
PHARMACOGENOMICS & DRUG SAFETY

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Philosophy

- Importance of inter-individual variation in drug response
 - Life threatening adverse drug reactions
 - Lack of desired therapeutic effect
- Inheritance is an important factor responsible for individual variation in drug response
 - Besides : age, sex, comedications, underlying disease ...
- Questions and applications of the knowledge of a patient's DNA sequence
 - Maximize efficacy
 - Target patients that are likely to respond
 - Avoid Adverse Drug Reactions (ADR)

... goal of individualized drug therapy

- Bridging the tolerability gap between healthy volunteers and patients ?

- Among the solutions what about pharmacogenetics and pharmacogenomics ?

Definitions

. Drug Safety

- Detection and prevention of adverse-drug-reactions
- Pharmacovigilance : spontaneous reports; individual cases; other (PMS)
- Toxicology in early phases of drug development (MTD)

. Pharmacogenomics (PGx)

- Genetic basis for individual variations in response to therapeutics
- Single nucleotide polymorphisms (SNPs) - single - base variations at a unique physical locations among different individuals - are the most frequent polymorphisms in the Human Genome

goals : 1) aid in target discovery

2) prioritize and optimize lead compounds

3) evaluate efficacy and safety

4) stratify patients enrolled in clinical trials

5) create predictive and diagnostic tests

The life of a drug

Clinical steps (Phase I – II – III Studies)
(IND)* → (NDA)**

Drug on the market

- . - 50% NDA in 2002-2003 vs 1996-1997
- . investment in research spending x 2.5 fold
- . 80% IND fail it to market
- . Cost of NCE = US \$ 800 million ; 50 % for RCT
- . Failure rate in phase III = 50 %
(SAFETY; EFFICACY; INDUSTRIALIZATION)

- Pharmacovigilance
- Pharmacoepidemiology
- Post-marketing surveillance

VALID BIOMARKERS

- . Gefitinib (K) *EGFR* gene (success 10%)
- . Atomoxetine (ADHD) - *CYP2D6* (dosage)

DROP-OUTS

- Cox 2-inhibitors : vascular events
- SSRIs : suicide in children

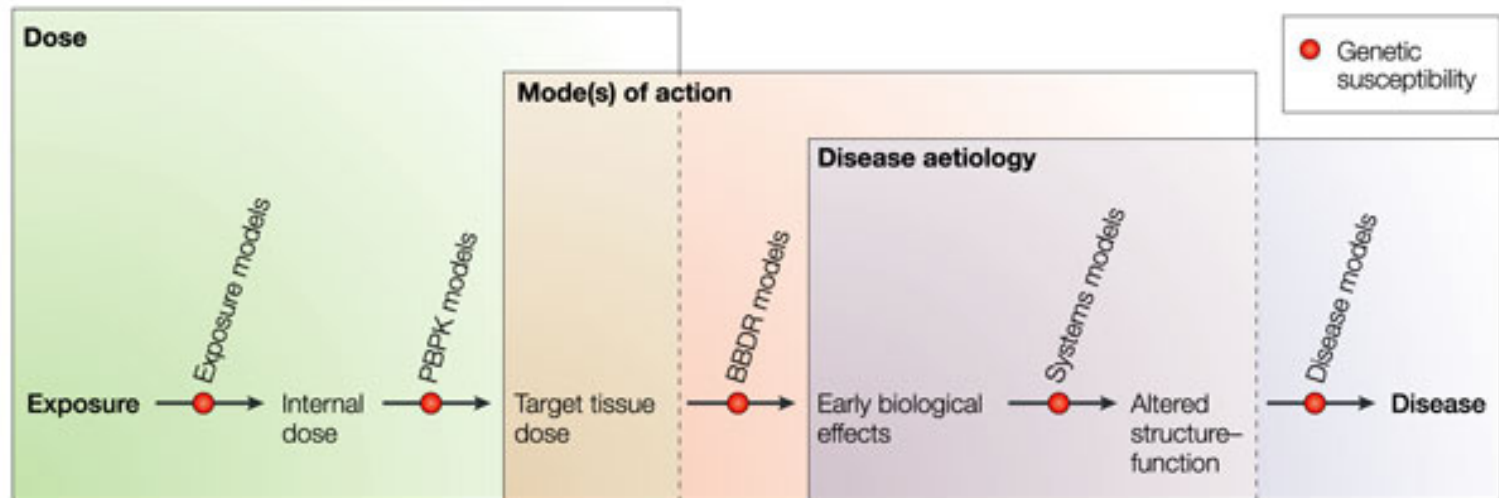
ADRs IN HOSPITALS

- 2 million/year ; 5 % of admissions
- 100,000 deaths/year

IND* = Investigational NewDrug applications ; ADR = Adverse Drug Reaction

NDA** = New Drug Application

GTx : from exposure to disease & treatment outcome



Types of ADR

Characteristics of type A and type B adverse drug reactions

Characteristic	Type A	Type B
Dose dependency	Usually shows a good relationship	No simple relationship
Predictable from known pharmacology	Yes	Not usually
Host factors	Genetic factors might be important	Dependent on (usually uncharacterized) host factors
Frequency	Common	Uncommon
Severity	Variable, but usually mild	Variable, proportionately more severe
Clinical burden	Higt morbidity and low mortality	Higt morbidity and mortality
Overall proportion of adverse drug reactions	80%	20%
First detection	Phases I-III	Usually Phase IV, occasionally Phase III
Animal models	Usually reproducible in animals	No known animal models

ADR & HLA

HLA & adverse drug reactions ^{A,B}

Drug	Adverse reaction	HLA association
Carbamazepine	Severe hypersensitivity reactions	DR3, DQ2
Clozapine	Agranulocytosis	B38, DR4, DQ3
Dipyrrone	Agranulocytosis	A24, B7, DQ1
Gold	Proteinuria, dermatological reactions, thrombocytopenia	DR3
Hydralazine	SLE	DR4
Levamisole	Agranulocytosis	B27
Oxicam	Toxic epidermal necrolysis	A2, B12
Penicillamine	Penicillamine toxicity	DR3
Sulfonamides	Toxic epidermal necrolysis	A29, B12, DR7

^A The list of HLA associations is not exhaustive. Importantly, most of these findings are based on single studies, and therefore do need replication.

^B Abbreviations : HLA, Human Leukocyte Antigen ; SLE, Systemic Lupus Erthematosus

Pharmacogenetics (GPt) : a historical step

Pharmacogenetics (PGt)

■ BACKGROUNDS

- Twin studies
- Drug levels or metabolism (PK) are inherited as Mendelian traits
- Use of « probe drugs »
- Phenotypic variations

■ ICONS OF PGX

- N-acetyl-transferase (*NAT2*)
 - Isoniazid
 - Caffeine
- Thiopurine S-methyltransferase (*TPMT*)
 - *TPMT^L* and *TPMT^H*
 - Individuals homozygous for *TPMT*3A* : great risk for myelosuppression (↓ dose 1/10) with 6 mercaptopurine
- Cytochrome P4502D6
 - Debrisoquine
 - Poor, extensive, ultra rapid metabolisers

ADR & PGt

Pharmacogenetic defects in enzymes that lead to undesirable pharmacodynamic adverse effects

Enzyme defect	Drug	Adverse reaction
Glucose-6-phosphate dehydrogenase deficiency	Primaquine, sulfonamides, dapsone, nitrofurantoin	Haemolytic anaemia
Methaemoglobin reductase deficiency	Nitrites, dapsone	Methaemoglobinaemia, haemolysis
Porphobilinogen deaminase deficiency (acute intermittent porphyria)	Barbiturates, estrogens, alcohol, anticonvulsants and sulfonamides	Acute porphyric crises
Acetylcholinesterase	Anticholinesterase agents	Neurotoxicity

ADR et enzymes

Phase II drug metabolizing enzyme gene polymorphisms & putative adverse drug reactions

Phase II enzyme	Drug	Adverse reaction
Plasma butyrylcholinesterase	Succinylcholine	Prolonged apnoea
N-acetyltransferase	Sulfonamides Amonafide Procainamide, hydralazine, isoniazid	Hypersensitivity Myelotoxicity SLE
Thiopurine methyltransferase	6-Mercaptopurine, azathioprine	Myelotoxicity, treatment- related second tumours
Dihydropyrimidine dehydrogenase	5-Fluorouracil	Myelotoxicity
UDP glucuronosyl transferase 1A1	Irinotecan	Diarrhoea, Myelosuppression

Abbreviation : SLE, Systemic Lupus Erthematosus

Pharmacogenomics :
more informative than GPT

Genotype > Phenotype ?

Pharmacogenomics (PGx): genomic science

■ Definitions

- Effects of inheritance on PK and PD pathways involving multiple gene products
- Development of expression profiling and high-throughput DNA sequencing and genotyping

■ Question

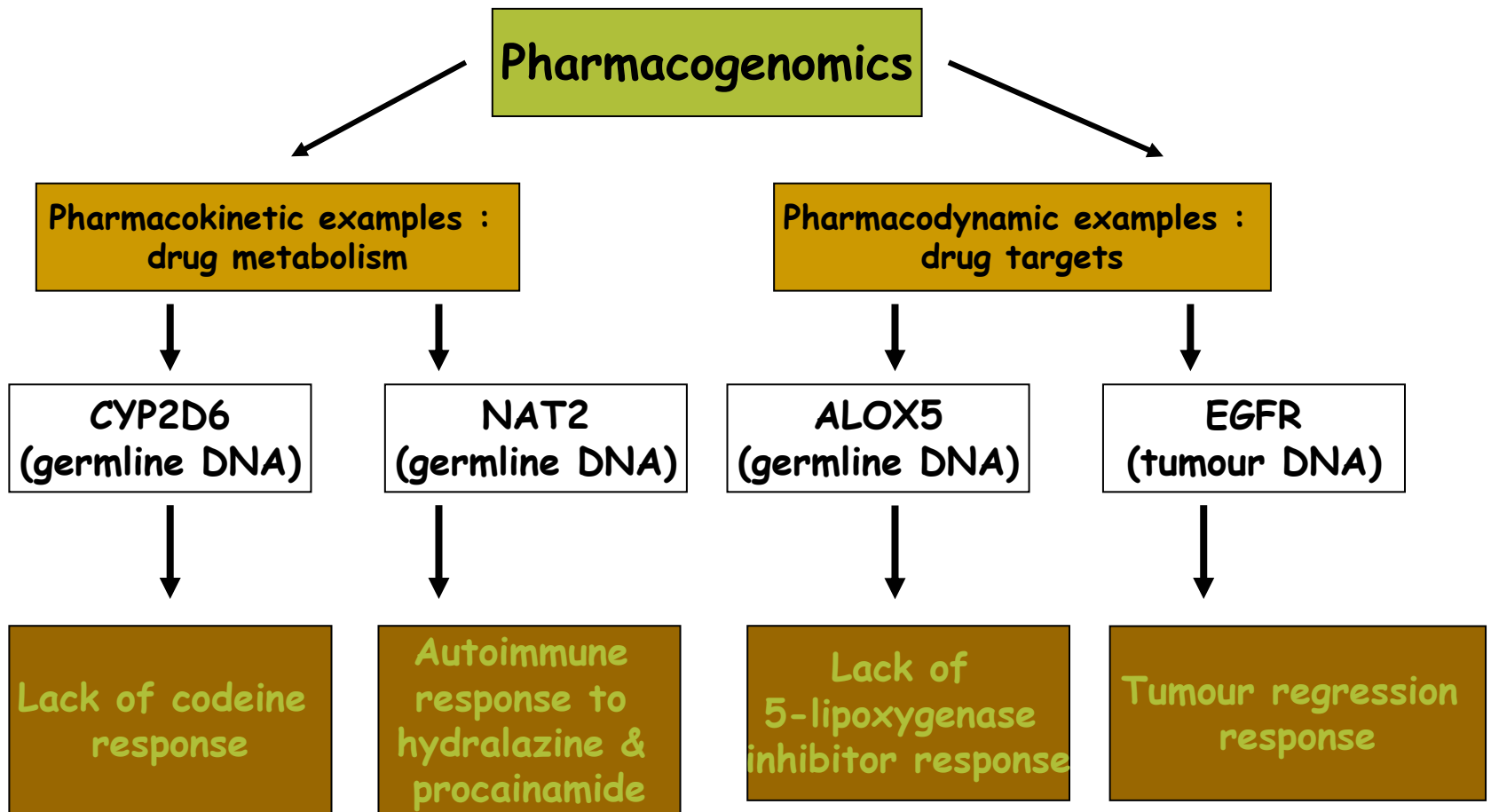
- Translation of this information to the bedside

■ Results

- Application are limited to a few tests and to rare academic referral centers
 - The monogenic model does not apply to the majority of drugs
 - Necessity to simultaneously study genes encoding a variety of proteins involved in PK + PD (large number of polymorphisms, haplotypes, genome-wide scans)
 - Trials designed to test pharmacogenomic hypotheses

... pharmacoproteomic, pharmacometabolomic ...

PGx : a new tool in medicine



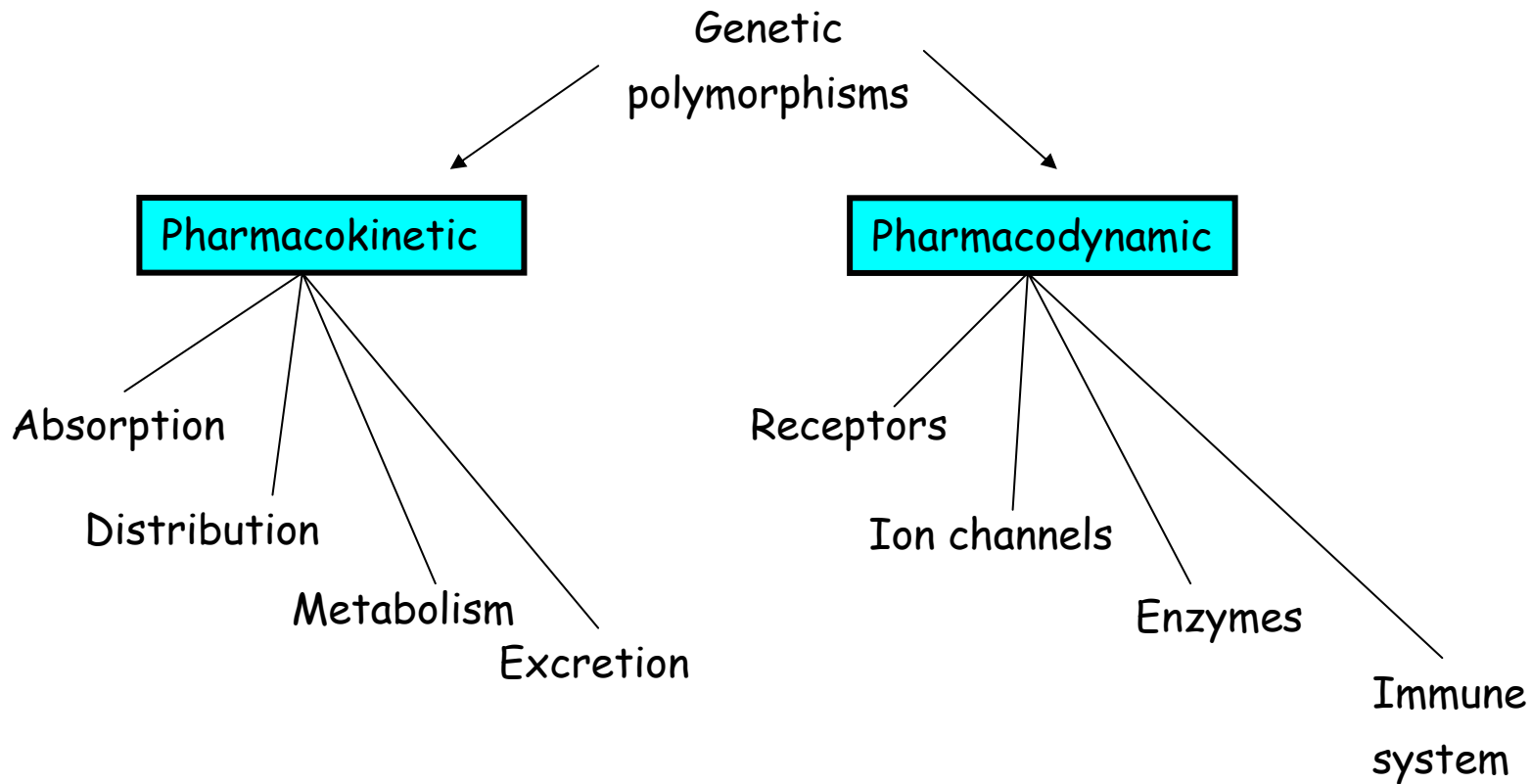
Pharmacogenomics/personalised medicine

Single Nucleotid Polymorphism (SNPs)

« know yourself »

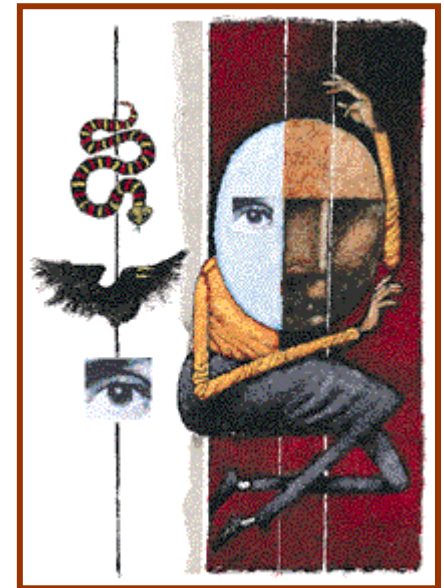
- **SNPs :**
 - Single base differences in the DNA sequence observed between individuals
 - Simplest form of DNA polymorphism
- **SNPs impact :**
 - Vast majority of SNPs are biologically silent
 - If present in the promotor : affect gene expression
 - If within the gene itself : impact on protein function
- **Consequence**
 - Correlate information from patients DNA with their response to a medicine (beneficial/adverse)
 - Linkage disequilibrium
 - Degree of association between the genotype and the phenotype
 - Pharmacogenomic stratification of patients in RCT

PGt versus PGx in monitoring ?



Examples

- Codein intoxication
- Torsades de Pointes (TdP)
- HIV & antiretroviral drugs
- 5-FU
- Clinical trials

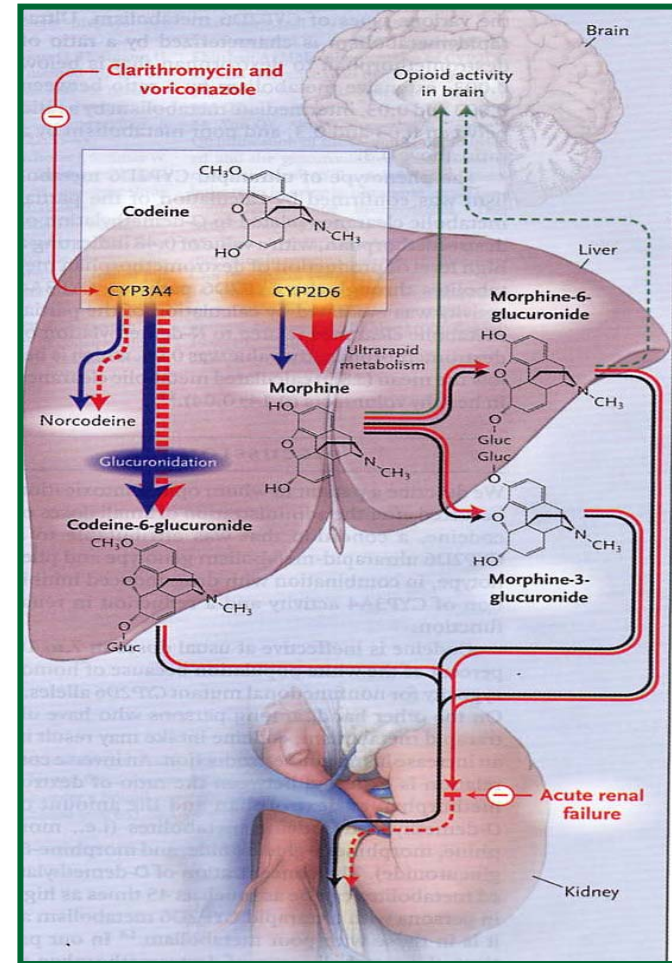


PGt & PGx : an explanation of a severe adverse effect

- **An example** : codeine intoxication associated with ultra rapid *CYP2D6* metabolism (Gasche Y et al, *NEJM* 2004 ; 351 : 2827)
 - Oral codeine (25 mg tid) in a 62 yo man in a context of pneumonia and comedication
 - 4 days later deterioration of consciousness ; unresponsiveness ; miotic pupils
 - Recovery 2 days later
- **Biological management**
 - Blood levels of codeine, morphine, metabolites
 - Duplication or multiduplication of the *CYP2D6* gene
 - *CYP2D6* and *CYP3A4* phenotype
 - CYP phenotypic activity (probe drug : dextromethorphan 25 mg)

Results : opioid intoxication

- *CYP2D6* ultrarapid-metabolism genotype and phenotype
- Drug-induced inhibition of *CYP3A4* activity



... diagnosis based on PGt, PGx and drug monitoring

Drug - induced torsades de pointes (TdP) tools of predictivity ?

1. Significant iatrogenic cause of morbidity and mortality (10-17%)
 2. Major reason for the withdrawal of a number of drugs from the market
 3. Wide variety of drugs are incriminated : pimozide, terfenadine, thioridazine, cisapride, astemizole...
 4. QT interval as a surrogate of TdP
 5. Congenital LQTS (1/5000 in USA)
 - Brugada syndrome - nocturnal sudden death sodium channel anomaly (SCN5A)
 - « formes frustes » (↓repolariation reserve)
 6. Ion - channels and voltage - gated potassium channels are the arrhythmia - related pharmacological targets
-

Long-QT syndrome : genes, locations, currents

Gene	Chromosomal location	Current
KCNQ1	11p 15.5	I _{ks}
KCNE1	21q22.1	I _{ks}
KCNE2	21q22.1	I _{kr}
KCNH2	7q35-36	I _{kr}
KCNQ1	11p15	I _{ks}
SCN5A	3p21-24	I _{na}
KCNJ2	17q23	I _{kir2,1}
ANK2	(sodium pump, Na/Ca exchanger, IP3)	

. Problem of modifier gene(s) : same *HERG* (*KCNH2*) and variable clinical expression

. Role of diseases : cardiomyopathy, diabetes, cirrhosis, automic failure...

. Major role of *CYP* polymorphisms : 2B6 (methadone), 2C19 (nelfinavir, citalopram), 2D6 (antipsychotics, antidepressants), 3A4 (antirhythmics, tacrolimus, halofantrine...)

PGt and PGx in TdP

- PGt and PGx are recommended in individual cases and clinical research
- Intensive study of specially targeted subjects/patients enrolled in phase I, II, III studies (ie : QT outliers)
- Post-hoc analysis (DNA banks)
- Question of ethnicity
 - Japan : LQTS 1/1164 school children; Brugada syndrome, SCN5A mutation 12 %
- **TECHNIQUES**
 - PCR based high-throughput assay detecting SNPs in CYP, NAT2, TMPT, UGT1A1, MDE1
 - Potassium channels : genes and protein encoding

Ref :

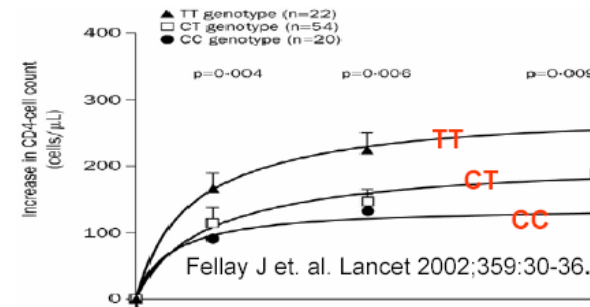
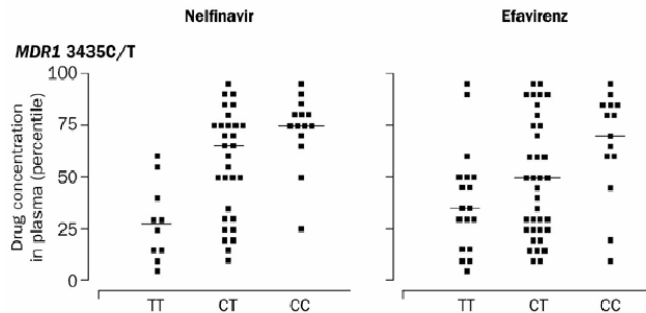
Shah RR, *Drug Safety* 2004; 27 : 145-172

Finlayson et al, *Eur J. Pharmacol* 2004; 500 : 126-142

HIV : antiretroviral drugs

■ Drugs :

- nucleoside reverse transcriptase inhibitors (Abacavir, Zidovudine)
- non nucleoside reverse transcriptase inhibitors (Efavirenz)
- protease inhibitors (Ritonavir, Nelfinavir)



ANTIRETROVIRAL DRUGS : PLASMA LEVELS AND GENOTYPES

■ Toxicity :

- mitochondrial toxicity : myopathy
- hypersensitivity : skin rashes, hepatitis ...
- lipodystrophy : fat atrophy, TGR ...
- miscellaneous : CNS symptoms, hemorrhage, interactions ...

Beyond serum drug monitoring ...

ADR & antiretroviral drugs & PGx

■ Mitochondria :

- Mitochondrial DNA levels : ↓ if hyperlactatemia
- CAG repeat mutations in the second exon DNA polymerase- α gene (POL G) : peripheral neuropathy, lactic acidosis

■ Lipodystrophy :

- TNF- α : -238 allele = susceptibility factor

■ Hypersensitivity :

- Abacavir :
HLA-B region HLA-B* 5701
TNF- α (308 G \rightarrow A polymorphism)

Fluorouracile (5-FU) : technical problem

- 5-FU : use in adenocarcinoma
pro-drug
- Dihydropyrimidine deshydrogenase (DPD) : 5-FU catabolites
- Toxicity of 5-FU : Cardiac, CNS, hematology
due to a total or partial deficit in DPD
- Problem : measurement of DPD activity
 - Lymphocyte DPD not correlated with liver DPD
 - Observed in 50 % of ADR

Conclusion : DPD genotype is a necessity

Benefits of PGx in clinical trials

- 1. Reduction of drug development time
 - Efficacy/Safety in specific populations
 - 2. Optimization of clinical utility
 - Linkage between subtypes and E/S
 - 3. Reduction of time to market
 - Specificity to the predicted population
 - 4. Explanation of response & identification of groups at risk
 - 5. Increased reimbursement
 - By differentiated responder (R) and non-R populations
-

Clinical trials & PMS

Development

Phase II trials



Phase III studies



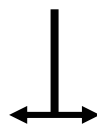
Approval of medicine for marketing



Initial post-launch surveillance

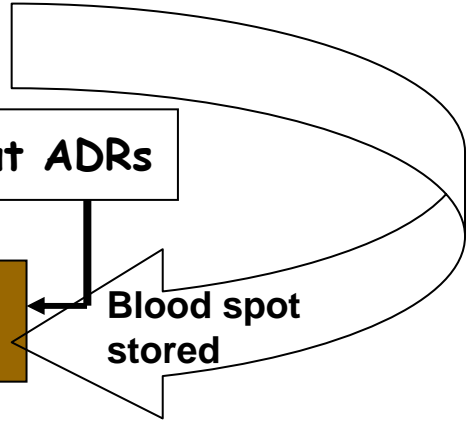
Patients taking the new drug

Patients with ADRs



Patients without ADRs

DNA comparison (eg comprehensive genome scan)



Genetic markers

Summarizing the data on ADR & Pharmacogenomics

Summarizing ADR & PGx

- Listing and tables !
- What can we do with those data ?
 - Contemplation ?
 - Dissemination ?
 - Practical use ?
 - Drug-surveillance strategies
 - Systematic blood spot + storage
 - Analysis of individual cases
 - Problems of specificity

« One drug fits all » → « Personalized therapy »

Predominantly monogenic pharmacogenetic disorders

Nerbert DW, Vesell ES, *Eur J.Pharmacol* 2004 ; 500 : 267-280

Glucose - 6 - phosphate deshydrogenase deficiency	G6PD
Isoniazid slow N-acetylation	NAT2
Sensitivity to alcohol	ALDM2
P - glycoprotein transporter defect	ABCBI
Dihydropyrimidine deshydrogenase deficiency	DPYD
Dopamine transporter defect	SLC6A3
Calcium channel defect	CACN1A
Serotonin transporter defect	SLC6A4
Caffeine 3 - demethylase defect	CYP1A2
Cyclophosphamide metabolism deficiency	CYPZB6

Clinical consequences for *CYP2D6*-PMs

Debrisoquine	Postural hypotension
Sparteine	Oxytocic effects
Perphenazine	Extrapyramidal symptoms
Flecainide	Ventricular tachyarrhythmias
Perhexiline	Neuropathy and hepatotoxicity
Phenformin	Lactic acidosis
Propafenone	CNS toxicity
Metoprolol	Hypotension
Terikalant	↑ QT interval
L-tryptophan	Eosinophilia – myalgia syndrome
Indoramin	Sedation

ADR & PGx

	Drug/drug class	Adverse effect	Gene or protein
Cardiovascular drugs	Heparin ACE inhibitors Procainamide, hydralazine Antiarrhythmics	Heparin-induced thrombocytopenia Drug-induced cough Drug-induced lupus QT prolongation & Torsades de Pointes	<i>FCRII</i> <i>BDKRB</i> <i>NAT</i> <i>HERG, K, LQT1, Mink, M, RP1</i>
Psychoactive drugs	Warfarin Antipsychotics Ethanol Levodopa	Bleeding risk Tardive dyskinesia, acute akathisia Alcohol dependence Drug-induced dyskinesias, drug-induced hallucinations	<i>CYP2C9</i> <i>ADRD2, ADRD3, ADRD5, CYP2D6</i> <i>ADH3</i> <i>Parkin, ADRD5</i>
Other	Thiopurines Thiopurines Abacavir Erythromycin, terfenadine, etc Anesthetics Oral contraceptives	Myelosuppression Secondary tumors Hypersensitivity QT prolongation & Torsades de Pointes Malignant hyperthermia Drug-induced venous thrombosis	<i>TPMT</i> <i>TPMT</i> <i>MHC proteins</i> <i>HERG, K, LQT1, Mink, M, RP1</i> <i>RYR1</i> <i>F2, F5</i>

Examples of adverse drug effects associated with genetic variability

Abbreviations : ACE, angiotensin converting enzyme ; MHC, major histocompatibility complex

The limits of technology

Technologies for SNP analysis

1. **Mass spectrometry**

Allele specific products identification

2. **High - through put micro array technologies**

DNA alterations such as SNPs, insertions and deletions can be identified

3. **Microsphere technology**

Direct hybridizations, oligonucleotide ligation and primer extension assay formates

4. **Invader assay**

Genotyping (SNPs) without PCR amplification FRET - based genotyping method

5. **Bioinformatics tools**

« discovery manager »

<http://pubs.acs.org/subscribe/journalmdd/v04>

CYP2D6 in routine ?

- Procedures not available
- Typing is expensive
- Many of the drugs have a wide therapeutic index ,
ADR mild and amenable to dose reduction
- Routine typing not shown to be cost effective

... prospective RCT: clinically and cost effective

CYP2C9 & warfarin in routine ?

- Anticoagulants (AC) response depends on R-warfarin (CYP1A2 ; CYP3A4)
- Sensitivity to AC : vitamin K & tyroide disease
- Mutations in clotting factors (prothrombin) might alter AC
- Genotype required within 24 hours of admission to hospital

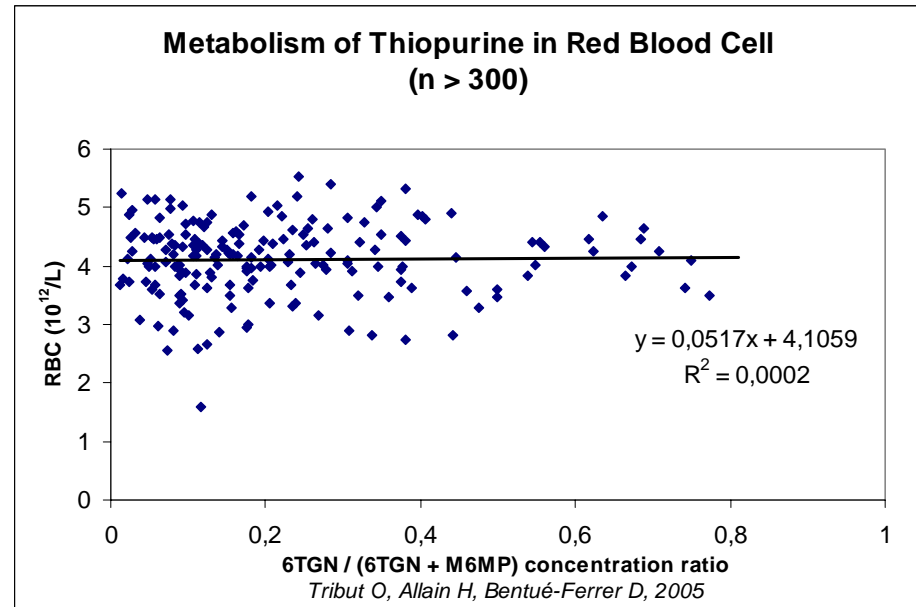
... prospective RCT: include confounding factors

PGx drug metabolism & pain medicine : recommendations *

- 1. Check whether the drug is metabolized by a polymorphic drug metabolizing enzyme
- 2. Consequence of this polymorphism (PM) ?
- 3. Prevalence of the PM alleles in the patient population (ethnicity) ?
- 4. Consider an alternate drug not subject to PM-enzyme
- 5. Advise the patient to carefully monitor ADR
- 6. Avoid drug inhibitors of the PM enzymes in question
- 7. PGx : cause of the ADR
- 8. If no response to treatment : Therapeutic Drug Monitoring
- 9. Think about speed of metabolism (UR, LM, EM) : dosage ?

Other practical attitude

- **TPMT** : prospective genotyping



- **Transporters** : Pgp/ *ABCB1* (multi-drugs resistance gene) : digoxin, protease inhibitors, cyclosporin toxicity ?
- **Receptors** : D3/tardive dyskinesia ; malignant hyperthermia after anaesthetics. Ryanodine receptor ??

Law - Regulations

Example of hepatotoxicity

- Most drugs induce hepatotoxicity. Rare events
 - PGx can identify population at risk so contraindications (drug labeling)
 - PGx and PGT are not required by FDA... mainly because nobody reaches « causation conclusions »
 - Drug maker duty ?
 - Testimony about PGT and PGx should be excluded in a trial about an alleged liver injury; studies must « fit the case » (poorly suited for courtroom opinions)
 - Ethics : creation of extensive genetic database; role of ethnicity...
-

General attitude

- Pharmacogenomic detection of rare ADR : not practical
 - Prospective storage of samples and evaluation in phase IV when a problem has been identified
 - RCT to examine both the clinical and cost-effectiveness of prospective genotyping
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Conclusion

Doctors are men who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing

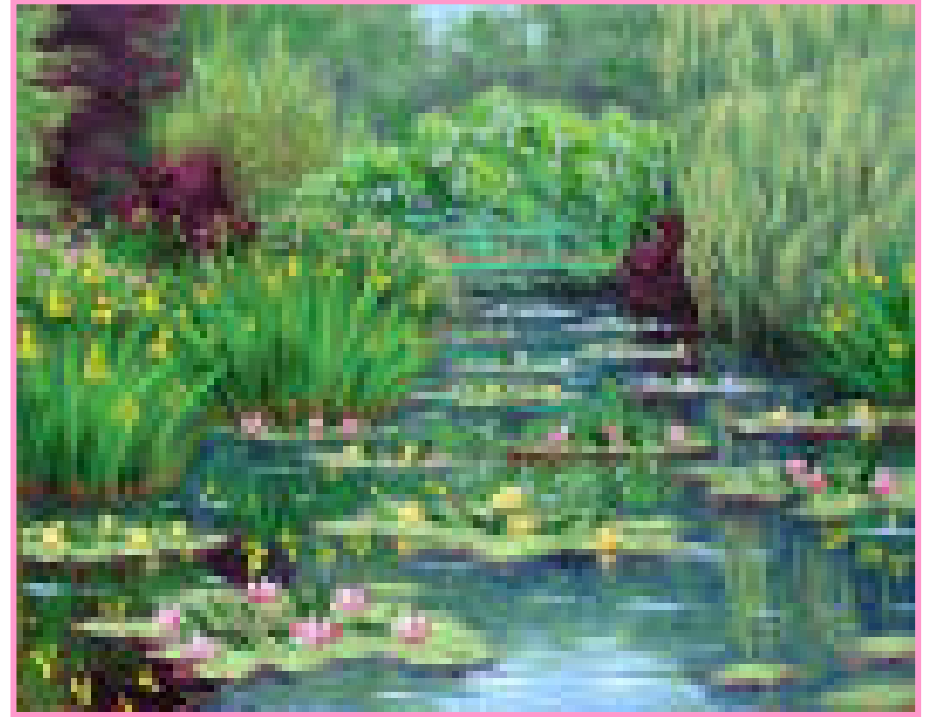
Voltaire (1694-1778)



- Pharmacogenomics can improve the situation if translated at the bedside and widespread
- Trends towards transcriptomics (gene transcripts), metabonomics (metabolite profiling), proteomics (proteins encoded by genome) ...

Thanks to the team ...

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