

# **Clinical Pharmacogenomics**

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# Outline

- Germline Genomics
- Candidate Gene Pharmacogenomics
  - Drug Absorption
  - Elimination
  - Effect
- Pathway Pharmacogenomics
- Genome Wide Studies

# Ten Drugs and Their Available Pharmacogenetic Tests December 2008

- Abacavir
- Imatinib
- 5-Fluorouracil
- Clozapine
- QT-prolonging Drugs
- Irinotecan
- Azathioprine and Mercaptopurine
- Warfarin
- Carbamazepine
- HLA-B\*5701
- BCR-ABL
- DPYD-TYMS
- 2 SNPs in HLA-DQB1
- Familion™
- UGT1A1
- TPMT
- CYP2C9 and VKCoR
- HLA-B\*1502

# The Genomic Revolution



Why Genomics?

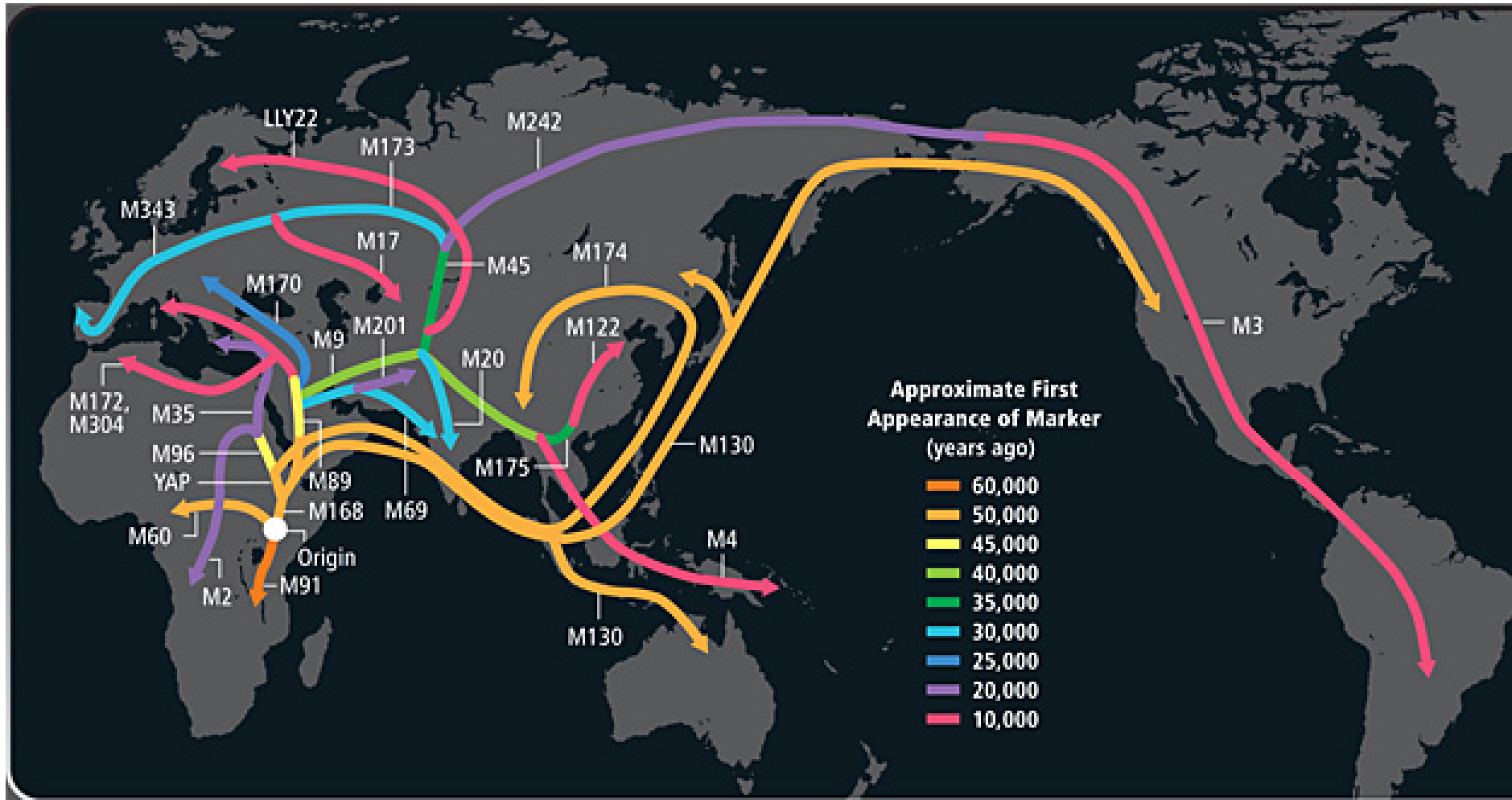
The Genome Map is available on the web, to anyone, free.

The Human Hapmap is available on the web to anyone, free.

DNA is very stable

DNA can be amplified

# Human Migration out of Africa



# MICROSATELLITES

Microsatellite

G AGT AGT AGT AGT AGT C

5 copies

T C C G AGT AGT AGT C T C T

3 copies

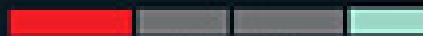
C G AGT AGT AGT AGT C T C

4 copies

# COPY NUMBER VARIANTS



Reference genome slice



Deletion



Duplication

# SNP Variability in The Human Genome

## July 2008

- 2.85 billion base pairs
- ~22,000 genes
- 1.7% of the genome codes for protein
- 3.3% of the genome is as conserved as the 1.7% that codes for protein
- On average 1 SNP/1.2kb
- 10 - 15 million SNPs that occur at  $> 1\%$  frequency
- ~450,000 SNPs in MCS (Multiply Conserved Regions)
- Copy number variations exist in 5-7.5% of the germline genome
- Most tumor DNA sequence is identical to that of the host
- **4-5% of the genome is in areas with high copy number variation**

# SNP Variability In Exons

- ~150,000 SNPs in known exons
- 48,451 non-synonymous SNPs
- 1113 introduce a stop codon
- 104 disrupt an existing STOP



# PharmGKB as a source of Candidate Genes and Pathways

PharmGKB: The Pharmacogenetics and Pharmacogenomics Knowledge Base - Mozilla Firefox

http://www.pharmgkb.org/

PharmGKB  
The Pharmacogenetics and Pharmacogenomics Knowledge Base

Search PharmGKB: ?

Home Search Submit Help PGRN Contributors Sign In Feedback

PharmGKB curates information that establishes knowledge about the relationships among drugs, diseases and genes, including their variations and gene products. Our mission is to catalyze pharmacogenomics research.

Click for more information

genotyped genes 608	variants of interest 360	drugs 546	pathways 53	diseases 542	important PGx genes VIP 36	SNP arrays 2	downloads & services
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Search PharmGKB: ?

e.g. a gene ("CYP2B6"), drug ("meperidine") or disease ("pulmonary embolism")

**What's New?**

- New PharmGKB Initiative: Variants of Interest (Click on Second Home Page Icon to View!)
- For Submitters: Status of Submission to dbSNP is shown on your group's submission page!

See the [archives](#) for more.

**Curator's Favorite Papers**

- [Pharmacogenetics of ACE inhibition in stable coronary artery disease](#) CO PD GN
- [Pharmacogenetics of P450 oxidoreductase](#) PK GN
- [Regulatory SNP in VKORC1 affects gene expression and warfarin dose requirement](#) FA GN

Updated 6/30/08. See the [archives](#) for more.

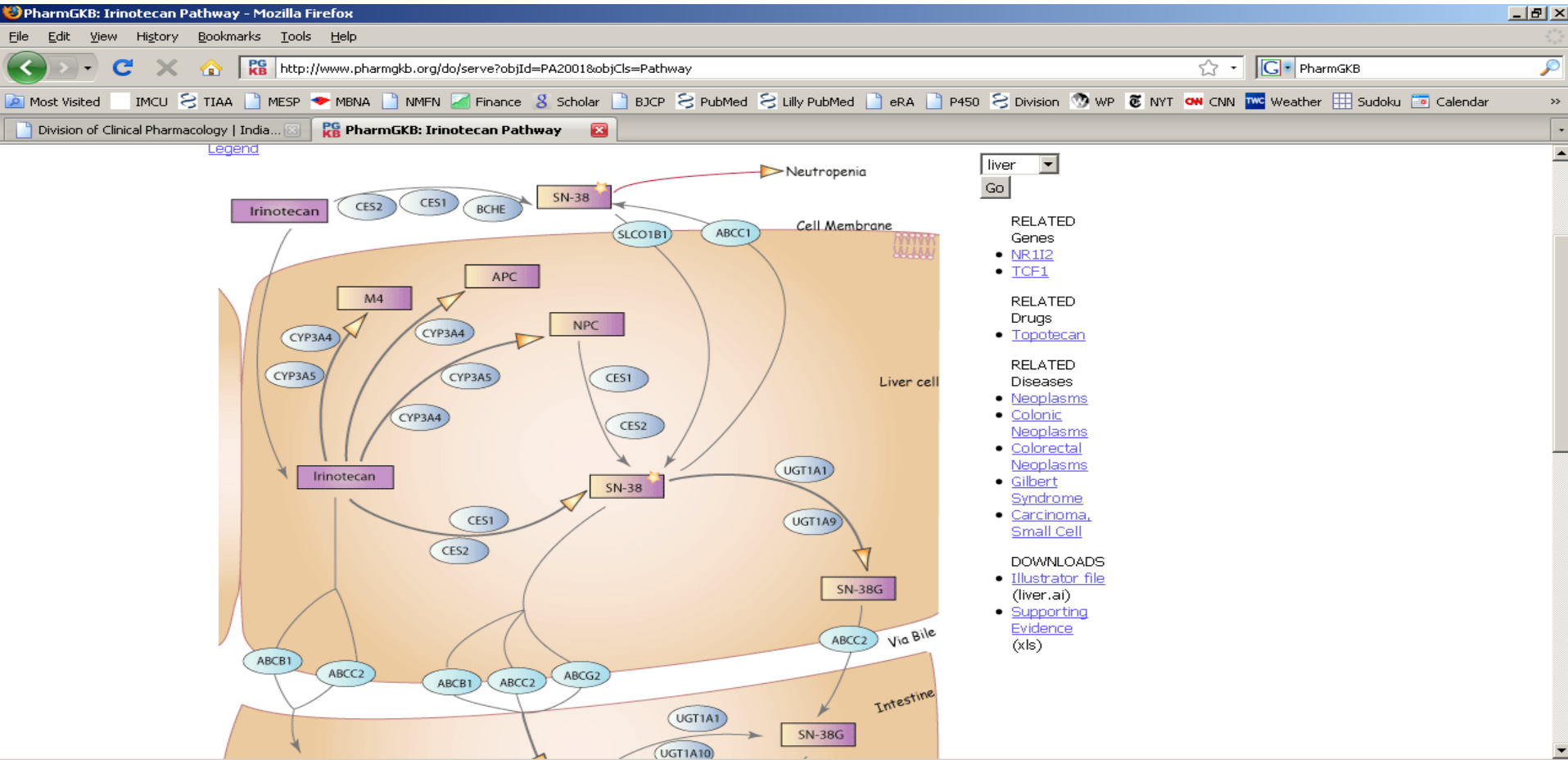
**PGx Information Flow**

Items in the flow chart below are clickable:

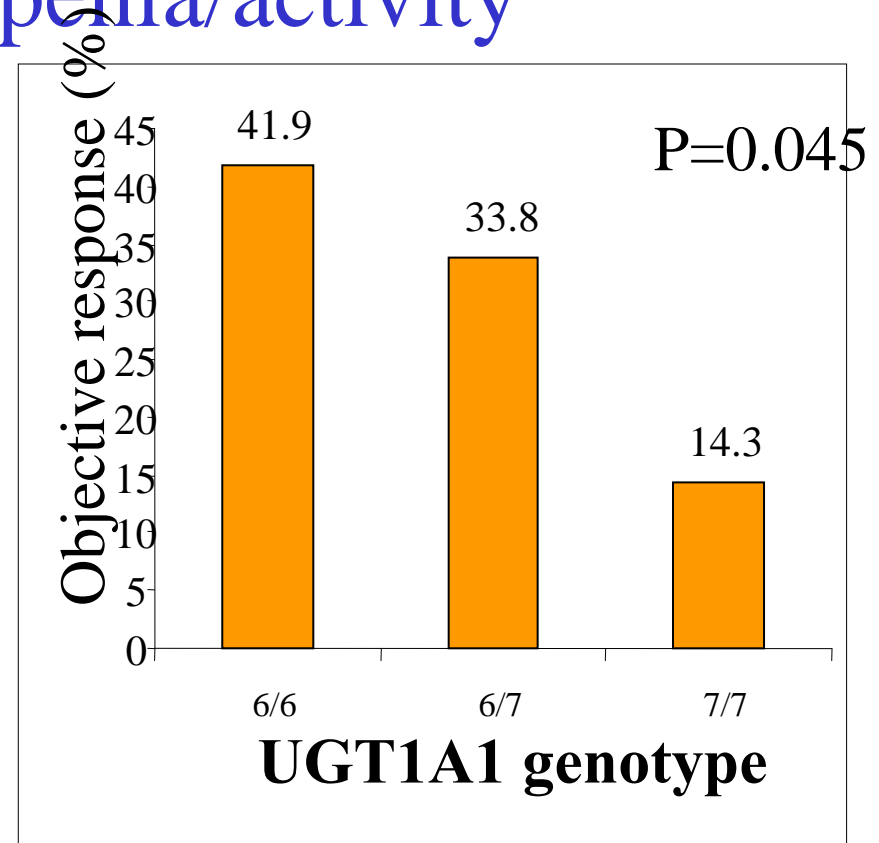
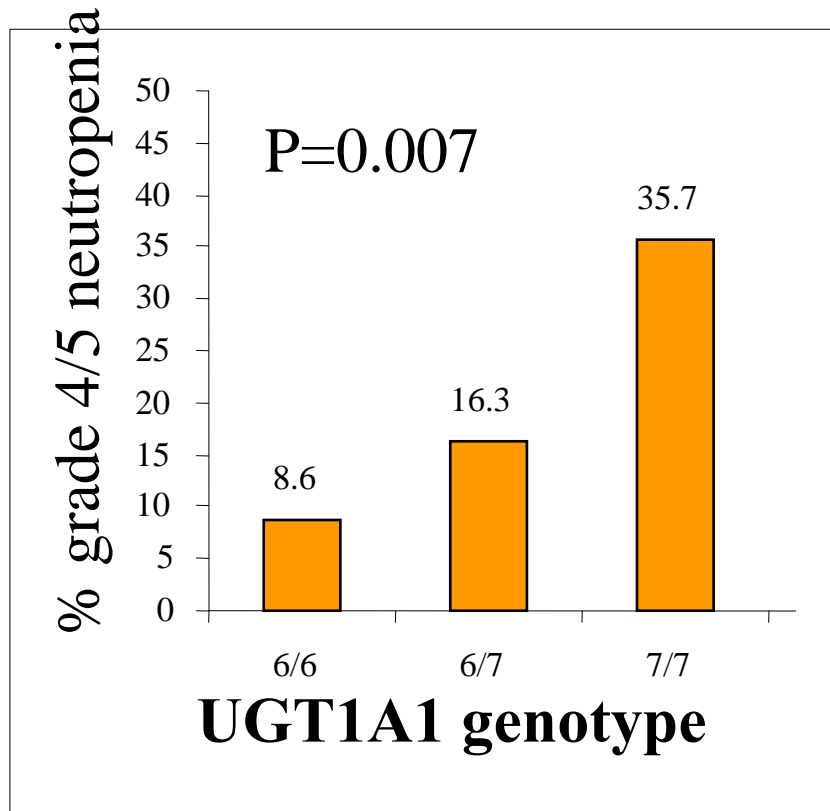
```

graph TD
    Variants[Variants GN] --> Genes[Genes PK]
    Genes --> Drugs[Drugs]
    Drugs --> PK[PK: Absorption, Distribution, Metabolism, Excretion]
    Genes --> CO[CO: Clinical Outcome]
    Genes --> PD[PD: Pharmacodynamics]
    Genes --> DR[Drug Responses]
  
```

# PharmGKB Irinotecan Pathway



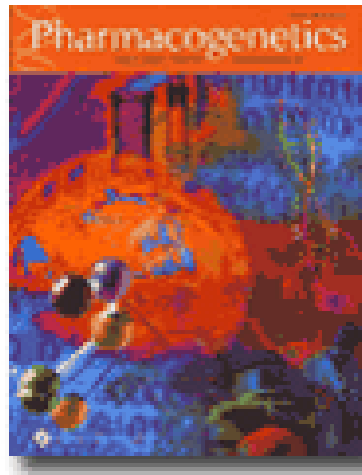
# UGT1A1 TA repeat genotype alters irinotecan neutropenia/activity



N=524

McLeod H. et al, 2003.

# Pharmacogenomic Journals, 2008



**Pharmacogenomics**

July 2002 Vol. 3 No. 4

Reviews & Reports

- Current pharmacogenomic approaches to clinical drug development
- Pharmacogenetic diagnosis of cytochrome P450 polymorphisms
- The DR-ACE genotype and cardiovascular disease
- DNA microarray technology and antimicrobial drug discovery
- Human polymorphic proteins
- Quantitative gene profiling
- Phase display and pharmacogenomics
- Therapeutic target discovery using Caenorhabditis elegans
- Large-scale SNP scoring from unamplified genomic DNA
- Approaches to allele frequency determination

# Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

SNPs that change clinical outcome



SNPs that change drug response



SNPs that change pharmacokinetics



SNPs that change activity *in vitro*



Non-conservative amino acid changes



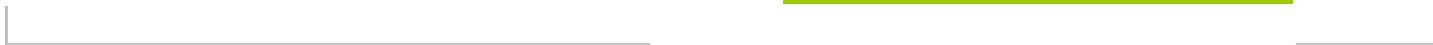
Non-synonymous SNPs in exons



Exon-based changes

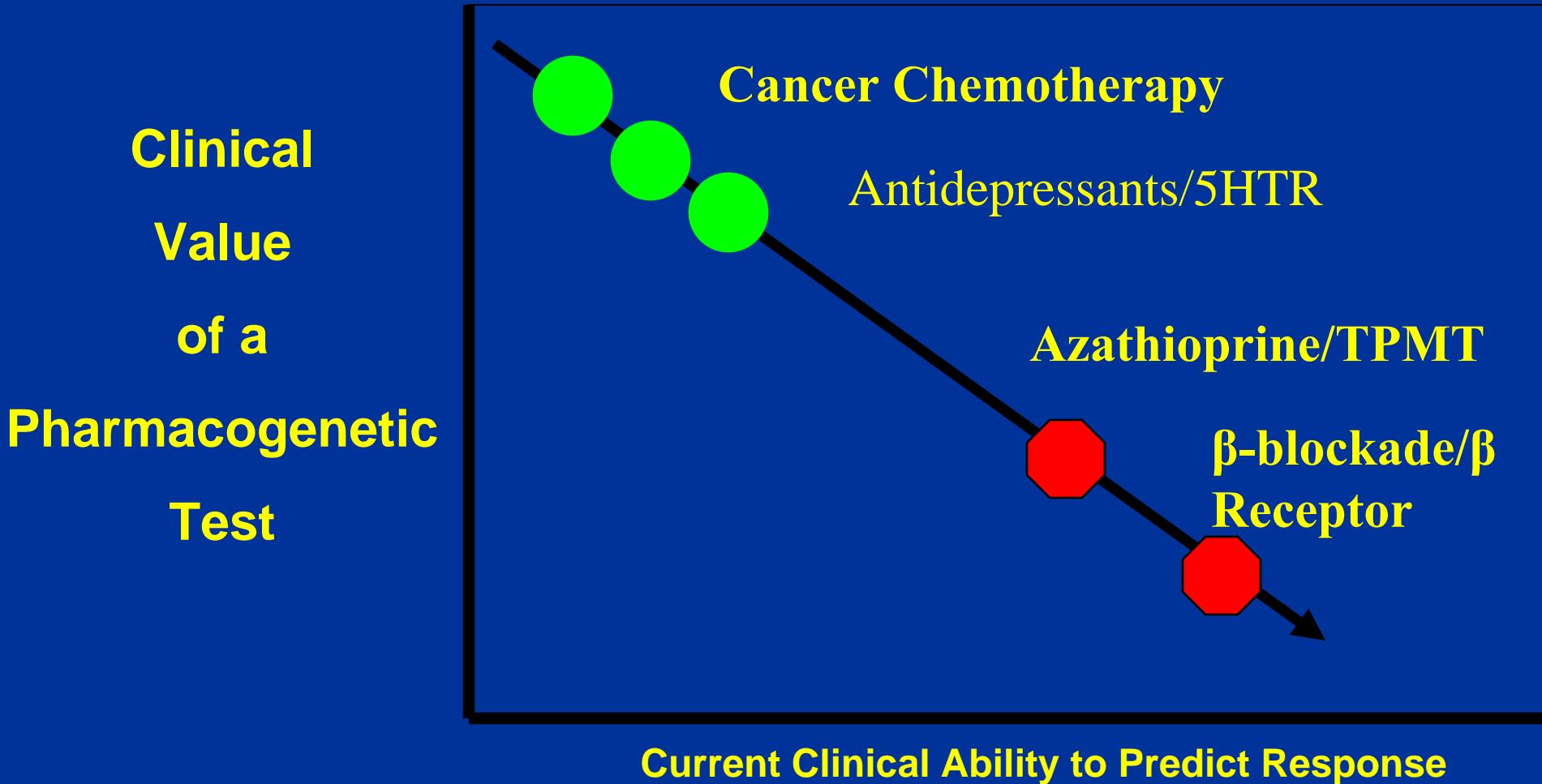


All SNPs




# Pharmacogenetic Principle 1:

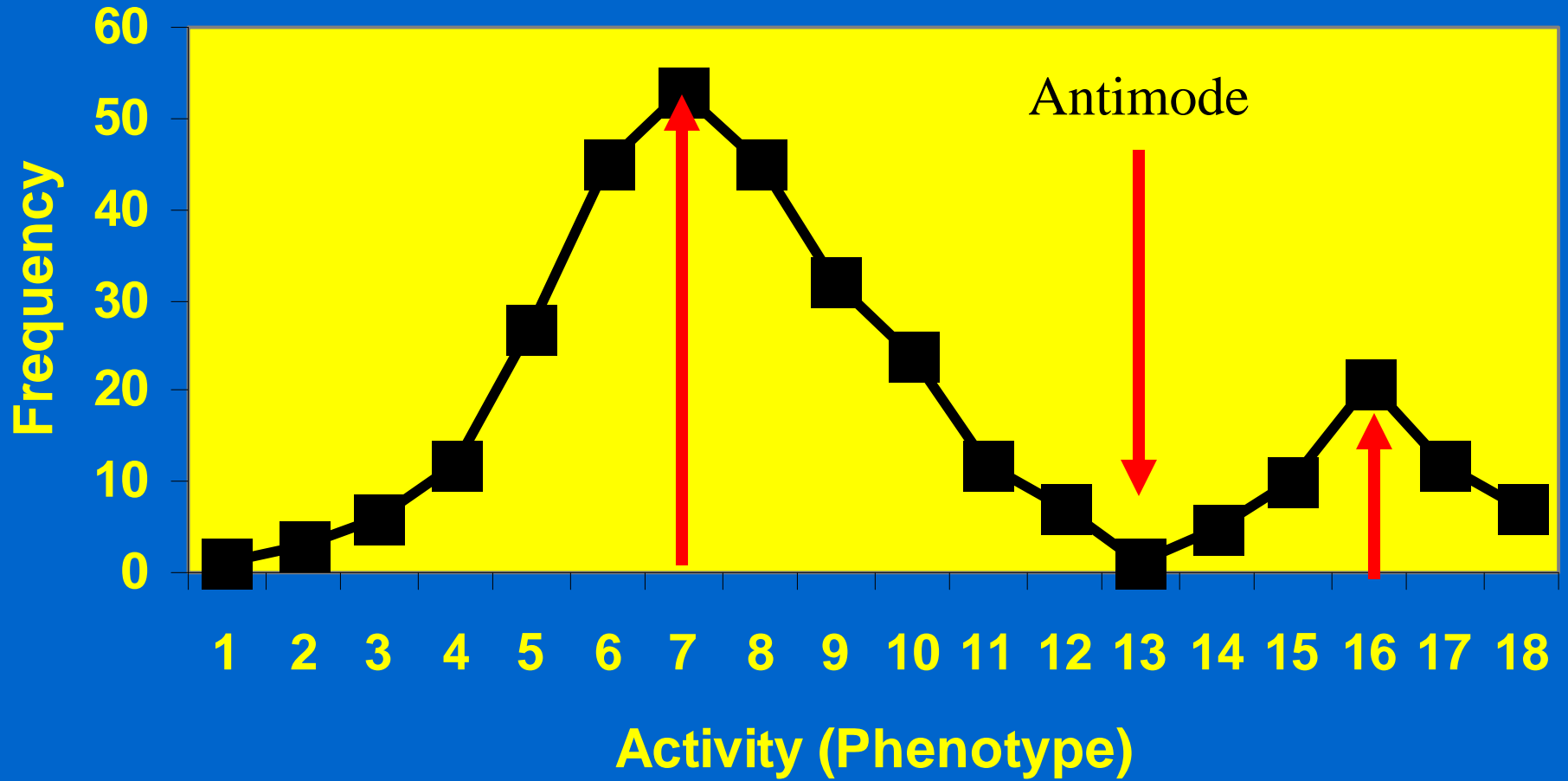
Value Decreases when Current Predictive Ability is High



# Methods in Pharmacogenetics

- SNP discovery:
    - Candidate gene approach
    - Pathway approach
    - Genome Wide Arrays
    - Next Generation Sequencing
  - Identification of gene and variants
  - Development of a genetic test for DNA variants
  - Correlation between genotype and phenotype
  - Validation
  - **Application in Clinical Practice**
- 

# Polymorphic Distribution

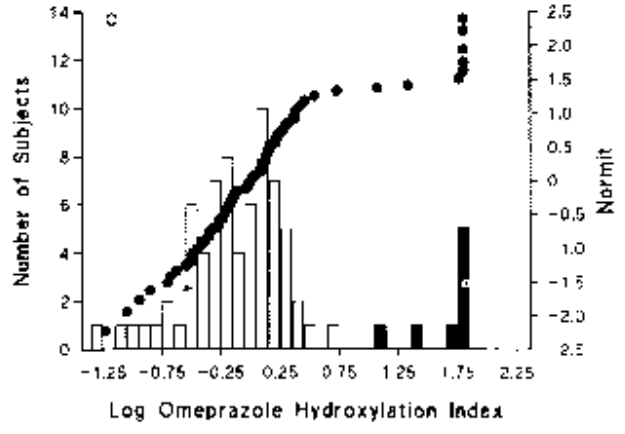
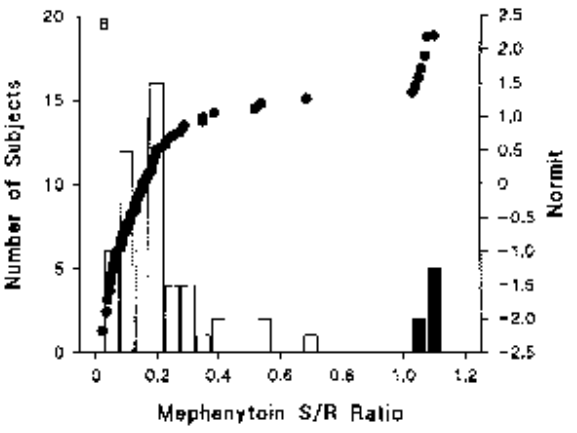
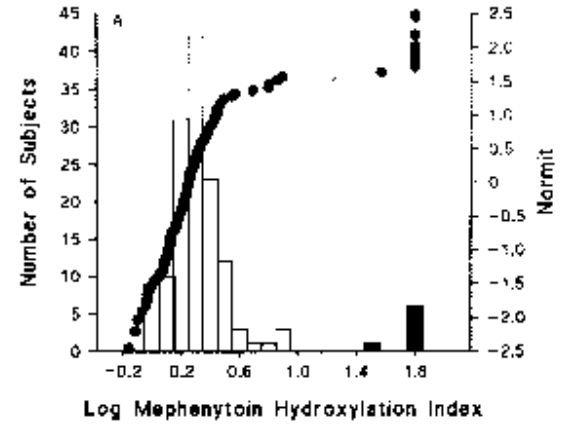




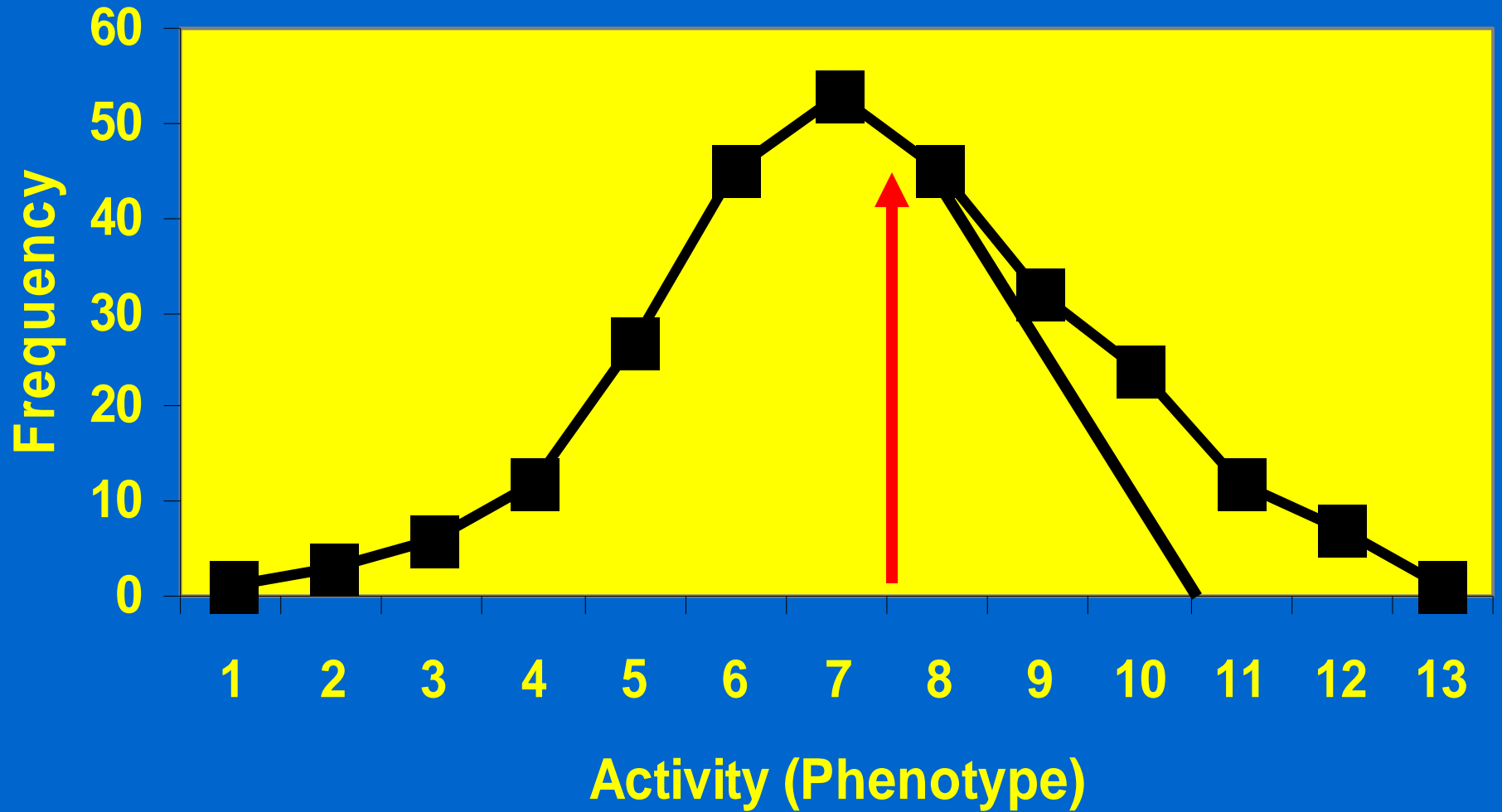
# The Value of Normit Distribution Plots:

## Population Distribution of CYP2C19 phenotype

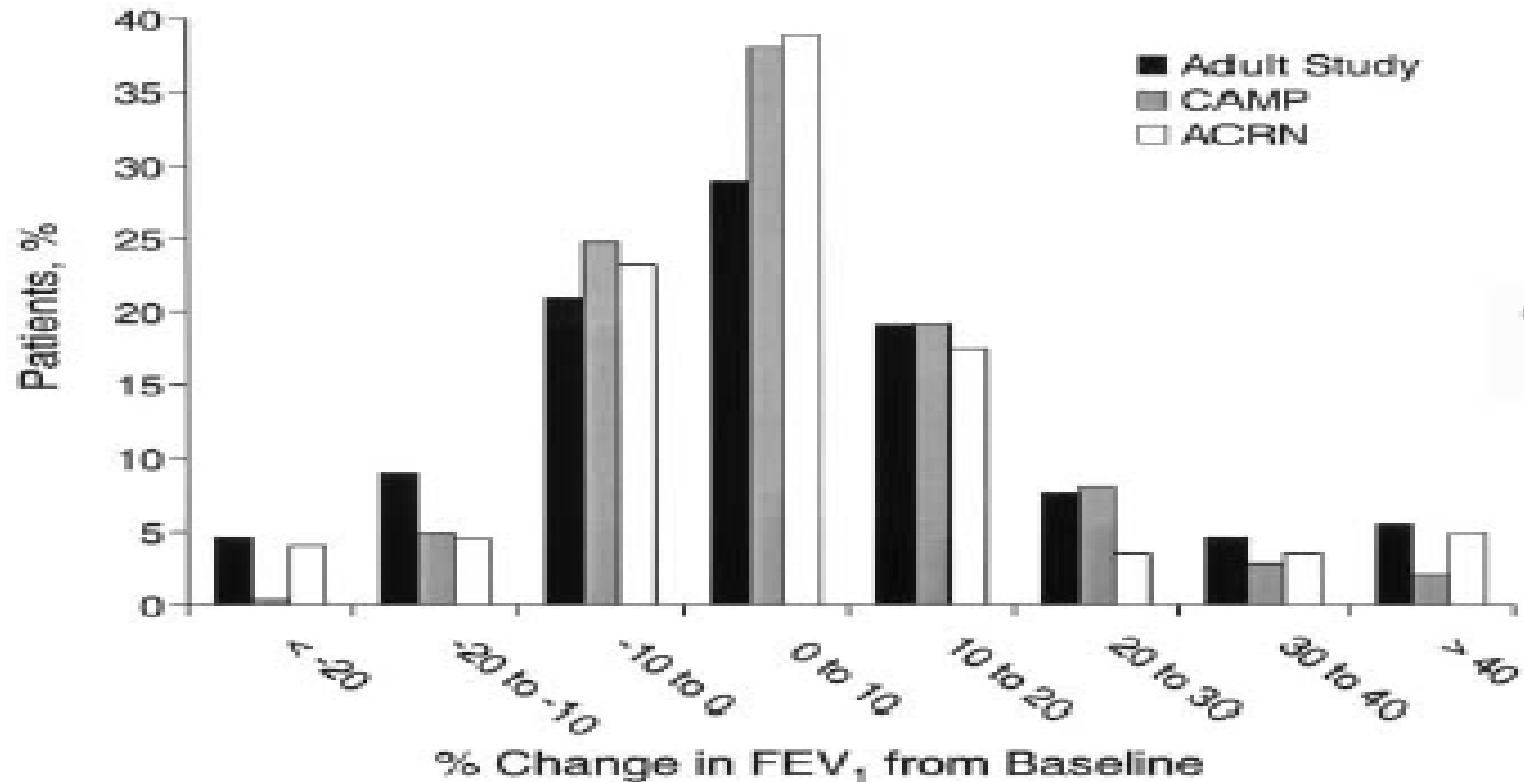
*Flockhart et al: Clin Pharmacol Ther 1995;57:662-669*



# Skewed Distribution



# Example 1 of a Skewed Distribution: Heterogeneity in response to Inhaled Corticosteroids

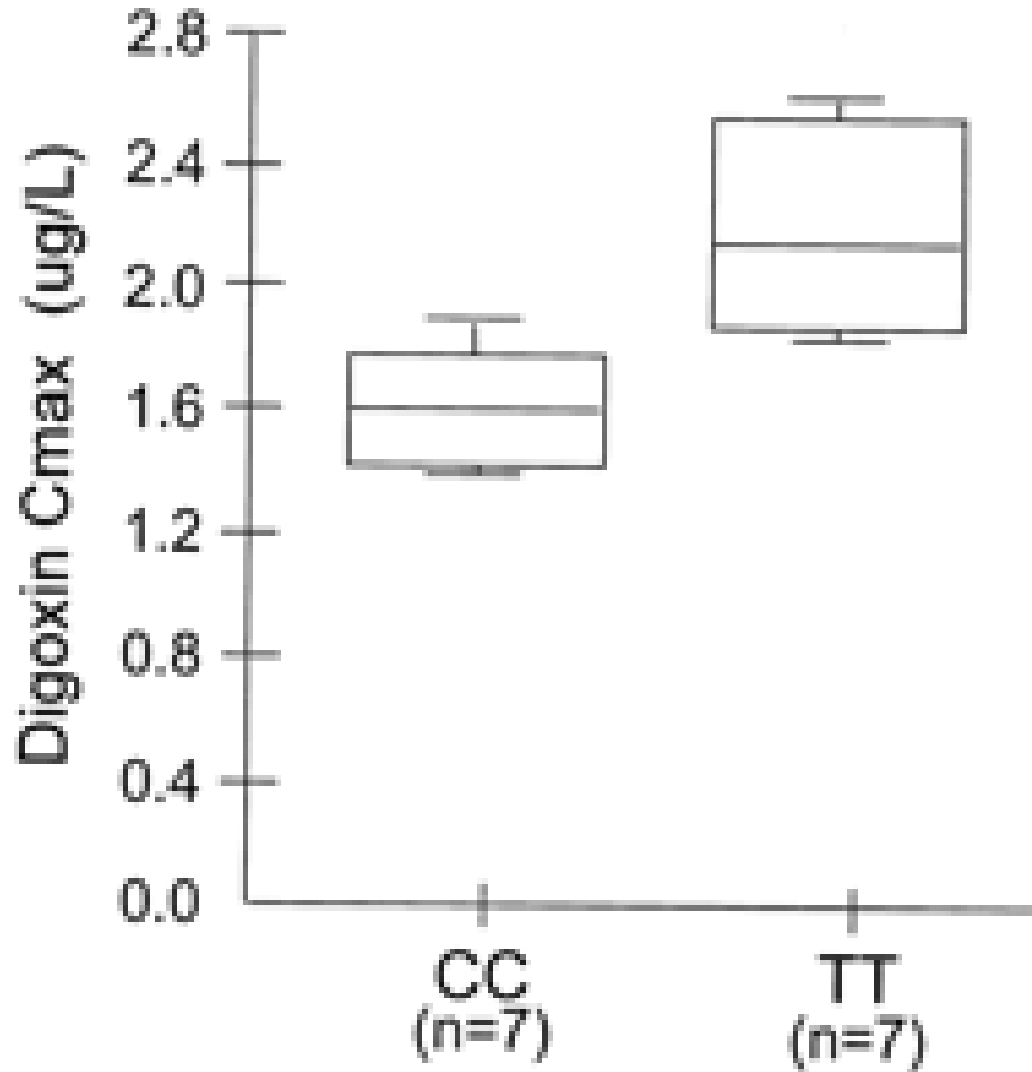


# Lessons

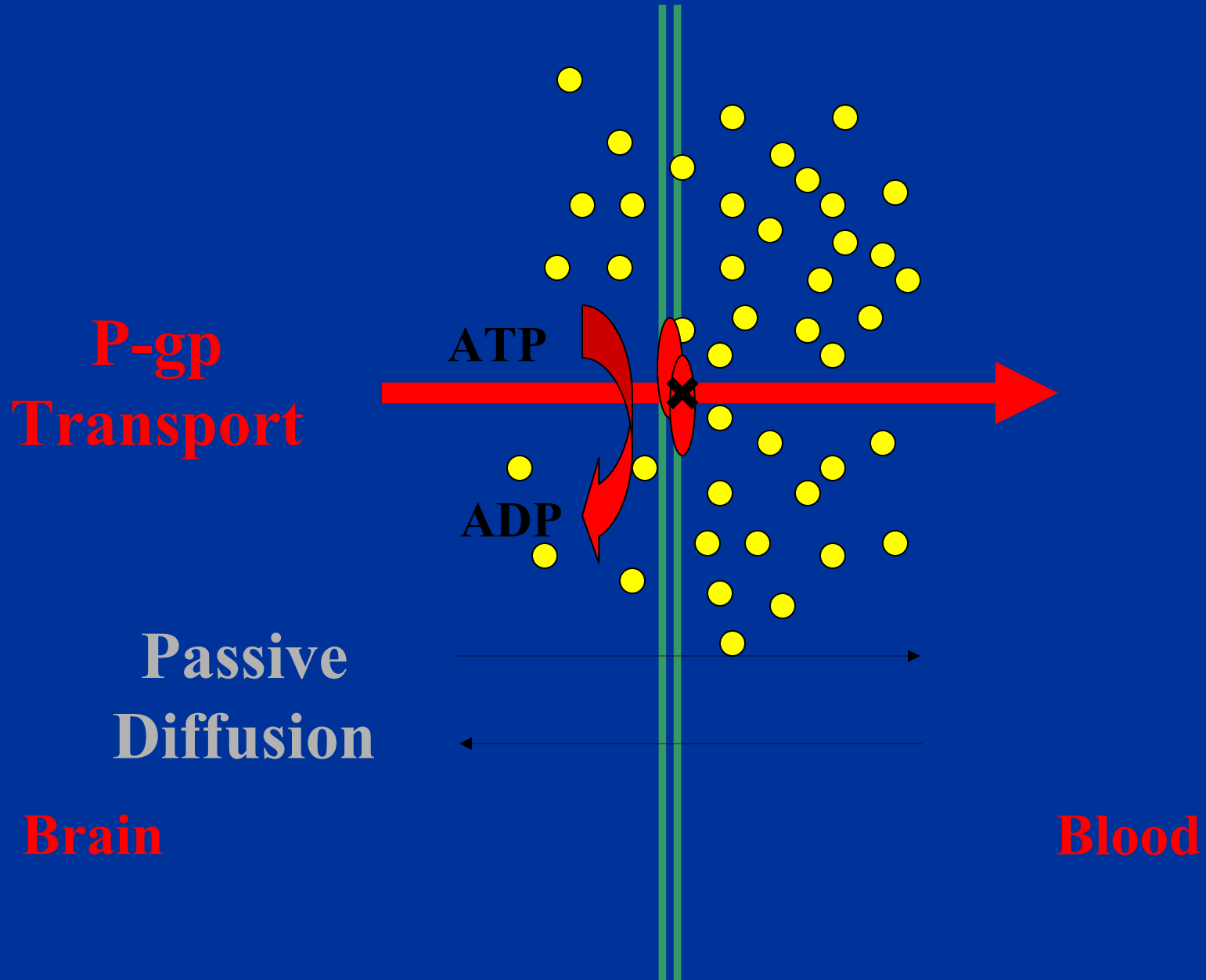
- Germline genetic variation is a potentially valuable biomarker for many drug effects
- Extremes of phenotype are often viewed as “discardable data”, but outliers (patients or events) should be viewed as important research stimuli
- Drug effects on populations can obscure effects on individual patients. A significant proportion of people may be harmed by a beneficial drug.

# Genetics and Drug Absorption

0.25 mg of digoxin po at steady state



# Digoxin Transport across the Blood-Brain Barrier



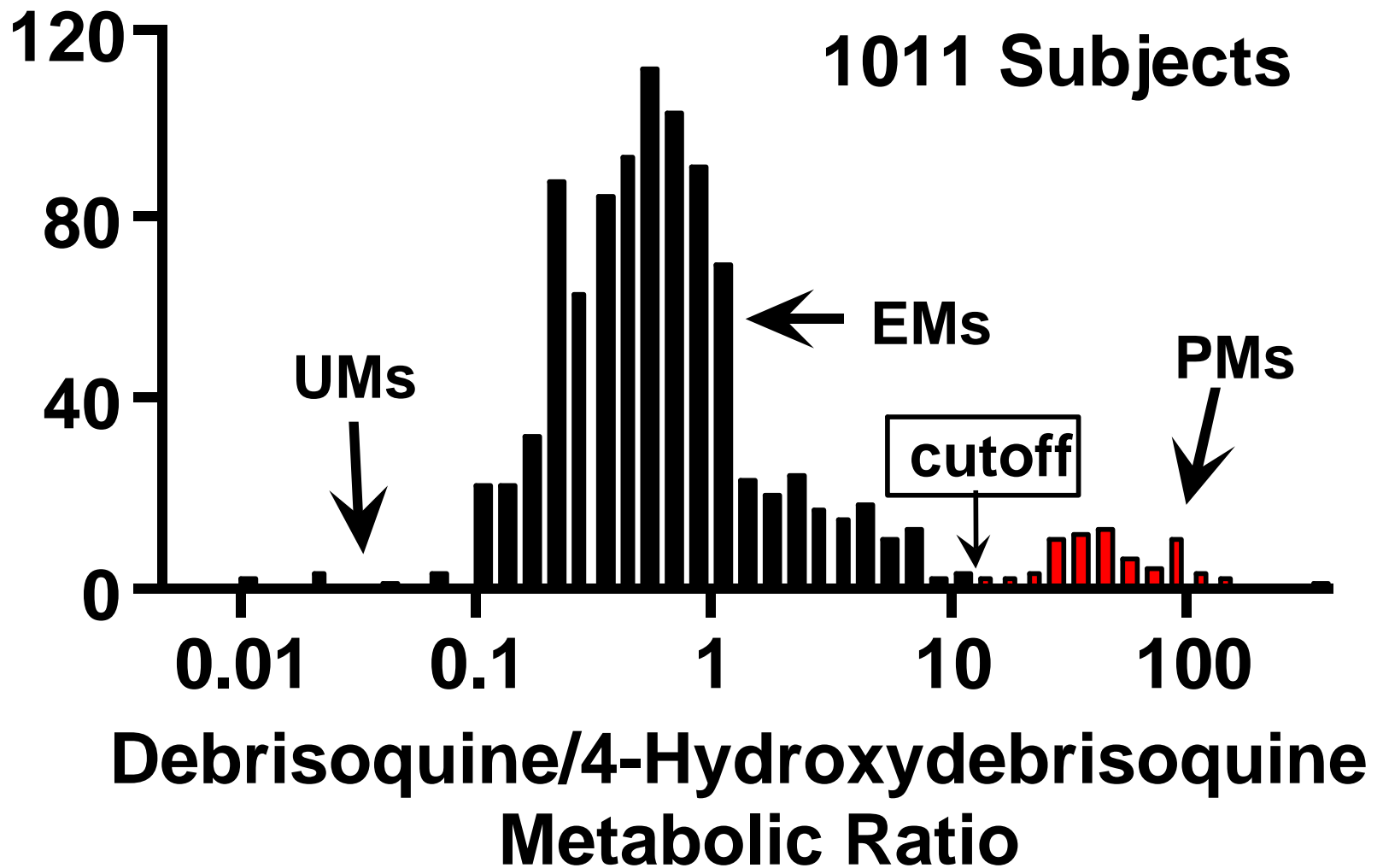
# Genetics and Drug Elimination



# Cytochrome P450 2D6

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

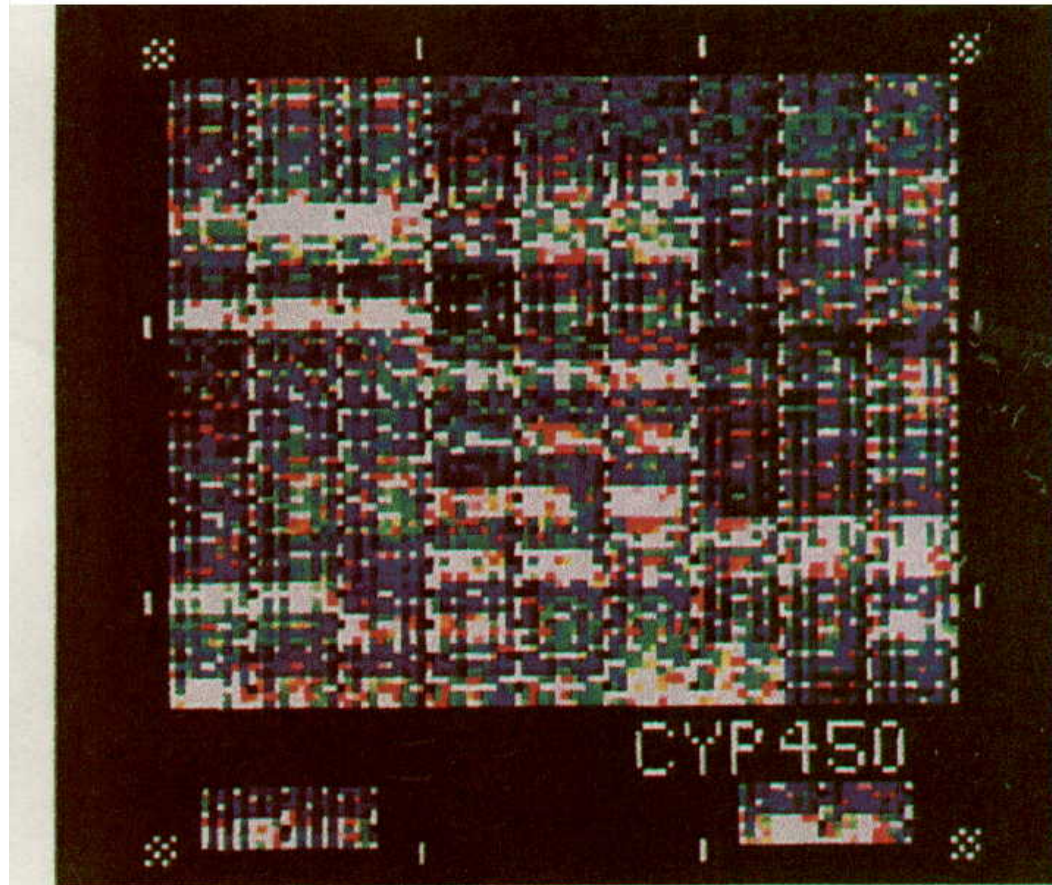
# CYP2D6 Pharmacogenetics



# *CYP2D6* Alleles

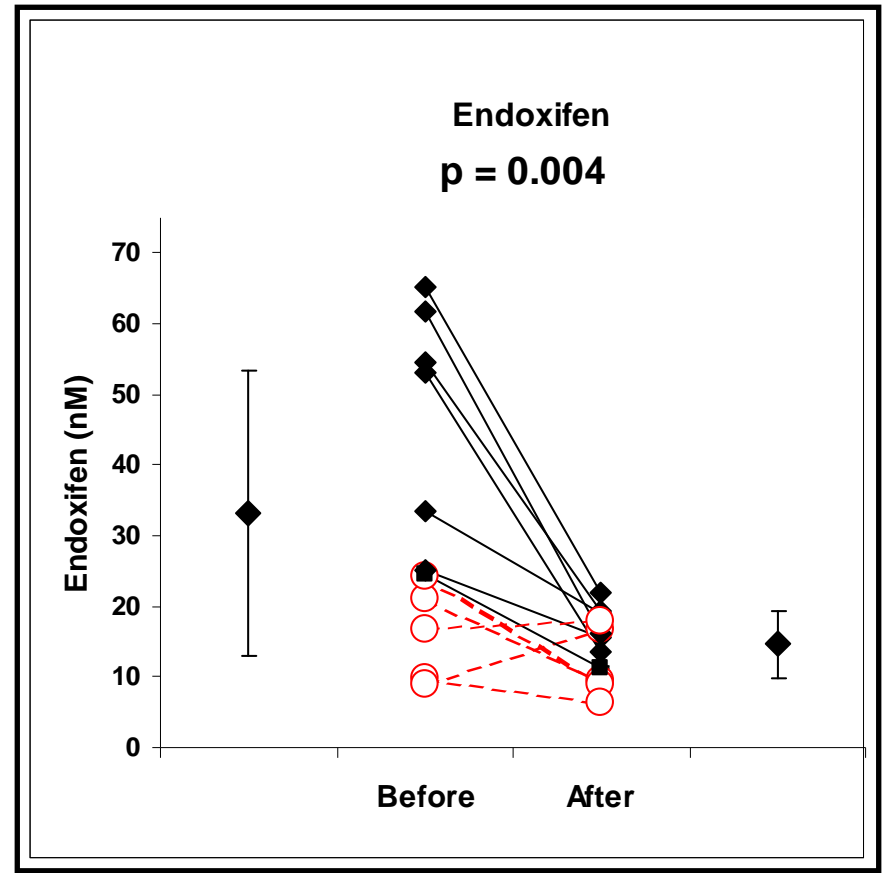
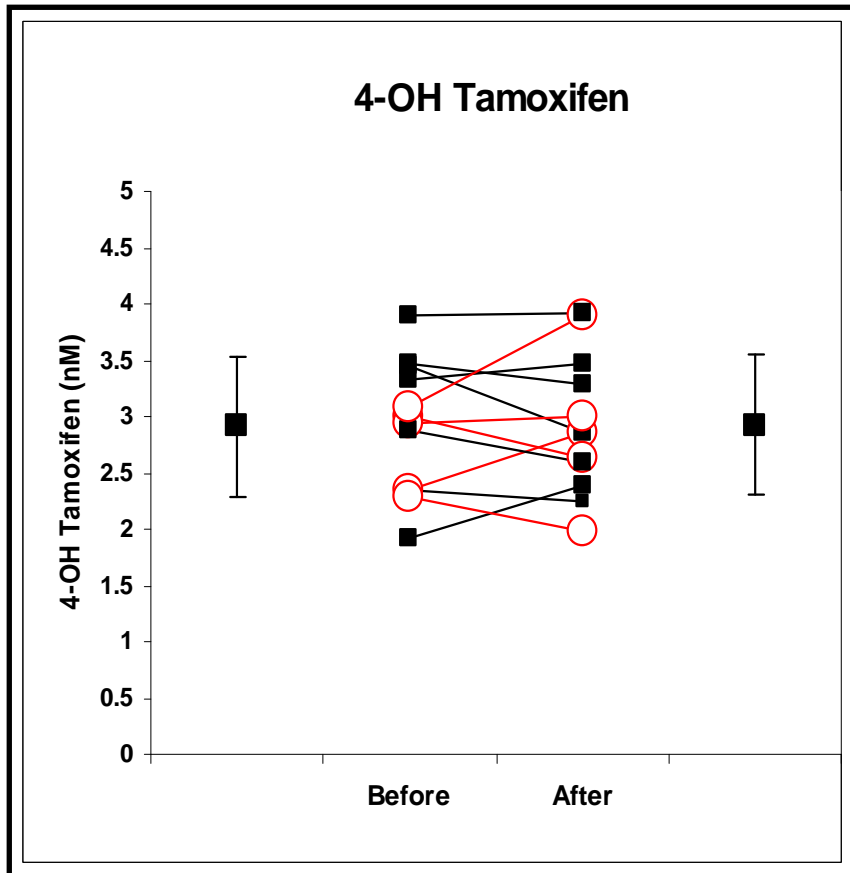
- 69 as of December, 2008
- 24 alleles have no activity
- 6 have decreased activity
- \*1, \*2, \*4 and many others have copy number polymorphisms
- The \*2 variant can have 1,2,3,4,5 or 13 copies i.e increased activity

# Oligonucleotide array for cytochrome P450 genotyping

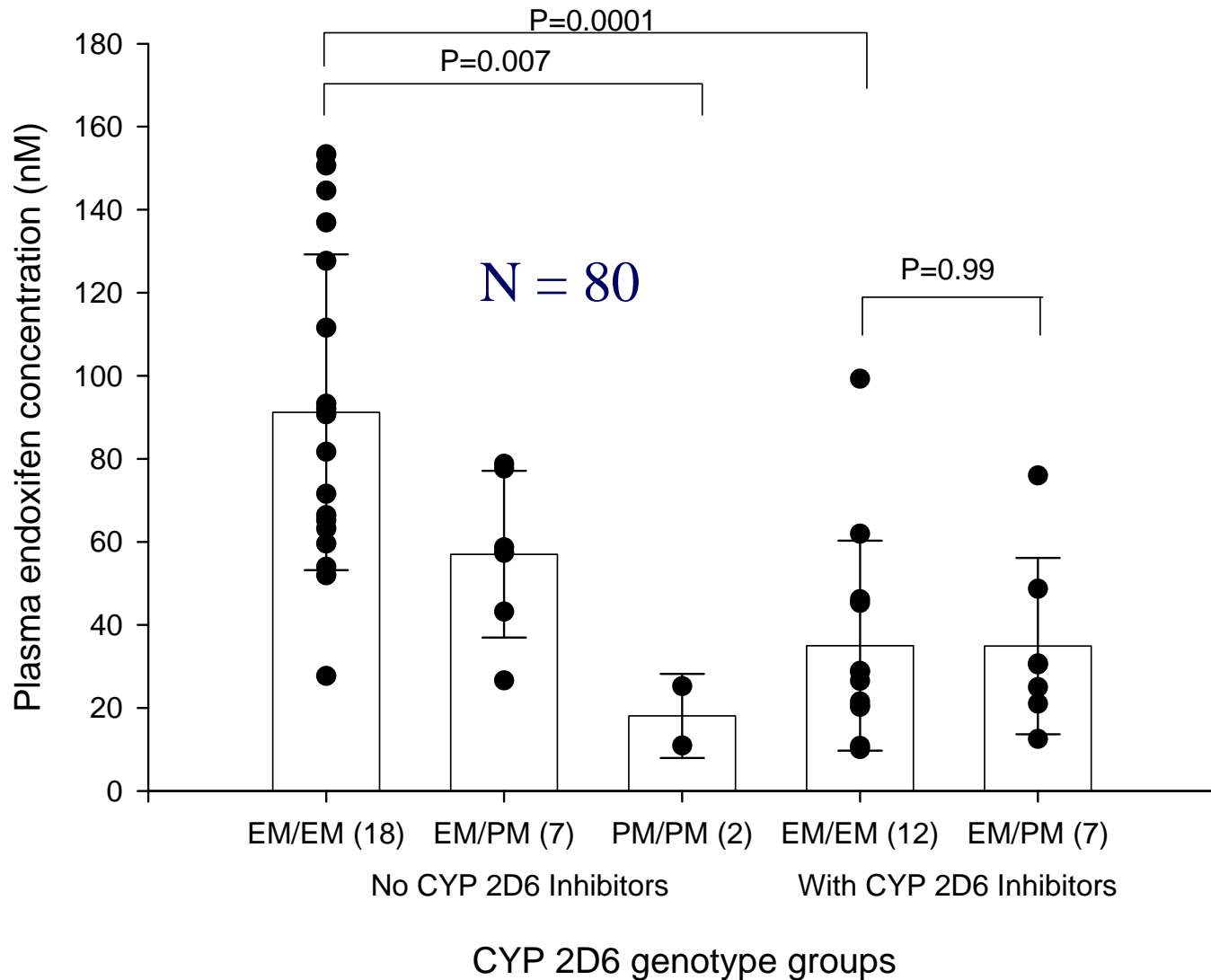


From: Flockhart DA and Webb DJ. *Lancet* End of Year Review for Clinical Pharmacology, 1998.

# Paroxetine and CYP2D6 genotype change the plasma concentrations of endoxifen



# CYP2D6 variant genotype and CYP2D6 inhibitors lower [Endoxifen]



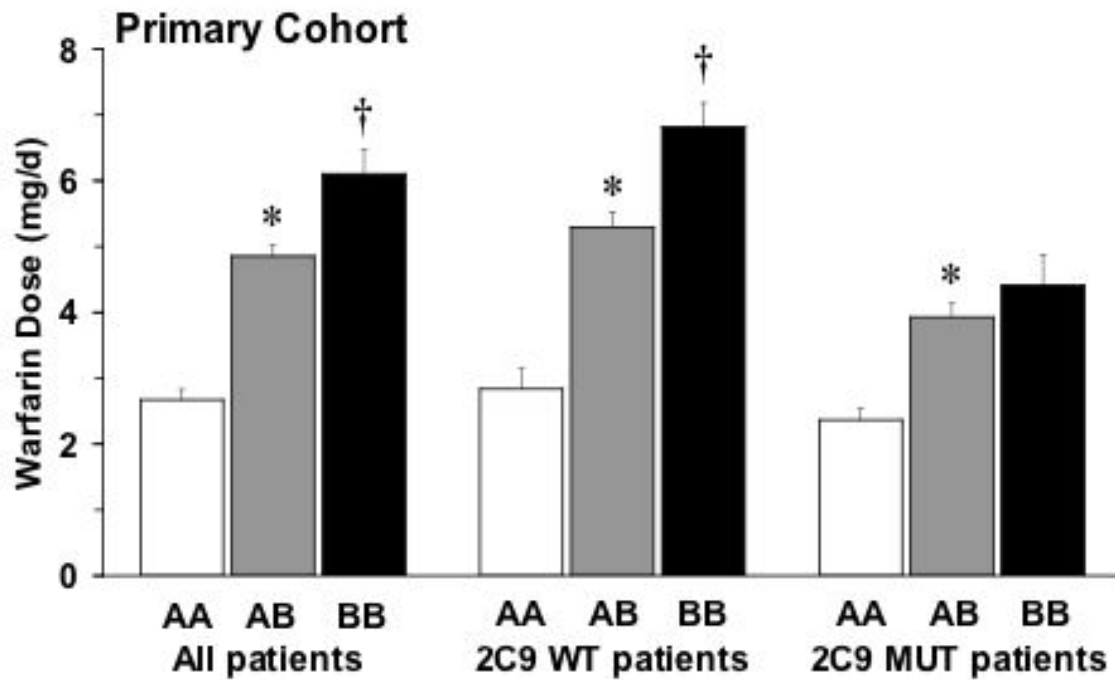
# Methods

- 225 Charts were reviewed at each randomizing site to ascertain medication history
  - Potent CYP2D6 inhibitors: Fluoxetine and paroxetine
  - Moderate CYP2D6 inhibitors: Sertraline, cimetidine, amiodarone, doxepin, ticlopidine, or haloperidol
  - Duration of coadministration: <1, 1-2, 2-3, 3-4 and 4-5 years
- Statistics: Log rank test and Cox modeling

# Lessons from CYP Pharmacogenetics

- Multiple genetic tests of one gene may be needed to accurately predict phenotype
- Gene duplication in the germline exists
- The environment in the form of Drug Interactions can mimic a genetic change

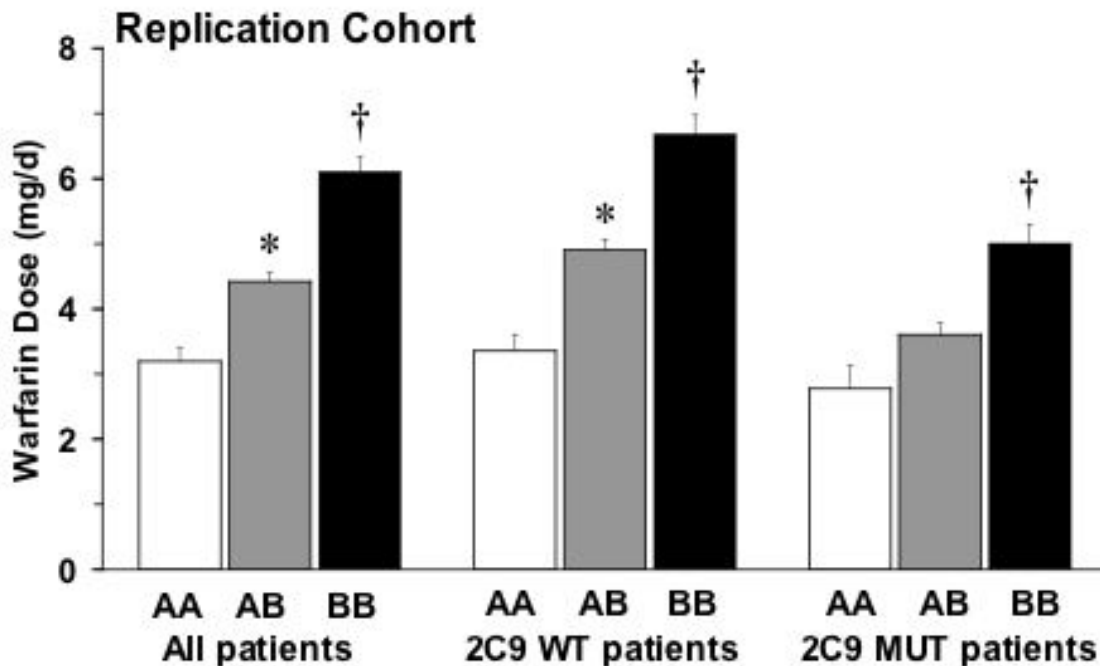




## ***VKORC1* Haplotype and CYP2C9 Genotype changed Warfarin Dose**

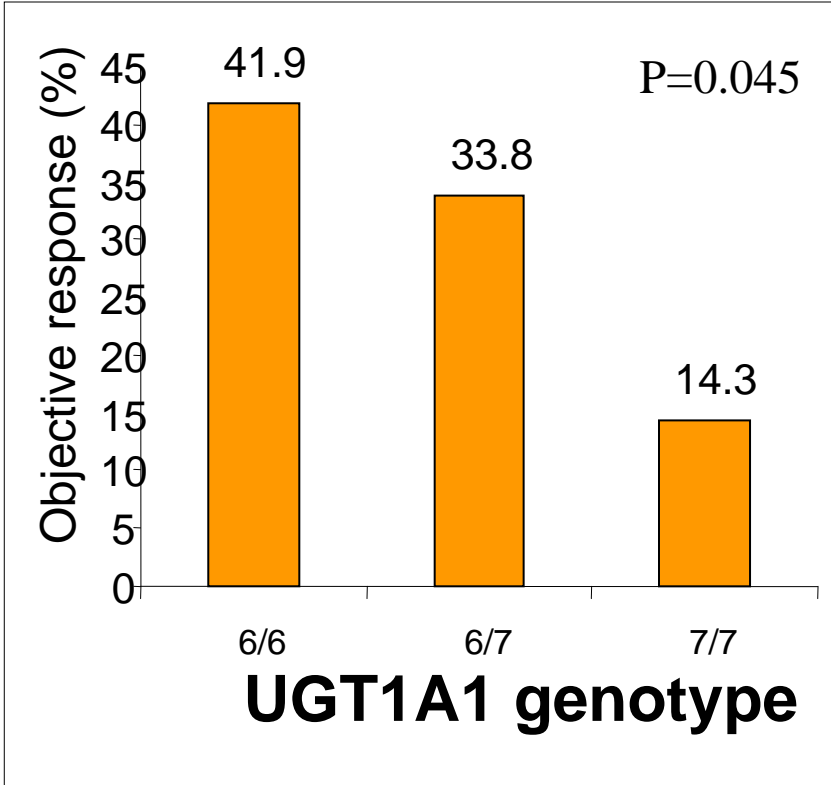
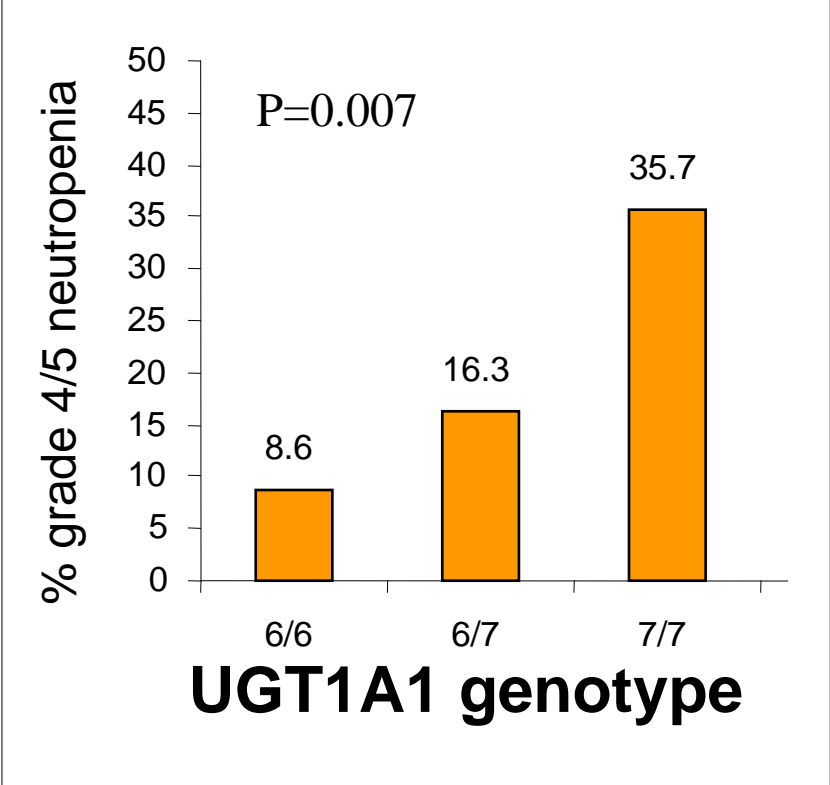
Primary cohort: UW (N=185);

Replication cohort: Wash U (N=368).



All participants were Caucasian.

# Phase II matters too: UGT1A1 TA repeat genotype alters irinotecan neutropenia/activity



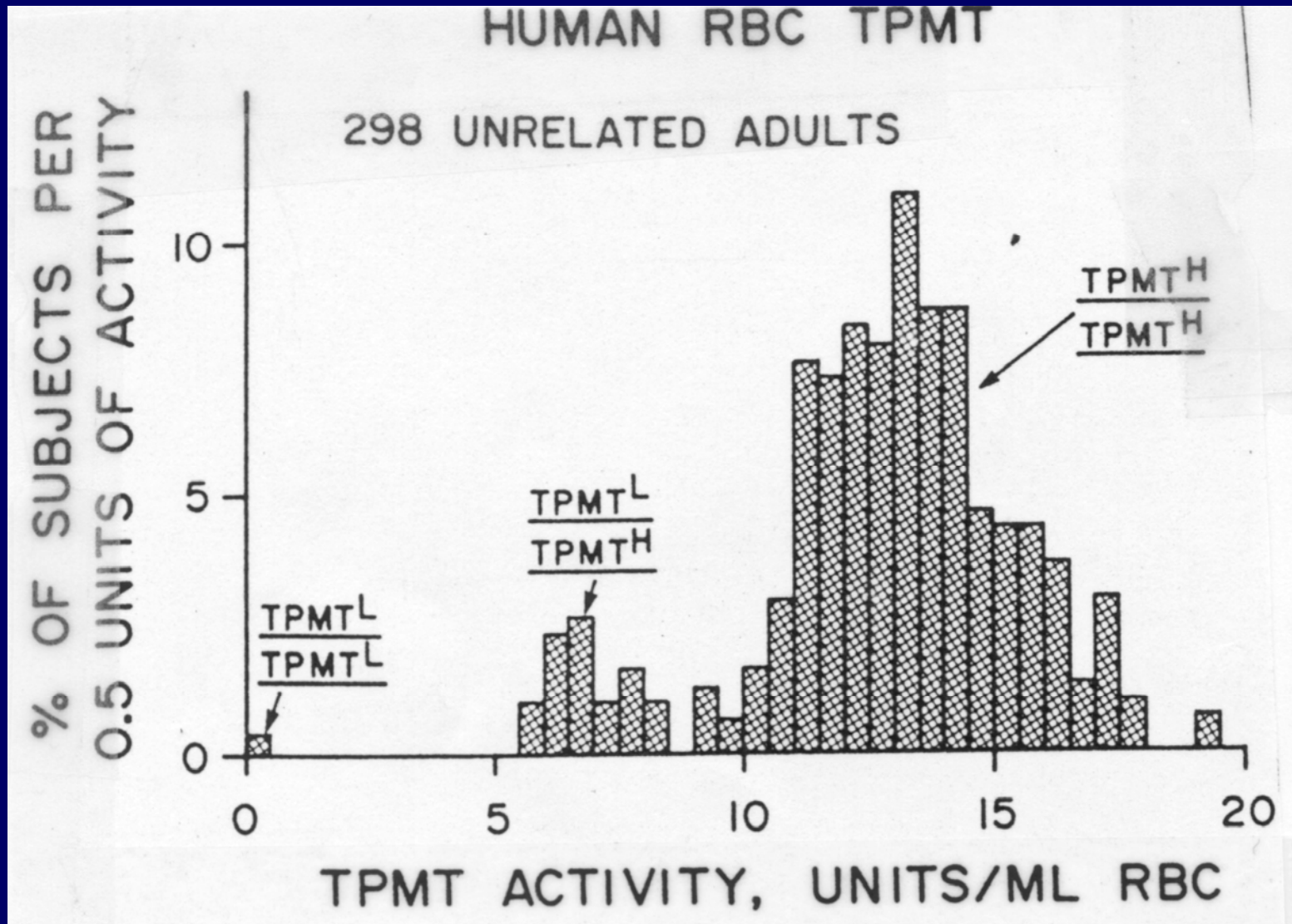
N=524

McLeod H. et al, 2003.

# Thiopurine Methyl Transferase

- Homozygous mutants are 0.2% of Caucasian Populations
- Heterozygotes are ~ 10%
- Homozygous wild type is 90%
  - Metabolism of Azathioprine
  - 6-Mercaptopurine

# Thiopurine Methyl Transferase Deficiency


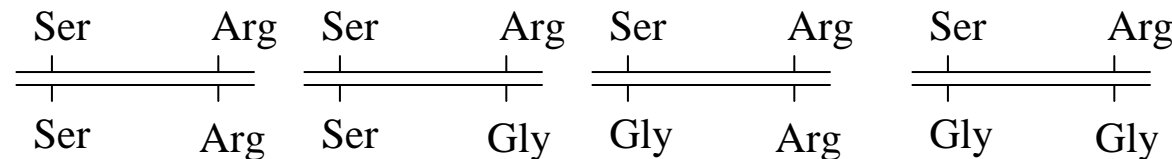

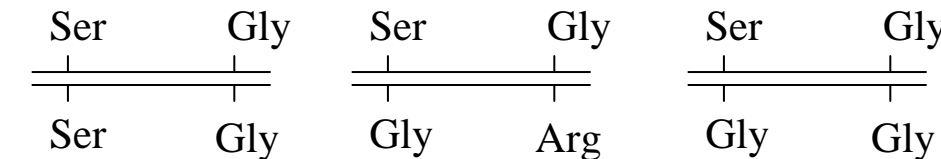

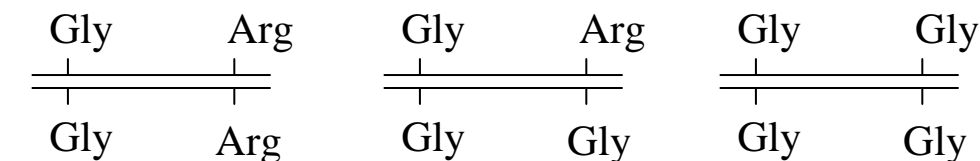


From: Weinshilboum et al. JPET;222:174-81. 1982

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

- G-proteins
- Angiotensin II receptor and angiotensinogen
- Angiotensin converting enzyme
- $\beta_2$ receptor
- Dopamine D<sub>4</sub> receptor
- Endothelial NO synthase
- 5HT<sub>4</sub>receptor

# 2SNPs: 10 possible haplotypes

Haplotypes	Diploypes
	
	
	

Ying-Hong Wang PhD,

Indiana University School of Medicine

# Observed $\beta_1$ AR Haplotypes in Caucasians and African American Women (WISE study)

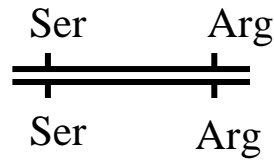
Haplotype	Frequency (C)	Frequency (AA)
AC (Ser49/Arg389)	0.65 (0.64)	0.42 (0.42)
AG (Ser49/Gly389)	0.26 (0.25)	0.36 (0.28)
GC (Gly49/Arg389)	0.09 (0.08)	0.22 (0.18)
GG (Gly49/Gly389)	0 (0.03)	0 (0.12)

Terra et al. *Clin. Pharmacol. Ther.* 71:70 (2002)

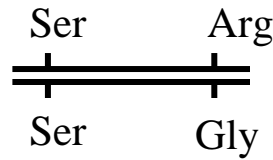
**Of 10 theoretical diplotypes, only 4 were present in the study**

Haplotypes

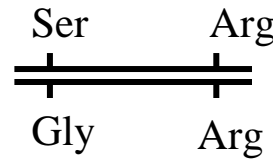
population  
Diploypes



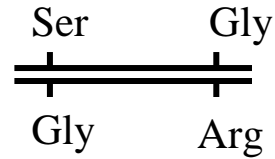
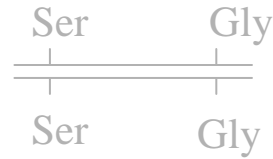
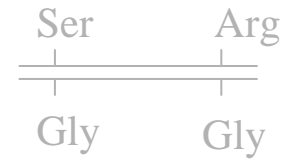
**SR/SR**



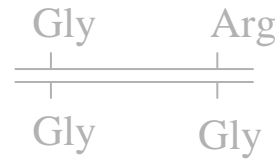
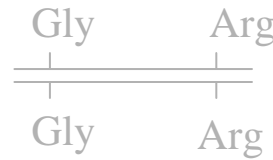
**SR/SG**



**SR/GR**

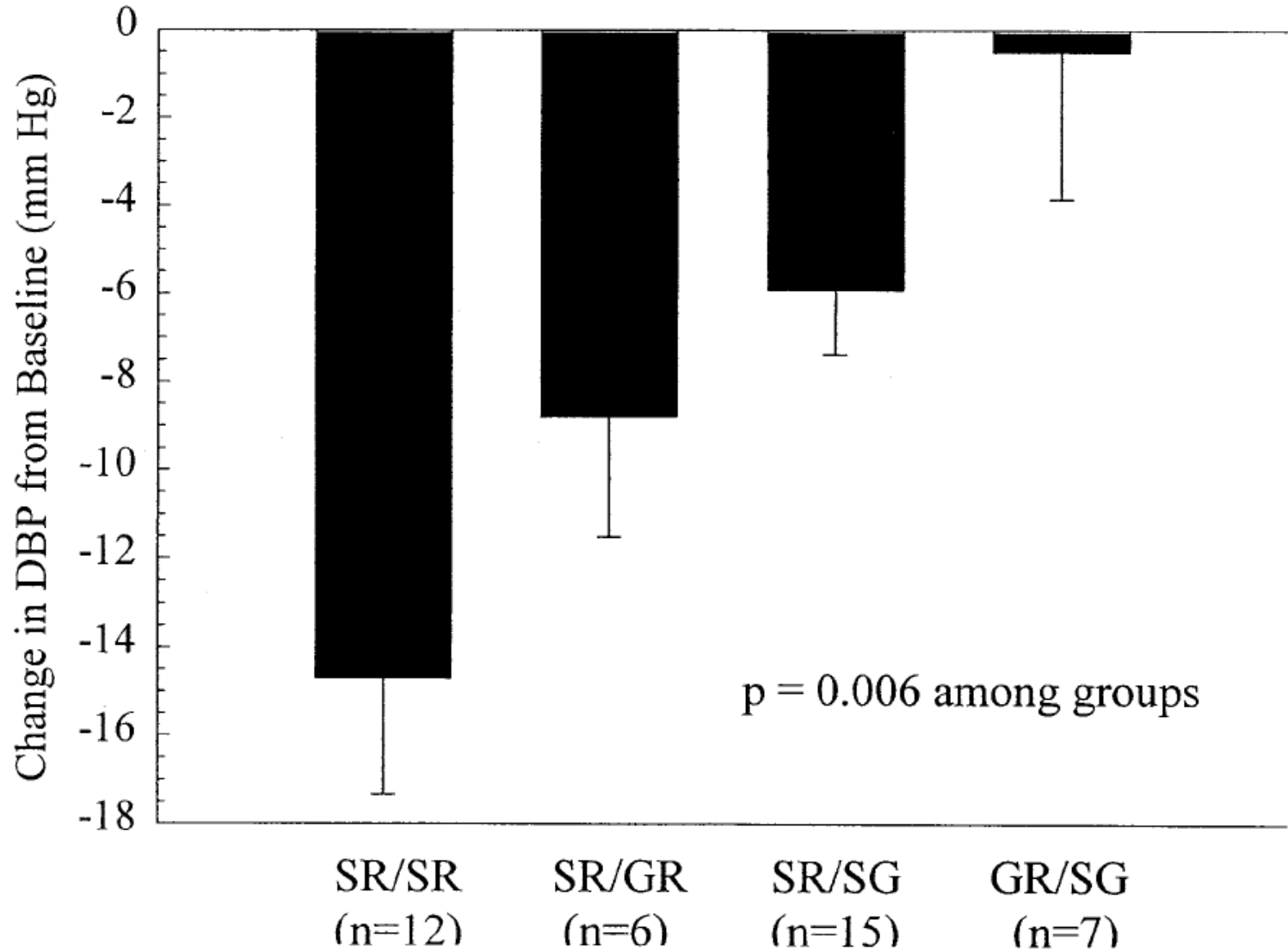


**SG/GR**





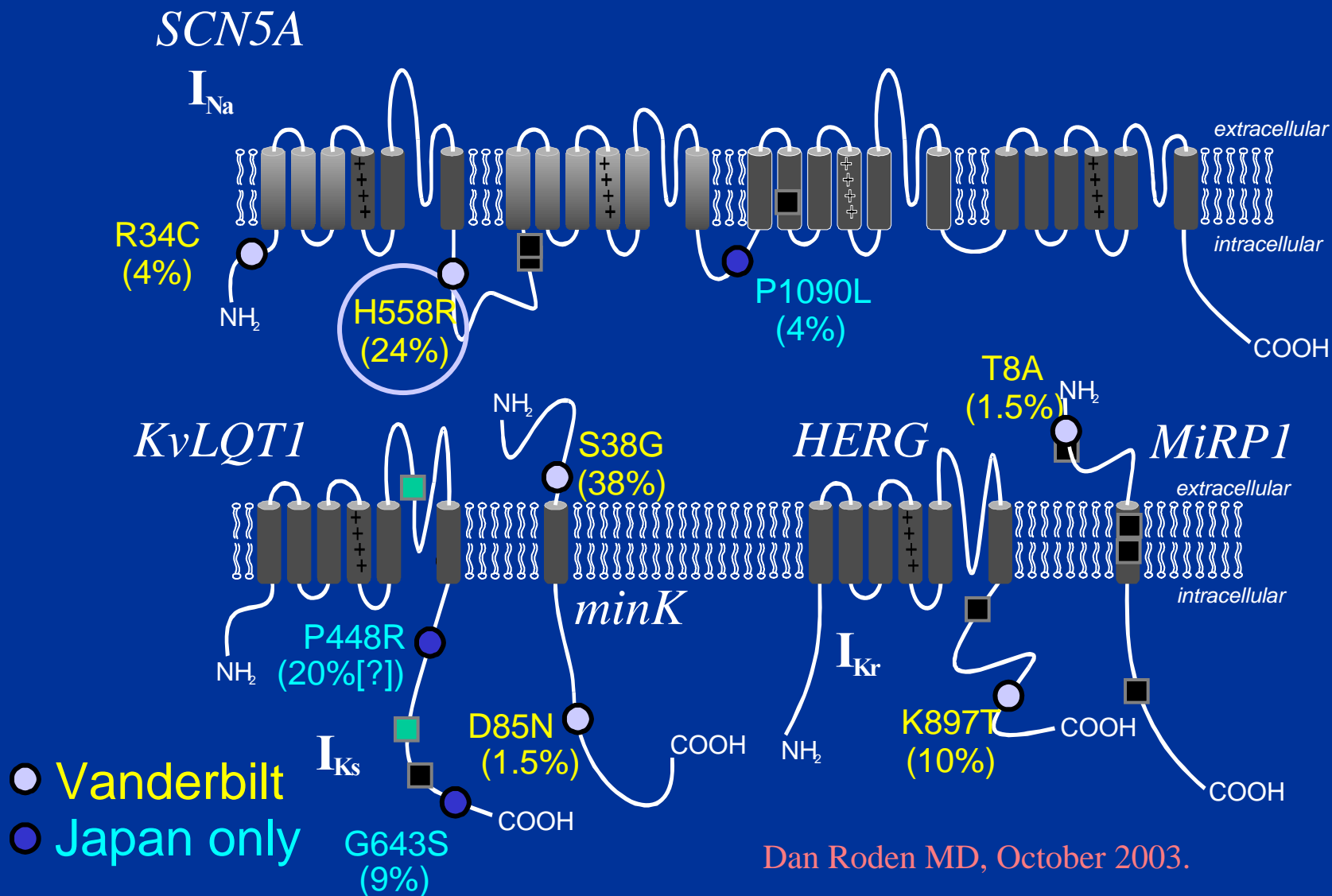
## Diplotype predicts Beta-blocker effect



Lesson: Diplotype *may* be a better predictor of effect than Genotype

A SNP that tags a Haplotype (tagSNP) may be an economical means of screening

# Non-synonymous coding region polymorphisms in long QT disease genes



# Pharmacogenetic approach to angiogenesis biomarker discovery



## Essential Ingredients:

- 1). Genetic variability must have potential for biologic impact
- 2). Genetic variability must exist in drug disposition or destination
  - metabolizing enzymes/transporters/targets
- 3). Drug evaluated must be heterogeneous in outcome
  - mix of success and toxicity
- 4). Variability must be frequent
  - generalizability of results



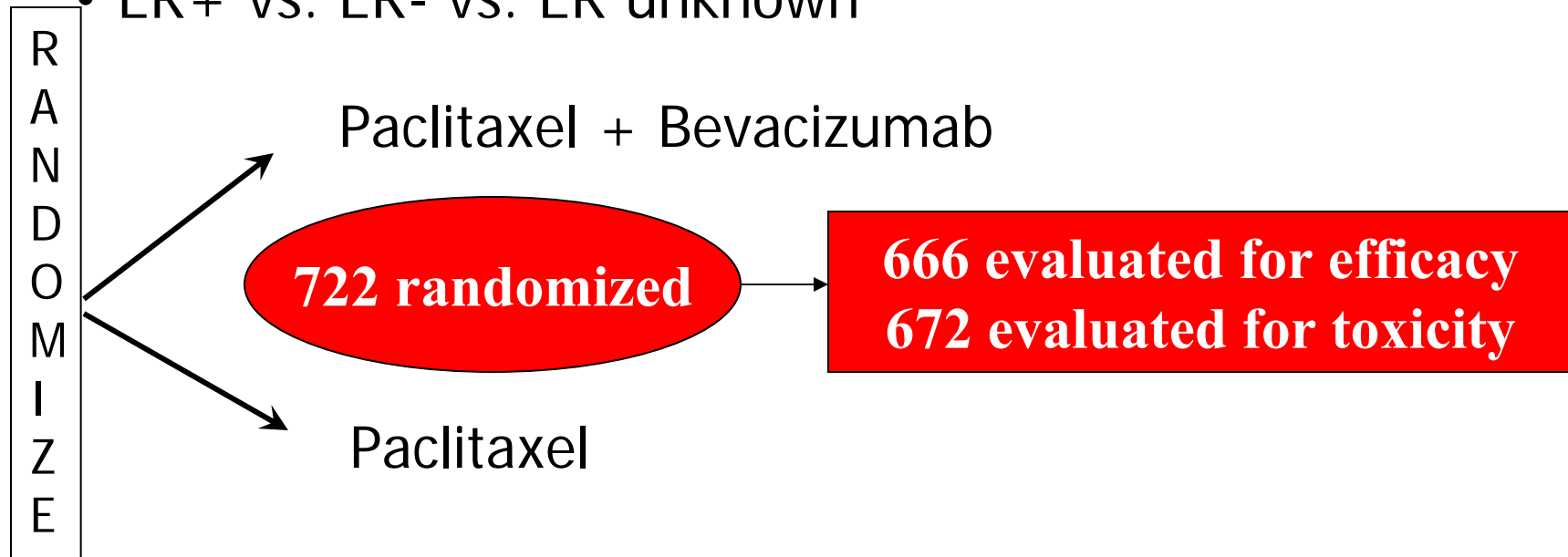
No Benefit  
No Toxicity

Benefit  
No Toxicity

# Bevacizumab in breast cancer-**E2100**: a model of therapeutic heterogeneity

Stratify:

- DFI  $\leq$  24 mos. vs.  $>$  24 mos.
- $<$  3 vs.  $\geq$  3 metastatic sites
- Adjuvant chemotherapy yes vs. no
- ER+ vs. ER- vs. ER unknown

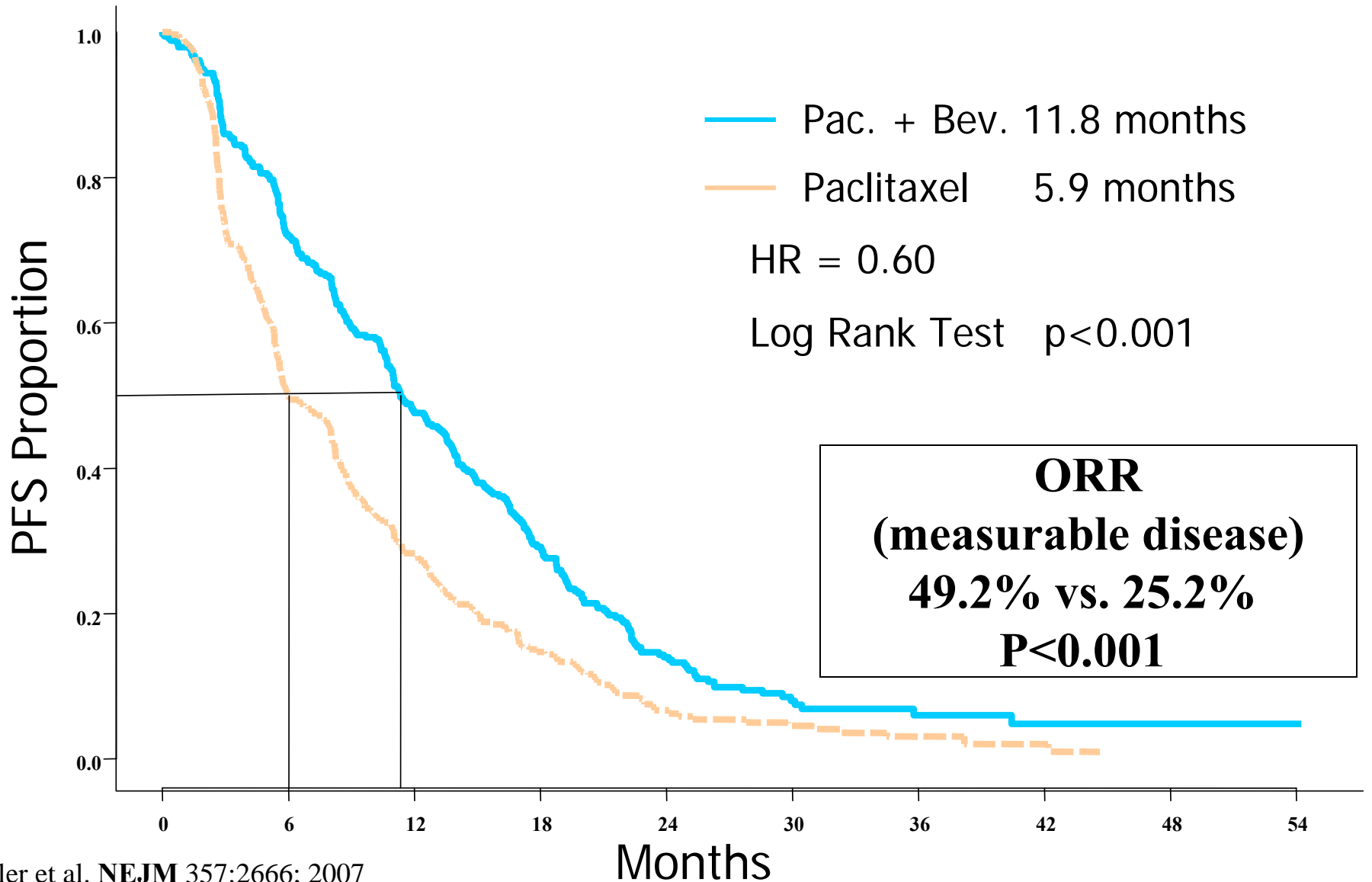


# Bevacizumab increased grade 3/4 toxicity

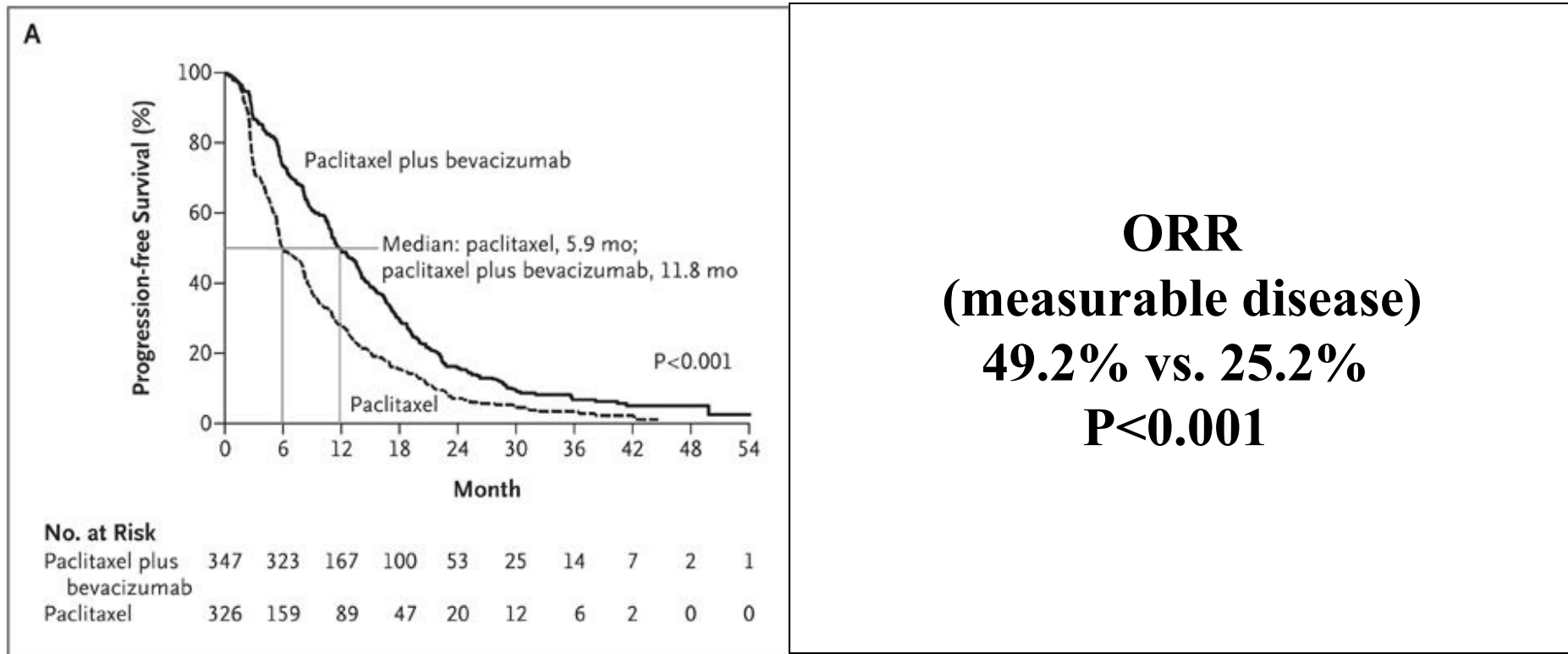
Serious, frequent, & unique      Serious but rare      Likely related to duration of taxane exposure

Toxicity	P (%)	P+B (%)	p-value
Infection	2.9	9.3	<0.001
Fatigue	4.9	9.1	0.04
Neuropathy	17.7	23.5	0.05
CNS ischemia	0	1.9	0.02
Headache	0	2.2	0.008
Proteinuria	0	3.5	<0.001
Hypertension	0	14.8%	<0.001

# Bevacizumab significantly improved PFS



# Improvement in PFS/ORR did not translate into OS benefit



**ORR**  
**(measurable disease)**  
**49.2% vs. 25.2%**  
**P<0.001**



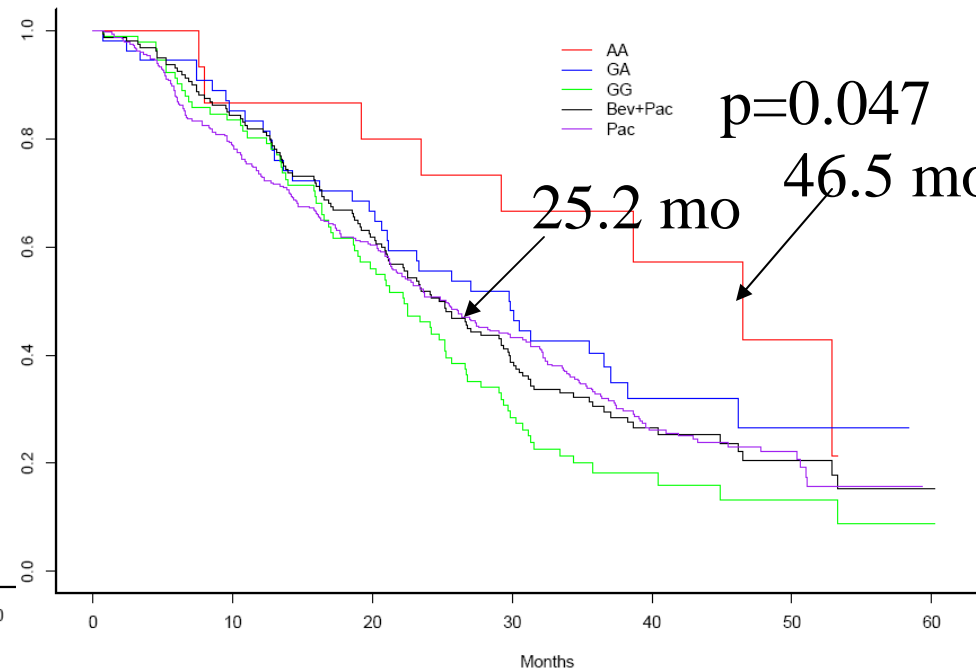
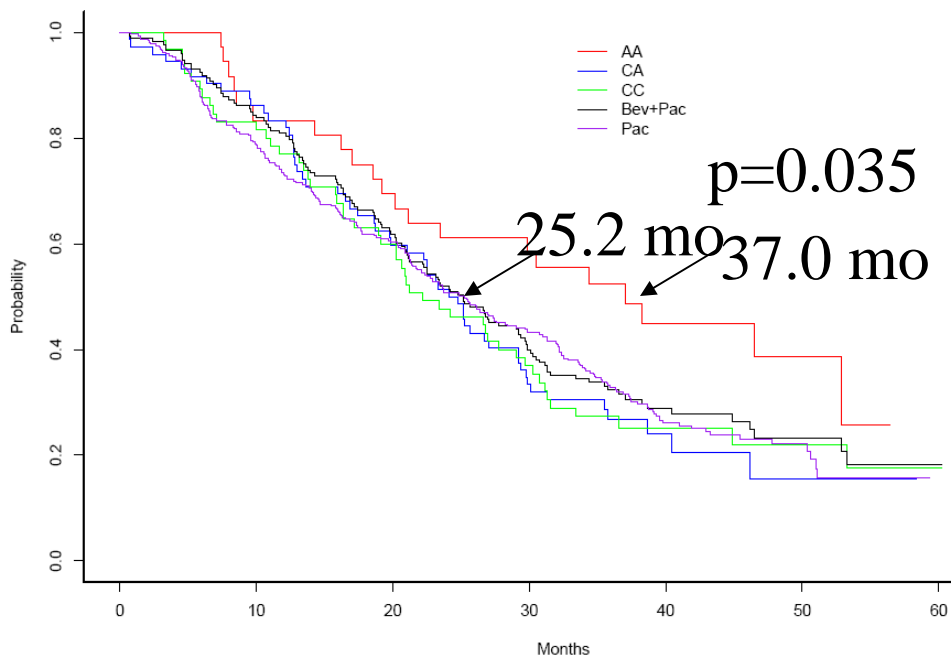


We need something better!

# VEGF -2578 AA & -1154 AA genotypes in combination arm outperformed control

OS for VEGF-2578C/A

OS for VEGF-1154G/A



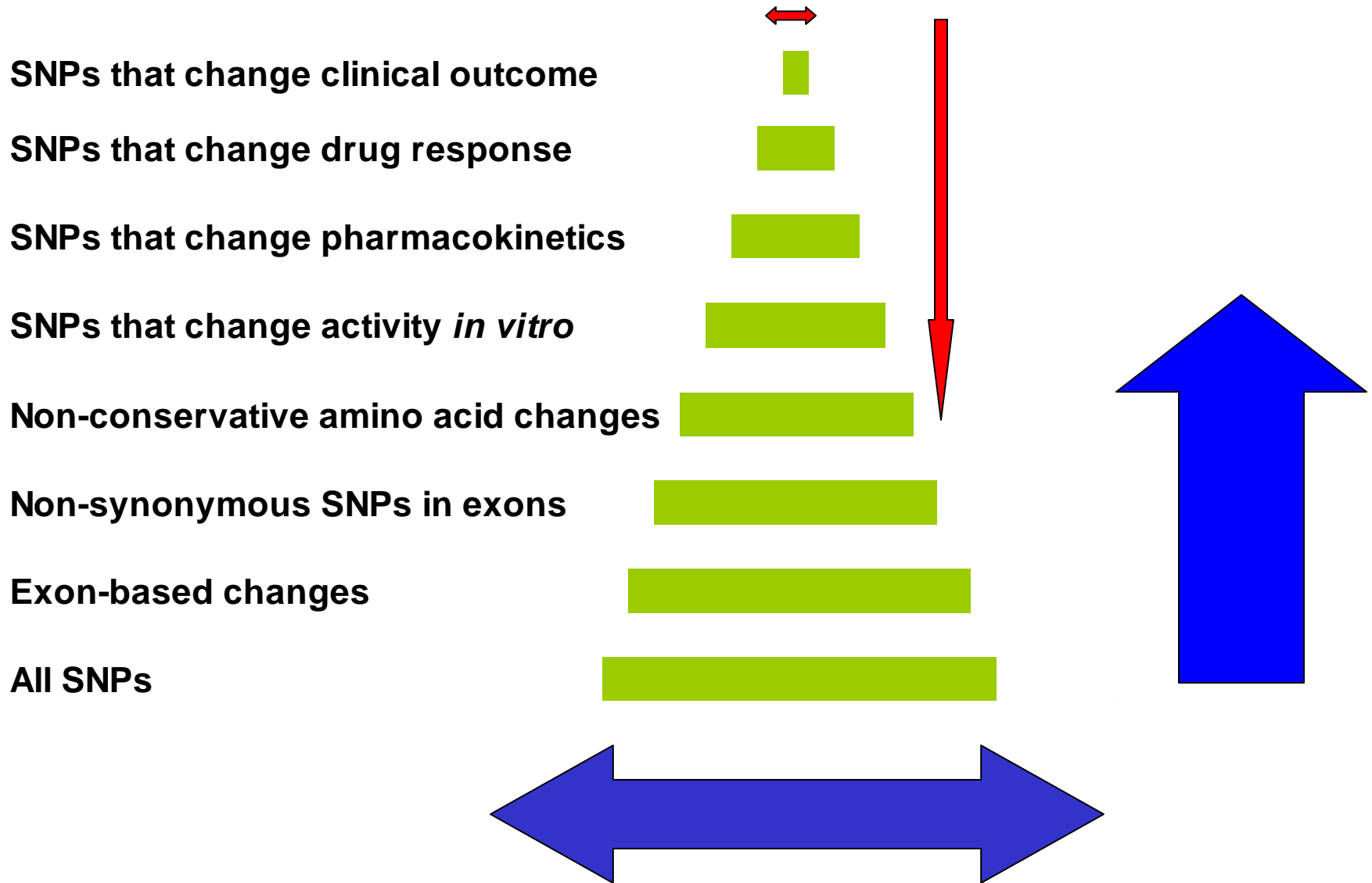
## Median OS

Control arm=25.2 mo  
 Combination arm=26.7 mo  
 Combination arm AA=37.0 mo

## Median OS

Control arm=25.2 mo  
 Combination arm=26.7 mo  
 Combination arm AA=46.5 mo

# Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

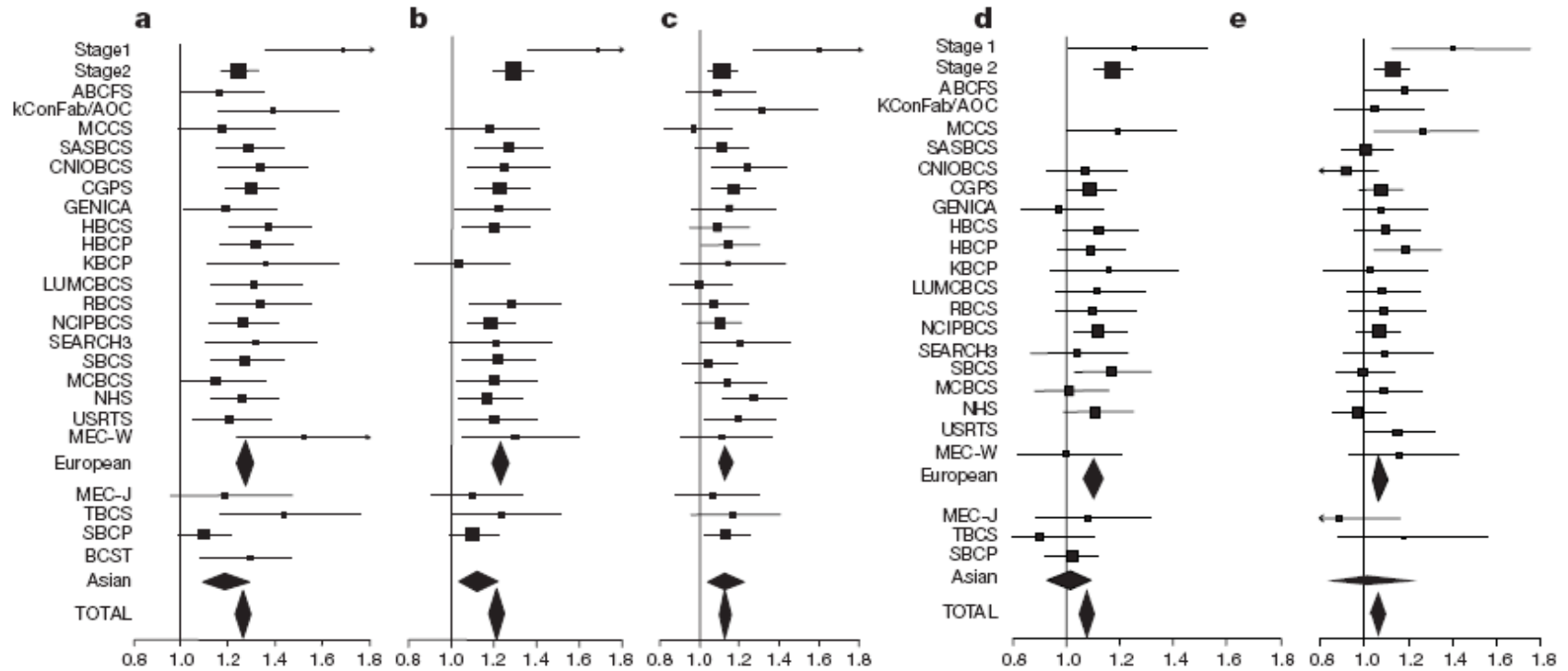


# Genome Wide SNP Arrays

- Affymetrix 6.0 Gen Chip Arrays
  - 906,000 SNPs
  - 1.8 million genetic markers
  - 946,000 copy number probes
- Illumina Infinium Bead Chips

# Genome-wide association study identifies novel breast cancer susceptibility loci

Nature May 27<sup>th</sup>, 2007



Published online before print November 22, 2006, 10.1101/gr.5629106

Genome Res. 16:1575-1584, 2006

©2006 by [Cold Spring Harbor Laboratory Press](#); ISSN 1088-9051/06 \$5.00

## Methods

# Genome-wide detection of human copy number variations using high-density DNA oligonucleotide arrays

Daisuke Komura<sup>1,2,8</sup>, Fan Shen<sup>3,8</sup>, Shumpei Ishikawa<sup>1,8</sup>, Karen R. Fitch<sup>3</sup>, Wenwei Chen<sup>3</sup>, Jane Zhang<sup>3</sup>, Guoying Liu<sup>3</sup>, Sigeo Ihara<sup>1</sup>, Hiroshi Nakamura<sup>1,2</sup>, Matthew E. Hurles<sup>4</sup>, Charles Lee<sup>5</sup>, Stephen W. Scherer<sup>6</sup>, Keith W. Jones<sup>3</sup>, Michael H. Shapero<sup>3</sup>, Jing Huang<sup>3,9</sup>, and Hiroyuki Aburatani<sup>1,7,9</sup>

<sup>1</sup> Research Center for Advanced Science and Technology, The University of Tokyo, Meguro, Tokyo 153-8904, Japan; <sup>2</sup> Department of Advanced Interdisciplinary Studies, Graduate School of Engineering, The University of Tokyo, Bunkyo-ku, Tokyo 113-8656, Japan; <sup>3</sup> Affymetrix, Inc., Santa Clara, California 95051, USA; <sup>4</sup> The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom; <sup>5</sup> Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA; <sup>6</sup> The Centre for Applied Genomics and Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, M5G 1L7, Canada; <sup>7</sup> Japan Science and Technology Agency, Kawaguchi, Saitama, 332-0012, Japan

# Copy Number Variation screening:

- “There is a decreased level of linkage disequilibrium between CNVs and SNPs, suggesting that SNPs are not an ideal surrogate for CNVs in association studies. This implies that CNVs need to be assessed independently in whole-genome association studies. “

# Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

SNPs that change clinical outcome

SNPs that change drug response

SNPs that change pharmacokinetics

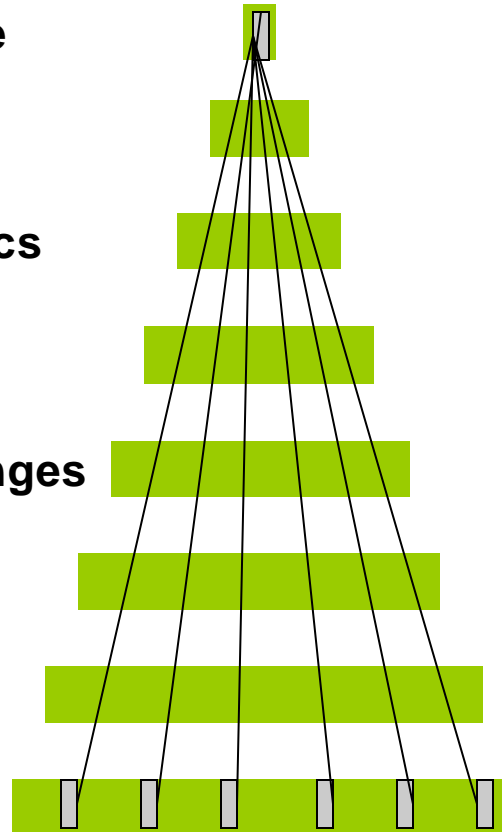
SNPs that change activity *in vitro*

Non-conservative amino acid changes

Non-synonymous SNPs in exons


Exon-based changes

All SNPs





# An International Community of Genomic Analysts: <http://dchip.forum5.com>



## Microarray Data Analysis Forums

Microarray data analysis using dChip and ComBat

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**Illumina Array Data Mgmt**  
Solutions to manage & analyze BeadStudio data  
[www.GenoLogics.com](http://www.GenoLogics.com)


**Whole Genome SNP Analysis**  
Rapidly analyze Illumina, Affymetrix whole genome microarray data.  
[www.goldenhelix.com/SNP\\_Variation/](http://www.goldenhelix.com/SNP_Variation/)

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### Read Illumina SNP or expression array data

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Author	Message
<b>Ich</b> Site Admin <b>Offline</b>  Joined: 23 May 2007 Posts: 600 Location: Dana-Farber Cancer Institute, Boston, MA	<p>Posted: Wed May 23, 2007 10:09 pm Post subject: Read Illumina SNP or expression array data <a href="#">quote</a></p> <p>Alicia,</p> <p>You may try the "Analysis/Get external data" function. A proper dchip gene info file should be prepared from Illumina's annotation data.</p> <p>Cheng</p> <p>dChip manual link: <a href="http://www.dchip.org/snp.htm#external">http://www.dchip.org/snp.htm#external</a></p> <p>--- In <a href="mailto:dchip@yahooogroups.com">dchip@yahooogroups.com</a>, "Alicia Chung" &lt;achung@...&gt; wrote: Thu May 10, 2007 1:52 pm</p>

Done

Start | Ask and ye shall... | BBC NEWS | Microarray D... | 2008 Lecture fin... | 2008 lecture fin... | Flockhart Br J Cl... | Skype™ - dadnp... | 7:10 AM

# Current Methods for Pharmacogenetic Testing

- By phenotype: metabolic probe drug or Western blot or Immunohistochemistry
- By PCR with mutation-specific endonuclease
- By PCR and allele-specific hybridization
- By oligonucleotide chip hybridization
- By laser lithography - guided oligonucleotide chip hybridization.
- By rapid throughput pyrosequencing
- Taqman probe screening
- By genome wide SNP array
- By rapid, robust and high throughput full sequencing
- By including accurate quantitative tests of CNV.

# Conclusions

- Candidate gene pharmacogenetic testing is migrating beyond industry phase 1 trials into clinical practice
- Multiple candidate gene /pathway testing has begun with warfarin
- No germline genome wide patterns predictive of drug effect have yet become clinically useful
  - Stay tuned!

# Ten Drugs and Their Available Pharmacogenetic Tests December 2008

- Abacavir
- Imatinib
- 5-Fluorouracil
- Clozapine
- QT-prolonging Drugs
- Irinotecan
- Azathioprine and Mercaptopurine
- Warfarin
- Carbamazepine
- HLA-B\*5701
- BCR-ABL
- DPYD-TYMS
- 2 SNPs in HLA-DQB1
- Familion™
- UGT1A1
- TPMT
- CYP2C9 and VKCoR
- HLA-B\*1502

# Pharmacogenetics Websites

- [www.pharmgkb.org](http://www.pharmgkb.org)
- The SNP consortium: <http://brie2.cshl.org>
- The Human Genome:  
[www.ncbi.nlm.nih.gov/genome/guide/H\\_sapiens.html](http://www.ncbi.nlm.nih.gov/genome/guide/H_sapiens.html)
- CYP alleles: [www.imm.ki.se/CYPalleles/](http://www.imm.ki.se/CYPalleles/)
- Drug Interactions: [www.drug-interactions.com](http://www.drug-interactions.com)