Jumping Into Your Gene Pool: Understanding Genetic Test Results

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Acknowledgment

• American Society of Clinical Oncologists Curriculum - educational materials used for this session (plus a few of my own!) Look for ASCO

• Understanding Gene Testing - National Cancer Institute, 1996
Chromosomes, DNA, and Genes

Adapted from Understanding Gene Testing, NIH, 1995
Genes are on chromosomes
The Human Genome

23 pairs of chromosomes made of 700 million base pairs

Extragenic DNA
- Repetitive sequences
- Control regions
- Spacer DNA between genes
- Function mostly unknown

30% = the exome

20,000-25,000 genes
The Cell Cycle

- M (mitosis)
- G1 (cell growth)
- G2
- S (synthesis)
- G0 (resting)
Mitosis and Meiosis

Mitosis

Meiosis (germ cells only)
Genetic Code

A **codon** is made of 3 base pairs

64 codons total

1 codon (AUG) encodes methionine and starts translation of all proteins

61 codons encode 20 amino acids (redundant code)

3 codons stop protein translation

AUG

Met

UAA

STOP
DNA Transcription and Translation

DNA → mRNA → Growing chain of amino acids → Ribosome → Protein

Nuclear membrane → Cell membrane

Adapted from Understanding Gene Testing, NIH, 1995
Gene Structure

Promoter

RNA transcription start site

Splice sites

Stop site

Exon 1

Intron

Exon 2

Intron

Exon 3

5' end

3' end

mRNA

Exon 1

Exon 2

Exon 3
RNA Processing

- DNA
- Exon
- Intron
- Exon
- Intron
- Exon

Primary mRNA

- Transcription
- Processing

Mature mRNA

- Translation

Protein
Alleles: What are they!? 

Alleles: variant forms of the same gene (A a)
Autosomal Recessive Inheritance

- Two germline mutations (one from each parent) to express the condition
- Equally transmitted by men and women
Your Dr. wants to “phase” your family. Why?

Child’s result

Is it this? or This?
Carrier Frequency

Prevalence of an altered disease gene in a given population

Carrier frequency = 20%
Founder Effect

A high frequency of a specific gene mutation in a population founded by a small ancestral group.

1. Original population
2. Marked population decrease, migration, or isolation
3. Generations later
Environmental Factors Affecting Genes

Modifier genes  \rightarrow  Carcinogens

Response to DNA damage

Hormonal/reproductive factors

Not every altered gene has the same effect on each person that inherits it
Genotype/Phenotype Correlation

*Different mutations in the same gene cause different effects*

- Where is the genetic change in the sequence?
- What kind of change is it?
Variations in DNA sequence: What kinds are there?

A **variant** is a change in the normal base pair sequence

Can cause a change that does NOT alter protein function

OR

We might not be able to tell if the change is harmful

OR

Can be a change that makes a protein not work
Disease-Associated (Pathogenic) Variants Alter Protein Function

Functional protein

Nonfunctional or missing protein
# Point Mutations

<table>
<thead>
<tr>
<th>Type</th>
<th>Example</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>THE BIG RED DOG RAN OUT.</td>
</tr>
<tr>
<td>Missense</td>
<td>THE BIG RAD DOG RAN OUT.</td>
</tr>
<tr>
<td>Nonsense</td>
<td>THE BIG RED.</td>
</tr>
<tr>
<td>Frameshift (deletion)</td>
<td>THE BRE DDO GRA.</td>
</tr>
<tr>
<td>Frameshift (insertion)</td>
<td>THE BIG RED ZDO GRA.</td>
</tr>
</tbody>
</table>

**Point mutation:** a change in a single base pair
Silent Sequence Variants

Sequence variant: a base pair change that does not change the amino acid sequence

Adapted from Campbell NA (ed). Biology, 2nd ed, 1990
Missense Mutations

Missense: changes to a codon for another amino acid (can be harmful or neutral)

Adapted from Campbell NA (ed). Biology, 2nd ed, 1990
Nonsense Mutations

Nonsense: change from an amino acid codon to a stop codon, producing a shortened protein

Adapted from Campbell NA (ed). Biology, 2nd ed, 1990
Frameshift Mutations

Frameshift: insertion or deletion of base pairs, producing a stop codon downstream and (usually) shortened protein

Adapted from Campbell NA (ed). Biology, 2nd ed, 1990
Splice-Site Mutations

Splice-site mutation: a change that results in altered RNA sequence
Other Types of Mutations

- Mutations in regulatory regions of the gene
- Large deletions or insertions
- Chromosome translocations or inversions
Deciphering Clinical Gene Tests
Many terms are used to describe sequencing methods that fall under the umbrella term “next generation sequencing”.

https://m1.healio.com/~media/images/education-lab/learning-sites/learn-genomics-vs2/img/nextgensequencing.jpg
Preparing DNA for Analysis

Blood sample → Centrifuge and extract DNA from white blood cells → DNA for analysis
Polymerase Chain Reaction (PCR)

1. Isolate and denature DNA
2. Anneal and extend primers
3. Repeat as necessary
4. Amplified segments

Sequence to be amplified
Principle of Microarray (Chip) Assay

Prehybridization

Synthetic DNA probes

Posthybridization

Probes with hybridized DNA
Pathogenic: associated with known disease state
Likely pathogenic: strong evidence in favor of pathogenicity
Uncertain (“VUS”): limited and/or conflicting evidence of association with disease
Likely benign: strong evidence against pathogenicity
Benign: very strong evidence against pathogenicity
What kinds of evidence?

• Published variant associated with condition
• Segregation in a family or in others with the condition
• Variant is very rare in an appropriate control population
• Loss of gene function is proven
• Prediction based on data tools
• Variant highly likely to not yield protein
Summary

• FAOD conditions follow an autosomal recessive pattern of inheritance
• Parental testing is needed to “phase” the child’s result
• Results can be ambiguous (VUS) but often can be clarified by assessment of phenotype (how the child presented biochemically)