene	Patient population (number of patients)	Response
Pravastatin <sup>1</sup>	Cholesterol ester transfer protein	Men with coronary atherosclerosis (n=807)	Carriers of two S1 alleles benefited most from pravastatin
Formoterol <sup>2</sup>	β <sub>2</sub> adrenoceptor	Moderately severe stable asthmatics (n=22)	Homozygous Gly16/Gly16 significantly more prone to desensitisation than Arg16/Arg16 homozygous patients