Pharmacogenetics
Introduction

• Some individuals can be especially sensitive to the effects of a particular drug whereas others can be quite resistant.

• Such individual variation can be the result of factors which are not genetic.

• For example, both the young and elderly are very sensitive to morphine and its derivatives, as are persons with liver disease.

• Individual differences in response to drugs in humans are, however, often genetically determined.
Definition

• The term *pharmacogenetics* was introduced by Vogel in 1959
  – for the study of genetically determined variations that are revealed solely by the effects of drugs.
  – strictly exclude those hereditary disorders in which symptoms are usually precipitated or aggravated by drugs.
Pharmacology: Pharmacokinetics and Pharmacodynamics

• Pharmacokinetics
  – What the body does to the drug.
  – Time course of [drug] in blood and tissues
  – Drug metabolizing enzymes (DME):
    • Prodrugs => active drugs
    • Active drugs => inactive compounds
  – Drug transporters
    • Influence intracellular drug concentrations; much less well-studied.

• Pharmacodynamics
  – What the drug does to the body.
  – The action of drugs including beneficial and toxic effects.
    • Targets of drug therapy: intended and unintended
Drug metabolism

- Intake
- Absorption
- Distribution
- Drug–cell interaction
- Breakdown
- Excretion
Biochemical modification

• The actual breakdown process, which usually takes place in the liver, varies with different drugs.
  – Completely oxidized to CO₂
  – Excreted in modified forms
  – Increase solubility resulting in them being more readily excreted
• Conjugation
  – Involves union with the carbohydrate glucoronic acid.
  – Occurs primarily in the liver
  – Elimination of morphine and its derivatives, such as codeine
• Acetylation
  – Modified by the introduction of an acetyl group into the molecule
  – E.g. Isoniazid used in the treatment of tuberculosis and other drugs including sulphonamides.

![Diagram of acetylation of Isoniazid to Acetyl-isoniazid](image.png)

Fig. 11.2
Acetylation of the antituberculosis drug isoniazid.
This figure shows the paths that are taken by the anti-epileptic drug phenytoin and the angiotensin-converting enzyme (ACE) inhibitor imidapril in the human body. Phenytoin is absorbed into the bloodstream at the gut and circulated through the liver to the brain. It crosses the blood–brain barrier where it binds and inhibits its target, neuronal sodium channels. It is pumped back out across the blood–brain barrier into the bloodstream by multidrug resistance protein 1 (MDR1, also known as ABCB1) efflux pumps. Note that MDR1 efflux pumps are also active in the gut, where they promote drug excretion (not shown). At the liver, phenytoin is metabolized by the cytochrome P450 enzymes CYP2C9 and CYP2C19, and it is eliminated through the kidneys. Imidapril is a PRO-DRUG. After its absorption from the gut into the bloodstream it is hydroxylated in the liver to the active metabolite imidaprilat. Imidaprilat binds and inhibits ACE in the plasma. Imidaprilat is also eliminated through the kidneys.
Outline of Action of Endoxifen on Breast Cancer Tissues

Tamoxifen

Liver + other Tissues

N-Desmethyl Tamoxifen

4-Hydroxy Tamoxifen

Endoxifen (END)

END-Estrogen Receptor (END-ER)

Partial Degradation of ER

Altered Gene Regulation

Altered Cell Function

Inhibition of Cell Proliferation

Inhibition of tumor growth, size, and metastasis

Breast Cancer Cell
Kinetics of Drug Metabolism

• Drug response based on dose
  – Amount
  – Rate being metabolized
  – Variability in response
  – Continuous and discontinuous
Fig. 11.3
Various types of response to different drugs consistent with poly and monogenic control of drug metabolism. (A) Continuous var multifactorial control of drug metabolism, (B) discontinuous bimodal variation, (C) discontinuous trimodal variation.
Genetic variations: The Effects of Drugs

- Isoniazid in TB
  - Metabolism distinguished two groups: Rapid and slow inactivators
  - Involvement of N-acetyltransferase
  - Side-effects such as Systemic Lupus Erythematosus (SLE) in some individuals
  - Three genes NAT1, NAT2 and NATP (pseudogene)
  - Modify risk to cancers
• **Succinylcholine sensitivity**
  – Muscular relaxation
  – Plasma enzyme pseudocholinesterase

• **Glucose-6-Phosphate Dehydrogenase variants**
  – Quinine
  – G6PD deficiency
• **Coumarin metabolism**
  – Anticoagulant drugs
  – Discontinuous variation

• **Malignant hyperthermia**
  – Rare complication of anaesthesia
  – Muscle rigidity
  – Increased temperature
• **Phenylbutazone metabolism**  
  – Arthritis

• **Debrisoquinone metabolism**  
  – Hypertension

• **Alcohol metabolism**  
  – Alcohol dehydrogenase
Evolutionary Origin of Variation

### Table 11.1 Ethnic variations in some pharmacogenetic disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Ethnic group</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow acetylation</td>
<td>Europeans</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Orientals</td>
<td>10</td>
</tr>
<tr>
<td>Pseudocholinesterase variants</td>
<td>Europeans</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Eskimos</td>
<td>1–2</td>
</tr>
<tr>
<td>G6PD deficiency</td>
<td>N. Europeans</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>S. Europeans</td>
<td>up to 25</td>
</tr>
<tr>
<td></td>
<td>Afro-Caribbeans</td>
<td>10</td>
</tr>
<tr>
<td>Atypical ADH</td>
<td>Europeans</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Orientals</td>
<td>85</td>
</tr>
</tbody>
</table>
Hereditary disorders with altered drug response

- Porphyra variegata
- Haemoglobinopathies
- Gout
- Crigler-Najjar Syndrome
- Non-insulin dependent diabetes
Pharmacogenomics

• Defined as the development of new drugs from the discovery of new genes and DNA sequence information from the Human Genome project
  – Inherited variation results in functional variation
  – Therapeutic and adverse responses to a drug
Pharmacogenetics and Pharmacogenomics: Loose Definitions

• Pharmacogenetics
  – Individual variation in drug metabolism and distribution
  – Single gene

• Pharmacogenomics
  – Pharmacogenetics + variation among individuals in drug targets and disease mechanism
  – Multiple genes

“The molecular study of genetic factors that determine drug efficacy and toxicity”
Pharmacogenomics - Positive and Negative Overtones

• Negative
  – “Personalized Medicine” vs Public Health

• Positive
  – “Personalized Medicine” vs Impersonal Medicine
Prediction: Pharmacogenomics will precede genomics in the clinic

- Genotypic-phenotypic relationships easier to detect
- Genetic variation is often caused by a single gene or a relatively small number of well-studied genes.
  - Drug metabolizing enzymes
  - Drug targets (e.g. receptors)
- Drug metabolizing enzymes are more variable than other genes.
- Experimentation (drug administration) is possible.
- Drug toxicity has medical-legal implications.
Financial Incentives for Pharmacogenomics

• Pharmaceutical industry
  Pharmacogenomics => market segmentation, but:
  – Clinical trials may be easier to conduct.
  – May rescue drugs with rare genotype-dependent side effects.

• Diagnostic companies
  – Developing new tests

• Third-party payers
  – Potential increased cost-effectiveness:
    • Decreased toxicity
    • Increased efficacy
    • Less drug use
Adverse Drug Reactions (ADRs)

• Estimated ADRs in hospitalized patients (1994):
  – Serious: 6.7% (2.2 million)
  – Fatal: 0.3% (106,000)
  (Lazarou et al., JAMA 1998)

• Of the 27 drugs frequently cited in ADR studies:
  – 59% are metabolized by an enzyme with a variant allele known to cause poor metabolism vs. about 15% of other drugs
  (Phillips et al., JAMA 2001)
Ethical Concerns about Pharmacogenomics and “Individualized Medicine”

• Does not address more pressing concerns
  – Many countries lack access to basic needs and preventable measures
  – Industrialized countries: lifestyle and uneven access to health care

• Will create more “orphan” diseases and genotypes
  – Drugs targeted at most common genotypes
  – Drugs targeted at diseases that are easiest to treat
  – Drugs targeted at genotypes of wealthier groups

• Genetic privacy
  – Open the door to widespread genetic testing
Historical Examples

- G6PD deficiency
- Malignant hyperthermia
- Cytochrome P450 pathway
G6PD Deficiency (1956)

- African-American soldiers taking antimalarial drug, primaquine developed hemolytic anemia and found to have decreased G6PD activity in affected RBCs.
- Glucose-6-phosphate-dehydrogenase (G6PD) catalyzes 1st step in hexose monophosphate shunt leading to RBC’s only source of NADPH (reducing equivalent which protects against oxidative damage).

- 200-400 million people have G6PD variants that increase risk of hemolysis
- >70 point mutations have been reported.
- One common variant has near normal activity under but is associated with severe hemolysis when exposed to oxidant drugs.
Malignant Hyperthermia Susceptibility (MHS, 1962)

- High proportions of individuals in certain families died during general anesthesia.
  - Volatile anesthetics or depolarizing muscle relaxants => uncontrolled skeletal muscle hypermetabolism.
  - Sustained increased tone of voluntary muscles => muscle damage (rhabdomyolysis) and increased temperature
  - Other triggers may be severe exercise in hot conditions, neurolopetic drugs, high fever and infection.

- Has been mapped to multiple loci. RYR1 gene is most common.

- Many dominant mutations in the RYR1 gene are responsible making detection difficult.
Drug-Metabolizing Enzymes

Most DME have clinically relevant polymorphisms
Those with changes in drug effects are separated from pie.

Thiopurine S-Methyltransferase (TPMT): Phenotypes

TPMT: Genotypes

TPMT Genetics and Activity

• Follows Hardy-Weinberg expectations for a pair of autosomal co-dominant alleles for low and high activity
  – Allele frequencies
    • TMPT-low: 0.06
    • TMPT-high: 0.94
  – Phenotypic distribution
    • High: 0.94 ** 2
    • Intermediate: 2 * 0.06 * 0.94
    • Low: 0.06 ** 2
Clinical Significance of TPMT Activity

- Metabolism of 6-mercaptopurine (6MP), 6-thioguanine, azathioprine
- Used for treating leukemia, inflammatory bowel diseases, transplant patients
- Patients without enzymatic activity have high risk of severe bone marrow toxicity
- Homozygous wildtype have a decreased response to chemotherapy.
Measuring TPMT Activity

• Phenotypic or functional testing
  – Measure TPMT activity in RBCs (surrogate for drug-metabolizing tissues)
  – Not reliable in persons who have received RBC transfusions within previous 30-60 days.

• Genotypic testing
  – PCR amplification surrounding the 3 functionally important SNPs
N-Acetyl Transferase (NAT2) Polymorphism

- Slow acetylators are more likely to have side effects with the anti-TB drug, isoniazid (INH).
- Slow acetylators also acetylate other drugs more slowly including caffeine and sulfamethoxazole.
- Some polymorphisms have been demonstrated to result in the absence of catalytic activity in vitro.
- NAT2 polymorphisms may modulate the risk of cancer of lung, bladder, and colon owing to NAT2 acetylation of aromatic amines found in tobacco smoke and cooked foods.
NAT Polymorphisms and TB Treatment

- Isonicotinylhydrazine (INH) for 6-12 months reduces risk of active TB in persons with a positive TB skin test.

- But ~1/10,000 develop irreversible hepatic failure leading to death or hepatic transplantation.

- This complicates decisions about who should receive INH prevention and how they should be monitored.

- NAT polymorphisms are responsible for slow acetylation and appear to increase risk of hepatitis.
Cytochrome P450 Enzymes

• Act on:
  – Endogenous substrates
  – Xenobiotics including plant and fungal products, pollution, chemicals
  – Drugs (metabolize 50-60%)

• Typical reaction:
  \[ \text{NADPH}^+ + \text{H}^+ + \text{O}_2 + \text{RH} \rightarrow \text{NADP}^+ + \text{H}_2\text{O} + \text{R-OH} \]

• Ancient superfamily
• Expressed mainly in liver
• Nomenclature
  – Family: 40% homology (about 15 in humans)
  – Subfamily: 55% homology (about 30 in humans)
  – Individual genes (about 60 in humans)
  – Allelelic variants
## Cytochrome P450 Enzymes

<table>
<thead>
<tr>
<th>Family</th>
<th>No. sub families</th>
<th>No. genes</th>
<th>Substrates and functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1</td>
<td>2</td>
<td>3</td>
<td>Foreign chemicals, arachidonic acid, eicosanoids</td>
</tr>
<tr>
<td>CYP2</td>
<td>13</td>
<td>16</td>
<td>Foreign chemicals, arachidonic acid, eicosanoids</td>
</tr>
<tr>
<td>CYP3</td>
<td>1</td>
<td>4</td>
<td>Foreign chemicals, arachidonic acid, eicosanoids</td>
</tr>
<tr>
<td>CYP4</td>
<td>5</td>
<td>12</td>
<td>Fatty acids, arachidonic acid, eicosanoids</td>
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<tr>
<td>CYP5</td>
<td>1</td>
<td>1</td>
<td>Thromboxane A2 synthase</td>
</tr>
<tr>
<td>CYP7</td>
<td>2</td>
<td>2</td>
<td>Cholesterol, bile acid synthesis</td>
</tr>
<tr>
<td>CYP8</td>
<td>2</td>
<td>2</td>
<td>Prostacyclin synthase, bile acid synthesis</td>
</tr>
<tr>
<td>CYP11</td>
<td>2</td>
<td>3</td>
<td>Steroidogenesis</td>
</tr>
<tr>
<td>CYP17</td>
<td>1</td>
<td>1</td>
<td>Steroid 17α-hydroxylase</td>
</tr>
</tbody>
</table>

CYP19, 20, 21, 24, 26, 27, 39, 46, 51: steroid, vitamin D, vitamin A, cholesterol metabolism

[http://www.imm.ki.se/CYPalleles/]
## Cytochrome P450 Enzymes Most Commonly Involved in Drug Metabolism

<table>
<thead>
<tr>
<th>Gene</th>
<th>Site</th>
<th>Polymorphic</th>
<th>Inducible</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3A4/5</td>
<td>7q22.1</td>
<td>Regulatory regions</td>
<td>√</td>
<td>CYP3A are most abundant CYPs in liver and intestine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inter-individual differences in expression (especially 3A5)</td>
</tr>
<tr>
<td>2D6</td>
<td>22q13.1</td>
<td>√</td>
<td></td>
<td>Greatest number of variants</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wide range in activity.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5-10% are poor metabolizers</td>
</tr>
<tr>
<td>2C9</td>
<td>10q24</td>
<td>√</td>
<td>√</td>
<td>Involved in the metabolism of many drugs associated with adverse reactions (e.g. warfarin, antidepressants, phenytoin, …)</td>
</tr>
<tr>
<td>2C19</td>
<td>10q24</td>
<td>√</td>
<td>√</td>
<td>Involved in the metabolism of many drugs associated with adverse reactions (e.g. warfarin, antidepressants, phenytoin, …)</td>
</tr>
</tbody>
</table>
Pharmacogenetics of CYP2D6


Ratio of urine debrisoquin (probe drug) to 4-OH- debrisoquin
Pharmacogenetics of Nortriptyline

CYP2D6 Evolution

First description of stably amplified active gene in humans

Table 3 Major human polymorphic variant CYP2D6 alleles and their global distribution. For a complete list, see http://www.imm.ki.se/cypalleles/cyp2d6.htm

<table>
<thead>
<tr>
<th>Major variant alleles</th>
<th>Mutation</th>
<th>Consequence</th>
<th>Allele frequencies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caucasians</td>
</tr>
<tr>
<td>CYP2D6*2xN</td>
<td>Gene duplication/multiduplication</td>
<td>Increased enzyme activity</td>
<td>1–5</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>Defective splicing</td>
<td>Inactive enzyme</td>
<td>12–21</td>
</tr>
<tr>
<td>CYP2D6*5</td>
<td>Gene deletion</td>
<td>No enzyme</td>
<td>2–7</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>P34S, S486T</td>
<td>Unstable enzyme</td>
<td>1–2</td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>T107I, R296C, S486T</td>
<td>Altered affinity for substrates</td>
<td>0</td>
</tr>
</tbody>
</table>

For further allele frequencies in different populations, see Bradford.52

Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6: Clinical consequences, evolutionary aspects and functional diversity. Pharmacogenom J, 2005.
CYP2D6 Evolution

- CYP2D6 detoxifies alkaloids potentially increasing the number of plants able to provide useful food.

*Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6: Clinical consequences, evolutionary aspects and functional diversity. Pharmacogenom J, 2005.*
Complexities of Cyp2D6 Genotyping

- Duplications
- Deletion - 3-5% worldwide
- 2 pseudogenes
- More than 80 polymorphisms in coding or promoter regions
DNA Chip Based Technologies

AmpliChip CYP450 Array
- CYP2D6 and CYP2C19
- Multiplex long PCR
- 30 polymorphisms

CYP2D6
- promoter and coding
- 3.5-kb CYP2D6 deletion
- gene duplication-specific reactions

CYP2C19
- exons 4 and 5
- common polymorphisms

Koch WH. Technology Platforms for Pharmacogenomic Diagnostic Assays. Nat Drug Disc. 2004
CYP3A4: Variability in HIV-1 Protease Inhibitor Pharmacokinetics

Acosta EP et al. JAIDS supplement 2002
CYP3A4

• Abundant in liver and intestines and accounts for nearly 50% of CYP450 enzymes.

• Activity can vary markedly among members of a population but its distribution is continuous and unimodal => suggests that multiple genes are involved in its regulation.

• Constitutive variability is ~5-fold but can increase to 400-fold through induction and inhibition

• Examples:
  – Rifampin is a potent inducer; Ritonavir is a potent inhibitor
  – St Johns wort is an inducer, grapefruit juice is an inhibitor
Boosting refers to the practice of administering a PI with a low dose of ritonavir (another PI) which interferes with CYP3A metabolism.
Oral Erythromycin and the Risk of Sudden Death from Cardiac Causes

Drug Transporters

- Energy dependent export of xenobiotics to outside of cells.
- ATP-binding cassette (ABC) family of membrane transporters
  - 48 identified in humans
  - Belong to 7 families (ABCA-ABCG).
- MDR1 (ABCB1) encodes P-glycoprotein, most well-studied.
  - Intestines absorption and “blood-brain barrier”
  - Substrates include bilirubin, anticancer drugs, digoxin, immuno-suppressive agents, glucocorticoids, and HIV protease inhibitors.
  - Over-expression => multidrug resistance to cancer cells in vitro.
Polymorphic Sites in ABCB1

48 variants
13 coding
60 patterns in 247 individuals

Kroetz DL. Sequence diversity and haplotype structure in ABCB1. Pharmacogenetics 2003
Possible Clinical Significance of ABCB1 Variation

• Cancer chemotherapy (strong evidence)
  – Selection for cells with increased expression
• Drug-resistant epilepsy (weak evidence)
• Digoxin toxicity (weak evidence)
• HIV protease inhibitors (weak evidence)
• Preliminary associations with other diseases
  – Inflammatory bowel disease
ABCB1 and Drug-Resistant Epilepsy

Table 1. Summary of Genotype and Phenotype Data.*

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Total No.</th>
<th>ABCB1 3435 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no. (%)</td>
</tr>
<tr>
<td>Drug-resistant epilepsy</td>
<td>200</td>
<td>55 (27.5)</td>
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<tr>
<td>Drug-responsive epilepsy</td>
<td>115</td>
<td>18 (15.7)</td>
</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>37 (18.5)</td>
</tr>
</tbody>
</table>

* For all 315 patients with epilepsy, patients with drug-resistant epilepsy were more likely than those with drug-responsive epilepsy to have the CC genotype than the TT genotype ($\chi^2=7.65$, $P=0.006$). The results were similar when the analysis was restricted to the 297 white patients ($\chi^2=6.66$; $P=0.01$; odds ratio, 2.58; 95 percent confidence interval, 1.25 to 5.36).

- Not consistently replicated
- Position 3435 is noncoding but is associated with differences in activity:
  CC > CT > TT

Siddiqui et al. Multidrug resistant epilepsy and ABCB1 variants. NEJM 2003
Haplotypes may correlate better with function than individual mutations.

*Kroetz DL. Sequence diversity and haplotype structure in ABCB1. Pharmacogenetics 2003*
MutDB: ABCB1
<table>
<thead>
<tr>
<th>Source ID</th>
<th>AA Position</th>
<th>WT -&gt; MT</th>
<th>Sequence</th>
<th>Phenotype</th>
<th>Structure</th>
<th>PubMed</th>
<th>SIFT Score</th>
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<tbody>
<tr>
<td>SWISS:VAR_014704</td>
<td>21</td>
<td>N D</td>
<td>Swiss-Prot</td>
<td>in dbSNP:1805053</td>
<td>None</td>
<td>10790226 10716719 11240981</td>
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<td>DBSNP:rs9282564</td>
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<td>NCBI</td>
<td>In dbsnp.</td>
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<tr>
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<td>Swiss-Prot</td>
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<tr>
<td>SWISS:VAR_018351</td>
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<td>Swiss-Prot</td>
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<td>None</td>
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<tr>
<td>SWISS:VAR_015002</td>
<td>185</td>
<td>G V</td>
<td>Swiss-Prot</td>
<td>in a colchicine-selected multidrug-resistant cell line confers increased resistance to colchicine</td>
<td>None</td>
<td>2876781 9038218 2897240</td>
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<tr>
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<td>G V</td>
<td>NCBI</td>
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<tr>
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<td>S N</td>
<td>Swiss-Prot</td>
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<td>None</td>
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<tr>
<td>DBSNP:rs2229109</td>
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<td>S N</td>
<td>NCBI</td>
<td>In dbsnp.</td>
<td>None</td>
<td></td>
<td>0.44</td>
</tr>
</tbody>
</table>
PharmGKB (Pharmacogenomics Knowledge Base)

- Curated database of genotype and phenotype information
- Shared resource for researchers
- Developed at Stanford - Russ Altman and Teri Klein
Drug Target Variation: Pharmacodynamic Consequences

- HMG-CR (3-OH-3-methylglutaryl coenzyme A reductase)
  - Statin responsiveness at lowering LDL cholesterol

- ADRB1 (Adrenergic receptor-β-1)
  - Response to β-blockers for treating hypertension and heart disease

- Angiotensin converting enzyme (ACE)
  - Response to ACE-inhibitors for treating hypertension

- Dopamine D2 receptor gene
  - Response to antipsychotic medications

* Unintended target variation and toxicity: example LQTS
Statins and HMGCR Variants

Kajinami K. Pharmacogenomics of statin responsiveness. Am J Cardiol 2005
β2 adrenergic receptor variants (ADRB2)

- Guanine nucleotide binding protein (G-protein)
- Expressed in airway smooth muscle and target for the β-agonists used in treating bronchospasm.
- Expressed in blood vessel walls and target for antihypertensives
- 13 SNPs have been found within the gene and its upstream regulatory regions.
- The 13 SNPs have been organized into 12 haplotypes.
β2 Adrenergic Receptor Variants

Liggett SB. β2-Adrenergic Receptor Pharmacogenetics.
Am J Resp Crit Care Med 2000

63
Unintended Targets: Cardiac Ion Channel Blockade

- Prolongation of cardiac action potential predisposes to fatal arrhythmias.
- Prolongation is manifested by increased QT interval on an EKG.
- Results from block of the delayed rectifier $K^+$ channels
- Between 1990 – 2001, 11 non-cardiac drugs were withdrawn or given revised indications because they predisposed to fatal cardiac arrhythmias in some persons.
- More than 50 approved drugs may also cause arrhythmias in vulnerable persons.
Unintended Targets: Cardiac Ion Channel Blockade

A. Cardiac action potential: depolarization followed by repolarization: Na, K, Ca channels.

B. Normal QT interval

C. Prolonged QT interval

D. Torsade de Pointe

E. Ventricular fibrillation.

Anantharam et al. Pharmacogenetic Considerations in Diseases of Cardiac Ion Channels. 2003
Cardiac Arrhythmias: Genetic +/- Drugs

- Cardiac channel mutations
- Certain drugs
- Cardiac disease
  - Female sex
  - Low K+

Prolonged QT interval
Ventricular arrhythmias
Genetics of the Long-QT Syndrome

• LQT1-LQT12

• KvLQ1, HERG, SCN5A, ankyrin B, KCNE1, KCNE2, KCNJ2

• hERG (also KCNH2) – encodes rapidly acting K channel (IKr) is most commonly responsible for drug-related LQTS.

• Many different mutations reported

• Homozygotes often have high rates of sudden death
Genetics of Drug-Induced Arrhythmias

- Some mutations result in high risk of arrhythmia and sudden death
- Other mutations increase risk under certain conditions including drugs.
  - Mutations in a LQT gene were reported in 10-15% of 92 people with drug-associated LQTS (significantly greater than controls)
  - One polymorphism in hERG present in 2% of population leads to drug-induced LQTS during sulfamethoxaole treatment
Structural Basis for Drug-Induced LQTS

Mitcheson JS. Structural Basis for Drug-Induced LQTS. PNAS 2000
Structural Basis for Drug-Induced LQTS

Experimental approaches to screening compounds for hERG block
Computational approaches using homology models

Aranov. Predictive in silico modeling of hERG channel blockers. Drug Disc Today 2005
# PharmGKB: LQTS

## Drug-Induced Long QT Intervals

<table>
<thead>
<tr>
<th>Summary</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes Studied:</strong></td>
<td>ADRB1, ADRB2, KCNE1, KCNE2, KCNH2, KCNH1, SCN5A</td>
</tr>
<tr>
<td><strong>Drugs Studied:</strong></td>
<td>almokalant, amiodarone, amitriptyline, bretylium, bupivacaine, cisapride, disopyramide, doxifluridine, encaidine, fluconazole, haloperidol, hydroquinidine, isoflurane, itraconazole, ketoconazole, lithium, loratadine, metoclopramide, nortriptyline, procainamide, quinidine, sotalol, sulfamethoxazole, thioridazine hydrochloride, trimethoprim</td>
</tr>
<tr>
<td><strong>Diseases Studied:</strong></td>
<td>Arrhythmias, Long QT Syndrome, Torsades de Pointes</td>
</tr>
<tr>
<td><strong>Categories of Pharmacogenetic Knowledge:</strong></td>
<td>PD Pharmacodynamics and Drug Response</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Details</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Investigator:</strong></td>
<td>Dan Roden, MD</td>
</tr>
<tr>
<td><strong>Group:</strong></td>
<td>PAT</td>
</tr>
</tbody>
</table>
| **Description:** | Background: DNA variants appearing to predispose to drug-associated long QT syndrome (aLQTS) have been reported in congenital LQTS disease genes. However, the incidence of these genetic risk factors has not been systematically evaluated in a large set of patients with aLQTS. We have previously identified functionally-important DNA variants in genes encoding K+ channel ancillary subunits in 11% of an aLQTS cohort. Methods and results: The coding regions of the genes encoding the pore-forming channel proteins KvLQT1, HERG, and SCN5A were screened in (1) the same aLQTS cohort and (2) 126 controls. Four variants were identified in the KvLQT1 gene, two in the HERG gene, and none in SCN5A. The four identified variants were present in controls, two of which were found in single individuals (0.8%), and the other two in 2% and 1% of controls.

[Link to PharmGKB Accession ID: PA133866952](#) | Download Data |
<table>
<thead>
<tr>
<th><strong>Column Descriptions</strong></th>
<th><strong>Description</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject ID</strong></td>
<td>PharmGKB Accession IDs for subjects in the PharmGKB.</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Male, female, or unknown.</td>
</tr>
<tr>
<td><strong>Race/Ethnicity</strong></td>
<td>Self-reported information.</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Sample ages have been binned into 10-year ranges for anonymity.</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td>TDP: Torsades de pointes, LQT: QT interval prolongation to &gt;600 msec with drug, resolving to normal with drug withdrawal. No TDP control: Subject who tolerated a QT-prolonging antiarrhythmic without TDP or LQT for &gt; 1 month.</td>
</tr>
<tr>
<td><strong>Offending drug</strong></td>
<td>The drug that, in the opinion of the treating physician, was most likely to be implicated as the cause of TDP (Torsades de pointes) or LQT.</td>
</tr>
<tr>
<td><strong>Time between dosing and onset of diagnosis</strong></td>
<td>The time between initiation of offending drug and TDP (Torsades de pointes) or LQT.</td>
</tr>
<tr>
<td><strong>MAX QT/QTc (msec)</strong></td>
<td>Longest QT and rate-corrected QT (QTc) intervals recorded during drug, in msec.</td>
</tr>
<tr>
<td><strong>MIN QT/QTc (msec)</strong></td>
<td>Shortest QT and rate-corrected QT (QTc) intervals recorded prior to or following drug, in msec.</td>
</tr>
<tr>
<td><strong>Original rhythm</strong></td>
<td>The cardiac rhythm at the time of drug administration or for which the drug (if an antiarrhythmic) was administered. Ant: atrial fibrillation; AFIB: atrial flutter; AT: Atrial Tachycardia; VT: ventricular tachycardia; VF: ventricular fibrillation; WPW: Wolff-Parkinson-White syndrome; SVT: supraventricular tachycardia; NSR: normal sinus rhythm; PVCs: premature ventricular contractions; PACs: premature atrial contractions</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td>Symptoms at the time of the TDP/LQT episode.</td>
</tr>
<tr>
<td><strong>Serum potassium (mg/L)</strong></td>
<td>Serum potassium level, in mg/L, at the time of the TDP/LQT episode.</td>
</tr>
<tr>
<td><strong>Serum magnesium (mg/L)</strong></td>
<td>Serum magnesium, in mg/L, at the time of the TDP/LQT episode.</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td>History of hypertension, as diagnosed by history of treatment.</td>
</tr>
<tr>
<td><strong>Left ventricular hypertrophy</strong></td>
<td>Left ventricular hypertrophy, as diagnosed by echo or EKG.</td>
</tr>
<tr>
<td><strong>Congestive heart failure</strong></td>
<td>Congestive heart failure, as diagnosed by doctor.</td>
</tr>
</tbody>
</table>
LQTS: GeneTests

LQT 1

<table>
<thead>
<tr>
<th>Laboratories offering clinical testing:</th>
<th>Sequencing of entire coding region</th>
<th>Sequencing of selected exons</th>
<th>Mutation scanning</th>
<th>Targeted mutation analysis</th>
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</thead>
<tbody>
<tr>
<td>Academic Medical Center</td>
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<tr>
<td>DNA Diagnostics Laboratory</td>
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<tr>
<td>Amsterdam, Netherlands</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Marcel M.A.M. Mannens, PhD</td>
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<tr>
<td>Boston University School of Medicine</td>
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<tr>
<td>Center for Human Genetics</td>
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<tr>
<td>Boston, MA</td>
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<tr>
<td>Aubrey Milunsky, MD, DSc</td>
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<tr>
<td>Fondazione S. Maugeri, IRCCS</td>
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<tr>
<td>Molecular Cardiology Laboratories</td>
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<tr>
<td>Pavia, Italy</td>
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<tr>
<td>Silvia G Forni, MD, PhD</td>
<td></td>
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<tr>
<td>Genaissance Pharmaceuticals</td>
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<tr>
<td>The Familion (tm)Test</td>
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<tr>
<td>New Haven, CT</td>
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<td></td>
</tr>
<tr>
<td>Patricia D Murphy, PhD (Consultant); Jeffrey M Otto, PhD</td>
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<tr>
<td>Rikshospitalet</td>
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<tr>
<td>Medical Genetics Laboratory</td>
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<td>Oslo, Norway</td>
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<tr>
<td>Trond P Leren, MD, PhD; Knut Erik Berge, MD, PhD</td>
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<tr>
<td>University of Cologne</td>
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<tr>
<td>Institute of Human Genetics</td>
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<td>Cologne, Germany</td>
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<tr>
<td>Brunhilde Wirth, Prof. Dr.</td>
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</tbody>
</table>
LQTS: Genetic Testing

About FAMILION™

The FAMILION™ Test is a new genetic test that is designed to identify mutations in ion channel genes in patients and their family members with inherited cardiac channelopathies such as Long-Q-T Syndrome (LQTS) and Brugada Syndrome. There is also evidence that mutations in these same genes may cause a percentage of the cases of Familial Atrial Fibrillation, Short Syntroventricular Fibrillation, Progressive Cardiac Conduction Disease, Congenital Sick Sinus Syndrome and Sudden Infant Death Syndrome (SIDS).

Description of the Test

The FAMILION™ Test provides analysis of five major cardiac ion channel genes. This analysis includes sequence determination and variant detection i.e., mutations and polymorphism reading frames (ORF) and intronic sequences containing splice junction sites. Sequencing is performed in both forward and reverse directions using dye-terminator chemistries.

The following genes are analyzed:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ion Channel Encoded</th>
<th>Base-Pairs Analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>K</td>
<td>2,331</td>
</tr>
<tr>
<td>KCNH2</td>
<td>K</td>
<td>3,730</td>
</tr>
<tr>
<td>SCN5A</td>
<td>Na</td>
<td>6,571</td>
</tr>
<tr>
<td>KCNE2</td>
<td>K</td>
<td>390</td>
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<tr>
<td>KCNE2</td>
<td>K</td>
<td>372</td>
</tr>
</tbody>
</table>

The test can be ordered in three configurations:

- Comprehensive Cardiac Ion Channel Analysis provides analysis for variants in all five genes and is appropriate when there is a high index of suspicion of disease such as syncopal, prolonged QT interval, family history of sudden cardiac death and/or unexplained VTAF or TdP
- Shortarm Channel Analysis provides analysis for variants only for the SCN5A gene and is appropriate in cases of suspected Brugada Syndrome.
- Family Specific Analysis provides analysis of one or more mutations found in index case using either one of the above test configurations or confirmed results from another is appropriate for testing blood relatives.
Abacavir Hypersensitivity

• Abacavir is a highly potent HIV nucleoside RT inhibitor

• 5%-10% of patients receiving this drug develop a progressive hypersensitivity reaction that can be fatal if the drug is continued.

• In an Australian cohort, 14/18 (78%) patients with abacavir hypersensitivity had the allele HLA-B*5701 compared with 4/167 (2%) tolerant patients (p<0.001).

• In a case-control study in the U.S. (genetically more diverse population), 39/84 (46%) patients with abacavir hypersensitivity had the allele HLA-B57 compared with 4/113 (4%) matched controls who were tolerant (p<0.001).
Potential Impact of Pharmacogenomic Information to Reduce Drug Toxicity

• Medical need
  – High incidence of ADRs and wide drug use
  – High prevalence of variant alleles
  – Current methods for monitoring are inefficient

• Clinical utility
  – Sufficient evidence exists linking a genotype to a clinical outcome.

• Ease of use
  – Assay – rapid, inexpensive
  – Clinicians are able to interpret the results and use the information

Phillips KA. Pharmacogenomics and Reducing ADRs. JAMA 2001
Barriers to Implementation

• Correlation is not 100%
  – Polygenic influences
  – Environmental influences
    (Response to a drug usually shows normal distribution within a population)
• Require biological plausibility
  – Correlation may be with a haplotype but not with a specific mutation.
• Require clinical validation
  – False-positive, false-negatives, positive predictive value
  – Retrospective study
  – Prospective study
• May not be sufficient financial incentive to develop a test
  – Patents can create financial incentive but can also cause stagnation
## Table 11.2 Ecogenetics: genetic variation in susceptibility to environmental agents

<table>
<thead>
<tr>
<th>Environment agent</th>
<th>Genetic susceptibility</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV light</td>
<td>Fair complexion</td>
<td>Skin cancer</td>
</tr>
<tr>
<td>Drugs (see text)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fats</td>
<td>Hypercholesterolaemia</td>
<td>Atherosclerosis</td>
</tr>
<tr>
<td>Fava beans</td>
<td>G6PD deficiency</td>
<td>Favism</td>
</tr>
<tr>
<td>Gluten</td>
<td>Gluten sensitivity</td>
<td>Coeliac disease</td>
</tr>
<tr>
<td>Salt</td>
<td>Na–K pump defective</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Milk</td>
<td>Lactase deficiency</td>
<td>Lactose intolerant</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Atypical ADH</td>
<td>Alcoholism</td>
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<tr>
<td>Oxalates</td>
<td>Hyperoxaluria</td>
<td>Renal stones</td>
</tr>
<tr>
<td>Fortified flour</td>
<td>Haemochromatosis</td>
<td>Iron overload</td>
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<tr>
<td>Inhalants</td>
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<tr>
<td>Dust</td>
<td>$\alpha_1$-antitrypsin deficiency</td>
<td>Emphysema</td>
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<tr>
<td>Allergens</td>
<td>Atopy</td>
<td>Asthma</td>
</tr>
<tr>
<td>Infections</td>
<td>Defective immunity</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spondylitis?</td>
</tr>
</tbody>
</table>
Clinical applications of pharmacogenetics

- Prognosis
- Choosing the Right Drug
- Optimising the Dose
- Predicting Adverse Drug Reactions
- Monitoring Progress
- Profiling Infection + Cancer
- Drug Development
- Gene + Stem Cell Therapy
- Diagnosis