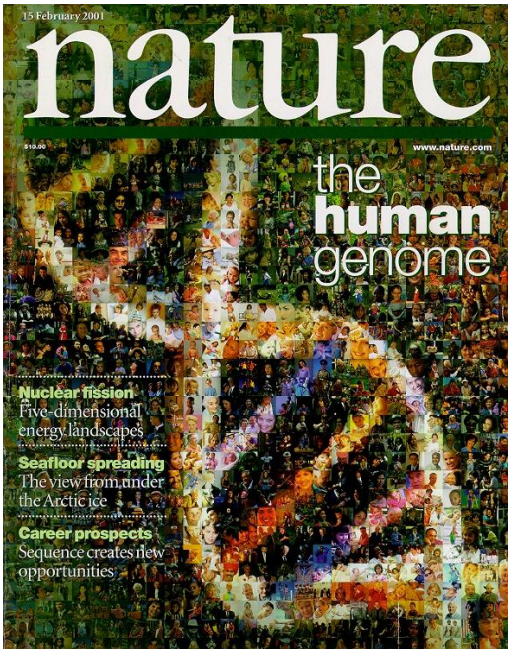
Clinical Pharmacogenomics

David A Flockhart MD, PhD
Director, Indiana Institute for Personalized Medicine
Professor of Medicine, Genetics and Pharmacology
Indiana University School of Medicine

Outline

- Germline Genomics
- Genome Wide Studies
- Candidate Gene Pharmacogenomics
  - Drug Absorption
  - Elimination
  - Effect
- Clinical Utility of Pharmacogenomics

The Genomic Revolution



Why Genomics?

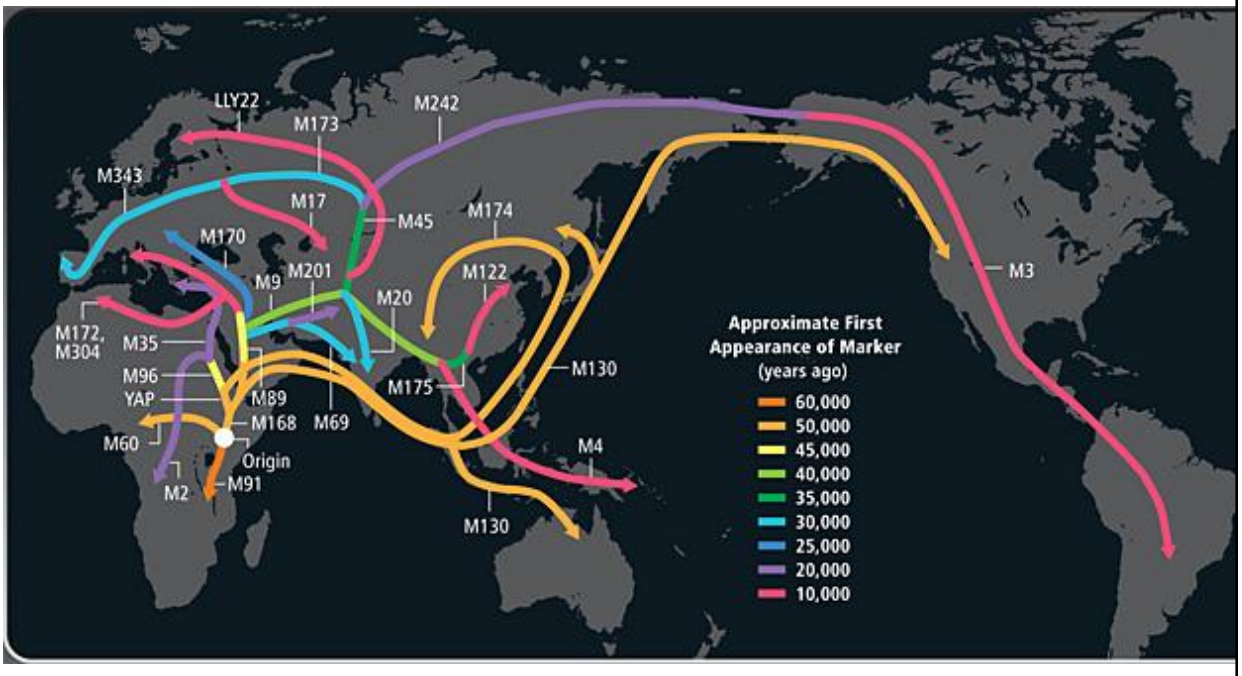
The Genome Map is available on the web, to anyone, free.

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

DNA is very stable

DNA can be amplified

Human Migration out of Africa



Scientific American, July 2008

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 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

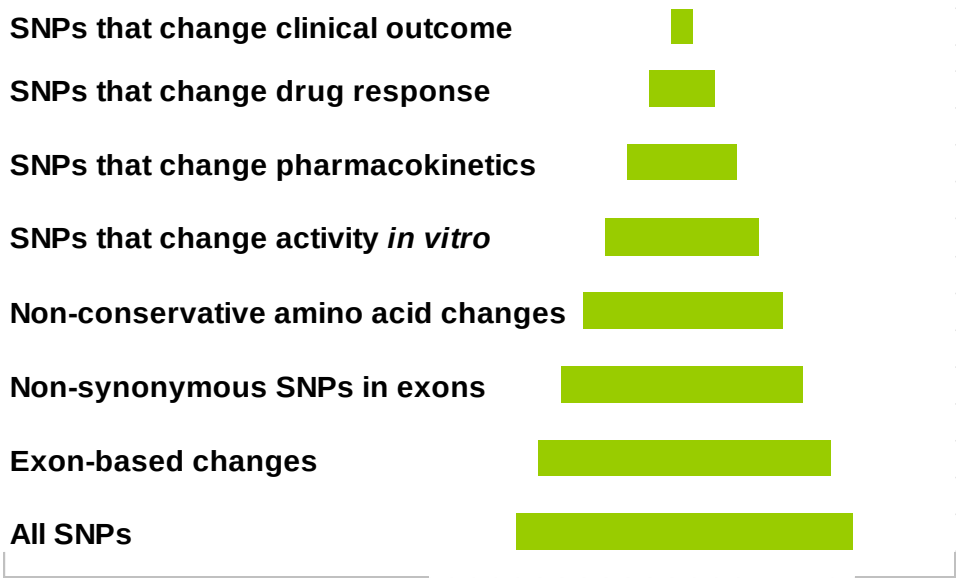
- ~150,000 SNPs in known exons
- 48,451 non-synonymous SNPs
- 1113 introduce a stop codon
- 104 disrupt an existing STOP

Pharmacogenomic Journals, 2010

Current Methods for PharmacogeneticTesting

- By phenotype: metabolic probe drug or Western blot or Immunohistochemistry
- By PCR with mutation-specific endonuclease
- By PCR and allele-specific hybrization
- 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.

Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

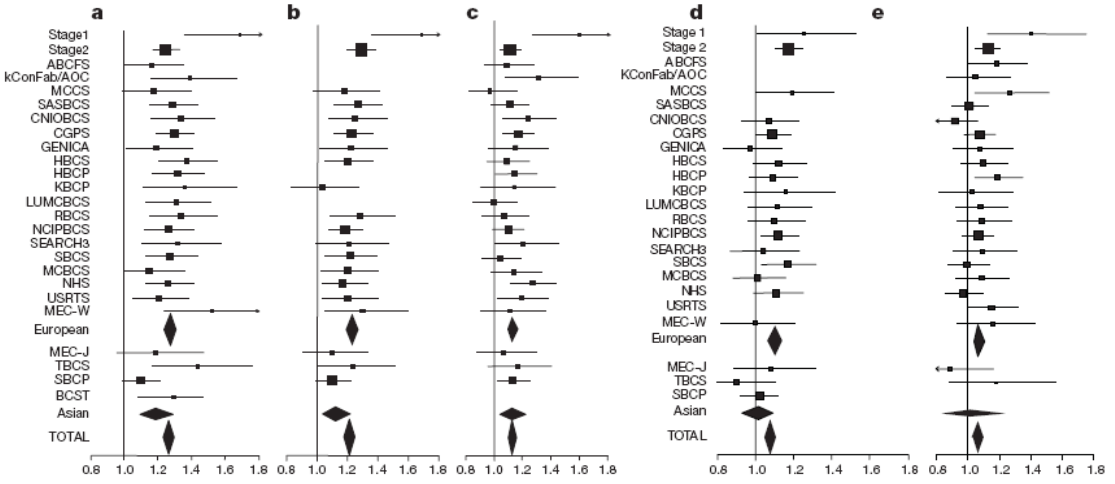


Genome Wide SNP Arrays

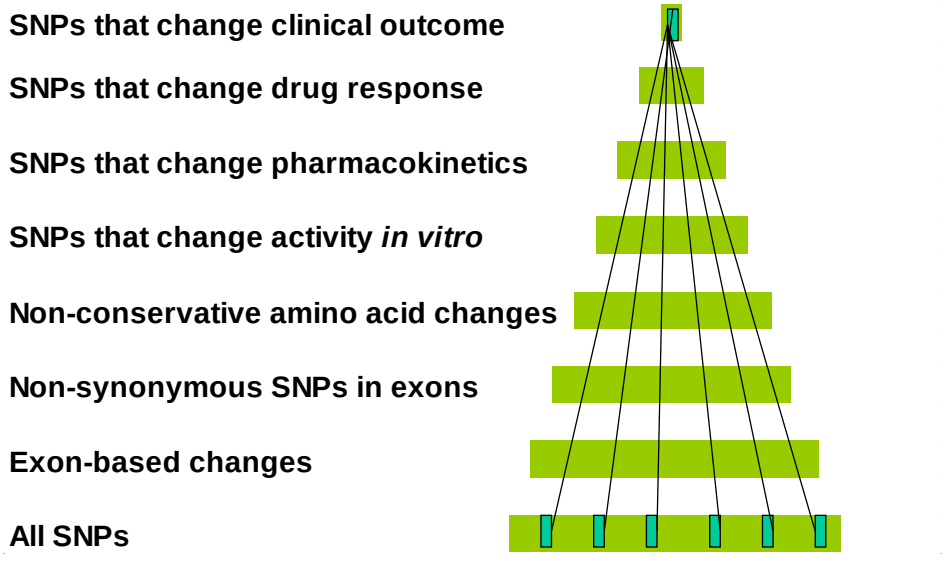
- Affymetrix 6.0 Gen Chip Arrays
  - 906,000 SNPs
  - 1.8 million genetic markers
  - 946,000 copy number probes
- Illumina Infinium Bead Chips
  - Soon to have 5,000,000 common and rare SNPS integrated with copy number variants

Genome-wide association study identifies novel breast cancer susceptibility loci

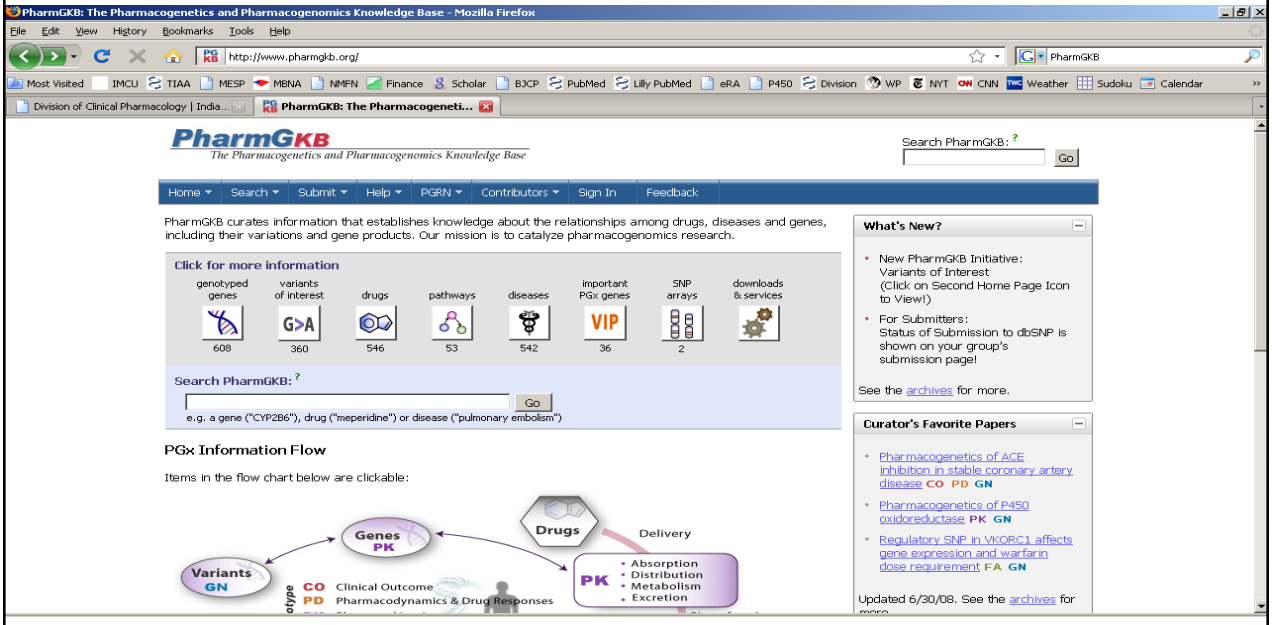
Nature May 27th, 2007



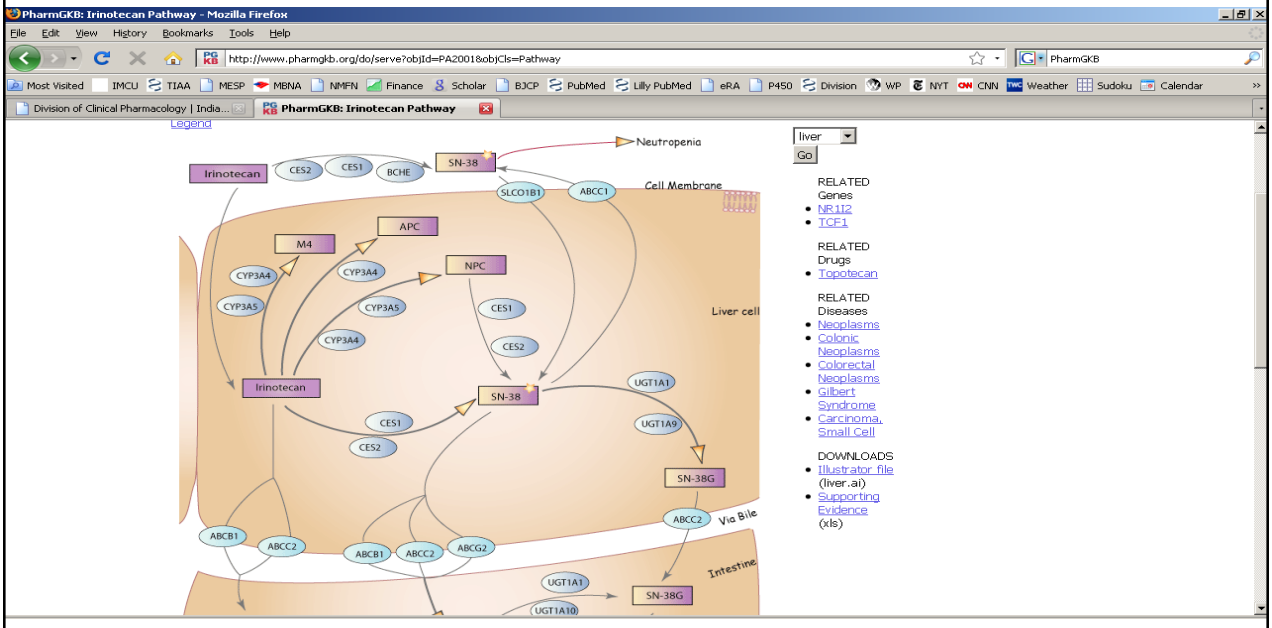
Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)



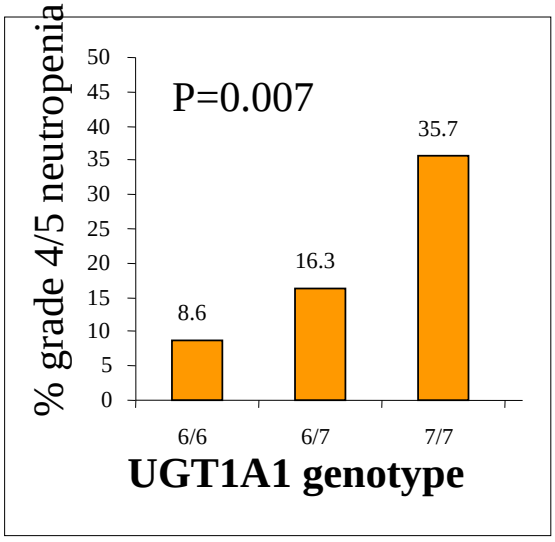
PharmGKB as a source of Candidate Genes and Pathways

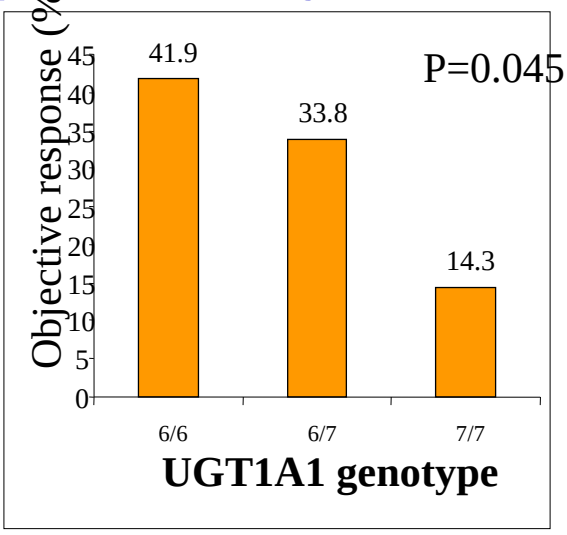


PharmGKB Irinotecan Pathway



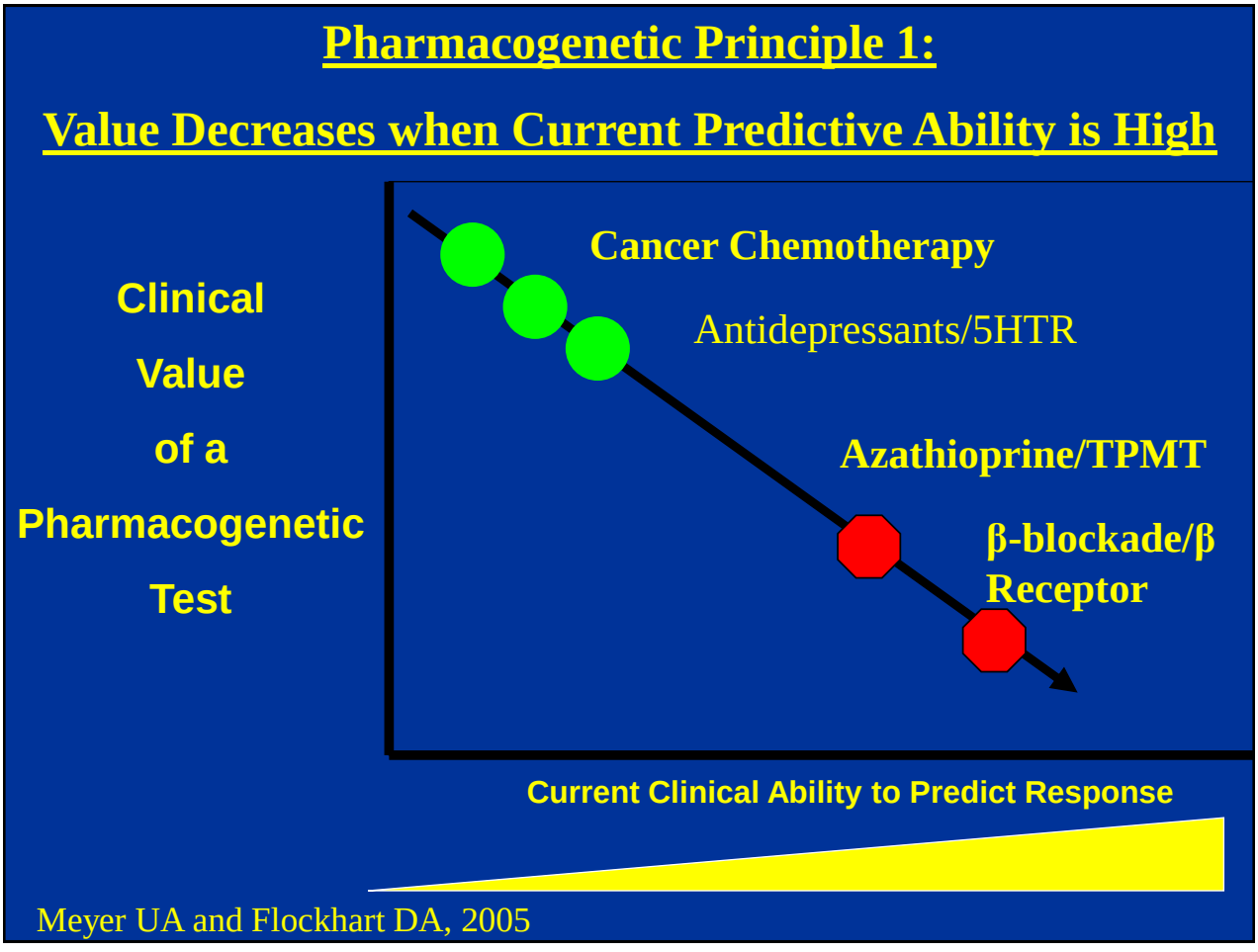
UGT1A1 TA repeat genotype alters irinotecan neutropenia/activity



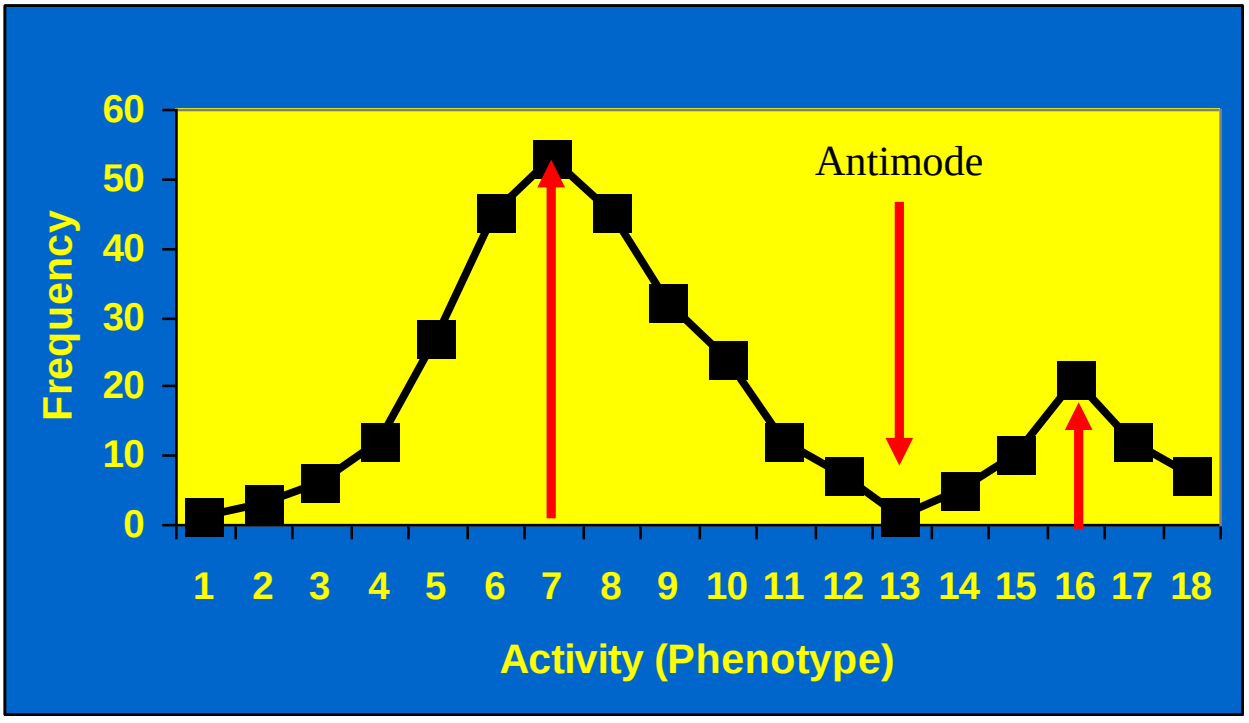


N=524

McLeod H. et al, 2003.

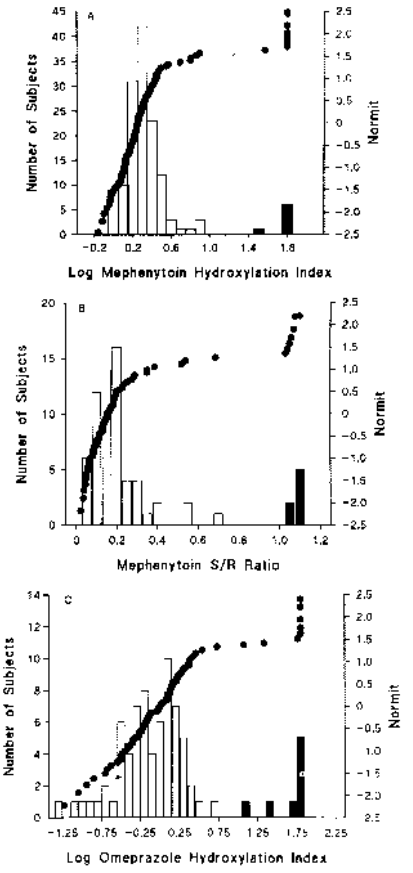

Pharmacogenetic Principle 1:
Value Decreases when Current Predictive Ability is High
Clinical Value of a Pharmacogenetic Test
Cancer Chemotherapy
Antidepressants/5HTR
Azathioprine/TPMT
β-blockade/β Receptor
Current Clinical Ability to Predict Response
Meyer UA and Flockhart DA, 2005

Polymorphic Distribution



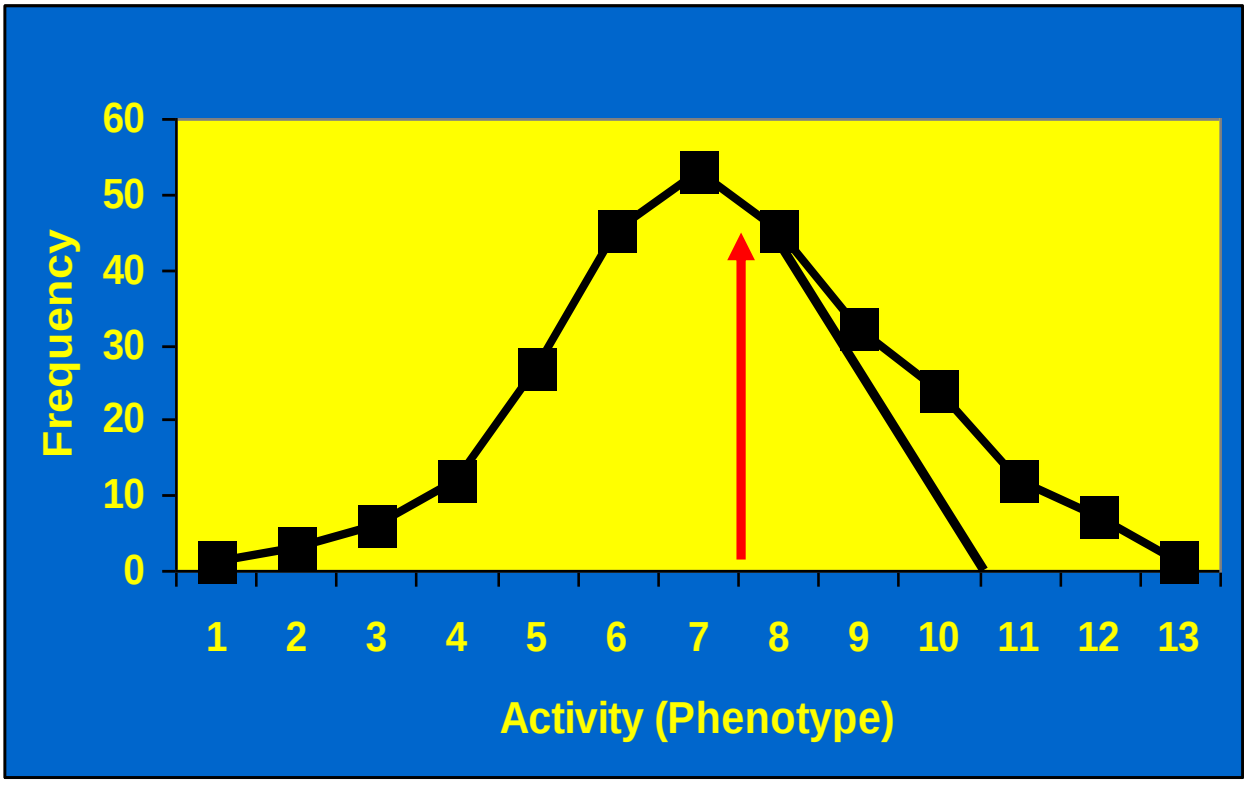
The Value of Normit Distribution Plots:

Population Distribution of CYP2C19 phenotype

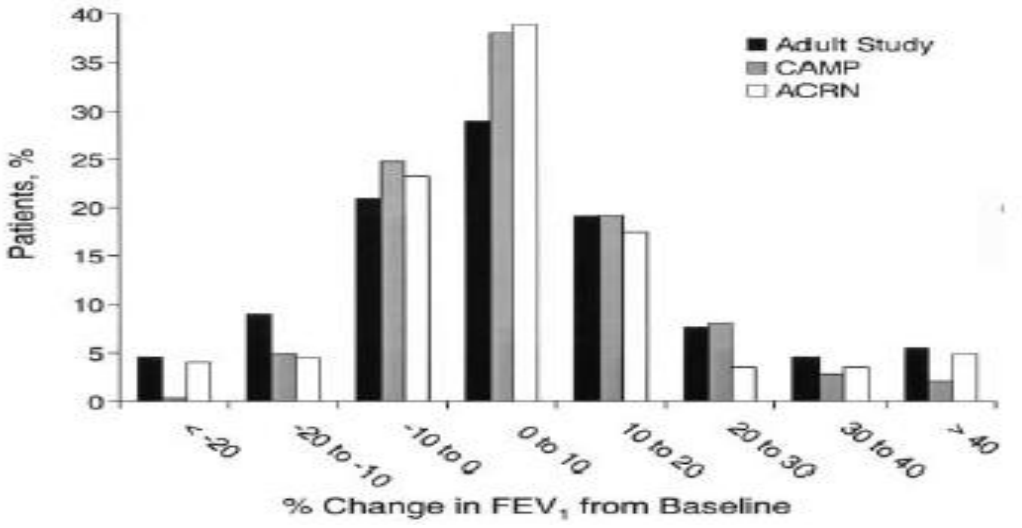


Flockhart et al: Clin Pharmacol Ther 1995;57:662-669

Skewed Distribution

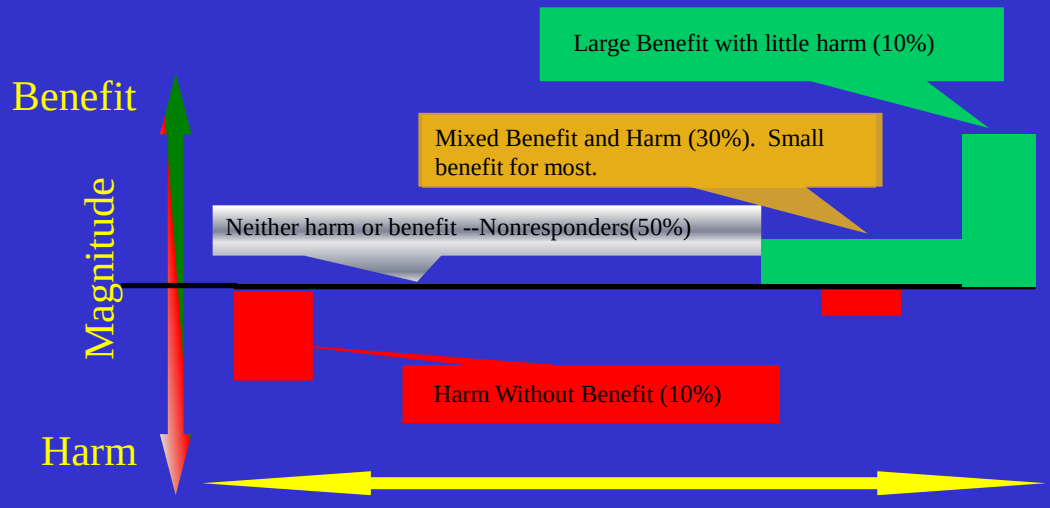


Example 1 of a Skewed Distribution: Heterogeneity in response to Inhaled Corticosteroids



Weiss ST et al. Hum Molec Genetics 2004; 13:1353-1359

The Problem with Mean Response Data : Heterogeneity in Response to Medicines In Clinical Trials



Frequency of various responses in the RCT treated population

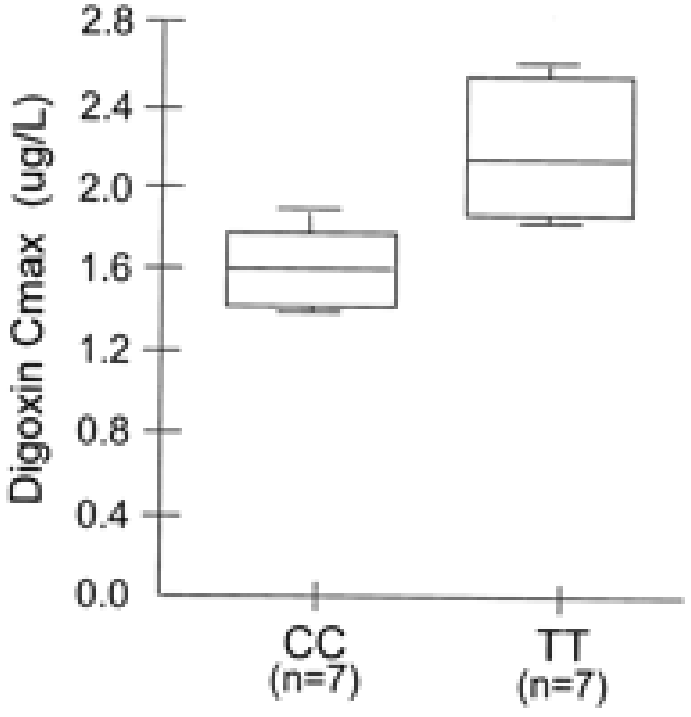
Barbara Evans et al. (2010)

Lessons

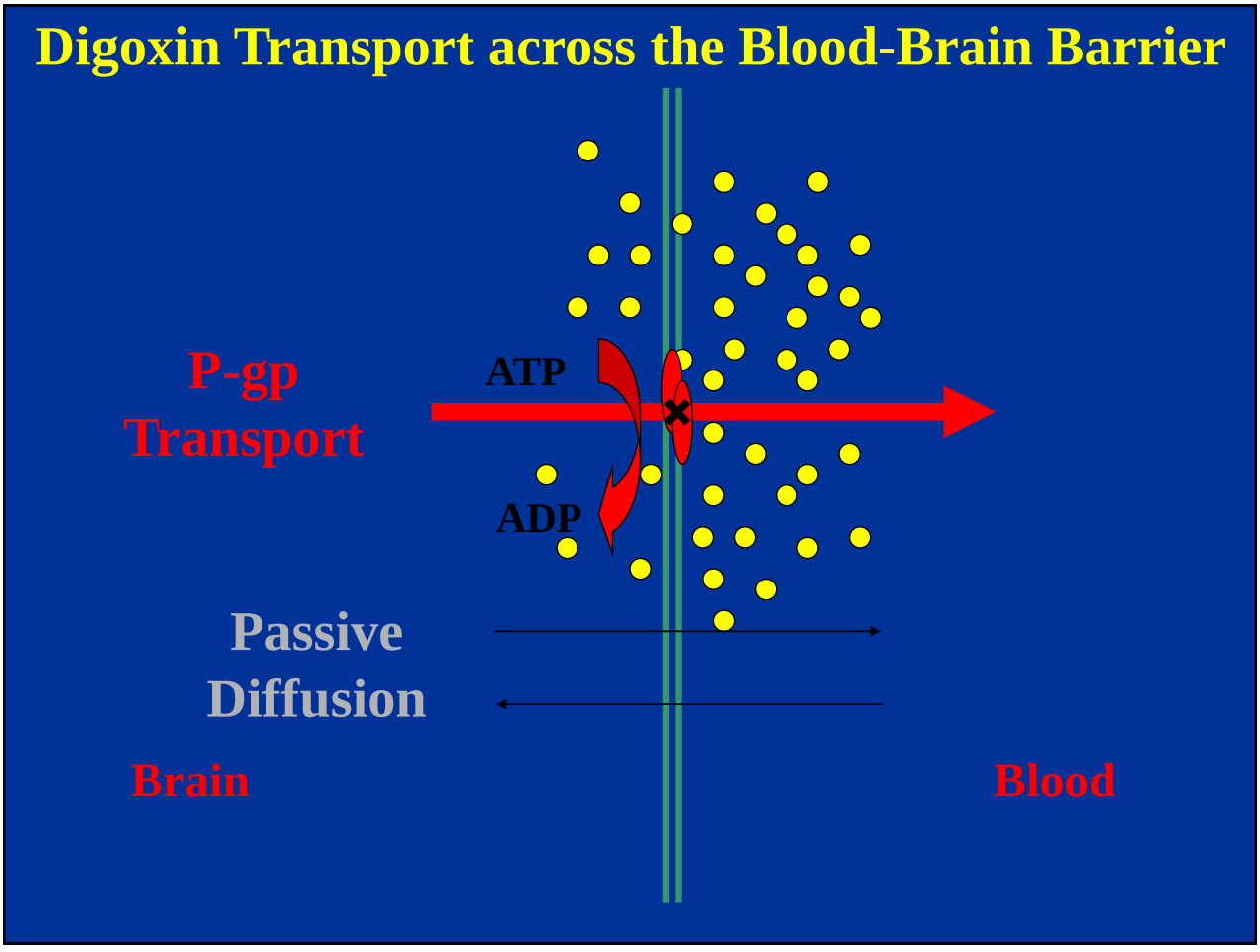
- Germline genetic variation is a potentially valuable biomarker for many drug effects
- Extremes of phenotype are often viewed as “discardable data”, but outliers (patients or events) should be viewed as important research stimuli
- Drug effects on populations can obscure effects on individual patients.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



Eichelbaum et al, Proc Nat Acad Sci, 2000:March

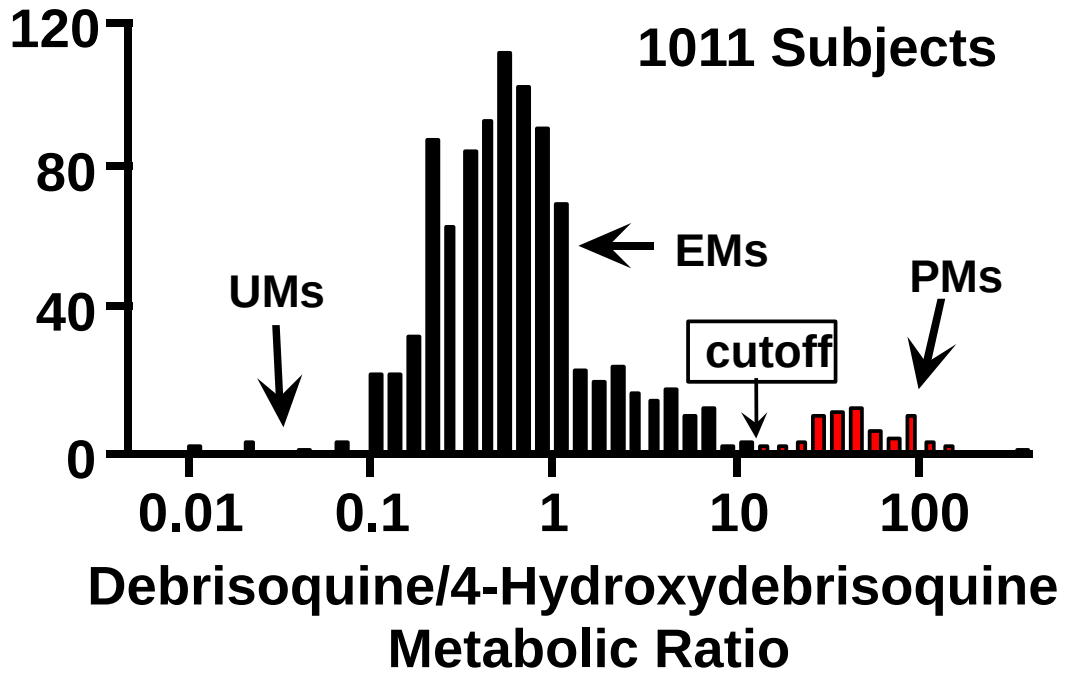

Digoxin Transport across the Blood-Brain Barrier
P-gp
Transport
ATP
ADP
Passive
Diffusion
Brain
Blood

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
  - β-blockers
  - tricyclic antidepressants
- Inhibited by:
  - fluoxetine
  - haloperidol
  - paroxetine
  - quinidine

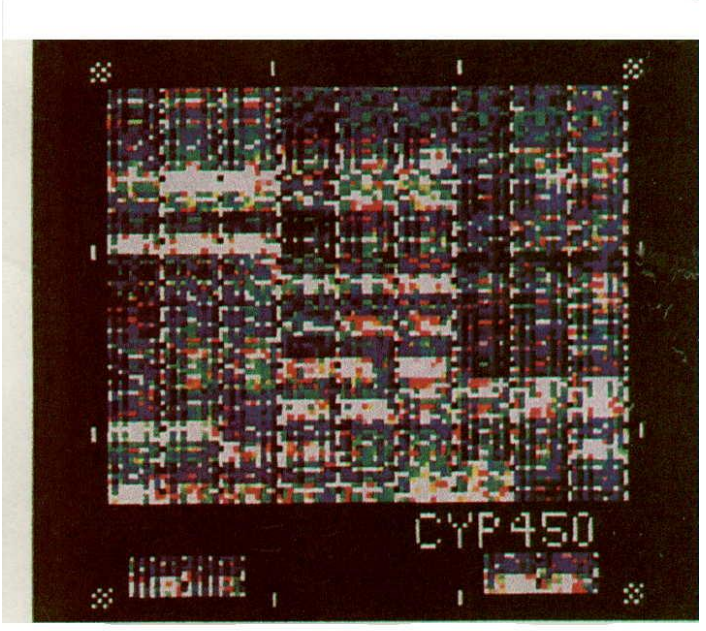
CYP2D6 Pharmacogenetics



CYP2D6 Alleles

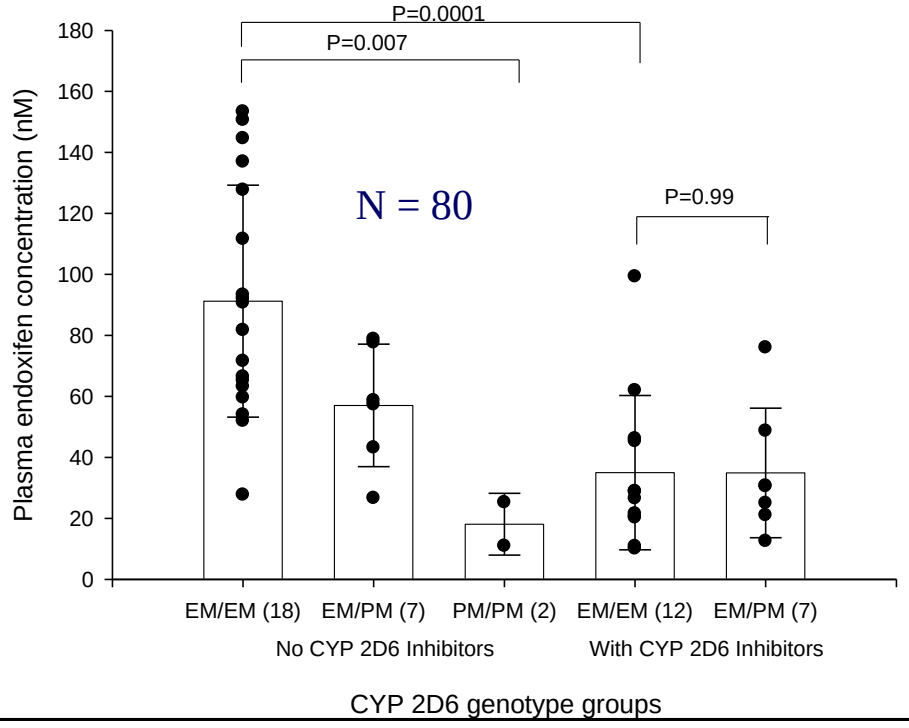
- 69 as of December, 2008
- 24 alleles have no activity
- 6 have decreased activity
- *1, *2, *4 and many others have copy number polymorphisms
- The *2 variant can have 1,2,3,4,5 or 13 copies i.e increased activity

Oligonucleotide array for cytochrome P450 genotesting



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

CYP2D6 variant genotype and CYP2D6 inhibitors lower [Endoxifen]

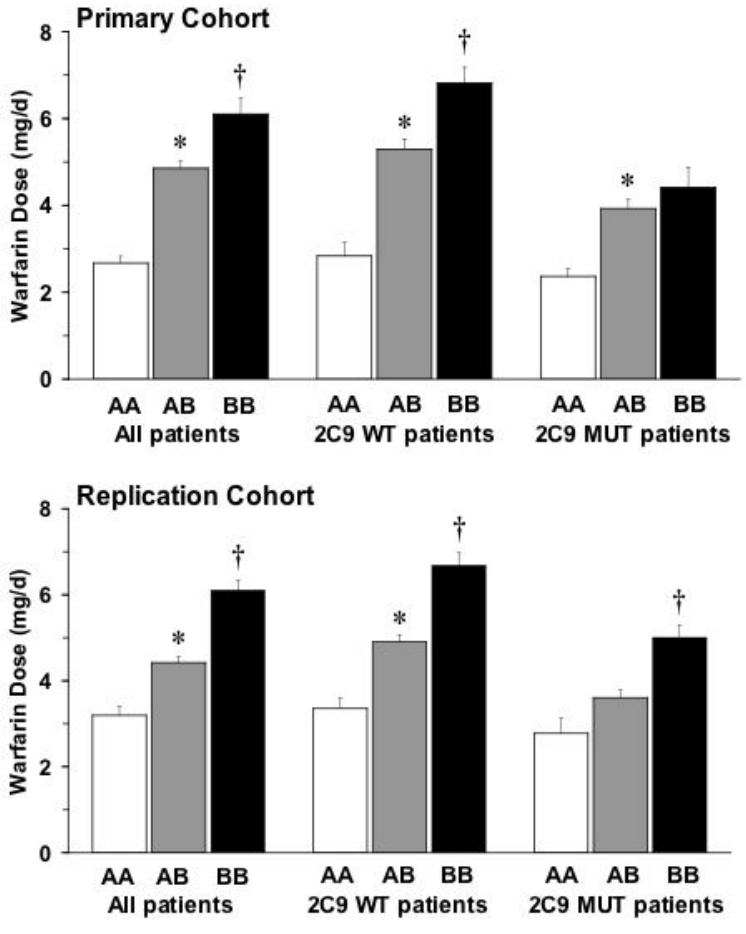


Lessons from CYP Pharmacogenetics

- 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 mimick a genetic change

Metrics of Clinical Biomarker Value

- Analytical Validity
  - Is the genetic test robust in the lab?
- Clinical Validity
  - Does the test predict a clinical event?
- Clinical Utility
  - Would the test change what you do?



VKORC1 Haplotype and CYP2C9 Genotype changed Warfarin Dose

Primary cohort: UW (N=185);

Replication cohort: Wash U (N=368).

All participants were Caucasian.

Rieder et al. N. Eng J. Med 2005;352: 2285-2293[

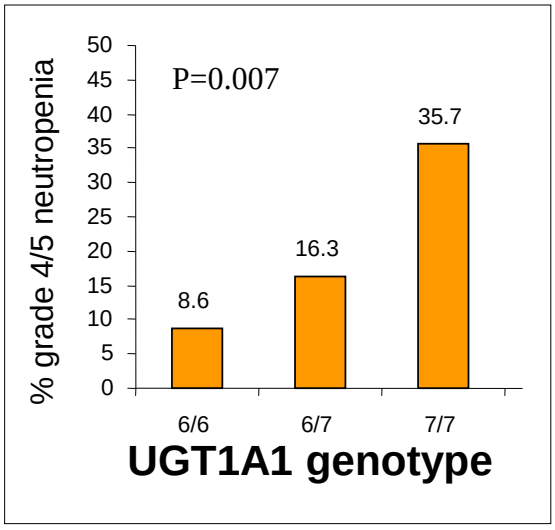
Warfarin Pharmacogenomic Testing

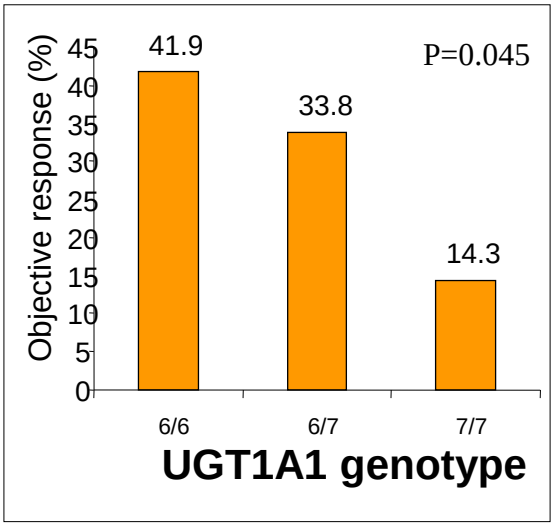
- Analytical Validity OK
- Clinical Validity OK
- Clinical Utility

– limited because a viable alternative (INR) is available in many, but not all practice settings.

- Not in widespread use.

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





N=524

McLeod H. et al, 2003.

Irinotecan Pharmacogenomic Testing

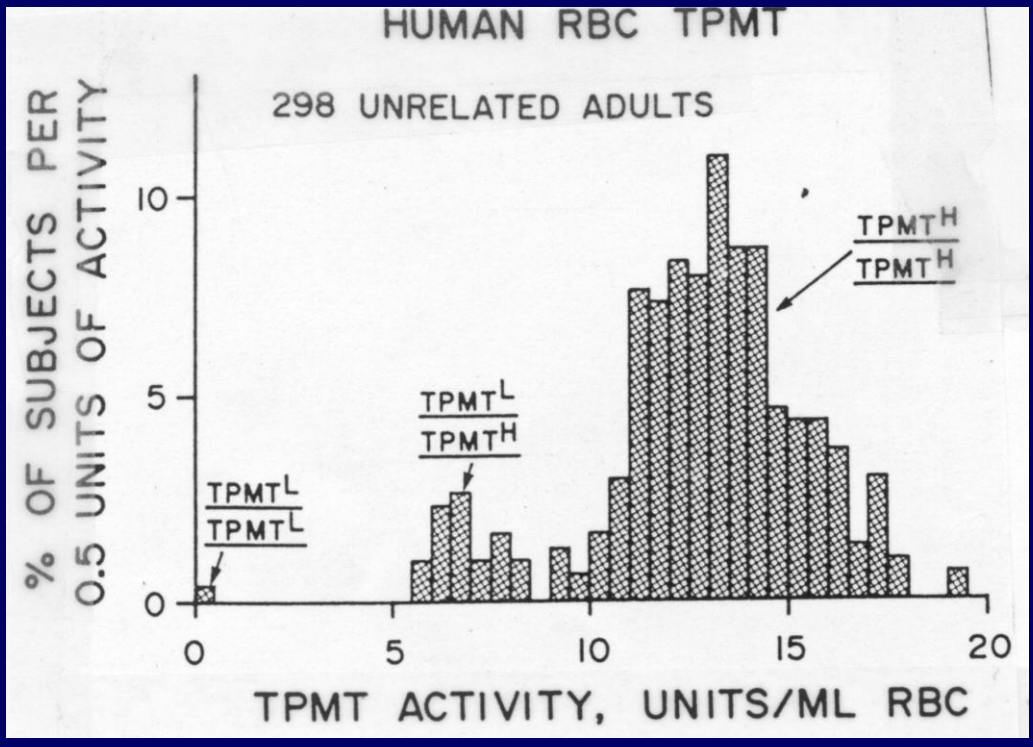
- Analytical Validity OK
- Clinical Validity OK
- Clinical Utility unclear because the tests value is limited to specific dosing regimes

- Not in widespread use

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

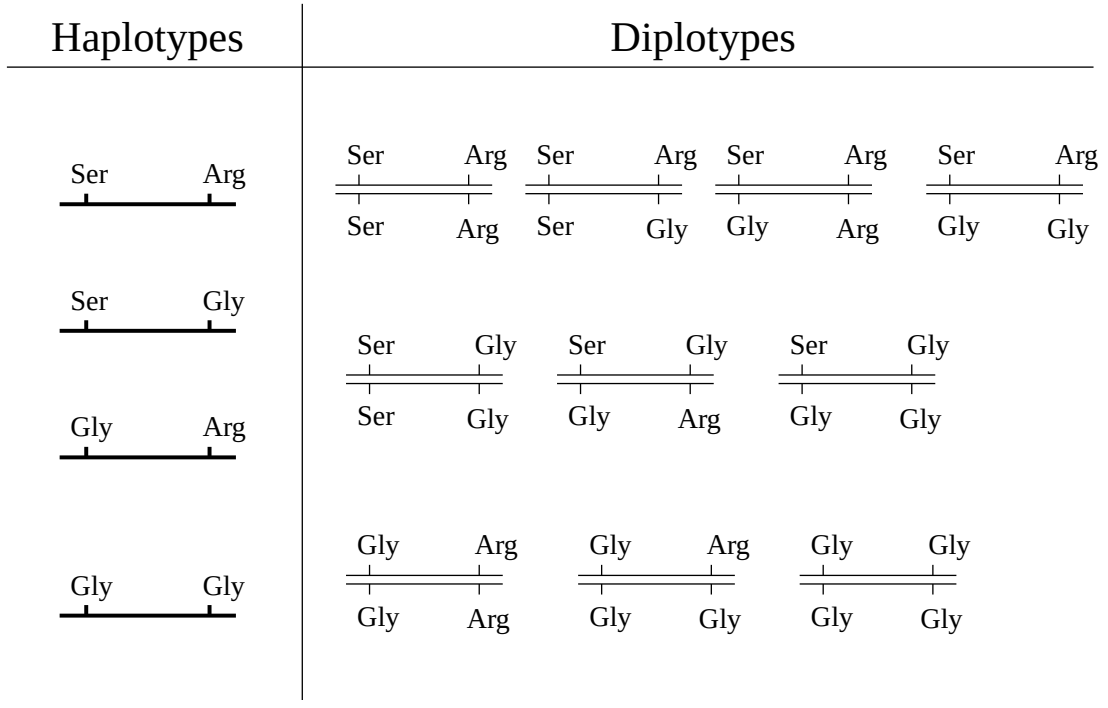


From: Weinshilboum et al. JPET;222:174-81. 1982

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_4 receptor
- Endothelial NO synthase
- $5HT_4$receptor

2SNPs: 10 possible hapoltypes



Ying-Hong Wang PhD,

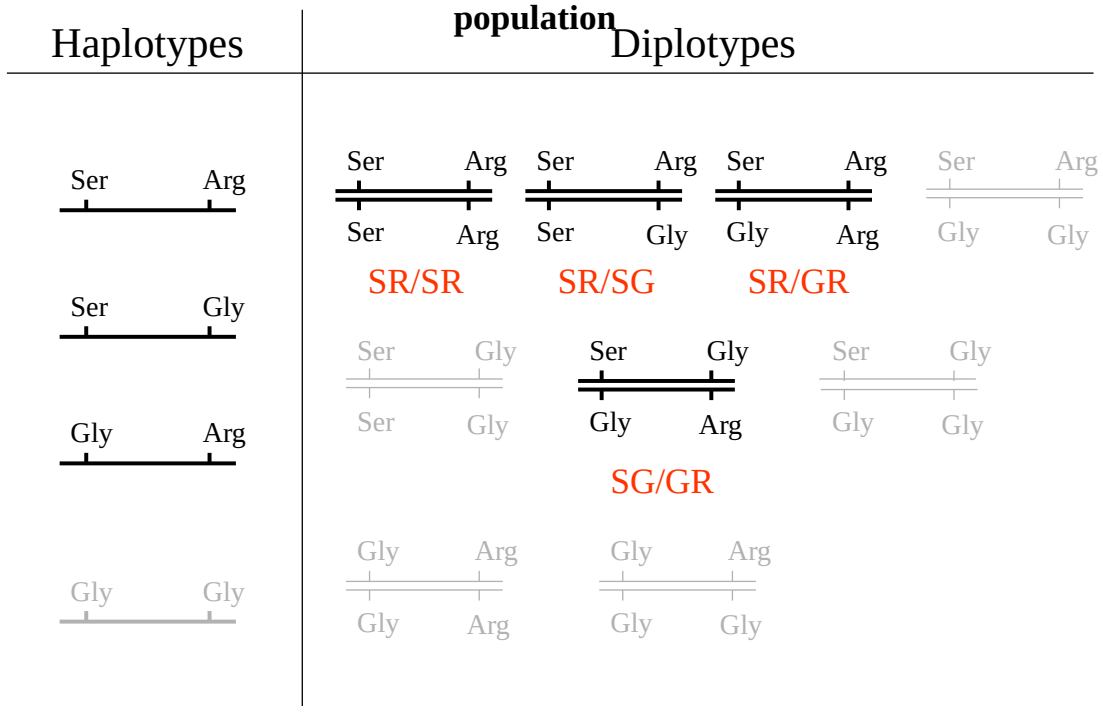
Indiana University School of Medicine

Observed β_1AR 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)

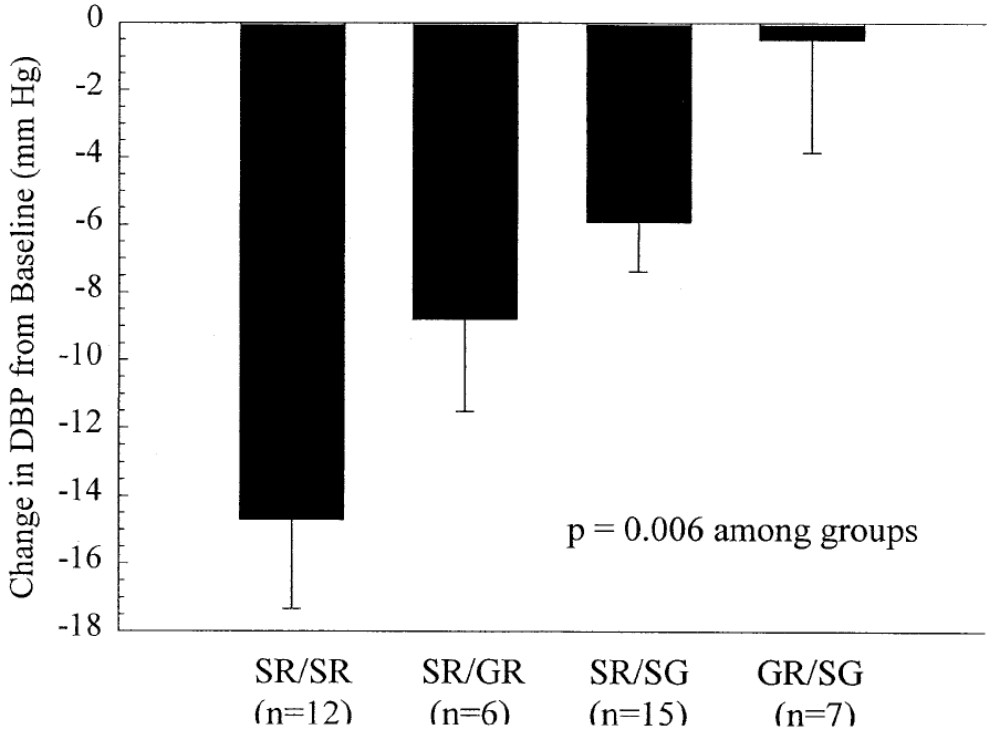
Terra et al. *Clin. Pharmacol. Ther*. 71:70 (2002)

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



Ying-Hong Wang PhD,
Indiana University School of Medicine

Diplotype predicts Beta-blocker effect

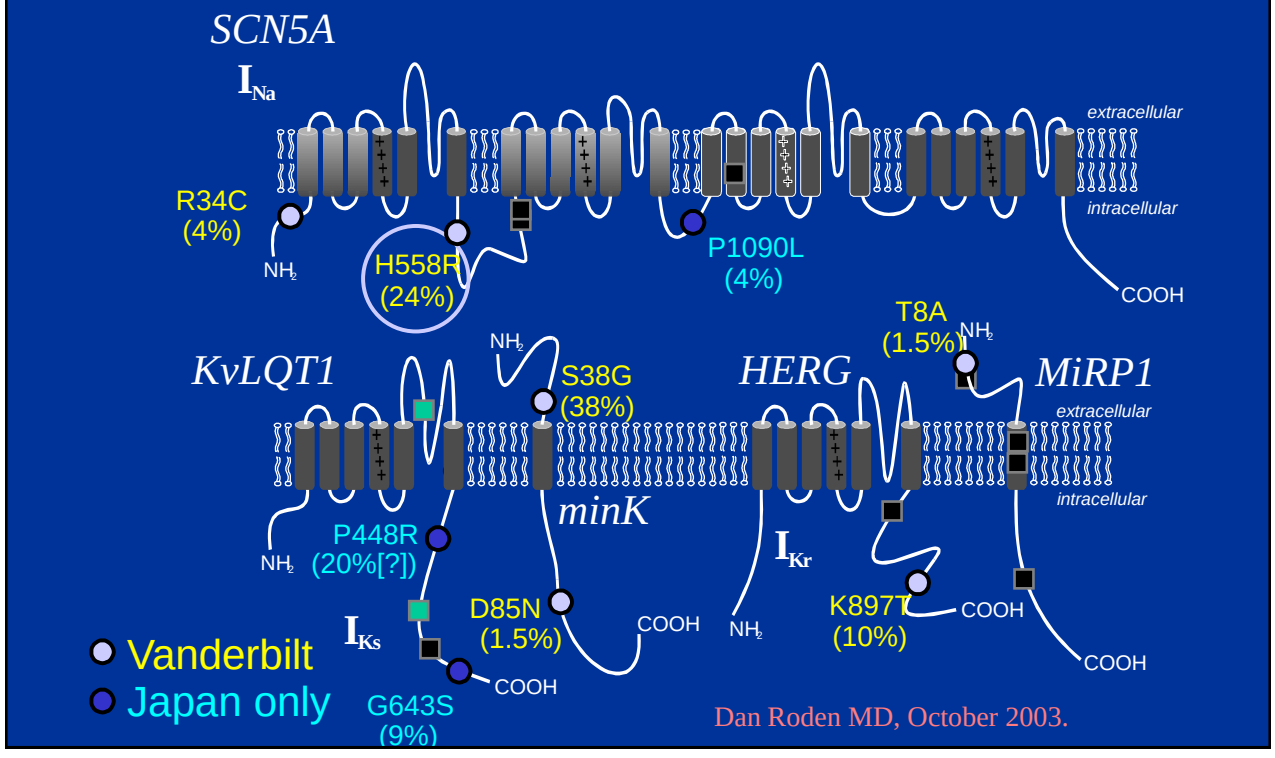


Johnson et al. Clin Pharmacol & Ther. 2003,74:44-52.

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

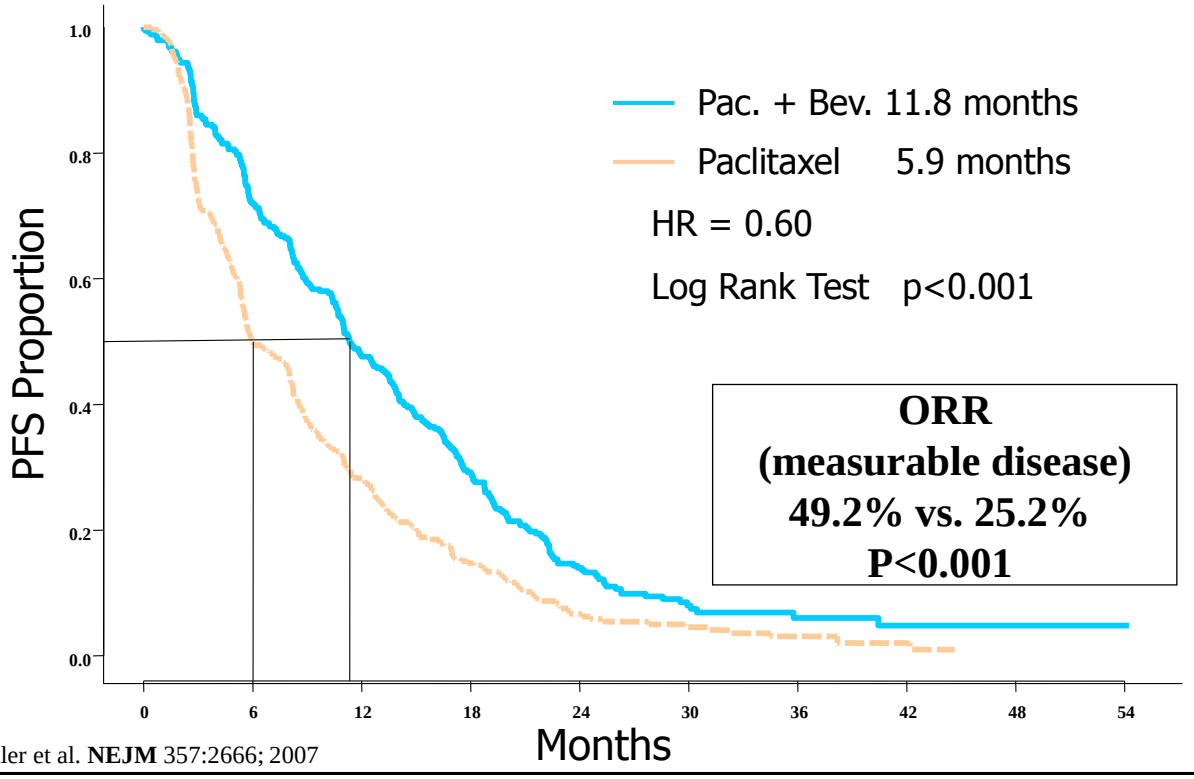
Non-synonymous coding region polymorphisms in long QT disease genes



Biotech Pharmacogenomics

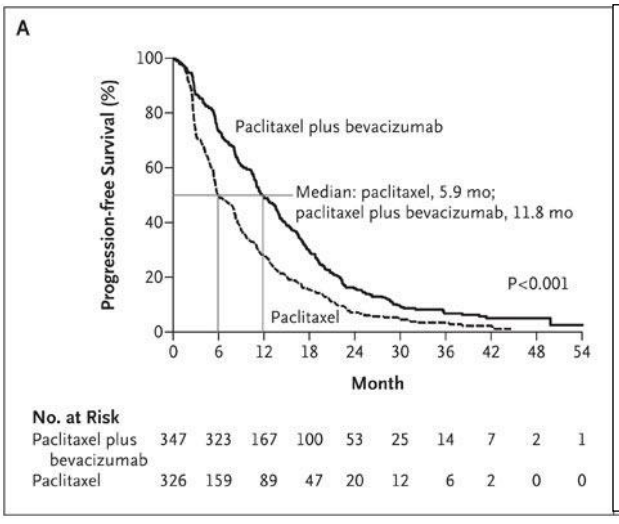
- Bevacizumab (Avastin™)
  - Interferon and IL 28b
  - Erlotinib and K-Ras

Bevacizumab significantly improved PFS



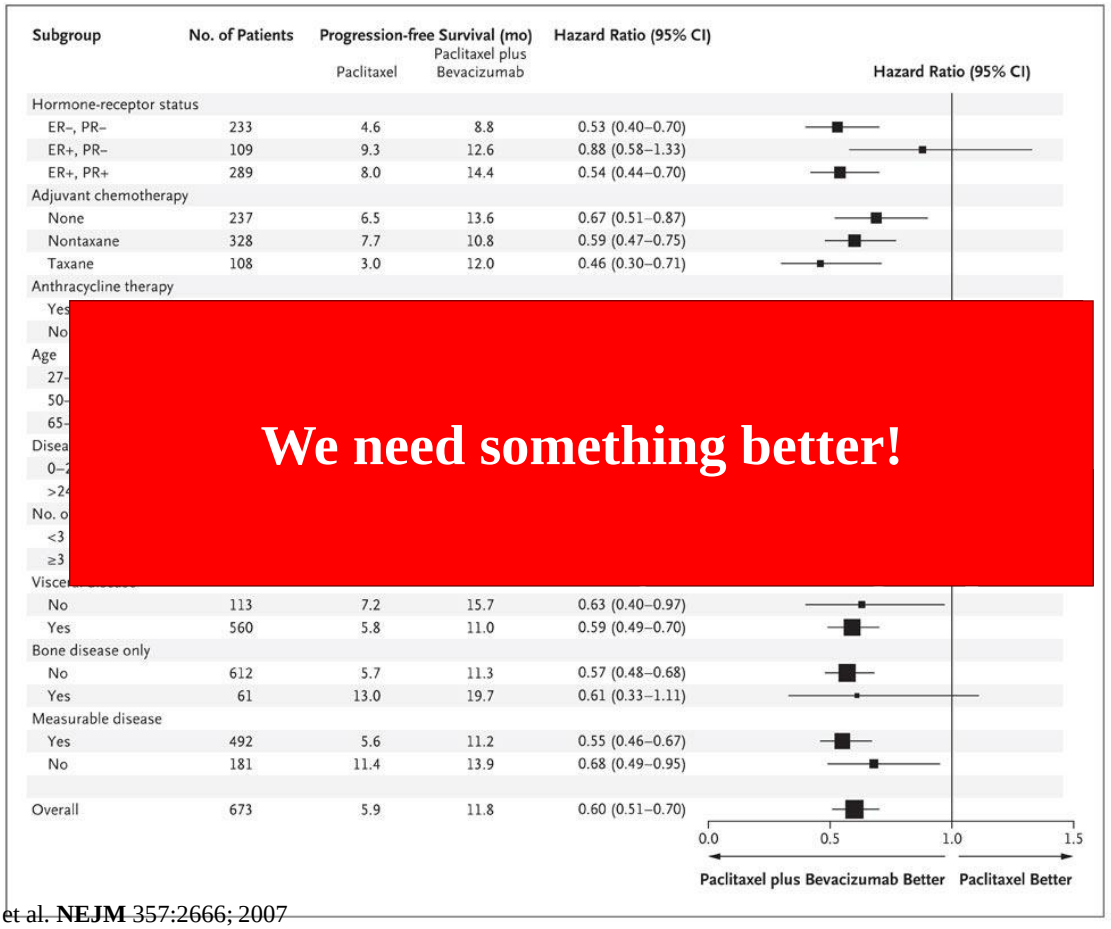
Miller et al. **NEJM** 357:2666; 2007

Improvement in PFS/ORR did not translate into OS benefit



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

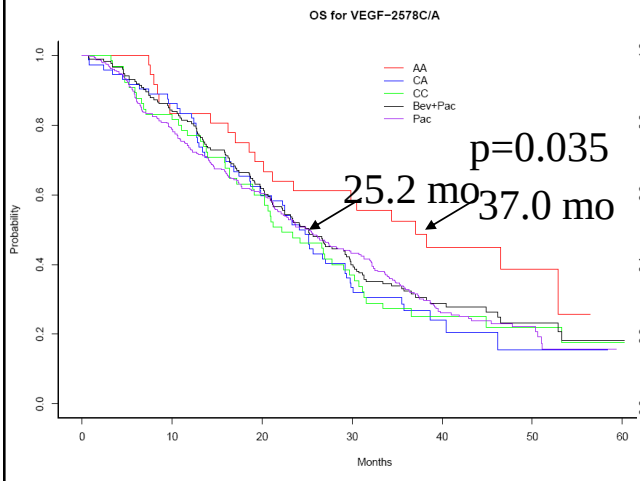
Miller et al. **NEJM** 357:2666; 2007

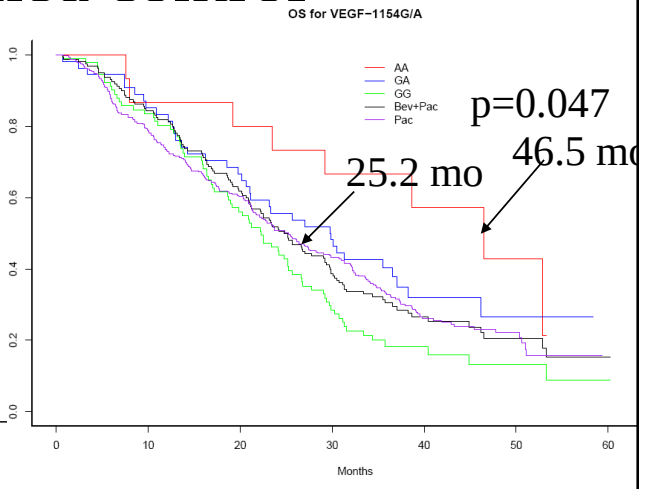


We need something better!

Miller et al. **NEJM** 357:2666; 2007

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





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

Bevacizumab increased grade 3/4 toxicity

Serious but rare

Serious, frequent, & unique

Likely related to duration of taxane exp

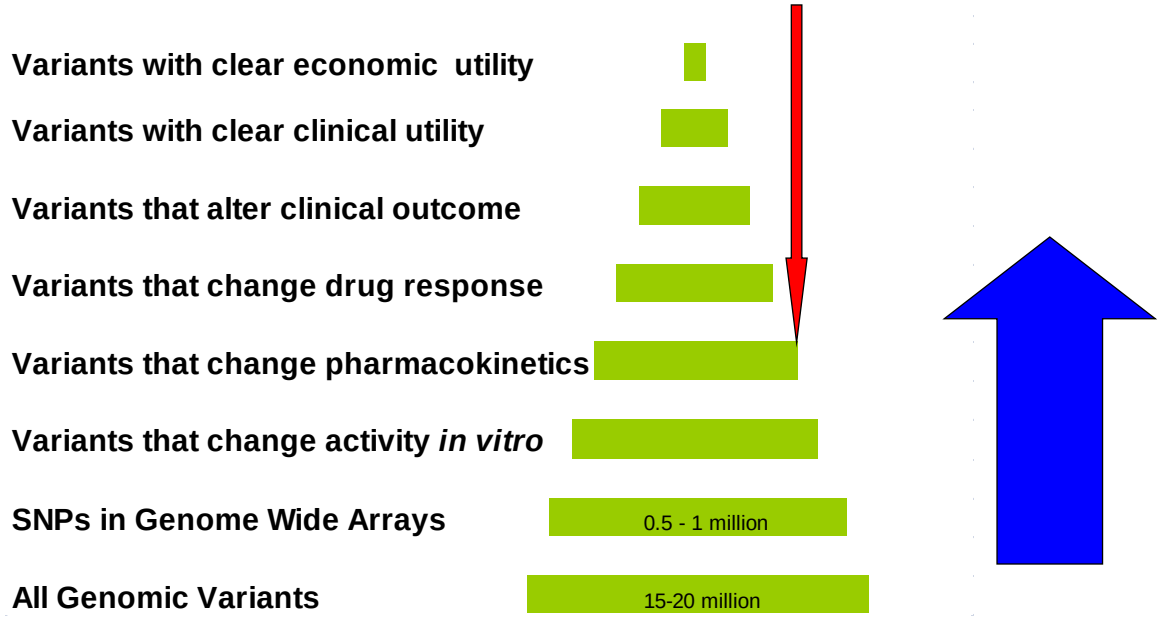
Toxicity	P (%)	P+B (%)	p-value
Infection	2.9	9.3	<0.001
Fatigue	4.9	9.1	0.04
Neuropathy	17.7	23.5	0.05
CNS ischemia	0	1.9	0.02
Headache	0	2.2	0.008
Proteinuria	0	3.5	<0.001
Hypertension	0	14.8%	<0.001

Miller et al. **NEJM** 357:2666; 2007

Fourteen Drugs and Their Available Pharmacogenetic Tests December 2010

- **Abacavir**
- Clopidogrel
- **Tamoxifen**
- metformin
- **Imatinib**
- 5-Fluorouracil
- **Clozapine**
- QT-prolonging Drugs
- **Irinotecan**
- Azathioprine and Mercaptopurine
- **Warfarin**
- Carbamazepine
- **Interferon**
- **HLA *B5701**
- CYP2C19
- **CYP2D6**
- OATP3
- **BCR-ABL**
- DPYD-TYMS
- **2 SNPs in HLA-DQB1**
- Familion™
- **UGT1A1**
- TPMT
- **CYP2C9 and VKCoR**
- HLA-B* 1502
- **IL 28b**

Hierarchy of Value for Pharmacogenomic Information



Summary

- Pharmacogenomic testing is now being widely applied to some of the most wodely prescribed drugs
- Pharmacogenomic biomarkers require demonstration of clinical utility before widespread implementation
  - This has happened in very few cases to date
- Clinical pharmacogenomic predictive tests must provide real value over existing predictors
- Economic utility is often as important as clinical utility

Pharmacogenetics Websites

- www.pharmgkb.org
- The SNP consortium: http://brie2.cshl.org
- The Human Genome:
  www.ncbi.nlm.nih.gov/genome/guide/H_sapiens.html
- CYP alleles: www.imm.ki.se/CYPalleles/
- Drug Interactions: www.drug-interactions.com