Pharmacogenetics
Clinical implementation: a 7 year experience

Ron van Schaik
Associate Professor Pharmacogenetics
Eur Clin Chem / Advisor EMA - PGWG

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Pharmacogenetics Core Laboratory*
Dept. Clinical Chemistry
Erasmus MC Rotterdam

*IFCC Certified Reference Laboratory for Pharmacogenetics
Bringing pharmacogenetics to the clinic
“Here is my DNA sequence...”
(The New Yorker, 2000)
Hurdles to take.....

**Proof of Principle**  
*(Pharmacokinetics)*

**Proof of Efficacy**  
*(Pharmacodynamics)*

**Availability of a test**

**Alternative treatment available?**

**Cost effectiveness**

**Uptake in Guidelines**  
*(convincing clinicians)*
TPMT gene and 6-MP or Azathioprine therapy

UK questionnaire to 328 prescribers of AZA, 287 (70%) responded

TPMT enzyme testing: 67% (91% prior to therapy)
- dermatologists: 94%
- gastroenterologists: 60%
- rheumatologists: 47%

DNA testing: 5%
(not routinely available(?)):
- dermatologists: 2%
- gastroenterologists: 2%
- rheumatologists: 1%

(Faragher EA et al 2007 J Clin Pharmacy and Ther 32: 187-195)
TPMT genotyping for 6-MP or AZA

Own experiences:

**Dermatology:**
always request for TPMT genotyping before start of AZA therapy
→ taken up in guidelines!

**Gastro-Intestinal department:**
request for TPMT genotyping before start of AZA therapy

**Acute Lymphatic Leukemia:**
Competition with national protocol for measuring enzyme activity

Turning point: awareness that genotype results were available in 3 days and cost only € 79.
Reason for genotyping request:

Drug
Dose
Conc.
Co-med.

Screening prior to therapy
High blood levels
Low blood levels
No effect
Side effects

Gene to be tested:

CYP1A2
CYP2B6
CYP2C8
CYP2C9
CYP2C19
CYP2D6 - (33 variants, AmpliChip)
CYP2D6 - (14 variants, DNA chip)
CYP2E1
CYP3A4
CYP3A5

ABCB1 (MDR-1)
DPYD
HLA-B*1502
HLA-A*3301
HLA-B*5701
IL-28B
SLCO1B1
TPMT
UGT1A1
Pseudocholinesterase (BChE) (4)

Unknown to me: please advice.
Other gene, being:

Consulted with:

Not invullen! T.b.v. intero registratio AKC:

POR D
DNAnr

FIDNR
Monste mr
Drug: [Blank]
Dose: [Blank]
Conc.: [Blank]
Co-med.: [Blank]

Reason for genotyping request:
- Screening prior to therapy
- High blood levels
- Low blood levels
- No effect
- Side effects

Gene to be tested:
- CYP1A2
- CYP2B6
- CYP2C8
- CYP2C9
- CYP2C19
- CYP2D6 - (33 variants, AmpliChip)
- CYP2D6 - (14 variants, DNA chip)
- CYP2E1
- CYP3A4
- CYP3A5
- ABCB1 (MDR-1)
- DPYD
- HLA-B*1502
- HLA-A*3301
- HLA-B*5701
- IL-28B
- SLCO1B1
- TPMT
- UGT1A1
- Pseudocholinesterase (BChE) (4)

Unknown to me: please advice.
Other gene, being: CYP450

Consulted with:

Not invullen! T.b.v. interno registratio AKC:
- PORD
- DNAarr
- FIDNR
- Monstemr
PGx testing from 2005 → 2012
CYP2D6 & antidepressants
Psychiatry: Imipramine (antidepressive)

Imipramine  \xrightarrow{CYP2C19}  Desipramine  \xrightarrow{CYP2D6}  2OH desipramine

(Schenk et al 2008 Mol Psychiatry)
Psychiatry: Imipramine (antidepressive)

Imipramine $\xrightarrow{\text{CYP2C19}}$ Desipramine $\xrightarrow{\text{CYP2D6}}$ 2OH desipramine

Imipramine doses after reaching steady state

CYP2D6 genotyping: *3, *4, *5, *6,

(Schenk et al 2008 Mol Psychiatry)
Imipramine (tricyclic antidepressant)

Imipramine $\xrightarrow{CYP2C19}$ Desipramine $\xrightarrow{CYP2D6}$ 2OH desipramine

Imipramine doses after reaching steady state

n=11  n=69  n=90  n=11

(Schenk et al 2008 Mol Psychiatry)
CYP2D6 & Tamoxifen
Tamoxifen metabolism & breast cancer

Most effective component
CYP2D6 genotype and endoxifen levels

(Vt=*4 = deficient; Based on Jin et al 2005)
CYP2D6 genotype and adjuvant TAM (n=1,325)

(Schroth et al 2009 JAMA (Oct 7))
## Published Articles: contradictory results.....

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Genotyping</th>
<th>Endpoint</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goetz et al. JCO 2005</td>
<td>190</td>
<td>*4</td>
<td>TTR, RFS</td>
<td>+</td>
</tr>
<tr>
<td>Lim et al. JCO 2007</td>
<td>21</td>
<td>*10</td>
<td>TTP</td>
<td>+</td>
</tr>
<tr>
<td>Bijl et al. Breast Cancer Res Treat 2009</td>
<td>85</td>
<td>*4</td>
<td>OS</td>
<td>+</td>
</tr>
<tr>
<td>Xu et al. Ann Oncol 2008</td>
<td>152</td>
<td>*10</td>
<td>DFS</td>
<td>+</td>
</tr>
<tr>
<td>Stingl et al. Curr Med Res Opin 2010</td>
<td>496</td>
<td>*4</td>
<td>TTP, PFS</td>
<td>-</td>
</tr>
<tr>
<td><strong>Leyland-Jones et al. San Antonio 2010 (abstract)</strong></td>
<td>1243</td>
<td>*4</td>
<td>DFS</td>
<td>-</td>
</tr>
<tr>
<td>Okishiro et al. Cancer 2009</td>
<td>173</td>
<td>*3, *10</td>
<td>RFS</td>
<td>-</td>
</tr>
<tr>
<td>Toyama et al. JCO 2009</td>
<td>154</td>
<td>*10</td>
<td>OS</td>
<td>-</td>
</tr>
<tr>
<td>Dezentje et al. JCO 2010</td>
<td>747</td>
<td>?</td>
<td>DFS</td>
<td>-</td>
</tr>
<tr>
<td>Wegman et al. Breast Cancer Res 2005</td>
<td>76</td>
<td>*4</td>
<td>RR</td>
<td>invers</td>
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</tbody>
</table>
Current controversy.....

“Laboratory/Clin Pharmacology view”
1. CYP2D6 theoretically involved
2. Genotype proved to affect metabolism
3. Genotype proved to affect outcome

“Oncology view”
1. Not all studies confirm the effect of CYP2D6 on outcome
2. There has been no randomized controlled trial available

Ready for clinical implementation

Not ready for clinical implementation
CYP2C19 & Clopidogrel
Genetic Determinants of Response to Clopidogrel and Cardiovascular Events

Tabassome Simon, M.D., Ph.D., Céline Verstuyft, Pharm.D., Ph.D., Murielle Mary-Krause, Ph.D., Lina Quteineh, M.D., Elodie Drouet, M.Sc., Nicolas Méneveau, M.D., P. Gabriel Steg, M.D., Ph.D., Jean Ferrières, M.D., Nicolas Danchin, M.D., Ph.D., and Laurent Becquemont, M.D., Ph.D., for the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators

CONCLUSIONS

Among patients with an acute myocardial infarction who were receiving clopidogrel, those carrying CYP2C19 loss-of-function alleles had a higher rate of subsequent cardiovascular events than those who were not. This effect was particularly marked among the patients undergoing percutaneous coronary intervention. (ClinicalTrials.gov number, NCT00673036.)
Clopidogrel: needs activation by **CYP2C19** (3% PMs, 26% IMs)

Test for CYP2C19 variants:
- Negative → clopidogrel
- Positive → prasugrel

**Meta-analysis**
Geisler et al 2011 Pharmacol & Ther:
- CY2C19*2 carriers are at risk

**Meta-analysis**
Zabalza et al 2012 BMJ:
- Large studies fail to confirm risk

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**Antonius Hospital Nieuwegein**

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**Erasmus MC**
Hurdles to take…..

**Proof of Principle**  
(Pharmacokinetics?)

**Proof of Efficacy**  
(Do patients benefit?)

**Availability of a test**

**Alternative treatment available?**

**Cost effectiveness**

**Uptake in Guidelines**  
(convincing clinicians)
Cost-effectiveness CYP2C9/VKORC1 testing

Cost-effectiveness of using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation.

Eckman MH, Rosand J, Greenberg SM, Gage BF.
University of Cincinnati Medical Center, Cincinnati, OH 45267-0535, USA. mark.eckman@uc.edu

DESIGN: Markov state transition decision model.
DATA SOURCES: MEDLINE searches and bibliographies from relevant articles of literature published in English.
TARGET POPULATION: Outpatients or inpatients requiring initiation of warfarin therapy. The base case was a man age 60 years with newly diagnosed nonvalvular atrial fibrillation and no contraindications to warfarin therapy.
TIME HORIZON: Lifetime.
PERSPECTIVE: Societal.
INTERVENTION: Genotype-guided dosing consisting of genotyping for CYP2C9*2, CYP2C9*3, and/or VKORC1 versus standard warfarin induction.
OUTCOME MEASURES: Effectiveness was measured in quality-adjusted life dollars.

RESULTS: In the base case, genotype-guided dosing resulted in better outcomes, but at a relatively high cost. Overall, the marginal cost-effectiveness of testing exceeded $170 000 per QALY. On the basis of current data and cost of testing (about $400), there is only a 10% chance that genotype-guided dosing is likely to be cost-effective (that is, <$50 000 per QALY).
Sensitivity analyses revealed that for genetic testing to cost less than $50 000 per QALY, it would have to be available within 24 hours, and cost less than $200.

Cost of testing: $400 (!) (€300)

At Erasmus MC: €160

cost effective if available within 24 hours and costs < $200 (€160)

CONCLUSION: Warfarin-related genotyping is unlikely to be cost-effective for typical patients with nonvalvular atrial fibrillation, but may be cost-effective in patients at high risk for hemorrhage or meet the following optimistic criteria: prevent greater than 32% of major bleeding events, and cost less than $200.

genotyping is unlikely to be cost effective
The laboratory

current diagnostics for patient care
PGx testing in 2012

PGx requests 2012 (n=536)

- TPMT
- CYP2D6
- CYP2C19
- TPMT (Dermatology ALL, Crohns)
- HLA-B*5701 HIV
- CYP2C19 Psychiatry
- CYP2D6 Psychiatry
- HLA-B*5701
- BChE
- CYP3A4
- CYP3A5
- CYP2B6
- CYP1A2
- CYP2C9
- VKORC1
Pharmacogenetic testing in The Netherlands 2011

(6 labs: n=4,972)
Procedures in PGX diagnostics

Quality assurance:
- Threeway split of sample

DNA isolation in duplicate

SNP analysis on two different platforms

Quality:
- Participation in proficiency schemes

Speed:
- Results in 4 dagen (24 hrs possible)

Support:
- Specific dosing advice
Observations from 7 year two platform approach

- Rerun of 300 TaqMan samples (CYP3A5) by PCR-RFLP revealed 1% discrepancies. Sequencing proved the PCR-RFLP to be right.

- Amplichip missed twice a *6 in *1/*6 patients due to SNP under one of the primers. Detected because of discrepancy with TaqMan.

- 2 PCR-RFLP samples were called wrongly due to weak bands on gel: detected through comparing with TaqMan result.

- Luminex failed in 2/100 cases to give right genotype

- Discrepancy between direct sequencing and PCR-RFLP: sequencing was wrong (unequal allele amplification due to SNP close to seq-primer).

- Re-analysis of 6 reported TMPT*3B patients in 4 papers showed that none of these were actually TPMT*3B
Request: CYP2D6 genotyping

Problem: side effects on 150 mg/day imipramine

Material: EDTA blood


Result: CYP2D6*4/*4 (2 inactive alleles)

Interpretation: Poor Metabolizer

Advice: This genotype would fit with 30% of standard dose of imipramine

Extra info on SNPs tested, duplicate analysis, techniques used, frequencies of predicted phenotype, limitations of the test

Signed by Clinical Chemist and by Hospital Pharmacist
"Here is my sequence..."

(The New Yorker, 2000)

Royal Dutch Pharmacy (KNMP) initiative:

literature review by 15 experts

Rating evidence from literature and providing dose recommendations based on genotype

Chair: Dr. Vera Deneer

www.kennisbank.knmp.nl
**Drug/Small Molecule:**
imipramine

### Dutch Pharmacogenetics Working Group Guideline - imipramine, CYP2D6

The Royal Dutch Pharmacists Association - Pharmacogenetics Working Group has evaluated therapeutic dose recommendations for imipramine based on CYP2D6 genotype (PMID: 21412232). They recommend reducing the dose for poor and intermediate metabolizer patients, and selecting an alternative drug for ultra metabolizers.

<table>
<thead>
<tr>
<th>Phenotype (Genotype)</th>
<th>Therapeutic Dose Recommendation</th>
<th>Level of Evidence</th>
<th>Clinical Relevance</th>
</tr>
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<tbody>
<tr>
<td>PM (2 inactive alleles)</td>
<td>Reduce dose by 70% and monitor imipramine and desipramine plasma concentrations.</td>
<td>Published controlled studies of good quality relating to phenotyped and/or genotyped patients or healthy volunteers, and having relevant pharmacokinetic or clinical endpoints.</td>
<td>Clinical effect (S): long-standing discomfort (48-168 hr) without permanent injury e.g. failure of therapy with tricyclic antidepressants, atypical antipsychotic drugs; extrapyramidal side effects; parkinsonism; ADE resulting from increased bioavailability of tricyclic antidepressants, metoprolol, propafenone (central effects e.g. dizziness); INR 4.5-6.0; neutropenia 1.0-1.5x10⁹/l; leucopenia 2.0-3.0x10⁹/l; thrombocytopenia 50-75x10⁹/l</td>
</tr>
<tr>
<td>IM (2 decreased activity alleles, or 1 decreased activity allele)</td>
<td>Reduce dose by 30% and monitor imipramine and desipramine plasma concentrations.</td>
<td>Published controlled studies of good quality relating to phenotyped and/or genotyped patients or healthy volunteers, and having relevant pharmacokinetic or clinical endpoints.</td>
<td>Minor clinical effect (S): QTc prolongation (&lt;450 ms men, &lt;470 ms women); INR increase &lt; 4.5; Kinetic</td>
</tr>
</tbody>
</table>

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**www.pharmgkb.com**
Conclusions: hurdles in clinical practice

- Unawareness
- Competition with common practice
- Test accessibility
- Costs
- Turn-around-time
- Translation genotype to phenotype
- Difference in screening prior to therapy or diagnostic testing
- Convincing clinicians → uptake in guidelines (catch 22?)
Conclusions: hurdles in clinical practice (accompanying diagnostics)

- Unawareness
- Competition with common practice
- Test accessibility
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