Clinical genetic testing
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Case 1
- 54 y/o man of Italian descent
- 2001: Vascular aneurysm repaired in abdomen (details unknown)
- 2015: 3.6 cm. left iliac aneurysm; 2.5 cm right iliac aneurysm
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- MGM had a single sister, no known issues
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Physical Exam
- Normal inner canthal distance
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Objectives

- To review basic aspects of clinical genetics and new genetic testing technologies
- To recognize the difference between clinically useful and research-based genetic testing
- To recognize vascular EDS as an example of a monogenic condition where genetic testing directly impacts management

Medical Genetics: what we do

- Use molecular genetics to more accurately prevent, diagnose and treat disease
- Place Genetics in simple terms
- Manage expectations from a genetic test result
- How the test result will change management for the patient and family
- Medico-legal implications of genetic testing
Genetics: the basics

- **Penetrance** - the percent of individuals with a mutation who manifest disease (rarely 100%)
- **Expressivity** - the degree to which each individual with mutation is affected
- **Locus heterogeneity**: A single phenotype caused by mutations in genes at different chromosomal loci.
- **Allelic disorders**: different phenotypes caused by mutations in the same gene.

Genetics: the basics

- **Inheritance patterns:**
  - Autosomal dominant
  - Autosomal recessive
  - X-linked, male lethal (“dominant”)
  - X-linked “recessive” (but women can have symptoms)
  - Digenic (but can be gene + another locus)
  - Triallelic
  - Imprinted
  - Somatic
Genetic Tests:

- Karyotype: image of chromosomes
- Microarray: Allows for detection of small deletions or duplications across the genome using special probes
- Sanger sequencing: traditional test for point mutations
- 2010: Next generation sequencing (clinical)
- 2012: Medical genome and exome tests

Microarray

- Technique for detection of very small deletions or duplications
- Two different techniques:
  1. CGH
     - Individual’s DNA is compared to control, detecting areas were signal is excessive (duplications) or lacking (deletions).
  2. SNP array
     - Key areas are sequenced and distributed across the genome, loss of heterozygosity can be detected.
**Microarray**

- Indicated in:
  - Multiple Congenital Anomalies
  - Unexplained developmental delay
  - Autism
  - If a complete gene deletion is identified in another test, to see the extent of the deletion
  - If is not indicated to "update" the result of a previous test

**Karyotypes**

- Traditional "photograph" of chromosomes in metaphase
- Useful in trying to rule out low level mosaicism (for example, mosaic 45,X in coarctation of the aorta).
- Important to follow-up microarray findings with karyotype (where is the duplication actually located?)
Next Generation sequencing

- Thousands of genes can be sequenced at the same time, for the same cost as a single gene.
- Interpretation is very complex
- Gene panels: designed by the lab, may have more targeted coverage
- Deletion/duplication detection techniques vary from lab to lab
- Therefore, panels vary greatly from lab to lab
- Pseudogenes: may result in false negatives.
Genetic Testing: Practical aspects

- Next-generation panels are more “practical”. Usually the price is the same or cheaper vs. selecting individual genes.

- The more genes, the more likely a VUS will be identified

- Insurance coverage: changing landscape. More coverage from private payers but often policies have “genetic exclusions”
Genetic Testing: Practical aspects

• Often require preauthorization, but labs may help with this.

• Pre-testing counseling and consent are very important (not a simple “positive or negative” test)

• GINA offers protection for health but not life insurance
Mutations

- Mutation = Pathogenic

- Polymorphism = Too frequent to be a mutation (2%) 
  
  MTHFR

Mutations –types

- Point
  - Missense mutations: change one of one amino acid for another
  - Nonsense: change of an AA for a “stop” codon
  - " Silent": does not result in an amino acid change
  - Splice-site: alters final mRNA processing

- Insertion, Deletion and In/del
  - Usually results in an early stop codon with no protein translated
Mutations – clinical types

- **Variant**: Any deviation from the reference DNA
  - Pathogenic: Variant is recognized as causing disease
  - Probably pathogenic: it is likely that the variant causes disease (Question: Would you recommend an abortion?)
  - Uncertain significance
  - Probably benign: it is probably it will not cause disease
  - Benign: Variant is recognized as NOT causing disease

Examples

- **CFTR**: c.1521_1523delCTT (p.Phe508del), pathogenic, cystic fibrosis, autosomal recessive
- **MSH2**: c.2047G>A (p.G683R), pathogenic, Lynch Sd, autosomal dominant.

Pathogenic vs Benign

- Cosegregates with disease
- Previously reported in affected individuals
- Absent from controls
- Alter protein function
- AA change in a conserved region
Exome and Genome Sequencing

- Exome sequencing: Looks at all 20,000 genes. –
  - About 40K-60K variants per individual.
  - Can be used to identify new conditions.
  - Trio: maximal utility
- Clinical exome: Looks only at the 4,600 associated with disease.
  - Does not require parental samples.
  - About 400 variants per individual after filtering

Panels, exome and genome

- In principle, all three use the same NGS technique.
- Specific panels have greater “coverage” of the genes of interest.
- In many cases, the full “library” is run but only the requested genes are reported.
Uncertain results

- As the number of genes in a panel increases, so does the likelihood of an uncertain result
- Managing uncertain results is the greatest challenge in current clinical practice

GWAS vs. Genome Sequencing

- **GWAS**: Genome-wide association studies
  - Uses SNPs that are equally distributed (~1.5 M)
  - Case-control study in which each SNP is a risk factor
  - SNPs are "tags" that point to a locus of interest
  - SNPs are not the "culprits"
- **Genome sequencing**:
  - Sequences every nucleotide (~3.2 B)
  - Sequence changes are the "culprits"
GWAS: CAD

- 33398 CAD Cases
- 75726 Controls
- Two different platforms: 500K and 2.5 M SNPs

Genetic testing: clinical utility

- Low clinical utility in testing for Risk Factor SNPs due to low OR (23AndMe, other platforms)
High clinical utility in testing for Mendelian Disorders

Can help establish diagnosis if broad differential (e.g., arrhythmias)

Can give prognosis in certain diseases (e.g., double hets with HCM)

Can guide management (e.g., lower threshold for surgery in Loeys-Dietz)

Testing at risk-relative (cost effective)

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Differential diagnosis:

- Loeys-Dietz Syndrome
- Vascular type EDS ("type IV")
- Heavy smoking

Ehlers Danlos Syndrome, vascular type

- Arterial aneurysms, dissection or rupture
- Intestinal rupture
- Uterine rupture during pregnancy
- Others: hypermobility, skin laxity, acrogeria, gingival recession, early onset varicose veins, …
Loeys Dietz Sd

- Originally discovered in a group of “atypical Marfan” patients
- Mutations in TGFBR1, TGFBR2, SMAD3, TGFBR2, TGFBR3
- Craniosynostosis, hypertelorism, bifid uvula
- Aortic dissection with relatively small diameters
- Arterial tortuosity and aneurysms
- Early osteoporosis (especially in SMAD3)
- Food allergies
- Possible benefit of losartan – RCT unlikely

Genetic Test results

TAADNext: Analyses of 22 Genes Associated with Thoracic Aortic Aneurysms and Dissections

<table>
<thead>
<tr>
<th>RESULT</th>
<th>CDH1</th>
<th>Variant, Likely Pathogenic: p.Ser178Thr</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMARY</td>
<td>POSITIVE: Likely Pathogenic Variant Detected</td>
<td></td>
</tr>
</tbody>
</table>

Interpretation:
- This individual is heterozygous for the p.Ser178Thr pathogenic variant in the CDH1 gene.
- This result is consistent with a diagnosis of Thoracic Aneurysm syndrome (TAS) vascular type.
- Transmission and severity of disease in this individual cannot be excluded.
- Genetic testing in family members can be helpful in identifying at-risk individuals.
- Genetic counseling is recommended for all individuals underreporter certain.

Management

- Avoid endovascular procedures: open repair
- Avoid colonoscopies!
- Genetic testing to FDRs
- Alpha/beta blocker: Preliminary evidence in Europe
- Continued screening of ascending aorta
**Had it been Loeys-Dietz...**

- Proceed with endovascular repair
- Annual surveillance of ascending aorta, surgery at 4.5 cm threshold
- Start losartan
- Genetic testing to FDRs/ if not possible the echos for surveillance

**Case 2**

- 20 y/o previously healthy man
- Presents with acute emesis
- Found to have calcium level of 7.6
- PTH level very low
- FH: Father with borderline low calcium. Paternal grandmother died from complications of intestinal obstruction
- PE: No hyperpigmented macules, no dysmorphic features, no skeletal anomalies
- Currently on three times a day high dose calcium supplement

**Case 2: differential diagnosis**

- CASR: Autosomal dominant hypocalcemia w/ low Mg
- PTH mutations: autosomal dominant or recessive, depending on the mutation – family fits AD. Relatively benign. Would respond to PTH supplement
- PTH1R mutations - would not respond to PTH
Case 2: differential diagnosis

- AIRE: Recessive polyglandular autoimmune. Could affect other glands
- GNAS: AD but with imprinting effects. May lead to insulin resistance
- 15 gene panel, but includes genes with single case reports

Case 2: Management

- VOUS! – Try to test parents, and their calcium
- Await further clarification as time goes by
- Cell studies? Not a clinical test, no validation
- Phenotype fits very well, so manage as AD hypocalcemia
- Start tracking magnesium

MOLECULAR GENETICS REPORT:

hyoparathyroidism Sequencing Panel

SUMMARY OF RESULTS: Heterozygous for a Variant of Uncertain Significance in CASR

RESULTS AND INTERPRETATIONS: This patient is heterozygous in the CASR gene for a sequence variant designated p.H444K. To our knowledge, this variant has not been reported in literature or public databases. The amino acid residue p.H444K has been highly conserved among evolution. The prediction programs PolyPhen-2: SIFT and MutationTaster predict the p.H444K change to be "benign", "deleterious", and "disease causing", respectively. Without further functional analysis, the significance is uncertain due to the absence of conclusive functional and genetic evidence (see notes below).
Key points

- DNA testing is full of pitfalls. It is not just another test.
- Pre-test genetic counselling
- However carefully selected clinical genetic testing can be very useful
- *Do not order MTHFR. Do not give clinical value to 23andMe*

Questions?