Pharmacogenomics: 2012

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“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
Beitrag zur Arzneimittellehre, 1849

The clinical problem
• Multiple active regimens for the treatment of most diseases
• Variation in response to therapy
• Unpredictable toxicity

$\text{\$\$\$\$\$\$\$\$\$\$\$\$\$\$}\$

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

Discovery Strategies

HapMap

Model systems

Expression array

Linkage

Association

Cases

Controls
We are only beginning to try!

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

10/50 had $\geq$ 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 $1 \times 10^4$ / 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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>h²</th>
</tr>
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<tbody>
<tr>
<td>Tema</td>
<td>4</td>
<td>63.51</td>
</tr>
<tr>
<td>Epi</td>
<td>1</td>
<td>50.48</td>
</tr>
<tr>
<td>Carb</td>
<td>1</td>
<td>46.44</td>
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<td>Topa</td>
<td>1</td>
<td>46.12</td>
</tr>
<tr>
<td>Taxa</td>
<td>1</td>
<td>45.95</td>
</tr>
<tr>
<td>Ida</td>
<td>1</td>
<td>45.83</td>
</tr>
<tr>
<td>Cetox</td>
<td>2</td>
<td>43.8</td>
</tr>
<tr>
<td>Hydro</td>
<td>2</td>
<td>43.22</td>
</tr>
<tr>
<td>Cyra</td>
<td>1</td>
<td>41.74</td>
</tr>
<tr>
<td>Trep</td>
<td>1</td>
<td>41.28</td>
</tr>
<tr>
<td>Daur</td>
<td>1</td>
<td>37.74</td>
</tr>
<tr>
<td>Nca</td>
<td>1</td>
<td>36.98</td>
</tr>
<tr>
<td>Zeri</td>
<td>1</td>
<td>36.75</td>
</tr>
<tr>
<td>Dox</td>
<td>1</td>
<td>36.1</td>
</tr>
<tr>
<td>Acra</td>
<td>1</td>
<td>34.12</td>
</tr>
<tr>
<td>Amba</td>
<td>4</td>
<td>31.17</td>
</tr>
<tr>
<td>Zec</td>
<td>1</td>
<td>30.3</td>
</tr>
</tbody>
</table>

Maximum heritability over all doses

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
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2012 Estimated US Cancer Cases*

*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.

Source: American Cancer Society, 2005.
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/m² 1-hr IV, followed by Carboplatin AUC 5* IV
Repeat q 3 wk for up to 6 cycles

Paclitaxel 175 mg/m² 3-hr IV, followed by Carboplatin AUC 5* IV
Repeat q 3 wk for up to 6 cycles

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

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+

<table>
<thead>
<tr>
<th>Grade</th>
<th>Docetaxel–carboplatin arm (n = 537)†</th>
<th>Paclitaxel–carboplatin arm (n = 532)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>8</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>78</td>
<td>&lt;.001†</td>
</tr>
</tbody>
</table>

*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

Chemotherapy Neurotoxicity: Candidate Gene Approach
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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Base Change</th>
<th>Corrected P-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>Risk Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs139887</td>
<td>SOX10</td>
<td>C-&gt;G</td>
<td>0.001</td>
<td>2.87</td>
<td>(1.4361, 5.7530)</td>
<td>CG</td>
</tr>
<tr>
<td>rs2849380</td>
<td>BCL2</td>
<td>A-&gt;G</td>
<td>0.013</td>
<td>4.08</td>
<td>(1.3254, 10.8975)</td>
<td>AA</td>
</tr>
<tr>
<td>rs544093</td>
<td>OPRM1</td>
<td>A-&gt;C</td>
<td>0.013</td>
<td>2.25</td>
<td>(1.2365, 4.0841)</td>
<td>AA</td>
</tr>
<tr>
<td>rs879207</td>
<td>TRPV1</td>
<td>A-&gt;G</td>
<td>0.002</td>
<td>2.31</td>
<td>(1.6467, 3.6787)</td>
<td>AG</td>
</tr>
</tbody>
</table>

Table 2: Percent PAR for each SNP and joint PAR

<table>
<thead>
<tr>
<th>SNP</th>
<th>PAR (%)</th>
<th>rs2849380</th>
<th>rs544093</th>
<th>rs879207</th>
<th>All SNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs139887</td>
<td>45.8</td>
<td>9.1</td>
<td>50.2</td>
<td>38.4</td>
<td>84.9</td>
</tr>
</tbody>
</table>
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)
What needs to be done to determine hope vs hype?

• Find the 'right' biomarkers

• Validate in robust datasets

• Apply them!
Tamoxifen Metabolism

Endoxifen

Relapse-free Survival

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)

Relapse-free Survival

- 2-year RFS
  - EM 98%
  - IM 92%
  - PM 68%

- Log Rank
  - P=0.009

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

N=119

Endoxifen concentration (ng/ml)

Start of study 4 months on study

P=0.84

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
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
Background: Source of data for patient therapy selection

Best option: individual

Good: relevant geographic/ethnic/racial population

Worst: inferred world population

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
Optimal dose for each patient differs by TPMT genotype

Reference Freq. = x, Country Freq. = y

Legend
- $y < 0.5x$
- $0.5x \leq y < x$
- $x \leq y < 1.5x$
- $y \geq 1.5x$

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CYP2C19 allele frequency
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

Example:
Population Genotypes > “risk threshold” for TYMS, NAT2, MTHFR, but not TPMT
First Line Therapy
Methotrexate (MTX)

RA patient

MTX + corticosteroids

TYMS

MTX

MTX

MTHFR

677C

677T

Add post treatment folic acid

Second Line Therapy
Azathioprine (AZA)
Sulfasalazine (SSZ)

Therapeutic options

NAT2 variant

TPMT

Risk of SSZ induced neutropenia

AZA

RA

patient

MTX

+ corticosteroids

TYMS

MTX

MTHFR

677C

677T

Increased risk of treatment failure
Increased risk of toxicity

Increased risk of treatment failure
Increased risk of toxicity

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.
Pharmacogenomic examples-2012

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- HER2-*neu*—trastuzumab**
- C-kit mutations—imatinib mesylate**
- Epidermal growth factor receptor mutations—gefitinib
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- Identify low utility (KRAS)
- Dose selection (CYP2C9, CYP2C19)
- 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

Health Economics
- $$$ $$$
- Health system integration

Medical informatics
- IF THEN
- Research assay to Clinical assay

Changing old habits

Routine Clinical Use
Warfarin Package Insert

Table 1: Range of Expected Therapeutic Warfarin Doses Based on CYP2C9 and VKORC1 Genotypes

<table>
<thead>
<tr>
<th>VKORC1</th>
<th>CYP2C9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1/*1</td>
</tr>
<tr>
<td>GG</td>
<td>5-7 mg</td>
</tr>
<tr>
<td>AG</td>
<td>5-7 mg</td>
</tr>
<tr>
<td>AA</td>
<td>3-4 mg</td>
</tr>
</tbody>
</table>

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 with 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
Translational science: The steps to success

Step I: Discovery
Step II: Validation
Step III: Integration into practice
Step IV: Integration into policy

Boring!
We now have new audiences

- **Past**
  - Ourselves
  - 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\textsuperscript{o} 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)

Thank you to the PGENIUSES!
Because everybody's therapy is not your body's therapy.