Genetics and Toxicity

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Objectives

1. Identify genotypes that place patients at increased risk for toxicity following various exposures.

2. Describe the metabolic consequences and clinical manifestations of patients with genetic predisposition to toxicity following exposure to certain agents.

3. Recognize when genetic testing can be useful and should be performed.
Patient MS

• Previously healthy male
• Admitted for acute appendicitis
• During the surgery became hypertensive, hyperthermic, and had severe rhabdomyolysis
• Diagnosed clinically with malignant hyperthermia
Malignant Hyperthermia

- Autosomal dominantly inherited
- No symptoms unless exposed to certain medications
- Can be lethal if not properly recognized and treated
- Subsequence genetic evaluation demonstrated a mutation in RYR1
Baby CP

• Four week old baby girl comes into the Emergency Room, sleepy and difficult to arouse and sweaty

• She has been feeding breast milk/formula and has been well except for constipation

• Blood tests show a glucose of 35 mg/dl (low) with other results being normal
Baby CP

• On initial hospitalization she received IV glucose and responded well with normalized glucose

• She did well until 6 months of age when the babysitter saw her sweaty and “zoned out” after a bottle of apple juice. In retrospect, mother had treated her constipation with prune juice before the first episode of hypoglycemia

• In the emergency room she was found to have a glucose of 30 mg/dl and responded to IV glucose

• She had no hepatomegaly
Metabolism of Fructose

- Phosphorylated and split into two 3-carbon fragments
- DHAP is ready to continue on in glycolysis
- Glyceraldehyde is phosphorylated to GAP
Genetics of Hereditary Fructose Intolerance

- Autosomal recessive
- Prevalence of 1:20,000 in Europe
- Often clinically silent
- Avoidance of fructose, sucrose, sorbitol (hidden in lots of foods)
- Vitamin C supplementation
- Supportive care when necessary
  - IV glucose
- Normal growth and development with good metabolic control
- As children grow older, they are able to avoid foods that make them feel ill
Baby OT

• Full term baby boy who was doing well until immediately prior to discharge at ~ 30 hours of life

• He became lethargic requiring stimulation for feeding

• His temperature fell to 97 and he became progressively more lethargic

• Initial labs showed a blood urea nitrogen of 3 (low)
Baby OT

• An ammonia was measured and was 1503 mg/dl
• Diagnostic metabolic testing was performed and confirmed a urea cycle defect
OTC Deficiency

- Symptoms include lethargy, vomiting, hyperammonemia, coma, rapidly progressing to death.
- May present with an infant male with lethargy, hypothermia who stops breathing.
- Encephalopathy leads to cerebral edema due to intraglial accumulation of glutamine.
What is the biochemical signature of OTC deficiency?

- Low citrulline and high orotic acid
- Increased glutamine and decreased arginine
Urea Cycle
Treatment of OTC Deficiency

- Restriction of dietary protein intake.
- Arginine becomes an essential amino acid and must be supplemented.
- Sodium phenylbutyrate activates synthesis of phenylacetylglutamine to serve and nitrogen waste product.
- Liver transplantation is curative
- Even if you are able to treat infants without symptoms from birth, still residual developmental delays
Treatment of Urea Cycle Disorders

(a) Benzoic acid

(b) Phenylacetic acid

\[
\begin{align*}
\text{COOH} & \quad \xrightarrow{\text{CoA ATP}} \quad \text{Benzoyl CoA} & \quad \xrightarrow{\text{Glycine}} & \quad \text{NHCH}_2\text{COOH} \\
\text{CH}_2\text{COOH} & \quad \xrightarrow{\text{CoA ATP}} \quad \text{Phenylacetyl CoA} & & \quad \text{NHCH}_2\text{COOH}
\end{align*}
\]
Types of Genetic Tests

• Metabolic testing
  • Best performed when decompensated
  • Ammonia, glucose, amino acids, organic acids, acylcarnitine profile, CSF for neurotransmitters

• Molecular genetic targeted tests
  • Sequencing of a specific gene when there is a high index of suspicion
  • Pharmacogenetics

• Panel gene testing
  • Sequencing of several genes simultaneously when the clinical diagnosis is suggestive of one of a group of disorders (glycogen storage disorder, mitochondrial disorder)

• Whole exome sequencing
  • Covers all your bases when you really don’t know
Genetic Test Results

• Positive
• Negative
• Variant of uncertain clinical significance
wpod?am fkew cu.gjhklf four sjckfo qw score
and void m$%d jkkk yp@mvjckd fkseo
cbqw,oijwsfm du seven years ago dllfkk*wqm
fkkd xmmcnfyruuei our skkdj$fmvjkjdfk&%wo
qppalfkkf qa.q.d eiidty forefathers brought jjd
qpoooekfjg vbxzg dsf forth a
Exome sequencing

• Sequencing of the exome (all coding exons of all genes)
  • ~1.5% of the genome (30Mb)
  • ~20,500 genes
• Capture of the exons
• Sequence using NextGen technology
• Generates a massive amount of data which needs to be filtered
Indications for Clinical Exome Sequencing

- Patients who have undergone an extensive diagnostic odyssey, with no molecular basis identified
  - Individual gene tests negative
  - Targeted panels negative
- Patients with a clinical phenotype that could be explained by one of many, many genes (ID/cognitive disability/developmental delay) where sequencing each individual gene is prohibitive
- Higher yield if familial condition and/or consanguinity
- Severe disorder in a child with no known family history
- Results can be obtained STAT when necessary within a week
- Can be done post mortem as a molecular autopsy
DNA Banking

- DNA sample stored for future use
- Can be saved for many years
- Alternative for uninformative families
- Consider when testing is currently unavailable
  - especially for patient at risk for premature death
“Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy”

Paracelsus
Drug Levels Determined by

• Absorption
• Distribution
• Metabolism
• Excretion
## Factors that affect drug levels/effects

<table>
<thead>
<tr>
<th>Patient factors</th>
<th>Pathophysiology</th>
<th>Environment</th>
<th>Genetics</th>
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<tr>
<td>Age</td>
<td>Liver Function</td>
<td>Drug Therapy</td>
<td>Drug-metabolizing Enzymes</td>
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<td>Sex</td>
<td>Kidney Function</td>
<td>Smoking Status</td>
<td>Drug transporters</td>
</tr>
<tr>
<td>Weight</td>
<td>Cardiovascular Function</td>
<td>Alcohol Consumption</td>
<td>Drug Receptors</td>
</tr>
<tr>
<td>BMI</td>
<td>Lung Function</td>
<td>Nutrition</td>
<td>Ion Channels</td>
</tr>
<tr>
<td>Other diseases</td>
<td>Pollutants</td>
<td>Target enzymes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Occupation</td>
<td>Signal transduction</td>
<td></td>
</tr>
</tbody>
</table>
Genes Involved in Phase I and II of drug metabolism
CYP2D6

- Metabolizes 25% of drugs
- 57 enzymes
- CYP1, CYP2, and CYP3 oxidize most drugs
- Different phenotypes
  - Poor metabolizers
  - Extensive metabolizers
  - Ultrarapid metabolizers
- 80 genetic variants, but 6 account for 95% of variation
- Ultrarapid metabolizers have gene amplifications (2-13 extra copies) and may require much high doses of medication for efficacy
- Poor metabolizers are more likely to have problems with drug interactions.
- Codeine is a prodrug and must be converted to active morphine
  - Significant variation in dose required and some issues with post-partum dosing
Drug Transporters

• Efflux transporters: ABC cassette family
• Multidrug resistance proteins
• Bile salt export pump
• Organic anion transporters
• Organic cation transporters
• Peptide transporters
Clinical Pharmacogenetics

- FDA-approved medications ($n = 1,200$)
  - Affected by actionable pharmacogenes: 7%
  - Not affected by actionable pharmacogenes: 93%

- Prescriptions in the United States ($n = 4$ billion)
  - Affected by actionable pharmacogenes: 18%
  - Not affected by actionable pharmacogenes: 82%
Alcohol Dehydrogenase (ADH)

- Oxidases ethanol to acetaldehyde which is metabolized by Aldehyde Dehydrogenase (ALDH)
  - Variants in ALDH genes (ALDH2) are also related with alcohol dependence
Glucose 6 Phosphate Dehydrogenase

• X linked
• Most common enzyme deficiency
• Enzyme in pentose phosphate pathway and necessary to make reducing equivalents important in the red blood cells with oxidative stress
• Increased sensitivity to primaquine (anti-malarial) resulting in acute hemolytic anemia
• Newborn screening in some programs
Acute Intermittent Porphyria

• Autosomal dominant
• Intermittent neurological symptoms
• Due to a deficiency or porphobilinogen deaminase (needed to make heme)
• Due to a mutation that alters regulation of gene expression by ~50%
• Usually asymptomatic until a drug is used that decreases heme concentrations (barbiturates)
Isoniazid

- Treatment of tuberculosis
- Enzyme NAT2 acetylates the drug
- Slow metabolizers (acetylators) have increased neuropathy
Succinylcholine

• Neuromuscular relaxant
• 1/3500 Caucasians is homozygous for an abnormal butyrylcholinesterase producing reduced ability to metabolize succinylcholine
• Differences in cholinesterase activity lead to prolonged drug effects after anesthesia
### Examples of Effects of Gene Polymorphisms on Drug Response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Enzyme/Target</th>
<th>Drug</th>
<th>Clinical Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>Cytochrome P450 2D6</td>
<td>Codeine</td>
<td>Individuals homozygous for an inactivating mutation do not metabolize codeine to morphine and thus experience no analgesic effect</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Cytochrome P450 2C9</td>
<td>Warfarin</td>
<td>Individuals heterozygous for a polymorphism need a lower dose of warfarin to maintain anticoagulation</td>
</tr>
<tr>
<td>NAT2</td>
<td>N-Acetyl transferase 2</td>
<td>Isoniazid</td>
<td>Individuals homozygous for &quot;slow-acetylation&quot; polymorphisms are more susceptible to isoniazid toxicity</td>
</tr>
<tr>
<td>TPMT</td>
<td>Thiopurine-S-methyltransferase</td>
<td>Azathioprine</td>
<td>Individuals homozygous for an inactivating mutation develop severe toxicity if treated with standard doses of azathioprine</td>
</tr>
<tr>
<td>ADRB2</td>
<td>β-Adrenergic receptor</td>
<td>Albuterol</td>
<td>Individuals heterozygous for a polymorphism get worse with regular use of albuterol</td>
</tr>
<tr>
<td>KCNE2</td>
<td>Potassium channel, voltage-gated</td>
<td>Clarithromycin</td>
<td>Individuals heterozygous for a polymorphism are more susceptible to life-threatening arrhythmias</td>
</tr>
<tr>
<td>SUR1</td>
<td>Sulfonylurea receptor 1</td>
<td>Sulfonylureas</td>
<td>Individuals heterozygous for polymorphism exhibit diminished sensitivity to sulfonylurea-stimulated insulin secretion</td>
</tr>
<tr>
<td>F5</td>
<td>Coagulation factor V (Leiden)</td>
<td>Oral contraceptives</td>
<td>Individuals heterozygous for a polymorphism are at increased risk for venous thrombosis</td>
</tr>
<tr>
<td>Gene</td>
<td>Drug</td>
<td>Implication</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Thiopurine methyltransferase</td>
<td>6MP</td>
<td>Homozygotes require 10% drug dose to avoid bone marrow suppression</td>
<td></td>
</tr>
<tr>
<td>UGT1A1</td>
<td>Irinotecan</td>
<td>Increased toxicity with standard dosing</td>
<td></td>
</tr>
<tr>
<td>N-acetyltransferase</td>
<td>Isoniazid, hydralazine, procainamide</td>
<td>Slow acetylators may have increased toxicity</td>
<td></td>
</tr>
<tr>
<td>Dihydropyrimidine dehydrogenase</td>
<td>5-FU</td>
<td>toxicity leads to seizures, psychomotor retardation</td>
<td></td>
</tr>
<tr>
<td>SULT1A1</td>
<td>Tamoxifen</td>
<td>Alters dosing</td>
<td></td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Several drugs</td>
<td>Alters dosing</td>
<td></td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methotrexate</td>
<td>T677 increases toxicity</td>
<td></td>
</tr>
<tr>
<td>VKORC1</td>
<td>Warfarin</td>
<td>Alters dosing</td>
<td></td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Warfarin</td>
<td>Alters dosing</td>
<td></td>
</tr>
</tbody>
</table>
Comparison of Diagnostic Test Criteria of a Selection of PGx Tests and non-PGx Tests Used in Clinical Practice

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Biomarker</th>
<th>Associated Effect</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGx tests</td>
<td>Carrier of a CYP2C9 and VKORC1 polymorphism</td>
<td>Acenocoumarol-induced overanticoagulation (INR&gt;6)</td>
<td>226</td>
<td>0.48</td>
<td>0.81</td>
<td>0.20</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>5-lipoxygenase (Alox5) genotype</td>
<td>Response to leukotriene antagonist ABT761</td>
<td>221</td>
<td>1</td>
<td>0.17</td>
<td>0.52</td>
<td>1</td>
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<tr>
<td></td>
<td>UGT1A1-3156AA genotype</td>
<td>Grade 4 neutropenia and irinotecan in whites</td>
<td>66</td>
<td>0.50</td>
<td>0.96</td>
<td>0.60</td>
<td>0.95</td>
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<tr>
<td></td>
<td>β1 receptor Arg389Arg genotype</td>
<td>Reduction in daytime diastolic blood pressure</td>
<td>40</td>
<td>0.78</td>
<td>0.82</td>
<td>0.78</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>HLA-B*5701 genotype</td>
<td>Hypersensitivity to abacavir in whites</td>
<td>1821</td>
<td>0.46-0.94</td>
<td>0.90-0.98</td>
<td>0.19-0.81</td>
<td>0.97-0.99</td>
</tr>
<tr>
<td>Non-PGx tests used in clinical practice</td>
<td>Prostate specific antigen (&gt;4.0 ng/ml)</td>
<td>Prostate cancer</td>
<td>284</td>
<td>0.68-0.75</td>
<td>0.6-0.71</td>
<td>0.51-0.54</td>
<td>0.73-0.87</td>
</tr>
<tr>
<td></td>
<td>Troponin T (&gt; 0.1 ng/ml)</td>
<td>Acute myocardial infarction</td>
<td>773</td>
<td>0.94</td>
<td>0.89</td>
<td>0.36</td>
<td>1</td>
</tr>
</tbody>
</table>

The Industry Pharmacogenomics Working Group (I-PWG) is a voluntary and informal association of pharmaceutical companies engaged in research in the science of pharmacogenomics. The Group was initially established in response to regulatory requests for non-competitive information from industry about such research. The Group provides information, sometimes in the form of publications in peer-reviewed journals, and sponsors educational and informational programs intended to promote a better public understanding of pharmacogenomic research. The Group’s discussions, activities, and programs are open and transparent and are limited exclusively to non-competitive matters. Topics include educational, informational, ethical, legal, and regulatory matters. Organizational and procedural rules have been adopted by the Group and by the companies they represent.

The I-PWG follows closely the activities of the US Food and Drug Administration (FDA), European Medicines Evaluation Agency (EMEA) other regulators and policy groups to ensure that its activities are relevant to their programs and needs. Among other steps, The I-PWG seeks to engage these bodies in discussion and information sharing and asks their continued assistance in identifying non-competitive issues about which the Group can provide information or other support.
Clinical Implementation

CPIC guideline number of drugs metabolized by gene

Abacavir
Allopurinol
Carbamazepine
Phenytoin
Amitriptyline
Citalopram, escitalopram
Clopidogrel
Warfarin
Codeine
Azathioprine
Mercaptopurine
Fluorouracil (DPYP)
Ivacaftor
Rasburicase
Simvastatin
Tacrolimus
Peginterferon alfa-2a/2b
Ribavirin
Barriers in Clinical Implementation

• Education of HCPs / EMR decision support
• Logistics of ordering and insurance coverage
• Cost effectiveness
  • There are discussions and contradictory studies
Conclusion

• Inborn errors of metabolism should be considered in circumstances of hypoglycemia, lactic acidosis, metabolic acidosis, acute liver failure, hyperammonemia, especially in children

• Toxic effects of drugs may be dependent on the genome of the host

• Genetic testing is available to clinically determine if there is a genetic contribution to the clinical symptoms, and a benign family history should not dissuade you from genetic testing

• Call your local geneticist if in doubt