Updates on Issues in Molecular Genetics Testing

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November 19, 2009
Overview

HDGC

- Inheritance
- Molecular Pathology
- Clinical Criteria and Findings
- Molecular Techniques
- Results
Hereditary diffuse gastric cancer

- Gastric cancer is the second most common cause of cancer death worldwide

- Two types:
  - Intestinal type
  - Diffuse type (DGC)

- 90% sporadic, 10% have familial clustering
- 1-3% of all GC have an AD pattern
- The majority of families with AD clustering are associated with DGC
Autosomal dominant inheritance

- Affected person has at least one affected parent
- Affects either sex
- Transmitted by either sex
- Risk of being affected child after an affected with unaffected mating: 50%
**CDH1**

- HDGC is the only known cancer syndrome dominated by GC
- 30-40% of patients with HDGC have a *CDH1* mutation

**CDH1:**
- Tumor suppressor gene
- Adhesion protein e-cadherin
- Isolated, mucin filled tumor cells
- Decreased expression
- LOF

Criteria for testing

- A patient with both DGC and lobular breast cancer
- ≥ Two DGC in one family, one younger than age 50
- A patient age < 35 with DGC
- Multiple cases of lobular breast cancer in first degree relatives

Original criteria: International Gastric Cancer Linkage Consortium (IGCLC, 1999)
AD inheritance, reduced penetrance
Clinical course, therapy, diagnosis

- All 11 cousins who inherited the *CDH1* mutation went ahead with the gastrectomy.
- Endoscopy, random biopsies, stool tests and stomach PET and CT scans -- all negative.
- After gastrectomy, pathology review revealed hundreds of early tumors in 9 cases.

- “The genetic legacy no longer writes the family's history.”
CDH1 mutation distribution
Which method to choose?

1. SEQUENCING ANALYSIS PLUS DEL/DUP TESTING
2. SEQUENCING ANALYSIS
3. SEQUENCE BASED SCREENING
4. MUTATION PANEL
Allele drop out

- Failure of a genotyping method to detect one of the two germline alleles

- For PCR-based methods, this may be caused by a polymorphism within a primer binding site

- For autosomal dominant conditions the primary danger of allele dropout is a false negative result

- For autosomal recessive conditions, allele dropout may lead to a false positive or to a false negative result
Primer design

- Known SNP data should be incorporated into the design of PCR-based assays whenever possible, but especially in cases of highly polymorphic genes for which an incorrect test result may have devastating consequences for patient care.

- Primers binding to regions with known SNPs should not be incorporated into clinical \textit{CDH1} sequencing assays
The earliest PCR cycles use a high annealing temperature.
Increased specificity and sensitivity.
Example, Ta of 67°C ± 47°C (-1°C per cycle) for 21 cycles, followed by 18 more cycles at 47°C.
Sequence analysis: example 1

B.

2634 C>T

- A single nucleotide change: what does it mean?
- Gly878Gly in exon 15
- Synonymous, protein unchanged

- Some sequence changes, e.g. missense mutations, can be difficult to interpret without functional studies
Sequence analysis: example II

B.

- A frameshift mutation
- THE DOG CAN EAT AND RUN =
- THE DOG CAN ATA NDR
- Premature termination
Sample considerations

- Peripheral blood
- Tumor tissue
- Paraffin embedded tissue (AMP Poster)
- Whole gene sequencing (16 exons)
- Single exon sequencing
Overview

Identity

- Paternity
- Maternal Cell Contamination
- Specimen Questions
- Molecular Testing
- Results (Examples)
Applications of identity testing

- AABB accredited relationship testing
- Maternal cell contamination testing in prenatal samples (amnio, CVS)
- “Floaters” in surgical pathology
- Specimen mix-ups
Prenatal diagnostic testing

- CVS and AF sampling are invasive procedures
- Irreplaceable, small samples
- Lab needs to verify that the tissue is fetal
- Need excellent turn-around-time
- Requires highly accurate results

CVS = chorionic villus sampling, AF = amniotic fluid
MCC risks

- Interpretation errors of diagnostic tests, including aneuploidy FISH and molecular tests
- Low levels matter and MCC cannot be reliably assessed by eye
- FISH alone will not detect MCC when the fetus is female
- FISH may seem mosaic when all cells are abnormal
- CVS has a higher risk than AF

MCC = maternal cell contamination
MCC risk factors

- Less experienced clinicians
- Technique used during sample collection

Risk reduction:
- Discard first 2-5 mL of the sample
- Obtain the actual sample with second syringe
- Minimize number of needle passes
- Use ultrasound guidance
- Avoid placenta penetration
- Sample processing
MCC testing in the U.S.

- Number of labs performing MCC testing
- Number of identity markers used
- Lower level of detection

J Mol Diagn 2007; 9: 394-400
Direct AF or direct CVS

MCC testing

MCC Negative

Diagnostic prenatal testing

Negative test result

Positive test result

Report result

Confirm with culture

Report
MCC testing example

- Multiplex fluorescent-PCR, capillary electrophoresis, and GeneScan analysis of nine tetranucleotide repeat markers and X/Y chromosome analysis (ABI AmpF/STR Profiler Plus assay)

- Simultaneous with the prenatal sample, analysis on a maternal blood sample is performed

- Each test initially performed on direct AF or CVS is followed by analysis of a cultured sample if MCC could not be ruled out
MCC take home points

- MCC testing of fetal samples is considered the standard of care
- Requires lower limit of detection of <5%
- Ideally multiple markers are used to reduce uninformative tests and increase the chance of detecting MCC
- Culture capabilities are necessary
Specimen identity example

- Bone marrow biopsies from a 55 yo woman and a 52 yo male
- Specimens had been processed by Bouin’s fixation, decalcification, and were paraffin embedded => poor DNA yield
- Aspirate and biopsy findings did not match
- Amelogenin marker (X,Y) is low molecular weight
Molecular testing as a QA measure

- QC measures to avoid specimen misidentification should be in place in all surgical pathology laboratories
- If suspected misidentification cannot be ruled out, molecular means should be pursued
- Threshold should be low and close to routine practice
- In the vast majority of cases, molecular testing provides accurate and definitive results and provides quality assurance for physician and patient
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**Acknowledgements:**

- Franklin Mullins
- Jim Zehnder
- Jim Ford
- Lisa Dietz
- Marla Lay
- Nicki Chun
- Angela Bartley
- Raj Mariappan
- Sarah Cherny