Personalized Medicine 101: Pharmacogenetics as a method for Improving Patient Outcomes

A. Pharmacogenetics vs Pharmacogenomics

1. **Pharmacogenetics** = inherited variations in individual drug effects; single gene interactions with drugs
   
   Includes:
   
   - Disposition
   - Safety
   - Tolerability
   - Efficacy

2. **Pharmacogenomics** = large population studies of all the many different genes that determine drug effects.

It is important to note that the terms “pharmacogenetics” and “pharmacogenomics” are sometimes used interchangeably, simply due to the fact that the term “genomics” is more contemporary “buzz word” than “genetics”.

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**Rare vs. common variants**

<table>
<thead>
<tr>
<th>Smaller effect; multiple variants</th>
<th>PHARMACOGENOMICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large single variant effect</td>
<td>PHARMACOGENETICS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single gene</th>
<th>Small number of genes</th>
<th>Complex biologic pathway</th>
<th>Whole genome</th>
</tr>
</thead>
</table>

*Pharmacogenetics Research Network Investigators, Ann Int Med, 2006*
3. The promise of pharmacogenetics/genomics is PERSONALIZED MEDICINE

Drug therapy tailored to a patient’s unique genetic makeup, especially with regards to:

a. Choice of the drug
b. Choice of the dosing regimen

B. Human Genome Overview

1. Sequence completed in 2001
2. 3 billion bases of DNA
3. Divided into 23 chromosomes
4. In females, all diploid (two copies)
5. In males, X and Y are haploid (one copy each)
6. ~30,000 Genes

C. Chromosomes

1. Every human cell with the exception of gametes contains 23 chromosomes
2. Carry all the genetic coding for all the proteins in every cell
3. Consist of DNA tightly wound around special protein structures called histones

It is important to note that red blood cells (RBCs) and platelets do NOT have chromosomal DNA, since these “cells” are derived from progenitor cells in the bone marrow. Interestingly, it has been estimated that RBCs are made at a rate of 2 million per second in humans. Even though RBCs and platelets lack a nucleus and chromosomal DNA, a “DNA sample” can still be derived from a blood sample due to the presence of other nucleated white blood cells in the blood (i.e. neutrophils, eosinophils, lymphocytes, monocytes, etc.).
D. Structure of DNA

DNA is comprised of a string of 4 nucleotide bases (A, G, T and C) that are linked together in a double helix. Bases on opposite strands are always matched A-T and C-G.

E. Structure of Genes

A segment of DNA containing all of the information needed to encode for one protein is called a gene.
F. Transcription and Translation

G. Genomic variations

1. Single Nucleotide Polymorphisms (SNPs)
2. Variable Number Tandem Repeats (VNTR)
3. Insertions/Deletions
4. SNPs Upclose
   a. Polymorphisms:
      i. common variation in DNA, often defined as greater than 1% in general population
      ii. occur on average every 1331 bp, although frequency can be much greater in a given gene
      iii. estimated to be ~11 million polymorphisms in the human genome
   b. influence expression

\[
\begin{align*}
5' &= \text{CATGTACCTGGGCCG-3'} \\
3' &= \text{GTACATGGACCCGGC-5'} \\
5' &= \text{CATGTACC GGGCCG-3'} \\
3' &= \text{GTACATGGGCCC CGGC-5'}
\end{align*}
\]
c. Coding Polymorphisms are further classified as:
   
   i. Non-synonymous (missense) – results in translation of a different amino acid
   
   ii. Synonymous (sense) – results in the translation of the same amino acid
   
   iii. Nonsense – results in the insertion of a stop codon

   d. Mainly used to characterize genetic differences between individuals

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H. SNP Application to Drug Therapy…. “Personalized Medicine”

Main goals of Personalized Medicine are to

- Prevent adverse drug reactions (ADRs)
- Improve drug efficacy

1. Adverse Drug Reactions (ADRs)
   
   i. Over 770,000 people are injured or die each year in hospitals from ADRs
   
   ii. Cost the U.S. health care system between $1.5 and $5.6 billion per year

      Source: Agency for Health Care Quality (AHRQ)

2. **Pharmacokinetic Considerations**: Drug Metabolism

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[Diagram of Drug Metabolism]

- Drug → Phase I (Oxidation, Reduction, And/or hydrolysis)
- Phase I → Oxidation, Reduction And/or hydrolysis
- Phase II → Conjugation Products
Table 1*: Representative Drug Metabolizing Enzymes SNPs

<table>
<thead>
<tr>
<th>CYP Family</th>
<th>Allele</th>
<th>Nucleotide Change</th>
<th>Enzyme Activity Change</th>
<th>Associated Drug Concentration Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>CYP1A2*1C</td>
<td>-3860 G&gt;C</td>
<td>Decreases</td>
<td>Increases</td>
</tr>
<tr>
<td>2C9</td>
<td>CYP2C9*3A</td>
<td>1075 A&gt;C</td>
<td>Decreases</td>
<td>Increases</td>
</tr>
<tr>
<td>3A4</td>
<td>CYP3A4*18A</td>
<td>878 T&gt;C</td>
<td>Increases</td>
<td>Decreases</td>
</tr>
</tbody>
</table>

*Only three representative CYP families, a single SNP example. Complete list can be found at http://cypalleles.ki.se/.

The CYP Families

Proportion of drugs metabolized by the major P450 enzymes

- CYP 3A
  - most abundantly expressed in human liver
  - CYP 3A4 most abundant form
  - CYP 3A7 most abundant in fetal liver &
    and thought to be involved in steroid metabolism
c. CYP 2D6 Polymorphisms

- Over 70 Single Nucleotide Polymorphisms (SNPs)
- Over 65 drugs metabolized by CYP2D6 inc. tricyclic antidepressants, neuroleptics, serotonin reuptake inhibitors, antiarrythmics, b-adrenergic agonists, opiates

- 4 Phenotypic Subpopulations
  PM – poor metabolizers, adverse drug effects
  IM – Intermediate metabolizers
  EM – Extensive metabolizers
  UM – Ultra-rapid metabolizers, usual drug doses ineffective; exaggerated response if metabolite is active (exaggerated response to codeine, formation of morphine increased)

- Frequency varies with racial background
- CYP2D6*4 is the most common variant allele in Caucasians with a population frequency of ~20%.

TCA Example
d. CYP2C9

- Encodes the p450 enzyme that metabolizes the anticoagulant warfarin

i. Warfarin

a. antagonist at vitamin K epoxide reductase

i. required to maintain levels of reduced vitamin K, which allows carboxylation of glutamate receptors on coagulation factors

b. there is wide inter-individual variability in therapeutic efficacy

i. 0.5 mg/day – 50 mg/day

e. CYP2C19

i. Only two SNPs have been identified thus far

CYP2C19*2 and CYP2C19*3

ii. Each results in a non-functional protein product

individuals homozygous for either CYP2C19*2 or CYP2C19*3 have no functional enzyme

results in increased drug levels and improved therapeutic outcome

iii. Clopidogrel Bioactivation

![Clopidogrel Structure]
### Table 2. Genetic Basis for Adverse Drug Responses in Drug Metabolism.

<table>
<thead>
<tr>
<th>Adverse Drug Response Type</th>
<th>Effect of SNP on Metabolic Enzyme</th>
<th>Effect on Peak Drug Plasma Concentration</th>
<th>Adverse Drug Response</th>
<th>Remediation of Adverse Drug Response Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decreased Clearance</strong></td>
<td>(1) SNP in promoter region of gene causes decreased enzyme expression. (2) SNP in coding region of gene causes altered enzyme activity.</td>
<td>Upon normal dosing, peak plasma concentrations will exceed normal levels due to decreased metabolic capability of the patient.</td>
<td>Risk of drug-induced toxicity due to inadvertent overdosing of patient.</td>
<td>Decrease the drug dose or choose an alternate drug therapy.</td>
</tr>
<tr>
<td><strong>Increased Clearance</strong></td>
<td>SNP in promoter region of gene causes increased enzyme expression and/or inducibility.</td>
<td>Upon normal dosing, peak plasma concentrations will not reach efficacy levels normal due to increased metabolic capability of the patient.</td>
<td>Risk of undermedication due to increased drug metabolism.</td>
<td>Increase the drug dose or choose an alternate drug therapy.</td>
</tr>
<tr>
<td><strong>Decreased Bioactivation</strong></td>
<td>(1) SNP in promoter region of gene causes decreased enzyme expression. (2) SNP in coding region of gene causes altered enzyme activity.</td>
<td>The efficacy of the drug depends upon oxidative enzyme-mediated bioactivation to be effective.</td>
<td>Risk of undermedication due to the absence of bioactivation of the prodrug.</td>
<td>Choose an alternate drug therapy.</td>
</tr>
</tbody>
</table>

### iv. Drugs that require P450 bioactivation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Active Metabolite</th>
<th>CYP450 Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>Morphine</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>Dihydrocodeine</td>
<td>Dihydromorphone</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>Tramadol</td>
<td>O-desmethyltramadol</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Unidentified</td>
<td>CYP2C19</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Nortriptyline</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Norfluoxetine</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Desmethyl Diazepam</td>
<td>CYP2C19, CYP3A4</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Carbamazepine-10,11-epoxide</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>Desmethylchlordiazepoxide</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Desipramine</td>
<td>CYP2C19, CYP2D6</td>
</tr>
<tr>
<td>Isosorbide Dinitrate</td>
<td>Isosorbide 5-mononitrate</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Morphine</td>
<td>Morphine-6-glucuronide</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>Prazepam</td>
<td>Desmethyl-diazepam</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Prednisolone</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Primidone</td>
<td>Phenobarbital</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Norverapamil</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Zidovudine triphosphate</td>
<td>CYP3A4, CYP2A6</td>
</tr>
<tr>
<td>Theophylline</td>
<td>1-methylxanthine and 3-methylxanthine</td>
<td>CYP1A2</td>
</tr>
<tr>
<td>Citalopram</td>
<td>desmethylcitalopram</td>
<td>CYP3A4, CYP2C19</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>O-desmethylvenlafaxine</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Quinidine</td>
<td>O-desmethylquinidine</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>4-hydroxycyclo-phosphamide</td>
<td>CYP3A4, CYP2B6</td>
</tr>
</tbody>
</table>
b. **Pharmacodynamic Considerations**

General overview

Classic dose-response curves. Based on EC\(_{50}\), the potency of the drugs are in the following order A > B > C. However, drugs A and B have the same efficacy both reach the same Emax. Drug C is less efficacious than both drugs A and B.

\(\beta_2\)-adrenergic receptor (\(\beta_2\)AR)

- 12 polymorphisms reported in the ADRB2 coding region
- Gly16 allele predisposes patients to nocturnal asthma and asthma severity
- Non-synonymous SNPs encoding for either Arg or Gly at position have been linked to altered responses to short acting \(\beta_2\)AR agonists (Gly better response than Arg)
I. Example Drugs Requiring Pharmacogenetic Testing

<table>
<thead>
<tr>
<th>Drug</th>
<th>Genetic Screening</th>
</tr>
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<tbody>
<tr>
<td>Warfarin</td>
<td>CYP2C9, VKORC</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>CYP2C19</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Thiopurine methyltransferase</td>
</tr>
<tr>
<td>6-mercaptopurine</td>
<td>Thiopurine methyltransferase</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>UGT1A1*28 homozygosity</td>
</tr>
<tr>
<td>5-flurouracil</td>
<td>Dihydropyrimidine dehydrogenase</td>
</tr>
<tr>
<td>Abacavir</td>
<td>HLA-B*5701</td>
</tr>
</tbody>
</table>

J. Limitations to implementation

1) High-Throughput DNA Analysis Technology: Costs, Data Standards and Future Technologies.
2) Information Management: Access, Security and System Structures.
3) Genomics & Genetics Education: Physicians, Pharmacists, Nurses and Consumers.
4) Point-of-Care Utilization of Genomics: Physician’s Office, Hospital, Pharmacy and Consumer.
6) Electronic Health Record Management and Utilization.
7) Translational Research: Establishing Linkages Between Allelic Information and Healthcare Outcomes.

K. References