Retinitis pigmentosa - novel treatments and challenges

Strategies for Genetic Screening in Patients with Inherited Retinal Dystrophies

Tamar Ben-Yosef
Strategies for genetic screening in patients with Inherited Retinal Dystrophies

Tamar Ben-Yosef, Ph.D.

Inherited Retinal Dystrophies (IRDs)

- Clinical and genetic heterogeneity
- Over 200 genes identified

Cone

Rod

Cone Dystrophy

Achromatopsia

Cone-Rod Dystrophy

Retinitis Pigmentosa (Rod-Cone Dystrophy)

Leber Congenital Amaurosis
retinitis pigmentosa (RP)

- The most common form of Inherited Retinal Dystrophy
- One of the most **genetically heterogenous** conditions in humans

### Why is genetic screening important?

**Prevention**
- Genetic testing and counseling in high-risk families and populations

**Prognosis**
- Genotype-phenotype correlations

**Treatment**
- Identification of patient groups with shared genetic diagnoses, who can be recruited to clinical trials for evaluation of various treatment strategies

**Research**
- Identification of novel causative genes and mechanisms
Challenges in Genetic Analysis of IRD patients

- Over 200 causative genes identified
- The contribution of each of these genes to the overall prevalence of the disease is relatively small
- In 40-50% of IRD patients the underlying genes are yet to be identified

How should we assess a DNA sample of an IRD patient?
Test for founder mutations

Are there known founder mutation/s in the patient’s ethnic group?

A founder mutation: A mutation that proliferates in a kinship or community from a single identifiable ancestor.

Examples:
- Ashkenazi Jews
- Old Order Amish
- Finland

Examples for IRD founder mutations in various populations:

<table>
<thead>
<tr>
<th>population</th>
<th>phenotype</th>
<th>gene</th>
<th>mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ashkenazi Jews</td>
<td>Type 3 Usher syndrome</td>
<td>CLRN1</td>
<td>p.N48K</td>
</tr>
<tr>
<td>Spain</td>
<td>RP/CRD</td>
<td>CERKL</td>
<td>p.R257X</td>
</tr>
<tr>
<td>Dutch and Belgians</td>
<td>RP</td>
<td>FAM161A</td>
<td>p.R437X</td>
</tr>
<tr>
<td>Japan</td>
<td>RP</td>
<td>EYS</td>
<td>c.4957dupA</td>
</tr>
<tr>
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<tr>
<td>yes</td>
<td>no</td>
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<tr>
<th>Does the patient have a specific phenotype with one (or a few) relatively small causative gene?</th>
<th>Sanger sequencing</th>
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<tbody>
<tr>
<td>yes</td>
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</table>

**A specific phenotype with one (or a few) relatively small causative gene**

**Sanger sequencing of causative gene**

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**Positive Examples:**

- Congenital Stationary Night Blindness (XL): **NYX** (2 exons)
- Retinoschisis (XL): **RS1** (6 exons)
- Best Disease (AD): **BEST1** (11 exons/10 coding)

**Negative examples:**

- Stargardt disease: **ARCA4** (50 exons)
- Type 2 Usher syndrome: **USH2A** (72 exons)
Test for founder mutations

Are there known founder mutation/s in the patient’s ethnic group?

no

Does the patient have a specific phenotype with one (or a few) relatively small causative gene?

Sanger sequencing

yes

Does the patient have a phenotype with many and/or large causative genes?

NGS (WES, TNGS)

yes

no

negative

A phenotype with many and/or large causative genes

Next Generation Sequencing (NGS)

- Whole Genome Sequencing (WGS)
  Sequencing of the entire genome

- Whole Exome Sequencing (WES)
  Sequencing of exons only (including exon/intron borders)

- Targeted Next Generation Sequencing (TNGS)
  Direct sequencing of selected regions in the genome
Advantages and disadvantages of different NGS approaches

**WGS**

- Sequencing both coding and noncoding regions of the genome
- Detects all types of genomic variations
- Large amount of data - 3.5M variants (complex analysis)
- Expensive ($2,000/sample)

Nowadays performed mainly on a research-basis

**WES or TNGS: filtration strategies**

- Pipeline: commercial/ in-house/ online (wANNOVAR)

- Filtering criteria:
  - Variant quality and depth (avoid artifacts)
  - Variation effect (nonsense, missense, indels, splice-site)
  - Frequency in population (rare variants only)
  - Genotype (homozygous/heterozygous) – according to expected mode of inheritance
Negative results?

• Coverage analysis

• Use a different pipeline

**J Med Genet.** 2016 Sep;53(9):600-7. doi: 10.1136/jmedgenet-2016-103825. Identification of genomic deletions causing inherited retinal degenerations by coverage analysis of whole exome sequencing data. Khateb S1, Hanany M1, Khalajeh A1, Beryozkin A1, Meyer S1, Abu-Diab A1, Abu Turkv E1, Mizrahi-Meissonnier L1, Lieberman S2, Ben-Yosef T1, Banin E1, Sharon D1.

An example of a homozygous deletion of 3 exons which were not covered by WES
Test for founder mutations

Are there known founder mutation/s in the patient’s ethnic group?

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no

Does the patient have a phenotype with many and/or large causative genes?

yes

Sanger sequencing

WES, TNGS

report

CNV analysis, WGS

Summary

- Inherited Retinal Dystrophies (IRDs) are highly heterogeneous
- Genetic testing of IRD patients is important
- Genetic testing of IRD patients is challenging
- A step-by-step process combining both specific and wide-scale screening is cost-effective
thank you