Biomarkers in Precision Medicine: Their Deployment to Improve Clinical Trial Efficiency

Nov 15, 2016

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We are different!

In length, weight, talents, interests, IQ........ and.....

in drug response!

Two individuals do not respond the same to a certain drug treatment and a lot of these differences are due to genetic variation, in total about 30-40 % of the overall interindividual variations.
Adverse Drug Reactions (ADRs)

ADRs were between the 4th-6th commonest cause of death in the US in 1994
Lazarou et al, JAMA, 1998

About 3% of the 548 new drugs approved by the FDA between 1975-1999 withdrawn because of safety problems
Lasser et al, JAMA, 2002

- 20 % of readmissions to the hospital and 30 % of admissions of elderly are caused by ADRs
- ADRs cause an average 2 days prolonged hospital visit
- USD 2,500/patient, USD 100 billion in USA/year
Sim and Ingelman-Sundberg, The Pharmacogenomics J. 2011
Interindividual differences in drug action

Absorption / Excretion
- Slow
- Rapid
- Slow
- Rapid

Drug-drug interactions

Drug-food interactions

Metabolism
- Poor
- Efficient
- Ultrarapid

Receptor interactions
- Poor
- Efficient

Drug-drug interactions

Kidney function

Personalized medicine, reasons for clinically important interindividual response differences

Drug interactions
- Enzyme competition
- Target interactions

Genetic variation
- Drug metabolism
- Drug transport
- Drug targets

Health factors
- Pathophysiology
- BMI
- Age
- Lifestyle

Epigenetic variation
- Drug metabolism
- Drug transport
- Drug targets

Biomarkers
- Somatic mutations
- Circulating biomarkers

40-50%
25-40%
20-30%
unknown

Ingelman-Sundberg *J Intern Med*, 2015
Genetic variation causing different efficiency of drug metabolism

- Poor metabolisers (PM)
  - Two defect genes

- Intermediate metabolisers (IM)
  - One defect gene or two less active genes

- Extensive metabolisers (EM)
  - Two normal genes

- Ultrarapid metabolisers (UM)
  - Gene duplication on one allele
Subjects who do not respond correctly

Patients

Normal responders

Adverse drug reactions or lack of response

Rapid drug metabolism, too low drug concentration, no response

Too slow metabolism, too high drug concentrations, adverse reactions

Immune-mediated adverse reactions

Formation of toxic metabolites, adverse reactions

Inappropriate interactions with drug targets, adverse reactions, no response
Major pharmacogenomic biomarkers

HLA alleles → Enzymes → Transporters → Targets → Somatic genome

155 (15 %) of medical products by EMA and 118 medicines according to FDA contain pharmacogenomic labels

ABL, ALK, BRAF, EGRF, HER2, KRAS, KIT, MET
Treatment with the antiepileptic drug carbamazepine.

Risk for hypersensitivity syndrome in HLAB*1501 carriers.

Treatment with codeine CYP2D6 ultrarapid metabolisers – risk for morphine intoxication.

Treatment with codeine CYP2D6 poor metabolisers – no analgetic effect.

Treatment with the anticoagulant drug clopidogrel after heart infarction.

CYP2C19*2 carriers: no/poor effect - increased risk for additional CV problems.

Treatment with lipid lowering statins.

Risk for myopathy in SLCO1B1*5 carriers treated with high doses of statins.

Treatment with the anticoagulant warfarin.

High risk for bleedings among CYP2C9*3 carriers.

Problems with inter-individual difference in response to drug treatment.

Treatment with the anticoagulant warfarin.

Problems with inter-individual difference in response to drug treatment.

Treatment with the antiepileptic drug carbamazepine.
Some additional examples of polymorphically influenced drug response

<table>
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<tr>
<th>Drug</th>
<th>Treatment</th>
<th>Odd effect</th>
<th>Freq</th>
<th>Genetic variation</th>
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<tr>
<td>Abacavir</td>
<td>AntiHIV</td>
<td>Hypersensitivity</td>
<td>5-7 %</td>
<td>HLA-B genotype</td>
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<td>5-fluorouracil</td>
<td>Cancer</td>
<td>Toxicity</td>
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<td>DPYD*2</td>
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<td>Herceptin</td>
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<td>Response</td>
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<td>Her2 (ERBB2) rec expr</td>
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<td>Irinotecan</td>
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<td>Interferon</td>
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<td>Response</td>
<td>30%</td>
<td>IL28B (IFN-γ-3)</td>
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<td>Iressa</td>
<td>Lung cancer</td>
<td>Response</td>
<td>10%</td>
<td>EGFR-TyrKinase</td>
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<td>6-Mercaptopurines</td>
<td>ALL, MbCrohn</td>
<td>Leukopenia</td>
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<td>Simvastatin</td>
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<td>Warfarin</td>
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<td>Bleedings</td>
<td>12 %</td>
<td>CYP2C9/VKORC1</td>
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Some HLA allele associations reported with serious adverse drug reactions since 2000

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<td>DQA1*02</td>
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<tr>
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Overlaps of pharmacogenomic labels

- Abacavir
- Afatinib
- Alectinib
- Anastrozole
-Arsenic trioxide
-Atazanavir
-Azathioprine
-Bosutinib
-Brentuximab vedotin
-Carbamazepine
-Carglumic acid
-Ceritinib
-Cetuximab
-Cholic acid
-Clopidogrel
-Cobimetinib
-Crizotinib
-Dabrafenib
-Dasatinib
-Denileukin diftitox
-Dextromethorphan
-Divalproex sodium
-Eliglustat
-Elosulfase alfa
-Erlotinib
-Ethinyl estradiol
-Everolimus
-Exemestane
-Fulvestrant
-Gefitinib
-Ibritumomab
-Ibrutinib
-Imatinib
-Ivacaftor
-Lapatinib
-Letrozole
-Lomitapide
-Maraviroc
-Mercaptopurine
-Nilotinib
-Norelgestromin
-Olaparib
-Osimertinib
-Oxcarbazepine
-Panitumumab
-Pegloticase
-Pembrolizumab
-Pertuzumab
-Phenylacetic acid
-Pimozide
-Ponatinib
-Rasburicase
-Rituximab
-Sodium benzoate
-Sodium phenylbutyrate
-Tetrabenazine
-Trametinib
-Trastuzumab
-Trastuzumab emtansine
-Tretinoin
-Vandetanib
-Velaglucerase alfa
-Vemurafenib
**New guideline by EMA**

By the European Medicines Agency (EMA)

**Guideline on good pharmacogenomic practice**

*Draft*

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
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<tbody>
<tr>
<td>Agreed by Pharmacogenomics Working Party</td>
<td>21 March 2016</td>
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<tr>
<td>Adopted by CHMP for release for consultation</td>
<td>28 April 2016</td>
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<tr>
<td>Start of public consultation</td>
<td>1 May 2016</td>
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<tr>
<td>End of consultation (deadline for comments)</td>
<td>16 September 2016</td>
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• €15 million, H2020, 10 EU countries

• Implement pre-emptive PGx testing in a real world clinical setting across 7 EU sites

• Evaluate patient outcome and cost effectiveness using solid scientific methodology

• Start 1-1-2016, 5 yr

• Consortium members
  • H-J Guchelaar (Coordinator), JJ Swen, M Kriek, LUMC
  • M Pirmohamed, R Turner, UOL
  • J Stingl, FDMD
  • M Ingelmann-Sundberg, KI
  • M Karlsson, S Jönsson, PBUU
  • M Schwab, E Schaeffeler, IKP
  • VHM Deneer STZH
  • M Samwald, G Sunder-Plassmann, MUWV
  • M van Rhenen, KC Cheung, KNMP
  • C Mitropoulou, GHXF
  • D Steinberger, BIOL
  • CL Davila Fajardo, SAS
  • G Patrinos, UPAT
  • V Dolžan, ULMF
  • A Cambon-Thomsen, UPS
  • G Toffoli, E Cecchin, CROA
Provide suitable genotyping technology for the selected panel of variants

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<td>VKORC1</td>
<td>VKORC1_c.174-136C&gt;T</td>
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<td>UGT1A1</td>
<td>UGT1A1*28</td>
<td>rs8175347</td>
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<td>HLA</td>
<td>HLA-A*04</td>
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<tr>
<td>HLA</td>
<td>HLA-A<em>8</em>5701</td>
<td>rs35599367</td>
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<tr>
<td>CYP3A4</td>
<td>CYP3A4*22</td>
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</tr>
</tbody>
</table>
Drug treatment at 7 different EU centra are subjected to conventional or pharmacogenetic assisted treatment.

Costs and effectiveness between the two treatment regimens are compared.

Patients

N=4,000

N=4,000
• The U-PGx project will provide both quantitative and qualitative evidence for a new model of PGx guided personalized medicine.

• U-PGx will provide physicians, pharmacists, nursing staff and patients with the necessary tools to bridge the gap from individual genotype to personalized prescription allowing them to truly embrace the concept of personalized medicine.
Drug metabolizing enzymes and population migration, background to the polymorphisms we see today
Genetic drift and genetic selection

Selection based on environment, e.g.:
- Infections
- Climate
- Diet

Evolution of human P450 genes
Parallel evolution of the CYP2D genes in mice and humans
Forest experiment

Without grandmother information

With grandmother information
Insect selection based on P450 gene inducibility
Adaptive alterations in CYP genes following shift of host by insects

Myzus persicae

Microsatellite amplification of 100 fold in CYP6CY3 of the polyphagous aphid adapted to feed on tobacco. The enzyme is active in metabolism of nicotine derivatives

Bass et al., *PNAS* 110, 19460, 2013
The human situation: starting in Ethiopia

Subject for severe starvation periods killing millions of people in each event in the past

CYP2D6 metabolises plant toxins
Alleles with duplicated and multiduplicated CYP2D6*2 genes

Genetic adaptation to the environment among CYPs

Insecticide resistance in Drosophila (1930-1960)

Daborn et al *Science* 2002; **297**:2253
Amichot et al *Eur J Biochem* 2004; **271**:1250

Alkaloid resistance in CYP2D6 (10000-5000 BC)

Ingelman-Sundberg, *The Pharmacogenomics J*, 2005
Dietary stress induced *CYP2D6* gene duplications in Ethiopia

- **60,000 years ago**
- **6,000 Years ago**

Selection for *CYP2D6* gene duplications

Population expansion

Food constraint in relation to the size of the population
Global distribution of duplicated *CYP2D6* alleles

Mainly *CYP2D6*\(^*2xN\)

Ingelman Sundberg, 2005, Sistonen et al., 2007
Rare genetic variants might be of clinical importance in specific geographical regions.

Lauschke VM, Ingelman-Sundberg M. *Pharmacogenomics* 2016 Jun 1:0.


Lauschke VM, Ingelman-Sundberg M. *Trends Pharmacol Sci.* 2016;37:85-6;

Rare variant of CYP3A4 (CYP3A4*20)

CYP3A4*1

−GGACTTCTTCAACCAGAAAAACCCCGTTGTTCTAAAGGTTGAGTCAAGGGATGGCACCCTAAGTGAGCCCTGA
G L L Q P E K P V V L K V E S R D G T V S G A *

CYP3A4*20

−GGACTTCTTCAACCAGAAAAACCCCGTTGTTCTAAAGGTTGAG
G L L Q P E K T R C S K G *

Found in a clinical trial in one single participant with South American origin

CYP3A4 polymorphism is very rare in general, but...

Geographical distribution of CYP3A4*20 allele

Impact of CYP3A4 variants on neuropathy grade and paclitaxel treatment modification

Neuropathy grade in patients with different CYP3A4 activity

- CYP3A4 Loss-of-function (n=4)
- CYP3A4 Missense (n=3)
- CYP3A4 Wild type (n=229)

Treatment modification due to neuropathy among patients with different CYP3A4 activity

- CYP3A4 Loss-of-function (n=4)
- CYP3A4 Missense (n=3)
- CYP3A4 Wild type (n=229)

*1/*20  *1/*25  *1/*27  *1/*8

New sequencing projects

- AstraZeneca 500,000 DNA samples; later 2 million genome sequences
- Obama precision medicine initiative 1 million American volunteers
- UK 100,000 genome project
Pharmacogenomics in the NGS era

• The problem of rare variants
• The problem of clinical implementations
• The problem of nomenclature
Analyses of genes encoding transporters, phase II enzymes and nuclear receptors from WGS and exome sequencing databases

Variant composition of all pharmacogenes analysed

Very rare variants per kbp in different classes of genes

Each enzyme and transporter has many different variants

Each individual has up to 3 different mutations per gene
Number of genetic variants of transporters

Distribution of genetic variations between Europeans and Africans


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Variants per individual

Europe ■ Africa ■

ABC transporters

SLC transporters

Rare variants among CYPs, contribution to the overall variability in different CYP genes

Conclusion

The analyses of rare SNPs reveal that about 30-40% of all genetically determined variability in drug pharmacokinetics is governed by rare variants.
Every individual has many many personal mutations not shared by others

Sequencing of the coding region from 60 706 human genomes reveal one nucleotide variant every eight bases in exomes (!)

Nature August 16, 2016: 536, 285

More than half of the new ~7.5 million variants found are seen only once!!
How is the real impact of previously undetermined SNPs?

The same drug is given to monozygotic (identical genetics) or dizygotic (genetics like siblings) and the drug pharmacokinetics is determined.

The difference between the paired individuals is determined and the extent of the genetically determined differences in drug pharmacokinetics can be calculated.
Genetically determined variation in drug PK in monozygotic and dizygotic twins

Matthaei et al *CPT* 98: 611; 2015
Conclusion

Only 40% of genetically inherited metoprolol and toremfide PK studied in monozygotic vs dizygotic twins are predicted by common ADME genetic variants

(Matthaei et al., CPT, 2015)
ClinVar & Variant Nomenclature

Local ID

HGVS-like

607008.0001
985A>G
985A>G (K304E)
985A>G (K329E)
A985G
ACADM, LYS304GLU
K304E
K304E (985 A->G)
K304E (K329E)
K304E only
K329E
K329E(985A>G)
LYS304GLU
c.985A>G
c.985A>G (p.K304E)
c.985A>G (p.Lys304Glu)
c985A>G
p.K304E
p.Lys329Glu
includes: K304E (985A>G)
Mutation c.985A>G (p.K304E)
previously known as p.Lys329Glu
Analysis of ACADM 985A>G mutation

HGVS

Multiplicity in assemblies, transcripts, legacy conventions for numbering systems, abbreviations for amino acids, formats

Unstructured text

Slide from Bob Freimuth and Donna Maglott

www.aaps.org

#AAPS2016
Rare variant problems

- The impact of rare variants cannot be experimentally validated for all variants detected.
- Clinical trials validating the rare variants are impossible to conduct.
- The possibility to provide specific guidelines approved by the regulatory agencies for their use is does not exist.
- Haplotype assignment for rare SNPs creates a problem.
- Hitherto unknow to which extent WGS will provide a better prediction for decisive SNPs for drug response as compared to WES.
- A nomeclature for variant alleles that is useful in applied settings is lacking.
- Implementation of NGS-based comprehensive genotyping into clinical practice requires more reliable functionality assessment algorithms.
Handling of new rare genetic variants, database expansion

NGS based pharmacogenomic analyses

Patient gDNA

Whole genome sequencing

Data applied to pharmacogenes of interest

Genes

A

Silent mutations

No action

B

Missense mutations

Loss of function mutations

In silico prediction of functionality of mutations

Construction of a gene functionality score

Prediction based on gene functionality, not SNPs
For results from outliers which cannot be explained by known variants, additional sequencing and validation of the variants will be carried out.

Lauschke and Ingelman-Sundberg, *Pharmacogenomics* 2016
New approach for personalized strategy

Patient

Whole genome/exome analyses

Identify mutations in the genes of interest for the drug therapy

In silico assisted classification of the functionality score of the different genes of interest

Based on patients specific gene functionality score drug dose and type is selected

*Individualized drug therapy*
During drug development

- Identify all outliers
- Make genotyping of outliers by extensive commercial platforms
- For remaining unexplained outliers make WES/WGS analyses and try to interpret data.
- Store DNA for future analyses
Novel in vitro system for studying drug metabolism and toxicity

Use of the 3D hepatic systems

PHH Spheroids, proteomic analyses

Whole proteome analysis

Analyses of drug metabolism; Metabolites formed in the 3D spheroid system as compared to 2D hepatocytes
Classes of diseases to be mimicked in spheroids

Enhanced by inflammation + TNFα

Enhanced by macrophages + Kupffer + LPS

Virus enhanced toxicity (XIM) + Virus

Steatosis via e.g. mitochondria

Cholestasis, ′ + bile acids
Chronic toxicity in PHH spheroids

A

Viability (%) vs. Amiodarone (µM)

B

Viability (%) vs. Bosentan (µM)

C

Viability (%) vs. Diclofenac (µM)

D

Viability (%) vs. Fialuridine (µM)

E

Viability (%) vs. Tolcapone (µM)

- Green: 48h
- Red: 7 days
- Blue: 28 days

<table>
<thead>
<tr>
<th>Compound</th>
<th>PHH 2D 24h</th>
<th>48h</th>
<th>7d</th>
<th>28d</th>
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</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>24.6</td>
<td>&gt;100</td>
<td>6.5</td>
<td>1.6</td>
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<tr>
<td>Bosentan</td>
<td>219</td>
<td>&gt;250</td>
<td>69.5</td>
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<td>Diclofenac</td>
<td>233</td>
<td>190.8</td>
<td>56.8</td>
<td>45.9</td>
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<tr>
<td>Fialuridine</td>
<td>&gt;300*</td>
<td>&gt;100</td>
<td>0.7</td>
<td>0.1</td>
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<tr>
<td>Tolcapone</td>
<td>19.8</td>
<td>18.8</td>
<td>9.4</td>
<td>5.6</td>
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</tbody>
</table>

EC50 (µM)

- > 30 x C_max
- 1-30 x C_max
- < 1 x C_max
Spheroid specific detection of fialuridine toxicity and CYP3A4 induction by a drug candidate.

- **FIAU** $c_{\text{max}}$ in man *in vivo* = 1μM
- EC$_{50}$ > 100μM
- EC$_{50}$ = 0.7μM
- EC$_{50}$ = 0.1μM

**Viability (%)**

**Fold change CYP3A4 expression (relative to DMSO)**

- DMSO
- Cmp
- Rifampicin
- Phenobarbital
Drug target validations

Proof-of-principle identification of ADME-miRNA pairs

Spheroids can be efficiently transfected with nucleic acid-based inhibitors (antagomiRs)

Down regulation of miR-103 by antagomirs in hepatocytes results in increased levels of CYP2C8
Steatotic models

Lipid accumulation via steatotic compounds

Cyclosporine A (CsA)

Amiodarone (AMD)

Induction of phospholipidosis
NAFLD models

<table>
<thead>
<tr>
<th></th>
<th>Control medium</th>
<th>2x serum</th>
<th>Glc+Frc+ insulin</th>
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<tbody>
<tr>
<td>20 days</td>
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</tr>
<tr>
<td>Steatosis</td>
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</tr>
</tbody>
</table>

Steatosis induced by nutritional perturbations
Drugs whose actions are dependent on genetic variability are labeled with recommendations regarding altered prescriptions in genetically different parts of the populations; FDA have 118 labelled drugs, EMA 155.
Colleagues, collaborators and sponsors

Prof. Bo-Göran Ericzon
Dr. Ewa Ellis

Volker Lauschke
Mikael Kozyra
Delilah Hendriks
Catherine Bell
Olof Beck
Åsa Nordling
Sabine Vorrink