Clinical Pharmacogenetics

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Georgetown University Medical Center
Pharmacogenetics

The study of heredity as it relates to the absorption, distribution, elimination and action of medicines:

A tool to limit variability and individualize therapy
Pharmacogenomics

The study of the human genome, and its structure as relates to genes involved in drug absorption, action and elimination:

A tool for drug discovery
Mechanisms of Inherited Genetic Variability

(All are in germ line DNA or mitochondrial DNA)

Single nucleotide polymorphisms (SNPs)
Deletions
Duplications
Early History of Pharmacogenetics

- 1932: First Inherited Difference in an Inherited Response to a Chemical: Inability to Taste Phenylthiourea.
- Motulsky: 1957 “inheritance might explain many individual differences in the efficacy of drugs and in the occurrence of adverse drug reactions”
- 1959: Friedrich Vogel :“Pharmacogenetics: the Role of Genetics in Drug Response”
- 1959: Genetic influence on isoniazid blood concentrations
- 1964: Genetic variation in ethanol metabolism
- 1977: CYP2D6 polymorphism
Methods in Pharmacogenetics

• Family and twin studies to confirm genetic characteristics (dominant, recessive, Mendelian, maternal etc.)

• Population distribution analysis with Normit plots using a valid probe to detect phenotypic polymorphism (> 1% of population)

• Identification of gene and mutations

• Development of a genetic test for mutations in DNA

• Correlation between genotype and phenotype
Phenylthiourea Nontaster Trait

- 800 families including 2043 children
- Serial Dilution Testing
- Mendelian Inheritance
- US prevalence of the nontaster trait = 30%.
Genetically Polymorphic Trimethylaminuria

From: Thithapandha, A.
A pharmacogenetic study of trimethylaminuria in orientals
Pharmacogenetics (1997) 7, 497–501
N-Acetylation Polymorphism
NAT-2

• Late 1940’s: Peripheral Neuropathy noted in patients treated for tuberculosis.

Incidence of the Slow Acetylator NAT-2 phenotype

- 50% among Caucasians
- 50% among Africans
- 90% among Japanese
- 20% among Egyptians
Properties of an ideal pharmacogenetic probe for phenotype

- Specific for the trait in question
- Sensitive
- Easy to assay
- Clinically benign
NAT-2 substrates
(All have been used as probes)

- Caffeine
- Dapsone
- Hydralazine
- Isoniazid
- Procainamide
Clinical relevance of the NAT-2 polymorphism

• Higher isoniazid levels, greater neuropathy in slow acetylators
• Faster ANA appearance with procainamide in slow acetylators
• Lack of N-Acetyl Procainamide in slow acetylators, and therefore lack of K-channel blockade
Examples of Genetic Effects on Human Drug Absorption, Action and Elimination

- **Absorption:**
  - Alcohol Dehydrogenase
  - Cytochrome P450 3A5
  - Cytochrome P450 2C19

- **Action**
  - Angiotensin II receptor
  - $\alpha_2$ receptor
  - Dopamine D4 receptor
  - Endothelial NO synthase
  - 5HT$_4$ receptor
  - Glucose 6 phosphate dehydrogenase
Examples of Genetic Effects on Human Drug Absorption, Action and Elimination (continued)

- Cytochrome P450 2A6
- Cytochrome P450 2C9
- Cytochrome P450 2C19
- Cytochrome P450 2D6
- Regulation of cytochrome P450 3A4
- Dihydropyridine Dehydrogenase (DPD)
- UDP-Glucuronyl Transferase 1A1 (UGT 1A1)
- Glutathione - S - Transferase (GST)
- Thiopurine methyl transferase (TPMT)
- Flavin Mono-Oxygenase 3 (FMO-3)
- Multidrug Resistance Transporter (MRP)
Examples of genetic effects on Drug Absorption in Humans

(Phenotype ahead of the genotype)
Aldehyde Dehydrogenase
Genetics

• 10 human ALDH genes
• 13 different alleles
• autosomal dominant trait because of lack of catalytic activity if one subunit of the tetramer is inactive
• ALDH2 deficiency results in build up of toxic acetaldehyde
• Absent in up to 45% of Chinese, not at all in Caucasians or Africans
Population Distribution of CYP2C19 phenotype

Balian et al:
Clin Pharmacol Ther
1995;57:662-669
Genotyping of CYP2C19

Distribution of CYP2C19 genotypes

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>wt Allele</th>
<th>m1 Allele</th>
<th>m2 Allele</th>
<th>Calculated&lt;sup&gt;§&lt;/sup&gt; (observed) PM genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>European-Americans</td>
<td>105</td>
<td>0.87</td>
<td>0.13</td>
<td>0</td>
<td>2% (2%)</td>
</tr>
<tr>
<td>Saudi Arabians</td>
<td>97</td>
<td>0.85</td>
<td>0.15</td>
<td>0</td>
<td>2% (2%)</td>
</tr>
<tr>
<td>Japanese</td>
<td>53</td>
<td>0.67&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.23&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.104</td>
<td>11% (15%)</td>
</tr>
<tr>
<td>Filipinos</td>
<td>52</td>
<td>0.54&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;def&lt;/sup&gt;</td>
<td>0.077</td>
<td>21% (23%)</td>
</tr>
<tr>
<td>Chinese-Taiwanese</td>
<td>118</td>
<td>0.63&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.055</td>
<td>14% (15%)</td>
</tr>
<tr>
<td>African-Americans (NC)</td>
<td>108</td>
<td>0.75&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.25&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0</td>
<td>6% (7%)</td>
</tr>
</tbody>
</table>

Examples of CYP2C19 substrates

- Amitryptyline, Imipramine
- Citalopram
- Diazepam
- Nelfinavir
- Omeprazole, Lansoprazole
- Mephenytoin
- Phenytoin
- Proguanil
Clinical relevance of the CYP2C19 polymorphism

- Increased toxicity with citalopram in PMs
- Prolonged sedation by diazepam in PMs
- Increased sedation by mephenytoin in PMs
- Altered metabolic saturation threshold for phenytoin in PMs
- Decreased activity of proguanil in PMs
Examples of genetic effects on drug action in humans

(Genotype ahead of the phenotype)
Glucose 6 Phosphate Dehydrogenase:


- Deficient in 1 in 10 Africans
- X-linked recessive
- Primaquine induces hemolysis, as does:
  - Quinine, quinidine
  - Sulfonamides
  - Dapsone, methylene blue
Examples of Human Receptors shown to be genetically polymorphic with possible alterations in clinical phenotype:

- Angiotensin II receptor
- Angiotensin converting enzyme
- $\beta_2$ receptor
- Dopamine D$_4$ receptor
- Endothelial NO synthase
- 5HT$_4$ receptor
Not all mutations in DNA have a phenotypic consequence (most do not)
Not all genetic polymorphisms have a clinical consequence
Examples of genetic effects on drug elimination in humans
Population Distribution of CYP2D6 phenotype using Dextromethorphan

From Woosley et al:
CYP2D6 Substrate Probes used to Determine Phenotype

• Dextromethorphan
• Sparteine
• Debrisoquin
• S- Metoprolol
• Bufarol (in vitro)
Cytochrome P450 2D6

- Absent in 7% of Caucasians
- Hyperactive in up to 30% of East Africans
- Catalyzes primary metabolism of:
  - codeine
  - β-blockers
  - tricyclic antidepressants
- Inhibited by:
  - fluoxetine
  - haloperidol
  - paroxetine
  - quinidine
Effect of CYP2D6 on timolol pharmacokinetics and pharmacodynamics

From: Wood AJJ et al. JAMA 1995;274:1611-1613
Dihydropyridine Dehydrogenase

• Absent in ~ 3% of Caucasians
• Responsible for metabolism of 5-fluorouracil
• 80-90% of 5-FU is metabolized, 10 - 20% is renal
• Deficient patients treated with conventional doses of 5-FU experience diarrhea, stomatitis, mucositis, myelosuppression and neurotoxicity.
Dihydropyridine Dehydrogenase

Marked Increase in tissue 5-FU levels → Toxicity to bone marrow and intestines

Tegafur (FT) → Liver

5-FU → NADPH → (HBr) → α-Fluoro-β-alanine

H2-5-FU → NADP+ → H2-BVU

Gut flora

Sorivudine (SRV) → NADPH

Inactivated DPD

Okuda et al. Eighteen deaths due to an interaction with DPD. JPET 1998;287:791-809
UGT 1A1

- Responsible for Gilbert’s
- absent in ~15% of Caucasians
- < 5% Asians
- > 50% of Africans
- > 50% of Hispanics
Sulfonamide Hypersensitivity Reactions

- 50% Prevalence HIV patients
- 1% Prevalence in healthy normals
Glutathione-S-Transferase

- 55% of Caucasians have no GSTM1
- 15% of Caucasians have no GSTT1
Glutathione S-transferase (GSTM1) null genotype and sulphonamide intolerance in acquired immunodeficiency syndrome

Claudine Deloménie¹, Pascale Mathelier-Fusade², Sandrine Longuemaux¹, Willy Rozenbaum³, Francisque Leynadier², Rajagopal Krishnamoorthy¹ and Jean-Marie Dupret¹*

Pharmacogenetics (1997) 7, 519–520

<table>
<thead>
<tr>
<th></th>
<th>Number of subjects</th>
<th>GSTM1*0/*0 Number (%)</th>
<th>GSTT1*0/*0 Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control individuals</td>
<td>205</td>
<td>115 (56)</td>
<td>39 (19)</td>
</tr>
<tr>
<td>AIDS patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphamamide intolerants⁶</td>
<td>36</td>
<td>26 (72)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Sulphamamide tolerant</td>
<td>44</td>
<td>21 (48)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Total of AIDS patients</td>
<td>80</td>
<td>47 (59)</td>
<td>11 (14)</td>
</tr>
</tbody>
</table>

⁶ Odds ratio and 95% confidence interval for sulphamamide hypersensitivity reactions among AIDS patients with the GSTM1 null genotype is 2.8 [95% CI, 1.1–7.3]. No association was observed with the GSTT1 null genotype (OR = 1.0; [95% CI, 0.3–3.7]).
Thiopurinemethyltransferase (TPMT)
Current Methods for genetic testing

- By phenotype: metabolic probe drug or Western blot
- By PCR with mutation-specific endonuclease
- By PCR and allele-specific hybridization
- By oligonucleotide chip hybridization
- By laser lithography - guided oligonucleotide chip hybridization.
Reality Check

• No genetic test, based on DNA testing is currently approved by the United States Food and Drug Administration, for the treatment of patients.
• The CYP2D6 polymorphism was first described in 1977, genetic tests have been available since 1986
Clinical Pharmacogenetics
Summary Pearls

- A good phenotyping probe is critical
- Genetic tests need validation just as any other tests
- A potent inhibitor can mimic a genetic polymorphism
- Not all genetic polymorphisms have a phenotypic correlate, or clinical effect
- The clinical relevance of genetic polymorphisms is greatest with drugs of narrow therapeutic range, but not confined to them