Inherited Disorders and Pharmacogenetics

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Introduction – Inherited Disorders

- Mendelian
- Autosomal dominant, Autosomal recessive, X-linked
- Each disorder caused by one or a few (or more) genes
- Diagnostic, prognostic, impact patient management
Benefits to genetic testing for inherited disorders

- Confirmed diagnosis in clinically suspected cases
- Accurate diagnosis for genetic disorders with phenotypic overlap with other genetic and non-genetic disorders
- Patient management implications
- Allows for testing of at-risk family members (MEDPED)
- In some cases, patient will be more adherent to treatment regimen if they know there is a genetic basis for their clinical presentation
- Gene-tailored management strategies
  - Treatment
  - Imaging
  - Intervention
  - Surgery
Inherited Disorders: Examples

- Cystic fibrosis
  - Carrier screening
  - Diagnostic test
- Duchenne muscular dystrophy
- Factor V Leiden thrombophilia
- Fragile X syndrome
- Hereditary hemochromatosis
- Marfan syndrome
- Prader-Willi/Angelman syndrome
- Spinal muscular atrophy
- Tay Sachs disease
- Hereditary cancers
Introduction – Pharmacogenetics

- a.k.a. Pharmacogenomics
- A component of individualized medicine
- Focuses on how genetic factors influence individual responses to different medications
  - Efficacy, side-effects, adverse events
- Clinical goals
  - Minimize adverse drug events (ADEs)
  - Maximize drug efficacy
  - Testing used as an aid to clinicians in determining therapeutic strategy
- “The Right drug, for the Right patient, at the Right dose, the First time”
How are PGx and Inherited Disorders Different?

- Inherited disorder testing involves looking for pathogenic variants that cause the disorder
  - Oftentimes, the variants are not known in advance
    - Sequencing
  - Genetic test results will often have implications for family members
  - Laboratory director must be board certified in genetics
- PGx testing involves looking for usually benign (i.e. non-disease causing) variants associated with altered response to medication
  - “Polymorphisms”
  - Most of the time, the variants are known in advance
    - Targeted variant analysis
  - Genetic test results will sometimes have implications for family members
  - Test results are generally needed in a much more rapid timeframe compared to inherited disorders
Determining whether genetic testing might benefit your patient population

- Will the results of a genetic test
  - Confirm a diagnosis?
  - Change a treatment plan?
  - Give information about prognosis and management?
  - Allow presymptomatic testing?
  - Provide an accurate assessment of genetic risk?
Setting up a Molecular Lab

- Space requirements
- Equipment
- Personnel
- Testing Menu
- Centralized or de-centralized
Space Requirements

- Space needs and lab design are dependent on multiple variables
  - Test menu
  - Number of technologists/personnel
  - Types of methods utilized
    - PCR, targeted mutation analysis, sequencing, large deletion/duplication detection methods, etc.
Space Requirements

- Start-up lab
  - 2-3 techs
  - Several PCR-based assays
  - 500-1000 sq ft dedicated space

- More established lab
  - 5+ techs
  - More diversified menu of testing
  - At least 1000-1500 sq ft dedicated space
Resources and Facilities

- Temperature-dependent equipment must be maintained at optimal temperatures and monitored and documented at appropriate intervals.
- The laboratory must be compliant with all relevant safety codes.
- The laboratory must participate in mandated laboratory inspection as required by state and federal regulations.
- All laboratory equipment should be maintained at appropriate intervals.
- Records of maintenance and repair must be kept as long as the equipment is in use.
- Adequate facilities for record storage must be available.
- Laboratories may subcontract to another laboratory as long as the subcontracting laboratory meets all applicable guidelines and standards including CLIA ‘88.
  - The identity of the subcontracting laboratory and contribution to the test must be clearly indicated in the report.

Standards and Guidelines for Clinical Genetics Laboratories, American College of Medical Genetics, www.acmg.net
Organization formats

- Special requirements for PCR-performing labs
- Unidirectional work-flow
- Physically separate labs
  - Reagent prep
  - Specimen receipt and prep
  - PCR set-up and amplification
  - Amplified DNA product analysis
- At a minimum, separate pre- and post-PCR areas
Reagent Prep area

- If LDT PCR-based methods are used, reagent prep must be separated from other areas of lab
- If commercial kits are used, less stringent physical separation since reagents are already prepped
- Dedicated equipment
- Master mixes prepared and aliquotted
- Periodic QC to rule-out contamination
Specimen Receipt and Accessioning Area

- Isolated from testing areas
- Original specimen containers accepted
  - Avoid splitting or pouring off original specimens
- Gloves, lab coats, safety glasses by accessioning personnel
- Assign unique identifiers – barcoding
- No freeze-thaw cycles
- Laboratory should have written criteria for acceptance/rejection of specimens
- Designated area for retrieval by testing personnel
Specimen Submission and Handling

- Provide users with instructions on specimen collection, handling, transport, and submission
  - Specimen stability and criteria for rejection of specimens
- Specimen containers arriving in the laboratory must include two identifiers (e.g. patient’s name, date of birth, lab number, etc.)
  - Date of specimen collection should be included
- Specimen transport and handling must be in accordance with OSHA guidelines
- Intake information to accompany the specimen must include sufficient clinical information to ensure appropriate and accurate testing and interpretation of results
- Informed consent should be obtained as required by law and professional standards
- The laboratory should retain the original patient sample until all testing is completed and the report has been signed out
- De-identified patient specimens can be reused for QA/QC and for test development as allowed by the IRB

1. Standards and Guidelines for Clinical Genetics Laboratories, American College of Medical Genetics, [www.acmg.net](http://www.acmg.net)
2. Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions, CDC, 2009
Specimen Prep Area

- DNA extraction equipment will depend on
  - Specimen type
  - Volume of samples received
  - Turn-around-time
  - Molecular weight of DNA needed for testing
    - Some tests require high MW DNA
- Need to establish cut-off times for
  - Receipt of samples in relation to time of DNA extraction
  - DNA extraction in relation to test set-up
- RNA extraction?
- Tissue testing
- Analysis of quality, quantity/concentration, and size of nucleic acid can be performed by spectrophotometry, fluorescent dyes, and electrophoresis
Specimen Type—Inherited Disorders

- Whole blood (usually EDTA)
- Buccal or saliva
- Other specimens to consider
  - Prenatal specimens and/or Products of conception?
    - Require facilities to grow the cells and extract DNA
    - Maternal cell contamination analysis methodology required
- Blood spots
- Tumor tissue
Specimen Type – PGx

- Whole blood (usually EDTA)
- Buccal
  - Convenience sample
  - May be useful for transplant patients
  - May not work as well for high complexity analyses
- Saliva
  - Convenience sample
Nucleic Acid Storage

- Best stored as multiple aliquots in separate tubes
- Avoid freeze/thaw cycles
- Purified DNA should be stored in TE buffer at
  - 4°C for <1 week
  - -20°C for longer term storage
  - -80°C for indefinite storage
PCR Set-up and Amplification

- PCR is utilized in most tests for inherited disorders and PGx
- Involves amplification of the targeted region of interest
- End result in an exponential increase in total number of target DNA copies
- Contamination can be a problem if proper cautionary measures are not taken
Isolate potentially contaminating aerosols

- Unidirectional work-flow: never bring amplified DNA into the PCR setup area
- Use a positive displacement pipet or aerosol-resistant pipet tips
- Controlled airflow: positive or neutral pressure in the labs
  - Post-amplification lab should have negative pressure
- Vestibules and magnetic interlocks
- Exhaust to outside air from post-amplification area
- HEPA filters of outside air drawn into facility to minimize potential of recirculation of contaminants
- UV light fixtures for nightly treatment of work surfaces
- Dead air boxes with UV lights for individual PCR set-ups
Post-PCR

- Analysis of amplified DNA
- Targeted variant analysis
  - TaqMan RT-PCR
  - Bead-based capture/fluorescence detection (e.g. Luminex)
  - LightCycler
  - Capillary electrophoresis to detect size-based variants
- Sequencing
- Copy Number Variant analysis
  - MLPA
  - Southern
  - arrayCGH
Records

- All patient test records must be accessible to the laboratory director
- Files should be retrievable by both patient name and a second unique identifier
- Laboratory records should only be released with appropriate authorization
- Critical records of genetic testing are kept for 20 years (CLIA says 25 years)

Standards and Guidelines for Clinical Genetics Laboratories, American College of Medical Genetics, www.acmg.net
Personnel

- Staff size
  - Sufficient staff must be available to ensure accuracy of results with prompt and proficient performance of tests and reporting of results

- Laboratory Director and/or Laboratory Technical Supervisor
  - Doctoral degree with at least 2 years of postdoctoral training in clinical laboratory subspecialty
  - American Board of Medical Genetics certification or eligibility
  - American Board of Pathology subspecialty certification in MGP
  - Canadian College of Medical Geneticists certification or eligibility
  - Laboratory director must be on site regularly (at least weekly)

- Laboratory or General Supervisor (molecular genetics)
  - Must have at least 3 years of experience in a clinical molecular genetics laboratory

Standards and Guidelines for Clinical Genetics Laboratories, American College of Medical Genetics, www.acmg.net
Personnel

- Clinical Laboratory Technologist/Technician
  - Undergraduate degree in relevant field and 5+ years relevant lab experience
- Laboratory personnel must
  - Maintain an ongoing QA/quality improvement program
  - Be compliant with all applicable laboratory regulations
  - Have appropriate education, experience, and training
  - Establish and maintain appropriate procedures to ensure the privacy and security of patient identity and all patient information

Standards and Guidelines for Clinical Genetics Laboratories, American College of Medical Genetics, www.acmg.net
Testing Menu – Inherited Disorders

- Considerations
  - Market
  - Local patient needs
  - Potential volume

- Methodology
  - Targeted variant analysis or whole gene analysis
  - Which types of variants do you need to detect?
    - Point mutations
    - Large gene deletions/duplications
      - Usually requires higher molecular weight DNA
  - Does gene methylation need to be detected?

- Mode of inheritance
  - Impact on analysis and reporting
  - Carrier testing vs. diagnostic testing for autosomal recessive and X-linked
  - Gender-specific comments for X-linked
Inherited Disorder Test Example: Cystic Fibrosis (CFTR)

**Background**

- Cystic Fibrosis is one of the most common autosomal recessive disease in the Caucasian population
- 1:2500 – 1:3300 live births
- Characterized by viscous mucous in the lungs, involvement of digestive and reproductive systems and sweat glands (excess salt loss)
- Pulmonary disease is a critical factor in prognosis/survival
  - Pulmonary infections (recurrent, persistent) lead to respiratory failure
- Neonatal meconium ileus occurs in 10-20% of newborns with CF
- Pancreatic sufficient and insufficient forms exist
- Overall survival of CF is approx 30 years
- Due to mutations in CFTR
Inherited Disorder Test Example: Cystic Fibrosis (*CFTR*)

- **CFTR**
  - ATP-binding cassette family of transporters
  - Abnormal protein results in defective electrolyte transport and chloride ion transport in epithelial cells
  - Over 1500 mutations in the gene
  - Major mutation, deltaF508, 31-72% of CF mutations (depending on ethnicity/race)
- ACMG recommended carrier screening panel (23 mutations)
  - Based on mutation frequency in non-Hispanic Caucasians and Ashkenazi Jewish population
  - Includes mutations that have ≥ 0.1% frequency in general population
Clinical applications for tests
- Carrier screening (Preconceptual and Prenatal Carrier Screening)
- Diagnostic
- Familial mutation testing

Relatively high volume

Methods
- Targeted mutation analysis
- Sequencing

Interpretation
- Important to know ethnicity of patient being tested in order to provide residual risk calculation for patients who test negative
Testing Menu – Pharmacogenetics

- **Considerations**
  - Review the FDA website of biomarkers, Pharm GKB list of drug-gene pairs, and the literature
  - Focus on pharmacogenomic targets which are broadly supported by the literature, FDA, Pharm GKB and which actually help patients
  - Market

- **Ranking process**
  - Drug toxicity/risk to patient
  - Literature support
  - Reliability of testing
  - Range of use among medical specialties
  - Volume of drug use
  - Reimbursement for testing
  - Existence of protocol/practice guidelines
  - Acceptability of the drug-gene pair among staff
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Therapeutic Area</th>
<th>Drugs</th>
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<tr>
<td>CYP2C9</td>
<td>Analgesics</td>
<td>Celecoxib</td>
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<td>Psychiatry</td>
<td>Fluvoxamine</td>
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<td>Hematology</td>
<td>Warfarin</td>
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<td>CYP2C19</td>
<td>Musculoskeletal</td>
<td>Carisoprodol</td>
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<td></td>
<td>Psychiatry</td>
<td>Citalopram, Diazepam, Fluvoxamine</td>
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<td>Neurology</td>
<td>Clobazam</td>
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<td>Cardiovascular</td>
<td>Clopidogrel, Prasugrel</td>
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<td>Gastroenterology</td>
<td>Esomeprazole, Omeprazole, Pantoprazole</td>
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<tr>
<td>CYP2D6</td>
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<td>Citalopram, Fluoxetine, Paroxetine, Venlafaxine, (numerous)</td>
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<tr>
<td></td>
<td>Cardiovascular</td>
<td>Carvedilol, Metoprolol, Pranolol, (others)</td>
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<td>Cevimeline</td>
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<td>Codeine</td>
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<td>Carbamazepine, Phenytoin</td>
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<td>Abacavir</td>
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<td>Oncology</td>
<td>Irinotecan, Nilotinib</td>
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<td>Pulmonary</td>
<td>Indacterol</td>
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<td>VKORC1</td>
<td>Hematology</td>
<td>Warfarin</td>
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Source: www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm
Testing Menu – Pharmacogenetics

- Considerations
  - Market
  - What does FDA recommend?
  - Potential volume
  - Local patient needs
  - Laboratory ability to deliver rapid turn-around-time for tests requiring rapid TAT

- Methodology
  - Usually targeted variant analysis
  - Kits are available for some tests
    - Want to ensure that the variants included in the kits are appropriate for the patient population being tested
Background

- Commonly used anti-platelet agent given as a prodrug
- Activated by CYP enzymes
- Used to reduce atherosclerotic events (MI, stroke, vascular death) in patients with ACSs and/or following PCI
- Often given as alternative to aspirin or in combination with aspirin (dual anti-platelet therapy)
- Highly variable response to clopidogrel (efficacious in some, not efficacious in others)
  - Due to many variables
    - Underdosing
    - Interactions with CYP inhibitors and substrates
      - e.g. lipophilic statins, calcium antagonists, PPIs
    - Genetics

PGx Test Example: **CYP2C19 for Clopidogrel**
PGx Test Example: 
**CYP2C19** for Clopidogrel

- **CYP2C19** *2 and *3 loss of function alleles
  - *2: Caucasians, 15%; Blacks, 15%; Asian 29-35%; Mexican Americans, 19%
  - *3: Asian 1-9%
- Associated with lower levels of active metabolite of clopidogrel
- Associated with marked decrease in platelet responsiveness to clopidogrel (higher on-treatment platelet aggregation)
- Associated with increased rate of subsequent cardiovascular events and death
  - Relative 53% increased risk of death from CV causes, MI, or stroke
  - 3-fold increased risk of stent thrombosis
  - Higher risks for heterozygote and homozygote *2 carriers
PGx Test Example: 
CYP2C19 for Clopidogrel

March 2010
FDA label update: Clopidogrel response in CYP2C19 poor metabolizers
PGx Test Example: *CYP2C19* for Clopidogrel

- Which variants (alleles) do you want to test for
  - If patient population is primarily Caucasian and Asian, consider *2, *3, and *17 (rapid metabolizer)
  - If patient population has a high percentage of Blacks, consider performing a more expanded analysis

- Keep in mind that CYP2C19 PGx testing is relevant for medications other than clopidogrel:
  - Anticonvulsants: mephenytoin, diazepam, phenytoin, primidone
  - Antidepressants: amitriptyline, citalopram, S-citalopram, clomipramine
  - Antineoplastics: cyclophosphamide
  - Antiretrovirals: nelfinavir
  - Proton pump inhibitors: lansoprazole, omeprazole, pantoprazole
  - Miscellaneous drugs: progesterone, propranolol, R-warfarin (less active isomer), proguanil, diazepam
PGx Test Example: 
*UGT1A1* for Irinotecan

- UGT1A1 is primary enzyme involved in glucuronidation of irinotecan
- Irinotecan is a camptothecin analogue widely used for treatment of solid tumors
  - Severe, occasionally fatal, leukopenia/neutropenia or diarrhea
  - Linked to *UGT1A1* polymorphisms
- FDA approved revisions to safety labeling on irinotecan to recommend reduced dosing in patients with specific *UGT1A1* genotypes
WARNINGS

Patients with Reduced UGT1A1 Activity.

Individuals who are homozygous for the UGT1A1*28 allele are at increased risk for neutropenia following initiation of CAMPTOSAR treatment. A reduced initial dose should be considered for patients known to be homozygous for the UGT1A1*28 allele (see DOSAGE & ADMINISTRATION). Heterozygous patients (carriers of one variant allele and one wild-type allele which results in intermediate UGT1A1 activity) may be at increased risk for neutropenia; however, clinical results have been variable and such patients have been shown to tolerate normal starting doses.
PGx Test Example: *UGT1A1* for Irinotecan

- Irinotecan activated by carboxylesterases
- SN-38 is active drug
  - Blocks DNA replication
- SN-38 inactivated by glucuronidation (*UGT1A1*)
- SN-38 conjugate secreted into bile and the intestines
Multiple polymorphisms associated with altered activity of UGT1A1 enzyme

Promoter (TA)n repeat region
- Enzyme expression inversely related to number of TA repeats
- Wild-type = 6 TA repeats
- TA5 – normal to possibly increased transcriptional activity
- TA7 (*28) and TA8 (*37) – decreased activity (approx 50%)
  - TA7 allele frequency
    - 0.33-0.4 in Caucasians
    - 0.48 in Africans
    - 0.14 in Chinese
    - 0.17 in Japanese
- TA5 and TA8 are found more frequently in African/Black populations

PGx Test Example: UGT1A1 for Irinotecan
UGT1A1 Promoter Repeats

TA₅ (*36)  TATATATATA

TA₆ (*1)   TATATATATA

TA₇ (*28)  TATATATATATA

TA₈ (*37)  TATATATATATATA

Exon 1   Exon 2   Exon 3   Exon 4   Exon 5
PGx Test Example: $UGT1A1$ for Irinotecan

- Which alleles do you test for?
  - TA repeat region
  - Entire gene?

- Methodology
  - Need method that can accurately detect difference between 5, 6, 7, and 8 TA repeats
Organization formats

- Centralized options
  - Core R&D
  - Core clinical labs
    - DNA/RNA extraction/sample prep
    - Sanger sequencing
    - Next generation sequencing
    - Copy number variant analysis (e.g. arrayCGH)
- Decentralized options
  - Specialty focused
    - Heme/Onc
    - Inherited disorders
    - PGx
    - Infectious disease
Additional Resources

- Laboratory general
  - American College of Medical Genetics (ACMG) laboratory standards and guidelines
  - Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions (Centers for Disease Control)
  - CLSI guidelines
  - CAP Molecular Checklist

- Inherited disorders
  - Clinical practice guidelines by ACMG
  - Genetic tests offered by other laboratories; GeneReviews: Genetests.org

- Pharmacogenetics
  - PharmGKB (PGx Knowledgebase)
  - CPIC (Clinical Practice Implementation Consortium) guidelines
  - FDA website
    - [www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm](http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm)