Clinical Pharmacogenomics

David A Flockhart MD, PhD
Chief, Division of Clinical Pharmacology
Professor of Medicine, Genetics and Pharmacology
Indiana University School of Medicine

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?

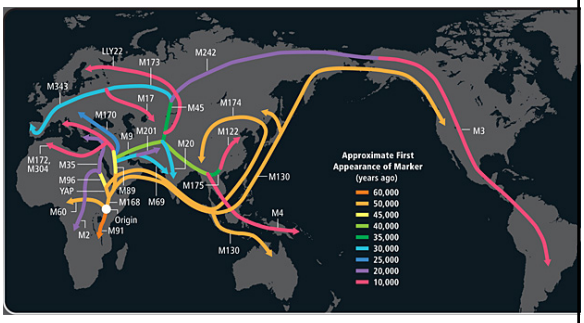
The Genome Map is available on the web, to anyone, free.

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

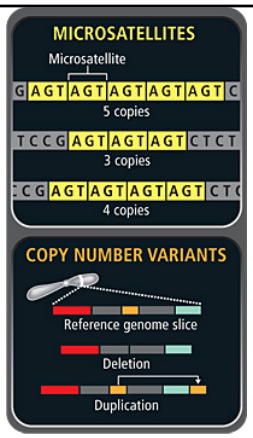
DNA is very stable

DNA can be amplified

Human Migration out of Africa



Scientific American, July 2008



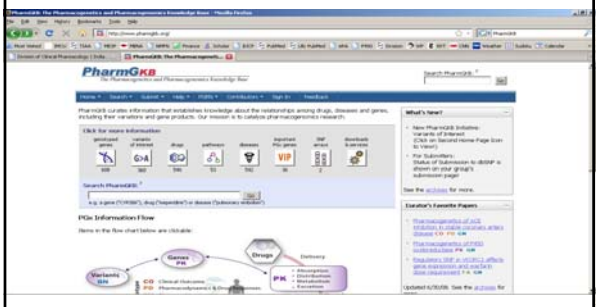
SNP Variability in The Human Genome July 2008

- 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 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**

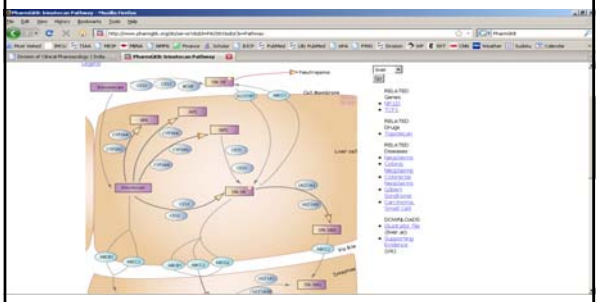
SNP Variability In Exons

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

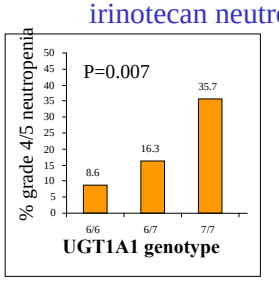
PharmGKB as a source of Candidate Genes and Pathways

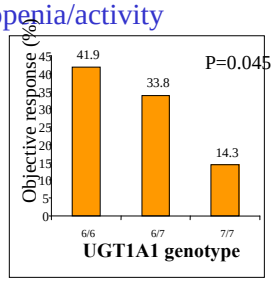


PharmGKB Irinotecan Pathway



UGT1A1 TA repeat genotype alters irinotecan neutropenia/activity



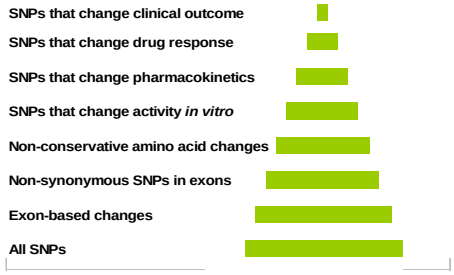


N=524

McLeod H. et al, 2003.

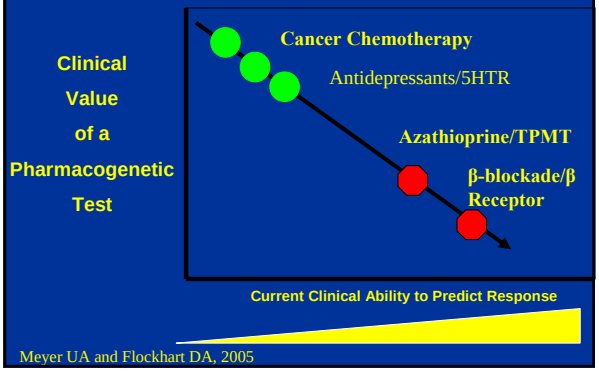
Pharmacogenomic Journals, 2008

Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)



Pharmacogenetic Principle 1:

Value Decreases when Current Predictive Ability is High

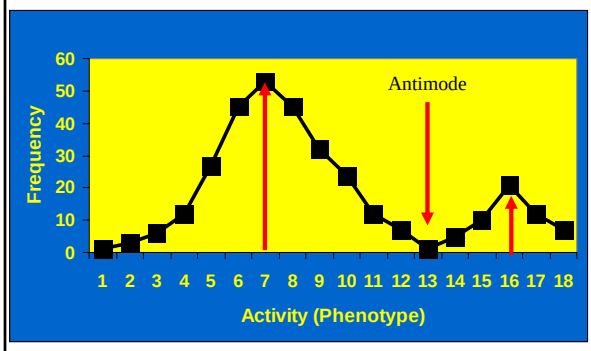


Meyer UA and Flockhart DA, 2005

Methods in Pharmacogenetics

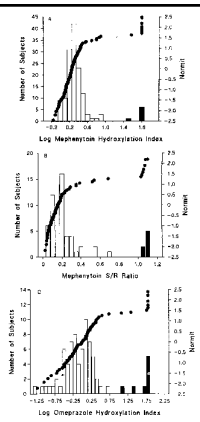
- SNP discovery:
  - Candidate gene approach
  - Pathway approach
  - Genome Wide Arrays
  - Next Generation Sequencing
- Identification of gene and variants
- Development of a genetic test for DNA variants
- Correlation between genotype and phenotype
- Validation
- **Application in Clinical Practice**

Polymorphic Distribution



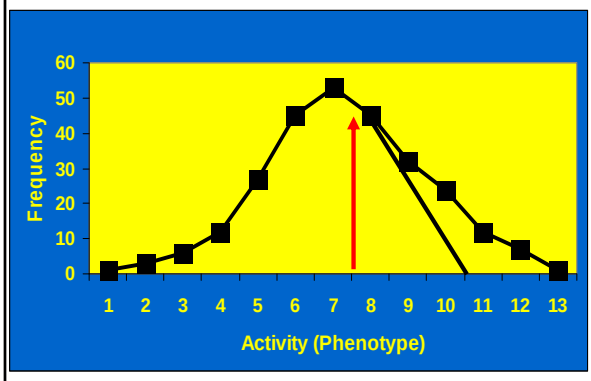
The Value of Normit Distribution Plots:

Population Distribution of CYP2C19 phenotype

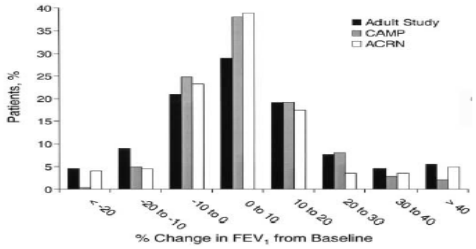


Flockhart et al: Clin Pharmacol Ther 1995;57:662-669

Skewed Distribution



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



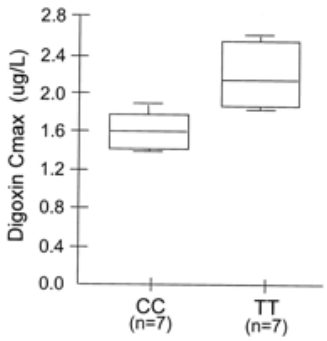
Weiss ST et al. Hum Molec Genetics 2004; 13:1353-1359

Lessons

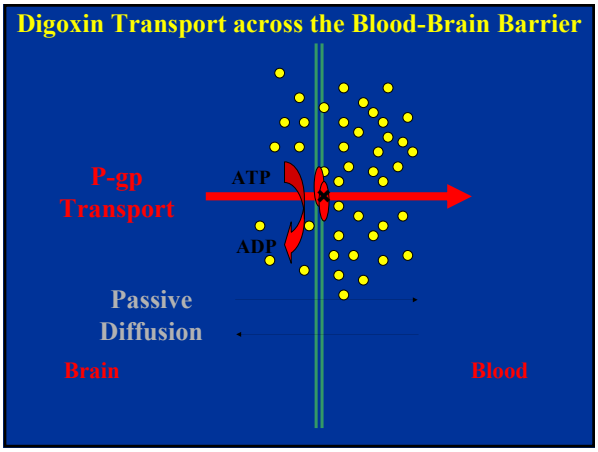
- Germline genetic variation is a potentially valuable biomarket for many drug effects
- Extremes of phenotype are often viewed as "discardable data", but outliers (patients or events) should be viewed as important research stimuli
- Drug effects on populations can obscure effects on individual patients.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

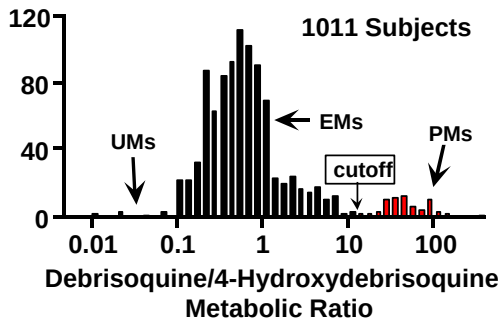


Genetics and Drug Elimination

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

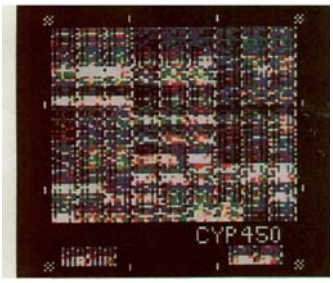
CYP2D6 Pharmacogenetics



CYP2D6 Alleles

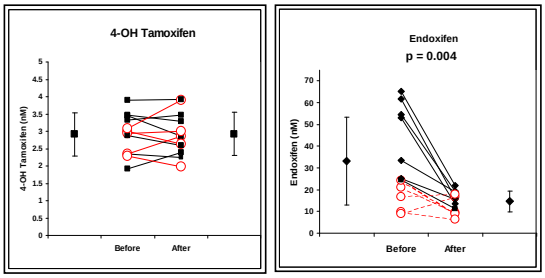
- 69 as of December, 2008
- 24 alleles have no activity
- 6 have decreased activity
- *1, *2, *4 and many others have copy number polymorphisms
- The *2 variant can have 1,2,3,4,5 or 13 copies i.e increased activity

Oligonucleotide array for cytochrome P450 genotesting



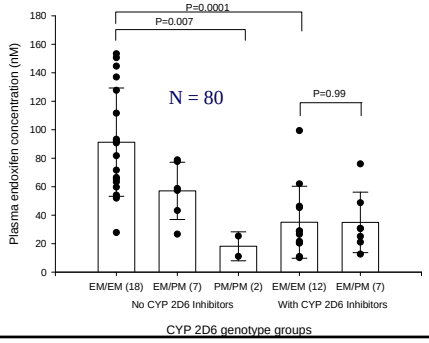
From: Flockhart DA and Webb DJ. *Lancet* End of Year Review for Clinical Pharmacology. 1998.

Paroxetine and CYP2D6 genotype change the plasma concentrations of endoxifen



Flockhart *et al.* 2003

CYP2D6 variant genotype and CYP2D6 inhibitors lower [Endoxifen]



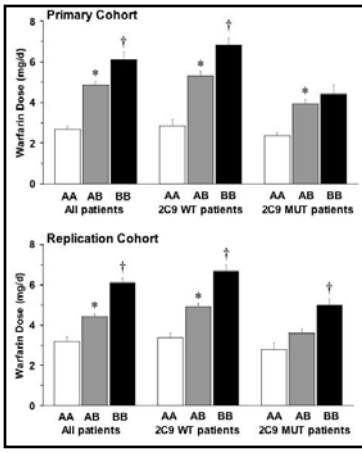
Methods

- 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

CP1229323-10

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



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

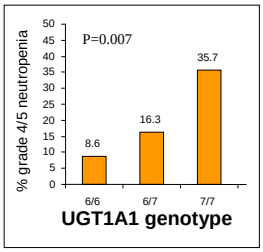
Primary cohort: UW (N=185);

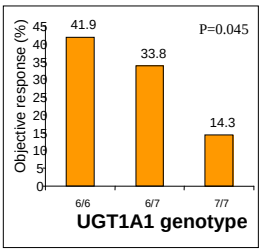
Replication cohort: Wash U (N=368).

All participants were Caucasian.

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

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





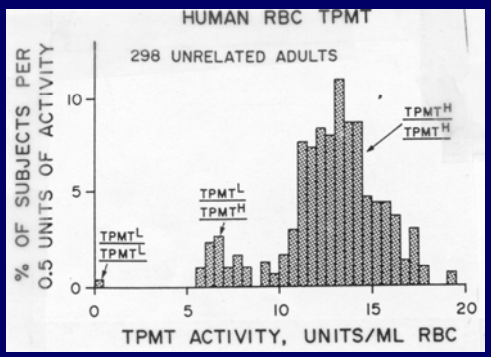
N=524

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

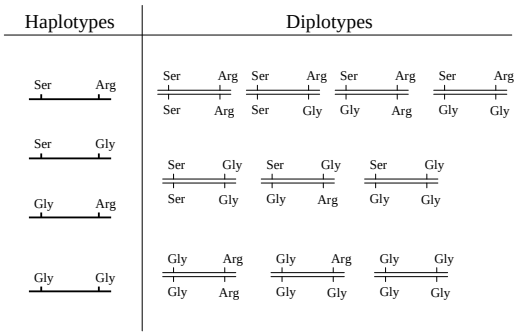


From: Weinshilboum et al. JPET;222:174-81. 1982

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

2SNPs: 10 possible hapoltypes



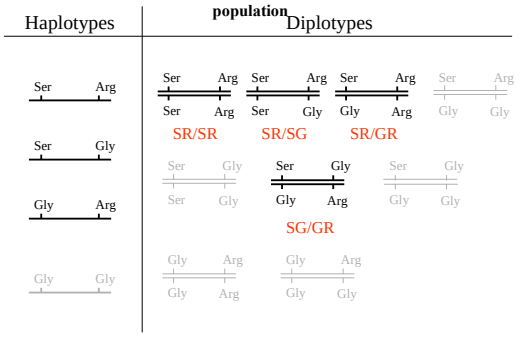
Ying-Hong Wang PhD,
Indiana University School of Medicine

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)

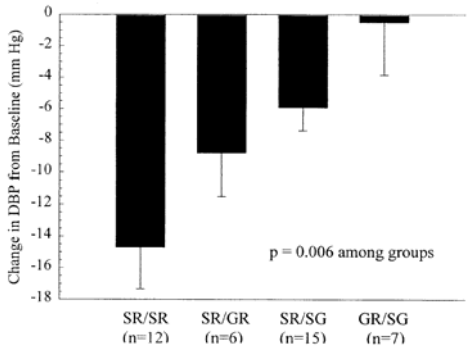
Terra et al. *Clin. Pharmacol. Ther*. 71:70 (2002)

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

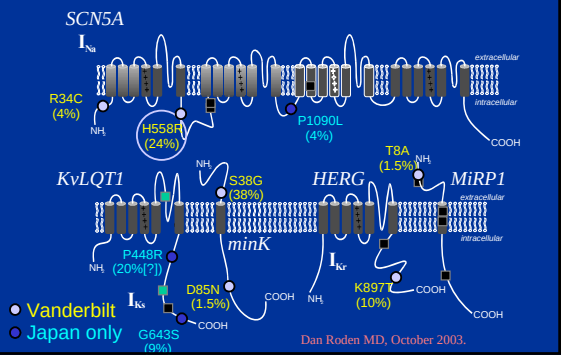


Johnson et al. Clin Pharmacol & Ther. 2003,74:44-52.

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



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

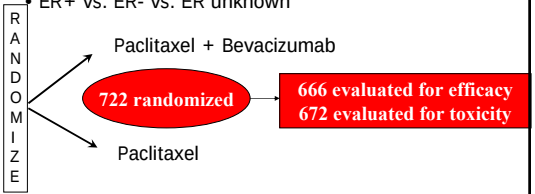
3).Drug evaluated must be heterogeneous in outcome
- -mix of success and toxicity

4).Variability must be frequent
- -generalizability of results

Walgren et al. *JCO* 2005;23:7342-7349

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

Stratify:
- DFI ≤ 24 mos. vs. > 24 mos.
- < 3 vs. ≥ 3 metastatic sites
- Adjuvant chemotherapy yes vs. no
- ER+ vs. ER- vs. ER unknown



Bevacizumab increased grade 3/4 toxicity

Serious, frequent, & unique

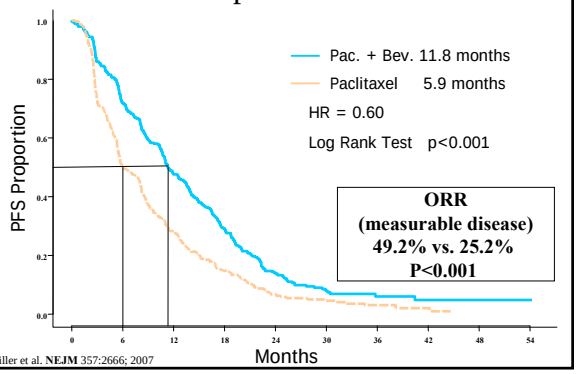
Serious but rare

Likely related to duration of taxane 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

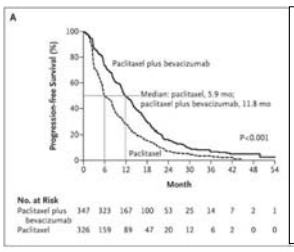
Miller et al. **NEJM** 357:2666; 2007

Bevacizumab significantly improved PFS



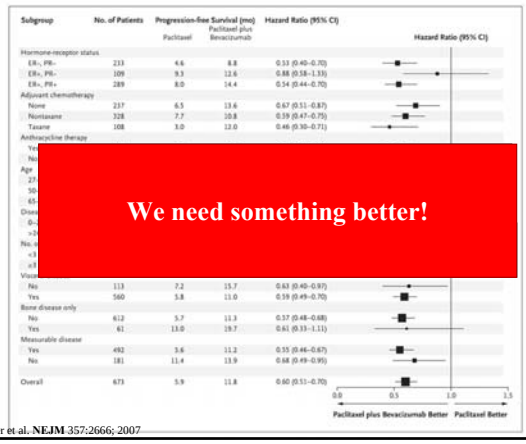
Miller et al. **NEJM** 357:2666; 2007

Improvement in PFS/ORR did not translate into OS benefit



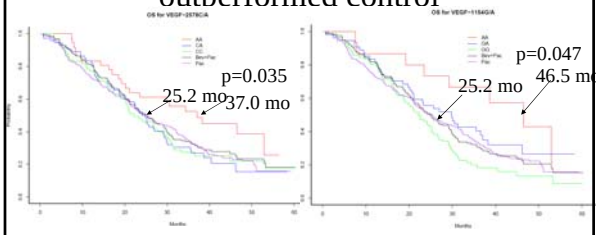
ORR
(measurable disease)
49.2% vs. 25.2%
P<0.001

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



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

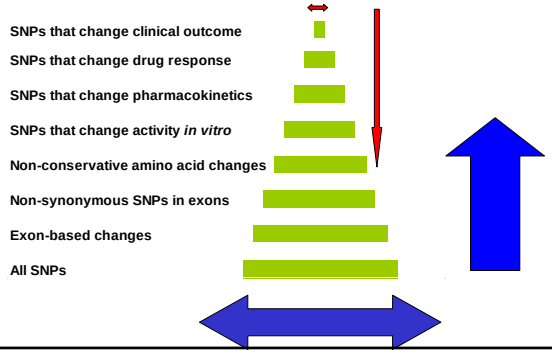
VEGF -2578 AA & -1154 AA genotypes in combination arm outperformed control



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

Hierarchy of Pharmacogenetic Information from Single Nucleotide Polymorphisms (SNPs)

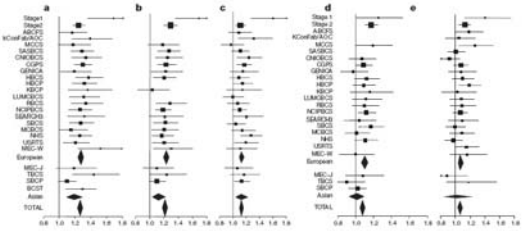


Genome Wide SNP Arrays

- 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 27th, 2007



GENOME RESEARCH

Cloning for the future... BioLabs

HOME HELP FEEDBACK SUBSCRIPTIONS ARCHIVE SEARCH TABLE OF CONTENTS

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

Genome Res. 16:1575-1584, 2006

Methods

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

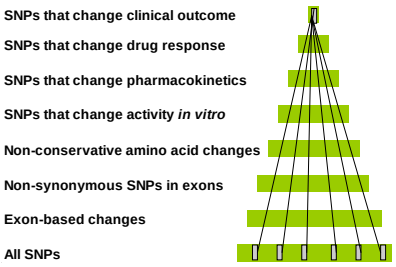
Daisuke Komura[1,2,8], Fan Shen[3,8], Shunpei Ishikawa[1,8], Karen R. Fitch[3], Wenwei Chen[3], Jane Zhang[3], Guoying Liu[3], Sigeo Ihara[1], Hiroshi Nakamura[1,2], Matthew E. Hurles[4], Charles Lee[5], Stephen W. Scherer[6], Keith W. Jones[3], Michael H. Shapero[3], Jing Huang[3,9], and Hiroyuki Aburatani[1,7,9]

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



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

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.

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
- 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