Pharmacogenomics: The Future of Pharmacy

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Objectives

• Define pharmacogenomics.

• Discuss how pharmacogenomics relates to the practice of pharmacy.

• List the known genetic variations that may impact drug therapy.

“If it were not for the great variability among individuals, medicine might as well be a science, not an art.”

Sir William Osler, Physician

History

• 1865 Gregor Mendel
  – Developed the principles of genetics and inheritance through his experiments with pea plants

• 1953 Watson and Crick
  – Determined the structure of deoxyribonucleic acid (DNA)

• 2003 Human Genome Project
  – An international research project to sequence and map all the genes in the human body


Human Genome Project

• Final results were published in 2003

• 20,500 human genes identified

• Determine the sequence of 3 billion base pairs

• 0.1% of DNA differs from one person to the next (about 3 million bases)
**Human Genome Project**

- Researchers have applied the findings from the human genome project in 3 major ways:
  - Determining the order, or “sequence,” of all the bases in our genome’s DNA
  - Mapping the locations of genes for major sections of all our chromosomes
  - Producing linkage maps, through which inherited traits (such as those for genetic disease) can be tracked over generations

**Pharmacogenomics (PGx)**

- A science that examines the inherited variations in genes that dictate drug response and explores the ways these variations can be used to predict whether a patient will have a good response to a drug, a bad response to a drug, or no response at all

  - **Pharmacogenetics** refers to the study of inherited differences which causes variation in drug metabolism and response

**Single Nucleotide Polymorphism**

- **Single Nucleotide Polymorphism (SNPs)** are small genetic changes or variations that can occur within a patient’s DNA sequence
  - Substitution
  - Insertions or deletions
  - Duplication/repeats
  - Point mutations

**Definitions**

- **Polymorphism**- variation in the DNA that is present at an allele frequency of ≥1% in a population
- **Homozygous**- 2 identical alleles for a trait
- **Heterozygous**- 2 different alleles for a trait
- **Wild Type**- most commonly occurring allele or 1st sequenced allele

**Definitions**

- **Poor metabolizer (PM)**, person who metabolizes a drug slower than others, 2 non-functional/mutant alleles
- **Intermediate metabolizer (IM)**- once functional allele and one mutant allele
- **Normal metabolizer (NM)**- normal metabolism of medications, homozygous for normal alleles
- **Ultrarapid metabolizer (UM)/ Extensive Metabolizer (EM)**- metabolizes drugs faster than most, duplicate or multiple copies of the normal allele

**Benefits of Pharmacogenomics**

- Optimize drug therapy and limit drug toxicity based on an individual’s genetic make-up

- **Patient group**
  - Drug toxic but beneficial
  - Drug toxic and NOT beneficial
  - Drug NOT toxic and beneficial

- **Same diagnosis, same prescription**
  - Drug toxic but NOT beneficial
  - Drug NOT toxic and beneficial
Question

1. How has the Human Genome Project influenced the practice of medicine?
   a) Develop the field of pharmacogenomics
   b) Enhance the field of biotechnology
   c) Helped identify the genetic influences that predispose a patient or patient population to a disease state
   d) Lead the medicine community closer to individualized medicine
   e) All of the above

Present Use of Genetic Information

Present Use of Genetic Data

• Sex-linked disorders
  – Colorblindness
  – Hemophilia
• Autosomal dominant or recessive disorders
  – Sickle Cell Disease
  – Cystic Fibrosis
  – Huntington’s disease
  – Marfan’s syndrome
• Trisomy 21 Down’s syndrome
• Antiretroviral therapy

Abacavir

• Nucleoside Reverse Transcriptase Inhibitor (NRTI) for treatment of HIV
• Hypersensitivity reaction:
  – Rash and/or fever
  – Abrupt onset of nausea/vomiting/diarrhea
  – Respiratory symptoms: tachypnea, cough, pharyngitis
• HLA-B*5701 allele has demonstrated a positive predictive value for the development of hypersensitivity reaction
  – Affects 5-8% of patients within the first 6 weeks of treatment
• Test for HLA-B*5701 prior to beginning abacavir

Chemotherapy

• Goal: individualize cancer chemotherapy to maximize efficacy and minimize toxicity
• 2 genomes to consider:
  – Germline genome of the patient
    • Example: UGT1A1 polymorphism in patients with irinotecan may effect toxicity
  – Somatic genome of the tumor
    • Example: gefitinib efficacy for over expression of EGFR on tumors

Targeted Chemotherapy

• Monoclonal antibodies- antibody mediated cellular toxicity directed at a specific antigen that is expressed on the cancer cell
  – Derived from mouse and human proteins
• May attach to the specific antigen alone or deliver a toxin, radioisotope, or cytokine to the cancer cell
Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Alemtuzumab</td>
<td>CD52</td>
<td>B-cell CLL</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>VEGF ligand</td>
<td>Colon, NSCLC, Renal Cell cancer, Glioblastoma</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>EGFR-1, IgG1 monoclonal antibody</td>
<td>Colorectal cancer, Head &amp; Neck Cancer</td>
</tr>
<tr>
<td>Gemtuzumab</td>
<td>CD33</td>
<td>AML</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>NHL</td>
</tr>
<tr>
<td>Ibritumomab</td>
<td>CD20</td>
<td>NHL, CLL</td>
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<tr>
<td>Ofatumumab</td>
<td>CD20 +, IgG1-kappa monoclonal antibody</td>
<td>CLL</td>
</tr>
<tr>
<td>Panitumumab</td>
<td>EGFR-1, IgG2 monoclonal antibody</td>
<td>Colorectal cancer, Head &amp; Neck Cancer</td>
</tr>
<tr>
<td>Tositumomab</td>
<td>CD20</td>
<td>NHL</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Her2/neu</td>
<td>Breast Cancer</td>
</tr>
</tbody>
</table>

Targeted Chemotherapy

- Tyrosine Kinase Inhibitors (TKI)
  - Tyrosine kinase plays a role in modulating growth factor signaling
  - TKIs compete with the ATP binding site of the catalytic domain of several oncopgenic tyrosine kinases
  - Primary use: cancer chemotherapy
  - Benefit: Oral medications

TKI Target Treatment

<table>
<thead>
<tr>
<th>TKI</th>
<th>Target</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasatinib</td>
<td>Bcr-Abl</td>
<td>Ph+ CML &amp; ALL, CML</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR-TK</td>
<td>NSCLC</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>EGFR-TK</td>
<td>NSCLC</td>
</tr>
<tr>
<td>Imatinib</td>
<td>ABL, BCR-ABL, c-KIT &amp; PDGFR TK</td>
<td>Ph+ CML &amp; ALL, GIST</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>EGFR, HER1&amp;2</td>
<td>Breast Cancer</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>Bcr-Abl</td>
<td>Ph+ CML &amp; ALL, CML</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>PDGFR, VEGFR, c-KIT, fms, fms, c-KIT</td>
<td>Renal cell carcinoma</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>PDGFR, VEGFR, c-KIT, FLT3</td>
<td>GIST, NSCLC, Breast Cancer, Renal Cell Carcinoma</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR, Raf kinase</td>
<td>Renal Cell &amp; Hepatocellular Carcinoma</td>
</tr>
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Current & Future Applications for Pharmacogenomics

Patient Case

2. MW is a 68 year old woman who was recently diagnosed with breast cancer at her yearly mammogram screening and confirmed by core biopsy. Her tumor is stage IIIB (T2, N1, M0). Her physician prescribed trastuzumab therapy, followed by surgery and radiation. MW's friend, TC, was diagnosed with stage III breast cancer 3 months ago and is receiving chemotherapy with doxorubicin and cyclophosphamide. Why were their chemotherapy regimens different?

a) MW's cancer was "cured" with surgery and didn’t need chemotherapy
b) TC had estrogen receptor + breast cancer
c) MW’s tumor is positive for expression of the HER2/neu antigen and is receiving targeted neoadjuvant therapy
d) TC’s tumor is positive for expressing the VEGF receptor and her treatment is directed at VEGF

Drug Delivery
CYP2D6

• At least 48 gene variations and 53 alleles have been identified for the CYP2D6 gene
• 6 main genotypic variants:
  – CYP2D6*1 = wild type allele - normal activity
  – CYP2D6*2 = same activity as *1 but can duplicate or amplify itself
  • Both present in EM
  – CYP2D6*4 and CYP2D6*5: inactive enzyme and absence of enzyme
  • Present in PM
  – CYP2D6*10 and CYP2D6*17 single amino acid substitution - reduction of enzyme activity
  • Present in PM and people of Asian and African descent

Phenytoin

Wild type allele: CYP2C9*1

• 6 fold higher risk of breast cancer relapse

FDA

• Women

Tamoxifen

• Tamoxifen is a prodrug, which requires metabolism by CYP2D6 to its active metabolites
  – Endoxifen being the most active metabolite
• Women taking tamoxifen who had a CYP2D6 genetic variation or also took medications that inhibited CYP2D6, had a nearly 4-fold higher risk of breast cancer relapse compared to women who were EMs
• FDA recommendation to change the label of tamoxifen to incorporate the importance of genetic and drug-induced variation in CYP2D6
  – CYP2D6 PM would benefit from anastrozole, another aromatase inhibitor, which does not rely on 2D6 for activation

CYP2D6

<table>
<thead>
<tr>
<th>Drug</th>
<th>2D6<em>1 &amp; 2D6</em>2 (EM)</th>
<th>2D6<em>4 &amp; 2D6</em>5 (PM)</th>
<th>2D6<em>10 &amp; 2D6</em>17 (PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricyclic antidepressants; atomoxetine; antipsychotics; fluoxetine</td>
<td>Inadequate response</td>
<td>Higher</td>
<td>More Side Effects</td>
</tr>
<tr>
<td>Codiene, tramadol, and other opioids</td>
<td>Increase production of active metabolite</td>
<td>Inadequate response</td>
<td>Inadequate response</td>
</tr>
<tr>
<td>Beta-Blockers (metoprolol)</td>
<td>Less BP reducing effect; more frequent dosing</td>
<td>Increase side effects (hypotension, dizziness, etc)</td>
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</tr>
</tbody>
</table>

CYP2C9

• Medications with narrow therapeutic index that may be affected by CYP2C9 variations:
  – Warfarin
  – Phenytoin

• CYP2C9*2 and 2C9*3 – reduced function alleles
  – Higher risk for adverse events
  – Would not receive full effect of medication, if a prodrug

Warfarin

• Warfarin is a racemic mixture
  – S-warfarin is 3-5x as potent as R-warfarin, has a shorter half-life, and is metabolized predominantly by a CYP2C9
• Wild type allele: CYP2C9*1
• Reduced function alleles: CYP2C9*2 and CYP2C9*3
  – Metabolize warfarin slowly
  – Greater risk for bleeding
  – Lower warfarin dose requirements
  – Longer duration to dose stabilization
  – Present in 10-20% Caucasians and <5% in Asians and African Americans
### Warfarin

- Vitamin K epoxide reductase complex 1 (VKORC1) converts inactive vitamin K to active vitamin K
  - Vitamin K dependent clotting factors: II, VII, IX, X
- SNPs associated with VKORC1 include:
  - VKORC-1GA, VKORC1GG, and VKORC1AA
  - VKORC1-AA genotype require a lower warfarin dose
- Polymorphism leads to decreased VKORC1 enzyme, less vitamin K dependent clotting factors produced; a lower warfarin dose necessary to achieve therapeutic INR

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### CYP2C19

- Defective alleles:
  - CYP2C19*2 and 2C19*3 - inactive CYP2C19 enzymatic action and PM phenotype
- Medications affected:
  - Omeprazole - increase INR, 5x higher AUC
  - Clopidogrel
- People of Asian decent are more likely to be PM

### CYP3A4

- CYP3A4 metabolizes ~50% of prescribed medications and is the predominant CYP3A form in the liver
- Known CYP3A4 variations: 3A4*1B and 3A4*2
  - Conflict over implications of 3A4*1B polymorphism
    - Lower drug concentrations than with the wild type (CYP3A4*1)
  - Clinical implications of 3A substrates: cyclosporine, tacrolimus, ketoconazole, rifampin, etc

### CYP3A5

- CYP3A5 - best studied minor CYP3A isoform
  - The wild type allele, 3A5*, is found in lower concentrations than the variant allele
  - Heterozygous or homozygous for 3A5*1 displays higher clearance of drugs
  - Genetic variation: 3A5*3 - reduced metabolism of drugs
  - CYP3A5 polymorphism expression may have implications in predicting disease states

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### Warfarin

- Recent analysis found another CYP450 gene:
  - CYP4F2
- Variations in CYP4F2 shows decreased catabolism of the warfarin reaction
  - Patients with this variation may require larger doses of warfarin to reach their INR range
  - ~1mg/day more warfarin

### Clopidogrel

- CYP2C19*1= fully functional metabolism
- Primary reduced functional alleles:
  - CYP 2C19*2
  - CYP 2C19*3
  - *3 allele present primarily in Asian population
- CYP 2C19*17 = gain-of-function allele (EM)
  - Ultrarapid enzyme activity
  - Lower platelet aggregation
  - Increased risk of bleeding
- Variations in CYP2C19 activity:
  - Impaired antplatelet activity
  - Lower levels of the active clopidogrel metabolite
  - Higher rates of cardiovascular events
N-acetyltransferase

- N-acetyltransferase 2 (NAT2) acetylation is a hepatic drug-metabolizing enzyme
  - Responsible for the metabolism of isoniazid, hydralazine, sulfonamides, procainamide, caffeine
- Rapid acetylators
  - One or 2 highly-active alleles (NAT2*4 & NAT2*12)
  - Short half life & lower drug concentrations
- Slow acetylators
  - Two low activity alleles (NAT2*5A, NAT2*5B, NAT2*6A)

Irinotecan

- Irinotecan is metabolized to SN-38, the active metabolite, which is inactivated via glucuronidation by UGT1A1
- UGT1A1*28 is the most common polymorphism
  - Results in lower enzyme activity and glucuronidation
  - May lead to toxicity
    - Myelosuppression
    - Diarrhea
- UGT1A1*6 is a reduced function allele that is found in Japanese people

Thiopurine S-Methyltransferase (TPMT)

- TPMT and 6-MP
  - 16 variations are shown to cause reduced TPMT activity and 12 variations cause deficient TPMT activity
  - TPMT*2, TPMT*3A and TPMT*3C are most common mutant alleles responsible for decreased TPMT activity
    - Mutant alleles lead to increased drug in body and increased risk of developing bone marrow suppression and even death
  - Blood test that allows physicians to calculate the best dose of 6MP for

UDP-Glucuronosyl Transferase (UGT)

- UGT2B7 catalyzes the glucuronidation of many drugs, including morphine, NSAIDs, valproic acid, mycophenolate mofetil
- 2 variant alleles of UGT2B7: UGT2B7*1 and *2
  - UGT2B7*1 is found more in Japanese people
- There is a trend towards lower activity of the UGT2B7*2 allele, but has not shown to be significant

Dihydropyrimidine Dehydrogenase (DPD or DPYD)

- DPD is the primary catabolic enzyme that metabolizes 5-fluorouracil (5-FU)
  - DPYD encodes for DPD
- Greater than 30 SNPs and deletion mutation have been identified for DPYD
- DPYD*2A- low DPD enzymatic activity
  - Risk factor for severe mucositis and leukopenia
- Higher DPD enzymatic activity in Korean subjects and lower enzymatic activity in African Americans compared to Caucasians
Thymidylate Synthase (TYMS)

- TYMS is the primary target of 5-FU chemotherapy
  - Also a target of capecitabine (Oral prodrug of 5FU)
- The expression of TYMS is linked to cellular sensitivity or resistance to 5-FU
- Cells with higher TYMS expression in 3R/3R genotype - less sensitive to 5FU, poorer outcomes

P-glycoprotein

- P-glycoprotein transports toxic substances or metabolites out of cells
  - Encoded by the ABCB1 gene
- Polymorphism of the ABCB1 gene influences p-glycoprotein expression
  - TT, CC, or TC gene variations
    - TT: lower p-glycoprotein activity/expression (increased concentration of the drug in the body)
  - Polymorphism may serve as a marker for predicting plasma concentrations of some medications
    - Digoxin
    - Cyclosporine
    - HIV protease inhibitors
    - Chemotherapeutic agents

Question

3. How will pharmacogenomics change the practice of pharmacy?
   a) Pharmacists will interpret the results from patient’s genetic tests and work with physicians to prescribe the most beneficial medications for patients
   b) Patients will receive genetic testing prior to being prescribed any medication
   c) Allow pharmacists to write prescriptions based on patient’s genetic information
   d) Pharmacists will no longer be of use due to the **

Question

4. Patient RS was started on warfarin 5mg daily and is monitored weekly in the anticoagulation clinic. Each week RS’s INR is subtherapeutic and RS’s weekly warfarin dose is increased. Which genetic polymorphism could account for RS’s increased warfarin requirements?
   a. VKORC1-AA
   b. CYP2C19*1
   c. CYP4F2

Technology

- Roche’s Amplichip CYP450 Test
  - Provides point of care testing for genetic variations for the CYP2C19 and 2D6 genes
- BinaxNOW® G6PD (Glucose-6-Phosphate Dehydrogenase)
  - Used for determining normal from deficient G6PD activity levels in whole blood
  - Samples which determine the patient to be G6PD deficient should be assayed using a quantitative G6PD test method to verify the deficiency
- Spartan RX CYP2C19
  - Point of care test that will identify the CYP2C19*2 allele variation that impairs clopidogrel’s metabolism
  - Only available in Germany, Austria, and Switzerland - company plans a US regulatory filing in 2011.
Technology

- **Oncotype DX Colon Cancer Assay**
  - Patients newly diagnosed with Stage 2 colon cancer
  - 12 gene assay
  - Information about the risk for colon cancer recurrence
  - May help indicate the need for adjunctive chemotherapy

- **Oncotype DX Breast Cancer Assay**
  - Women with node-negative, estrogen-receptor-positive invasive breast cancer
  - Post-menopausal women with node-positive, hormone-receptor-positive invasive breast cancer
  - 21 gene assay
  - Provides a prediction of chemotherapy benefit and 10 year recurrence to aid in adjuvant treatment decisions

Economics of Pharmacogenomics

- **Product Development Challenges**
  - Longer duration of study enrollment, smaller patient population
  - Increase cost of trials
  - Shorter duration of market exclusivity
  - Smaller market for the medication

- **Reimbursement**
  - Pricy testing
  - Who will pay?
    - Insurance companies, individuals, government

Ethics Related to Pharmacogenomics

- Who should get tested?
- How will patients be stratified?
- How will the distribution of genetic information be regulated?
- Physician and pharmacist responsibility to prescribe and dispense medications based on patient's genetic make-up

Technology

- **TheraGuide SFU-** test for polymorphisms of TYMS and DPYD
- **BRACAnalysis-** detects an inherited mutation on the BRCA1 or BRCA2 gene that may increase the probability of developing hereditary breast or ovarian cancer
- Mayo Medical Laboratories recently introduced Warfarin Sensitivity, Genotype, which is used to identify variants in both the CYP2C9 and VKORC1 genes.

Economics of Pharmacogenomics

- Health care cost savings from ineffective or harmful medications
- As time goes on, the cost of tests and medications will likely diminish and they will become more affordable
- There is no incentive for drug companies to develop drugs that would single out a large portion of the population, when they could develop a “blockbuster drug” for the masses

Patient Fear

- Genetic discrimination
- Insurance coverage of tests
- Will my genetic information be secured?
- Who will have access to my genetic make-up?
Legislation

- Genetic Information Nondiscrimination Act (GINA) prohibits U.S. insurance companies and employers from discriminating on the basis of information derived from genetic tests
  - Passed May 21, 2008
  - GINA does not cover a condition someone is experiencing symptoms for, being treated for, or that has been diagnosed
  - GINA does not protect one from genetic discrimination in life, disability, or long-term-care insurance

References


In Conclusion

Today’s Method

Hope for the Future

References

Questions?