Pharmacogenetics: Foundations, Puzzles and Future Directions

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Pharmacology Update
November 1, 2019
Objectives

1. Provide an overview of the history of pharmacogenomics/genetics.
2. Review genetic terms and definitions that are relevant to Pharmacogenetics.
3. Understand how Pharmacogenetics impacts patients and therapeutic outcomes in some key therapeutic areas.
4. Discuss testing, interpretation and the social milieu in Pharmacogenetics.
5. Provide resources for further learning & understanding.
Pharmacogenomics/genetics

- Study of genetic variations in drug metabolism & action
- How genes interact with drugs
- Decrease SE, ADR
- Improve safety & adherence
- Science of personalized medicine!
What is a drug?

• Molecule
• Binds/interacts with **specific** molecules
• Creates a response [physiologic change]
• Person / Condition

(Alenghat & Golan, 2017)
Drugs & Their Effects: Safety

• “Five Rights of Medication Administration” / Prescribing

• Right patient; Right drug; Right dose; Right route; Right time

• Focus?

• Individual prescriber, nurse: narrow

• P.Genomics ➔ Right drug: First Time!

(Frederico, 2019)
Benefits of Understanding PGx

- We’re all slightly different!
- Patients respond differently because of PGx \(\Rightarrow\) variability in drug response
- “Drug Metabolizing Enzyme Superfamily”
- Genetic variants in: CYP450
- Receptors
- Drug Transporters [Serotonin]
Question for you

- Which clinical area do you find to be the most challenging as a prescriber, in terms of side effects, adherence & positive therapeutic response?

- Hypertension
- T2 Diabetes
- Mental Health [anxiety, depression]
- Hypothyroid
- Other [Oncology, Cardiology, Pain]
All patients with the same diagnosis

- No benefit, no toxicity
- + Benefit, no toxicity
- No benefit, + toxicity
- + Benefit, + toxicity

(Hicks & McLeod, 2017)
Genetic Variation

• Can affect the pharmacokinetics

• AND also

• The pharmacodynamics
• Drug targets and receptors
Genetic Polymorphisms

Pharmacokinetic
- Absorption
- Distribution
- Metabolism
- Excretion

Pharmacodynamic
- Receptors
- Ion Channels
- Enzymes
- Immune System
PGx: PK, PD & Drug Response

- PK – Variant that impacts metabolism of the drug
  - Would affect concentration

- PD – Genetic variation that binding of drug to receptor
  - This would do what to therapeutic efficacy?
  - ↓↓↓↓↓

Tantisira & Weiss, 2019
PGx & Pharmacodynamics

- Drug molecules have targets
- Receptors
- Neurotransmitters
- Organs
- Transporters
- Ion Channels

These are all impacted by the genome, SNPs and individual genetics
CYP 450: 6 primary enzymes-75% of all drugs

- **3A4** ~47% ***
- **2D6** – 25% *** ^^
- **2C9** – 13% ^^
- **1A2, 2E1, 2C19**^^ – 15%

*** = very common
^ ^^ = lots of genetic variability
ROLE OF CYP ENZYMES IN HEPATIC DRUG METABOLISM

RELATIVE HEPATIC CONTENT OF CYP ENZYMES

- CYP 2D6: 2%
- CYP 2C: 17%
- CYP 1A2: 12%
- CYP 3A4-5: 26%
- CYP2E1: 7%
- OTHER: 36%

% DRUGS METABOLIZED BY CYP ENZYMES

- CYP 2D6: 23%
- CYP 3A4-5: 33%
- CYP 2C9: 14%
- CYP 2C19: 11%
- CYP 1A2: 14%
- CYP2E1: 5%
Foundations: A bit of history

- When was the term ‘pharmacogenetics’ first used?
  - 1923
  - 1959
  - 1963
  - 1990
  - 2003
Foundations: A bit of history

- Pythagoras – 1st to recognize fava beans → illness in some 6th c B.C.
- Differing T-1/2 in twins & warfarin – monozygotic [same] | dizygotic
- Drug concentrations varied
- Observing & reporting unpredictable reactions in drug studies
- Technology, genome, molecular

Tantisira & Weiss, 2019
Terms & Definitions

- Gene
- Allele
- Phenotype | Genotype
- Polymorphism | SNP
- Haplotype
- Homozygous | Heterozygous
- Gene ➔ Protein
- Exon | Intron | Codon
- Promoter region
Chromosomes of the Human Genome
DNA contains the blueprint for proteins – “genetic code”
DNA (Deoxyribonucleic Acid)

Deoxyribonucleic Acid (DNA)

Chromosome
Base pairs:
Adenine
Thymine
Guanine
Cytosine
Promoter region helps mRNA

Codons: Start & Stop Signals

Gene

Start Codon

5' A U G C U C G G A U A C C G U C A U

Ribosome

mRNA

3' G U G U G C A G G C A U U C A U A A

Stop Codons

| UAA |
| UAG |
| UGA |

We inherit 1 allele from each parent
Polymorphism = diversity [SNP]

- Genome diversity | loss of function [or alteration]
- Place in the DNA sequence where there is 1 or 2 variations along the DNA
- Must occur 1 in 100 people [1%]
- Could be a single letter: C instead of T
- Can be a stretch of DNA present or absent
Polymorphism

Haplotype

- >2 variants inherited together
- So, it’s a set of DNA Variations
- Can be several alleles
- Or several SNPs
- They tend to be close together along the chromosome
Genotype $\rightarrow$ Phenotype

- Homozygous $*1/ *1$
- Heterozygous $*2 / *1$
Individual Variation w/the same drug taken = Due to PGx
**Inducer**
- Something that accelerates enzyme activity – drug or chemical
- Eg. Phenobarbital induces the activity of CYP3A4
- Leads to ↑ metabolism [metoprolol]
- Effect?
- ↓BP lowering

**Inhibitor**
- Molecule that ↓ enzyme activity
- Grapefruit juice inhibits CYP3A4
- So, a CYP3A4 substrate* will have slower metabolism

* Molecule that CYP3A4 breaks down
Genes, Drugs & Metabolizing

- CYP Superfamily
- Clopidogrel, Losartan, Codeine, Tamoxifen
- Warfarin
- Skin reactions & HLA B *5701 variant – abacavir: single gene – potentially fatal drug reaction
- Simvastatin myopathy – variant in a gene that codes for a transport molecule
- B-1 adrenergic receptor gene & HF

Roden, Russell, Kroemer & Stein, 2011
CYP variations from Polymorphisms

Ultra Rapid

• The 3 most common variants

Normal [Extensive]

• CYP 2C19 – highly variable

• Reduced function alleles of 2C19 impact clopidogrel [“Nonresponder”]

Poor

• CYP2D6 has >90 allele variants

• Substrates include:
  • Codeine
  • Nortriptyline
  • Tamoxifen

Tantisira & Weiss, 2019
CYP Enzyme Variability: A closer look

- **CYP 3A4** – wide variation in gene regulation & expression by ancestry

- **CYP2D6** – absent function [poor metabolizer] in:
  - 5-10 % of European & African pop.
  - Rarely found in Asian population

- **CYP2C19** – poor metabolizers much more common in Asian population

- **CYP3A5** – much greater expression of this in African American population

Roden, Russell, Kroemer & Stein, 2011
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Variant Allele(s)</th>
<th>Frequency of PMs</th>
<th>Drugs with Affected Metabolism</th>
<th>Consequence(s)</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD</td>
<td>G6PD A, G6PD A(−)</td>
<td>...</td>
<td>Primaquine, sulfones,</td>
<td>Hemolytic anemia</td>
<td>Cessation of sulfa drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sulfonamides, nitrofurans,</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>vitamin K analogues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAT2</td>
<td>NAT*5B</td>
<td>40–70% of</td>
<td>Isoniazid, sulfonamides,</td>
<td>Increased relative risk of cancers and drug toxicity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasians and</td>
<td>procarbazine, hydralazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>African-Americans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>CYP2D6*2A,</td>
<td>6–10% of</td>
<td>β-receptor antagonists,</td>
<td>Lack of analgesic effects from</td>
<td>Dosage adjustment</td>
</tr>
<tr>
<td></td>
<td>CYP2D6*3, *4, *5,</td>
<td>Caucasians, 2–5% of</td>
<td>antiarrhythmics, antidepressants,</td>
<td>codeine, standard</td>
<td>in PMs</td>
</tr>
<tr>
<td></td>
<td>*6, *10, *17</td>
<td>African-Americans, 1% of Asians</td>
<td>antipsychotics, morphine</td>
<td>antidepressant dosage ineffective</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asians</td>
<td>derivatives</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>CYP2C9<em>2, CYP2C9</em>3</td>
<td>6–8% of</td>
<td>Warfarin, phenytoin,</td>
<td>Increased bleeding episodes from</td>
<td>Dosage adjustment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasians</td>
<td>glipizide, tobutamide,</td>
<td>standard warfarin dose, low blood</td>
<td>needed for PMs to achieve optimal therapeutic benefit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>losartan, NSAIDs*</td>
<td>sugar levels in PMs</td>
<td></td>
</tr>
<tr>
<td>CYP2C19</td>
<td>CYP2C19*2,</td>
<td>3–5% of</td>
<td>S-mephénytoin, omeprazole,</td>
<td>Increased omeprazole AUC* and higher H.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CYP2C19*3</td>
<td>Caucasians, 12–23% of Asians</td>
<td>diazepam, propranolol,</td>
<td>pylori eradication rate in PMs,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>imipramine, amitriptyline</td>
<td>prolonged half-life of diazepam and increased risk of diazepam toxicity in Asians</td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>CYP3A4*1B</td>
<td>Under investigation</td>
<td>Under investigation</td>
<td>Increased CYP3A5*1 activity, loss of</td>
<td></td>
</tr>
<tr>
<td>CYP3A5</td>
<td>CYP3A5<em>1, CYP3A5</em>3, CYP3A5*6</td>
<td>Under investigation</td>
<td>Under investigation</td>
<td>CYP3A5<em>3 activity, lower CYP3A5</em>6 activity</td>
<td></td>
</tr>
</tbody>
</table>

*G6PD = glucose-6-phosphate dehydrogenase, NAT = N-acetyltransferase, CYP = cytochrome P-450 isoenzyme system.
*PMs = poor metabolizers.
*NSAIDs = nonsteroidal antiinflammatory drugs.
*AUC = area-under-the-concentration-time curve.

Source: Am J Health-Syst Pharm © 2002 American Society of Health-System Pharmacists
## Type of metabolizer & drugs

<table>
<thead>
<tr>
<th>Prodrug</th>
<th>Active Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Inactive form</td>
<td>• Is in an active form when taken</td>
</tr>
<tr>
<td>• Needs conversion to active form by enzymes to become therapeutic</td>
<td>• Needs enzymes to be changed into inactive form for eliminated</td>
</tr>
<tr>
<td>• Poor metabolizer – no therapeutic effect</td>
<td>• Poor metabolizer → drug builds up; too much drug</td>
</tr>
<tr>
<td>• Examples:</td>
<td>• Eg: Warfarin needs CYP2C9 to be eliminated</td>
</tr>
<tr>
<td>• Clopidogrel-needs CYP2C19</td>
<td></td>
</tr>
<tr>
<td>• Codeine – converted to active morphine by CYP 2D6</td>
<td></td>
</tr>
</tbody>
</table>
Example of CYP variation

- Codeine = prodrug
- No analgesia until metabolized by CYP2D6
- **Poor metabolizer?** No relief!

- Nortriptyline – is in its active form
- Needs to be metabolized to be cleared & excreted
- **Poor metabolizer?** Overdosed!

Tantisira & Weiss, 2019
Variation of CYP2D6: Effects
Genes, Drugs & Metabolizing

- Variations in:
  - Drug uptake, metabolism & elimination; Drug MOA
  - $\rightarrow$ variable drug concentration
  - $\rightarrow$ variation in action of drugs
  - QT prolongation
  - Dangerous/Fatal skin reactions
  - There is a continuum from SNP $\rightarrow$ $\rightarrow$ Multiple Biologic Pathways

(Roden, Russell, Kroemer, & Stein, 2011)
Spectrum of individual drug metabolizing types, based on CYP

- Poor Metabolizer (Homozygote)
- Intermediate Metabolizer (heterozygote)
- Normal {Extensive} Metabolizer (Wild type)
- Ultrarapid Metabolizer (Multiple copies or promoter variant)
- Rapid Metabolizer (many copies or promoter variant)
**Pharmacogenetic testing**

- Allows us to predict which type of metabolizer the patient is!

Two Main CYP2C9 SNPs Found in Patients

- The “star allele” [*1, *2, etc.]
- CYP2C9*1: individuals possess normal enzyme activity.
- CYP2C9*2: carriers exhibit a 30% decrease in activity.
- CYP2C9*3: as much as 90% decrease in their enzymatic activity.
  - Patients can carry two normal copies *1/*1, a normal copy and a polymorphic *1/*2, or could have both copies with polymorphism *2/*3.
Mercier, Peterson, & Issa, 2017

- **Normal Enzyme Activity:** *1/*1 (wild-type alleles) no detected mutations
- **Intermediate Enzyme Activity:** *1/*2 or *1/*3 one wild-type allele (*1) paired with an allele with decreased activity *2 (681G>A) or *3 (636G>A)
- **Normal or Increased Enzyme Activity:** *1/*17 or *17/*17 enhanced activity allele *17 (−806C>T) with or without a wild-type allele *1
- **Low or Absent Enzyme Activity:** Combination of mutated alleles *2/*2, *2/*3 or *3/*3 resulting in a poor metabolizer phenotype
Polymorphism & CYP2C19

- Substrates include:
  - Clopidogrel, Citalopram, TCAs
- Slow metabolizers get a therapeutic effect with about 50% of dose of normal metabolizer
- Genetic testing/analysis
- Screen for CYP2C19 variants
Clopidogrel (prodrug) \(\rightarrow\) Intestinal absorption (ABCB1) \(\rightarrow\) Esterases \(\rightarrow\) Inactive metabolites (~85%) \(\rightarrow\) Hepatic metabolism

- First oxidative step: CYP2C19, CYP1A2, CYP2B6*
- CYP3A4, CYP3A5, CYP2B6, CYP1A2**

- Second oxidative step: CYP3A4, CYP2B6, CYP2C19, CYP2C9*

\[
\text{2-oxo-clopidogrel} \quad \text{Esterases} \quad \text{Inactive metabolites}
\]

\[
\downarrow \\
\text{ACTIVE METABOLITE (~15%)}
\]

\[
\text{ADP} \quad \text{P2Y12} \quad \text{Gi} \quad \text{Inhibition} \quad \text{P2Y1} \quad \text{Gq} \quad \text{AC} \quad \text{ATP} \quad \text{cAMP} \quad \text{PKA} \quad \text{VASP-P} \quad \text{VASP}
\]

\[
\text{Inhibition} \\
\text{?} \\
\text{SECRETION} \\
\text{AGGREGATION} \\
\text{SECRETION} \\
\text{PLATELET}
\]

Mercier, Peterson, & Issa, 2017
Clopidogrel Metabolism and Polymorphism

- The CYP2C19 gene: nine exons; situated on chromosome 10.
- > 30 different SNPs identified for this gene.
- Genetic variants of the CYP2C19 gene ➔ normal, reduced, or absent enzyme activity
- Can directly lead to an overactive or inactive enzyme.

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- Or, can directly lead to an overactive enzyme.

<table>
<thead>
<tr>
<th>Predicted phenotype</th>
<th>Genotypes</th>
<th>Samples (number)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive metabolizer</td>
<td>*1/*1</td>
<td>428</td>
<td>40.72</td>
</tr>
<tr>
<td>Intermediate metabolizer</td>
<td>*1/*2</td>
<td>369</td>
<td>35.10</td>
</tr>
<tr>
<td></td>
<td>*1/*3</td>
<td>72</td>
<td>6.85</td>
</tr>
<tr>
<td>Poor metabolizer</td>
<td>*2/*2</td>
<td>77</td>
<td>7.32</td>
</tr>
<tr>
<td></td>
<td>*2/*3</td>
<td>59</td>
<td>5.61</td>
</tr>
<tr>
<td></td>
<td>*3/*3</td>
<td>1</td>
<td>0.10</td>
</tr>
<tr>
<td>Ultra-rapid metabolizer</td>
<td>*1/*17</td>
<td>45</td>
<td>4.30</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1,051</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Abbreviation: CYP2C19, cytochrome P450 2C19.
Inherited genetic polymorphisms associated with CYP2C19 have a high impact on the physiological responses to clopidogrel in patients.
Distribution of Mutations Affecting Metabolism of Clopidogrel

- Poor metabolizers (PM): 2% - 15% carry loss-of-function mutations (*2/*2, *2/*3, *3/*3) on both alleles resulting in significantly or lack of CYP2C19 activity.
- Ultrarapid metabolizers (URM): 5% to 30% have a gain-of-function mutation making CYP2C19 more active (*1/*17, *17/*17).
- Extensive metabolizers (EM): 35% to 50% carry a normal CYP2C19 gene (*1/*1) without any mutations.
- Intermediate metabolizers (IM): 18% to 45% have only one loss of function allele (*1/*2, *1/*3).

Mercier, Peterson, & Issa, 2017
FULL PRESCRIBING INFORMATION

WARNING: DIMINISHED ANTIPLATELET EFFECT IN PATIENTS WITH TWO LOSS-OF-FUNCTION ALLELES OF THE CYP2C19 GENE

The effectiveness of Plavix results from its antiplatelet activity, which is dependent on its conversion to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19 [see Warnings and Precautions (5.1), Clinical Pharmacology (12.3)]. Plavix at recommended doses forms less of the active metabolite and so has a reduced effect on platelet activity in patients who are homozygous for nonfunctional alleles of the CYP2C19 gene, (termed “CYP2C19 poor metabolizers”). Tests are available to identify patients who are CYP2C19 poor metabolizers [see Clinical Pharmacology (12.5)]. Consider use of another platelet P2Y12 inhibitor in patients identified as CYP2C19 poor metabolizers.
SLCO1B1 gene: on Chromosome 12
- Encodes for a drug transporter: V174A
- Polymorphism
  - rs4149056T>C, in SLCO1B1
  - *17 variant → ↓ function
- Homozygous for *17
- Increases systemic exposure to simvastatin and the risk of muscle toxicity.

https://cpicpgx.org/content/guideline/publication/simvastatin/2014/24918167.pdf
PGx Variability: Examples

- Warfarin – Drug Target: Vitamin K Receptor
- Dosing variability factors:
  - VKORC1 gene $\rightarrow$ 25% dose variability
  - Promoter variant
  - CYP2C9 expression $\rightarrow$ 9% variability
- Caucasian, African American, Asian
- Genetically-guided therapy
Warfarin variability

- Originally thought to be due to CYP2C9
- Stopped the study:
- VKORC1 was discovered
- [previously unknown, unidentified]
- Found to be the major contributor to variability

https://cpicpgx.org/content/guideline/publication/warfarin/2017/28198005.pdf
Warfarin: A Great Example

- Has 1 drug target [VKORC1 gene]
- Metabolized by several pathways

VKORC

- Enzyme that converts Vitamin K to active form in the liver [vitamin K epoxide reductase]
- Warfarin inhibits this conversion
- Polymorphisms → warfarin resistance & sensitivity

CYP2C9

- Primary metabolizing enzyme in liver
- >60 known variants!!!
Warfarin Pathway

https://cpicpgx.org/content/guideline/publication/warfarin/2017/28198005.pdf
PGx Testing: Relevance

- Preemptive Genotyping
- Reactive PGx testing
- Requirements
- Prior authorization
- Documentation/ progress note
- Treatment plan
- Personalized Medicine [Mayo]
Puzzles & Challenges in PGx Testing

- Standardizing of terms for reporting
- \*3 allele: “low function”; ‘low activity’
- “null”; “undetectable activity”
- Inconsistent terms \(\rightarrow\) confusion –
  - Lab designation of phenotype varies:
  - “TPMT homozygous deficient” or “TPMT low activity”
  - “DPYD defective” or “nonfunctional”
  - TPMT - needed for Azathioprine
  - DYPD - needed for 5-FU

Caudle et al., 2017
Challenges in PGx testing

- Difficulties with replication studies
- Multiple SNPs → study sample
- GWAS = Genome-wide association studies
CPIC: Guidance in PGx

- Clinical Pharmacogenetics Implementation Consortium (CPIC)
- International Group of Volunteers
- “Consortium”
- Interest in:
- Facilitating use of Pharmacogenetic tests for use in patient care

https://cpicpgx.org
CPIC: Guidance in PGx

• Guiding Framework:

• Clinicians will have patient genotypes as testing becomes more widespread

• How to interpret and utilize results

• CYP enzymes, alleles

• Created over 169 Guidelines

https://cpicpgx.org
Gene(s)/drug(s)

Gene already subject to CPIC guideline
- Actionable in other professional society guidelines
- Evaluate alternatives, evidence

Gene not yet subject to CPIC guideline
- Nominated by CPIC member or recommended by external group (e.g., FDA, EMA)
- Evaluate alternatives, evidence, degree of testing
- PharmGKB Annotation level 1A, 1B, 2A or 2B
- Mentioned in professional society guidelines but not actionable

CPIC level A or B: Prescribing action recommended; alternative therapies or dosing are highly likely to be effective and safe

CPIC level C: No prescribing change based on genetics; alternatives are unclear or evidence is weak but testing is common or gene is CPIC level A or B for other drugs

CPIC level D: PharmGKB annotation only; no prescribing action recommended; alternatives unclear or evidence is weak; testing is rare
PharmGKB

CPIC guideline or known clinical implementation

variant in PharmGKB VIP

Level 1a
Level 1b

Level 2a
Level 2b

Level 3

Level 4

Evidence

high

moderate

low

preliminary

https://www.pharmgkb.org/page/clinAnnLevels
PharmGKB

- Pharmacogenomics knowledge resource
- Clinical guidelines and drug labels
- Review/inform FDA drug labels containing pharmacogenomic information
- Support consortia examining important questions in pharmacogenomics
What needs to happen?

- Evidence needs to improve
- Payors need to adopt
- FDA
- Guidelines
- Clinicians need education
- Trial-and-Error approach
ELSI & PGx

- Ethical
- Legal
- Social
- Issues
- Patient privacy
- Protection
- Results in Genetic / Genomic Studies

GINA

- Genetic Information Nondiscrimination Act
- Passed in 2008
Population Pharmacogenomics

Public health

Projects to address the prevalence of PGx biomarkers in populations

Population-specific PGx biomarker panels

Genome-guided patient care

Drug development

Genome-enriched clinical studies

Patrinos, 2019
References


References


