

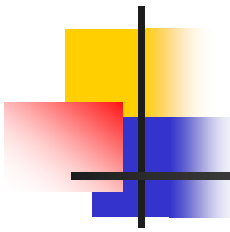


Individualized Medicine in Neurological Disease: Purpose, Potential and Promise

Gregory L. Kearns, Pharm.D., Ph.D.

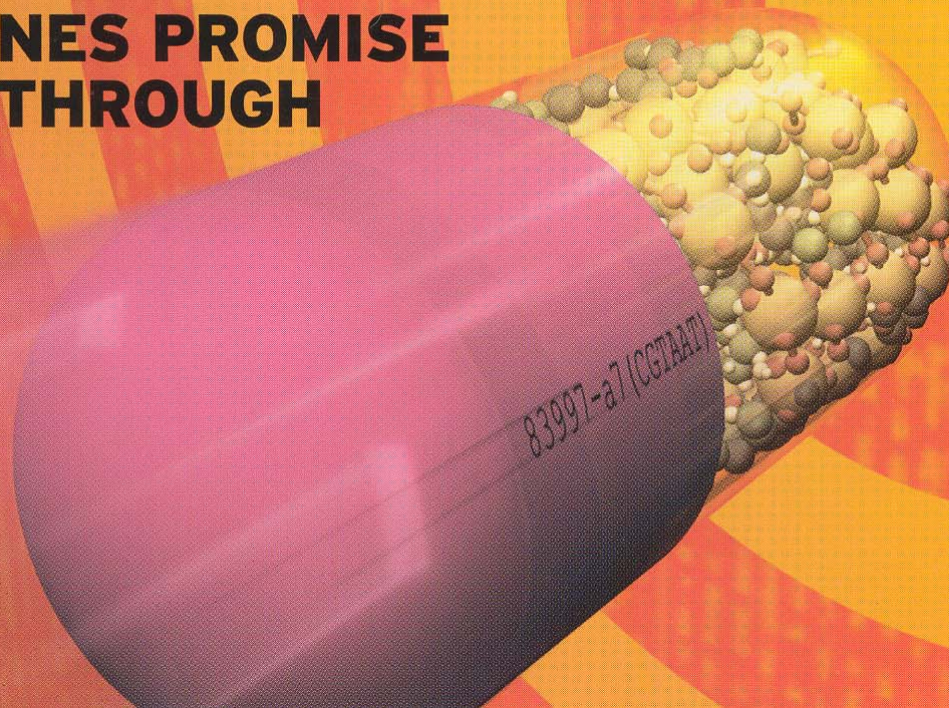
Marion Merrell Dow / Missouri Chair in Pediatric Pharmacology
Professor of Pediatrics and Pharmacology, University of Missouri –
Kansas City

Chief, Division of Pediatric Pharmacology and Medical Toxicology
Children's Mercy Hospitals and Clinics



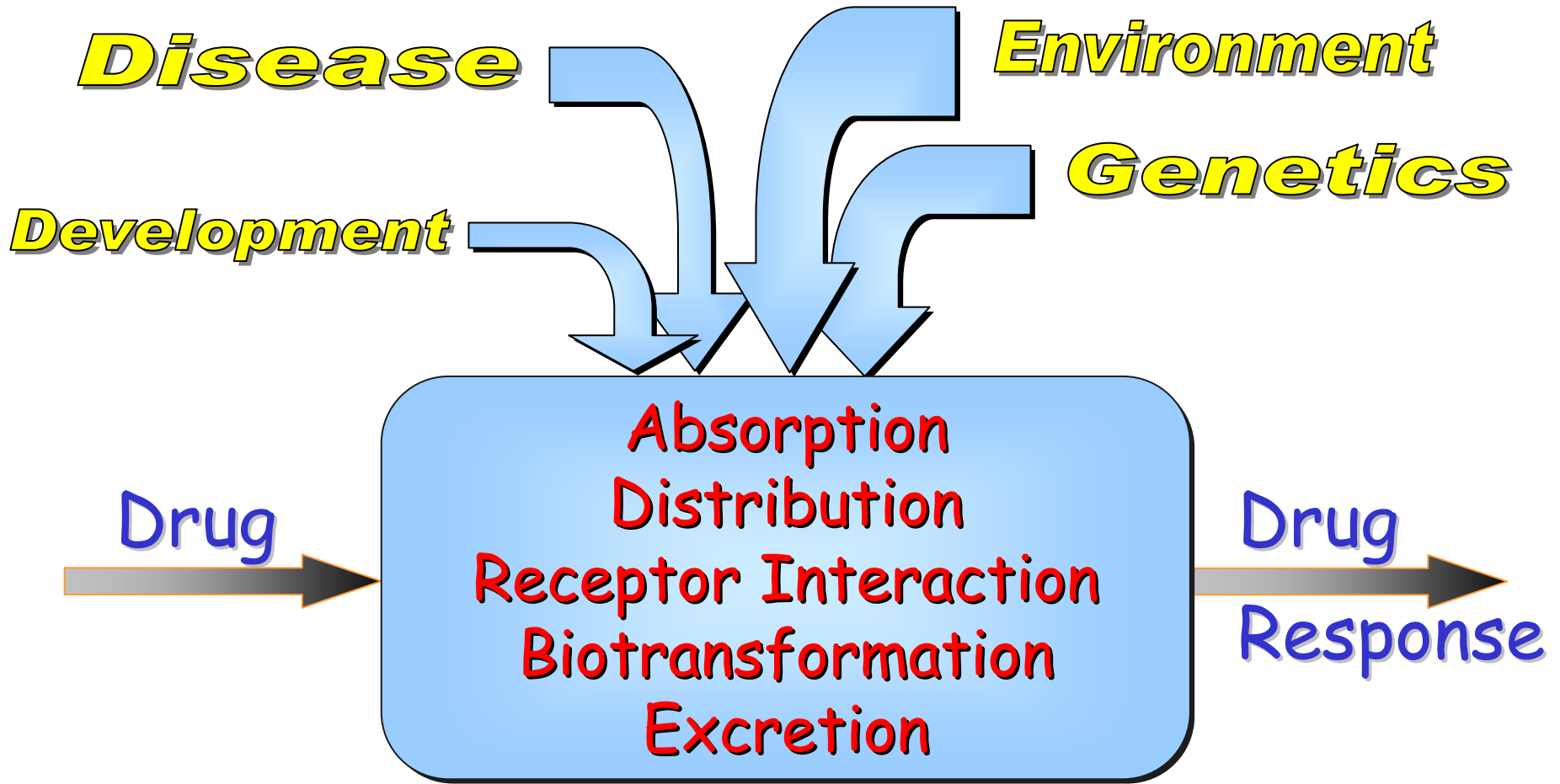
THIS DRUG'S FOR YOU

**NEW TARGETED
MEDICINES PROMISE
BREAKTHROUGH
CURES**



U.S. News and World Report, 14 January 2003

Variation in Drug Response



Definitions



Pharmacogenetics

Study of how variant forms of human genes contribute to inter-individual variability in drug response

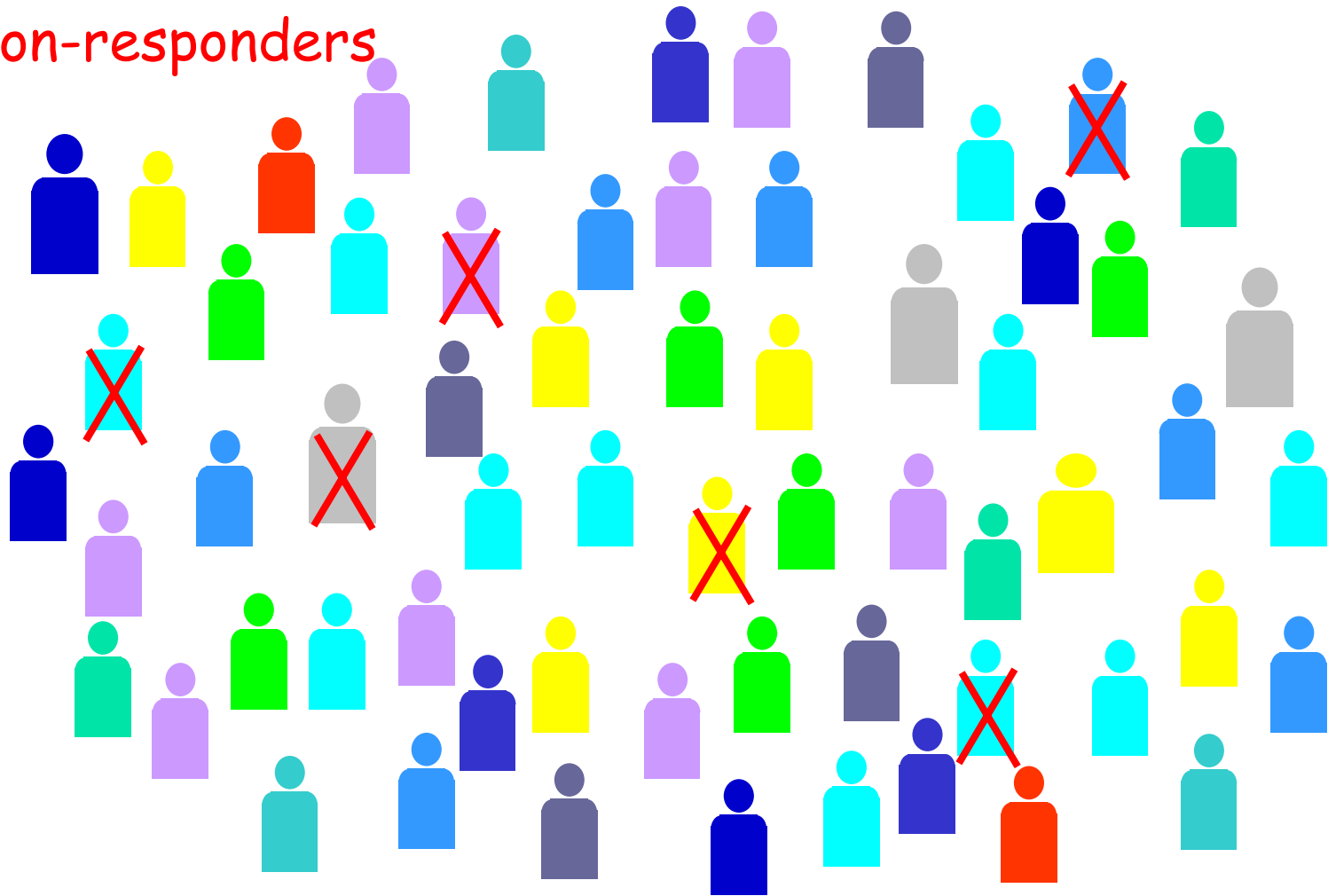
Single gene

Focus is at the level of the individual

“The right drug for the right patient”

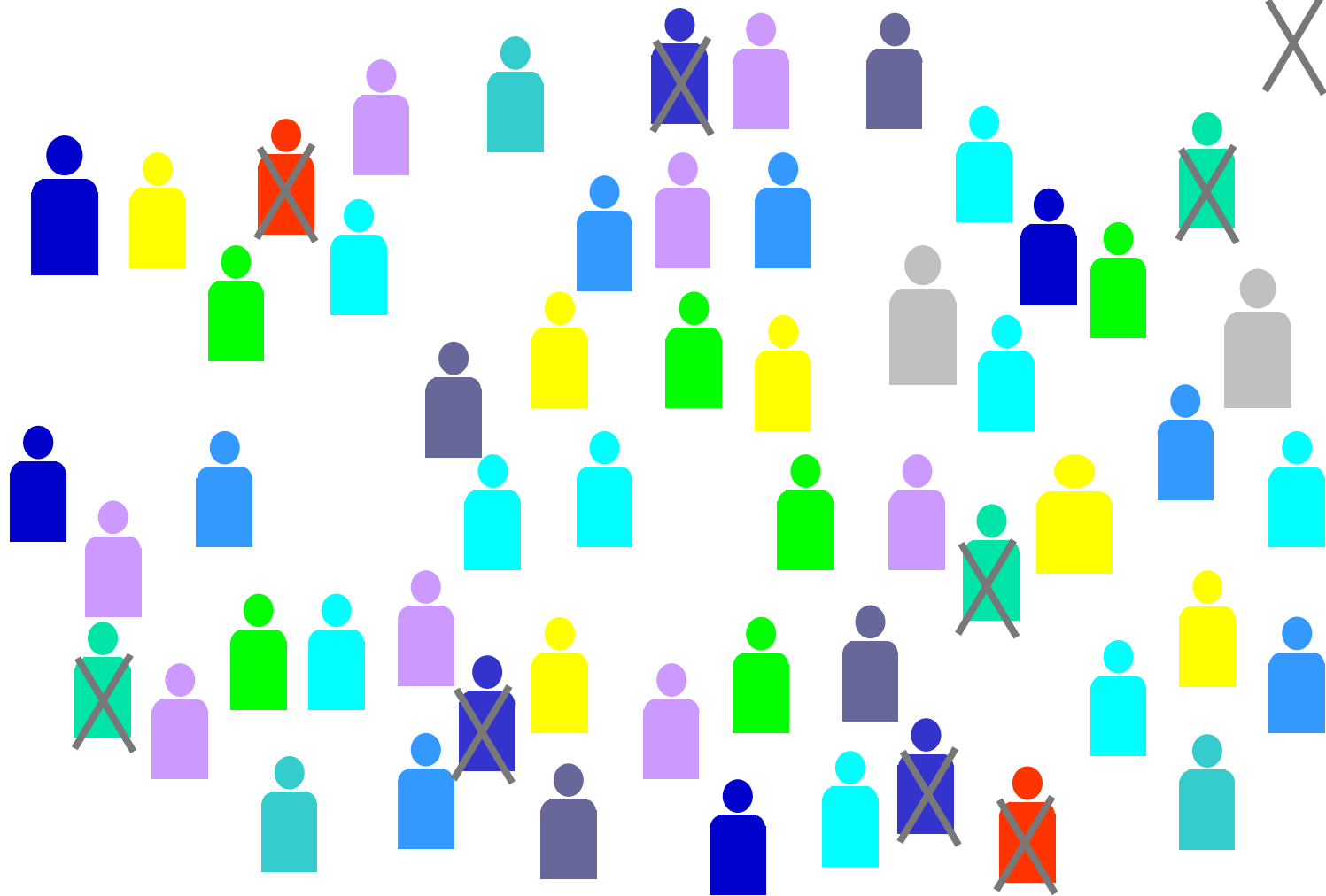
Pharmacogenetics

~~X~~ Non-responders



Pharmacogenetics

Side effects



Definitions



Pharmacogenomics

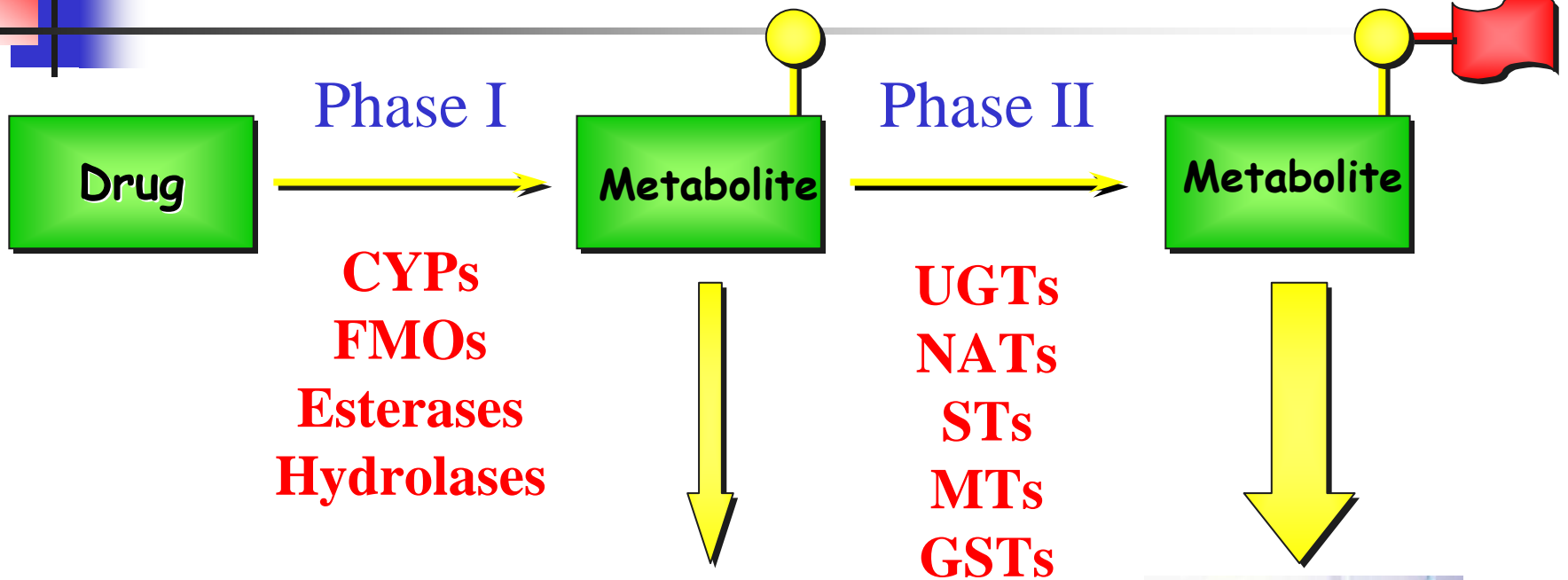
Characterization of the genome-wide response to small molecular molecules administered with therapeutic intent

“The right drug for the right disease”

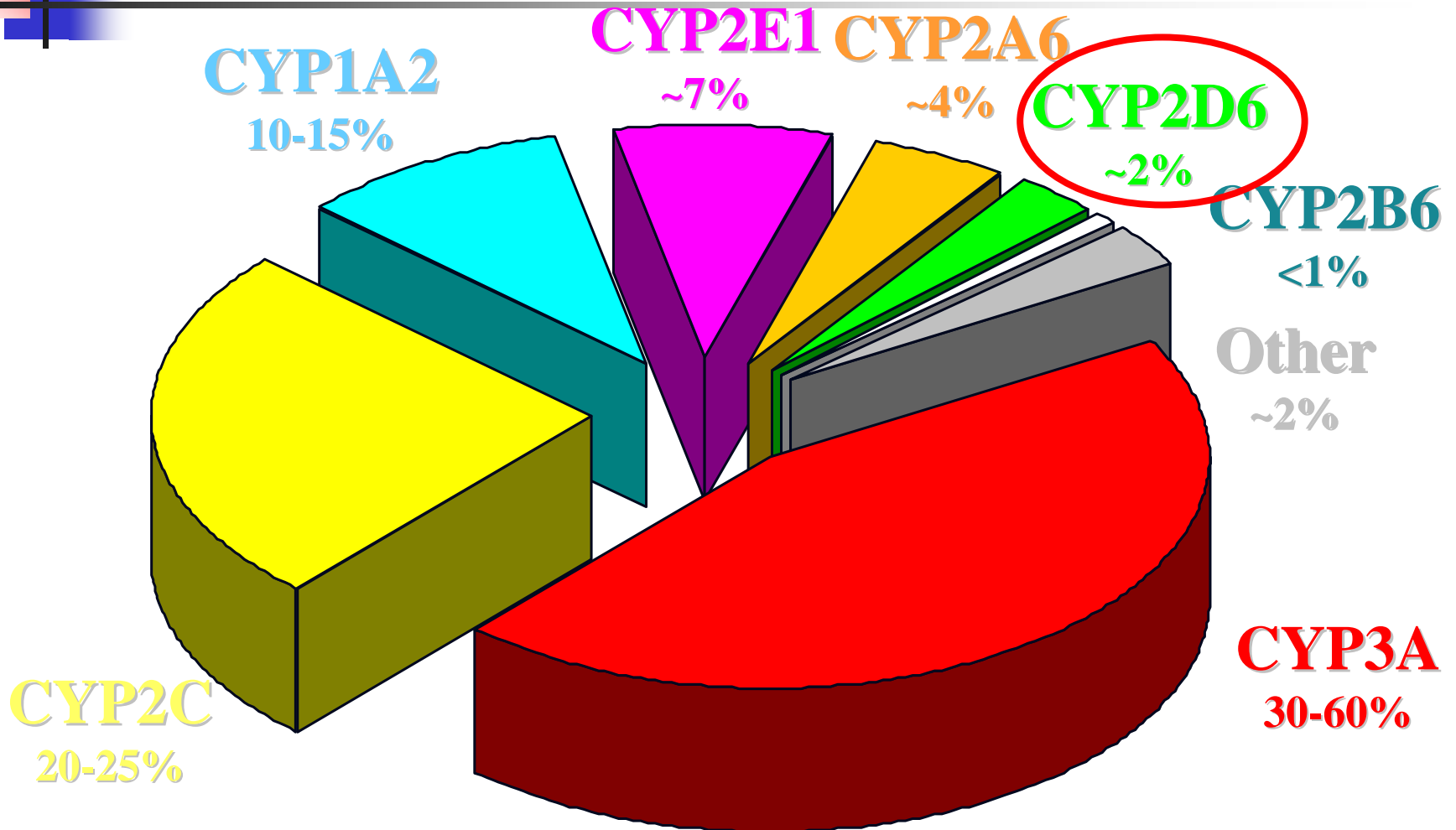
Study of how interacting systems of genes determine individual drug responses*

* Klein TE *et al. Pharmacogenomics J* (2001) 1:167-170

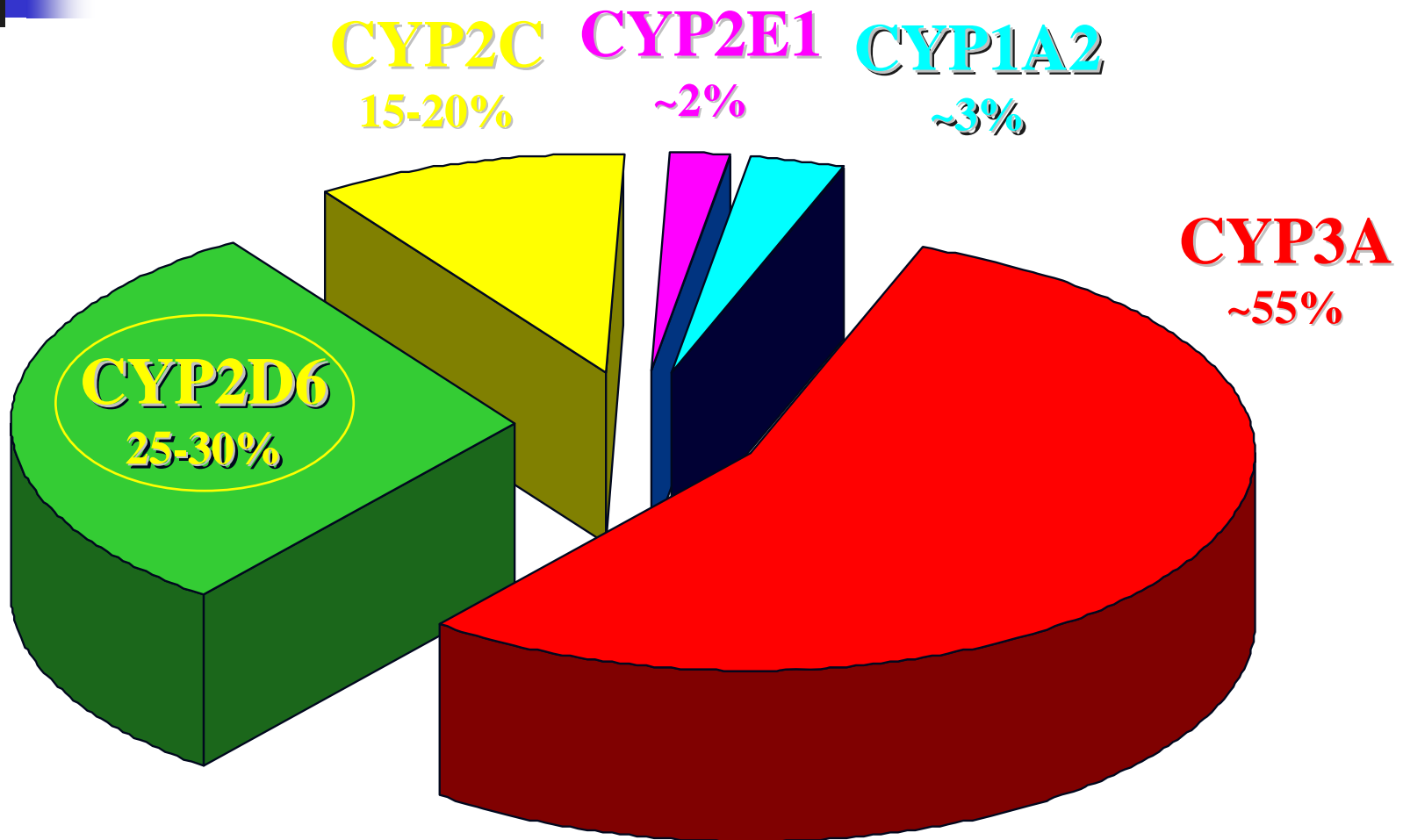
Drug Biotransformation



Relative CYP Composition of Adult Liver



Relative Contribution of CYP Isoforms to Drug Biotransformation





Medications Dependent Upon CYP2D6 for Metabolism

- ❖ Paroxetine
- ❖ Fluoxetine
- ❖ Imipramine
- ❖ Nortripyline
- ❖ Clozapine
- ❖ Risperidone
- ❖ Haloperidol
- ❖ Codeine
- ❖ Tramadol
- ❖ Dextromethorphan
- ❖ Perphenazine
- ❖ Atomoxetine
- ❖ *S*-metoprolol
- ❖ Propafenone
- ❖ Timolol
- ❖ Encainide
- ❖ Flecainide
- ❖ Mexilitene



CYP2D6 Pharmacogenetics

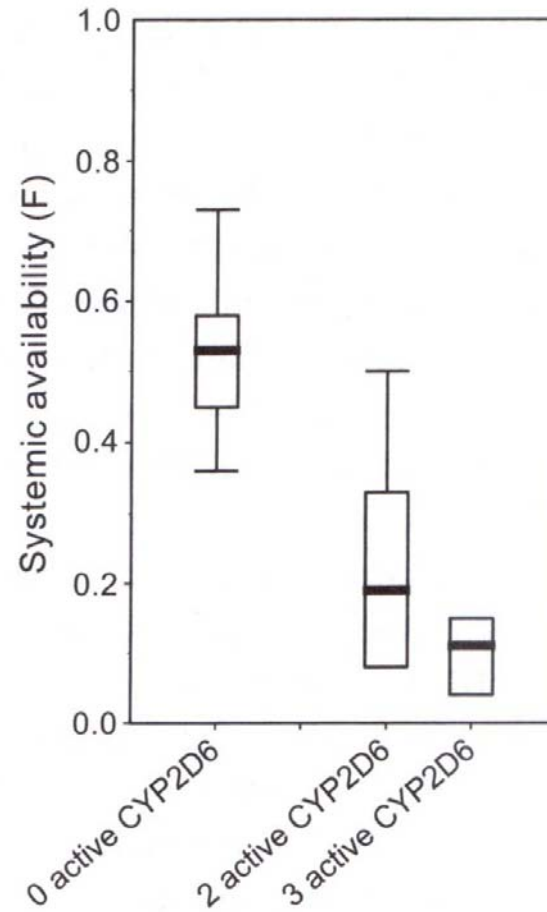
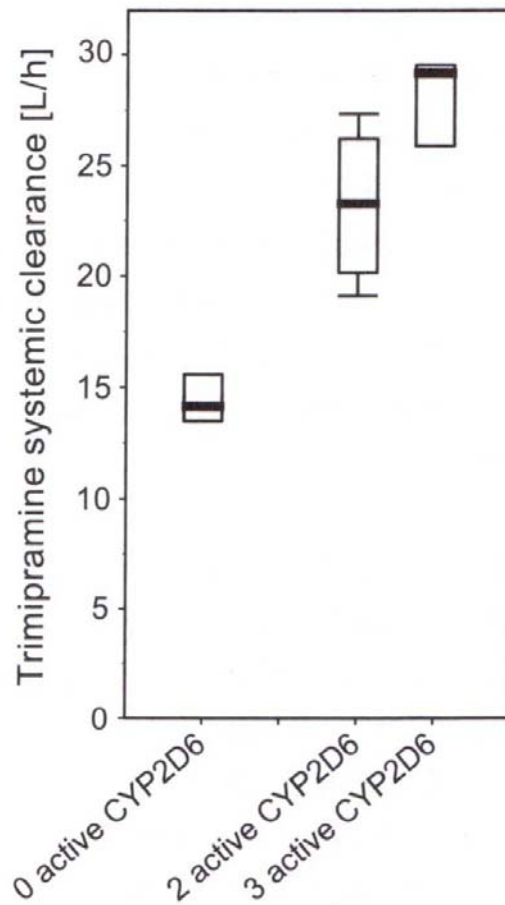
- ❖ CYP2D6 activity displays bimodal distribution in Caucasian subjects
- ❖ 5-10% of Caucasian population are deficient in CYP2D6 activity
- ❖ “Poor metabolizers” or “PMs” have two “inactive” forms (alleles) of the CYP2D6 gene
- ❖ PMs are at increased risk for concentration-dependent side effects with “normal” drug doses
- ❖ Some drugs may not work (codeine; tramadol)

CYP2D6 Pharmacogenetics



“Functional” overdose

CYP2D6 Pharmacogenetics: Impact on Trimipramine PK



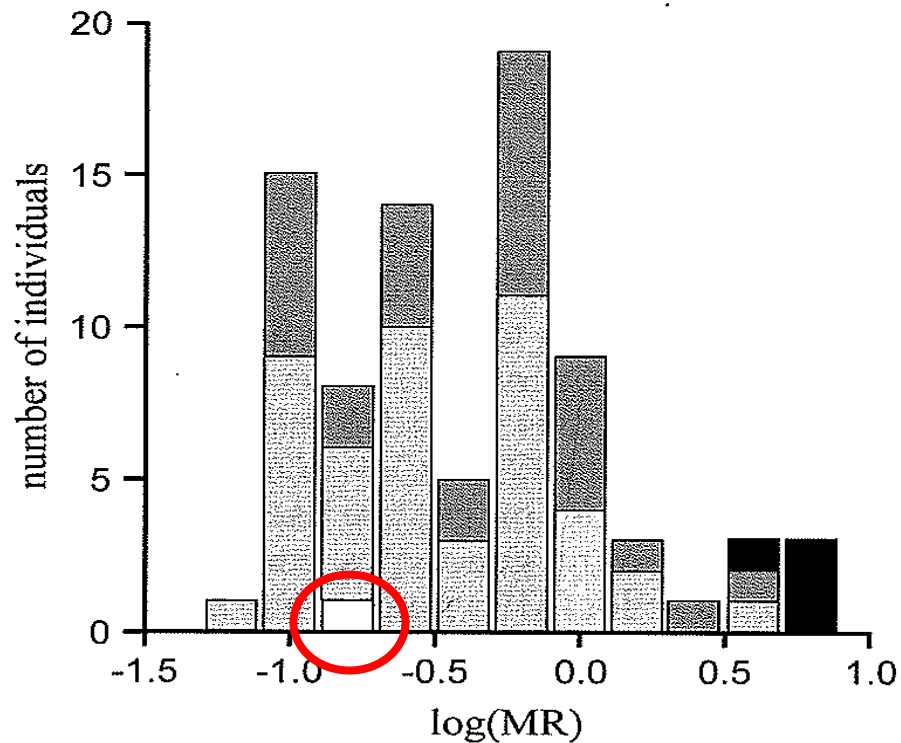


FIGURE 3. Histogram of the distribution of the $\log(\text{RISP}/9\text{-OH-RISP})$ [$\log(\text{MR})$] for 81 individuals treated with risperidone. White bar corresponds to an individual with *CYP2D6* gene multiplication, light gray bars correspond to individuals with 2 functional *CYP2D6* alleles, dark gray bars to individuals with 1 functional *CYP2D6* allele, and black bars to individuals without functional *CYP2D6* alleles.



Ethnic Differences in CYP2D6 Activity

Caucasian PMs: Bimodal distribution

- *3 "null" allele (no activity)
- *4 "null" allele (no activity)
- *5 "null" allele (no activity)
- *6 "null" allele (no activity)

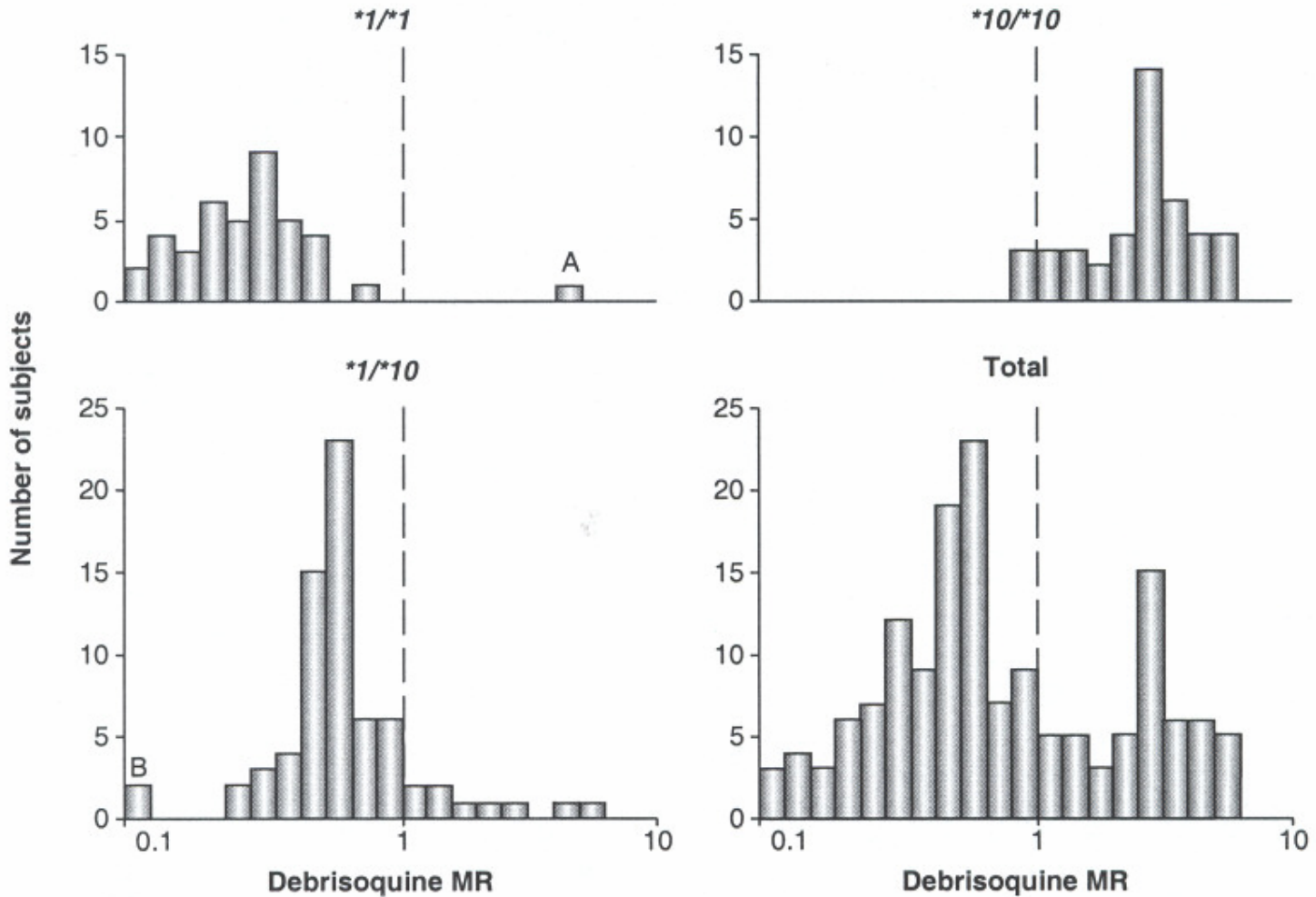
Asians: Lower population activity

- *10 "partial activity"

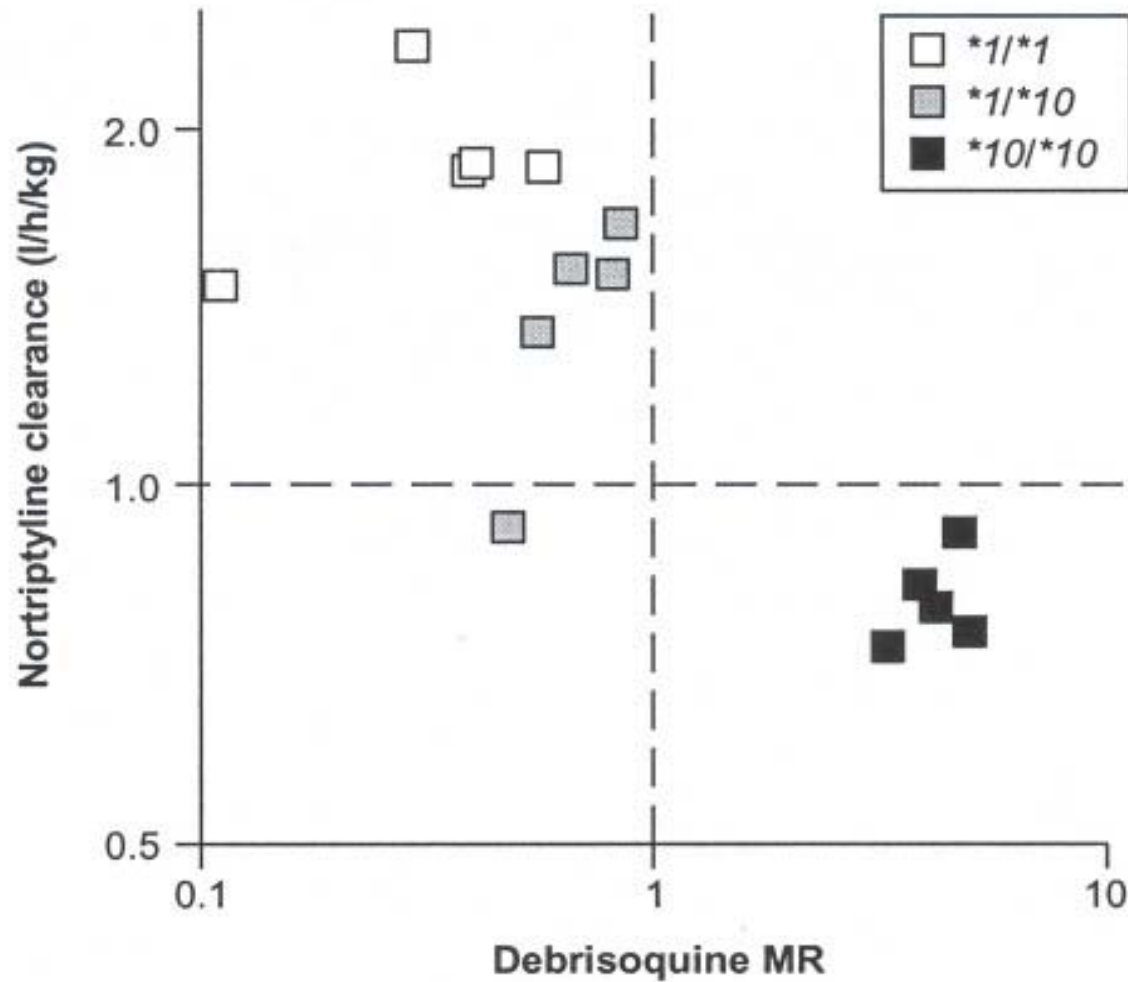
Africans: Lower population activity

- *17 "partial activity"
- *29 "reduced activity"

Impact of CYP2D6*10 on enzyme activity



Impact of CYP2D*10 on Nortriptyline Disposition



Population Histograms

Caucasian n = 204

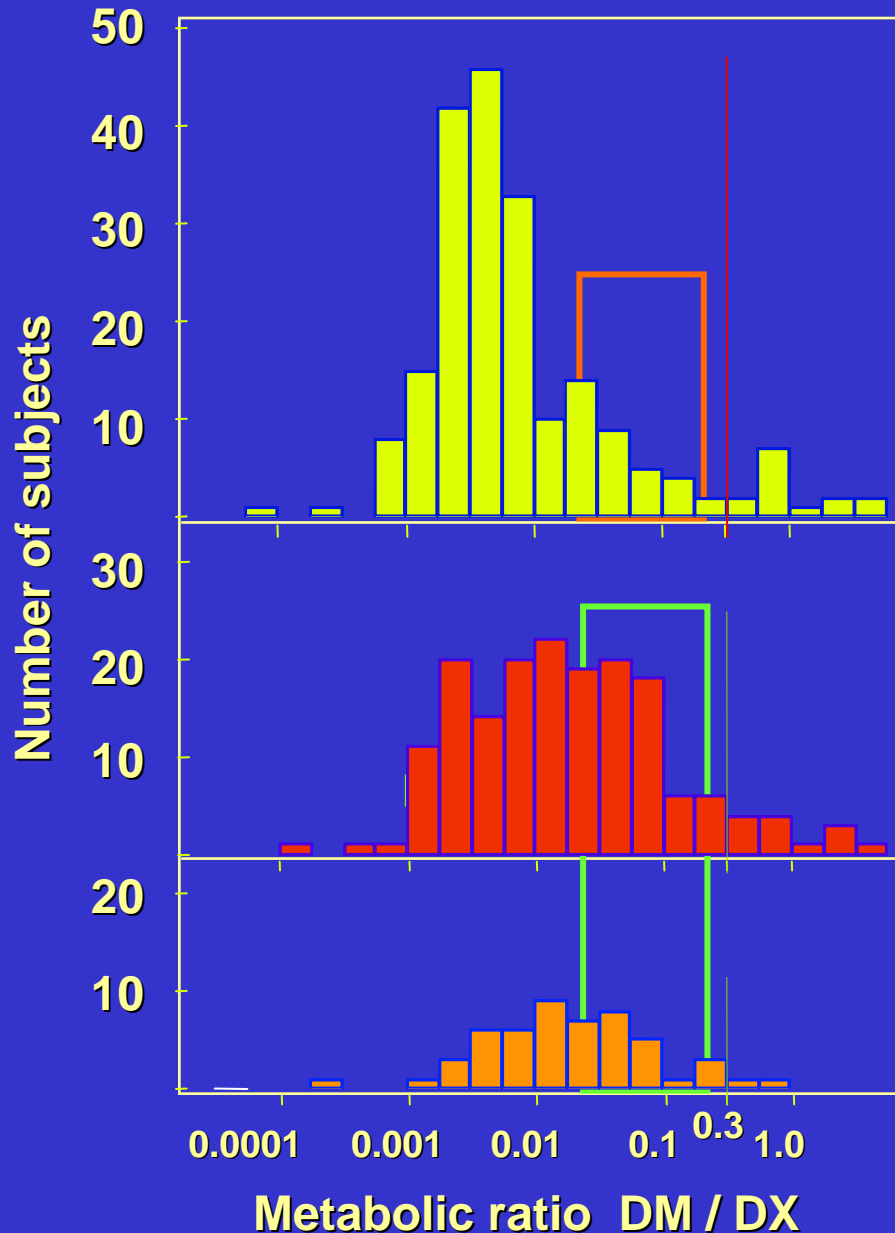
Median MR = 0.0044

AA group 1 n = 173

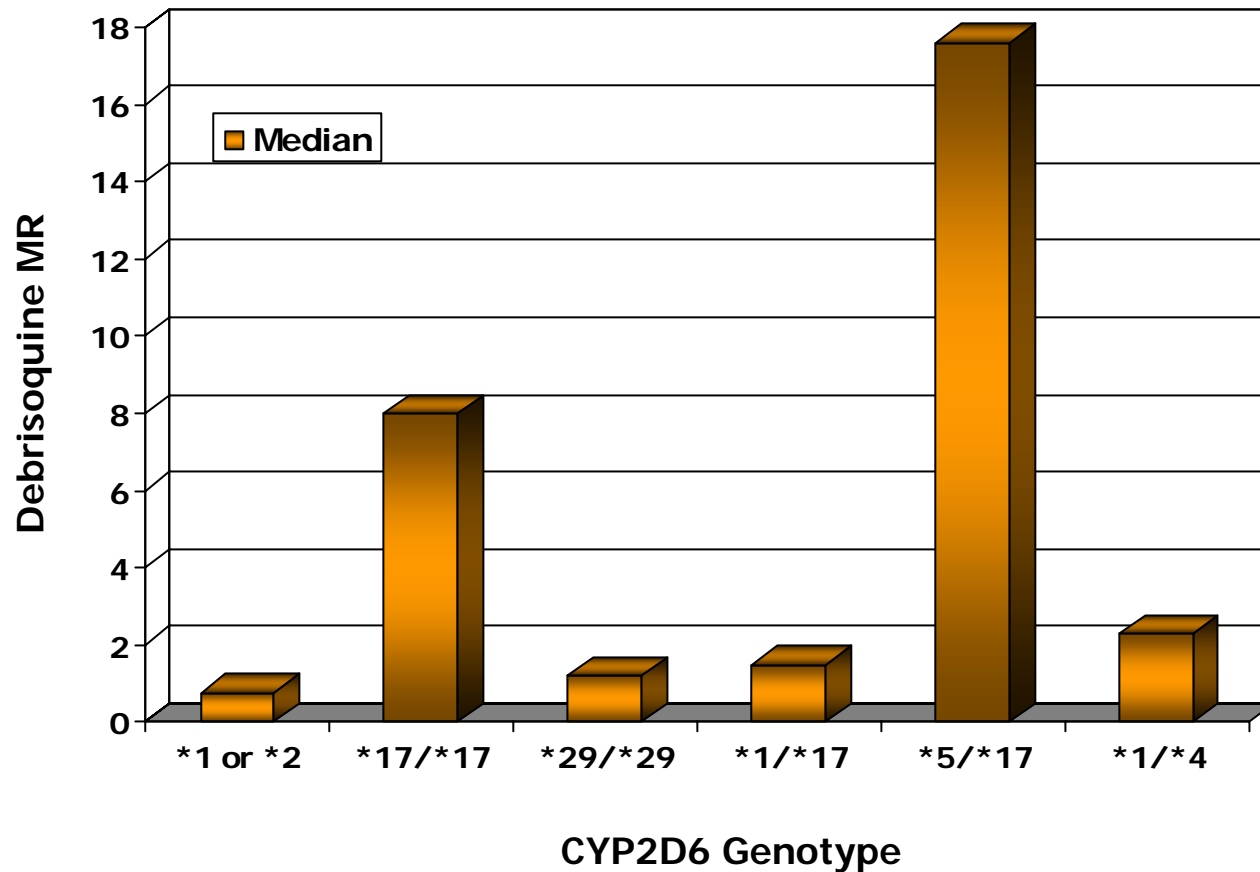
Median MR = 0.016

AA group 2 n = 52

Median MR = 0.0164



Impact of CYP2D6*17 allele on enzyme activity in Tanzanians



Grading of CYP2D6 genotypes traditionally associated with the extensive metabolizer phenotype

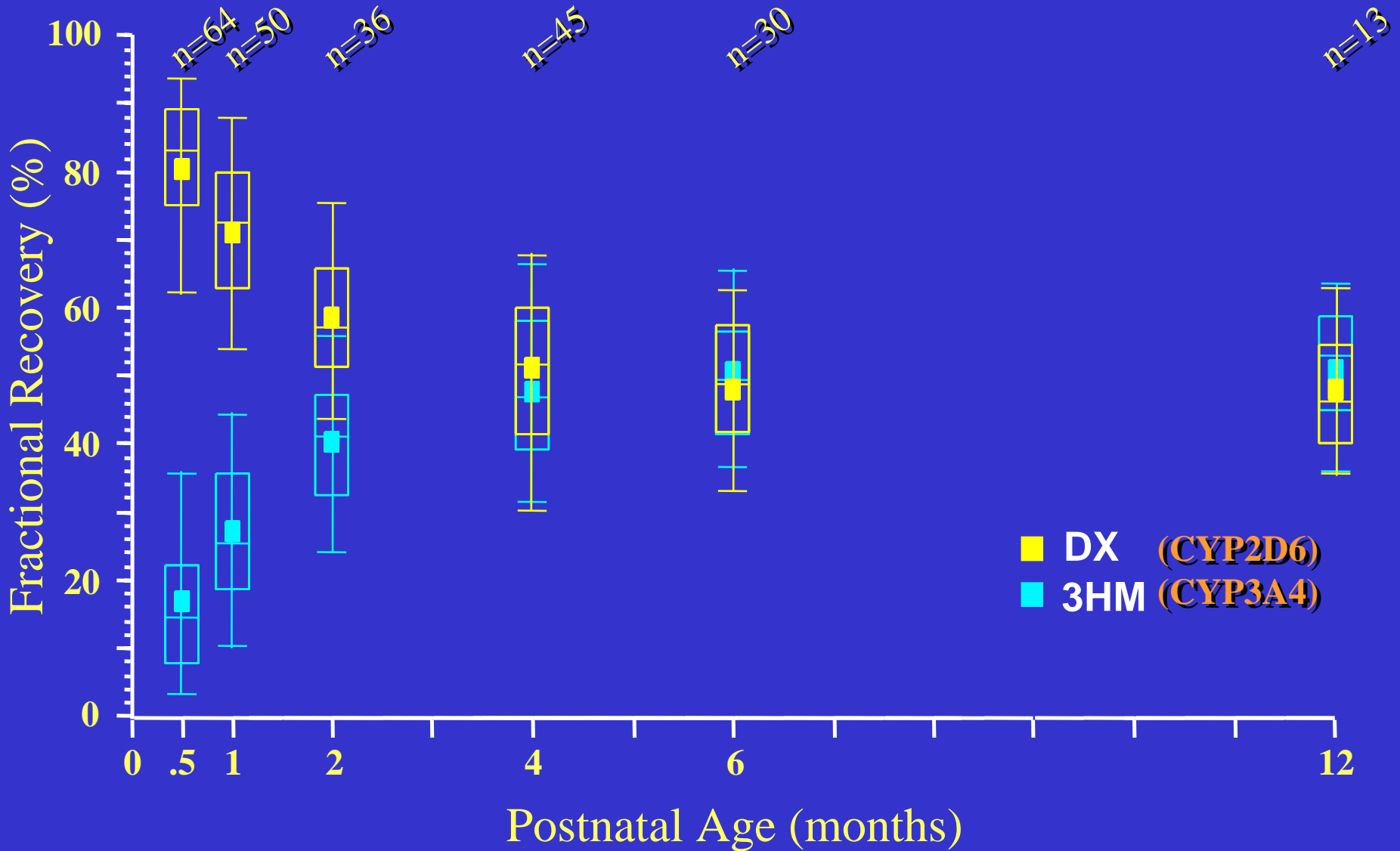
(Valdes R, Jr., Linder MW. *Clin Chem* 2004;50:1498-99)

Genotypes associated with EM phenotype	Representative genotype	Measured functional gene dose*	Semiquantitative gene dose
Active / Active	*1/*1	Reference point	2.0
Active / Intermediate	*1/*41	1.43	1.5
Active / Inactive	*1/*4	1.02	1.0
Intermediate / Inactive	*41/*4	0.73	0.5

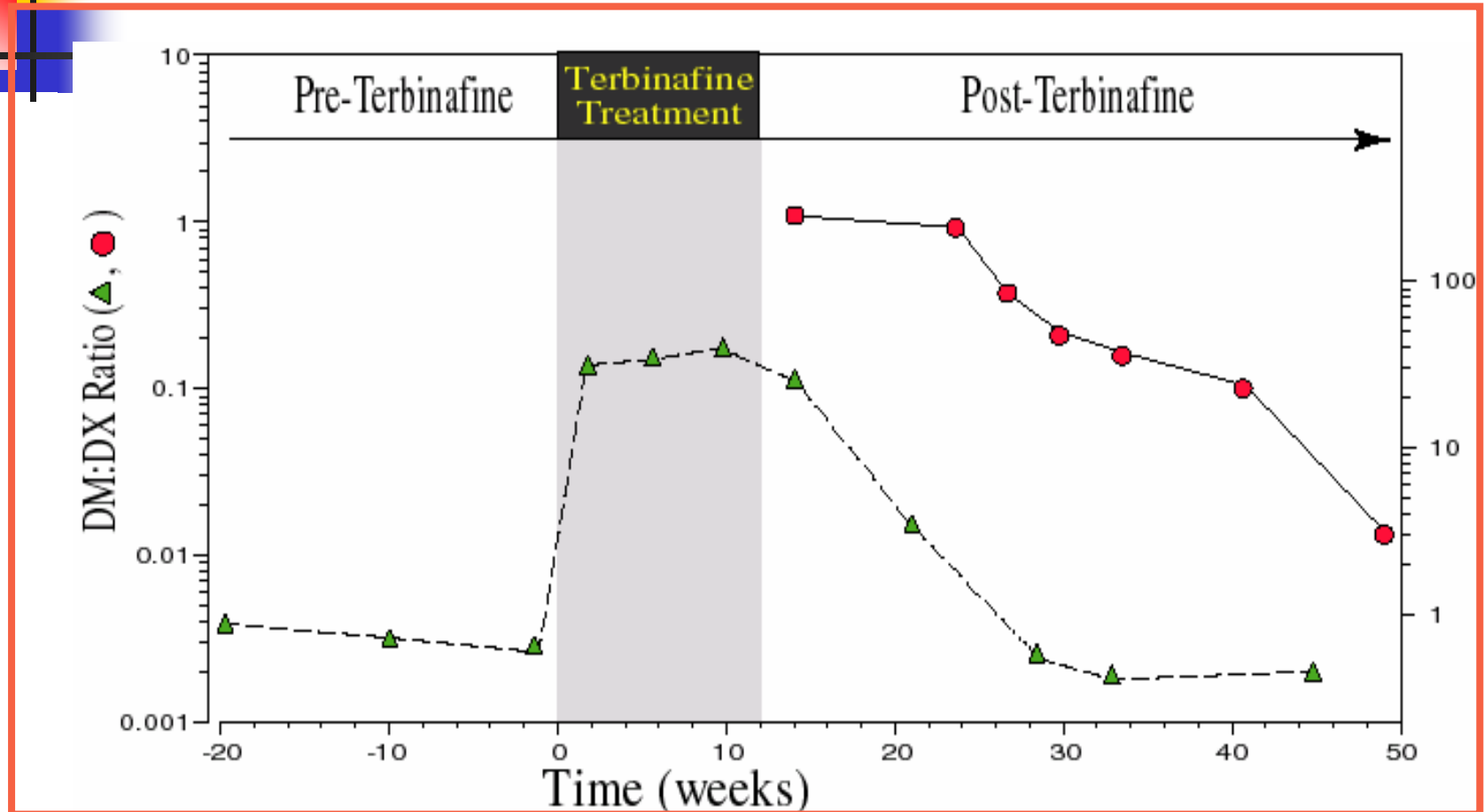
*Determined from relative change in steady state plasma concentrations attributed to a given allele (eg., Cp ~ 63% higher with CYP2D6*10 allele vs. *1 or *2, therefore $*1/*10 = 2 / (1+0.63) = 1.23$)

Fractional Recovery of Major DM Metabolites

(Leeder JS, et al., 2002)



Terbinafine - *in vivo*



Abdel-Rahman *et al.* 1999 DMD 27, 770

Abdel-Rahman *et al.* 1999 CPT 65, 465



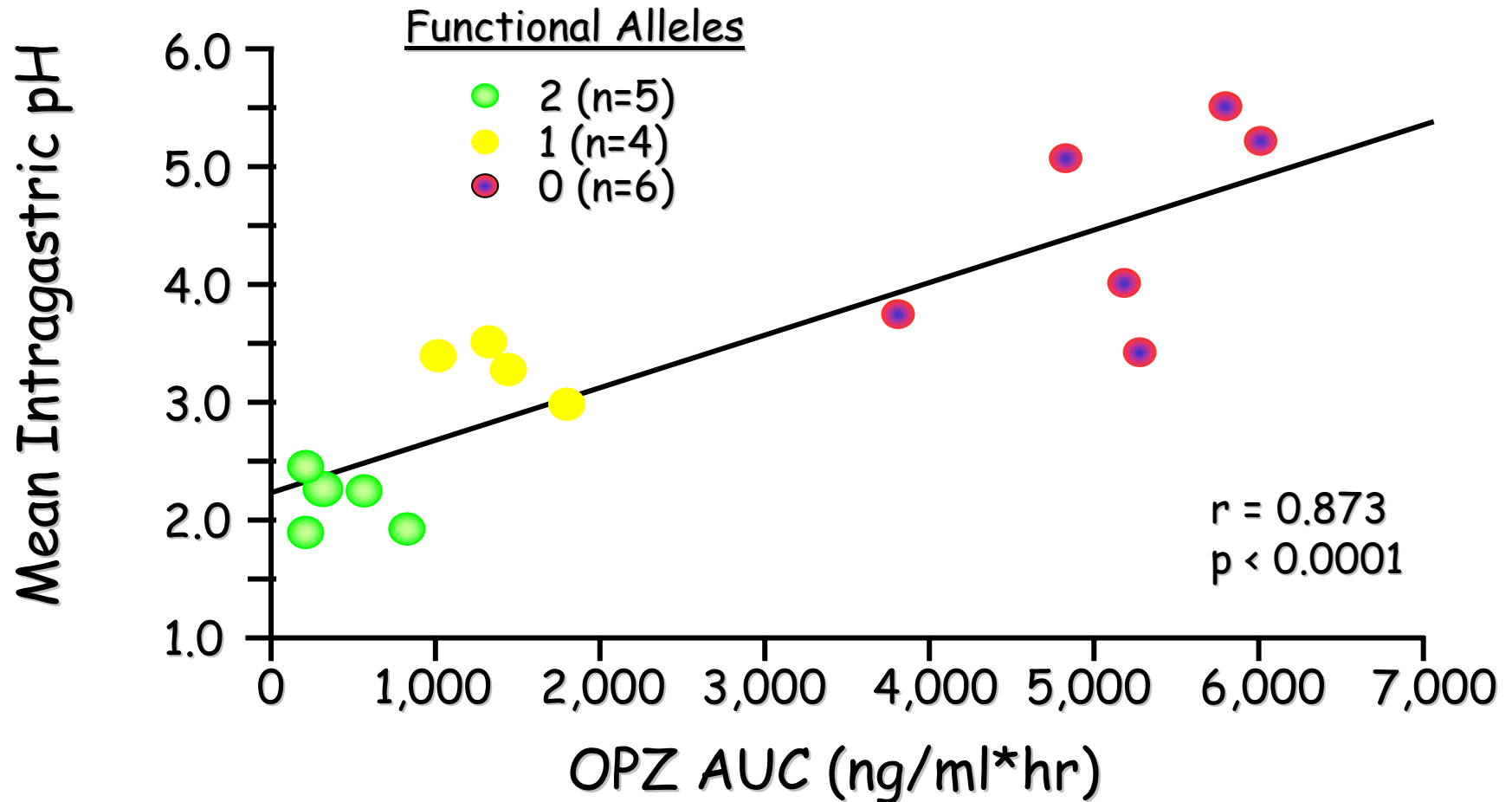
CYP2C19 Pharmacogenetics

- 1984: Unusual sedation in a subject receiving anticonvulsant mephenytion
- Impaired 4-hydroxylation of *S*-mephenytoin
- Affects 2-5% of Caucasians; 20-25% of Asians
- Affected drugs include the PPIs omeprazole, lansoprazole, pantoprazole, diazepam
- Major clinical consequence at present related to PPI pharmacodynamics and efficacy

CYP2C19 Pharmacogenetics

(Sagar M, et al. Gastroenterology 2000;119:670-676)

Omeprazole PK (After a Single 20 mg Oral Dose)



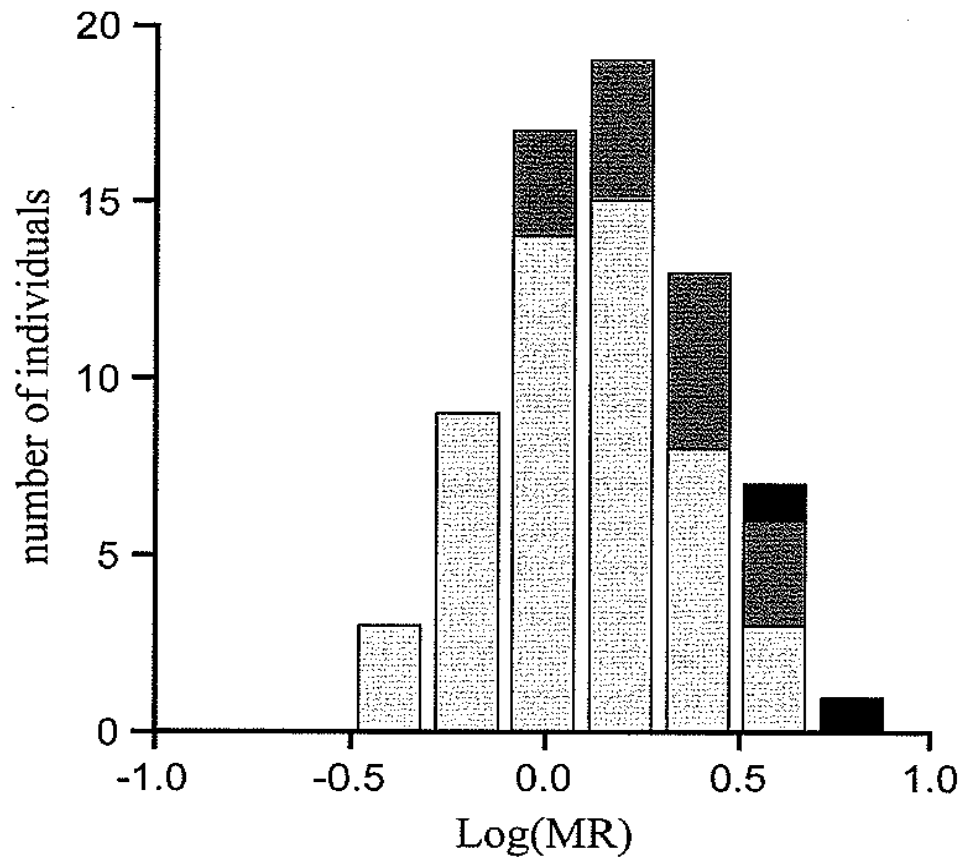


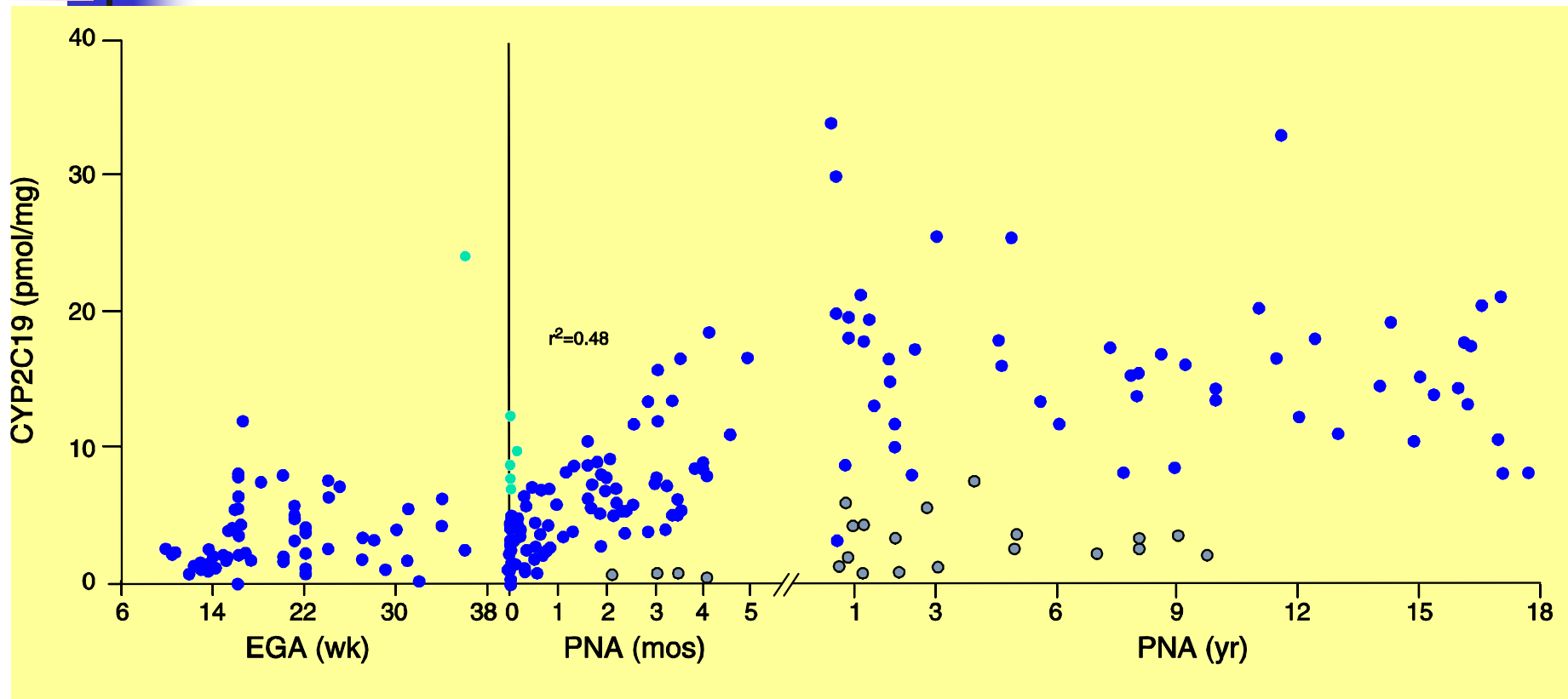
FIGURE 2. Histogram of the distribution of the $\log(\text{AMI}/\text{NORT})$ [$\log(\text{MR})$] for 69 individuals treated with amitriptyline. Light gray bars correspond to individuals with 2 functional *CYP2C19* alleles, dark gray bars to individuals with 1 functional *CYP2C19* allele, and black bars to individuals without functional *CYP2C19* alleles.

CYP2C19 Genotype Frequencies

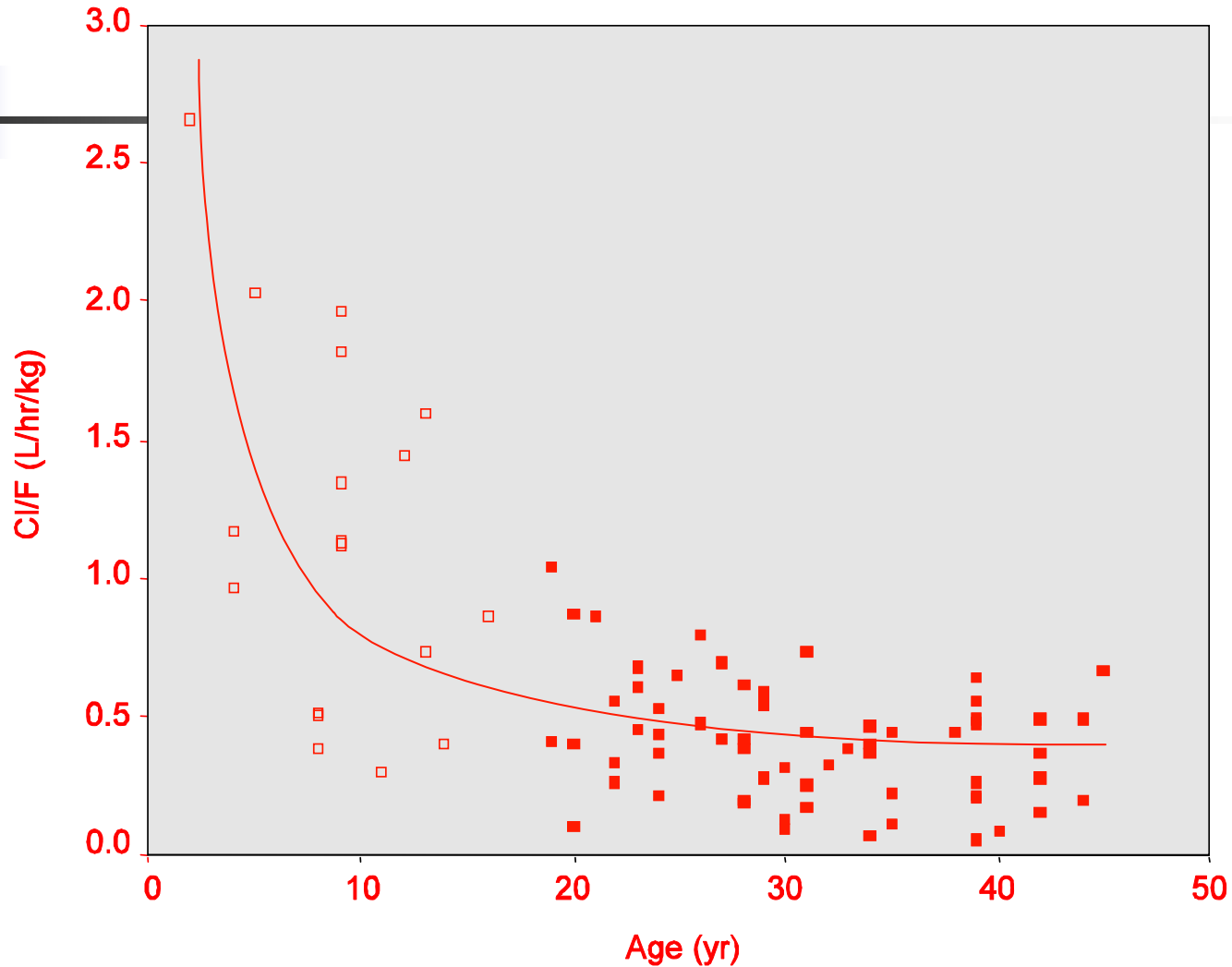
Table 3. CYP2C19 genotype frequencies by population.

Population	Genotype (%)			Sum
	*2/*2	*2/*3	*3/*3	
East Asian				
Chinese (Han)	13.42	2.72	0.55	16.6
Filipinos	15.21	2.73	0.49	18.4
Japanese	8.27	3.56	1.53	13.3
Koreans	4.36	2.43	1.36	8.15
Thais	7.09	0.62	0.05	7.77
Caucasian				
Swedish	1.2	0	0	1.2
Swedish	3.8	0	0	3.8
Danish	3.8	0	0	3.8
Portuguese	1.3	0	0	1.3
Australian	3.0	0	0	3.0

Developmental Alterations in CYP2C19 Expression

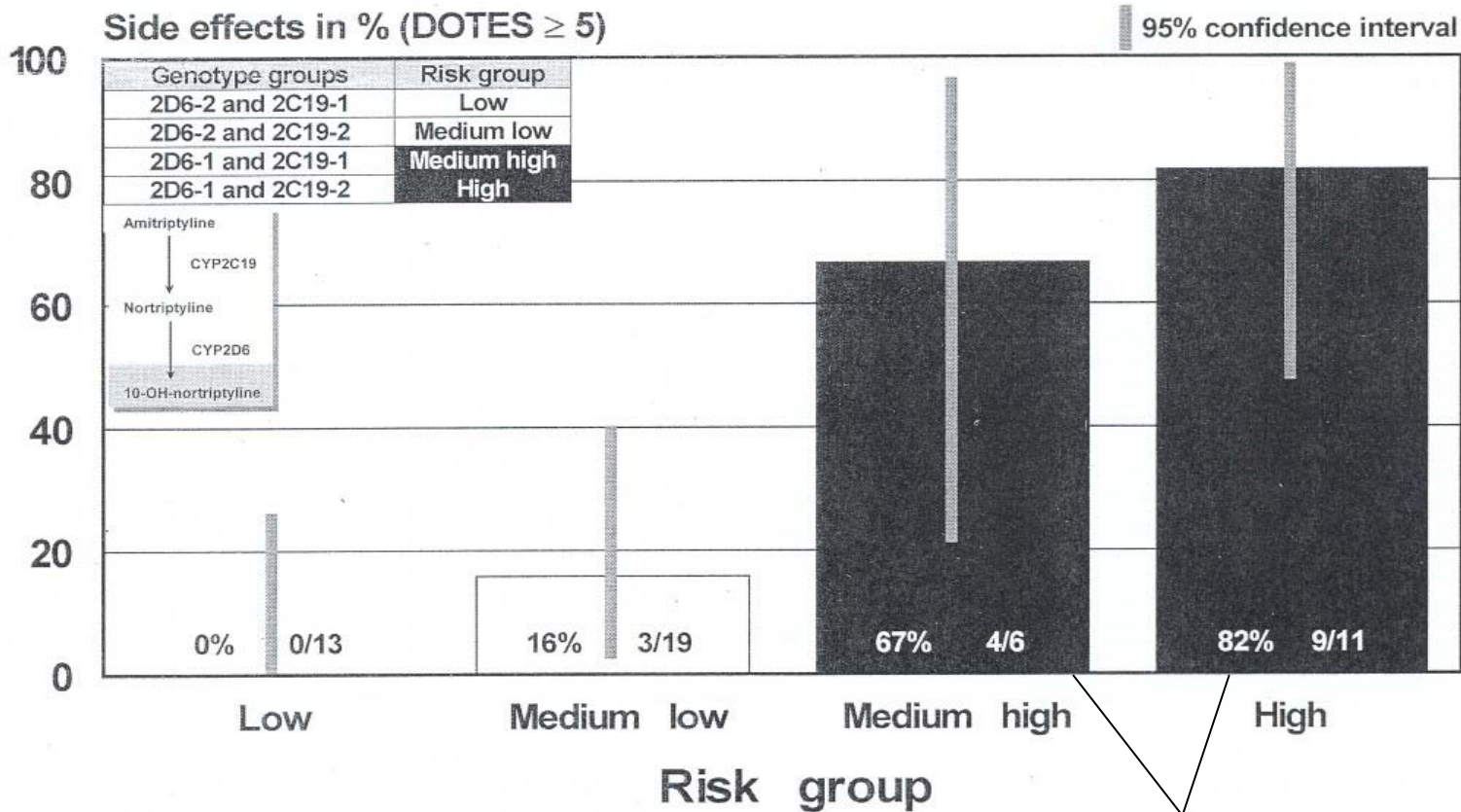


Age Effect on Omeprazole Oral Clearance



From Kearns GL, et al. *J Clin Pharmacol* 2003;43:840-48 & data on file, Santarus, Corp.

Risk of side effects from amitriptyline in relation to the CYP2D6 and CYP2C19 genotype



1 active CYP2D6 allele



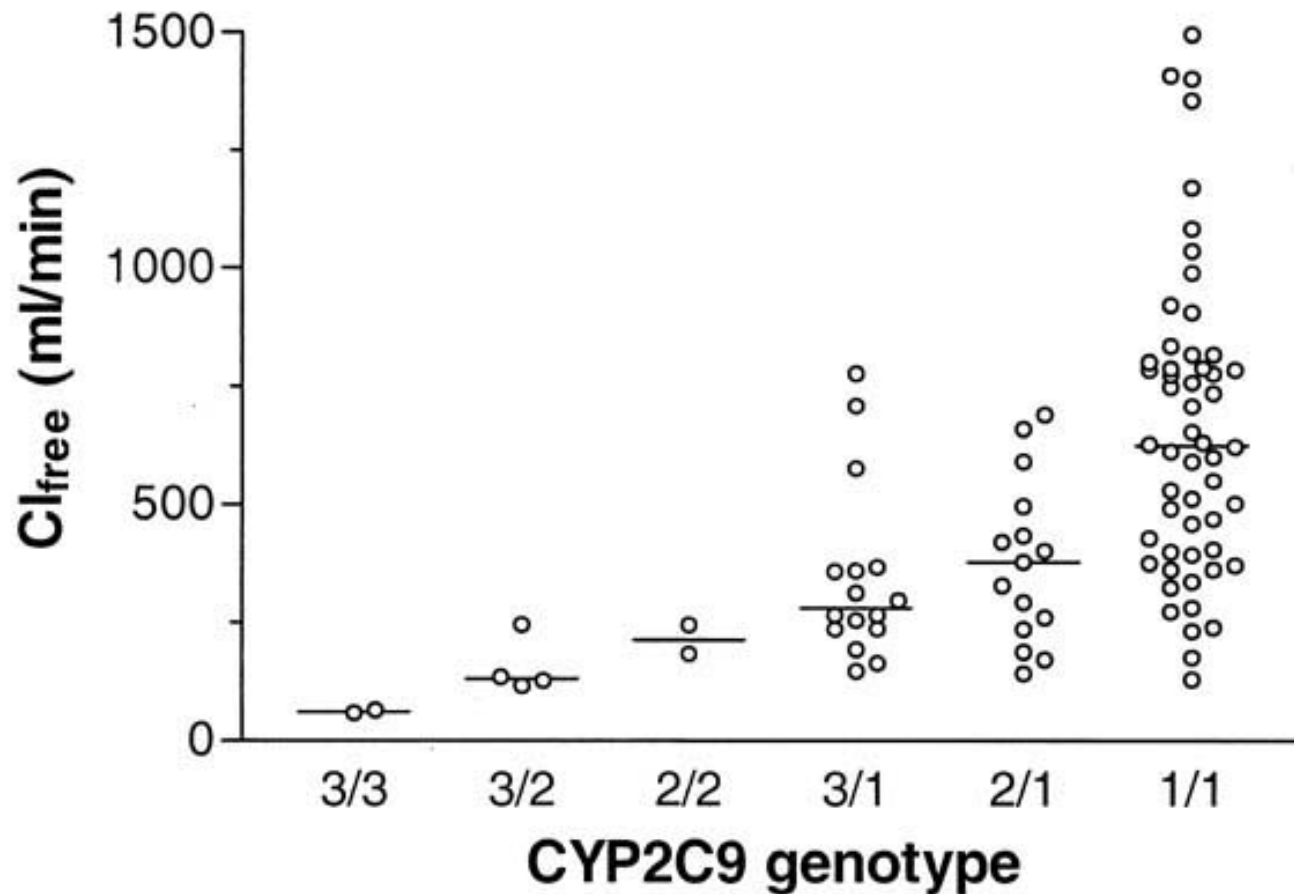
CYP2C9 Pharmacogenetics

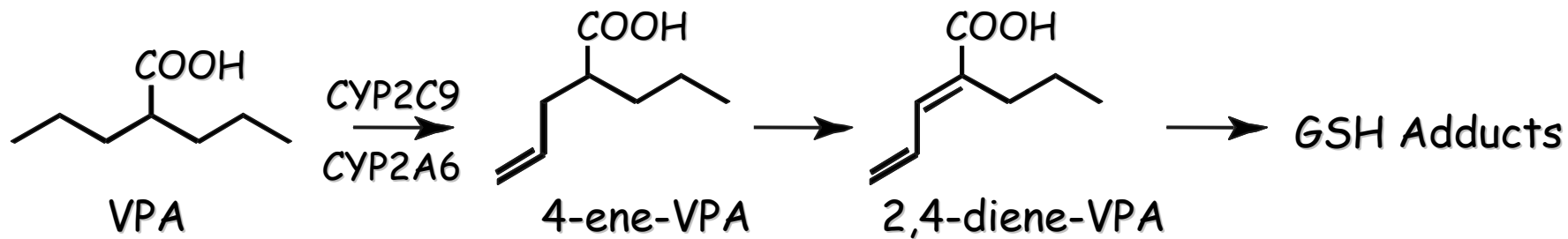
Several allelic variants:

CYP2C9*1	"wild-type"	
CYP2C9*2	Arg144Cys	18% activity
CYP2C9*3	Ile359Leu	5% activity
CYP2C9*4	Ile359Thr	
CYP2C9*5	Asp360Glu	10% activity
CYP2C9*6	Frameshift	0% activity

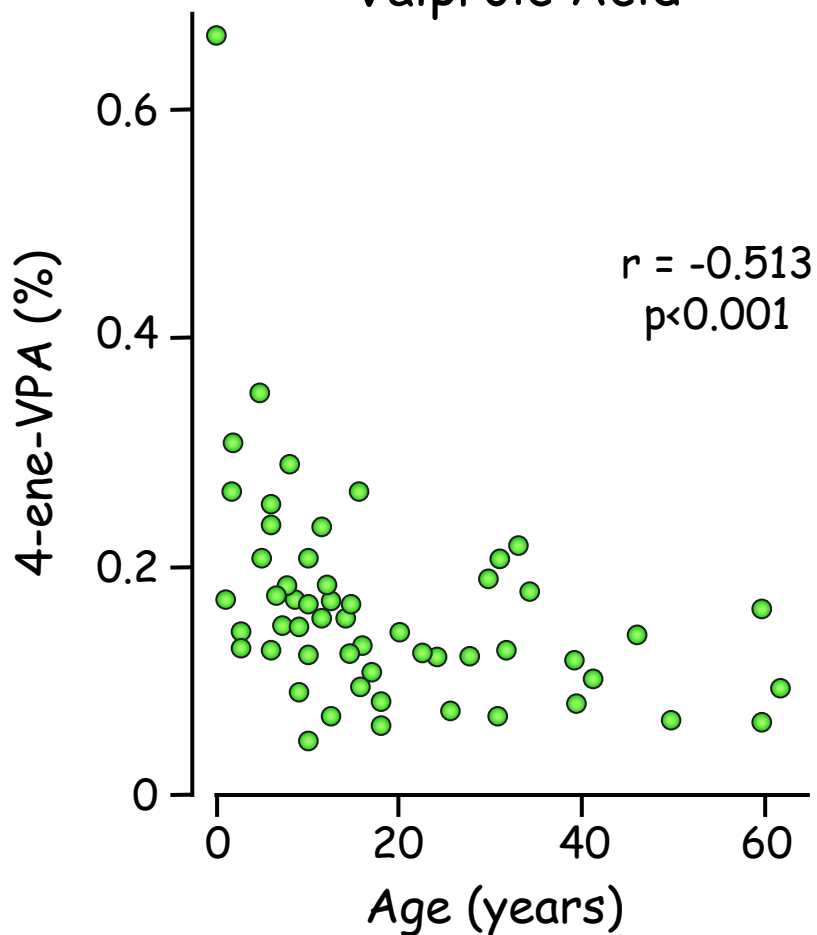
CYP2C9 Pharmacogenetics

S-warfarin

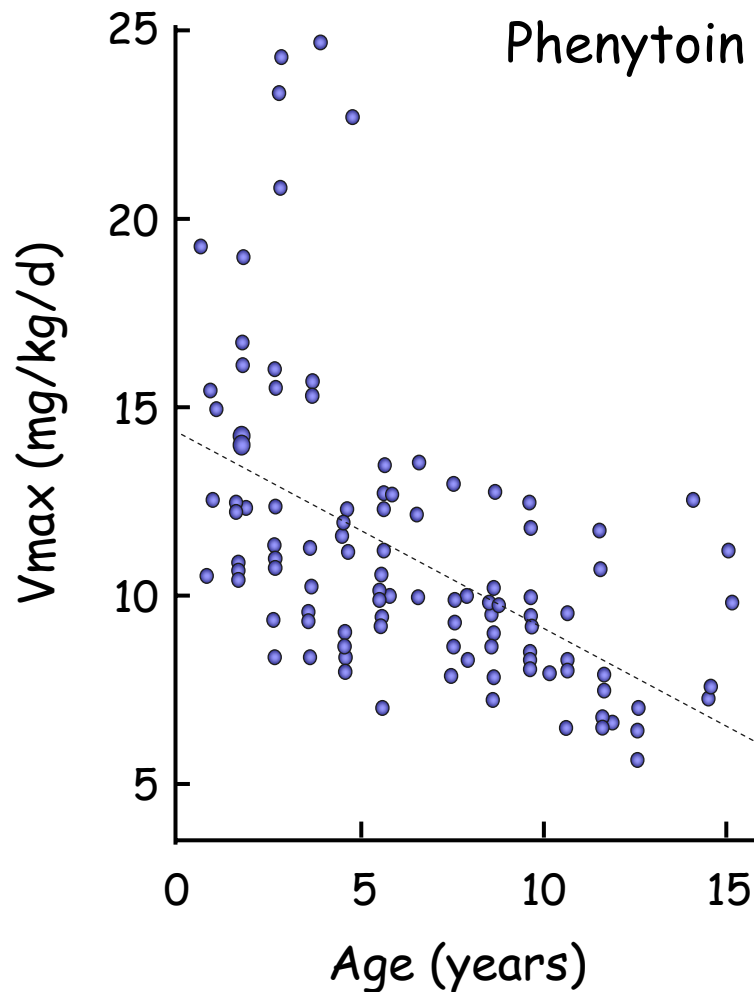




Valproic Acid



Phenytoin





Pharmacogenetics of Receptors

- Allelic variants of receptors affect ligand binding or signal transduction; promoter region variants affect level of expression
- Major investigation has focused on associations with disease *i.e.* obesity and eating disorders, ADD and ADHD, obsessive-compulsive disorders, drug dependence and addiction, schizophrenia, asthma
- Polymorphisms identified in opiate receptors (μ and δ), serotonin receptors, dopamine receptors, β -adrenergic receptors *etc.*

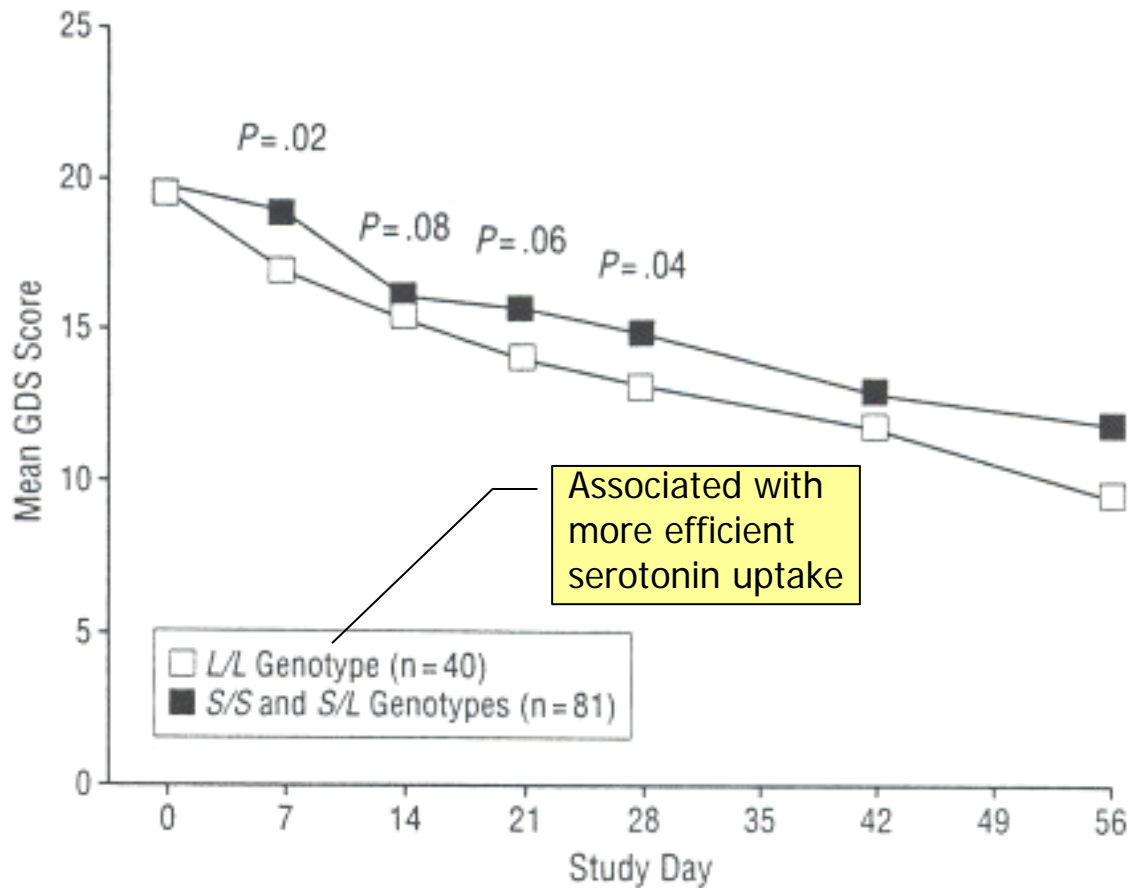
Lack of association between serotonin receptor (5-HT_{2A} T102C) polymorphism and clozapine response

TABLE 1. Studies of the Association Between the Serotonin 5-HT_{2A} T102C Polymorphism and Response to Antipsychotic Drugs

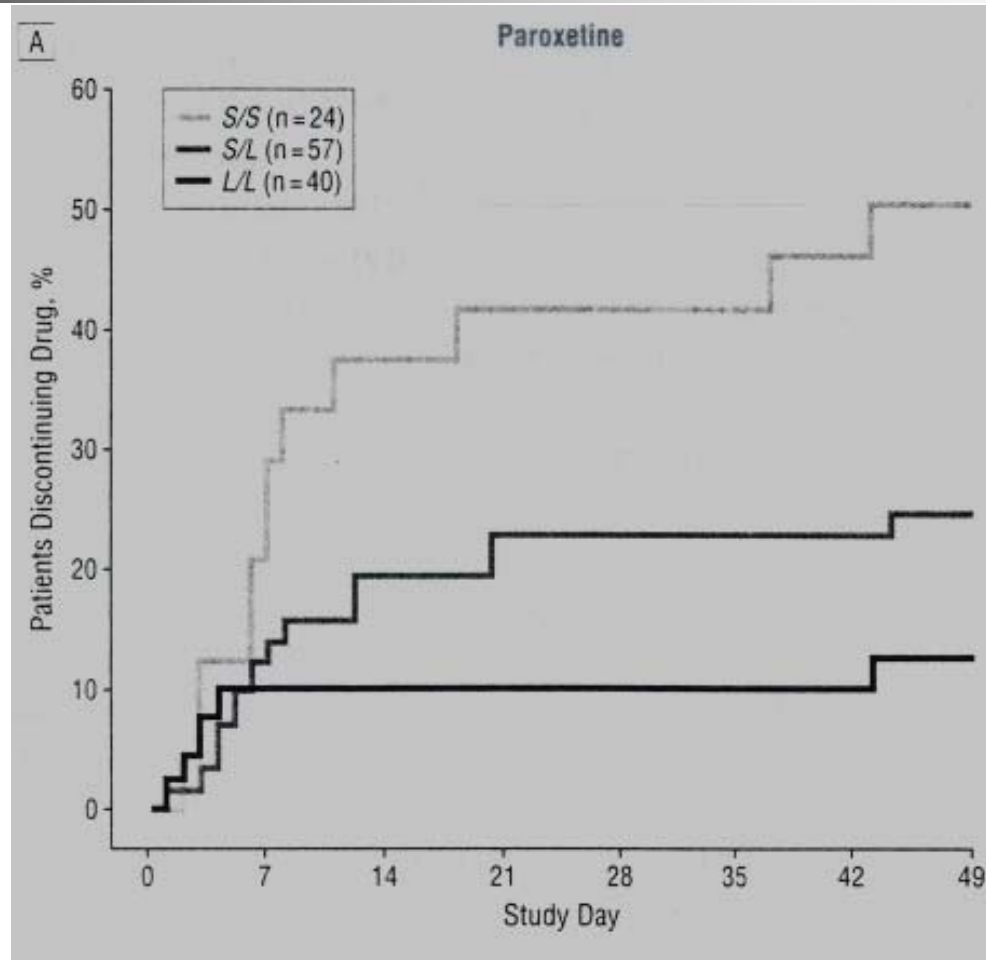
Study	Year	Subjects	Medication	Design	Outcome Measures	Result
Arranz et al. (31)	1995	149 west European patients with schizophrenia	Clozapine	Retrospective assessment of 12 weeks of treatment	Global Assessment Scale	C102/C102 genotype more frequent in nonresponders (53%) than responders (26%)
Masellis et al. (40)	1995	126 U.S. patients with schizophrenia	Clozapine	6-month trial after washout	Brief Psychiatric Rating Scale (BPRS)	No association
Nothen et al. (35)	1995	146 German patients with schizophrenia	Clozapine	Retrospective assessment after at least 28 days of treatment	Categorical assessment of response	No association
Malhotra et al. (29)	1996	70 U.S. patients with schizophrenia or schizoaffective disorder	Clozapine	10-week trial	BPRS	No association
Masellis et al. (37)	1998	185 U.S. patients with schizophrenia	Clozapine	6-month trial after washout	BPRS at 6 months plus Clinical Global Impression scale	No association
Lin et al. (36)	1999	97 Chinese patients with schizophrenia	Clozapine	8 weeks of treatment	BPRS	No association
Lane et al. (39)	2002	100 Chinese patients with schizophrenia	Risperidone	6 weeks of treatment after washout	Positive and Negative Syndrome Scale	C102/C102 genotype associated with better response

Effects of the “long/short” polymorphism in the serotonin transporter gene (*SLC6A4*) on paroxetine response in depression

B

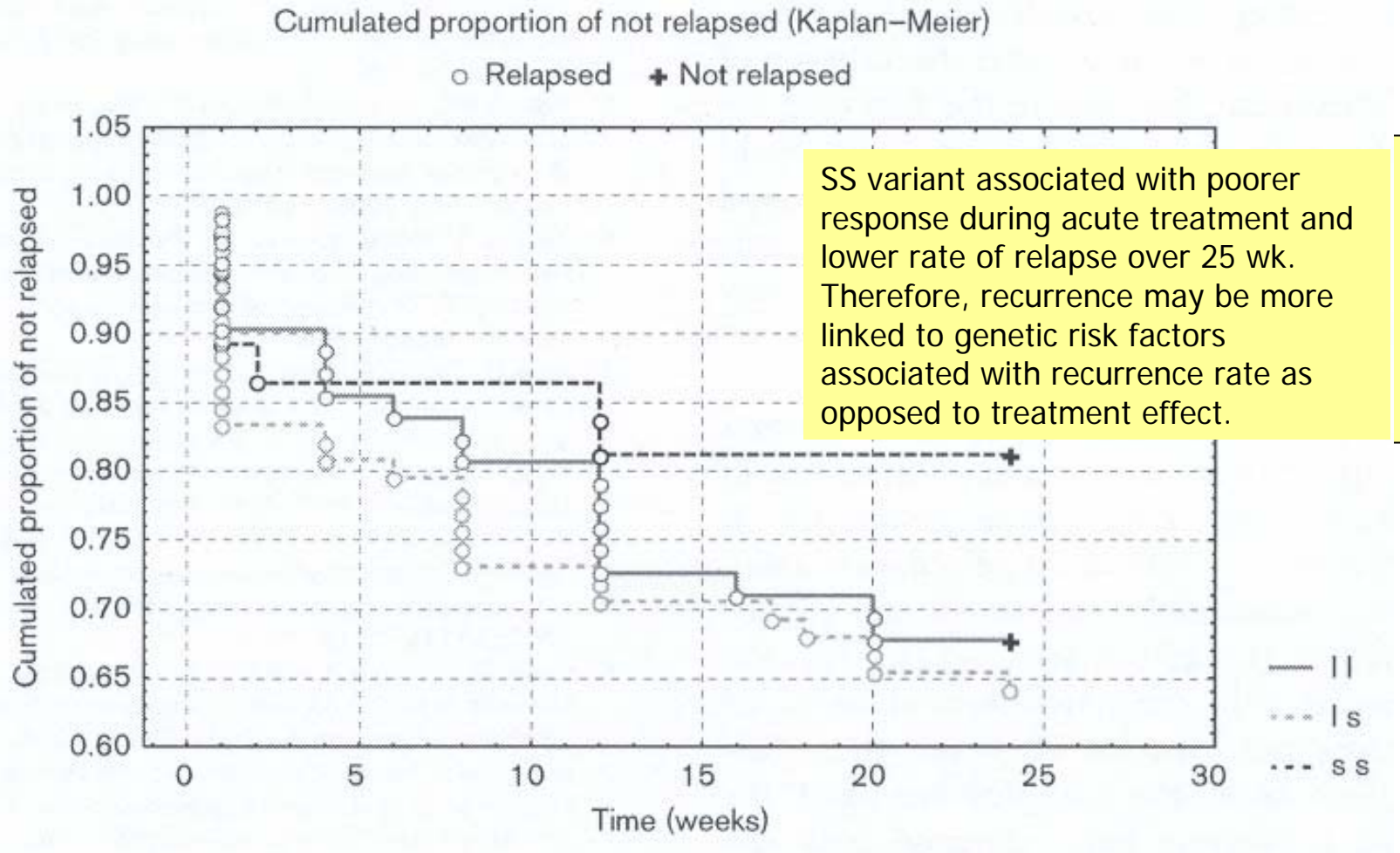


Survival curves showing paroxetine discontinuations due to adverse events in association with 5HTTLPR genotype in geriatric patients with depression



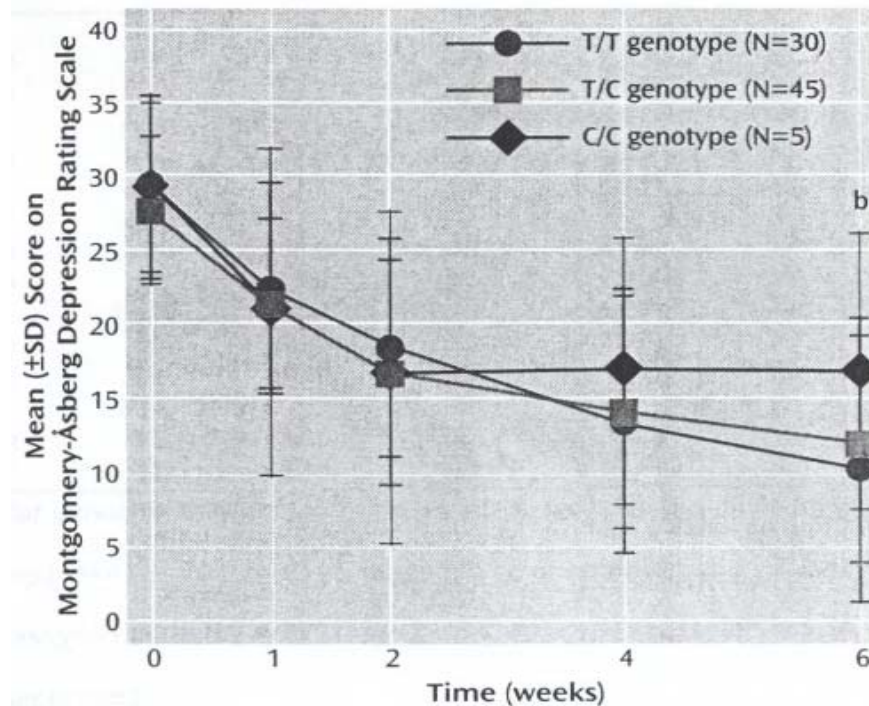
Murphy GM, et al. *Arch Gen Psychiatry* 2004;61:1163-1169

Functional polymorphisms in the serotonin transporter (SERTPR) gene: Impact on outcome of SSRI treatment



Effect of Genetic Polymorphism on Norepinephrine Transporters (NET) in Depression Treatment with Milnacipran

FIGURE 1. Montgomery-Åsberg Depression Rating Scale Scores During 6 Weeks of Treatment With Milnacipran in Three NET T-182C Genotype Groups^a



Association of SCN1A genotype with carbamazepine dose in epilepsy

Demonstrates significance of alternative splicing (IVS5-91 G>A) in the human sodium channel

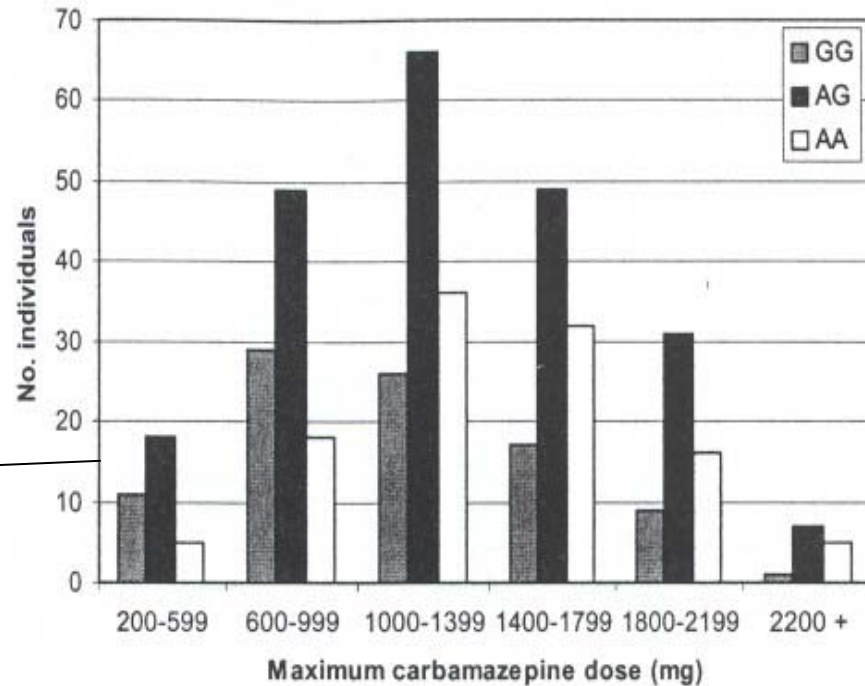


Fig. 3. Distribution of maximum carbamazepine doses for each *SCN1A* IVS5-91 G>A genotype.



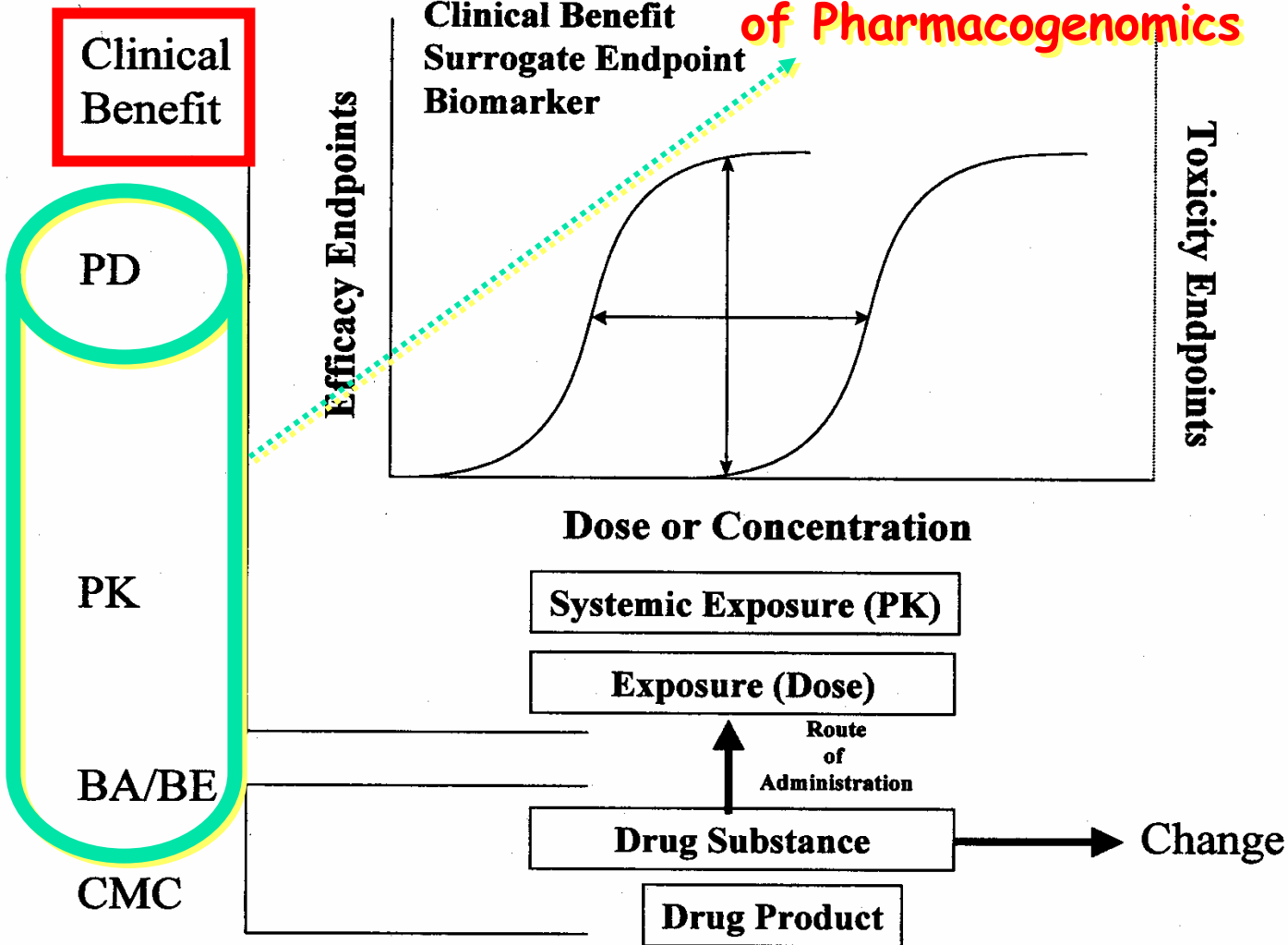
Relatively Little Known About ...

- Ontogeny of receptor systems
 - Serotonergic receptors and re-uptake pumps
 - Dopaminergic receptors and re-uptake pumps
- Ontogeny of other drug transporters
- Ontogeny of signal transduction systems
- Ontogeny of ion channels

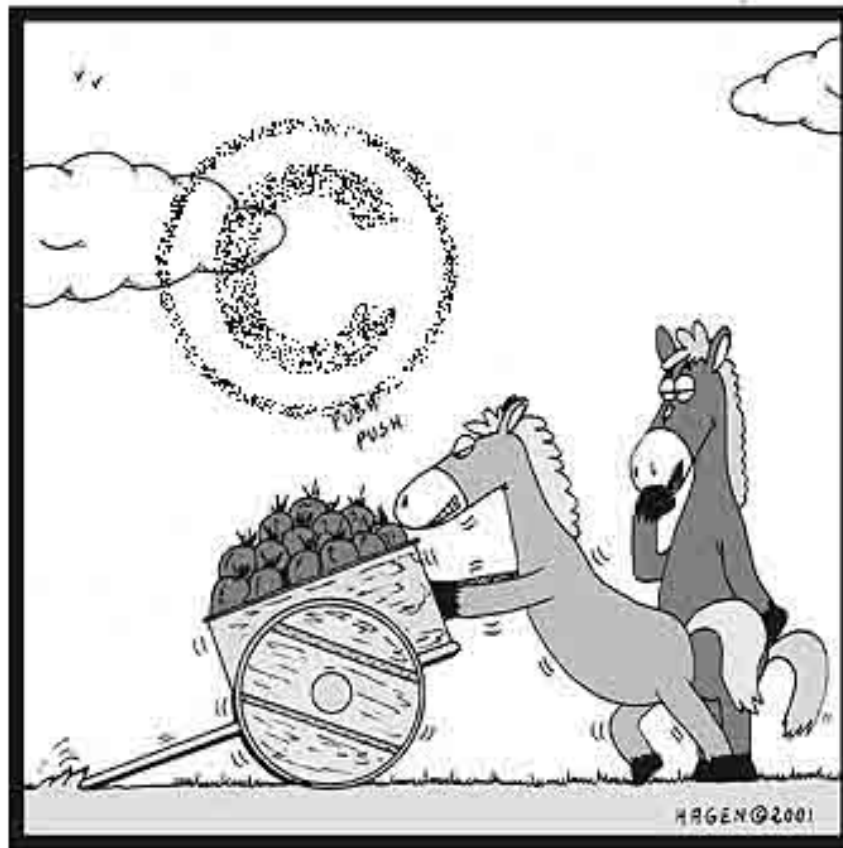


Optimal Dose \updownarrow Therapeutic Window \longleftrightarrow

Areas for Integration of Pharmacogenomics



Clinical Pharmacogenomics circa 2005...



Hang on... We must be doing something wrong...
How does the saying go again?

AmpliChip CYP450 Test

FDA recently cleared the *AmpliChip CYP450 Test* (Roche), which analyzes blood-derived DNA to detect genetic variations in the activity of cytochrome P450 (CYP) enzymes CYP2D6 and CYP2C19, and determines the metabolizer status of the patient. The test is intended to help guide clinicians in prescribing individualized drug therapy. About 25% of all drugs, including many antidepressants and antipsychotics, are substrates of either CYP2D6 or CYP2C19. The test is being promoted initially to psychiatrists.



FOR IMMEDIATE RELEASE

P05-53

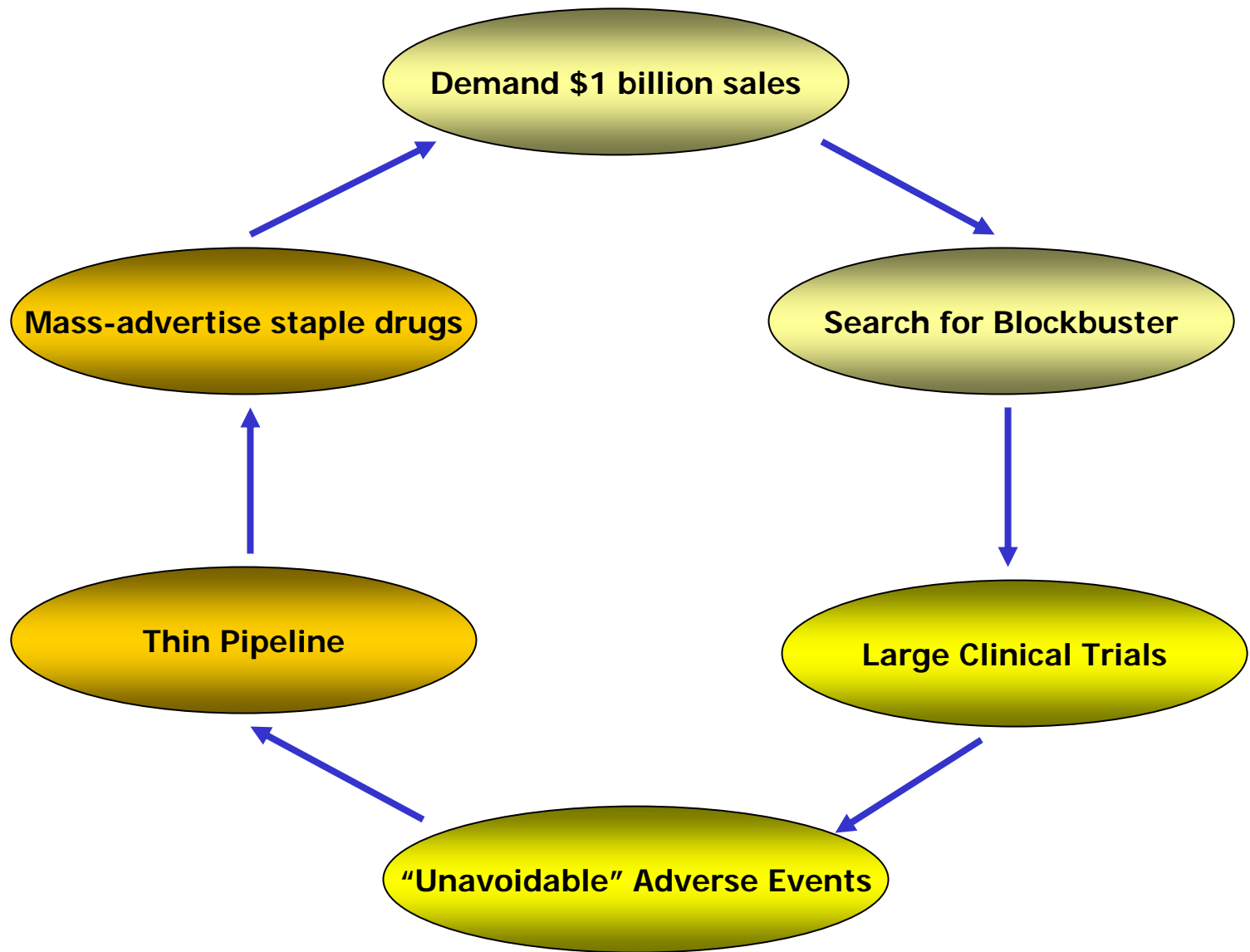
FDA News

August 22, 2005

Today, FDA cleared for marketing a new blood test that will help doctors make personalized drug treatment decisions for some patients. The Invader UGT1A1 Molecular Assay detects variations in a gene that affects how certain drugs are broken down and cleared by the body. Doctors can use this information to help determine the right drug dosage for individual patients, and minimize harmful drug reactions.

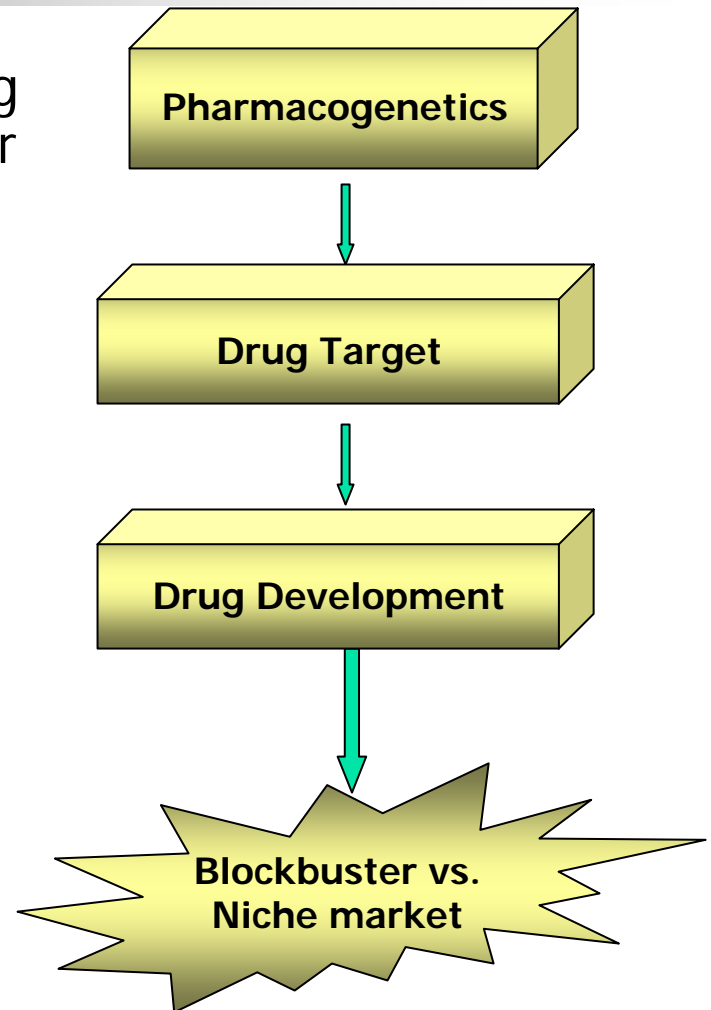
"This test represents the power of DNA-based testing to provide individualized medical care," said Daniel Schultz, MD, Director of FDA's Center for Devices and Radiological Health. "These technologies can significantly improve patient management and reduce the risk of ineffective or even harmful drug therapy by telling doctors how to individualize drug dosing."

The Invader assay joins a growing list of genetic tests used by physicians to personalize treatment decisions, including the Roche AmpliChip, used to individualize dosage of antidepressants, antipsychotics, beta-blockers, and some chemotherapy drugs, and TRUGENE HIV-1 Genotyping Kit, used to detect variations in the genome of the human immunodeficiency virus that make the virus resistant to some anti-retroviral drugs.



Incorporation of Pharmacogenetics in Drug Discovery and Development

- Current cost of bringing new drug to market appx. \$880 million over 15 years
- Incorporation of PGx could save companies over \$400 million and 1.6 years of development time
- Key benefit of PGx - ability to start research in human populations
- Early application is to identify drug targets by conducting case-control association studies where populations are segregated by age, disease, etc.



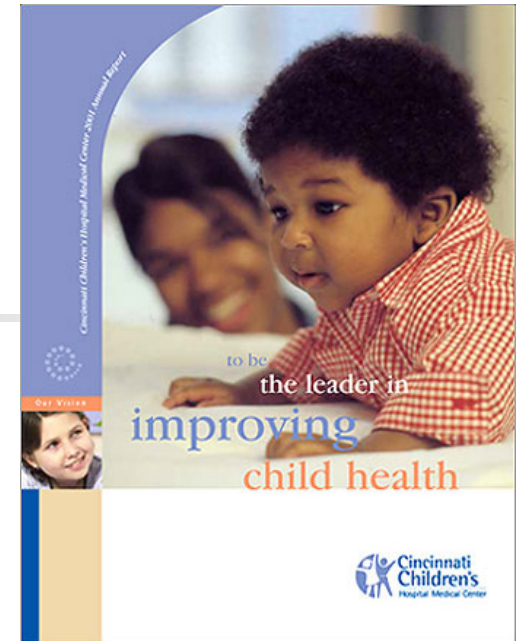


Genetic Pharmacology Service

Building a Future of Personalized Medicine for Children and Adults Through Pharmacogenetics

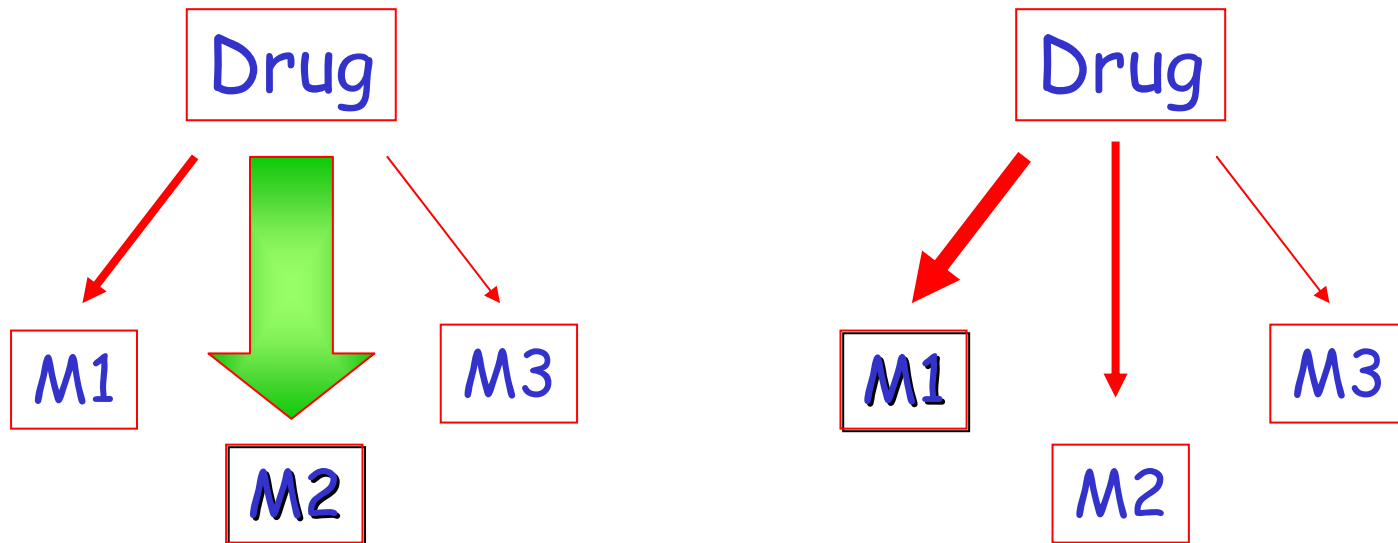
Scientific evidence indicates that **pharmacogenetics** -- the study of drug interactions with an individual's genetic makeup -- may improve patient safety. Pharmacogenetic testing for some medications adds genetic information to the usual considerations of patient age, weight, disease process, use of other medications, health behaviors and environment. The additional information gained from pharmacogenetics can help physicians and nurse practitioners choose and dose medication that best meets the needs of the individual patient.

<http://www.cincinnatichildrens.org/svc/alpha/g/gps/>



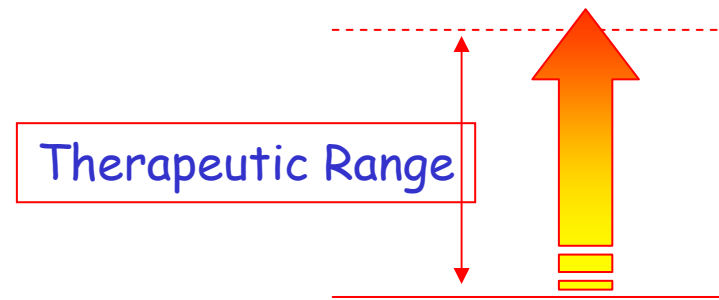
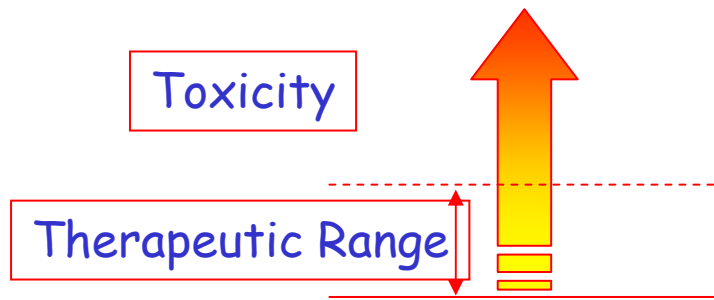
When is genetically defective metabolism of a drug clinically relevant?

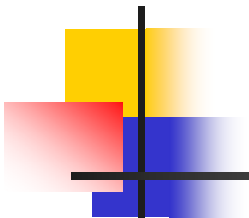
1. The defective metabolic pathway is quantitatively significant in determining the overall fate of the drug in the body



When is genetically defective metabolism of a drug clinically relevant?

1. The defective metabolic pathway is quantitatively significant in determining the overall fate of the drug in the body
2. The drug displays a narrow therapeutic range





When is genetically defective metabolism of a drug clinically relevant?

1. The defective metabolic pathway is quantitatively significant in determining the overall fate of the drug in the body
2. The drug displays a narrow therapeutic range
3. The drug's therapeutic and toxic effects can be readily assessed and titrated by clinical monitoring
4. Drug is widely used in clinical practice
5. Therapeutic alternatives are limited or absent



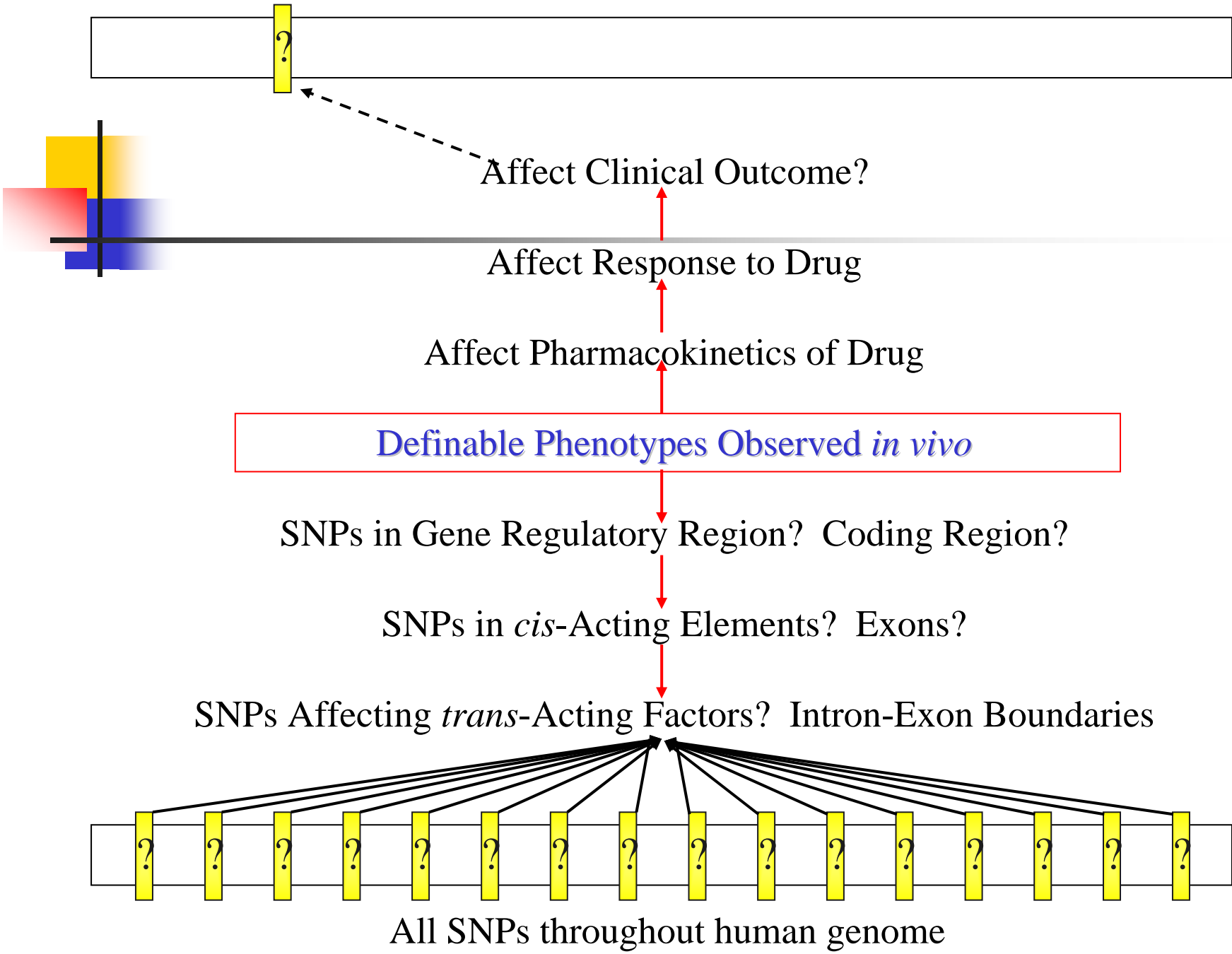
Several Potential Goals for Clinical Pharmacogenetic Testing

- The sub-division of common diseases into different molecular sub-types which may be more or less susceptible to specific treatments.
- To evolve more logical approaches to dosage, efficacy and the prevention of adverse reactions by analyzing the genetic basis for differences in the pharmacokinetic or pharmacodynamic properties of drugs.
- To identify genetic susceptibility to various common diseases that, although not directly related to drug metabolism, offer targets for pharmacological intervention.



Genotype to Phenotype to Activity: The Holy Grail of Clinical Pharmacogenetics

- Function follows form
 - Prediction of protein activity spectrum from genotype (ie., lumping and splitting)
- Must establish quantitative link between phenotype and genotype
- Must translate phenotype into accurate, quantitative reflection of protein (eg., enzyme, transporter) activity
- Need to translate the genetic information provided by the “marker” into a quantifiable and reliable altered dosing scheme



Affect Clinical Outcome?

Affect Response to Drug

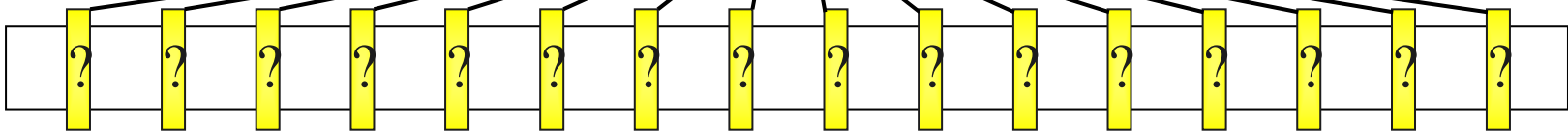
Affect Pharmacokinetics of Drug

Definable Phenotypes Observed *in vivo*

SNPs in Gene Regulatory Region? Coding Region?

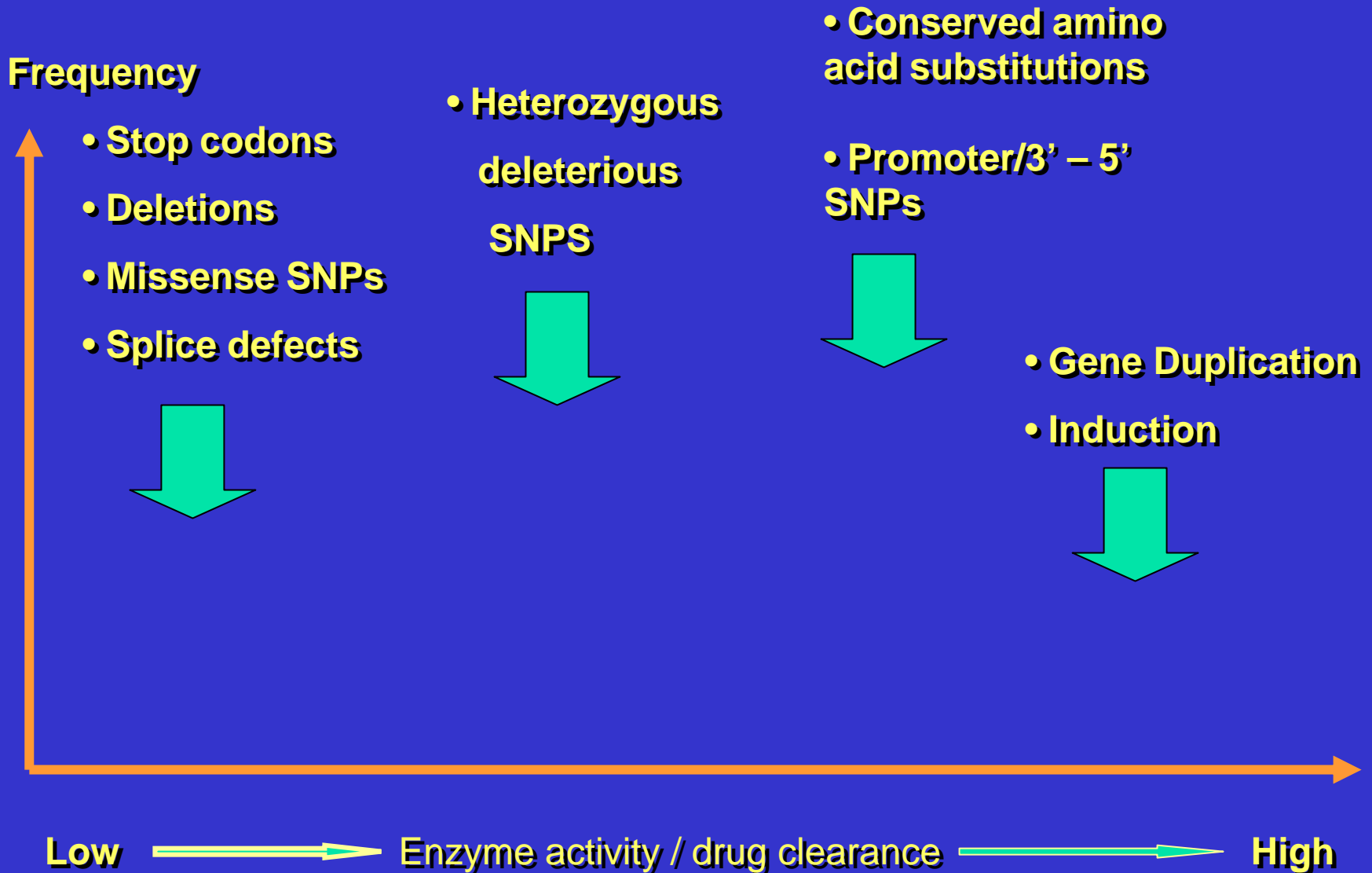
SNPs in *cis*-Acting Elements? Exons?

SNPs Affecting *trans*-Acting Factors? Intron-Exon Boundaries



All SNPs throughout human genome

Possible Phenotypes and Genotypes of Pharmacologic Significance



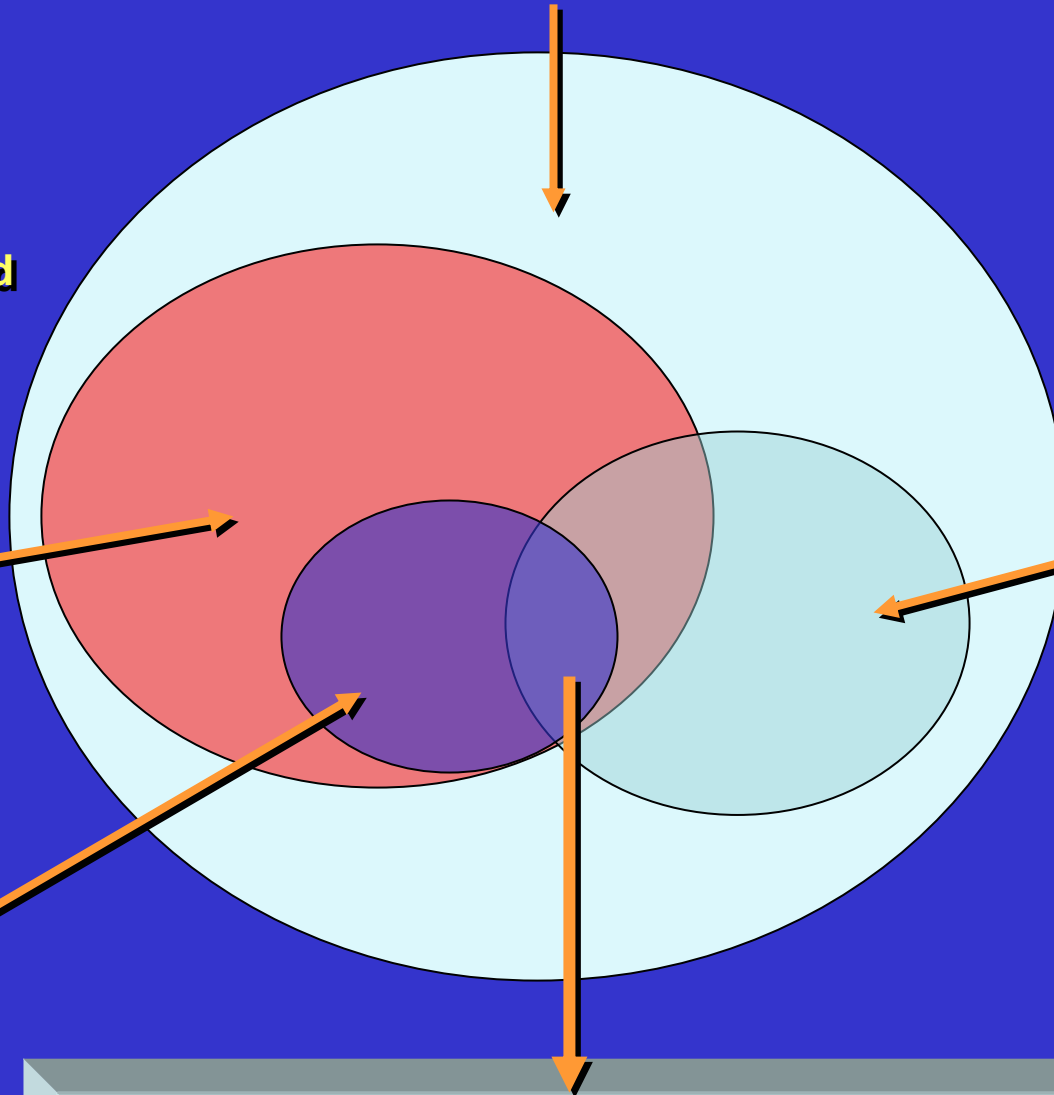
All Genes in Genome

Genes expressed in tissues with relevance to Absorption, Distribution, Metabolism &/or Excretion

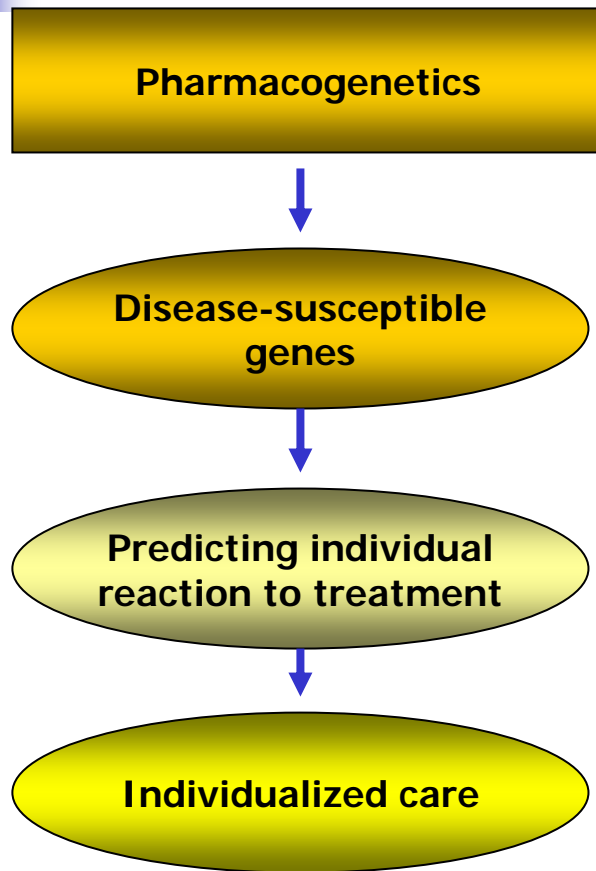
Set of genes responsible for modulation of drug-receptor Interaction

Set of genes capable of modulation by development, disease, drugs and/or environmental factors

Set of genes prioritized for pharmacogenomic evaluation and use for individualizing therapy



Individualized Medicine in 2005: What is apparently clear to date...



- NAT2 – hydralazine-associated SLE
- VKORC1 – warfarin-associated hemorrhage
- G6PD – primaquine-associated hemolysis
- HERG – quinidine-associated arrhythmia
- CYP2D6 – codeine, tramadol, antidepressant-associated efficacy and AE
- *Bcr/abl* – Glivec treatment of CML
- HER2 – Herceptin efficacy in breast cancer
- TPMT – 6MP and azathioprine-associated anemia

Utility of Pharmacogenetics in Clinical Medicine Circa 2004.....

<u>Application</u>	<u>Example DMEs and Therapeutic Drugs</u>
Evaluation of adverse drug reactions	CYP2C9 (warfarin, phenytoin), CYP2D6 (SSRIs), CYP2C19 (omeprazole), TPMT (6-mercaptopurine, azathioprine)
<i>A priori</i> selection of therapeutic dosing regimen	CYP2C9 (warfarin), CYP2D6 (SSRIs, atomoxetine), TPMT (6-mercaptopurine, azathioprine)
Interpretation of variability in PK/PD	CYP2C19 (proton pump inhibitors), CYP2D6 (tricyclic antidepressants, SSRIs, codeine), CYP3A4/5 (midazolam, alfentanil, nifedipine, cisapride), UGT2B7 (morphine), UGT1A6 (salicylates, β -blockers, methyldopa, acetaminophen), UGT2B15 (oxazepam), UGT2B17 (lorazepam), TPMT (6-mercaptopurine, azathioprine), NAT2 (isoniazid, procainamide, hydralazine), COMT (levodopa)

What information is needed to interpret pharmacogenetic data in the context of therapeutic decision making?

- Accurate genotyping information for ALL relevant genes
- Full access to patient medical record
- Full access to the patient (eg., drug-diet history)
- Comprehensive knowledge of the clinical pharmacology for drug(s) of interest
- Ability to integrate medical – pharmacologic – genetic information in a clinical “systems biology” context
- Ability to effectively translate all information into an effective medical decision





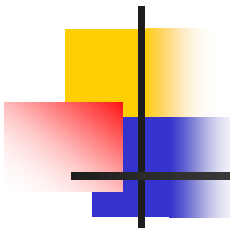
Pharmacogenomics Education: An Evolutionary Process....

- NHLBI \$1.2mil initiative (over 3 yr) to develop short courses on Application of Genomics and Proteomics to Complex Heart, Lung, Blood and Sleep Disorders
- “Clinicians lag behind pharmacists and diagnostic scientists” (*Moridani, AJPE 2004*)
 - 60% of 28 US colleges and schools of pharmacy offered at least six hours of pharmacogenomics-related courses
 - Established clinicians receive sporadic instruction. Psychiatrists are most open, followed by Oncologists
- A “minority” of medical schools in Europe and North America include pharmacogenomics as part of their core pharmacology courses (*Gurwitz D, et al. Pharmacogenomics J 2005;5:221-25*)
- Expanding symposia / workshops on individualized medicine

Clinical Pharmacogenetics: Questions Concerning its Value to Individualized Medicine in 2005

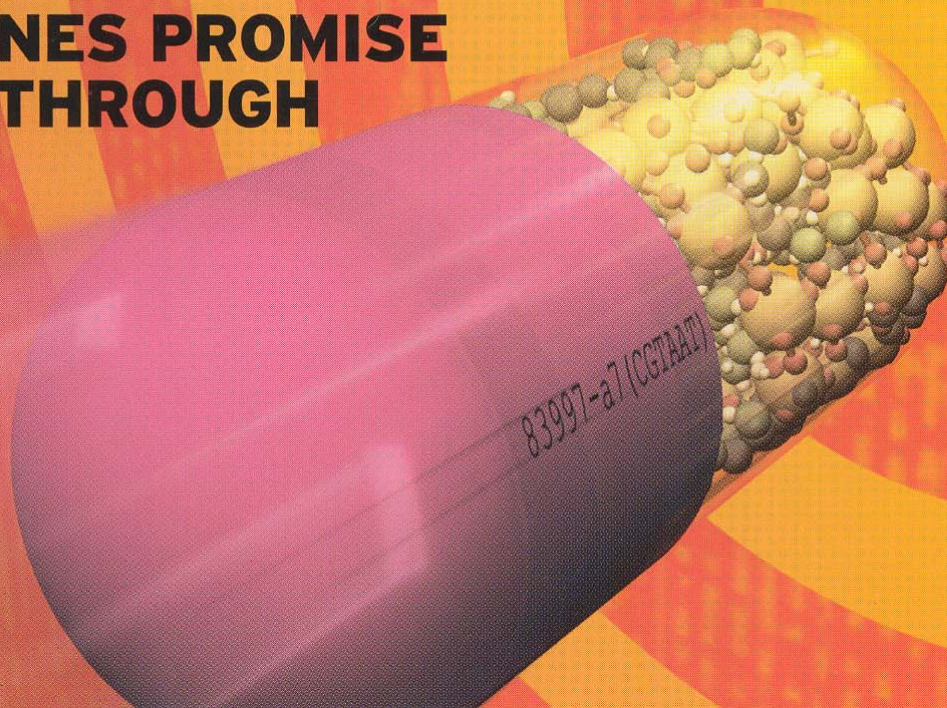
- The performance of testing: who, what, where, how
 - Retrospective vs. prospective
- How should testing be used and by whom?
 - Physicians
 - Clinical Pharmacologists
 - Clinical Chemists
 - Genetics Counselors
- Who determines the benefit of testing and by what criteria?





THIS DRUG'S FOR YOU

**NEW TARGETED
MEDICINES PROMISE
BREAKTHROUGH
CURES**



U.S. News and World Report, 14 January 2003