Importance of Clinical Information for Optimal Genetic Test Selection and Interpretation

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Learning Objectives

• Understand the relevance of clinical information for genetic testing
• Appreciate the clinical and financial importance of pre-analytical genetic test review
• Appreciate the significance of clinical information in genetic test interpretation
• Understand the role genetic counselors can play in the pre and post analytical test review

2009 CDC Report

• Published recommendations for best practices in molecular genetic testing for heritable diseases
• More errors occur in pre and post analytic phases than in the analytic process itself
• Inappropriate test selection underlines many pre analytic errors
• Study of APC gene testing found testing unwarranted in 17% of cases
• Labs should:
  – Help HCPs with appropriate test selection
  – Instruct HCPs on patient information needed for proper testing and interpretation
  – Be available for consultations with HCPs for test selection/interpretation
Additional Concerns in Preanalytic Phase

- Informed consent- including potential implication of results for other family members
- Establishing policies to assess and correct problems

Analytic Errors

- Already regulated by CLIA
- Rare specimen handling and analysis errors occur in 0.06 to 0.12% of samples with 100,000 tests

Post Analytic Errors

- Errors in report preparation and interpretation
  - Result from HCP’s poor understanding of limitations of molecular genetic tests and proper interpretation
- Problems with content, completeness and interpretation of reports
Test Order Review at ARUP Labs

• All heritable molecular sequencing and deletion/duplication tests
• Selected cytogenetic and biochemical assays
• GCs collected test review data between April 2010 through Dec 2011 (21 months)- excluded biochemical and cytogenetic assays

Health Care Savings from Molecular Test Modifications

• 86 tests modified /month (includes test cancellations and additions)

• Average Cost Savings/ month >$60,000 (specifically from cancelation of erroneous tests)

• Savings to hospitals, insurers and patients
  → $720,000 dollars annually

Misorders Comprise ~28% of Complex Molecular Genetic Tests

• 35% Cancelled incorrect test ordered correct one
• 26% Cancelled incorrect test but could not order correct one
• 14% Cancelled full gene sequencing & added targeted panel
• 13% Cancelled sequencing & ordered familial mutation
• 7% Cancelled incorrect and facilitated send out
• 5% Cancelled duplicate test order
35% Cancelled Incorrect Test and Ordered Correct One

- Ordered HHT FGA- (hereditary hemorrhagic telangiectasia) and wanted HH Panel (hereditary hemochromatosis)
- Ordered alpha globin sequencing but needed alpha thalassemia 7 deletion panel
- Ordered Rett syndrome FGA (MECP2) and wanted RET (MEN2)
- Ordered Lynch syndrome (MSH2) but needed Lynch syndrome (MSH6)

26% Cancelled Incorrect Test but Could not Change it to Correct One

- GALT testing ordered when actually wanted Aspergillus Galactomannan
- Factor 8 or 9 gene sequencing when actually desired factor 8 or 9 activity

14% Cancelled Full Gene Sequencing & Added Targeted Panel

- CFTR full gene sequencing ordered on a routine OB patient
- ACMG recommends 23 mutation panel
- Sequencing will detect many VUS
- TAT with sequencing much longer (weeks vs days with panel)
- Cost is more than 10 times higher
13% Cancelled Full Gene Sequencing & Ordered Familial Mutation

- Common mistake especially with AD and XL disorders
  - RET, HHT, PTEN, F8, F9, Alport, FAP
  - Instead of Lynch syndrome MLH1, MSH2, MSH6 or PMS2 full sequencing; order targeted SEQ FSM

Other Misorders

- 7% Cancelled incorrect test and facilitated send out
  - Neurofibromatosis D0 canceled; sequencing sent out
- 5% Cancelled duplicate test order
  - Detected same test previously performed
  - Rarely needed in genetic testing unless r/o sample switch or result does not correlate with symptoms
  - HCP usually could not locate previous results

Health Care Savings From Molecular Genetic Test Cancellations Alone

- Over $60,000 a month
- Approximately $720,000 savings annually
Top Tests Cancelled by Volume

- Cystic fibrosis sequencing and del/dup- 17%
- Alpha globin sequencing- 58%
- NF type 1, deletion/duplication- 87%
- Lynch syndrome gene sequencing/deldup- 8%
- Sequencing for known familial mutation- 12%

Performing Test Order Reviews

- Must have clinical history to understand why test was ordered
- Most labs performing molecular genetic tests request clinical information on test requisitions or consent forms
- ARUP creates custom patient history forms for each test

Helpful Information to Request

- Contact info for ordering HCP and practice type
- Patient symptoms
- Supporting laboratory results
- Family history
- DNA results of affected family members
- Test practitioner intended to order
Ex. Lynch Syndrome MSH2 Sequencing and Deletion/Duplication Ordered

- No info provided
- Contact ordering HCP
- Learn that pt has a brother with Lynch sx
- Ask HCP to call pt and see if he can get records of brother’s DNA test result
- Learn that brother has MSH6 c.242G>A
- Change test to targeted sequencing for MSH6
Lessons from Lynch Case

• Wrong test would have been run wasting >$1000
• Interpretation would indicate no pathogenic mutations detected in gene
• Appropriate screening for individual at high risk for Lynch syndrome would not be offered

Ex 2. Cystic Fibrosis

• Autosomal recessive
• Two mutations in *CFTR-*cystic fibrosis transmembrane regulator
• ACOG recommends CF mutation panel with 23 mutations be offered to OB patients
• Panel detection rate varies with ethnicity
  – Caucasian 89%  African American 65%
  – Hispanic 73%  Asian 55%

Ex 2; CFTR Sequencing

• 26 year old female
• No clinical info provided
• Ordering health care provider- OB/GYN
• Call HCP to document reason for testing
  – Routine OB screen; no symptoms or fam hx
• Cancel sequencing and order CF panel
• Cost savings >$1000
Ex 3. CFTR Sequencing

- Newborn with no clinical info provided
- Call HCP
- Learn that African American infant has an affected full brother
- Encourage getting a copy of brother’s DNA result
- F508/del exons 7-8

Infant at Risk for CF

- F508del would be detected by sequencing but expensive way to detect it (just need panel)
- Deletion of exons 7-8 would NOT be detected by sequencing; requires a del/dup test
- CFTR sequencing would have resulted in detecting only one of the infant’s two mutations delaying critical dx and treatment
- Also would have resulted in wasting >$1000

Ex 4; FBN1 Sequencing

- 1 year old asymptomatic male
- Contact primary care physician
- FOB has clinical dx of Marfan Sx but no molecular diagnostic confirmation
- Finding no FBN1 mutations would not rule out dx
- Extracted DNA and encouraged PCP to refer FOB to geneticist or test him for FBN1 mutation first
- FOB tested negative for FBN1 Seq and Dup/Del
- Cancelled test on his son
Hemophilia A

- Incidence 1 in 4000 male births
- Spontaneous joint or deep tissue bleeding
- F8 Deficiency
  - Severe; <1% activity
  - Moderate; 1-5% activity
  - Mild 6-35% activity
- F8 gene mutations
  - 51% Inversions
  - 43% Sequence Variants
  - 6% Large Del/dups

Factor 8 Sequencing

- 25 year old female
- Factor 8 sequencing is ordered
- Patient history shows; maternal uncle died of severe hemophilia A
- Cancel sequencing and order inversion with reflex to sequencing with reflex to del/dup

F8 Reflex Testing

- 5 year old mildly affected boy with factor 8 deficiency (10% of normal activity)
- Inversion, reflex to sequencing reflex to DD ordered
- Given mildly affected status; sequencing is best choice
Putting Test Review into Practice in Large Reference Laboratories

- Laboratory GCs can create custom patient history forms for tests performed in house
- Lab extracts DNA on specific tests being held for review
- GC reviews order for best test selection
  - instructs lab to run as ordered
  - cancels and reorders correct test

Hospital Send Out Lab Test Review

- Require ordering HCP to provide clinical information with test order/complete a patient history form
- If patient history is not provided with test order, determine where sample is being sent and print off proper form and call HCP for info
- Pathologist or GC should review genetic send out tests for accuracy and necessity

Genetic Counselors: Ideal Professionals to Review Send Outs

- GCs are Masters trained individuals with specialized training in clinical medical genetics
- It is a terminal degree
- NSGC 2006 Scope of Practice: Item 7 ….Order tests and perform clinical assessments in accordance with local state and federal regulations
- Most genetic tests ordered by HCPs with little formal education in genetics
Genetic Counselors

- In 2013, 27 US GC training programs have full ABGC accreditation; 3 in Canada
- ~3000 in practice
- ~80% of GCs work directly with patients
- ~10% work in diagnostic laboratories

Common Indications for GC Referral

- High risk pregnancies (abnormal MSS or U/S)
- Consanguinity
- High risk ethnic groups
- Infertility or infant death
- Birth defects or mental retardation
- Heritable disorders
- Psychiatric conditions
- Familial cancer

The Genetic Counseling Process

- Review medical records & research
- Draw medical pedigree
- Perform risk assessments
- Explain medical & scientific information
- Discuss disease management, treatment & surveillance options
- Review testing options
- Facilitate decision-making process
Use of Clinical Information for Accurate Test Interpretation

- Clinical info on patient
- Relevant family history
- Affected relative’s test results

Information for Proper Test Interpretation

- Why is testing being performed?
  - Carrier screening
  - Rule out classic or atypical disease
- Is there a family history? If Yes,
  - Is relative symptomatic?
  - Relationship of patient to relative?
  - Relative’s mutation(s)?
- What is the patient’s ethnicity?

Case Example

- CF Mutation Panel: Four day old female
- Single mutation identified (R553X)

  - How should this be interpreted?
    - Symptomatic- suggest full gene sequencing?
    - Asymptomatic- infant is probably only a carrier?
Asymptomatic with Positive Family History

- Caucasian mother carries R553X; Hispanic father refused testing
- $1 \times \frac{1}{46} \times \frac{1}{4} = 1/184$ prior risk to be affected
- Bayesian to calculate residual risk to be affected after R553X mutation identified

Bayesian Analysis Needed for Risk Interpretation

<table>
<thead>
<tr>
<th></th>
<th>Father Passed Mutation</th>
<th>Father did Not Pass Mutation</th>
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<tbody>
<tr>
<td>(Infant Affected)</td>
<td></td>
<td>(Infant Unaffected)</td>
</tr>
<tr>
<td>Prior</td>
<td>$1/46 \times 1/2 = 1/92$</td>
<td>$91/92$</td>
</tr>
<tr>
<td>Conditional</td>
<td>27/100</td>
<td>1</td>
</tr>
<tr>
<td>Joint</td>
<td>27/2000</td>
<td>91/92</td>
</tr>
<tr>
<td>Posterior</td>
<td>$27/9200 \sim 1 \text{ in } 340$</td>
<td>$339/340$</td>
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Asymptomatic; Has family history

- Caucasian
- Affected full brother is a compound heterozygote for R553X and F508del
- Reassuring interp- patient appears to be just a carrier
Symptomatic Asian/Caucasian

- Meconium ileus
- Asian/Caucasian
- No family history of CF
- Recommend CFTR sequencing with reflex to deletion/duplication testing

Prenatal Testing for CF Using Panel

- Result: F508del het
- Clinical Info: Caucasian couple; neither has undergone CF screening; no fam hx of CF; no fetal anomalies noted
- Bayesian analysis used to calculate risk for fetus to be affected

Bayesian Analysis

<table>
<thead>
<tr>
<th></th>
<th>Affected</th>
<th>Not Affected</th>
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</thead>
<tbody>
<tr>
<td>Prior Risk to Be Affected</td>
<td>1/2500</td>
<td>2499/2500</td>
</tr>
<tr>
<td>Condition of finding one mutation</td>
<td>1/5</td>
<td>1/25</td>
</tr>
<tr>
<td>Joint</td>
<td>1/12,500</td>
<td>2499/62,500</td>
</tr>
<tr>
<td>Posterior</td>
<td>1/500</td>
<td>499/500</td>
</tr>
</tbody>
</table>
Prenatal Diagnosis

- 28 year old Caucasian
- Echogenic bowel with dilated loops
- Result: F508del het
- Assuming a prior risk of 1 in 10
- Bayesian calculation indicates a 36% (1 in 2.8) risk for CF in fetus

Bayesian Analysis

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<thead>
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</thead>
<tbody>
<tr>
<td>Prior</td>
<td>1/10</td>
<td>9/10</td>
</tr>
<tr>
<td>Conditional</td>
<td>1/5</td>
<td>1/25</td>
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<tr>
<td>Joint</td>
<td>5/250</td>
<td>9/250</td>
</tr>
<tr>
<td>Posterior</td>
<td>5/14</td>
<td>9/14</td>
</tr>
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MCAD Deficiency

- Autosomal recessive
- Inability to metabolize fat for energy
- May lead to sudden death
- One common mutation A985G is responsible for 90% of abnormal alleles
- Therefore, about 80% of affected individuals will be homozygous for the common mutation
Case 1: ACADM Panel

- 3 year old female
- One copy of A985G identified
- Clinical info:
  - Newborn brother just diagnosed with MCAD through newborn screening; compound heterozygote for A985G/1100delAGTT
- Interpretation: Pt is at least a carrier of MCAD and may be affected since 1100delAGTT is not tested on the panel
- Recommendation to add targeted sequencing

MCAD Case 2

- MCAD Pan and OA ordered on newborn girl
- MCAD Pan result: A985G het
- Clinical info: 3 year old full sibling died with GI illness and dehydration; found homozygous for A985G
- Interpretation: Patient is predicted to be unaffected carrier

Summary

- Reviewing genetic test orders results in significant cost-savings
- GCs are ideally trained to perform genetic test order reviews
- Clinical information is critical for test review and accurate result interpretation
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