Genetic Testing in Primary Care

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Faculty

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Learning Objectives

1. Develop a clear understanding of the two basic categories of genetic variation/mutation
2. Develop a basic understanding of the types of genetic testing used to identify these variants and the limitations of each methodology
Basic Training

• DNA
  – Chemical structure is made up of four different bases
    • A (Adenine)
    • C (Cytosine)
    • G (Guanine)
    • T (Thymine)
• DNA is converted into RNA and then translated into protein
• DNA bases are “read” in groups of three
• Each codon (three bases) is specific for a single amino acid
Basic Training

- A **gene** is a stretch of DNA sequence needed to make a functional product.
Basic Training

• Each gene has untranslated parts that help with “processing”
  • Introns
  • Promotors
  • Splice sites
  • Regulatory sequences
Basic Training

• A nuclear DNA strand is wound very tightly with proteins to form an independent structure called a chromosome.
Basic Training

• An individual’s complete DNA sequence, containing the entire genetic information, is referred to as the genome

• The exome is the coding region of the entire genome
Back to the mission...

• When ordering a genetic test, it is critical to understand what type of genetic change you want to detect

• Genetic change occurs in two main categories:
  – Dosage
  – Sequence
Dosage Disorders

• Correct gene dosage is critical for typical human development
• Example of gene “overdose” is Trisomy 21 Down syndrome
• Example of gene “underdose” is Cri-du-Chat syndrome, caused by deletion of part of chromosome 5
• Dosage disorders can affect many genes at once
# Dosage Disorders

<table>
<thead>
<tr>
<th>Name</th>
<th>Region</th>
<th>Type</th>
<th>Test to detect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down syndrome</td>
<td>21</td>
<td>Duplication</td>
<td>Karyotype</td>
</tr>
<tr>
<td>Turner</td>
<td>X</td>
<td>Deletion</td>
<td>Karyotype</td>
</tr>
<tr>
<td>DiGeorge/VCF</td>
<td>22q11.2</td>
<td>Deletion</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Wolf-Hirschhorn</td>
<td>4p16</td>
<td>Deletion</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Potocki-Lupski</td>
<td>17p11.2</td>
<td>Duplication</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Pallister-Killian</td>
<td>12p</td>
<td>Triplication</td>
<td>Karyotype</td>
</tr>
<tr>
<td>Cat-eye</td>
<td>22q11.1</td>
<td>Triplication</td>
<td>Karyotype</td>
</tr>
<tr>
<td>Steroid sulfatase deficiency (XL ichthyosis)</td>
<td>STS gene on Xp22</td>
<td>Deletion</td>
<td>FISH/CGH</td>
</tr>
<tr>
<td>Duchenne/Becker muscular dystrophy</td>
<td>Parts of the DMD gene</td>
<td>Deletion/duplication</td>
<td>Targeted array</td>
</tr>
</tbody>
</table>
Dosage Testing

• Tests used to detect large dosage changes include:
  – Karyotype (chromosome analysis)
  – FISH analysis
  – Comparative genomic hybridization (microarray)

• Tests used to detect small dosage changes include:
  – Exon-level array
  – DNA methylation analysis
Dosage Testing

• Chromosome analysis is performed using microscopes to look at cells during metaphase (when chromosomes are easiest to see)
Example

Patient #1 is a 10 year old girl with short stature and a broad neck

Patient #2 is a 10 year old girl with short stature and a broad neck
Dosage Testing

- FISH analysis is a targeted technique to look for the presence, absence, and/or relative location of a specific chromosomal area (FISH is not used to “fish” for a diagnosis!)
Example

Patient #3 is a four year old male with developmental delay and hyperphagia

Patient #4 is a four year old male with developmental delay and hyperphagia
Dosage Testing

- Microarray CGH is used to detect sub-microscopic dosage changes but does not look inside individual genes
  - Exon-level targeted microarray can see dosage changes within exons of a specific gene
Dosage Testing

• Microarray compares the amount of probe from your patient with that of a control
Example

Patient #5 is a two year old male with ichthyosis

Patient #6 is a two year old male with ichthyosis
Gene Inactivation

• If your light won’t turn on, there can be more than one reason
  – For example, if you don’t have a light bulb (deletion), you don’t get any light
Gene Inactivation

- However, even if you have all the parts of a lamp but you can’t plug it in because the outlet is covered (gene methylation), you don’t get any light either
Dosage by Inactivation

• Some genes are physically present in the correct dosage but are inactivated by methylation.

• Many genes are supposed to be methylated but abnormal methylation can cause disease.

Methylation changes are also known as epigenetics.
Disorders Best Detected by Methylation Analysis

- Prader-Willi syndrome
- Angelman syndrome
- Beckwith-Wiedemann syndrome
- Russell-Silver syndrome
- UPD14
Sometimes you can’t use just one test!

• To fully understand a genetic change may take multiple methods
You found something!

Patient #9: Microarray shows a duplication

EXAMPLE 1:
Abnormal microarray showing DNA dosage gain on chromosome 17p11.2
But **where** is the duplication?

**FISH visualization demonstrates a duplication of 17p11.2.**

**FISH visualization demonstrates a marker chromosome 17p.**
You found something!

Patient #10: FISH for 4p shows a deletion
But how big is the deletion?

2.3 Mb deletion

12.1 Mb deletion
Detectable Range Comparison

- **Karyotyping**
- **FISH**
- **array-CGH** (Resolution depends on probe density)
- **MLPA**
- **Sequencing**

100Mb 10Mb 1Mb 100Kb 10Kb 1Kb 100bp 10bp 1bp
Sequence Variation

• Sequence changes usually affect only one gene
• Most disease-causing sequence changes (mutation) occur in the coding region, resulting in change to the protein structure
DNA Sequencing

• Gold standard for DNA testing; spells out DNA code
  – Very similar to a spell-checker program

• Limitations: why sequencing isn’t 100%
  – You only get data on what you sequence (=coding region)
  – If you only spell check one paragraph, you don’t know if there are errors in the rest of the text
  – You can only sequence what is there (no large deletions)
  – The spell-checker doesn’t tell you whether your sentence makes
  – The clinical significance of many sequence variants is unknown
  – Just because the spell-checker doesn’t recognize a word doesn’t mean it’s spelled incorrectly (proper names like “Zellmer”)

Know which test to order first!

• Most genetic diseases can be caused by either sequencing or dosage errors

• Examples:
  – Rett syndrome: 85% sequencing; 15% dosage
  – DMD: 85% dosage; 15% sequencing errors
  – Pelizaeus Merzbacher: 60% dosage; 25% seq
Interpreting the Results

• Nomenclature
  – Cytogenetic nomenclature
  – Molecular genetic nomenclature

• Significance
  – Polymorphisms
  – VUS (variant of unknown significance)
Cytogenetic Nomenclature

• Chromosome analysis
  – 46,XX or 46,XY (normal)
  – 47,XX,+21 means female with Down syndrome
  – 46,XX,del(3)(p12) means female with 46 chromosomes with a deletion of part of one chromosome 3 on the short arm (p) at band 12
  – 46,XY,dup(14)(q22q25) means a male with a duplication of part of one chromosome 14 on the long arm (q) involving bands 22 to 25
  – Other abbreviations include “t,” “inv,” “r” “mar” “der” and many more
Cytogenetic Nomenclature

- **Array CGH results**
  - arr (1-22,X)x2  (*normal female*)
  - arr(1-22)x2,(XY)x1  (*normal male*)
  - arr 4q28.3qter(134,293,639-qter)x3  (*duplication of 4q*)
  - arr 12q24.33qter(131,203,633-qter)x1  (*deletion of 12q*)

- **FISH results**
  - 46,XX.ish Xp22(SHOXx2),Xp11.1q11.1(DXZ1x2)[20] nuc ish(SHOX,DXZ1)x2[200]  (*normal*)
  - 46,XY.ish del(22)(q11.2q11.2)(HIRA-)[20] nuc ish(HIRAx1)[10]  (*22q deletion*)
Molecular Genetic Nomenclature

- All sequence variants are described at the DNA level, in relation to a coding reference sequence.
  - c.83G>A means the “G” that should be at the 83rd position has been changed to an “A”
- Sequence variants are also described at the protein level, in relation to the protein reference sequence.
  - p.Val312Ala or p.V312A means that the valine that should be the 312th amino acid has been changed to an alanine

Exon 11: ...GAGTCA

Exon 12: GT ... CCGTAT...

Intron: GT ... AG...

711 +1 +2...

-2 -1... 712
Types of Mutations

- **Silent mutations:** p.E315E (c.945G>A)
- **Missense mutations:** p.C282Y or p.Cys282Tyr.
- **Nonsense mutations:** p.W1282X or p.Trp1282Stop
  - End up with a truncated protein
- **Frameshift:** p.R97fs or p.Arg97fs (c.33delT)
  - Means that a base has been added or deleted which has thrown the reading frame out of whack and all amino acids after that point are wrong
WARNING:

• Not all genetic changes cause disease!
• There are many, many polymorphisms in the genome, in both dosage and sequence.
• 46,XY, inv(9)(p11q13) sounds significant but is found in many people and doesn’t cause problems – this is not a chromosome abnormality!
• If not previously reported as disease causing or benign and status is uncertain, these changes are called a “variant of unknown significance.”
• Family history and testing is the best way to figure out significance.
Patient #11: 6 year-old boy with DD and dysmorphic features

- Tested for panel of 96 XLID genes
- VUS detected in OFD1
- Determined not pathogenic based on presence in unaffected brother
Test Type Summary

• General (I don’t know precisely what I’m looking for)
  – Chromosome analysis
  – Microarray CGH
  – Multigene panels
    • Epilepsy (58 genes)
    • Developmental delay in males (90 genes)
Test type summary

• Specific (I’m concerned about a specific disorder)
  – FISH (for a specific microdeletion disorder)
  – Gene sequencing/dosage (must know which gene)
“Half” an Answer

• In recessive conditions, you need both copies of the gene to be altered in order to show symptoms.
• Many times testing reveals only one mutation.
• Where’s the other mutation?
  – A1: Maybe it’s deleted. Try exon-level del/dup analysis.
  – A2: The second mutation is in a non-coding region.
  – A3: Patient’s problem is due to a different gene.
One mutation in a recessive disorder

- Where’s the other mutation?
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**Patient 14:** 10-month-old Caucasian male with developmental delay, bilateral cherry red spots with retinal pallor and increased startle response. Positive enzyme testing for Tay Sachs.

- One copy c.1277_1278insTATC in HEXA
- Exon array shows partial deletion of other allele.
One mutation in a recessive disorder

- Where’s the other mutation?
  - A1: Maybe it’s deleted. Try exon-level del/dup analysis.
  - A2: The second mutation is in a non-coding region.
  - A3: Patient’s disease is due to a different gene.

**Patient #15:** 2 year-old girl with clinical presentation and specific lab findings consistent with recessive disease, HLH.

- Heterozygous for c.2346_2349 del GGAG (R782fsX793) in the UNC13-4 gene.
- This result in combination with clinical scenario and decreased NK function, supports a familial basis to this patient's disease.
One mutation in a recessive disorder

• Where’s the other mutation?
  – A1: Maybe it’s deleted. Try exon-level del/dup analysis.
  – A2: The second mutation is in a non-coding region.
  – A3: Patient’s problem is due to a different gene.

Case 6: 4 month old boy with failure to thrive, passed NBS for CF. CFTR mutation testing ordered.
• Positive for one copy deltaF508.
• Subsequent negative sweat chloride testing.
• Conclude baby is CF carrier (1/29 in general population).
Conclusions

• Karyotype, FISH, microarray, methylation testing, exon-level array and sequencing all detect different sizes and types of mutations.

• The correct test depends on disease/gene suspected; often more than one test is required.

• Interpretation is guided by type of mutation, clinical scenario, family studies, and may be unclear despite best efforts.

• May be complicated—contact your friendly lab genetic counselor for help!
Questions
Thank you for your participation!

For more information, please contact

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