Genetic Diagnosis, Disease Gene Discovery and Gene Therapy for Usher Syndrome

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Inherited Retinal Degenerations

- Inherited retinal degenerations (IRDs) are important causes of vision loss
  - Affect people of all ages
  - Diseases of photoreceptor and RPE cells of the retina
- Goal: to improve our understanding of these disorders so that therapies to prevent vision loss can be developed
- LCA2 and other gene therapy trials demonstrate potential for treatment of these disorders
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Outline

● Genetic diagnostic testing service of the Ocular Genomics Institute
  – Genetic Eye Disease (GEDi) Panel

● Research genetic studies for patients with Usher Syndrome

● Research directed towards developing gene therapies for genetic sub-types of Usher Syndrome
IRDs: Clinically Diverse

• Isolated (non-syndromic) diseases
  – Leber congenital amaurosis (LCA)
  – Retinitis pigmentosa (RP)
  – Congenital stationary night blindness (CSNB)
  – Cone dysfunction syndromes
  – Stargardt disease
  – Choroideremia
  – Macular dystrophies

• Systemic or syndromic disorders
  – Cilia diseases
    Alstrom, Bardet-Biedl, Joubert, Senior-Loken, Usher
  – Metabolic disorders
  – Mitochondrial disorders
  – Peroxisomal disorders
  – Neuronal lipid storage disorders
IRDs: Genetically Diverse

- **RetNet**: 191+ disease genes
  - Many causes of the same phenotype
  - Also overlap among genetic causes of clinical phenotypes
- Probably should classify diseases based on genetic cause

(Modified from Berger, et al 2010)
# Usher Syndrome

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
<th>Non syndromic form</th>
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<tr>
<td>Usher 1</td>
<td>MYO7A</td>
<td>Myosin VIIa</td>
<td>Actin-based motor protein</td>
<td>DFNB2</td>
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<td>USH1C</td>
<td>Harmonin</td>
<td>Scaffold protein</td>
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<td>CLRN1</td>
<td>Clarin 1</td>
<td>Cell adhesion</td>
<td>RP</td>
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</tbody>
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Genes and Mutations

- Genes = genetic information
  - Stored in “library” – nucleus of cells
  - 25,000 genes; think 25,000 books in library
    But, this library has two copies of each book
Genes and Mutations

- Genes have instructions for making proteins
- Proteins perform functions in cells
  - Such as response to light or sound
Genes and Mutations

- Mutations = mis-spellings
  - Spelling errors that change the meaning of words in a gene can cause disease
- Misspelled proteins don’t work correctly
  - e.g. can result in decreased vision or hearing
How do you find mutations?

- Human genome = 3 billion base pairs of DNA
- DNA sequence in any two people is 99.9% identical – only 0.1% is unique
  - But that’s still 3 million spelling differences between people
  - Which ones of these are normal variation, and which ones cause disease?
    - Including family members in genetic studies is important
    - Studying the functional effects of potential mutations is important
DNA Sequencing

- **Traditional Sequencing**
  - One gene at a time
  - Read two hundred books in the library, one at a time (but both copies…)

- **Next Generation Sequencing**
  - Hundreds to thousands of genes at once
  - Read both copies of two hundred books in the library all at once, automatically
  - Or, read 25,000 books (two copies) all at once, automatically
NGS for Inherited Eye Disease Genetics

- Accurate genetic diagnosis and identification of new disease genes are important steps toward developing gene therapies
- Selective exon capture/NGS for genetic diagnosis of patients with IRDs, glaucoma, optic neuropathy
- Exome sequencing and copy number variant analyses for new disease gene discovery
- Transcriptome analyses to identify novel genes and transcribed sequences expressed in the retina
Exon capture and NGS for genetic diagnosis

- **Genetic Eye Disease Panel, GEDi**
- **SureSelect, solution-based capture system**
  - 191 known IRD disease genes from RetNet (2013) ≈ 1 Mb
  - Additional genes associated with optic atrophy, glaucoma
  - Mitochondrial genome
- **Sequence Illumina MiSeq**
  - 12-15 samples/run
GEDi-R SureSelect

- Sequencing on Illumina MiSeq
  - Multiplex reactions (12-15X)
  - Paired end 150bp reads; ~80MB of sequence per patient

- 188 probands tested:
  - LCA, RP, CRD, CSNB, BBS, retinoschisis, Stargardt, Usher Syndrome
Prevalence of *USH1* gene mutations in MEEI Usher 1 patient cohort of 45 probands
New sequence variants identified
Importance of comprehensive IRD gene screening

- Year 2000
- USH1 cohort
- MYO7A screening
- 19 MYO7A patients
  - 11 cases with 2 mutant alleles
  - 8 cases with 1 mutant allele

- Year 2013
- GEDi screening of 8 patients with 1 mutant allele
CNVs as Pathogenic Alleles

- Copy number variants (CNVs) reported to cause up to 15% of pathogenic alleles in IRD disease genes
  - Larger scale insertions, deletions, duplications of genes or gene components
- aCGH for RetNet disease genes
  - Custom Agilent CGH array for GEDi genes
- Illumina Omni 2.5 SNP array for genome-wide CNV analysis
CNVs as Pathogenic Alleles

- Proband BGL 003-019
  - p.Cys3294Trp (c.9882C>G) in *USH2A* detected
  - GEDi aCGH detected a deletion of exon 27 in *USH2A*
    Causes frameshift, null allele
Research Genetic Studies

● What do we do for patients that have single mutations in USH genes?
  – CNV analyses
  – Sequence novel exons in USH genes
  – Sequence the USH genome

● What do we do for patients that don’t have any mutations in USH genes?
  – Exome sequencing
  – Genome sequencing
Novel Exons in USH Genes
Transcriptome Analyses

- Novel coding sequence in human retinal transcriptome
  - 79,915 novel junctions that consist of exon skipping, novel exons, and alternate splice sites
    - 19,637 novel internal exons
    - 7,006 (36%) preserve reading frame
      - Including 206 in the known IRD disease genes

- Find 106 novel multi-exon genes
  - Majority encode lincRNAs

(Farkas et al BMC Genomics 2013)
Novel IRD Disease Gene Exons
Example: Novel Usher Syndrome
Gene Exons
Usher Genome Sequencing

- Develop and test selective capture and Illumina sequencing of entire genomic regions of 10 Usher syndrome genes
  - Size: 3.6 Mb
  - Pros: High likelihood of identifying non-coding mutations in Usher genes
  - Cons: Need to develop a new test
Exome sequencing for new disease gene discovery

- Family-based and cohort studies
- Optimal exome capture:
  - Agilent v5 + UTR capture set
  - Mitochondrial content
  - Novel exons from human retinal transcriptome
- Sequence 2 sample per channel, Illumina HiSeq
  - >98% target sequences covered ≥10X
- Data analyses to identify candidate disease genes
LCA Family 047 – Data Filtering

EAP117: total

123,326 (108,899/14,327)

■ 34,406 (32,782/1,624)

■ 23,379 (21,762/1,617)

■ 15,656

■ 170 genes

■ 113 genes

■ 4 genes

Coding

Non-synonymous

Candidate SNPs/Genes

Variants in 1 of 4 genes predicted to be pathogenic
Variants in the NMNAT1 gene segregate with disease

(Falk/Zhang et al Nature Genetics 2012)
Exome sequencing doesn’t always work…

- Sequenced 39 families, solved 11
- Example: LCA Family 081
- Exome sequence: no putative disease gene identified
Updated Strategies

- Combination of optimal exome capture with CNV analyses
  - Optimal exome
  - Omni 2.5 SNP-based CNV detection
    (allows for QC, linkage analysis, homozygosity mapping)
  - Integrated informatic analysis

- Genome sequencing
Gene Therapies for Usher Syndrome

- UshStat for USH1B caused by mutations in *MYO7A*
  - Sanofi/Oxford Biomedica
- Ocular Genomics Institute/Berman Gund Lab:
  - *USH2A, GPR98*
  - Strategy
    Create mini-genes, test in AAV and lentiviral vectors
USH2A

- Coding sequence = 15609bp
- Protein has repetitive elements:
- There is precedent to for removing some of the repeated domains and retaining function:
  - The 14kb cDNA Duchene MD coding sequence was reduced to a therapeutic mini- (6.4kb) and microgene (3.7kb) with demonstrated efficacy in animal models for Duchene muscular dystrophy.
Summary

- NGS can facilitate genetic diagnostic testing and disease gene identification for IRDs such as Usher syndrome
  - Additional genetic studies are needed to identify non-coding mutations in known Usher genes, and identify novel Usher genes
- Transcriptome analyses show greater diversity of gene expression and splicing than previously appreciated
- Gene therapies for genetic sub-types of Usher syndrome are being developed
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