Learning Objectives

- Review the current status of the genetics of drug metabolism.
- Assess pharmacogenomics and how it may be a useful tool in understanding individual differences in pain perception.
- Determine whether genetic testing has any current clinical value in the management and control of pain.
44-year-old male presents with severe acute pain of 2 days’ duration
- Initially given NSAIDs (ibuprofen, then celecoxib) for pain
  - Pain not controlled
  - Serum creatinine began to increase
- Switched to opioid (codeine) with minimal pain relief
- Given tramadol IM with no improvement
- Assessed as “drug seeker”

### Physiology of Pain
- Pain functions to alert us to dangers in the environment
- Categories of pain:
  - Nociceptive pain
  - Inflammatory pain
  - Neuropathic pain
  - Functional pain

### Genomics & Pain: Many Layers
- Nociception – Neural perception
- Inflammation
  - Post-transcriptional
  - Modulation of inflammatory mediators
- Chronic pain susceptibility – Systems biology, “web” approach necessary
- **Pharmacogenomics** – Drug metabolism

### Pharmacogenomics: What Is It?
- The study of genetic variability and its relationship to an individual’s response to pharmaceutical drugs, OTC medications, and nonprescription drugs
- Or, more simply,
- Using genetic information to determine whether a drug will make you ill or well
Pharmacogenomics vs. Pharmacogenetics

- Pharmacogenetics: The study of the relationship between individual gene variants and variable drug effects.
- Pharmacogenomics: The study of the relationship between variants in a large collection of genes, up to the whole genome, and variable drug effects.

Pharmacogenomics & Pain: Key Enzymes

- Cytochrome p450 enzymes:
  - CYP2D6
  - CYP2C9
  - CYP2C19
- µ-Opioid receptor (OMPR1)
- Multidrug transporter (ABCB1)
- Catechol-O-methyltransferase (COMT)
- Uridine diphosphate-glucuronosyltransferase (UGT)
- Guanosine triphosphate cyclohydrolase (GCH1)

Two Major Pathways of Metabolism & Detoxification

- **Phase I**
  - Hydroxylation reactions
  - Cytochrome p450 enzymes
- **Phase II**
  - Conjugation reactions

- **Fat-Soluble Toxin** → **Activated Intermediate** → **Water-Soluble Compound**
Pharmacogenomics & Pain: In Practice

44-year-old male presents with severe dental pain of 2 days’ duration ...
- Initially given NSAIDs (ibuprofen, then celecoxib) for pain
  - Pain not controlled
  - Serum creatinine began to increase
- Switched to opioid with minimal pain relief
- Given tramadol IM with no improvement
- Assessed as “drug seeker”

Pharmacogenomics & Pain: In Practice

34-year-old female presents with chronic pain of 2 years’ duration, also taking silymarin
- Initially given NSAIDs (ibuprofen, then celecoxib) for pain
  - Pain not controlled
  - Serum creatinine began to increase
- Switched to TCA with minimal pain relief
- Given trial of codeine, developed difficulty breathing and was “out of it” for 2 days
- Deemed “hysterical” and referred to psych

Adverse Drug Reactions

- At least 6% of new hospital admissions are due to various adverse drug reactions
  - 2 million people in the U.S.
  - > 100,000 deaths annually
  - 5th leading cause of death
- Costs of drug-related morbidity and mortality is approximately $177 billion annually
- Medicine “for the masses” kills people!

Personalized Medicine = Biochemical Individuality
Roger Williams first coined the term “biochemical individuality” in 1956 to explain genetic variability in disease susceptibility, nutrient needs, and drug responsiveness among otherwise seemingly healthy people.


Biochemical Individuality ... Not a New Concept
“Some men have constitutions that are like wooded mountains running with springs, others like those with poor soil and little water, still others like land rich in pastures and marshes, and yet others like the bare dry earth of the plain.”

Hippocrates, 5th century BC

Biochemical Individuality: From Theory to Practice
“Our analysis of metabolic disease that affects cofactor binding, particularly as a result of polymorphic mutations, may present a novel rationale for high-dose vitamin therapy, perhaps hundreds of times the normal dietary reference intake (DRI) in some cases. … Feeding high doses of the vitamin raises the tissue cofactor concentrations and thereby increases the activity of the defective enzyme.”


Gene-Environment Fact Sheet
Virtually all human diseases result from the interaction of genetic susceptibility and modifiable environmental factors ...

- Variations in genetic makeup are associated with almost all disease.
- Genetic variations do not cause disease but rather influence a person’s susceptibility to environmental factors.
- Genetic information can be used to target interventions.
The Structure of DNA

- The structure of DNA is complementary: two long strands of sugar-phosphate polymers cross-linked by two bases
  - One a purine
  - The other a pyrimidine
  - Like rungs on a ladder
- Adenine always linking with thymine
- Guanine always linking with cytosine

Genomics Basics

- DNA sequence – The particular order of bases that spells out the genetic code
- Chromosomes – Paired packages of DNA segments within the nucleus of each cell; 23 pairs in humans
- Allele – One of the variant forms of a gene; one half of a gene pair
- Polymorphisms – Variations in the genetic code along the chromosome
3 Billion Letters of Code in the Human Genome

DNA Genetic Code Dictates Amino Acid Identity and Order

DNA Sequence Is the Genetic Code.

GCA AGA GAT AAT TGT... Growing Protein Chain

Single Nucleotide Polymorphism

![Diagram of DNA replication, transcription, and translation]

![Diagram illustrating the Central Dogma of Molecular Biology]

![Diagram explaining Single Nucleotide Polymorphism (SNP)]
Facts about Polymorphisms

- The most common type of polymorphism is a single point mutation in the genetic code, known as a single nucleotide polymorphism, mercifully abbreviated to SNP (“snip”).
- SNPs are quite common, occurring about once every 1000 nucleotides.
- The average person has about 3 million SNPs.

“You’re not ill yet, Mr. Blendell, but you’ve got potential.”
Two Major Pathways of Metabolism & Detoxification

- Fat-Soluble Toxin → Activated Intermediate → Water-Soluble Compound

**Phase I**
- Hydroxylation reactions
- Cytochrome p450 enzymes

**Phase II**
- Conjugation reactions

- COMT
- UGT – glucuronidation

**CYP2C9**
- Polymorphism associated with reduced hydroxylation activity
- Responsible for the metabolism of many drugs:
  - NSAIDs
  - Anti-depressants (SSRIs, TCAs)
  - Azole antifungals
  - Sildenafil (Viagra®)


**Definitions: Substrate**

An agent that is metabolized by an enzyme into a metabolic end product and eventually excreted from the body

www.druginteractions.com
**Definitions: Inhibitor**
An agent that interferes with the ability of an enzyme to metabolize a given substrate
- Usually a competitive inhibition
- Coadministration of inhibitors leads to rapid increases in the blood levels of substrates or may inhibit the activation of a “pro-drug”
- Silymarin/milk thistle – Beware!

**Definitions: Inducer**
- An agent that causes an increase in the production of the enzymes responsible for metabolizing a particular substrate
- Inducers generally lead to a gradual decrease (1–3 weeks) in the blood level of a substrate

**Definitions: Poor Metabolizer**
Metabolic variability can lead to decreased metabolism of the substrate and much higher than expected blood levels at standard dosing patterns
- Defined by particular SNPs
- Can be an effect of inhibitors

**Definitions: Ultra-Metabolizer**
Metabolic variability due to multiple copies of the “wild-type” enzyme, leading to much faster enzymatic activity and lower than expected blood levels of substrate at standard dosing patterns
### Isozyme Table

<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Substrate</th>
<th>Inhibitor</th>
<th>Inducer</th>
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<tbody>
<tr>
<td>CYP2C9</td>
<td>Amtriptyline</td>
<td>Antibiotics</td>
<td>Rifampin</td>
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<tr>
<td></td>
<td>Angiotensin II Blockers</td>
<td>Azole Antifungals</td>
<td>Secobarbital</td>
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<td></td>
<td>Ibuprofen</td>
<td>Antibiotics</td>
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<td>Diclofenac</td>
<td>Metronidazole</td>
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<td>Isoniazid</td>
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<td>Fluvoxamine</td>
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<td>Glipizide</td>
<td>Paroxetine</td>
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<td>Sertraline</td>
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<td>Warfarin</td>
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<td>Sulfur drugs</td>
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<td>Silymarin/ Silybin</td>
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<td>Imipramine</td>
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<td>Cimetidine</td>
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<td>Isoniazid</td>
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<td>Ritonavir</td>
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<td>Zafirlukast</td>
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<td>Torsemide</td>
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<td>THC (marijuana)</td>
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<td>Sildenafil</td>
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### Text for Case Presentation

#### 44-year-old male presents with severe acute pain of 2 days’ duration

- Initially given NSAIDs (ibuprofen, then celecoxib) for pain
  - Pain not controlled
  - Serum creatinine began to increase
- Switched to opioid (codeine) with minimal pain relief
- Given tramadol IM with no improvement
- Assessed as “drug seeker”

### CYP2C19

- Responsible for the metabolism of many drugs:
  - Antiepileptics
  - PPIs
  - SSRIs
  - Estrogen (Premarin®)
  - Propranolol
  - Prednisone
  - Warfarin
  - And more …
**CYP2C19**

- Polymorphism associated with impaired activity of 2C19
- Lower doses of medications required for therapeutic effectiveness
- Rule out CYP2C19 inhibitors!

**CYP2D6**

CYP2D6 is involved in the detoxification of approximately 20% of all prescribed medications and > 50% of all psychotropic medications, including:

- TCAs
- MAOIs
- SSRIs
- Opiates
- Antipsychotics
CYP2D6 Variants
Over 35 SNPs have been identified on the CYP2D6 gene, with complex algorithms to determine:
- Poor (slow) metabolizers
- Intermediate metabolizers
- Extensive (normal) metabolizers
- Ultra-rapid metabolizers

CYP2D6 Phenotypes by Race (%)

<table>
<thead>
<tr>
<th>Ethnicity/Race</th>
<th>CYP2D6 Poor Metabolizer</th>
<th>CYP2D6 Ultra-Rapid Metabolizer</th>
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</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>5–10%</td>
<td>1–10%</td>
</tr>
<tr>
<td>East Asian</td>
<td>1%</td>
<td>0–2%</td>
</tr>
<tr>
<td>African-American</td>
<td>1–2%</td>
<td>2%</td>
</tr>
<tr>
<td>North African &amp; Middle Eastern</td>
<td>2%</td>
<td>10–29%</td>
</tr>
<tr>
<td>Mexican American</td>
<td>3%</td>
<td>1%</td>
</tr>
</tbody>
</table>


COX-2 Pathway
- CYP2C9
- CYP2D6

Morphine Pathway
- CYP2D6
- UGT2B7
- OPRM1
- ABCs TBD
  - ABCB1
  - ABCC2
  - ABCC3
44-year-old male presents with severe acute pain of 2 days’ duration
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44-year-old male presents with severe acute pain of 2 days’ duration
- Initially given NSAIDs (ibuprofen, then celecoxib) for pain
  - Pain not controlled
  - Serum creatinine began to increase
- CYP2C9*3 = Poor metabolizer of NSAIDs
  - Greatly increased exposure to NSAIDs
  - CYP2C9 inhibitors to be used with caution
  - Celecoxib inhibits CYP2D6
  - Caution with drugs metabolized by CYP2D6

44-year-old male presents with severe acute pain of 2 days’ duration
- NSAIDs
  - Ibuprofen CYP2C9
  - Celecoxib CYP2C9
- Opioid (codeine) CYP2D6
- Tramadol CYP2D6

44-year-old male presents with severe acute pain of 2 days’ duration
- Switched to opioid (codeine) with minimal pain relief
- Given Tramadol IM with no improvement
- Both drugs are metabolized through CYP2D6; patient is a poor metabolizer
  - Decreased activation of codeine to morphine
    - 5–10% of Caucasian population will have little benefit
    - Morphine is more effective (downstream from CYP2D6)
  - Tramadol is also converted to its active metabolite via CYP2D6
CYP2D6: Poor Metabolizers
Poor metabolizers have decreased ability to hydroxylate a wide variety of drugs.
- Lower dosages may be required to prevent toxicity.
- Opioids and Tramadol require CYP2D6 for conversion to more active metabolites.
- Watch out for inhibitors ...

CYP2D6: Ultra-Rapid Metabolizers
Ultra-rapid metabolizers have increased ability to hydroxylate a wide variety of drugs.
- Higher dosages may be required to ensure efficacy of many drugs.
- Exaggerated pharmacologic effects, including opioid intoxication, can occur with normal doses of codeine.
- Watch out for inducers ...

44-Year-Old Male with Acute Pain: Personalized Medicine
- Avoid NSAIDs
  - Poor metabolism with CYP2C9
  - Further inhibit CYP2D6
- If opioids required, use oxycodone (or morphine)
  - Metabolized through CYP3A4, along with CYP2D6
  - Binds to μ-opioid receptor much more than codeine
- Evaluate sensitivity to pain
  - COMT – support with methyl groups
  - UGT – support with cruciferous vegetables and avoid PAH
  - GCH1 – support tetrahydrobiopterin (BH4) with folic acid and vitamin C
  - OMPR1

Two Major Pathways of Metabolism & Detoxification

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Phase I
- CYP2C9
- CYP2C19
- CYP2D6
- CYP3A4/5

Phase II
- COMT – methylation
- UGT – glucuronidation
Catechol-O-Methyltransferase

- COMT inactivates catecholamines and catechol drugs such as L-dopa.
- COMT is involved in the regulation of pain perception. Decreased activity leads to:
  - Increased pain sensitivity
  - Increased incidence of chronic pain and fibromyalgia syndrome
- Polymorphism is associated with low COMT activity (4–7x decreased for +/+).

COMT: Intervention

- Limit alcohol intake.
- Support methionine metabolism.
  - B vitamin support; ensure proper circulation of methionine production from homocysteine
  - SAMe supplementation
- Include antioxidants (quinones).
- Avoid excess weight and stress.

Uridine Diphosphate-Glucuronosyltransferase

- UGT glucuronidates opioids, increasing their binding affinity to opioid receptors.
- UGT is involved in the activation of analgesia. Decreased activity leads to decreased response to opioid pain medicine.
- Polymorphism is associated with lower UGT activity (2–20x decreased for +/+).

“We investigated the variation in the uridine diphosphate-glucuronosyltransferase 2B7 (UGT2B7) gene in patients receiving patient-controlled analgesia with morphine. UGT2B7 was sequenced in phenotypic extremes (n = 12) of the distribution of morphine-6-glucuronide/morphine plasma ratios. A new -161C/T promoter variant was in complete linkage disequilibrium with the 802C/T variant and was more frequent in low glucuronidators (P = .039). Both variants were genotyped in all patients (n = 86), and complete linkage disequilibrium was confirmed.”

“Trend analysis showed reduced morphine-6-glucuronide/morphine ratios in patients with T/T, C/T, and C/C genotypes (T/T > C/T > C/C) (P = .031). Morphine levels were lower in T/T patients (median, 18 ng/mL [range, 18-1490 ng/mL]) as compared with C/T and C/C patients combined (median, 66 ng/mL; range, 18-3995 ng/mL) (P = .04). Morphine-6-glucuronide and morphine-3-glucuronide concentrations were significantly lower in C/C patients (median, 18 ng/mL; range, 0-66 ng/mL; and median, 152 ng/mL; range, 30-434 ng/mL; respectively) compared with C/T and T/T patients combined (median, 43 ng/mL; range, 0-193 ng/mL; and median, 242 ng/mL; range, 33-1381 ng/mL; respectively) (P = .045 and P = .004, respectively).

Interindividual differences in morphine glucuronidation may be the result of genetic variation in UGT2B7, and further studies are indicated.”

**Gene Affects Pain Sensitivity**

- GCH1 is a rate-limiting enzyme for BH4 synthesis, which is a key modulator of neuropathic and inflammatory pain.
- BH4 is an essential cofactor for catecholamine, serotonin, and nitric oxide production.


**Identifying Genes Related to Neuropathic Pain**

**Gene microarrays**
- Rapid identification of genes expressed in damaged neurons
- Compared to genes expressed in non-damaged neurons

**Nerve Injury, GCH1 & BH4**

- Axonal injury and peripheral inflammation
  - BH4 concentrations increased in primary sensory neurons and in the dorsal root ganglion
  - Upregulation in GCH1
- Injecting BH4 into rats enhanced pain sensitivity
- Inhibiting de novo BH4 synthesis in rats increased neuropathic and inflammatory pain

GCH1 SNPs in Human Populations

- Specific variant of the gene that encodes for GCH1 protected against the risk of chronic pain in people who had surgery for a herniated disc.
- 28% of the people in the study had at least one copy of the pain-protective variant of the gene.
  - People with two copies of the protective version of GCH1 had lowest risk of developing chronic pain.
  - Those with one copy had an intermediate risk.
  - Those with no copy had the highest risk.


BH4 & GCH1 SNPs

- Changes in BH4 concentrations modify the sensitivity of the pain system.
- SNPs in the gene GCH1, which alter BH4 concentrations, alter both responses in healthy humans to noxious stimuli and the susceptibility of patients to develop persistent neuropathic pain.

Pain Protective Haplotype in GCH1

Involvement of BH4 in both inflammatory and neuropathic pain may explain why sensitivity to acute experimental pain is a predictor of postsurgical and eventually chronic pain.

GCH1 & Neuropathic Pain

- We inherit the extent to which we feel pain
  - Under normal conditions
  - After damage to the nervous system
- Identify subpopulations who are at risk for developing chronic pain
- Identify treatment options based on genotype
- Nutrigenomics: Appropriate antioxidants and anti-inflammatory supplements to influence gene expression and protect tissues
Sympathetic Nerve Pathway
Cholinergic Receptors
- CHRNA3
- CHRNA7
- CHRNB4
+ COMT
+ GCH1

COX-2 Pathway
- CYP2C9
- CYP2D6

Morphine Pathway
- CYP2D6
- UGT2B7
- OPRM1
- ABCs
- TBD
  - ABCB1
  - ABCC2
  - ABCC3

Post-Genomic Pain Management
- Identify the mechanism and molecular components rather than trial and error
- Personalize treatment with evaluation of genomic SNPs
  - Is it inflammation?
  - Is it increased sensation?
  - What medicines work well with this person?
- Support parallel pathways
- Avoid supplements and medicines that utilize pathways functioning abnormally
Pharmacogenomics & Pain: In Practice

34-year-old female presents with chronic pain of 2 years’ duration, also taking silymarin
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Pharmacogenomics & Pain Management

- Review the current status of the genetics of drug metabolism.
- Assess pharmacogenomics and how it may be a useful tool in understanding individual differences in pain perception.
- Determine whether genetic testing has any current clinical value in the management and control of pain.
Using Pharmacogenomics in Pain Management: Ready for Prime Time?

Patrick J. Hanaway, MD
Asheville, NC