Introduction to Genetics and Pharmacogenomics

Ching-Lung Cheung, PhD
Assistant Professor,
Department of Pharmacology and Pharmacy,
Centre for Genomic Sciences, HKU
Survey on pharmacogenomic knowledge

1. To evaluate pharmacogenomic knowledge among healthcare professionals

2. Hope to promote the knowledge in PGx among healthcare professionals
Overview of the lecture

1. Introduction to genetics
   • Types of genetic variations
   • Significance of genetic variation

2. Introduction to pharmacogenomics (PGx)
   • Role of PGx in PD/PK
   • Genes predispose to toxicities
   • Genes that influence disease susceptibility
Learning objectives

1. How to explain to patients?

2. Understand the importance of genetics in pharmacology

3. Genes that influence disease susceptibility vs. drug effect
Genetics

1. Base pairs
2. DNA (double helix)
3. Cell
4. Genes
5. Chromosome
6. Nucleus
Basic building block: Nucleotide (A/T/C/G):
DNA: a polymer of nucleotide
Allele: An allele is one of two or more versions of a gene. An individual inherits two alleles for each gene, one from each parent. Though the term allele was originally used to describe variation among genes, it now also refers to variation among non-coding DNA sequences.
Gene: The functional and physical unit of heredity passed from parent to offspring. Genes are pieces of DNA, and most genes contain the information for making a specific protein.
Basic Genetic Concepts & Terms: DNA

- **Mutation**: A mutation is defined as any change in a DNA sequence away from normal. This implies there is a normal allele that is prevalent in the population and that the mutation changes this to a rare and abnormal variant.

- **Polymorphism**: Polymorphism involves one of two or more variants of a particular DNA sequence.

- **Single nucleotide polymorphism (SNP)**: SNPs are a type of polymorphism involving variation of a single base pair.
Basic Genetic Concepts & Terms: DNA

• Trait: A trait is a specific characteristic of an organism.

• Susceptibility: Susceptibility is a condition of the body that increases the likelihood that the individual will develop a particular disease.

• Genetic association: The occurrence together in a population, more often than can be readily explained by chance, of two or more traits of which at least one is known to be genetic.
Types of genetic variations
Types of genetic variations

1. Point mutation (or variation)
   - One or few nucleotides change
   - Mutation, single nucleotide polymorphism, copy number variation, insertion, deletion, etc
   - E.g. Drug dose, severe adverse drug reaction

2. Chromosomal mutation (or variation)
   - Large scale change
   - Change in number of chromosome or arrangement of genes in chromosome
   - Deletion, duplication, inversion, translocation, aneuploidy, etc
   - E.g. Metabolic phenotype

3. Epigenetic variations: Histone modification, DNA methylation, etc
   (not covered in this lecture)
**Mutation vs. SNP**

- **Mutation:**
  - rare in nature and sudden change (*de novo*)
  - varies from that in the wild type

- **Single nucleotide polymorphism (SNP):**
  - validated to be frequent in a population
  - Rare (<1%) vs. common variant (≥1%)

- Rare variant and mutation often use interchangeably

- DNA: ~99.9% of the sequence are the same between different individuals (~3-10 millions genetic variations)
Genetic variations affecting protein

- Central dogma of molecular biology

- Regardless to point/chromosomal mutation (or variation), when mutation affects protein structure/function (e.g. nonsense, frameshift mutation) or presence of protein (chromosomal deletion, gene duplication), effect on cell (or health) is usually large
Genetic variations affecting protein

Genetic variation:

• Mutation, SNP, copy number variation, deletion, insertion, duplication/amplication

• Effect of genetic variation on a gene:

  • Loss-of-function
  • Gain-of-function
Genetic variations affecting protein

- Effect of genetic variation on a gene:

- Dominant negative: Mutation leads to alteration in gene product that acts antagonistically to the wild-type allele
FACT: many disease susceptibility loci or PGx markers are NOT located within genic regions

Why?
Gene expression is highly regulated

- Gene expression is NOT a binary (yes/no) event
- Is highly regulated, multiple copies of mRNA and polypeptides are produced at the same time
- Where are the switches/controls?

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Role of genetic variants

- Switches = Regulatory element
- promoter, enhancer, insulator, splicing site, etc
- Consensus sequence
Consensus sequence

- Consensus sequence: conserved sequence motif
- Regulatory protein (e.g. transcription factor) recognizes and binds to consensus sequence
- For example: runx2

\[
\begin{align*}
\text{5'}-&\text{ATGGGGTGAGGATATGC} \\
\text{5'}-&\text{ATGGGGTGAAAGTATGC}
\end{align*}
\]

- Not a binary event (efficiency)
Introduction to pharmacogenomics (PGx)
Precision medicine is an emerging approach for disease prevention and treatment that takes into account people’s individual variations in genes, environment, and lifestyle.
Precision medicine is an emerging approach for disease prevention and treatment that takes into account people’s individual variations in genes, environment, and lifestyle.

1. Disease prevention
2. Disease treatment
3. Individual variations in genes
4. Individual variations in environment
5. Individual variations in lifestyle
LONGER TERM GOALS

Create a research cohort of > 1 million American volunteers who will share genetic data, biological samples, and diet/lifestyle information, all linked to their electronic health records if they choose.

Pioneer a new model for doing science that emphasizes engaged participants, responsible data sharing, and privacy protection.

Research based upon the cohort data will:

• Advance pharmacogenomics, the right drug for the right patient at the right dose
• Identify new targets for treatment and prevention
• Test whether mobile devices can encourage healthy behaviors
• Lay scientific foundation for precision medicine for many diseases
Pharmacogenomics (PGx)

- Pharmacogenomics is a branch of pharmacology concerned with using DNA and amino acid sequence data to inform drug development and testing.

- An important application of pharmacogenomics is correlating individual genetic variation with drug responses.

the National Human Genome Research Institute
Which is the most desirable one?

- Conventional practice: one size fits all
Genetics in PGx

• The genetic differences likely to be of most relevance in drug development are those associated with genes in four broad categories:

1. Genes relevant to the drug’s PK
2. Genes that code for intended or unintended drug targets and other pathways related to the drug’s pharmacologic effect (PD);
3. Genes not directly related to a drug’s pharmacology that can predispose to toxicities such as immune reactions; and
4. Genes that influence disease susceptibility or progression
1. PGx and PK
ADME

Drug metabolism rates vary among patients.

Some patients metabolize a drug (does not include prodrug) so rapidly that therapeutically effective blood and tissue concentrations are not reached.

Some may be so slow that usual doses have toxic effects.

Cytochrome P-450 (CYP450) is the most important enzyme system of phase I metabolism, such as CYP1A2, CYP2A9, CYP2C19, CYP2D6, CYP3A4, etc.
Metabolic phenotype

- Metabolic phenotype: determined by the drug metabolizing enzyme activity

<table>
<thead>
<tr>
<th>Metabolic Phenotype</th>
<th>Rate of Metabolism</th>
<th>Plasma Drug Levels</th>
<th>Clinical Outcome</th>
<th>Individualized Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor metabolizer (PM)</td>
<td>None</td>
<td>Toxic</td>
<td>Side effects</td>
<td>Decrease dose to reduce toxicity</td>
</tr>
<tr>
<td>Intermediate metabolizer (IM)</td>
<td>Reduced</td>
<td>High</td>
<td>Sometimes side effects</td>
<td>Normal dose</td>
</tr>
<tr>
<td>Extensive metabolizer (EM)</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal response</td>
<td>Normal dose</td>
</tr>
<tr>
<td>Ultrarapid metabolizer (UM)</td>
<td>Rapid</td>
<td>Low</td>
<td>Reduced efficacy</td>
<td>Increase dose to increase efficacy</td>
</tr>
</tbody>
</table>

- Affected by genetic variation
CYP2D6

- Belong to cytochrome P450 (CYP) is a well-known super family of drug metabolizing enzymes involved in approximately 80% of drugs metabolism

- PM: no functional allele
- IM: one reduced + one non-functional allele
- EM: one full function + one non-functional/ one full function + one reduced function/ two full function/ two reduced function
- UM: more than two functional allele
## CYP2D6

<table>
<thead>
<tr>
<th>Functional Status</th>
<th>Activity Value</th>
<th>Alleles</th>
</tr>
</thead>
</table>

### Ethnic-specific genetic marker

<table>
<thead>
<tr>
<th>Slow metabolizers</th>
<th>Ultra-rapid metabolizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-10% Caucasians</td>
<td>0.80% Caucasians</td>
</tr>
<tr>
<td>&lt;1% Asians</td>
<td>21% Saudi Arabians</td>
</tr>
<tr>
<td>2% African-Americans</td>
<td>29% Ethiopians</td>
</tr>
</tbody>
</table>
Nomenclature

* indicate the allele (i.e. which genetic variation)

In drug metabolizing enzyme, allele is composed of a number of genetic variation (in form of haplotype)

<table>
<thead>
<tr>
<th>Allele Name</th>
<th>Defining Name/Change</th>
<th>Rs#</th>
<th>Comments</th>
<th>Enzyme Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*3A</td>
<td>g2549delA</td>
<td>rs35742686(-)</td>
<td>259frameshift</td>
<td>none</td>
</tr>
<tr>
<td>CYP2D6*3B</td>
<td>g1749A&gt;G, g2549delA</td>
<td>rs1135824(G); rs35742686(-)</td>
<td>N166D; 259frameshift</td>
<td>none</td>
</tr>
</tbody>
</table>
http://www.hgvs.org/mutnomen/figure_refseq.html
CYP2D6 and codeine

- Codeine is a prodrug to morphine after metabolizing by CYP2D6 in liver

- UM/ EM/ IM/ PM (select one) for CYP2D6 fail to experience an analgesic effect from codeine (1-min discussion)
2. PGx and PD
### Examples of PGx and targeted therapy in cancer

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic Area</th>
<th>HUGO Symbol</th>
<th>Referenced Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ado-Trastuzumab</td>
<td>Oncology</td>
<td>ERBB2</td>
<td>HER2 protein overexpression or gene amplification positive</td>
</tr>
<tr>
<td>Emtansine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afatinib</td>
<td>Oncology</td>
<td>EGFR</td>
<td>EGFR exon 19 deletion or exon 21 substitution (L858R) mutation positive</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>Oncology</td>
<td>BCR/ABL1</td>
<td>Philadelphia chromosome (t(9;22)) positive</td>
</tr>
<tr>
<td>Cetuximab (1)</td>
<td>Oncology</td>
<td>EGFR</td>
<td>EGFR protein expression positive</td>
</tr>
<tr>
<td>Cetuximab (2)</td>
<td>Oncology</td>
<td>KRAS</td>
<td>KRAS codon 12 and 13 mutation negative</td>
</tr>
<tr>
<td>Dabrafenib (1)</td>
<td>Oncology</td>
<td>BRAF</td>
<td>BRAF V600E mutation positive</td>
</tr>
<tr>
<td>Trametinib</td>
<td>Oncology</td>
<td>BRAF</td>
<td>BRAF V600E/K mutation positive</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Oncology</td>
<td>ERBB2</td>
<td>HER2 protein overexpression positive</td>
</tr>
</tbody>
</table>

Any non-cancer example?
• Denosumab (Prolia): monoclonal antibody against RANKL

• Whether expression of RANKL is related to treatment outcome has never been studied

• RANKL is a member in OPG/RANK/RANKL pathway

Unpublished data
To evaluate if serum and gene expression levels of OPG/RANK/RANKL pathway affect denosumab treatment outcome

- 50 osteoporotic patient receiving denosumab monotherapy
- Measured serum OPG, RANK, and RANKL, and mRNA expression of OPG, RANK, and RANKL in PBMC

What do you expect? (3-min discussion)
PGx and PD: Osteoporosis

- Higher the serum RANKL levels, lower the increase in BMD after 1 year use

Unpublished data
Serum RANKL is significantly correlated with mRNA expression in PBMC

Unpublished data
Introduction to genetic association study

- Case-control (e.g. diabetes) or quantitative trait (e.g. blood pressure)
- Association (correlation) with genotype (several mode of inheritance)
- GWAS: Genome-wide association study, study the association between few hundred thousand of SNP and trait-of-interest
- Candidate gene approach
Genome-wide association study

1. Design the study
2. Identify trait-of-interest
3. Recruit appropriate subjects
4. DNA extraction and genotyping (now usually >700K markers)
5. Trait measurement
6. Statistical analysis
• Metformin’s mechanism of action is largely unknown.
• Genome-wide association study identifies a PGx marker of metformin on A1c

Zhou et al., Nat Genet (2016)
The C allele of rs8192675 in the intron of SLC2A2, which encodes the facilitated glucose transporter GLUT2, was associated with a 0.17% (P = 6.6 × 10^{-14}) greater metformin induced reduction in A1c.

Zhou et al., Nat Genet (2016)
PGx and PD: Type 2 diabetes

Do you think it's clinically useful?

3 mins discussion
3. Genes predispose to toxicities
Types of allergic reactions

**TYPE I**
- Antibody-mediated reaction
- HLA plays an important role

**TYPE II**
- Cell-mediated reaction
- RBC lysis
- RBC removal by reticuloendothelial system

**TYPE III**
- Antibody-mediated reaction
- Immune complex deposition in tissues

**TYPE IV**
- Cell-mediated reaction
- Antigen presented
Example for type B ADR: SJS/TEN
Stevens-Johnson Syndrome (SJS)/ Toxic Epidermal Necrolysis (TEN)

1. Severe cutaneous adverse reaction of drugs (SCAR)
2. Mostly drug induced
3. Idiosyncratic in nature
4. Unpredictable until 2004
Genetic marker of carbamazepine induced SJS/TEN

• 2004: A strong association of CBZ-induced SJS with HLA-B*15:02 in Han Chinese was first uncovered in Taiwan

• 2007: The Taiwan and US FDA relabeled the drug info of CBZ and recommended a genetic screening of HLA-B*15:02 prior to starting CBZ in patients with Asian ancestry, particularly for those of Southeast Asian ancestry.
4. Genes that influence disease susceptibility or progression
Genetics of complex trait: BMD

- >60 genetic variations are associated with BMD
- Explain ~2% variance of BMD
- Genetic risk score (GRS): by adding all risk variants together

BMD GRS affects CaD intervention

- Women’s Health Initiative trial on CaD (N=5823)
- Clinical outcome: Fracture
- Median follow-up year: 6.5 yr

Wang et al., Am J Clin Nutr (2017)
BMD GRS affects CaD intervention

- Participants with higher GRS, no benefits from CaD on fracture prevention

- Why? (2-min discussion)

Wang et al., Am J Clin Nutr (2017)
Take home message
Take home message

1. Genetics play an important role in disease susceptibility

2. Genetic variation affects gene expression or function and hence pharmacology and drug effect

3. Genes that influence disease susceptibility or progression can affect drug effect
Learning objectives

1. How to explain to patients?

2. Understand the importance of genetics in pharmacology

3. Genes that influence disease susceptibility vs. drug effect
Next seminar

Pharmacogenomics for pharmacists

Seminar 1
Introduction to genetics and pharmacogenomics
By Dr. CL Cheung
Assistant Professor, HKU

Date 1: 4 Mar 2017 14:30-15:30
Venue: Room LE2, LG1/F Library Extension Building, HKU
OR
Date 2: 8 Mar 2017 19:30-20:30
Venue: Room CPD-2.19, 2/F Run Run Shaw Tower, HKU

Seminar 2
Clinical application of pharmacogenomics
By Dr. CL Cheung
Assistant Professor, HKU

Date 1: 18 Mar 2017 14:30-15:30
Venue: Room LE2, LG1/F Library Extension Building, HKU
OR
Date 2: 22 Mar 2017 19:30-20:30
Venue: Room CPD-2.19, 2/F Run Run Shaw Tower, HKU

A mobile APP for PGx and the training session will be offered in course!

website: http://www.hkuprecision.org/services.html
To register, please email to kchengyc@hku.hk
Or call us at 28315087 (Mr. Kelvin Cheng)
AppointMed Mobile APP

AppointMed, a Mobile Medical Helper

A mobile APP that improves medication compliance in Hong Kong citizens and provides a pharmacogenomic database and guideline to healthcare professionals.

- A FREE mobile APP of PGx
- PGx database
- Regularly update
- Clinical recommendation from FDA and Clinical Pharmacogenetics Implementation Consortium (CPIC)

Supported by HKU KE Fund
Thank you

Q and A

Comments?