The ‘Next’ in Next Generation Gene Sequencing Testing

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Disclosure

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Objectives

1. Know the difference between somatic and germline mutations
2. Describe the importance of taking a family history to determine the probability of hereditary risk of cancer
3. Understand the difference between pathogenic variants and variants of uncertain significance in genes
Genes

- Genes are units of genetic information
  - Instructions for our cells to function
  - Made up of DNA
- Genes are packaged into chromosomes
  - Humans have 46, or 23 pairs
  - One set from mom, one set from dad
- DNA
  - Bases A, G, T, & C
Normal Karyotypes

Karyotype: 46, XY

Karyotype: 46, XX
What is a Mutation?

A change within the gene that causes it to stop working properly

Sugar phosphate backbone

Bases
Germline vs. Somatic Mutations

- Mutations can occur in any cell. They only affect future generations if they occur in the cells that produce the gametes: these are “germinal” or “germ line” mutations.

- Mutations in other cells are rarely noticed, except in the case of cancer, where the mutated cell proliferates uncontrollably. Mutations in cells other than germ line are “somatic” mutations.
Somatic (Tumor) Mutations

• Mutations happen in all of our cells all the time
  – Chance during replication
  – Environmental Exposures
    • Chemicals
    • Radiation
    • Pesticides
Inheritance

Father: 46 chromosomes

Sperm: 23 chromosomes

Mother: 46 chromosomes

Egg: 23 chromosomes

Child: 46 chromosomes.
(23 chromosomes from the father and 23 chromosomes from the mother.)
Inheritance

• How many working copies of a gene do you need?
  – Varies by condition and gene
  – Sometimes one non-functional copy enough to cause disease
    • Autosomal dominant
  – Sometimes need two non-functional copies cause disease
    • Autosomal recessive
  – Non-functioning copy on X-chromosome
    • X-linked
Inheritance: Autosomal Dominant

**Autosomal Dominant Segregation**

- 1 in 3 chance (33%) that the child will be affected
- 1 in 3 chance (33%) that the child will not be affected

**Autosomal Dominant Inheritance**
Inheritance: Autosomal Recessive
Inheritance: X-Linked
Why Does Family History Matter?
History Gene Testing

• 1975 - Two groups, Frederick Sanger and colleagues, and Alan Maxam and Walter Gilbert, both develop rapid DNA sequencing methods.

• 1994 - BRCA1: chromosome 17

• 1995 - BRCA2: chromosome 13

• 2010 - Next Generation Sequencing available clinical basis.

• 2011 – Exome sequencing available clinical basis.
Recent Events Increase Gene Testing

• Supreme Court Ruling

• Affordable Care Act

• The Angelina Effect
Comparing Sequencing Technology

• **Sanger Sequencing**
  - Historical “gold standard”
  - 2 reads per base pair (forward and Reverse)

• **Next Generation Sequencing**
  - Massively parallel sequencing
  - 100s – 10000s of reads per base pair
NGS: Research → Clinical Testing
Seq Technologies: Advantages

• Sanger
  – Faster to run in lab = shorter TAT
  – Inexpensive for analyzing a single gene
  – Data is manageable (does not require bioinformatics involvement)

• NGS
  – More cost and time efficient when analysis of many genes is desired
  – Can detect some rare mutations better than Sanger
Seq Technologies: Limitations

- **Sanger**
  - Appreciable rate of *false negative* results (i.e. missed mutations) due to *allele dropout*
  - Expensive and time-consuming when analyzing many genes

- **NGS**
  - Longer analysis time (longer TAT) for single test compared to Sanger
  - Complexity and volume of data requires quality bioinformatics program
  - Appreciable rate of *false positive* results (i.e. seeing changes that aren’t real)
    - Sanger sequencing often used to confirm NGS findings
Panel Testing

• Specific syndromes (ie: Noonan syndrome)
• Cardiovascular
• Epilepsy
• X-Linked intellectual disabilities
• Cancer (breast, colon, paragangliomas)
Why Multi Gene Cancer Tests?

• Many patients with suggestive family histories test negative on genetic testing
  – Need for additional/expanded screening

• Overlapping phenotypes of different hereditary cancer syndromes

• Patients don’t meet classic guidelines for conditions
  – Variable expressivity and reduced penetrance

• **Many genes implicated in each cancer**
  – Testing multiple genes simultaneously can be more time and cost effective
BRCA1 and BRCA2 account for only about 50% of Hereditary Breast Cancer.
What have we been missing?

- BRCA1
- CHEK2
- RAD51C
- ATM
- NBN
- PA1B2
- PTEN
- RAD51D
- CDH1
- BARD1
- BRCA2
- BRIP1
- TP53
- RAD50
- STK11
Figure 1 Relative distribution of variants detected with NGS in 708 HBOC patients. Percentages were based on the number of time the gene was sequenced depending on the version of the capture design.
“Red Flags” of Hereditary Cancer

• Same type of cancer in multiple individuals

• Early age of diagnosis
  – Typically defined as under 50

• Multiple cancers in one person

• Rare cancers, or cancer in paired organs
Why should we care?

• If we can understand cancer genetics, we can:
  – High-risk breast screening for \textit{BRCA1, BRCA2, CDH1, PTEN, STK11,} and \textit{TP53} mutation carriers:
    • Clinical breast exams more frequently
    • Breast imaging with mammogram & breast MRI
    • Initiate screening at a younger age
  – Discuss the option of risk-reducing bilateral mastectomy with patients with a mutation
  – Avoid radiation treatment, if possible, for TP53 mutation carriers
Why should we care?

– Targeted surveillance and prevention options specific to the gene mutation and syndrome identified

– Identify at-risk family members with targeted genetic testing for identified family mutation and develop an individualized cancer screening and prevention program

– Assist couples in reproductive decision making (e.g. advise mutation carriers about assisted reproduction options including pre-implantation genetic diagnosis)
Gene Mutation Carriers and Pre-Implantation Genetic Diagnosis

• Early embryo from IVF is “biopsied” and DNA is analyzed before embryo transfer
• 90% carriers concerned about transmitting gene mutation to offspring
• 33% would consider PGD themselves and majority feel HCPs should discuss option with them
Possible Results of Genetic Testing

• Positive result: deleterious mutation identified

• Negative result: no mutation identified

• Variant of uncertain significance: a genetic change was identified, it is unclear at this point in time if this change leads to an increased cancer risk.

GREY vs. GRAY
Positive Result

• Test result reveals a pathogenic mutation or a variant that is likely pathogenic.

• Patient is at an increased risk to develop cancer.

• There are risk management options to detect cancer early or lower the risk to develop cancer.
Interpreting a Negative Result

*No identified mutation in family*

- Colon Ca, 52
- Colon Ca, 45
- Endometrial Ca, 47

*Family with a known mutation*

- Colon Ca, 52
- Colon Ca, 45
- Endometrial Ca, 47

37

$MSH2^-$

Inconclusive

True negative
Variants of Unknown Significance (VUS)

• A variation in a genetic sequence whose association with disease risk is unknown

• VUS’s are typically not used to make management recommendations

• The patient is left with an inconclusive result- not totally negative, but not a true positive

• Uncertainty can be difficult for both the client and the patient, especially as they are making treatment decisions
Working with a Genetic Specialist

- Geneticist
- Genetic Counselor
- Genetic Nurse Practitioner
Genetic Testing Covered by the Affordable Care Act

Why is BRCA testing covered? Covered preventive services are those that currently hold an “A” or “B” rating from the United States Preventive Services Task Force (USPSTF). The USPSTF rated Genetic Risk Assessment and BRCA Mutation Testing with a grade B in 2005 and reaffirmed that rating in 2013.
Genetic Testing Covered by the Affordable Care Act

- **When was this implemented?** The Affordable Care Act was passed by Congress and signed into law by the President on March 23, 2010. One provision of this health insurance reform is the coverage of certain preventive healthcare services with no cost-sharing (i.e. no out-of-pocket costs) to patients.

- “Preventive services that have strong scientific evidence of their health benefits must be covered and plans can no longer charge a patient a copayment, coinsurance or deductible for these services...”
Genetic Testing Covered by the Affordable Care Act

- In the 2013 USPSTF statement, no specific family history criteria are outlined. However, the guideline recommends that primary care providers screen women for family history to determine risk for a BRCA mutation. For women who have at least one family member with breast, ovarian, fallopian tube or peritoneal cancer, providers are encouraged to use a tool to elicit family history factors associated with an increased likelihood of a BRCA mutation, such as any of the following observed in a patient or their family:
  - Breast cancer diagnosis before age 50 years
  - Ovarian cancer
  - Bilateral breast cancer
  - Male breast cancer
  - Multiple cases of breast cancer in the family
  - Ashkenazi Jewish ancestry with a BRCA-related cancer
  - A known BRCA mutation in the family
Case Study #1

Sweden

- 60
- Prostate ca (60)
- Lt. IDC
- ER/PR neg.
- Her2 pos.

Ireland / Scotland

- 60
- Prostate ca (70s)
- d. 80

- 80
- Lung ca (80)
- Smoker

- 23
- Hodkin’s lymphoma (23)

BRCA1/2 sequencing deletion/duplication analyses

BRCA2 positive

c.1134delT
Case Study #2

Ashkenazi Jewish

d. heart

d. 65

78

81

OvaNext
23 gene

18

15

11

8

DCIS (45)

d. heart

d. 91

d. 40

d. Pancreatic ca

d. 64

d. Colorectal ca (64)

Ashkenazi Jewish

Neg. mammog.
No BSO

Ovarian Ca (69)

d. 69

Chek2
positive

c.1100delC

50

48

35

50

48

35

Ovarian Ca (69)

50

48

35

50

48

35

50

48

35

50

48

35
Case Study #3

CancerNext
28 gene

BRCA2 positive
c.5946delT

APC positive
p.I1307K

Lithuania / Austria
(Ashkenazi Jewish)

Poland / Romania
(Ashkenazi Jewish)

81

81

d. stroke

d. stroke

81

81

Gallbladder ca (81)
Prostate ca (72)
BRCA2 +

BrCa (40s)

Breast Ca (43)

Breast Ca (75)

Lung ca

Panc ca

Ovarian ca (40s)

Panc ca

Lymphoma (50s)

Pancreatic ca (70)

Pancreatic ca (70)

Panc ca

Ovarian ca (50s)

Old age

Throat ca (50s)

Old age

Breast ca (60s)

Breast ca (50s)

Throat ca (50s)

Lung ca smoker

Panc ca

MS3 BRCA-

Cutaneous angiosarcoma (78)

Panc ca

Gallbladder ca (81)

Prostate ca (72)

BRCA2 +

Precancerous polyp (38)

Precancerous polyp (38)

Pancreatic ca (37)

Pancreatic ca (37)

Pancreatic ca (37)

Pancreatic ca (37)

Pancreatic ca (37)

Pancreatic ca (37)
Case Study #4

Poland
Ashkenazi Jewish

France
Ashkenazi Jewish

Pancreatic ca (60)
d. 70

84

d. Colon ca (94)
Bladder ca (70s)
d. 94

84
d. Stroke

Bil. Breast ca (40s)
d. 80

Bil. Breast ca (48, 66)
Rt. ILC (60)
ER/PR/Her2 neg.
Melanoma (58)
d. 73

Bil. Breast ca (25, 50)
d. 80

Negative
Case Study #5

Ireland

- d. 80s
- d. Complications EtOH
- d. 85
- d. Colon cancer

BreastNext

- 18 gene

- d. 78
- d. Complications EtOH

Germany

- d. 60
- d. Colon cancer
- d. 68
- d. Breast ca (66)
- d. Ovarian Ca

Prostate ca (60s)

- 70

CHECK2 likely path.
p.R117G

MRE11A VUS
p.R604H

Breast ca (54)

- 58
- 57
- Breast ca (54)
Case Study #6

BRCA1/2 sequencing deletion/duplication analyses negative

France
Ashkenazi Jewish

MUTYH positive
p.G396D

Ireland / France

BreastNext
17 gene

France

Ashkenazi Jewish

- d. 50s
- d. 30s
- d. 65
- d. killed
- d. Bladder ca (65)
- d. Colon ca (85)
- d. 87

Diabetes mellitus

- d. 87

- d. 65

- d. 74

- d. Breast ca (56, 65, 73)
- Squamous / Basal cell carcinoma (60s)

smoker / work environment

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Questions?