# Pharmacogenomics: 2012

March, 2012

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University of North Carolina – Chapel Hill, NC

JOHNS HOPKINS
MEDICINE
CONTINUING MEDICAL EDUCATION

***Current Topics in Genome Analysis 2012***

***Howard McLeod***

***Gentris Corp: Consulting***
***Myriad Genetics: Consulting***

NATIONAL HUMAN GENOME RESEARCH INSTITUTE
Division of Intramural Research

**"A surgeon who uses the wrong side of the scalpel cuts her own fingers and not the patient;**

**if the same applied to drugs they would have been investigated very carefully a long time ago"**

***Rudolph Bucheim***
***Beitrage zur Arzneimittellehre, 1849***

The clinical problem

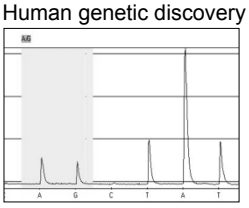
- Multiple active regimens for the treatment of most diseases
- Variation in response to therapy
- Unpredictable toxicity

$$$$$$$$$$$$

With choice comes decision

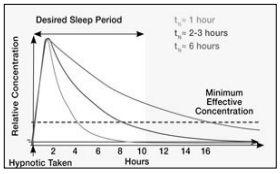
## Pharmacogenetics: what is your intent?

Human genetic discovery

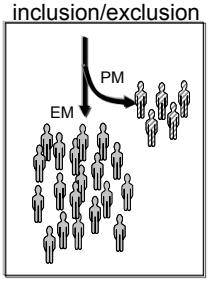


Drug Safety

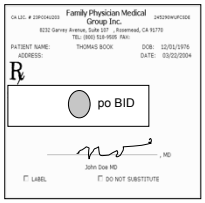
Explain variation in phenotype




Clinical trial inclusion/exclusion




Clinical practice




## Pharmacogenomic examples-2012

- *bcr/abl* or 9:22 translocation—imatinib mesylate*
- HER2-*neu*—trastuzumab**
- C-kit mutations—imatinib mesylate**
- Epidermal growth factor receptor mutations—gefitinib
- Thiopurine S-methyltransferase—mercaptopurine and azathioprine*
- UGT1A1-irinotecan**
- CYP2C9/VKORC1-warfarin*
- HLA-B*5701-abacavir *
- HLA-B*1502-carbamazepine *
- CYP2C19-clopidogrel
- IL28B-interferon
- Cytochrome P-450 (CYP) 2D6—5-HT3 receptor antagonists, antidepressants, ADHD drugs, and codeine derivatives, tamoxifen*

## Applications of pharmacogenetics

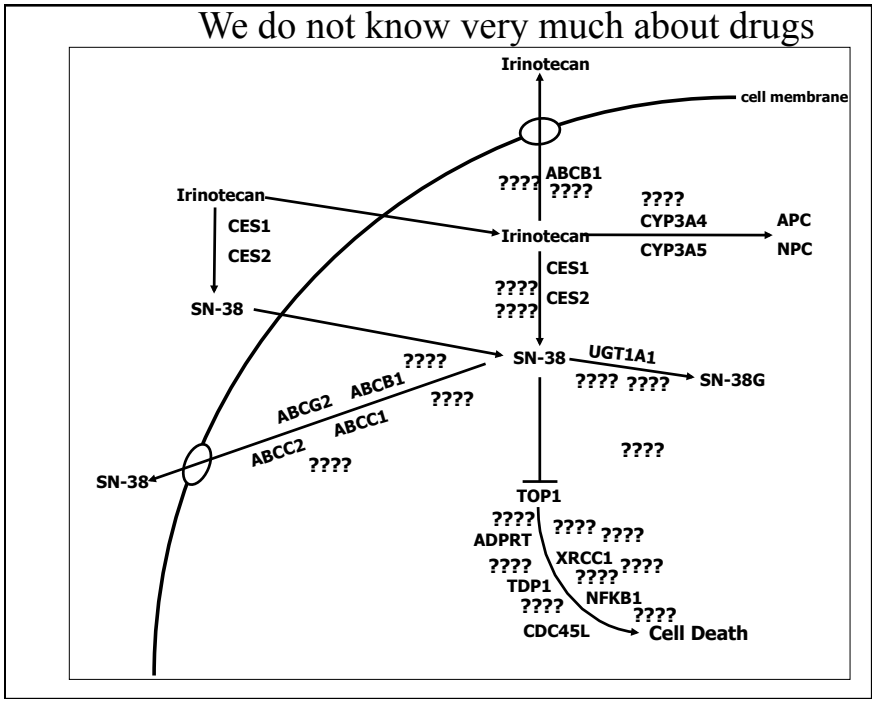
- Explanation for untoward event (DPYD, CYP2D6)
- Required for insurance coverage (KRAS, EGFR, ABL)
- Identify low utility (KRAS)
- Dose selection (CYP2C9, CYP2C19)
- Therapy selection (CYP2C19)
- Preemptive prediction (HLA-B*5701)

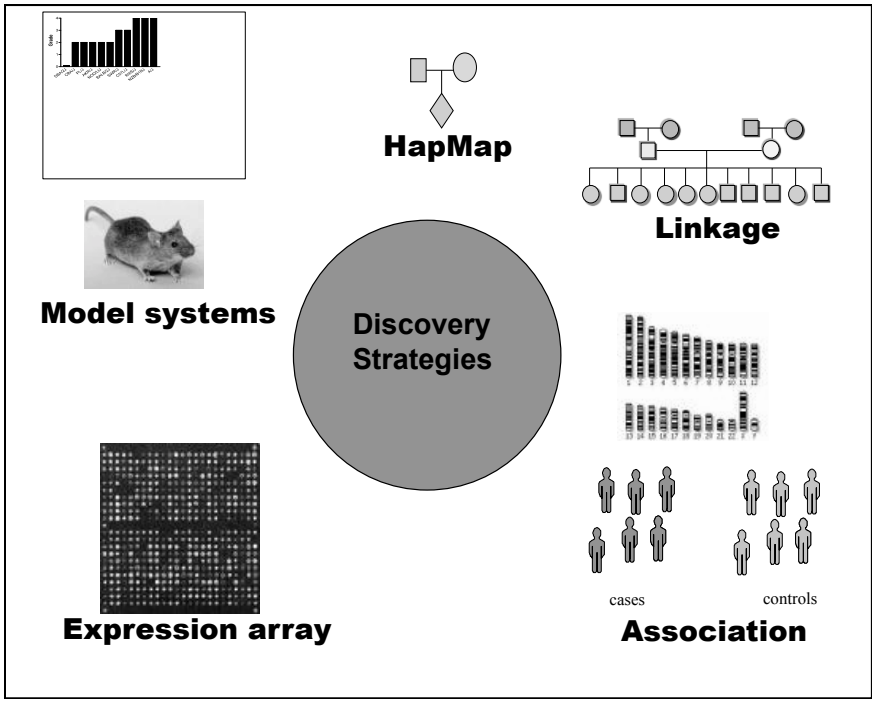
What needs to be done to determine hope vs hype?

•Find the 'right' biomarkers

•Validate in robust datasets

•Apply them!


We do not know very much about drugs
Irinotecan
cell membrane
ABCB1
????
????
Irinotecan
CES1
CES2
SN-38
Irinotecan
????
CYP3A4
CYP3A5
APC
NPC
CES1
????
CES2
????
SN-38
UGT1A1
SN-38G
????
????
????
ABCG2
ABCB1
ABCC1
ABCC2
????
????
SN-38
????
TOP1
????
ADPRT
????
????
XRCC1
????
????
TDP1
NFKB1
????
????
CDC45L
Cell Death


HapMap
Linkage
Model systems
Discovery Strategies
Expression array
cases
controls
Association

## We are only beginning to try!

- As of 3/10/12
  Drug-related phenotypes represented
  50/1196 GWA studies (4.1%)

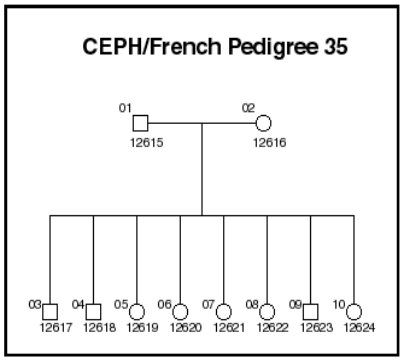
  10/50 had ≥ 500 'cases'

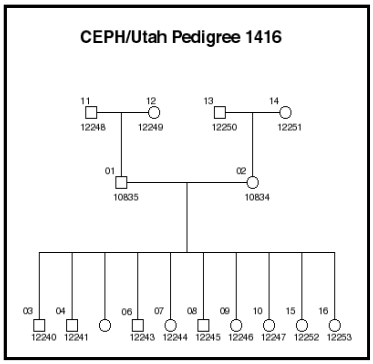
  15/50 (30%) found no significant 'hits'
  29/50 PGx studies had a replication cohort

  8 contributed to changes in FDA 'package insert'

## Centre d' Etude du Polymorphisme Human (CEPH) Cell lines

- Large, multigeneration pedigrees widely studied
- Immortalized lymphoblastoid cell lines



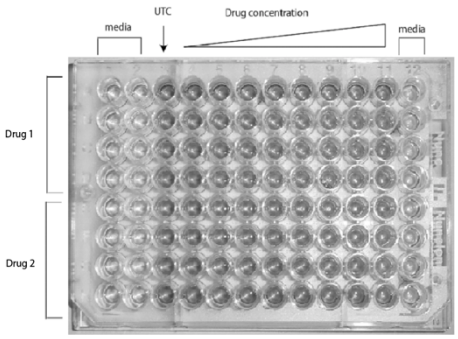



## Methodology

Cells counted, plated at 1 x $10^4$ / well

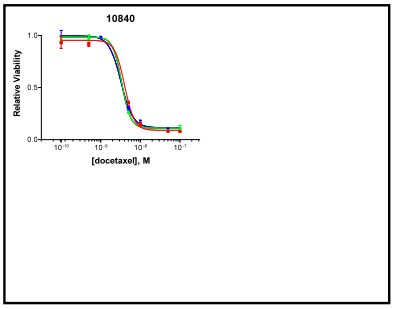
Cells incubated with increasing concentrations of drug

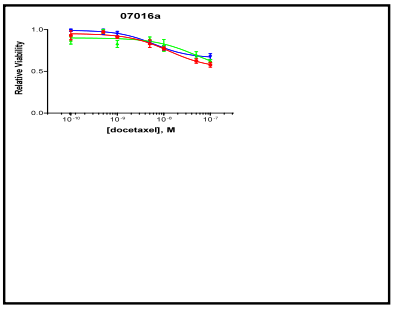
Alamar blue vital dye indicator added




Viability relative to untreated control calculated by spectrophotometry

## Significant Variation in Cellular Sensitivity to Docetaxel



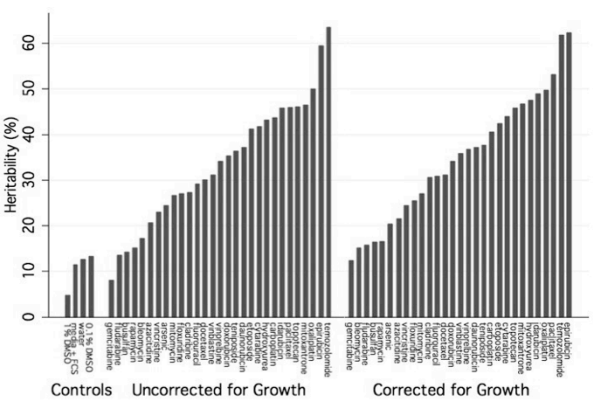



# 'CE-PH/F-DA' project

- 126 CEPH cell lines from 14 nuclear families
- All FDA approved cytotoxic drugs + new kinase inhibitors/MTOR/demethylation
- No antiestrogen or vitamin A analogues
- Evaluate degree of heritability, presence of QTL(s), and evidence for correlations between drug sensitivity patterns.

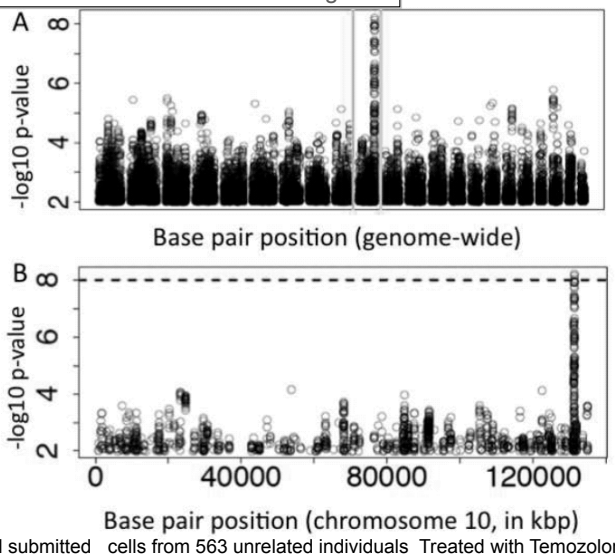
Maximum heritability over all doses

| Drug | Dose | $h^2$ |
|---|---|---|
| Temo | 4 | 63.51 |
| Epi | 1 | 59.48 |
| Oxal | 1 | 50.03 |
| Mitox | 1 | 46.46 |
| Topo | 1 | 46.12 |
| Pac | 1 | 45.91 |
| Ida | 1 | 45.83 |
| Carbo | 2 | 43.8 |
| Hydrox | 2 | 43.22 |
| Cytar | 1 | 41.74 |
| Etop | 1 | 41.26 |
| Daun | 1 | 37.14 |
| Ace | 1 | 36.98 |
| Teni | 3 | 36.37 |
| Dox | 1 | 35.3 |
| Vino | 1 | 34.13 |
| Vinb | 4 | 31.17 |
| Doc | 3 | 30.11 |



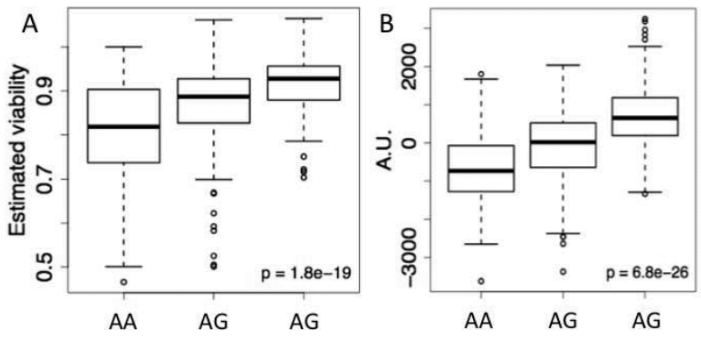

Peters et al 2011

In vitro GWAS as filter for candidate genes




Brown et al submitted cells from 563 unrelated individuals Treated with Temozolomide

MGMT SNP is associated with chemosensitivity and mRNA expression



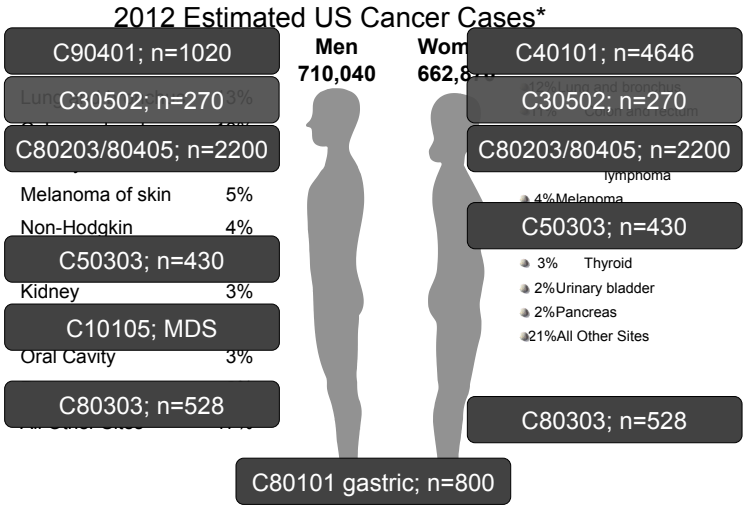

(A) Box plots for the estimated percent viability at 0.25 mmol by genotype for rs531572.
(B) Boxplots of *MGMT* transcript levels differ by rs531572 genotype

What needs to be done to determine hope vs hype?

•Find the 'right' biomarkers

•Validate in robust datasets

•Apply them!



## Docetaxel vs. Paclitaxel (Clinical data: SCOTROC1)

**Accrual of 1077 Pts With:**

- **Stage IC-IV epithelial ovarian cancer**
- **ECOG PS 0-2**
- **No prior history of CT or RT**

↓

**RANDOMISATION**

| | |
|---|---|
| **Docetaxel 75 mg/m$^2$ 1-hr IV, followed by Carboplatin AUC 5* IV**<br>**Repeat q 3 wk for up to 6 cycles** | **Paclitaxel 175 mg/m$^2$ 3-hr IV, followed by Carboplatin AUC 5* IV**<br>**Repeat q 3 wk for up to 6 cycles** |

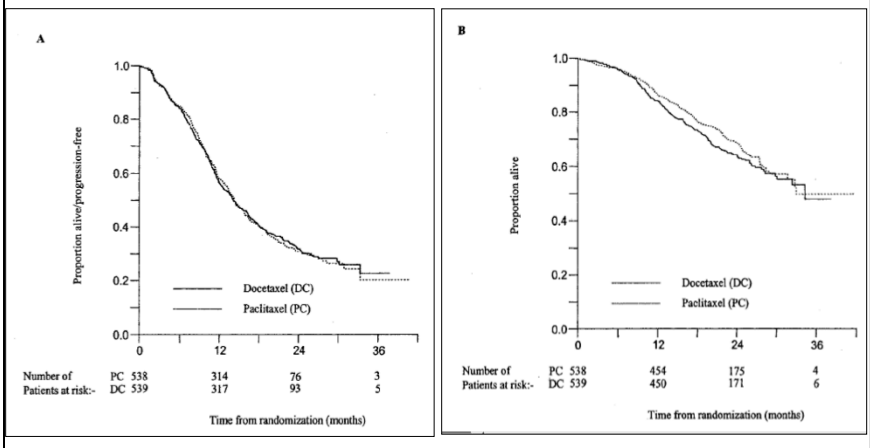
**Study End Points**
**Primary: progression-free survival**
**Secondary: response rate, overall survival, toxicity, QOL**

CANCER RESEARCH UK

scottish gynaecological cancer trials group

Sarah Glass, Alison Motsinger-Reif, Sharon Marsh, Bob Brown, Jim Paul

## Docetaxel vs. Paclitaxel (Clinical data: SCOTROC1)




Progression-free survival

Overall survival

**Vasey et al JNCI 2004**

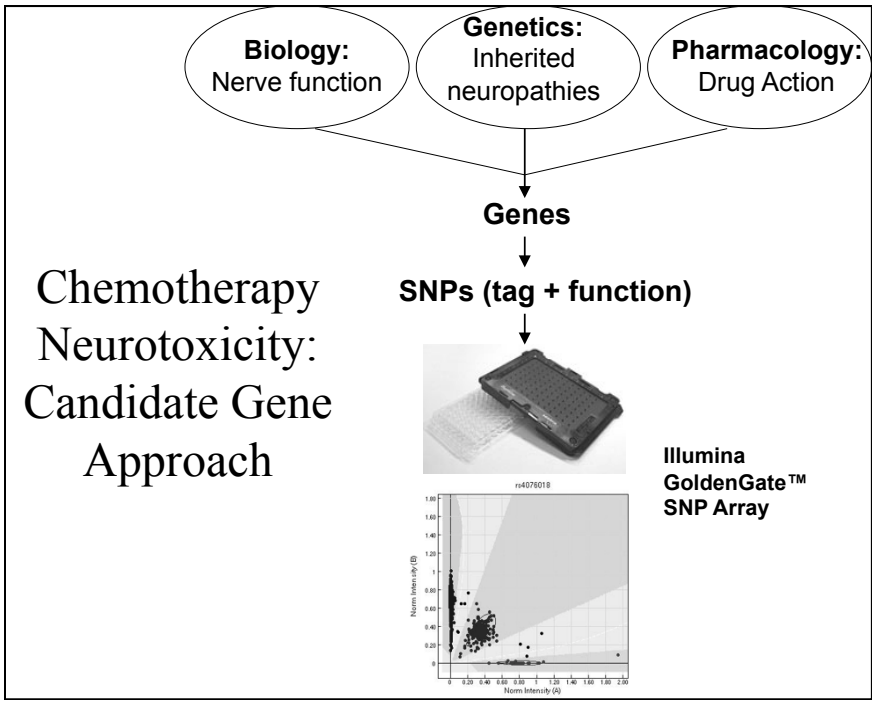
## Docetaxel vs. Paclitaxel (Clinical data: SCOTROC1)

**Table 5.** NCI–CTC neurotoxicity in the Scottish Randomised Trial in Ovarian Cancer 1*

| | % of patients | | |
|---|---|---|---|
| Grade | Docetaxel–carboplatin arm (n = 537)† | Paclitaxel–carboplatin arm (n = 532)‡ | *P* |
| Sensory‖ | | | |
| 1 | 35 | 48 | |
| 2 | 9 | 22 | |
| 3 | 2 | 8 | <.001‖ |
| 4 | 0 | 0 | |
| Total | 45 | 78 | <.001¶ |
| Motor¶ | | | |
| 1 | 6 | 9 | |
| 2 | 2 | 5 | |
| 3 | 1 | 2 | .005‖ |
| 4 | 0 | 0 | |
| Total | 9 | 16 | .001¶ |

*NCI–CTC = National Cancer Institute–Common Toxicity Criteria.
†Not available for two patients who died after one cycle.
‡Not available for one patient who died after one cycle.
§All statistical tests were two-sided. *P* value from Mann–Whitney *U* test.
‖Grades 1–4.
¶Total.

**Vasey et al JNCI 2004**



# The filtering of Neuro-risk genotypes

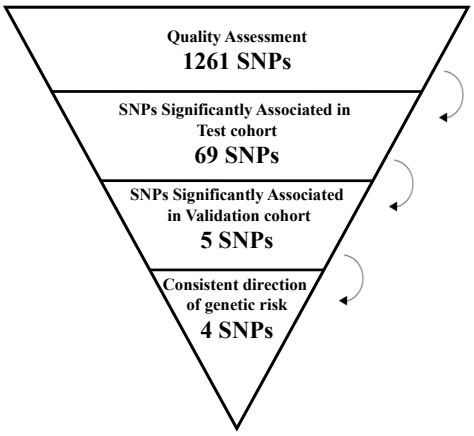



Figure 1: The workflow of the data analysis, represented by the narrowing number of SNPs at each stage of the analysis.

Table 1: SNPs significantly associated with severe neurotoxicity in the validation cohort

| SNP | Gene | Base Change | Corrected P-value | Odds Ratio | 95% CI | Risk Genotype |
|---|---|---|---|---|---|---|
| **rs139887** | SOX10 | C->G | 0.001 | 2.87 | (1.4361, 5.7530) | CG |
| **rs2849380** | BCL2 | A->G | 0.013 | 4.08 | (1.5254, 10.8975) | AA |
| **rs544093** | OPRM1 | A->C | 0.015 | 2.25 | (1.2365, 4.0841) | AA |
| **rs879207** | TRPV1 | A->G | 0.002 | 2.31 | (1.4467, 3.6767) | AG |

Table 2: Percent PAR for each SNP and joint PAR

| | rs139887 | rs2849380 | rs544093 | rs879207 | All SNPs |
|---|---|---|---|---|---|
| **PAR (%)** | **45.8** | **9.1** | **50.2** | **38.4** | **84.9** |

## Cumulative impact of Neuro-risk genotypes

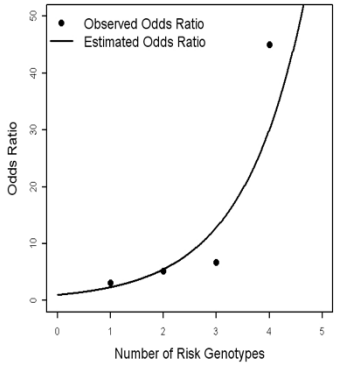



Figure 2: Number of Risk Genotypes by Predicted and Observed Odds Ratio

## Neuro-risk genotypes not associated with outcome

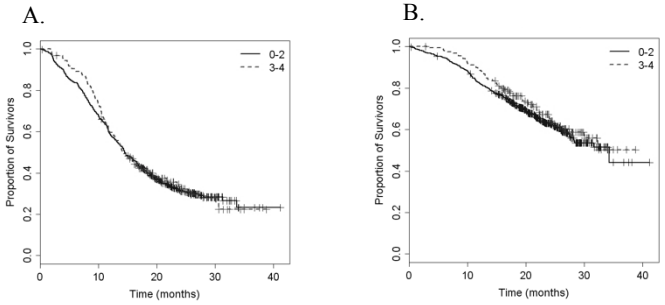
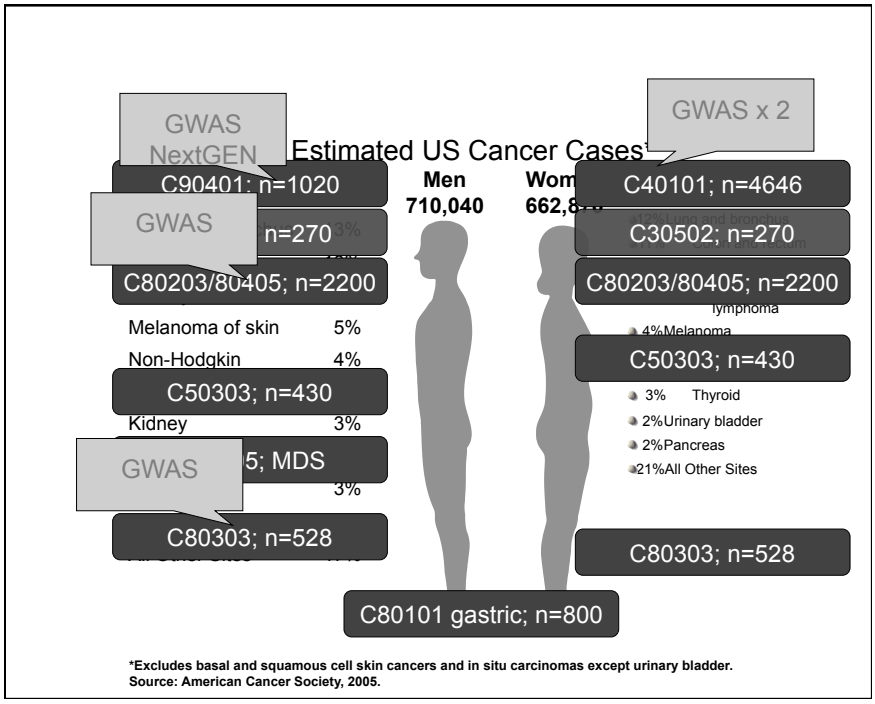



Figure 3: Relationship between genotype risk score (0-2 vs 3-4) and (A), progression free survival (p=0.75) or (B) overall survival (p=0.54)

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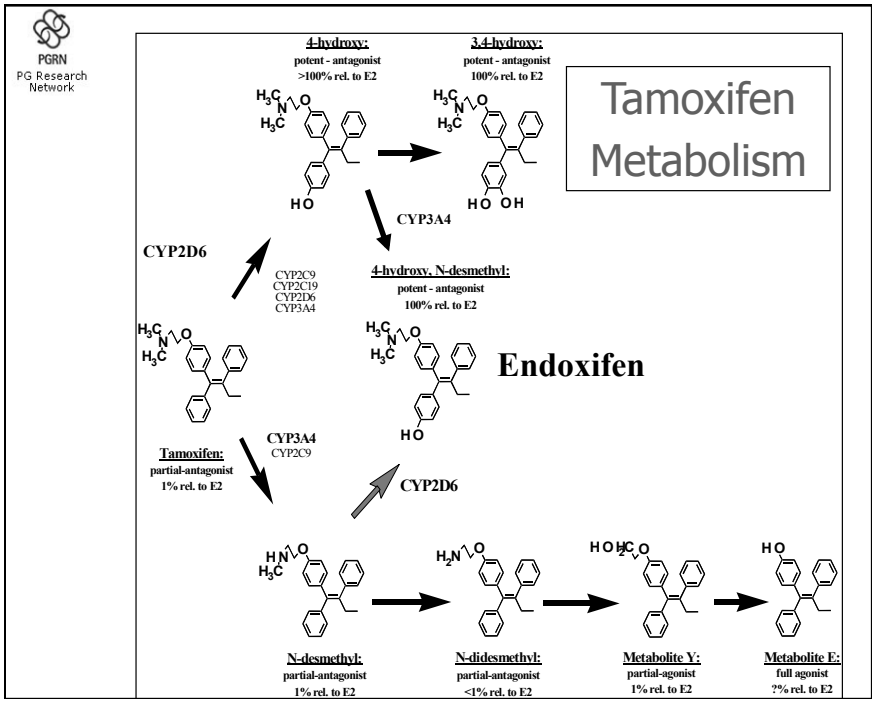
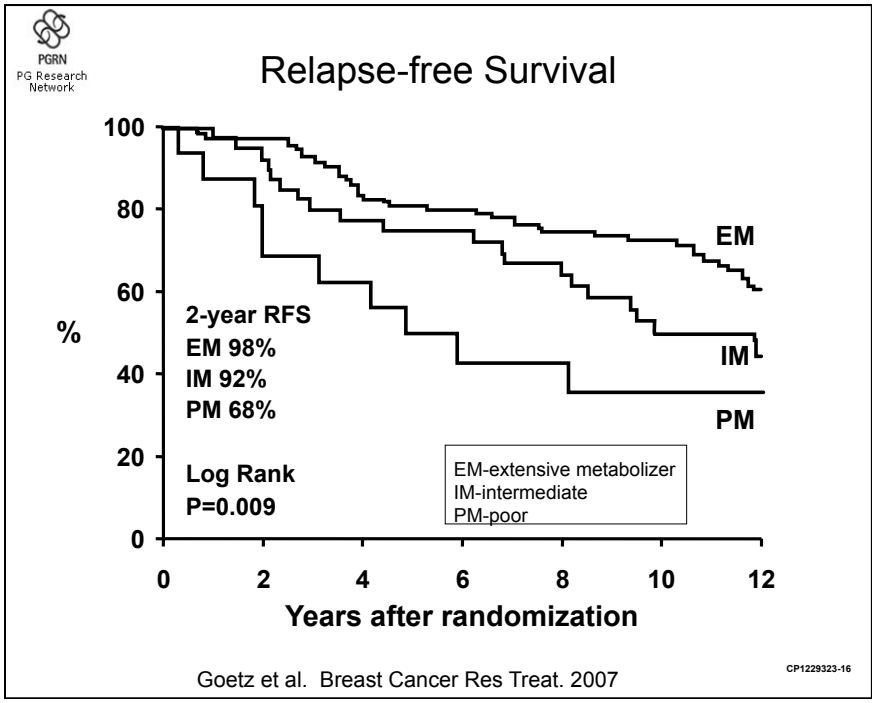


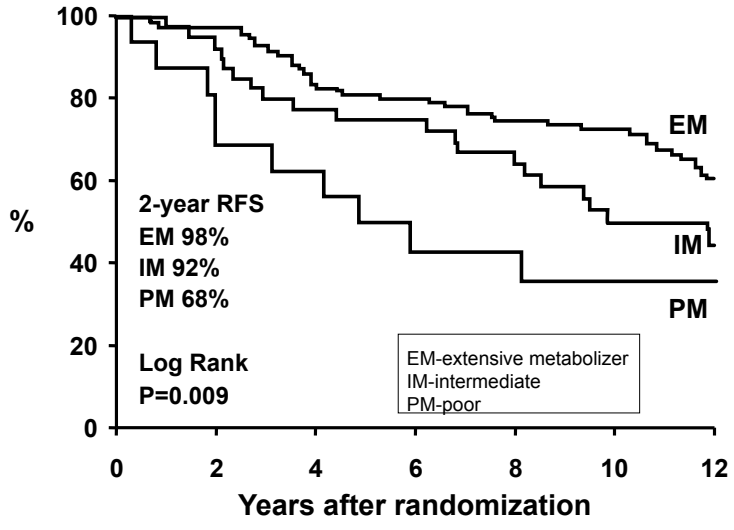

Goetz et al. Breast Cancer Res Treat. 2007

## Adjuvant Tamoxifen and CYP2D6

- **CYP2D6 associated with recurrence**
  - **Goetz et al. 2005, 2007 (USA)**
  - **Schroth et al. 2007 (Germany)**
  - **Kiyotani et al. 2008 (Japan)**
  - **Newman et al. 2008 (UK)**
  - **Xu et al. 2008 (China)**
  - **Okishiro et al. 2009 (Japan)**
  - **Ramon et al. 2009 (Spain)**
  - **Bijl et al. 2009 (Netherlands)**
  - **Schroth et al. 2009, 2010 (Germany, USA)**
  - **Fugisata et al. 2010 (Japan)**
  - **Lammers et al. 2010 (Netherlands)**
  - **Kiyotani et al. 2010 (Japan)**
  - **Thompson et al 2010 (UK)**
  - **Kiyotani et al 2012 (Japan)**
- **CYP2D6 not associated with recurrence**
  - **Wegman et al. 2005, 2007 (Sweden)**
  - **Nowell et al. 2005 (USA)**
  - **Abraham et al. 2010 (UK)**
  - **Goetz et al 2011 (USA)**
  - **Rae et al 2012 (UK)**
  - **Regan et al 2012 (USA/Europe)**

PGRN
PG Research Network

## Relapse-free Survival

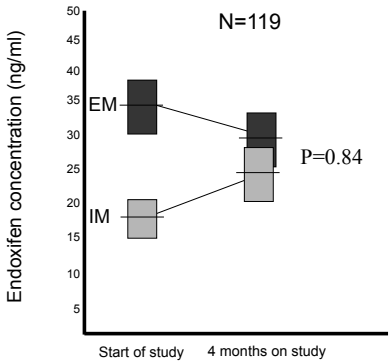



Goetz et al. Breast Cancer Res Treat. 2007

CP1229323-16

**CYP2D6-guided tamoxifen dosing normalizes endoxifen levels in IM patients**

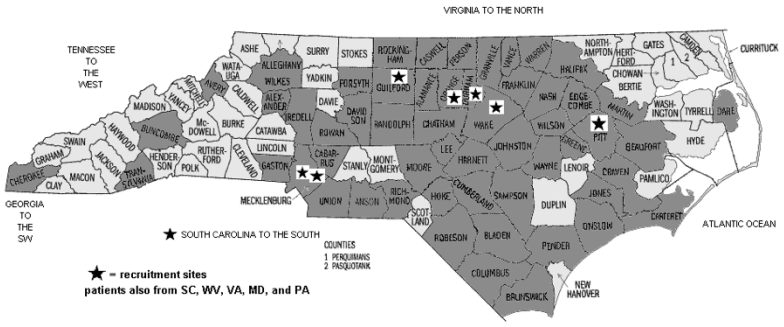
All patients on tamoxifen 20 mg/day for 4 months then
EM-20 mg
IM-change to 40 mg



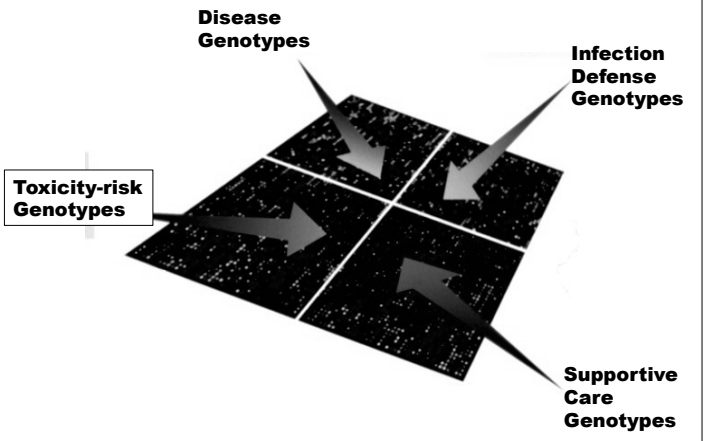

Irvin et al J Clin Oncol 2011

Study of 500 patients across NC is nearly completed, with oversampling of African American and Hispanic patient

## Implementation Science can be conducted where most patients are treated



## Comprehensive optimization of patient care



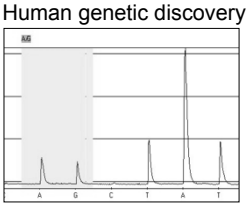

Does pharmacogenetics have relevance for public health?

Pharmacogenetics for Every Nation Initiative pgeni.org

PGENI Treating the Population. Impacting the World.

## Pharmacogenetics: what is your intent?

Human genetic discovery

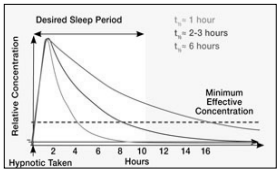



Drug Safety

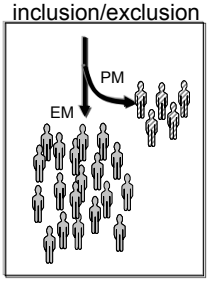
Public Policy



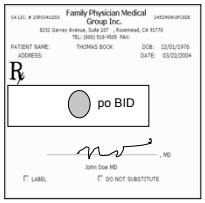
Explain variation in phenotype




Clinical trial inclusion/exclusion




Clinical practice




PGENI

- Modern medical therapy is a key component of improved health
- Selection of medications for each indication is a combination of clinical consensus, access/cost of drugs, and familiarity
- Medicine prioritization is a high stakes undertaking
- We need to use all available data

PGENI

**Background: Source of data for patient therapy selection**

Best option: individual

Good: relevant geographic/ ethnic/racial population

Worst: inferred world population



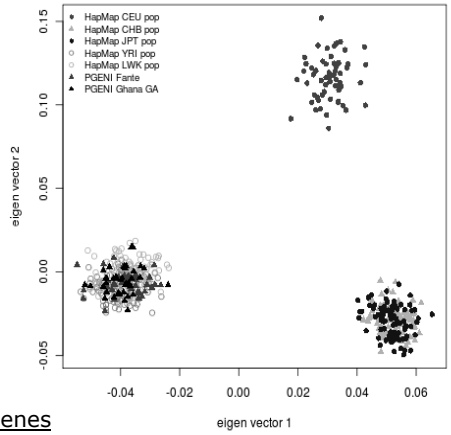
PGENI

Voltaire

- **"The best is the enemy of good."**,

## Continents are more similar than different

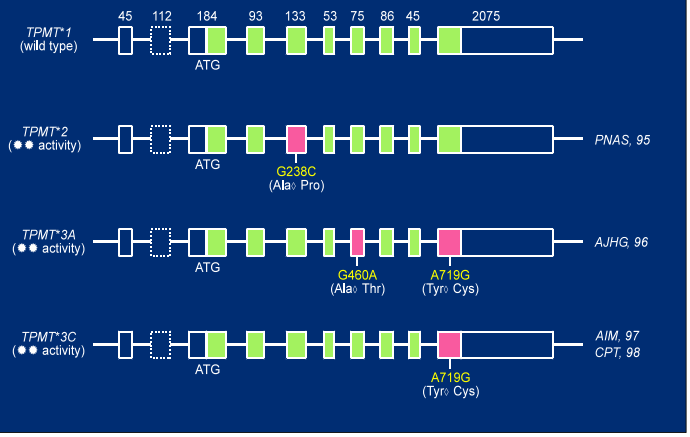
-but context is everything


1,936 functional mutations in 225 genes

PGENI Treating the Population. Impacting the World.

## Human TPMT Gene and Mutant Alleles

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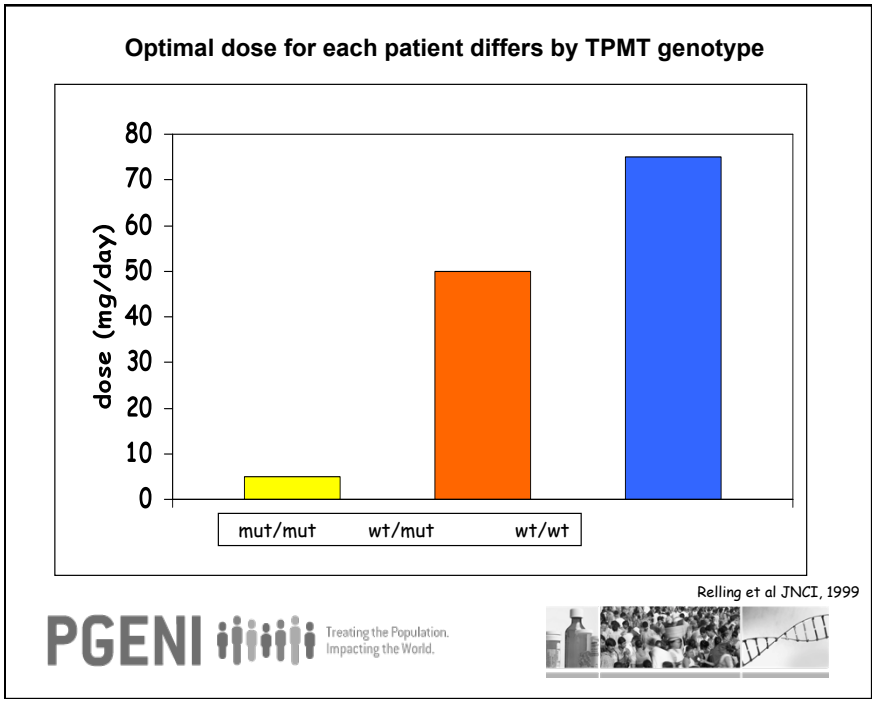
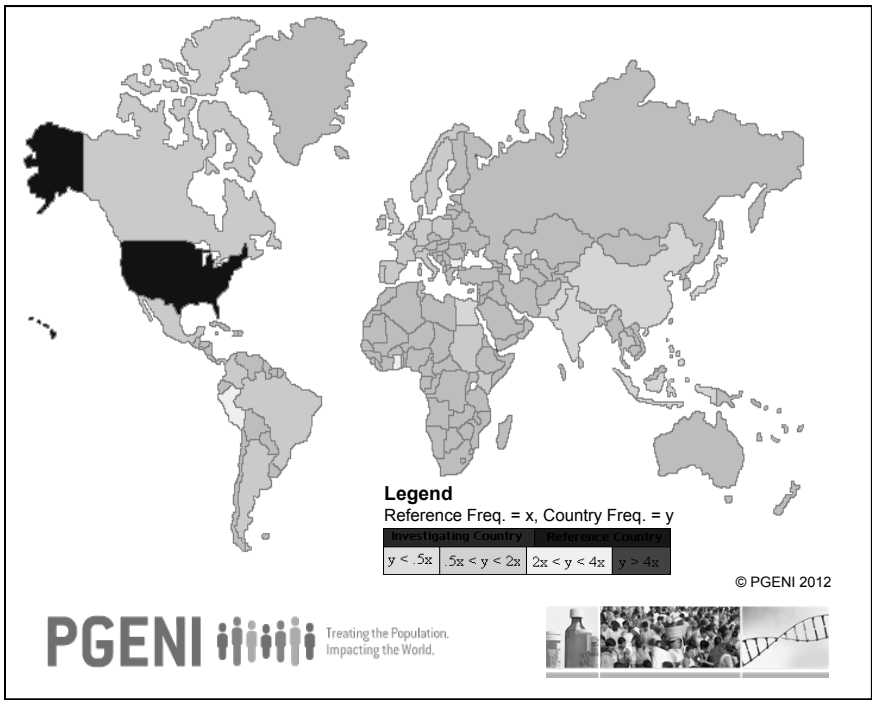

Optimal dose for each patient differs by TPMT genotype
dose (mg/day)
80
70
60
50
40
30
20
10
0
mut/mut
wt/mut
wt/wt
Relling et al JNCI, 1999
PGENI
Treating the Population.
Impacting the World.


Legend
Reference Freq. = x, Country Freq. = y
Investigating Country | Reference Country
y < .5x
.5x < y < 2x
2x < y < 4x
y > 4x
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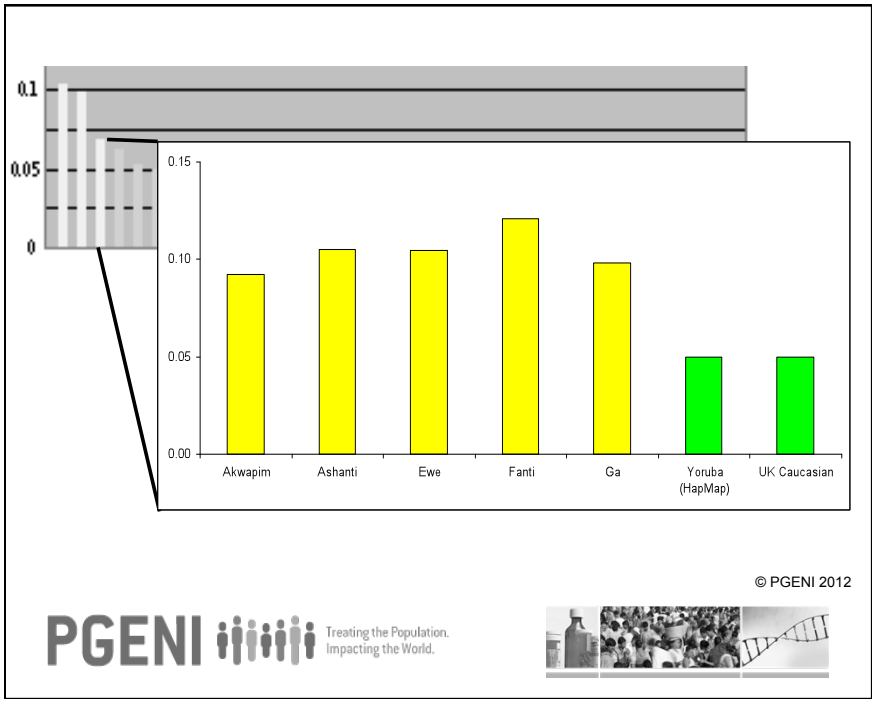
0.1
0.05
0
0.15
0.10
0.05
0.00
Akwapim
Ashanti
Ewe
Fanti
Ga
Yoruba (HapMap)
UK Caucasian
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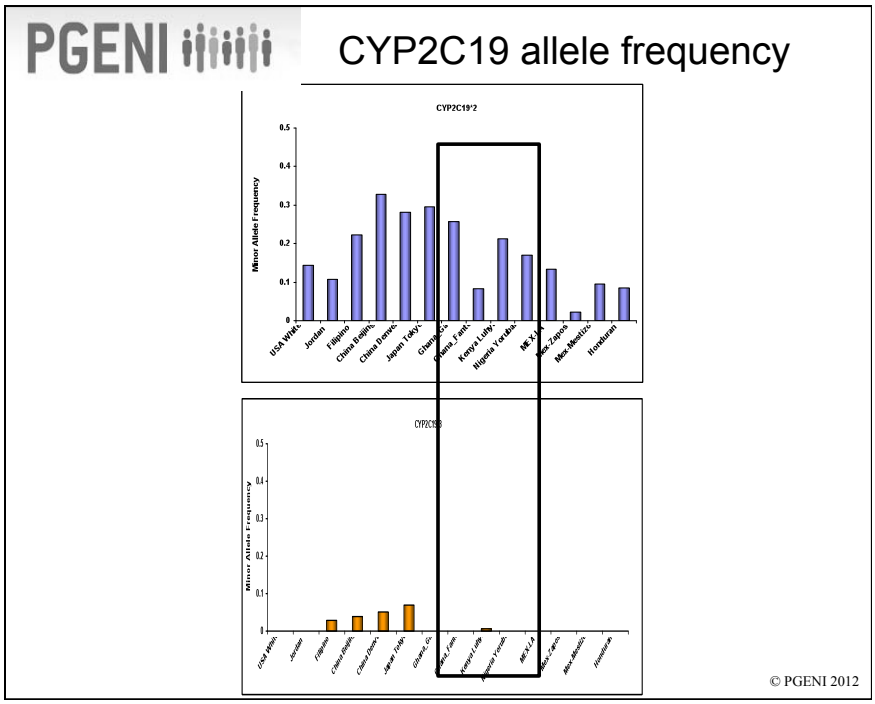
PGENI
CYP2C19 allele frequency
CYP2C19*2
Minor Allele Frequency
0.5
0.4
0.3
0.2
0.1
0
USA White
Jordan
Filipino
China Beijing
China Denver
Japan Tokyo
Ghana Ga
Ghana_Fanti
Kenya Luhya
Nigeria Yoruba
MEX LA
New Zapos
Mex Mestizo
Honduran
CYP2C19
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## Type of output

**Surveillance** - **identifying population subgroups at higher risk of toxicity or treatment failure**

**Prioritization** - **assisting the treatment selection from among WHO recommended therapies**



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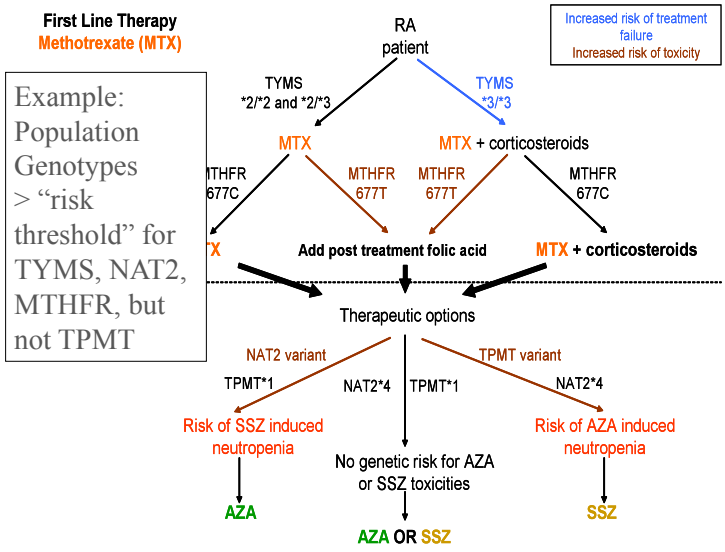
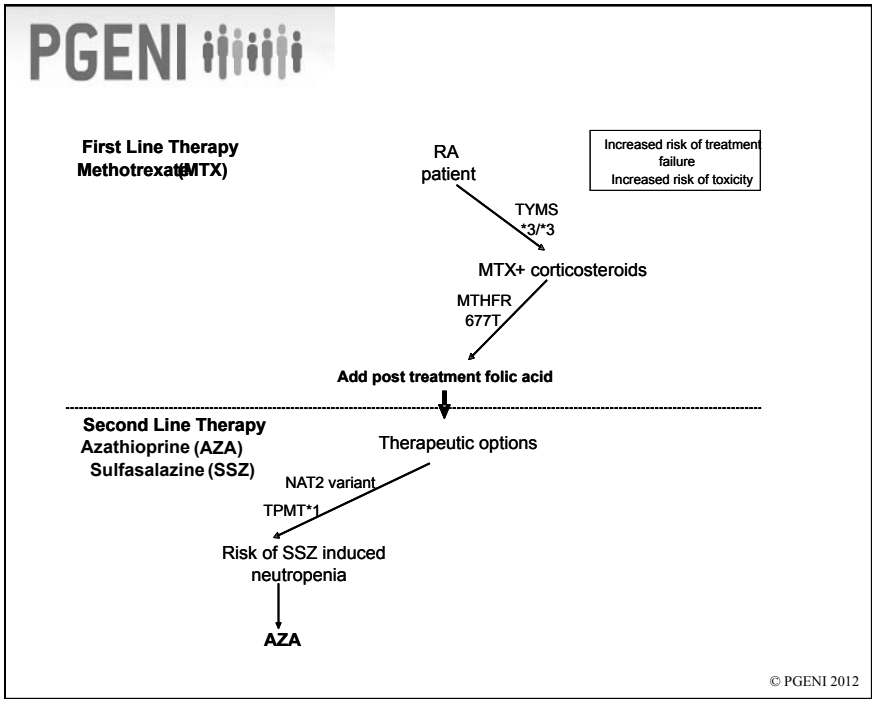
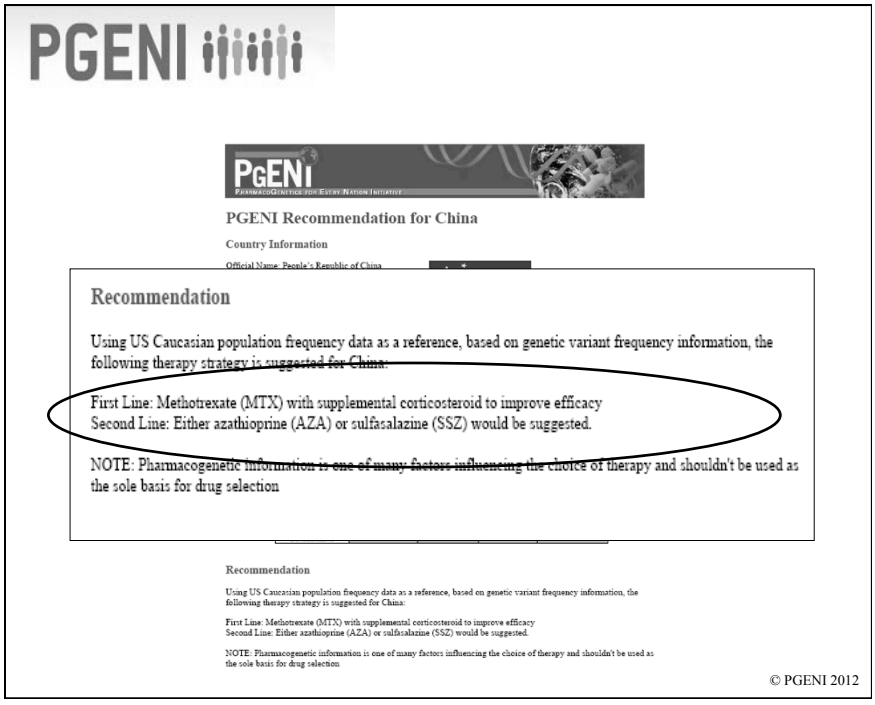



Example: Population Genotypes > "risk threshold" for TYMS, NAT2, MTHFR, but not TPMT


PGENI
First Line Therapy
Methotrexate (MTX)
RA patient
Increased risk of treatment failure
Increased risk of toxicity
TYMS *3/*3
MTX+ corticosteroids
MTHFR 677T
Add post treatment folic acid
Second Line Therapy
Azathioprine (AZA)
Sulfasalazine (SSZ)
Therapeutic options
NAT2 variant
TPMT*1
Risk of SSZ induced neutropenia
AZA
© PGENI 2012


PGENI
PGENI Recommendation for China
Country Information
Recommendation
Using US Caucasian population frequency data as a reference, based on genetic variant frequency information, the following therapy strategy is suggested for China:
First Line: Methotrexate (MTX) with supplemental corticosteroid to improve efficacy
Second Line: Either azathioprine (AZA) or sulfasalazine (SSZ) would be suggested.
NOTE: Pharmacogenetic information is one of many factors influencing the choice of therapy and shouldn't be used as the sole basis for drug selection
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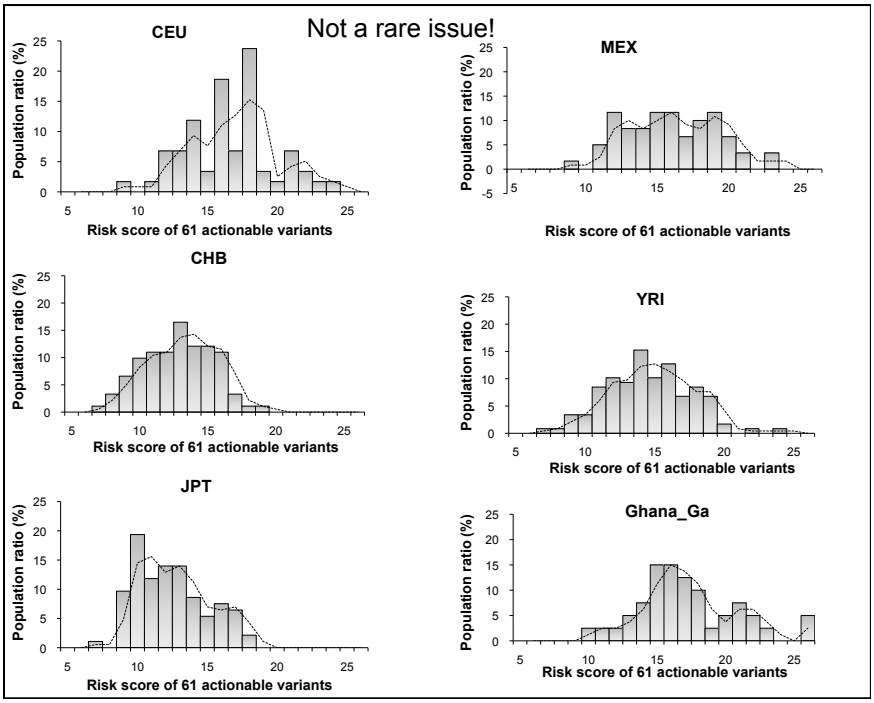
## Pharmacogenomic examples-2012

- *bcr/abl* or 9:22 translocation—imatinib mesylate*
- HER2-*neu*—trastuzumab**
- C-kit mutations—imatinib mesylate**
- Epidermal growth factor receptor mutations—gefitinib
- Thiopurine S-methyltransferase—mercaptopurine and azathioprine*
- UGT1A1-irinotecan**
- CYP2C9/VKORC1-warfarin*
- HLA-B*5701-abacavir *
- HLA-B*1502-carbamazepine *
- CYP2C19-clopidogrel
- IL28B-interferon
- Cytochrome P-450 (CYP) 2D6—5-HT3 receptor antagonists, antidepressants, ADHD drugs, and codeine derivatives, tamoxifen*

Not a rare issue!



## Applications of pharmacogenetics

- Explanation for untoward event (DPYD, CYP2D6)
- Required for insurance coverage (KRAS, EGFR, ABL)
- Identify low utility (KRAS)
- Dose selection (CYP2C9, CYP2C19)
- Therapy selection (CYP2C19)
- Preemptive prediction (HLA-B*5701)

## Applications of pharmacogenetics

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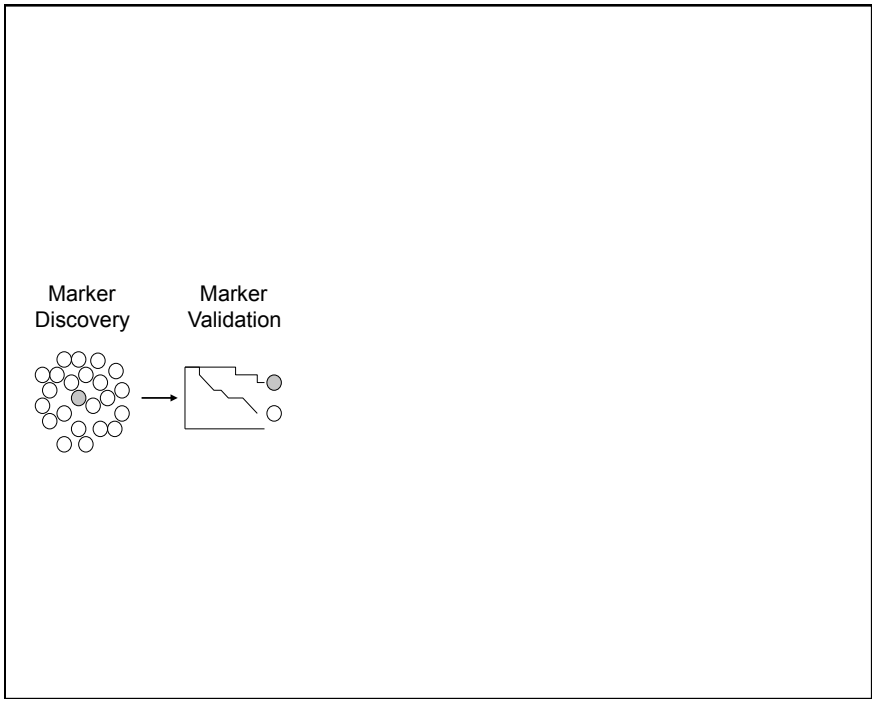
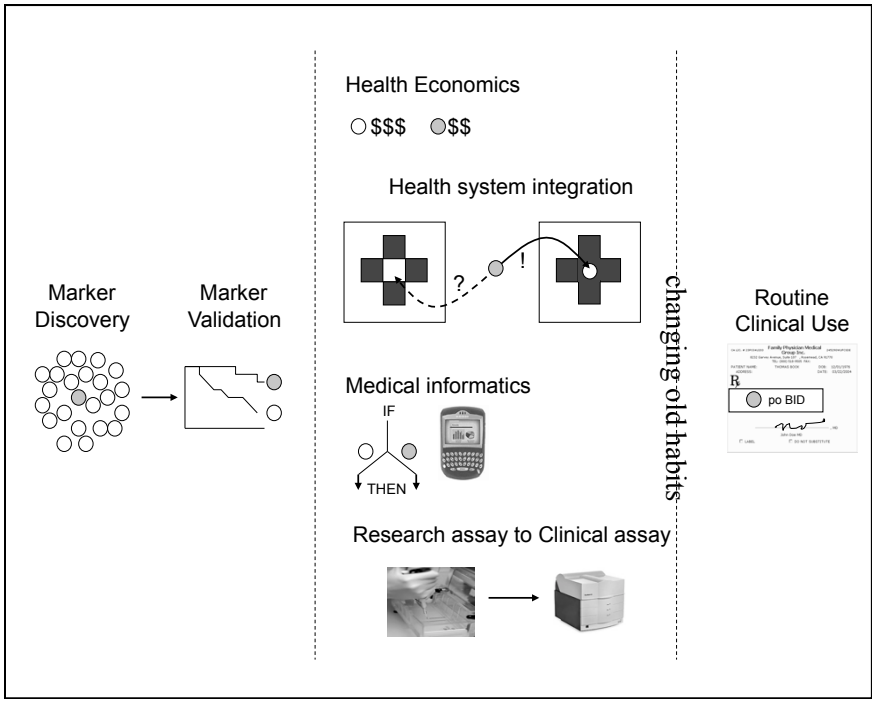
- Therapy selection (CYP2C19)
- Preemptive prediction (HLA-B*5701)

- Bundled care
- Patient safety
- 'bounce back' avoidance
- Pharmacy & Therapeutics committee
- National formulary
- Others…….

} Boring!


Marker Discovery
Marker Validation


Marker Discovery
Marker Validation
Health Economics
$$$ $$
Health system integration
?
!
Medical informatics
IF
THEN
Research assay to Clinical assay
changing old habits
Routine Clinical Use
po BID

# Warfarin Package Insert

Table 5: Range of Expected Therapeutic Warfarin Doses Based on CYP2C9 and VKORC1 Genotypes†

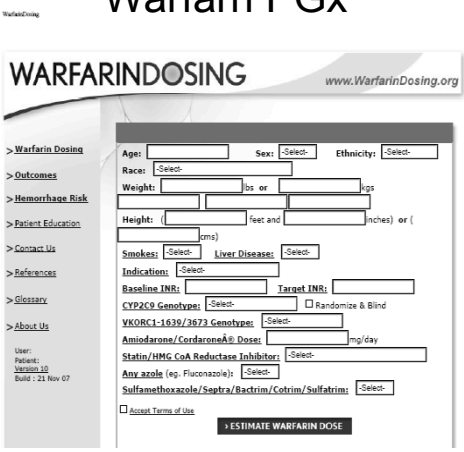
| VKORC1 | CYP2C9 | | | | | |
|---|---|---|---|---|---|---|
| | *1/*1 | *1/*2 | *1/*3 | *2/*2 | *2/*3 | *3/*3 |
| GG | 5-7 mg | 5-7 mg | 3-4 mg | 3-4 mg | 3-4 mg | 0.5-2 mg |
| AG | 5-7 mg | 3-4 mg | 3-4 mg | 3-4 mg | 0.5-2 mg | 0.5-2 mg |
| AA | 3-4 mg | 3-4 mg | 0.5-2 mg | 0.5-2 mg | 0.5-2 mg | 0.5-2 mg |

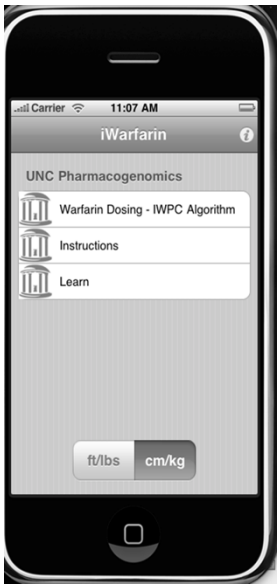
†Ranges are derived from multiple published clinical studies. Other clinical factors (e.g., age, race, body weight, sex, concomitant medications, and comorbidities) are generally accounted for along with genotype in the ranges expressed in the Table. VKORC1 –1639 G→A (rs9923231) variant is used in this table. Other co-inherited VKORC1 variants may also be important determinants of warfarin dose. Patients with CYP2C9 *1/*3, *2/*2, *2/*3 and *3/*3 may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen.

## Factors that Correlate w/ Warfarin Dose

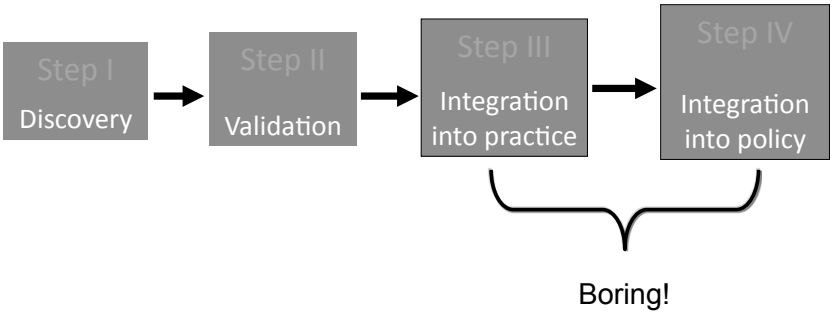
- Age
- Body surface area (BSA) or weight
- Amiodarone dose
- Other drugs (e.g. HMG CoA Reductase inhibitors)
- Target INR
- Race
- Sex
- Plasma vitamin K level
- Decompensated CHF or post-operative state
- CYP2C9 and VKORC1 genotype

## Tools to Implement Warfarin PGx




## Translational science: The steps to success

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## We now have new audiences

- Past
  - Ourself
  - Editors/reviewers
  - Study section
- Now
  - Clinic administrators
  - Payers
  - Patients

## We now have new (additional) endpoints

- Past
  - survival
  - Stent thrombosis
  - Severe neutropenia
- Now
  - Selection from amongst ‘equal’ therapies
  - Return on investment for medical home
  - Quality measures
  - Patient satisfaction

## I have ears, but cannot hear

- 44 year old white male (CSO at local biotech)
- AV block $2^0$ congenital heart disease
- Presents for placement of epicardial pacemaker
- Tells cardiologist, CT surgeon, anesthesiologist, and admitting team (cardiology fellow, resident, intern) that an executive physical revealed genetic data relevant to pain control and anticoagulation
- Adequate pain control (4/10) in recovery room on MS
- moved to CCU and switch to oxycodone during the night, waking up in severe pain (10/10), ignored x 24 hours
- Student and PharmD recognized CYP2D6 PM and patient was switched to hydromorphone (5/10)

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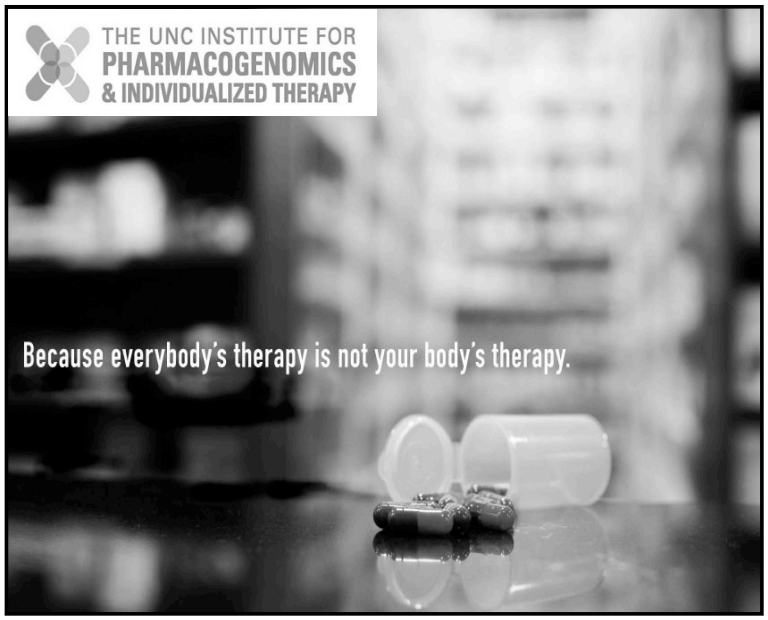
## Thank you to the PGENIUSES!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