

PHARMACOGENETICS
AND
PHARMACOGENOMICS

R. J. Desnick, M.D., Ph.D.

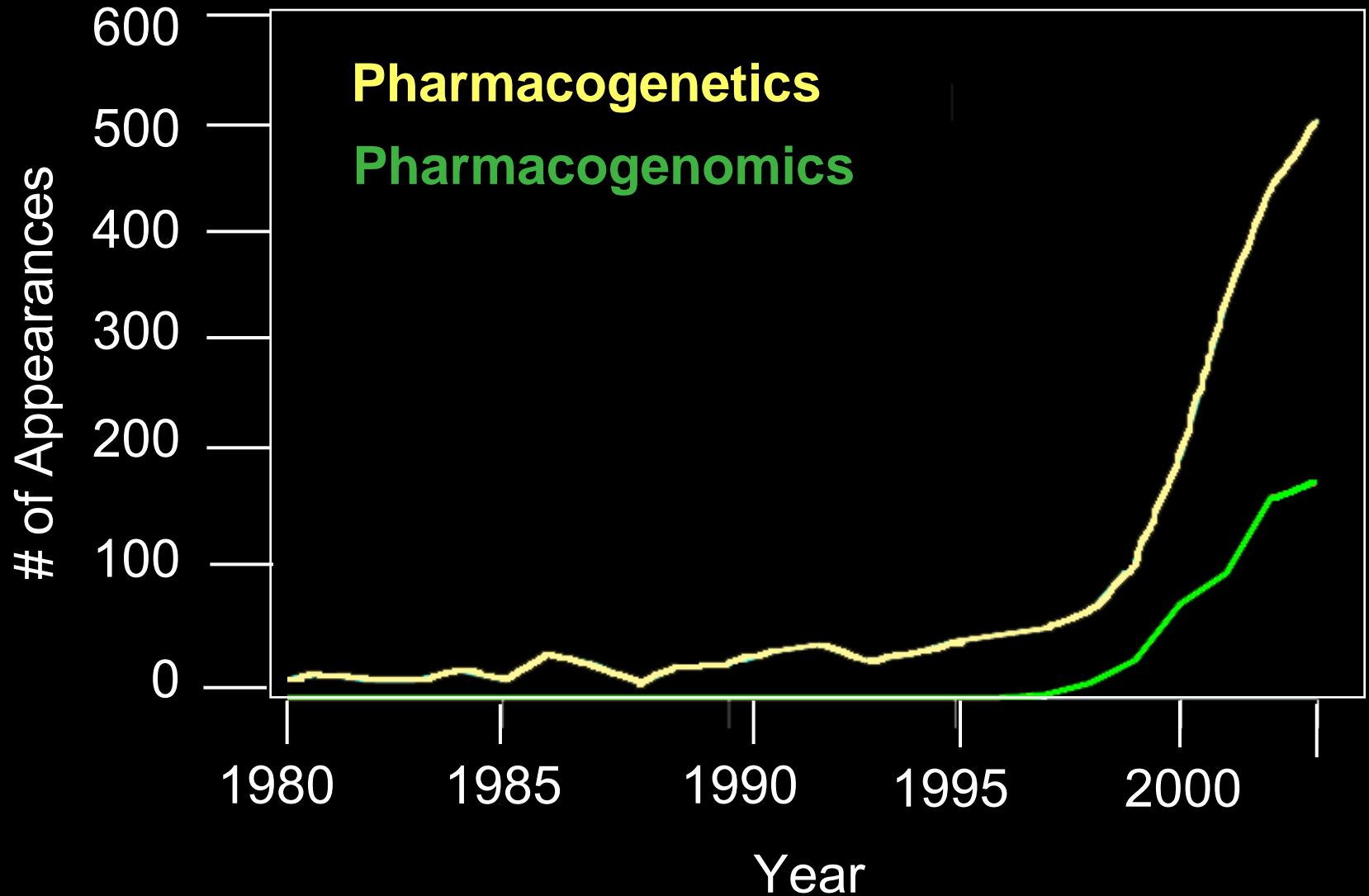
Professor and Chairman

Department of Genetics & Genomic Sciences

PHARMACOGENETICS

- **Pharmacogenetics** Is the Study of Inherited Differences (Variation) in Drugs Metabolism & Response
 - Genetic Variability Can Affect:
 - **Pharmacokinetics** - Plasma Clearance, Delivery of Drug or Metabolite to Target Cells,
 - **Pharmacodynamics** - The Relationship Between the Drug Concentration & Its Therapeutic Effect.
 - The Likelihood of an Adverse Reaction
 - **Genome-Tailored Drug Selection and Dosage**
-

APPEARANCE OF THE TERMS PHARMACOGENETICS & PHARMACOGENOMICS IN PUBLICATIONS IN PUBMED



PHARMACOGENETICS/GENOMICS

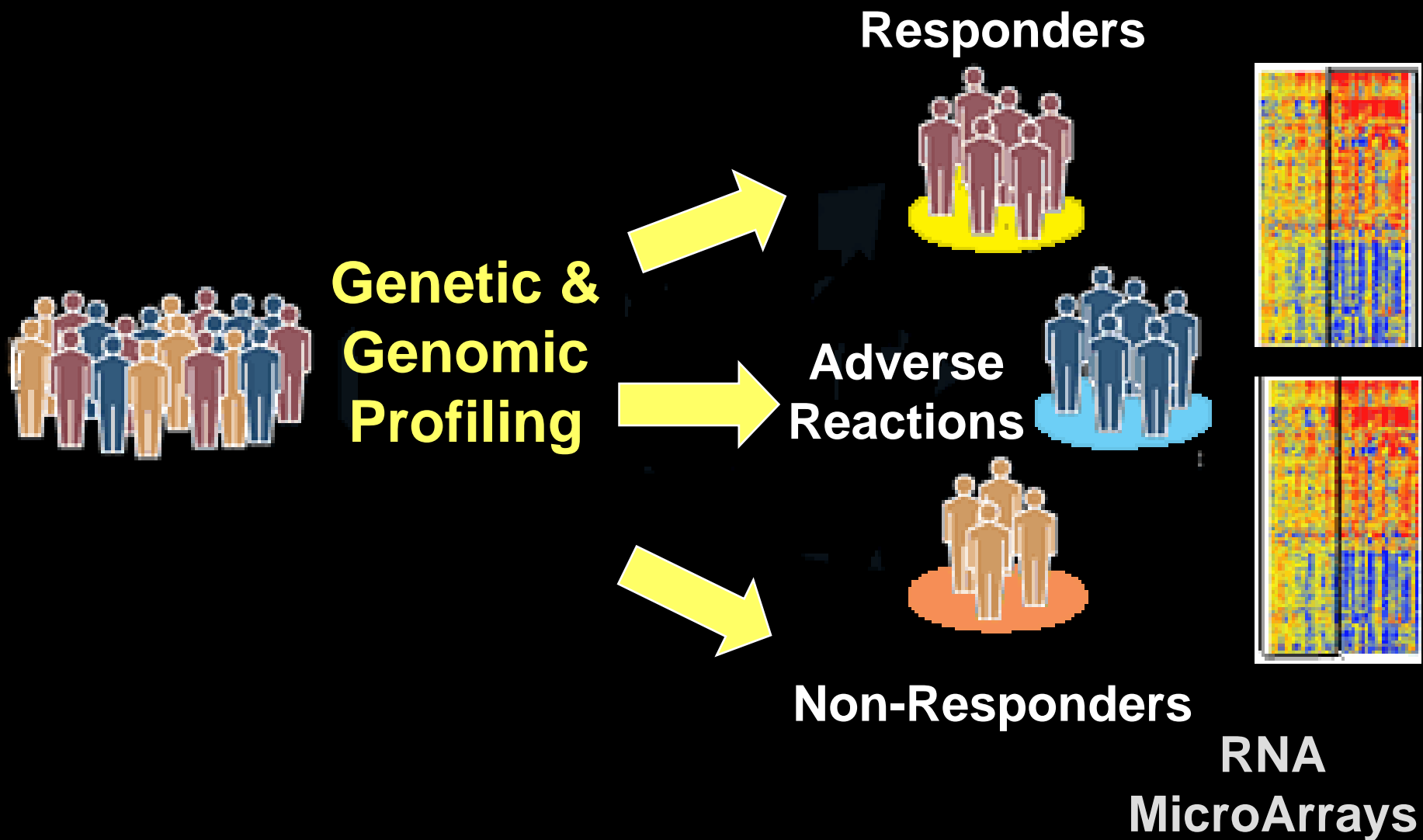
Recent Reviews

- Kingsmore et al., Genome-Wide Association Studies: Progress and Potential for Drug Discovery and Development. *Nature Reviews Drug Discovery* 7:221-30, 2008
- Brockmoller & Tzvetkov, Pharmacogenetics: Data, Concepts & Tools to Improve Drug Discovery & Drug Treatment *Eur J Clin Pharmacol* 64:133-57, 2008
- Mallal et al., HLA-B*5701 Screening for Hypersensitivity to Abacavir *N Engl J Med* 358: 568-79, 2008
- Goldstein et al., Potential Genetic Causes of Heterogeneity of Treatment Effects. *Am J of Med* 120:S21-S25, 2007
- Schmitz et al., Pharmacogenetics and Pharmacogenomics of Cholesterol-Lowering Therapy. *Curr Opin Lipidol* 18:164-173, 2007
- Giacomini et al., The Pharmacogenetics Research Network: From SNP Discovery to Clinical Drug Response. *Clin Pharmacol Ther* 18:328-45, 2007

BENEFITS OF PHARMACOGENETICS

- **Improve Drug Choices:**
 - Each Year, ~100,000 Americans Die of Adverse Reactions to Medicine & ~2 Million Are Hospitalized
 - Pharmacogenomics Will Predict Who's Likely to Have a Negative or Positive Reaction to a Drug
- **Safer Dosing Options:**
 - Testing of Genomic Variation Improve Determination of Correct Dose for Each Individual
- **Improvement in Drug Development:**
 - Permit Pharmaceutical Companies to Determine in Which Populations New Drugs Will Be Effective
- **Decrease Health Care Costs:**
 - Reduce Number of Deaths & Hospitalizations Due to Adverse Drug Reactions
 - Reduce Purchase of Drugs Which Are Ineffective in Certain Individuals Due to Genetic Variations
 - Speed Up Clinical Trials for New Drugs

REFINING THERAPEUTIC DECISIONS & PREDICTING DRUG EFFICACY



EFFICACY OF VARIOUS DRUGS

Class of Drug

**% Refractory
or Insufficient
Response**

Selective Serotonin Reuptake Inhibitors (Depression) 10-25

ACE Inhibitors (Hypertension, Proteinuria) 10-30

Beta Blockers (Cardiac) 15-25

Tricyclic Anti-Depressants (Depression) 20-50

HMG-CoA Reductase Inhibitors (Statins) 30-70

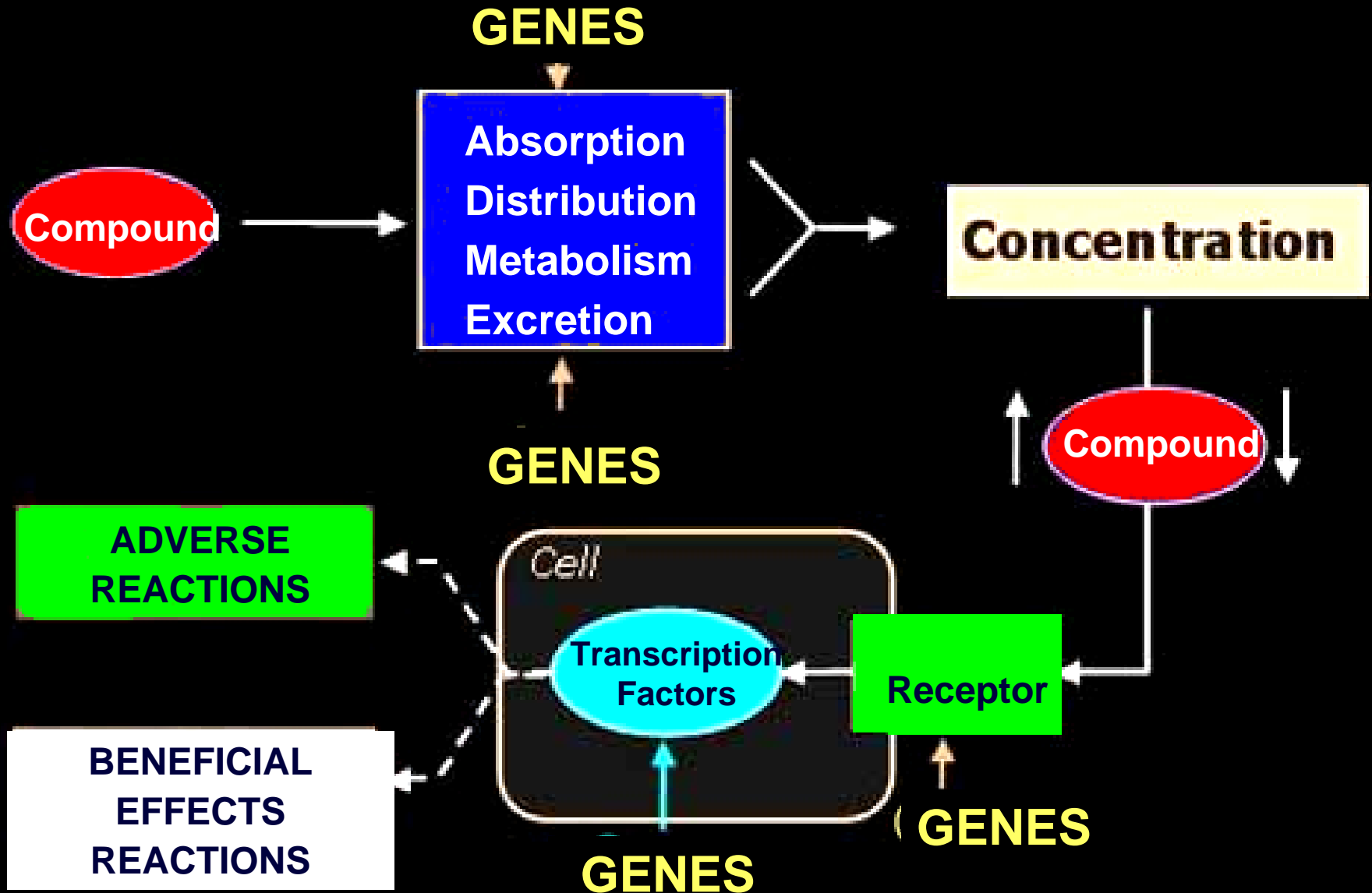
Beta 2-Agonists (Bronchodilators) 40-70

PRINCIPLES OF DRUG METABOLISM

Genetic Variation in Drug Metabolism Influences

- Absorption
- Distribution
- Receptor Interaction
- Biotransformation
- Metabolism
- Efficacy
- Elimination

GENES ARE INVOLVED IN DETERMINING DRUG EFFECTS



PRINCIPLES OF DRUG METABOLISM

Genetic Variation in Drug Metabolism Influences

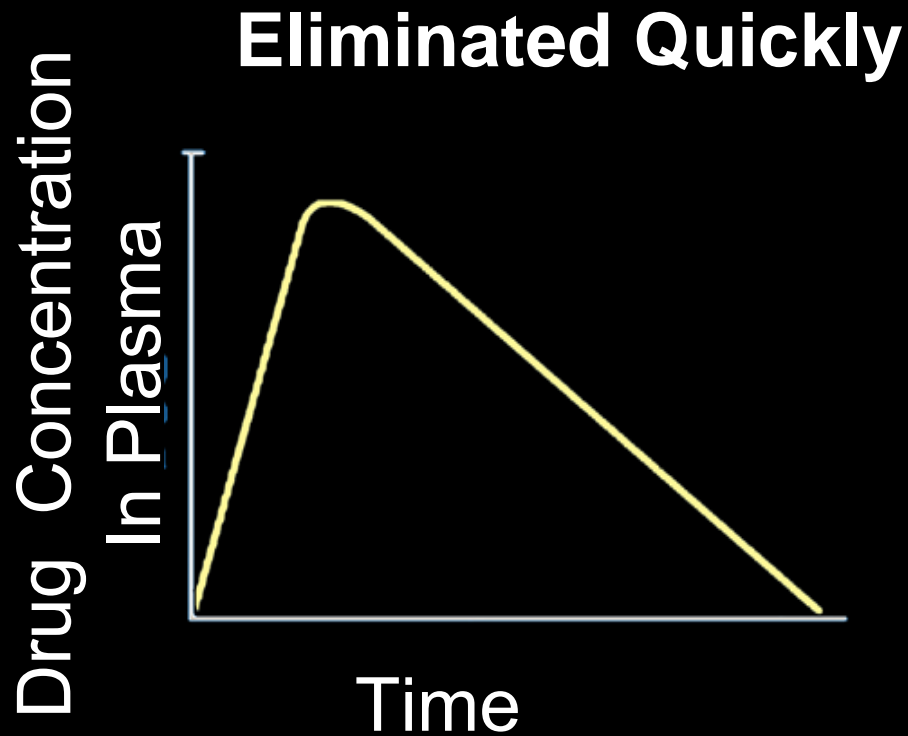
- **Pharmacokinetics:**

- Absorption
 - Distribution
 - Metabolism
 - Excretion
- ADME**

- **Pharmacodynamics:**

- Receptor Interactions
- Ion Channel Interactions
- Enzyme Interactions
- Signaling Pathway Interactions
- Immune System Interactions

PLASMA CLEARANCE

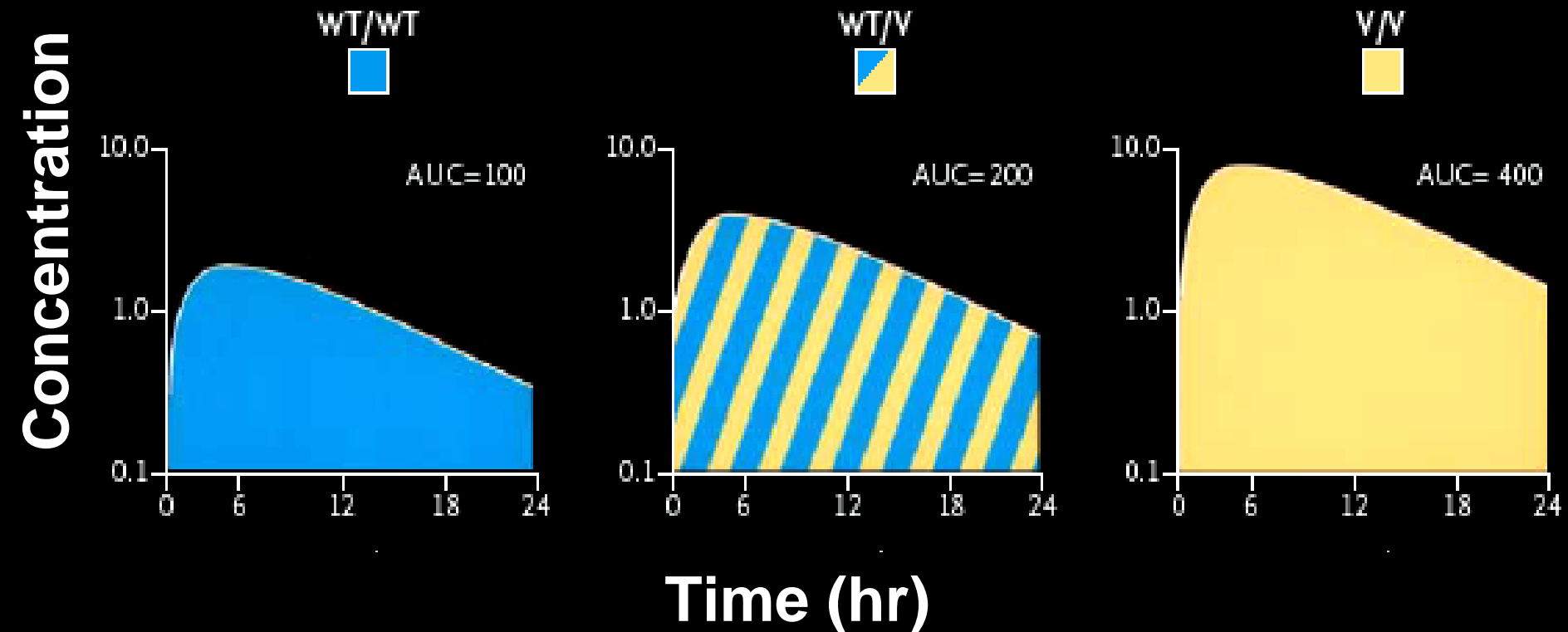


DRUG METABOLISM / DEGRADATION

Genotype vs Drug Concentration

AUC = Area Under the Curve

WT = Wild Type Allele; V = Variant Allele

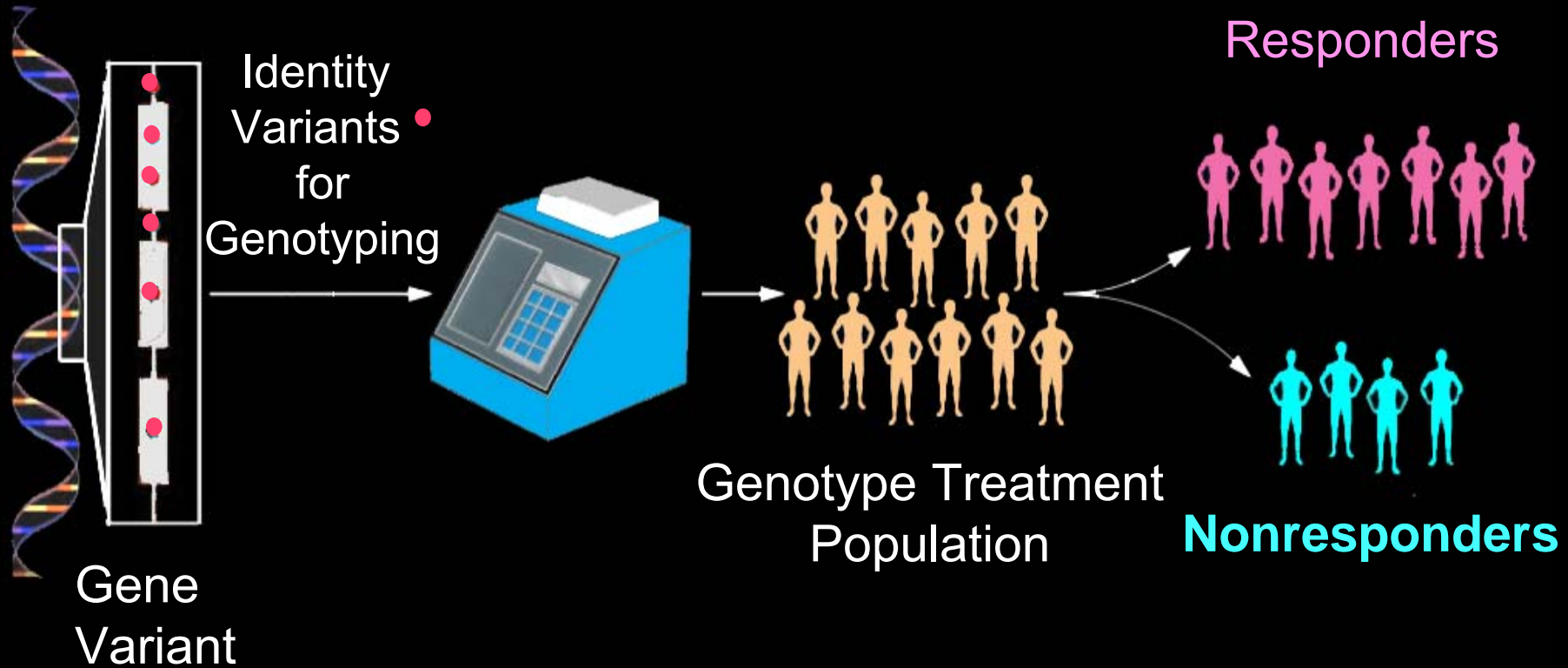


GENETIC VARIATION

- Sequence Differences in the Same Gene Are Called Alleles
- Sequence Differences Can Be in Coding Regions, Regulatory Regions (i.e., Promoters, Enhancers), Introns, at 5' or 3' Flanking Regions, etc
- If Alleles Are Present in **>1%** of Heterozygous Individuals in a Given Population, the Variation Is Called a **Polymorphism**
- If Alleles Are Present in **<1%** of Heterozygous Individuals in a Given Population, the Variations Are Called **Sequence Variants**

IDENTIFICATION OF PHARMACOGENETIC VARIANTS

Genome



ORIGINS OF PHARMACOGENETICS

- Three Discoveries in the 1950s Gave Rise to the Discipline of Pharmacogenetics:
 - Primquine Sensitivity (G-6-PD Deficiency)
 - The Slow Metabolism of Isoniazid (Acetylation Polymorphism & Tuberculosis)
 - Atypical Plasma Cholinesterase Giving Rise to Prolonged Effects of Succinylcholine (Respiratory Apnea)
-

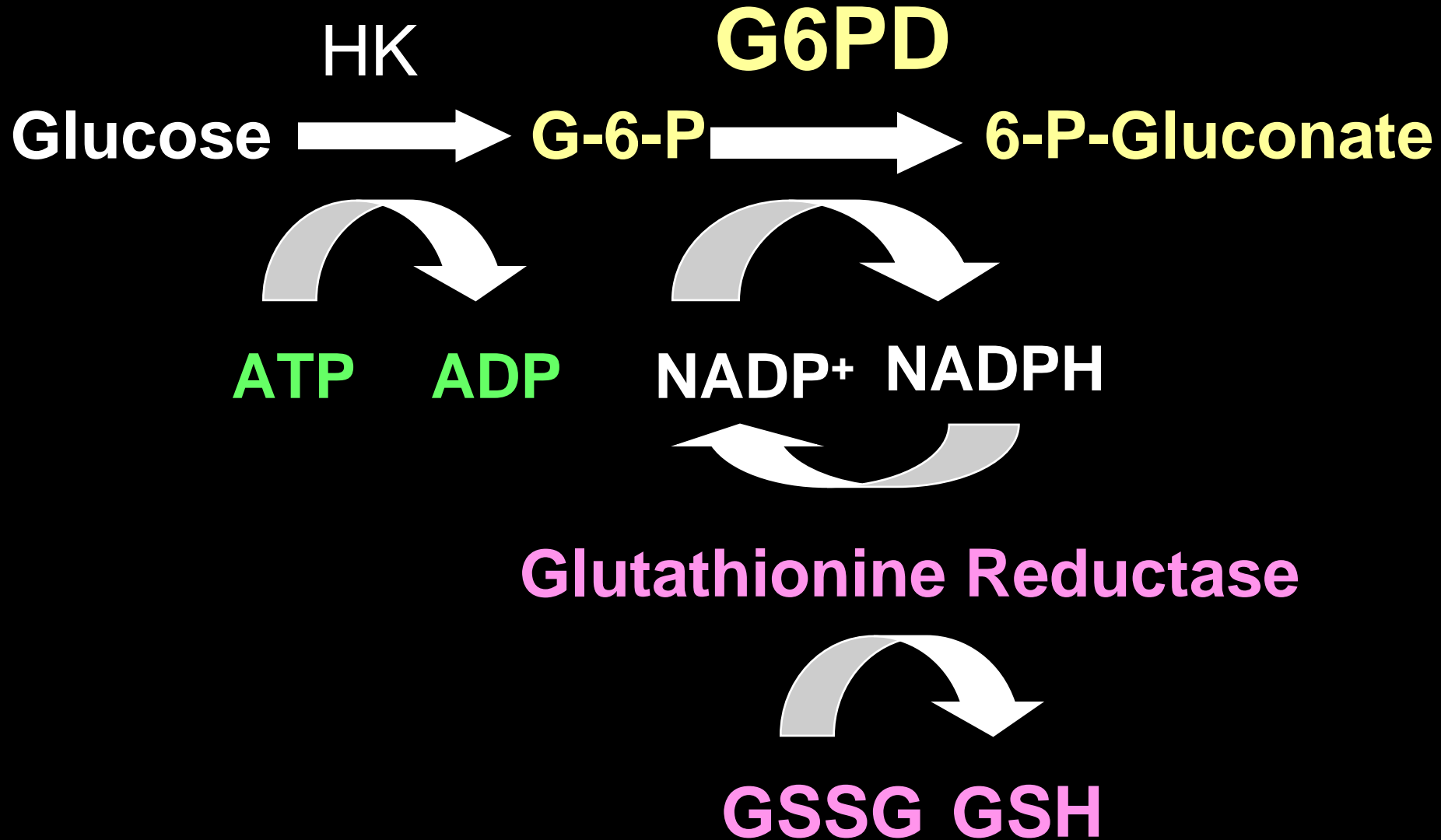
PHARMACOGENETICS: EXAMPLES OF ALTERED DRUG RESPONSE

Enzyme/Disease	Gene
• Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD Def)	G6PD
• <i>N</i> -Acetylation & Tuberculosis	NAT2
• Cytochrome P450 Enzyme – Drug Metabolism	CYP2D6
• Warfarin & Coagulation	CYP2C9 VKORC1
• Thiopurine S-Methyltransferase & Cancer	TPMT
• ACE Inhibitors, Antidepressants, Diabetes, Asthma, etc	

GLUCOSE -6- DEHYDROGENASE DEFICIENCY

- **Glucose-6-Dehydrogenase (G6PD):**
 - Enzyme in Hexose Monophosphate Shunt, A Principle Source of NADPH Generation
 - NADPH Needed to Reduce SH Groups on Glutathione (GS-SG →GSH) and Other Proteins
 - Many Drugs and Their Metabolites Can Put a Burden on GSH Levels and Can Lead to a GSH Deficiency in G6PD Deficient Patients
 - GSH Deficiency in Red Cells Results in:
Membrane Fragility → Hemolysis → Hemolytic Anemia

GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE



COMMON GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE VARIANTS

Variant	Enzyme Activity	Frequency
B	100%	Wild-Type
A	90%	1 in 5 US Black Males
A ⁻	15%	1 in 10 US Black Males
B ⁻	4%	Common in Mediterranean Area

SOME AGENTS THAT CAUSE HEMOLYSIS IN G-6-P D DEFICIENT INDIVIDUALS

Acetanilid

Phenylhydrazine

Sulfanilamide

Sulfacetamide

Sulfapyridine

Sulfamethoxypyridazine (Kynex)

Salicylazosulfapyridine (Azulfidine)

Thiazosulfone

Diaminodiphenylsulfone

Trinitroluene

Nitrofurazone (Furacin)

Nitrofurantion
(Furadantin)

Furazolidone

Furaltodone (Altofur)

Quinidine

Primaquine

Pamaquine

Pentaquine

Quinocide

Naphthalene

Neosalvarsan

Fava Beans

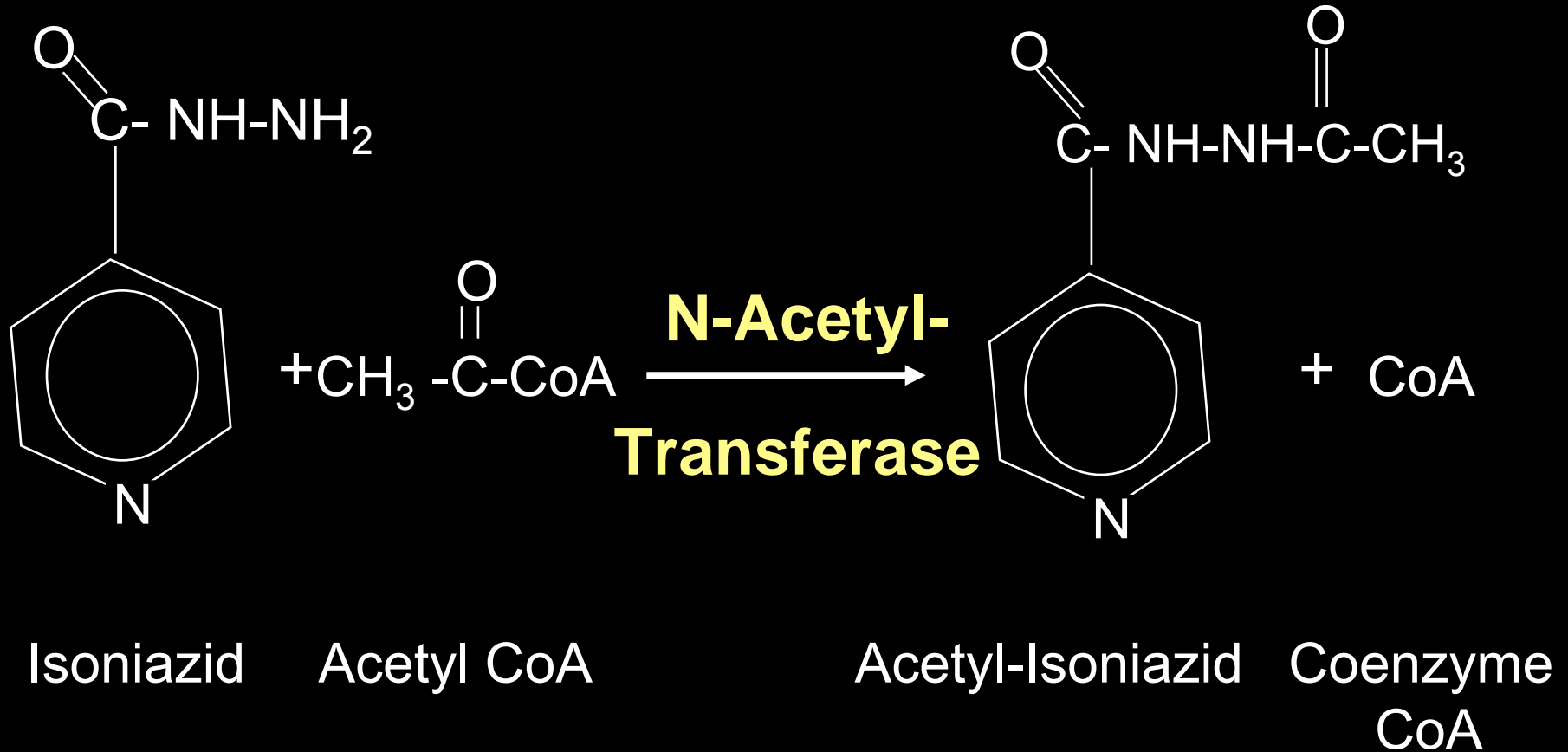
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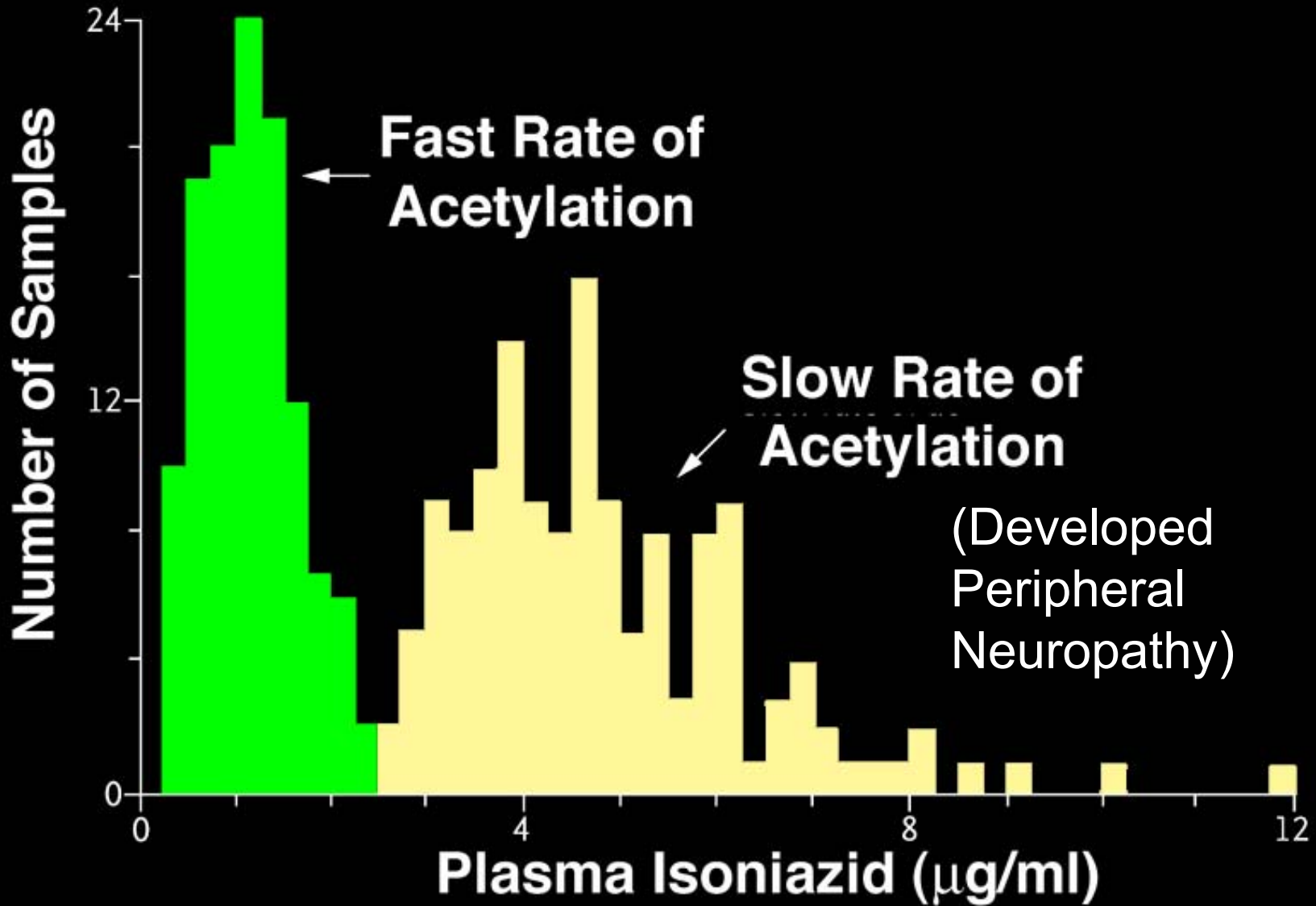
N - ACETYLATION AND TUBERCULOSIS

- Identified in the Late 1940's when Patients who Converted to a Positive Tuberculin Test Were Treated with the Drug Isoniazid
- High Incidence of Peripheral Neuropathy Among Patients Taking Isoniazid - Example of Therapeutic Drug Reaching Toxic Levels
- Isoniazid Is Cleared after Acetylation to Acetyl-Isoniazid by Liver *N*-Acetyltransferase (NAT2)

ACETYLATION OF ISONIAZID IS CATALYZED BY LIVER N-ACETYLTRANSFERASE (NAT2)



DISTRIBUTION OF PLASMA ISONIAZID LEVELS SIX HOURS AFTER AN ORAL DOSE OF ISONIAZID



RAPID & SLOW ACETYLATORS

- **Individuals Who Are Rapid Acetylators:**
 - Have Higher Failure Rate With Isoniazid Therapy for Tuberculosis
 - Require Larger Doses of Hydralazine to Control Hypertension and Dapsone to Treat Leprosy and Other Infections
- **Individuals who Are Slow Acetylators Have ↑ Risk of:**
 - Developing a Drug-Induced Systemic Lupus Erythematosus-Like Syndrome When Receiving Hydralazine
 - Hematological Adverse Reactions After Isoniazid Treatment
 - Idiosyncratic Adverse Responses to Sulfonamide Drugs
 - Bladder Cancer After Exposure to Carcinogenic Arylamines
 - Breast Cancer in Postmenopausal Female Smokers (4X↑)

MOLECULAR GENETICS OF N-ACETYLATION

- NAT2 Gene Has Several Alleles which Cause the Variation in Rate of Acetylation of Isoniazid:
 - **Rapid Acetylator**: Allele Considered Wild-Type
 - **Slow Acetylator**: Alleles Have Amino Acid Substitutions:
 - Caucasians (Common Mutant Alleles):
 - NAT2*5B (I114T, C481T & K268R) & NAT2*6A (G590A / C2872T)
 - Asians (Common Mutant Alleles):
 - NAT2*6A (G590A & C2872T) & NAT2*7A (G286R)
 - Slow Acetylators Are Homozygous for Slow Acetylator Alleles
 - Rapid Acetylators Are Heterozygous or Homozygous for the Rapid Acetylator Allele
 - Alleles Present in Different Frequencies in Different Populations

FREQUENCY OF SLOW NAT2 ALLELES IN DIFFERENT ETHNIC POPULATIONS

Population	Frequency (%)
Eskimo	23
South Pacific Islanders	35
Korean/Chinese/Japanese	37
North & South American Indians	50
African	71
Central & Western Asian	74
European	75
Egyptian	96

PHARMACOGENETICS: EXAMPLES OF ALTERED DRUG RESPONSE

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• ACE Inhibitors, Antidepressants, Diabetes, Asthma, etc	

CYTOCHROME P450 ENZYMES

- Cytochrome P450 Superfamily Responsible for Metabolism of Many Compounds
- At Least 55 Human CYP Genes Have Been Identified
- Four P450 Enzymes Involved in >80% of Over-the-Counter Medications:
 - CYP2C9, CYP2C19, CYP2D6, and CYP3A4
- Most Thoroughly Studied Pharmacogenetic Enzymes
- **Focus on CYP2D6**

POLYMORPHIC CYTOCHROME P450 GENES INVOLVED IN DRUG METABOLISM

Family	Gene	Alleles of Functional Significance	Drugs Metabolized (Selected)
CYP2	CYP2C19	↓ & 0 Activity Alleles	Antiepileptics, Antidepressants Antianxiety Drugs
CYP1	CYP1A2	↑, ↓ & 0 Activity Alleles	Acetaminophen Antifungals Cocaine Codeine Cyclosporine A Daizepam Erythromycin Cholesterol-Lowering Statins Taxol Warfarin

POLYMORPHIC CYTOCHROME P450 GENES INVOLVED IN DRUG METABOLISM

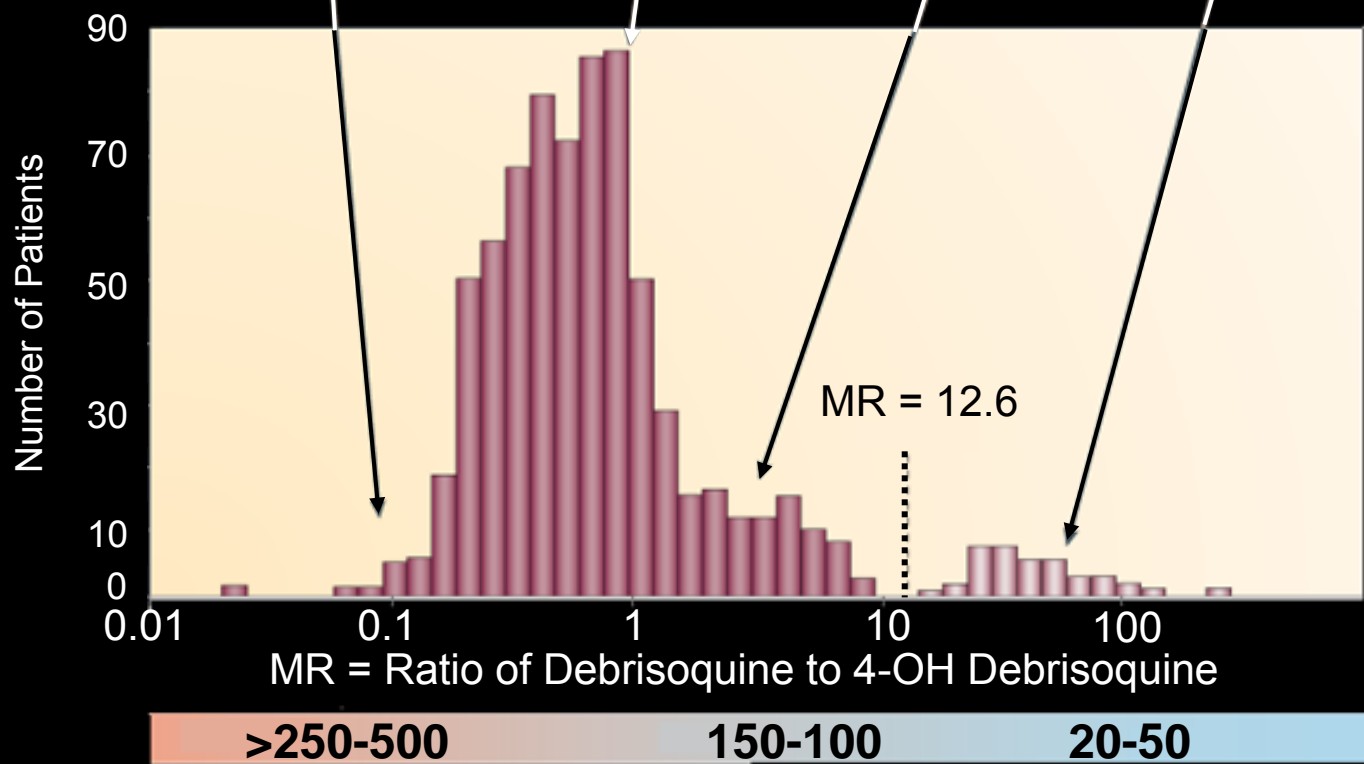
Family	Gene	Alleles of Functional Significance	Drugs Metabolized (Selected)
CYP3	CYP3A4	↑ & ↓ Activity Alleles	Caffeine Propranolol
CYP2	CYP2C9	↑, ↓ & 0 Activity Alleles	Angiotensin II Receptor Blockers Nonsteroidal Anti-inflammatory Metronidazole Oral Hypoglycemics Warfarin
	CYP2D6	↑, ↓ & 0 Activity Alleles	Antiarrhythmics Antidepressants Antipsychotics Beta-adrenergic blockers Narcotic Analgesics

HISTORY OF P450 CYP2D6

- Doctors Noticed in the 1970s that the Antihypertensive Agent Debrisoquine Caused an Unexpected High Incidence of Postural Hypotension, an Adverse Drug Reaction
- Individuals Who Became Hypotensive after Taking the Drug Had Slow Breakdown Rates (20 X Less)
- Slower Breakdown Resulted from Decreased Enzyme Activity
- Slower Metabolizers: ~6-10% of Caucasians, ~2% of Africans and <1% of Asians
- Gene Responsible Codes for a Microsomal Cytochrome P450 Monooxygenase, Called CYP2D6

GENOTYPE - PHENOTYPE RELATIONSHIPS OF THE CYP2D6 POLYMORPHISM

Genotype				
Phenotype	Ultrarapid Metabolizers	Extensive Metabolizers	Intermediate Metabolizers	Poor Metabolizers
Frequency (Caucasians)	5-10%	80-65%	10-15%	5-10%

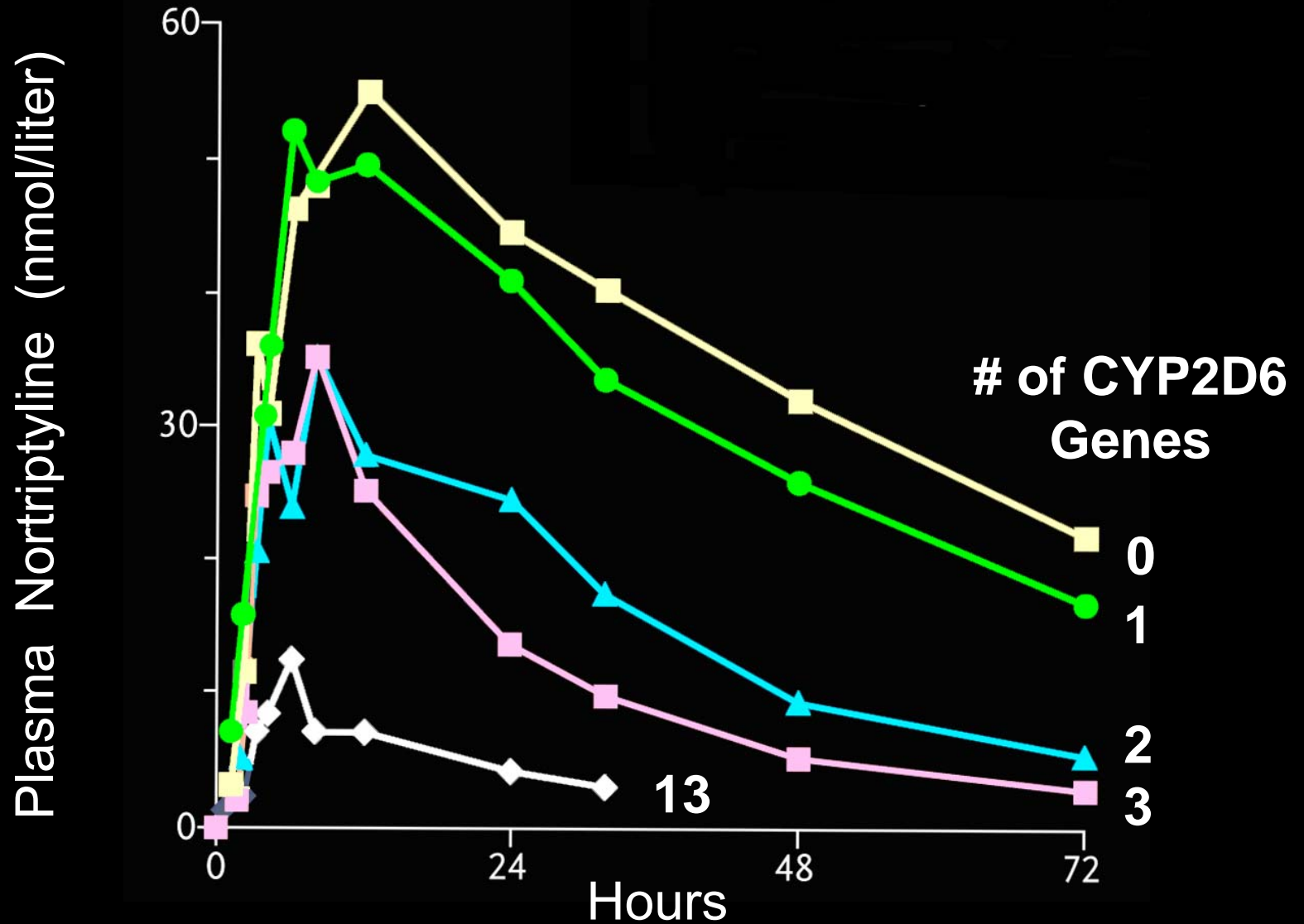


Antidepressive Drug Nortriptyline Dose Requirement (mg day⁻¹)

MOLECULAR GENETICS OF CYP2D6

- 14.8 Kb Gene Codes for 497 Amino Acids
- Enzyme in Endoplasmic Reticulum
- ~70 Alleles of CYP2D6 Have Been Identified, Nine of which Affect the Metabolism of ~25% of Drugs, Including Many Over-the-Counter Drugs
- Poor Metabolizers Have CYP2D6 Genes that Codes for Altered or Missing Proteins:
 - Common Mutations Include Splicing-Site, Large & Small Deletions
- Also Found “Ultra-Rapid Metabolizer” Individuals:
 - Due to Amplification of CYP2D6 Gene As Many As 13 Times
- Frequency of Alleles Varies in Different Populations

EFFECT OF INCREASING NUMBER OF FUNCTIONAL CYP2D6 GENES ON THE PLASMA NORTRIPTYLINE CONCENTRATION



SOME DRUGS WHOSE METABOLISM IS AFFECTED BY CYP2D6 VARIANTS

- Antiarrhythmics
 - β -Adrenergic Receptor Blockers
 - Neuroleptics
 - Tricyclic Antidepressants
 - Decongestants (Pseudoephedrine)
 - Importance: Drug Selection & Dose
-

FREQUENCY OF CYP2D6 ALLELES VARY IN DIFFERENT POPULATIONS

Slow
Metabolizers

Ultra-Rapid
Metabolizers

6-10 % Caucasians*

0.8% Caucasians

<1% Asians

21% Saudi Arabians

2% African-Americans

29% Ethiopians

* 4 Alleles Account for 90% of Slow Caucasian Metabolizers

AMPLICHIP CYP450 ARRAY –

With a Drop of Blood or Smear From Inside of Patient's Cheek, the Gene Chip Checks for 31 Variations in Two Genes (CYP2D6 and CYP2C19).



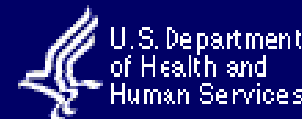
PHARMACOGENETICS: EXAMPLES OF ALTERED DRUG RESPONSE

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FDA RECOMMENDS WARFARIN LABEL REVISION TO INCLUDE INFORMATION ON CYP2C9 AND VKORC1 GENOTYPING



U.S. Food and Drug Administration



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FDA News

FOR IMMEDIATE RELEASE

August 16, 2007

Media Inquiries:

Karen Riley, 301-827-6242

Consumer Inquiries:

888-INFO-FDA

FDA Approves Updated Warfarin (Coumadin) Prescribing Information

New Genetic Information May Help Providers Improve Initial Dosing Estimates of the Anticoagulant for Individual Patients

WARFARIN (COUMADIN)

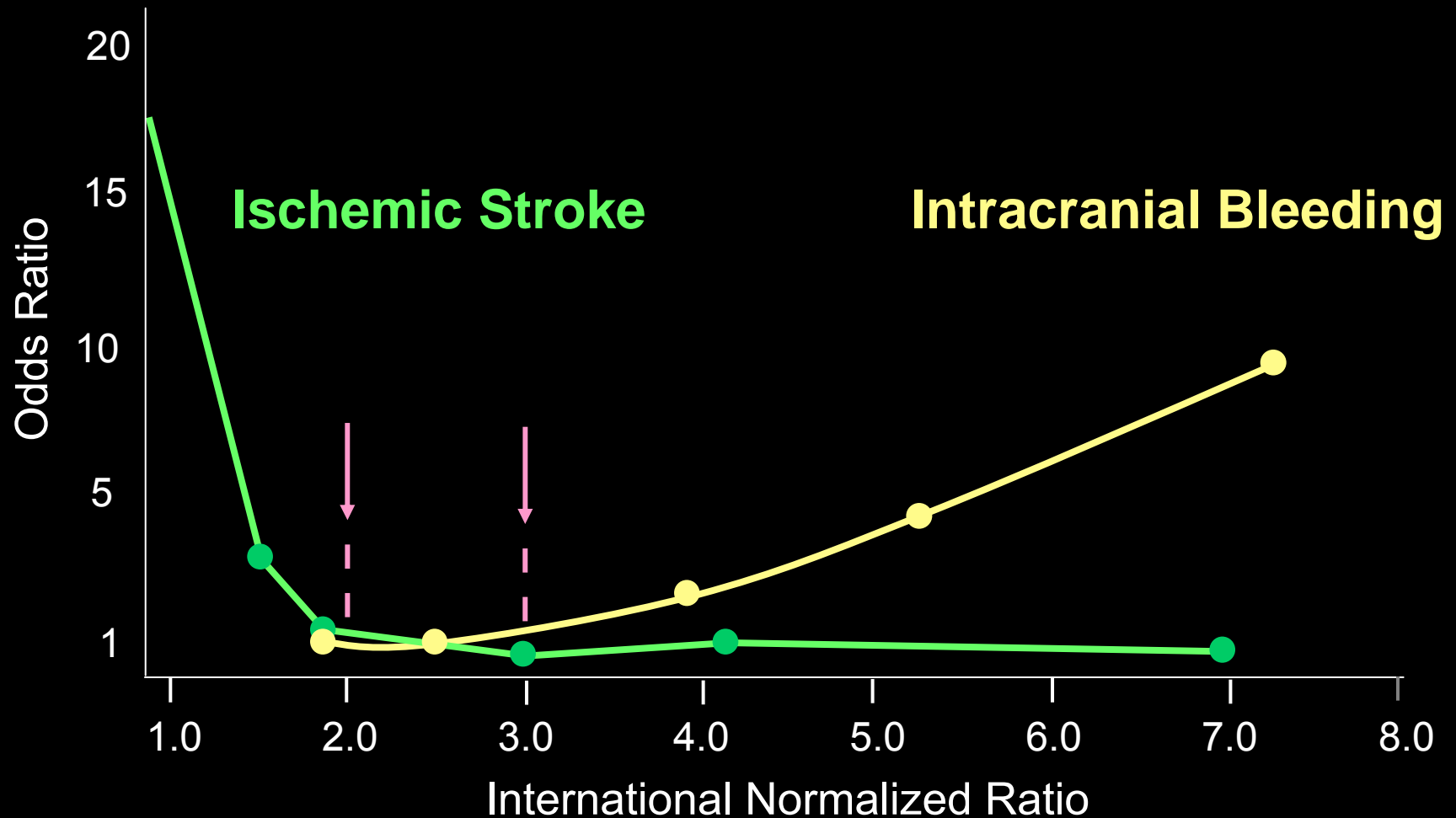
- **The Most Commonly Prescribed Anticoagulant**
- **Patients Maybe:**
 - Resistant - Need Higher Dose to Prevent Strokes
 - Sensitive - Need Lower Dose to Prevent CNS Bleeds
- **Warfarin Is Metabolised by the Cytochrome P450, CYP2C9**
 - SNPs in CYP2C9 Predict Lower Dose
- **Warfarin Metabolism Involves Vitamin K Epoxide Reductase (VKORC1).**
 - SNPs in VKORC1 Predict Higher or Lower Dose

WARFARIN (COUMADIN)

- The Most Commonly Prescribed Anticoagulant
- Targets Vitamin K, an Essential Cofactor for the Modification of Glutamic Acid to γ -Carboxyglutamate, in Coagulation Factors VIII, IX & Prothrombin by Vitamin K Dependent γ -Carboxylase
- Has a Narrow Therapeutic Index that Varies Widely Between Individuals & Requires Constant Monitoring & Adjustment
- Patients Maybe:
 - Resistant - Need Higher Dose to Prevent Strokes
 - Sensitive - Need Lower Dose to Prevent CNS Bleeds
- Warfarin Is Metabolized by the Cytochrome P450, CYP2C9
 - SNPS in CYP2C9 Predict Lower Dose
- Vitamin K Is Recycled by Vitamin K Epoxide Reductase (VKORC1)
 - SNPs in VKORC1 Predict Higher or Lower Dose

INTERNATIONAL NORMALIZED RATIO AT THE TIME OF STROKE OR BLEEDING

Efficacy and Safety of Warfarin



Fang MC, et al. Ann Intern Med 2004; 141:745.

Hylek EM, et al. N Engl J Med 1996; 335:540.

CYP 2C9 VARIANTS ALTER WARFARIN DOSE

- **CYP 2C9*2 Variant:**

- Has ~ 40% of Normal Enzyme Activity
- 2% of Caucasian Are Homozygous; ~20% Heterozygous
- Caucasian Heterozygotes Require 22% Lower Doses of Warfarin

- **CYP 2C9*3 Variant:**

- Has ~ 10% of Normal Enzyme Activity
- ~ 6-10% of Caucasian Population Are Heterozygous
- Caucasian Heterozygotes Require 38% Lower Doses of Warfarin

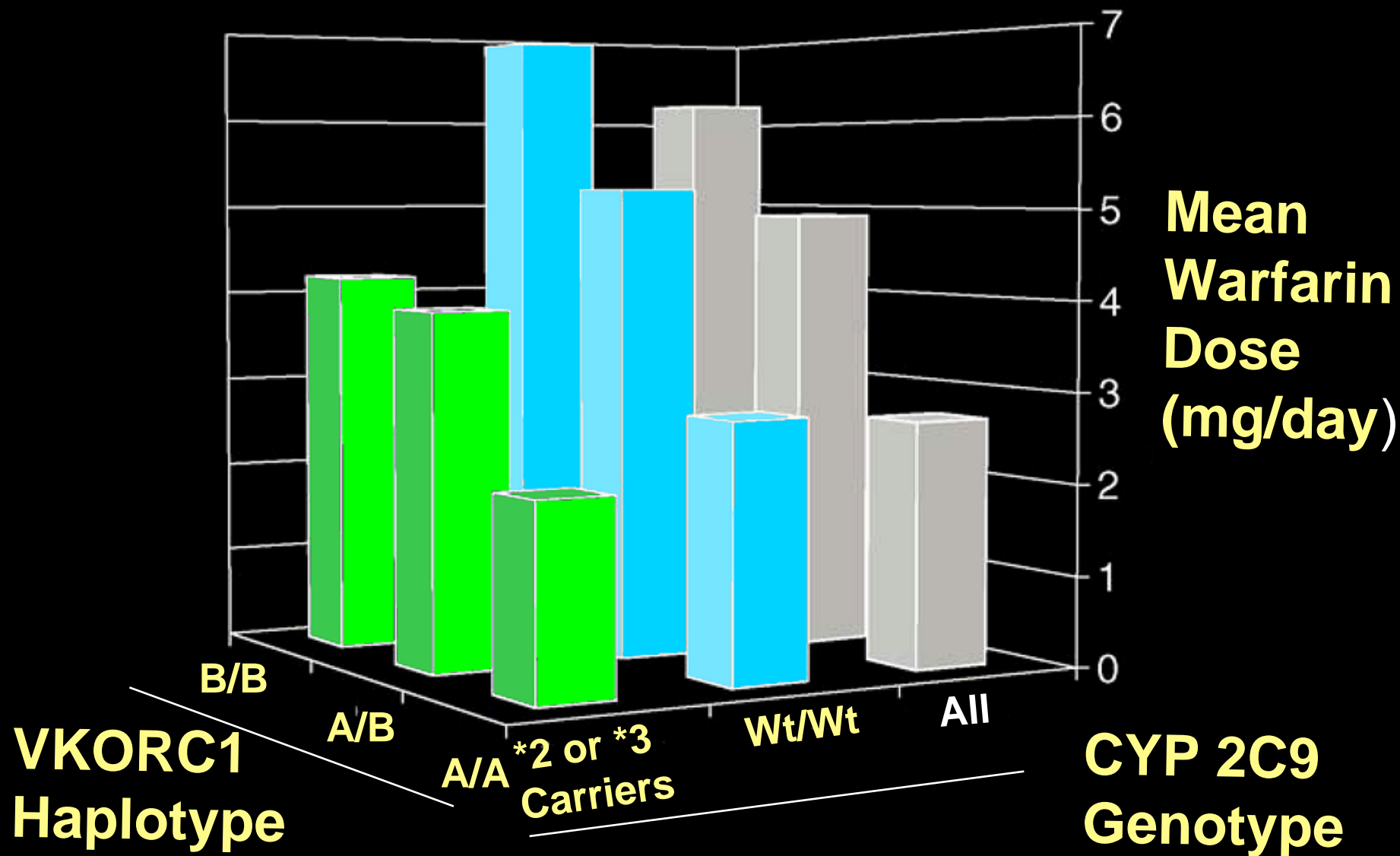
- Patients with Both Variants Experience 2-3 X Increased Risk of Bleeding when Beginning Warfarin

VKORC1 VARIANTS ALTER WARFARIN DOSE

- Two Common Vitamin K Epoxide Reductase Haplotypes (9 Variations in a Sequence of 10 Bases):
 - Haplotype A (Variants 1 & 2) is Associated with Lower Doses of Warfarin
 - Present in ~37% of Caucasians
 - Haplotype B (Variants 7,8 & 9) is Associated with Higher Doses of Warfarin
 - Present in ~58% of Caucasians
- Haplotypes A and B Explain >20% of the Variance in Warfarin Dose

Variant:	A/A	A/B	B/B
Warfarin Dose: (mg/Day)	2.7±0.2	4.9±0.2	6.2±0.3

EFFECT OF VKORC1 HAPLOTYPES & CYP2C9 GENOTYPES ON WARFARIN DOSE



PHARMACOGENETICS: EXAMPLES OF ALTERED DRUG RESPONSE

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• Thiopurine S-Methyltransferase & Cancer	TPMT
• ACE Inhibitors, Antidepressants, Diabetes, Asthma, etc	

TPMT AND CANCER

- Thiopurine S-Methyltransferase (TPMT) Catabolizes the Thiopurine Drugs Mercaptopurine and Azathiopurine which Are Used as Immunosuppressants and to Treat Cancer
 - 20 Years Ago It Was Found that Some Individuals Developed Severe Toxicity Including Myelosuppression
 - Further Studies Showed that The Caucasian Population Could Be Separated Into Three Groups Based on the Level of Red Cell TPMT Activity
-

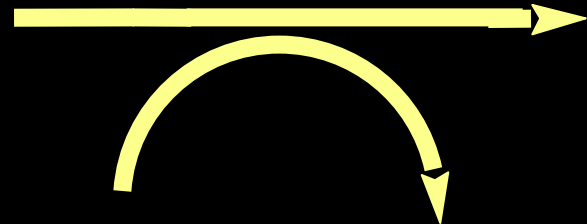
TPMT CATALYZED METHYLATION OF 6-MP

6-Mercaptopurine

6-Methylmercaptopurine



TPMT



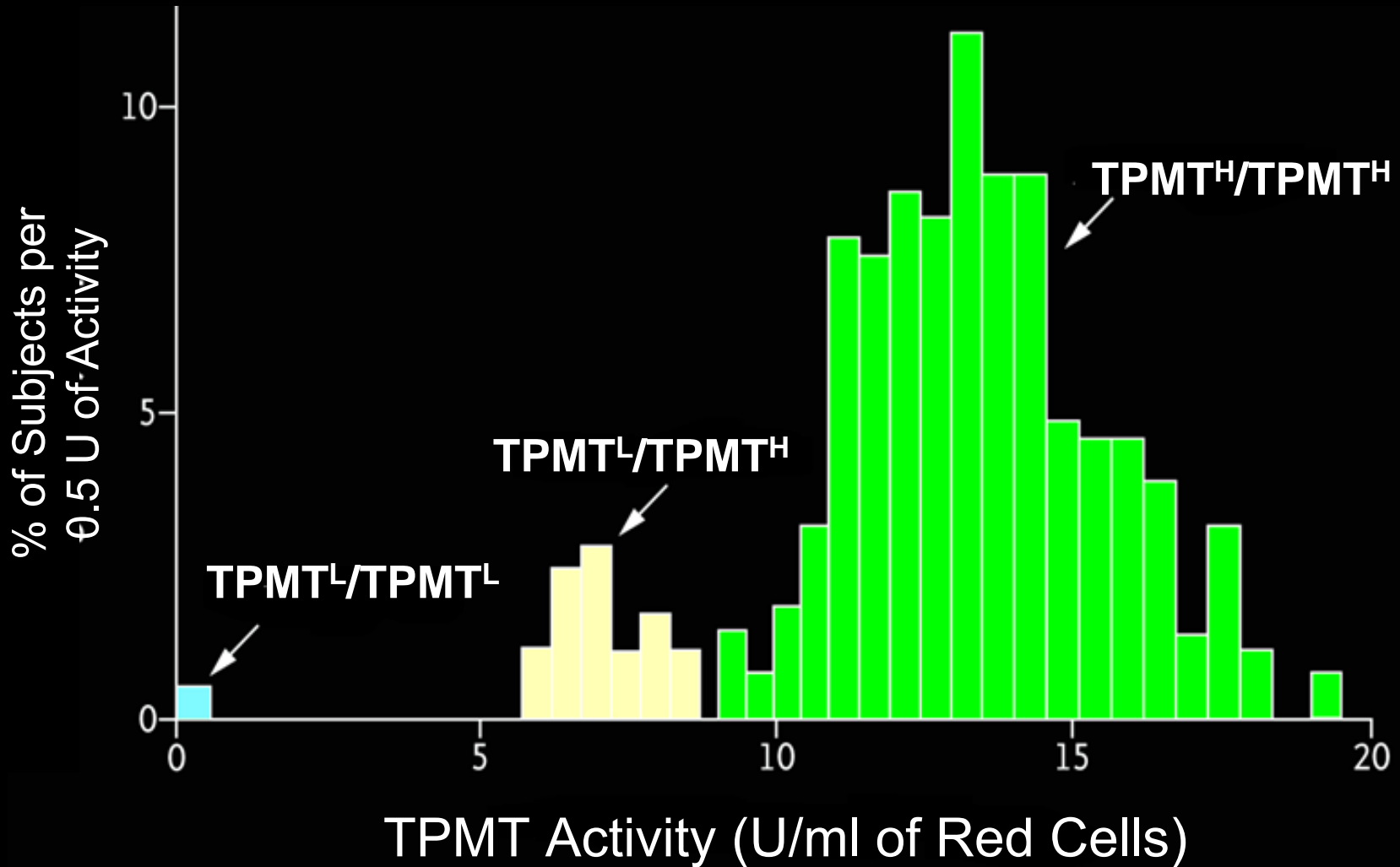
Active

Inactive



6-Thioguanine Nucleotides

DISTRIBUTION OF TPMT ACTIVITY



TPMT GENETIC POLYMORPHISMS: CLINICAL CONSEQUENCES

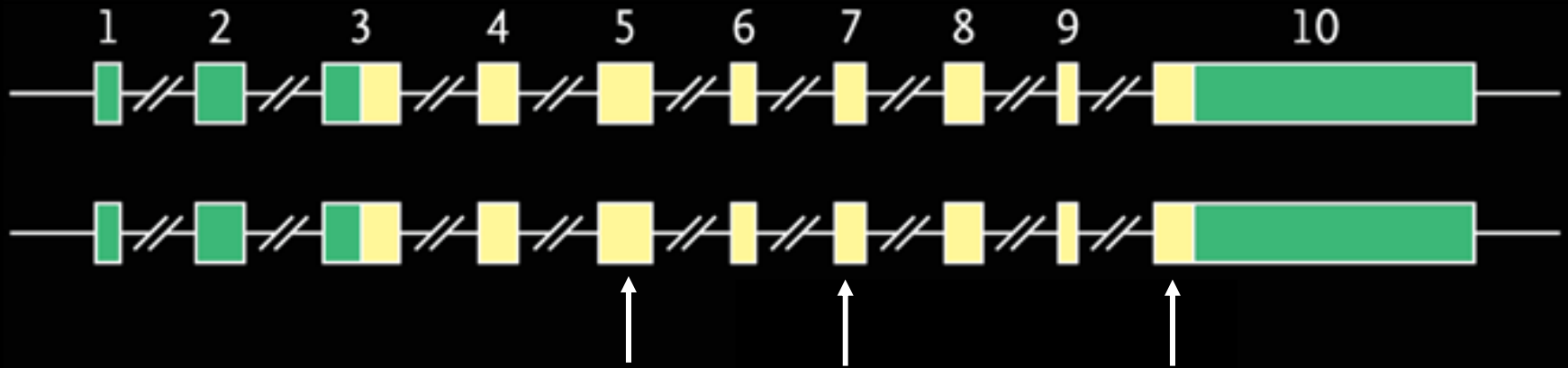
- **Low TPMT:**
 - Increased Thiopurine Toxicity
 - Increased Risk for Secondary Neoplasm
- **Very High TPMT:**
 - Decreased Therapeutic Effect

MOLECULAR GENETICS OF TPMT

Genotyping of TPMT By DNA Determined:

- High Activity: (Normal Allele)
 - ~90% Caucasians and African Americans
- Intermediate Activity: (Heterozygotes)
 - 10%
- Deficient:
 - 1 in 300
 - Several Mutations in Different Ethnic Groups

TPMT Gene: 34 Kb, 10 Exons, Codes for 245 Amino Acids



Common Mutations **G238C** **G460A** **A719G**

Allele	Mutation	Frequency/Ethnic Group
TPMT*1	Wild type	
TMPT*2	G238C	3-5% Caucasians, Most Common Mutant Allele in Asians, Africans and African- Americans
TMPT*3A	G460A/A719G	85% Caucasians
TMPT*3C	A719G	5-13% Caucasians

TPMT POLYMORPHISM

- Patients With Two Nonfunctional Variant Alleles Should Be Given 6-10% Of Standard Dose Of Thiopurines.
- Heterozygous Patients Can Usually Start On Full Doses But Are Significantly More Likely To Require Dose Reduction To Avoid Toxicity

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PHARMACOGENETICS: ACE INHIBITOR RESPONSE

Costa-Scharplatz et al., Cost-Effectiveness of Pharmacogenetic Testing to Predict Treatment Response to Angiotensin-Converting Enzyme Inhibitor. *Pharmacogenet Genomics* 17:359, 2007

- ACE Inhibitors (ACEi) Are Routinely Used in Patients With Cardio-Vascular and Renal Disorders
- The Response to Equivalent Doses of ACE Inhibitors Varies Considerably Among Individuals
- An ACE Gene Polymorphism (287 Bp Insertion in Intron 16) Accounts for ~50% of the Genetic Variance in Serum ACE Levels
 - ~20% of Individuals Have the 287 Bp Insertion
- Caucasian Patients With the 287 Bp Insertion Had a Poor Response to ACEi and Have a 1.4% Higher Risk of Developing End-Stage Renal Disease When Treated With ACEi

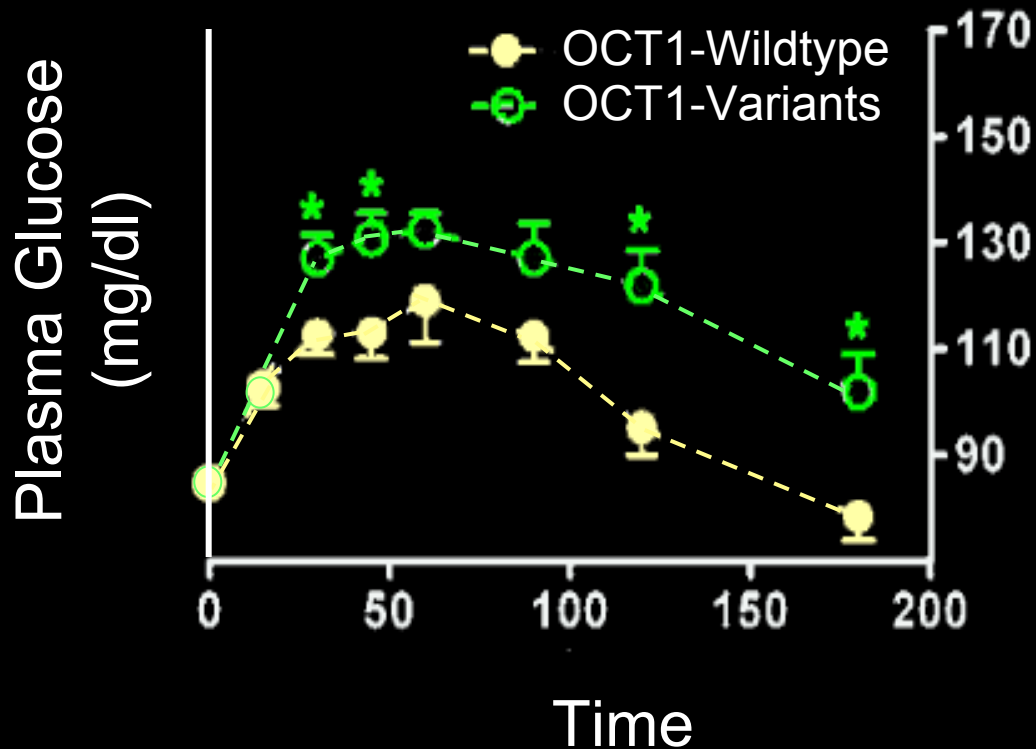
PHARMACOGENETICS: METFORMIN TREATMENT FOR TYPE 2 DIABETES

Shu et al., Effect of Genetic Variation in the Organic Cation
Transporter 1 (OCT1) on Metformin Aciton
J. Clin. Invest. 17:1422-1431, 2007

- Metformin Is a Widely Used Drug for Treating Type 2 Diabetes
- The Organic Cation Transporter 1 (OCT1) Is Involved in the Hepatic Uptake of Metformin
- Human OCT1 Is Highly Polymorphic:
 - OCT1-420del is found in ~20% of American Caucasians
 - OCT1-R61C Is Found in ~7% of American Caucasians
 - Polymorphism Has Reduced Activity for Metformin
- Individuals With the Oct1variants Have Reduced Therapeutic Response to Meformin

ORAL GLUCOSE TOLERANCE TEST AFTER METFORMIN TREATMENT IN HEALTHY INDIVIDUALS WITH OCT1 WILDTYPE & VARIANTS

Shu et al., Effect of Genetic Variation in the Organic Cation Transporter 1 (OCT1) on Metformin Aciton J. Clin. Invest.



PHARMACOGENETICS: ANTIDEPRESSANT THERAPY

Wilkie et al., Pharmacogenet Genomics 17:207-215, 2007

- Antidepressant Drugs Are Among the Most Commonly Prescribed Drugs
- Up to 40% of Patients Fail to Respond to Initial Therapy
- cAMP Cascade Is a Target for the Serotonin 5-Hydroxy Tryptamine and Noradrenaline Systems
 - Activation of Monoamine Receptors Following Antidepressant Treatment Leads to the Generation of cAMP via Adenylyl Cyclase Stimulation by G-Proteins
- A Splice-Site Polymorphism in Exon 10 of the G Protein β -Subunit Gene (GNB3 C825T) Has Functional Consequences
 - GNB3T Results in a 41 Amino Acid Deletion Which Inactivates the G-Protein
- GNB3T Is Significantly Associated With Lack of Remission of Depression and Lack of Response When the Patient Is Switched to a Second Antidepressant Drug

PHARMACOGENOMICS

- Pharmacogenomics Refers to the Investigation of Genetic Variants to Identify Those Involved in Effecting Drug Metabolism
 - Use Genomic Approaches to Identify Genes & Variants Involved in Drug Metabolism
 - Genome –Wide Association Studies
 - RNA Expression Arrays
 - Correlating Gene Expression or Single-Nucleotide Polymorphisms with a Drug's Efficacy or Toxicity
- Pharmacogenomics Is the Whole Genome Application of Pharmacogenetics

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)

A Single Nucleotide Polymorphism (SNP) Is a Nucleotide at a Specific Position in the Genome that Varies in Different Individuals

...CTAGATACG**A**ACTGCATC...
...CTAGATACG**G**ACTGCATC...

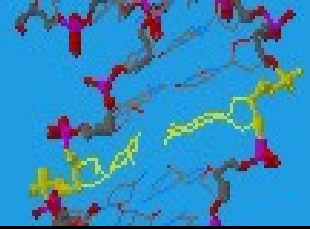
- Frequency: 1: 300 to 500 Nucleotides
- Widely Distributed Among All Chromosomes
- It is a Polymorphism when the Variant Occurs in at Least 1% of a Specific Population
- Useful for Linkage and Association Studies

SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs)



NCBI

Single Nucleotide Polymorphism

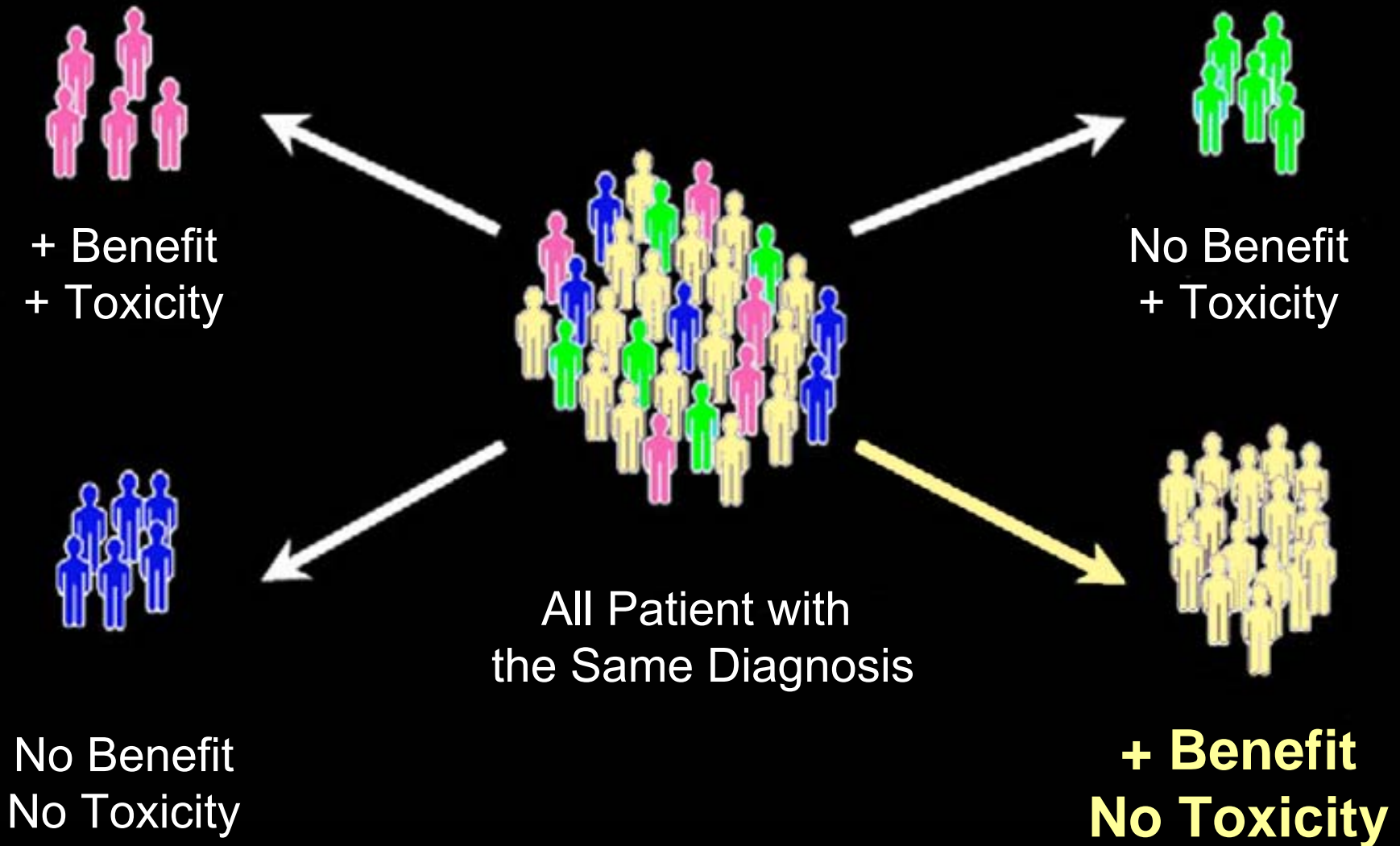


<http://www.ncbi.nlm.nih.gov/SNP/>

31,000,000 SNPs in Database (2/08)

11,880,000 Reference SNPs

PHARMACOGENOMICS AIMS TO IDENTIFY PATIENTS AT RISK FOR REDUCED RESPONSE TO THERAPY



IDENTIFICATION OF GENES FOR MENDELIAN & COMPLEX DISEASES

- **Candidate Gene Analysis:**
 - Select Candidate Genes Based on Disease Pathophysiology, Protein Partners, or Pathway Members
 - Analyze Candidate Genes by Sequencing or SNP Arrays
- **Genome-Wide Association Studies:**
 - Case-Control Studies Comparing Frequencies of 0.5 to 1 M SNPs Throughout the Genome

CANDIDATE GENE ANALYSIS

Case-Control Approach

SNP for Candidate

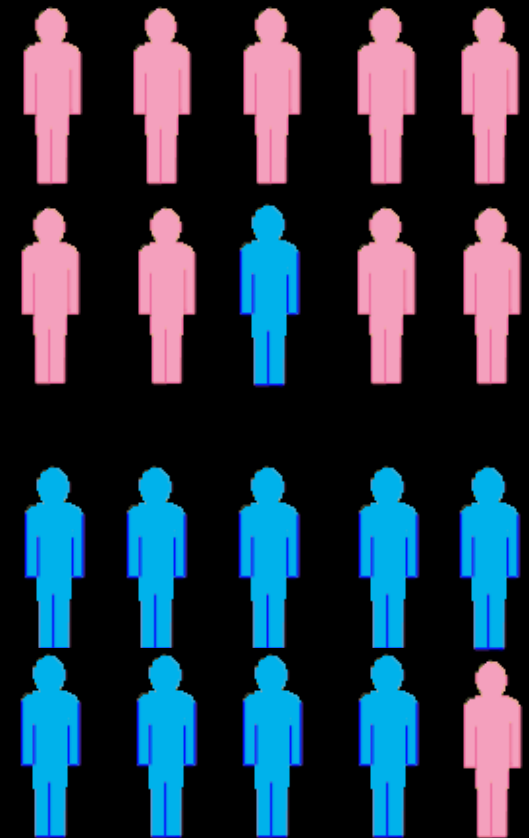
Gene A



Affected

SNP for Candidate

Gene B



Unaffected

SNP for Candidate B Correlates With Disease Phenotype

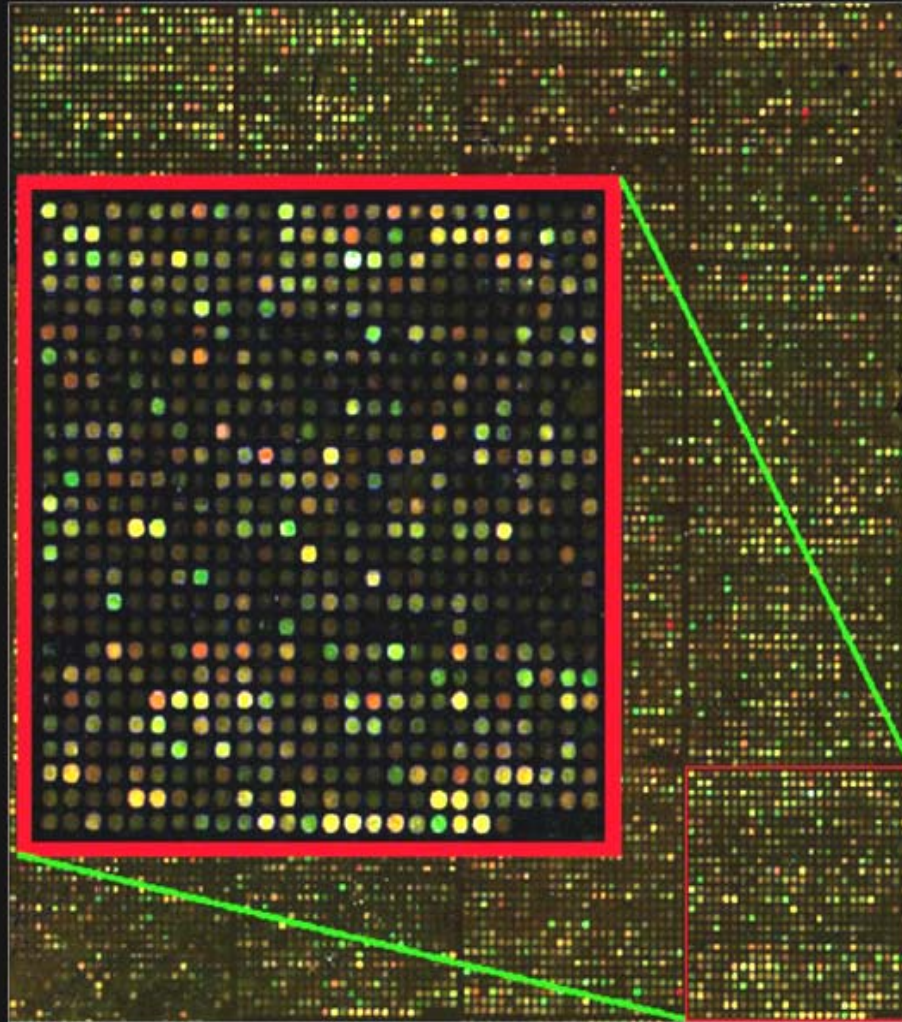
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GENOME-WIDE ASSOCIATION STUDY

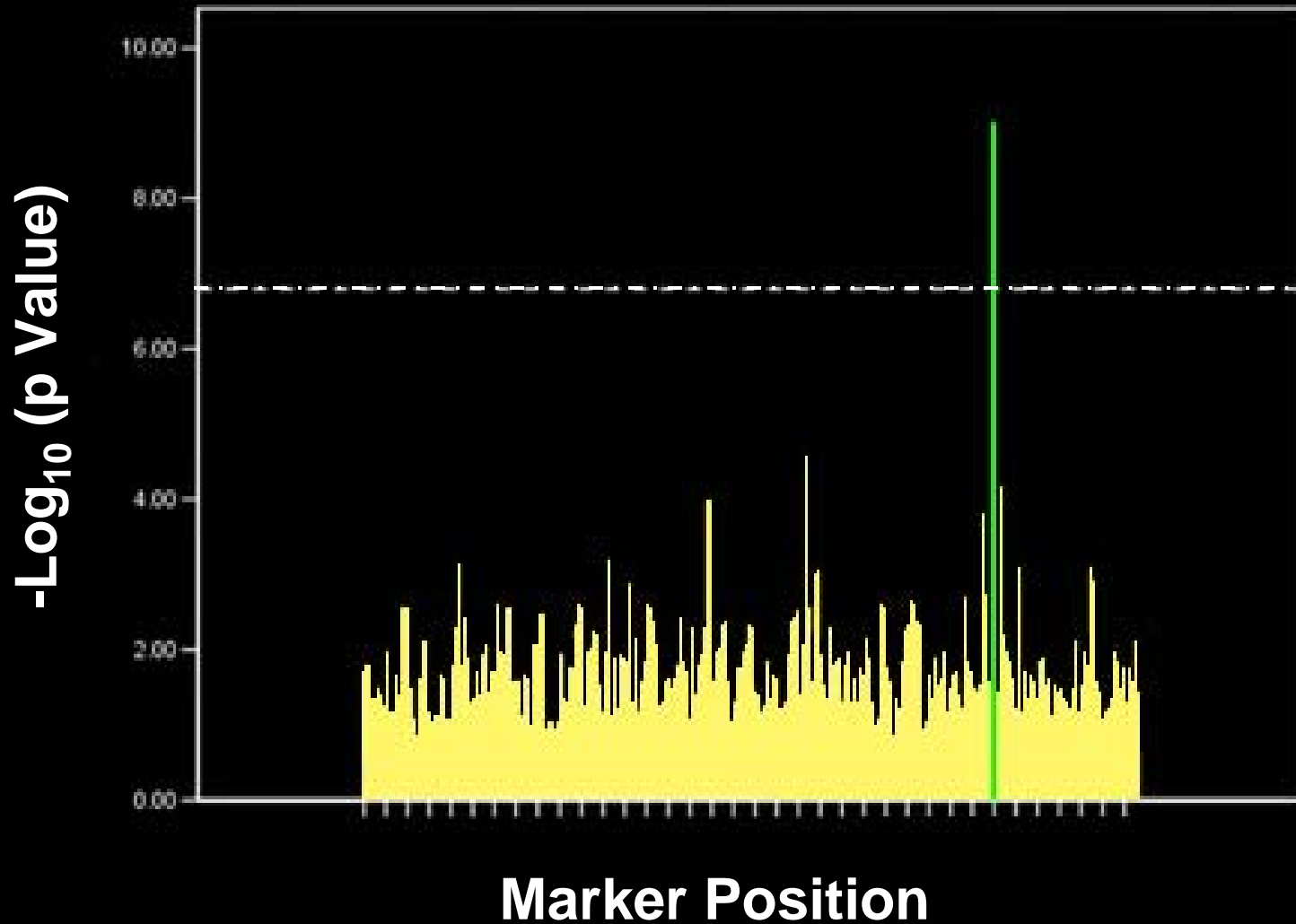
Case-Control Studies

DNA SNP MICROARRAY



GENOME-WIDE ASSOCIATION STUDY

Case-Control Studies



ELEVEN TYPE 2 DIABETES VARIANTS IDENTIFIED BY GENOME-WIDE ASSOCIATION STUDIES

Possible Targets

Example variant	Closest gene	Mode of identification	Previous evidence	Current evidence (p value)*	Additional evidence from human physiology	Odds ratio (per allele)*	RAF (UK)	N‡
rs1801282 (P12A)	PPARG	Candidate	Monogenic + drug target	2×10^{-6}	Nothing consistent	1.14 (1.08–1.20)	0.87	>20,000
rs5215 (E23K)	KCNJ11	Candidate	Monogenic + drug target	5×10^{-11}	Alters insulin secretion in general population	1.14 (1.10–1.19)	0.35	15,600
rs7901695	TCF7L2	Region-wide	None	1×10^{-48}	Alters insulin secretion in general population	1.37 (1.31–1.43)	0.31	2,760
rs4430796	TCF2	Candidate	Monogenic	8×10^{-18}	Nothing consistent	1.10 (1.07–1.14)	0.47	>20,000
rs10010131	WFS1	Candidate	Monogenic	1×10^{-7}	Nothing consistent	1.11 (1.08–1.16)	0.60	>20,000
rs1111875	HHEX-IDE	Genome-wide	Some, e.g. HHEX KO mouse has disrupted pancreatic development	7×10^{-17}	Early studies indicate altered insulin secretion in general population	1.15 (1.10–1.19)	0.65	12,800
rs13266634	SLC30A8	Genome-wide	None	1×10^{-18}	Early studies indicate altered insulin secretion in general population	1.15 (1.12–1.19)	0.69	14,400
rs10946398	CDKAL1	Genome-wide	None	2×10^{-18}	Early studies indicate altered insulin secretion in general population	1.14 (1.11–1.17)	0.32	16,200
rs10811661	CDKN2A-2B	Genome-wide	Some – CDKN2A KO mouse has reduced islet proliferation	8×10^{-15}	Nothing consistent	1.20 (1.14–1.25)	0.83	12,400
rs4402960	IGF2BP2	Genome-wide	Some — binds insulin-like growth factor mRNA	9×10^{-18}	Nothing consistent	1.14 (1.11–1.18)	0.32	16,200
rs8050136	FTO	Genome-wide	None	1×10^{-17}	Alters BMI in general population	1.17 (1.12–1.22)	0.40	10,400

GENOME-WIDE ASSOCIATION STUDIES

Age-Related Macular

Degeneration

Amyotrophic Lateral Sclerosis

Asthma

Atrial Fibrillation

Bipolar Disorder

Breast Cancer

Celiac Disease

Colorectal Cancer

Congestive Heart Failure

Coronary Artery Disease

Crohns Disease

Hypertension

Late-Onset Alzheimer Disease

Lung Cancer

Memory

Nicotine Dependence

Obesity

Parkinson Disease

Psoriasis

Prolonged QT Interval

Prostate Cancer

Rheumatoid Arthritis

Restless Leg Syndrome

Type 1 Diabetes

Type 2 Diabetes

Ulcerative Colitis

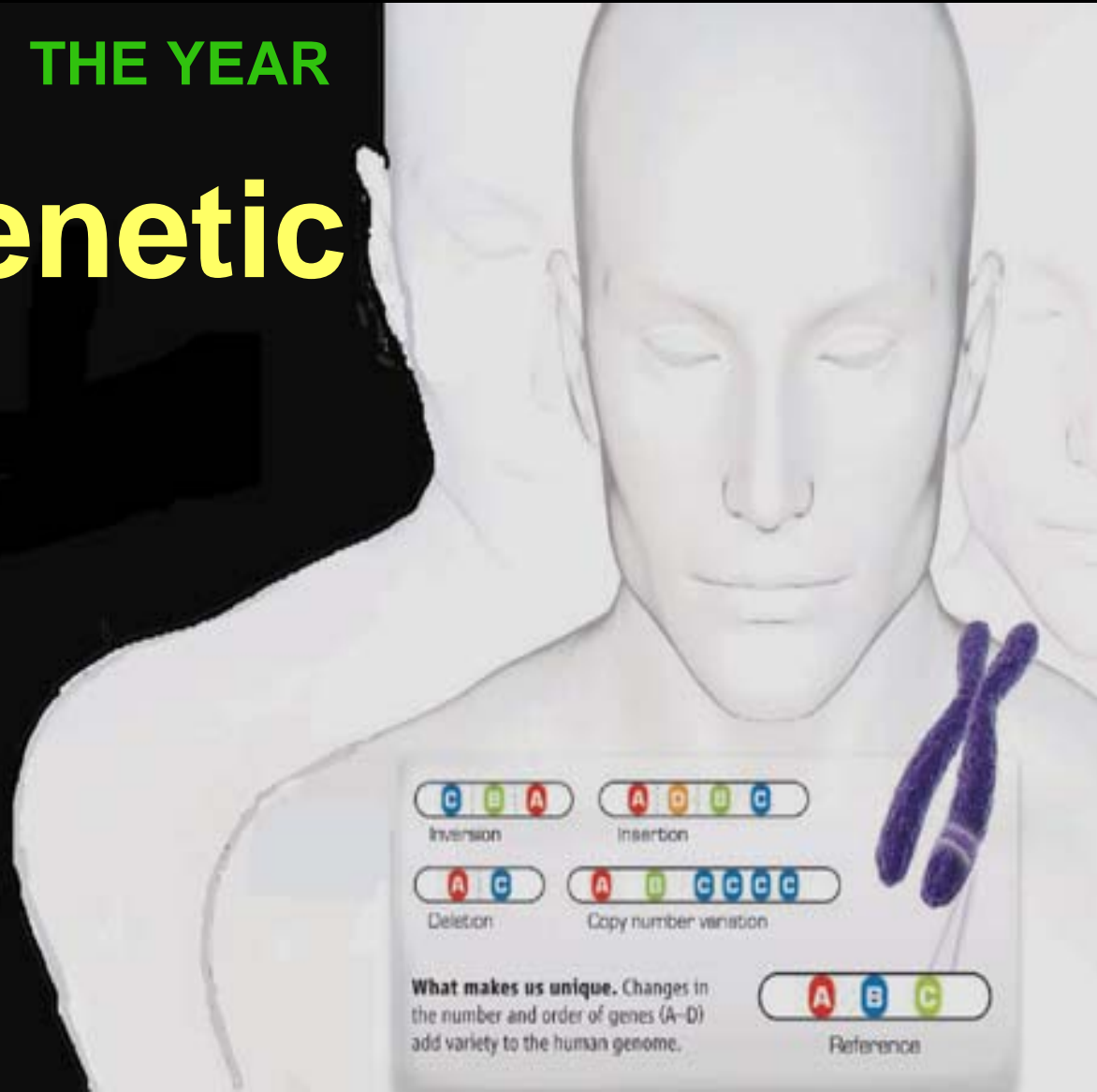
COPY NUMBER VARIATION (CNVs)

Science 318:1842, 2007

BREAK THROUGH OF THE YEAR

Human Genetic Variation

This Year Marked a Tipping Point, as Researchers Became Aware that Variation in Copy Number Affects More Bases than SNPs



HUMAN GENOME PROJECT - 2018

Genetic Disease Information

- **Single Gene Mendelian Disorders:**
 - Causative Genes
 - Common & Rare Mutations
 - Modifier Genes/Polymorphisms
- **Common “Complex” Diseases:**
 - Susceptibility/Predisposition Genes
 - Protective Genes
 - **Pharmacogenetic Variants**
 - Cancer Severity Profiling

PROMISE OF PHARMACOGENOMICS

Current State of Drug Development

Proportion of Patients
Who Respond to Drug



Patient Receiving Drug

Future Drug Development



Population of Patients with Given Disease:

All or Nearly All Respond to Different Drugs Depending on Genotype

USEFUL RESOURCES AND DATABASES FOR GENETIC-BASED STUDIES

- Genetic Association Database: An archive of human genetic association studies of complex diseases. <http://geneticassociationdb.nih.gov/>
- Schizophrenia Gene Database: An archive of genetic association studies performed on schizophrenia phenotypes. <http://www.schizophreniaforum.org/res/sczgene/default.asp>
- Online Mendelian Inheritance in Man: A catalogue of human genes and genetic disorders. <http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM&itool=toolbar>
- Human Gene Mutation Database. A catalogue of published gene lesions responsible for human inherited disease. <http://www.hgmd.cf.ac.uk/ac/index.php>
- Human Genome Variation Database: A catalogue of normal human gene and genome variation. <http://www.hgvbase.org/>
- dbSNP: A catalogue of human single nucleotide polymorphisms. <http://www.ncbi.nlm.nih.gov/projects/SNP/>
- GeneSNPs: A database of polymorphisms in human genes that are thought to have a role in susceptibility to environmental exposure. <http://www.genome.utah.edu/genesnps/>
- PharmGKB: A database of pharmacogenomics research. <http://www.pharmgkb.org/index.jsp>
- GeneCards: A database of human genes that includes genomic, proteomic and transcriptomic information, as well as orthologies, disease relationships, SNPs, gene expression and gene function. <http://www.genecards.org/>

LECTURE SUMMARY

- All Drug Effects Vary From Person to Person & All Drug Effects Are Influenced by Genes
 - Most Drug Responses Are Multifactorial
 - Genetic Polymorphisms of Single Genes Affect Numerous Drug-metabolism Enzymes. Some Polymorphisms Result in Adverse Reactions or Inefficacy of Drugs at Usual Doses
 - Genetic Polymorphisms of Drug Targets & Transporters Are Being Increasingly Recognized As Causing Variation in Drug Responses
 - The Frequency of Variation of Drug Effects Varies in Different Ethnic Populations
-



PHARMACOGENETIC BIOMARKERS AS PREDICTORS OF ADVERSE DRUG REACTIONS*

Gene or Allele	Relevant Drug (Disease)	Specificity	% of Pts with an Adverse Drug Reaction**
TMPT (Mutant)	6-Mercaptopurines (Cancer)	Very Good	1-10
UGT1A*28	Irtecan Colorectal Cancer)	Good	30-40
(CYP2C9 & VKORC1	Warfarin (Anticoagulant)	Good	5-40
CYP2D6 (Mutant)	Tricyclic Antidepressants	Relatively Good	5-7
HLA-B*5701	Abacavir (HIV)	Very Good	5-8
HLA-B* 1502	Carbamazepine (Biopolar /Epilepsy)	Very Good	10 (in Asians)
HLA-DRB1*07 & DQA1*02	Ximelagatran (Anticoagulant)	Good	5-7

* Ingelman-Sundberg, NEJM 358,637,2008; * *Affected Caucasians Except Where Noted

PHARMACOGENETICS: EXAMPLES OF ALTERED DRUG RESPONSE

Enzyme/Disease	Gene
• <i>N</i> -Acetylation & Tuberculosis	NAT2
• Cytochrome P450 Enzyme – Drug Metabolism	CYP2D6
• Warfarin & Coagulation	CYP2C9 VKORC1
• Thiopurine S-Methyltransferase & Cancer	TPMT
• ACE Inhibitors, Antidepressants, Diabetes, Asthma, etc	

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