Importance of Clinical Information for Optimal Genetic Test Selection and Interpretation

Chris Miller, MS, LCGC
ARUP Laboratories

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 underlies 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/interp

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

Morb Mortal Wkly Rep 2009;58(RR-6):1-37

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 DD 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

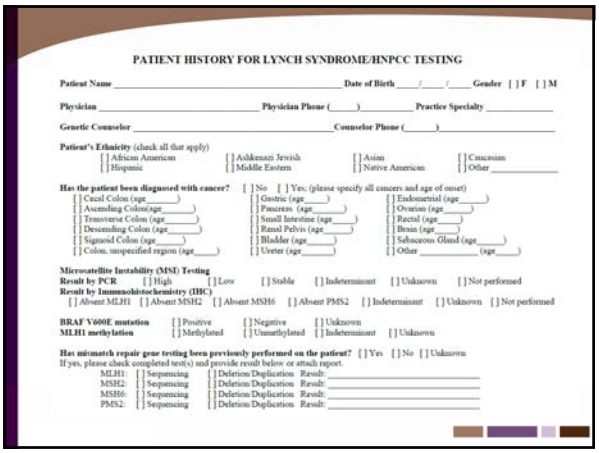
- Cystic fibrosis sequencing and del/dup- 17%
- Alpha globin sequencing- 58%
- NF type 1, deletion/duplication- 87%
- Lynch syndrome gene sequencing/deldup- 8%
- Sequencing for known familial mutation- 12%

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



PATIENT HISTORY FOR LYNCH SYNDROME/HNPCC TESTING

Patient Name _____ **Date of Birth** ____/____/____ **Gender** [ ] F [ ] M

Physician _____ **Physician Phone** (____)_____ **Practice Specialty** _____

Genetic Counselor _____ **Counselor Phone** (____)_____

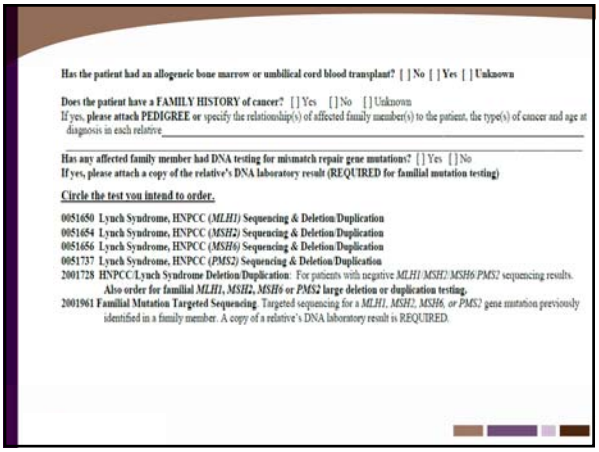
Patient's Ethnicity (check all that apply)
[ ] African American [ ] Ashkenazi Jewish [ ] Asian [ ] Caucasian
[ ] Hispanic [ ] Middle Eastern [ ] Native American [ ] Other _____

Has the patient been diagnosed with cancer? [ ] No [ ] Yes (please specify all cancers and age of onset)
[ ] Cecal Colon (age_____) [ ] Gastric (age_____) [ ] Endometrial (age_____)
[ ] Ascending Colon(age_____) [ ] Pancreas (age_____) [ ] Ovarian (age_____)
[ ] Transverse Colon (age_____) [ ] Small Intestine (age_____) [ ] Rectal (age_____)
[ ] Descending Colon (age_____) [ ] Renal Pelvis (age_____) [ ] Brain (age_____)
[ ] Sigmoid Colon (age_____) [ ] Bladder (age_____) [ ] Sebaceous Gland (age_____)
[ ] Colon, unspecified region (age_____) [ ] Ureter (age_____) [ ] Other _____ (age_____)

Microsatellite Instability (MSI) Testing
Result by PCR [ ] High [ ] Low [ ] Stable [ ] Indeterminant [ ] Unknown [ ] Not performed
Result by Immunohistochemistry (IHC)
[ ] Absent MLH1 [ ] Absent MSH2 [ ] Absent MSH6 [ ] Absent PMS2 [ ] Indeterminant [ ] Unknown [ ] Not performed

BRAF V600E mutation [ ] Positive [ ] Negative [ ] Unknown
MLH1 methylation [ ] Methylated [ ] Unmethylated [ ] Indeterminant [ ] Unknown

Has mismatch repair gene testing been previously performed on the patient? [ ] Yes [ ] No [ ] Unknown
If yes, please check completed test(s) and provide result below or attach report.
MLH1: [ ] Sequencing [ ] Deletion/Duplication Result: _____
MSH2: [ ] Sequencing [ ] Deletion/Duplication Result: _____
MSH6: [ ] Sequencing [ ] Deletion/Duplication Result: _____
PMS2: [ ] Sequencing [ ] Deletion/Duplication Result: _____



Has the patient had an allogeneic bone marrow or umbilical cord blood transplant? [ ] No [ ] Yes [ ] Unknown

Does the patient have a FAMILY HISTORY of cancer? [ ] Yes [ ] No [ ] Unknown
If yes, **please attach PEDIGREE** or specify the relationship(s) of affected family member(s) to the patient, the type(s) of cancer and age at diagnosis in each relative_____

Has any affected family member had DNA testing for mismatch repair gene mutations? [ ] Yes [ ] No
If yes, please attach a copy of the relative's DNA laboratory result (REQUIRED for familial mutation testing)

Circle the test you intend to order.

0051650 Lynch Syndrome, HNPCC (*MLH1*) Sequencing & Deletion/Duplication
0051654 Lynch Syndrome, HNPCC (*MSH2*) Sequencing & Deletion/Duplication
0051656 Lynch Syndrome, HNPCC (*MSH6*) Sequencing & Deletion/Duplication
0051737 Lynch Syndrome, HNPCC (*PMS2*) Sequencing & Deletion/Duplication
2001728 HNPCC/Lynch Syndrome Deletion/Duplication: For patients with negative *MLH1/MSH2/MSH6/PMS2* sequencing results.
Also order for familial *MLH1*, *MSH2*, *MSH6* or *PMS2* large deletion or duplication testing.
2001961 Familial Mutation Targeted Sequencing. Targeted sequencing for a *MLH1, MSH2, MSH6, or PMS2* gene mutation previously identified in a family member. A copy of a relative's DNA laboratory result is REQUIRED.

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

ARUP Institute for Learning

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 x 1/46 x ¼ = 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

	Father Passed Mutation	Father did Not Pass Mutation
	(Infant Affected)	(Infant Unaffected)
Prior	1/46X1/2= 1/92	91/92
Conditional	27/100	1
Joint	27/9200	91/92
Posterior	27 / 9127 ~ 1 in 340	339/340

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

	Affected	Not Affected
Prior Risk to Be Affected	1/2500	2499/2500
Condition of finding one mutation	1/5	1/25
Joint	1/12,500	2499/62,500
Posterior	1/500	499/500

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

	Affected	Not Affected
Prior	1/10	9/10
Conditional	1/5	1/25
Joint	5/250	9/250
Posterior	5/14	9/14

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/1100del AGTT
- 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

Acknowledgements

- Erin Baldwin
- Kim Hart
- Patti Krautscheid
- Danielle LaGrave
- Amanda Openshaw
- Tanya Tvrdik