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

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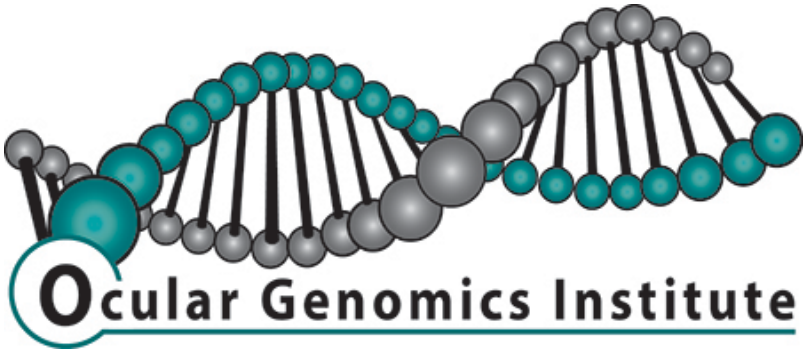

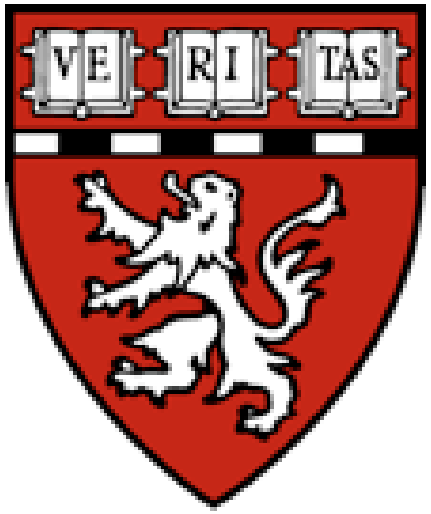

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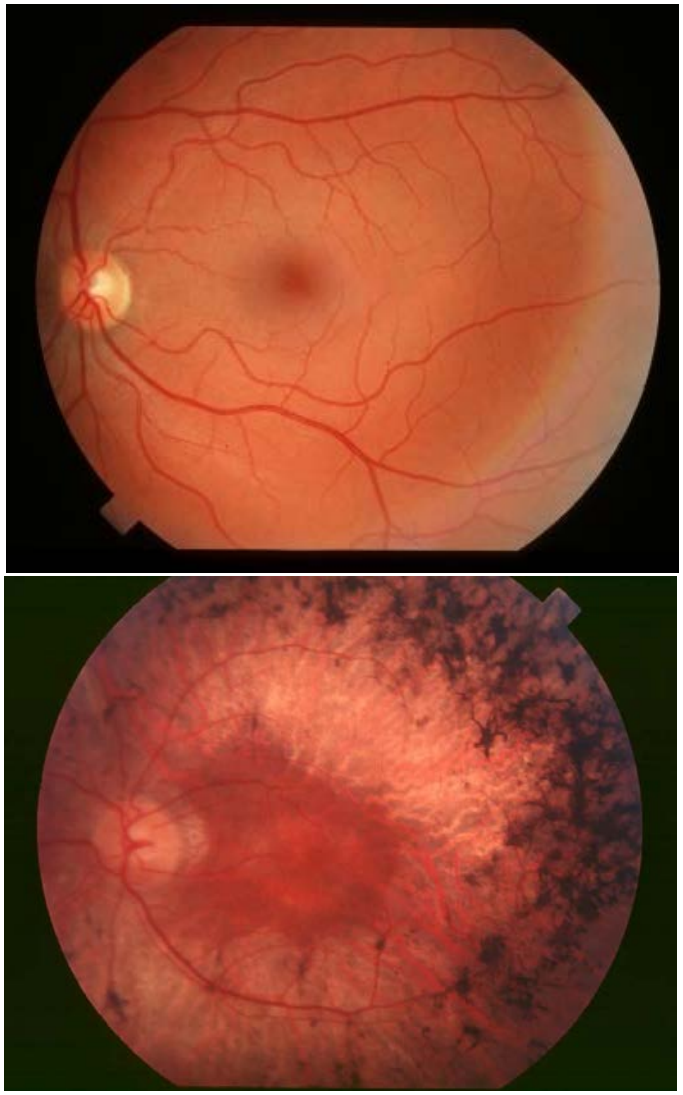
Inherited Retinal Degenerations

- 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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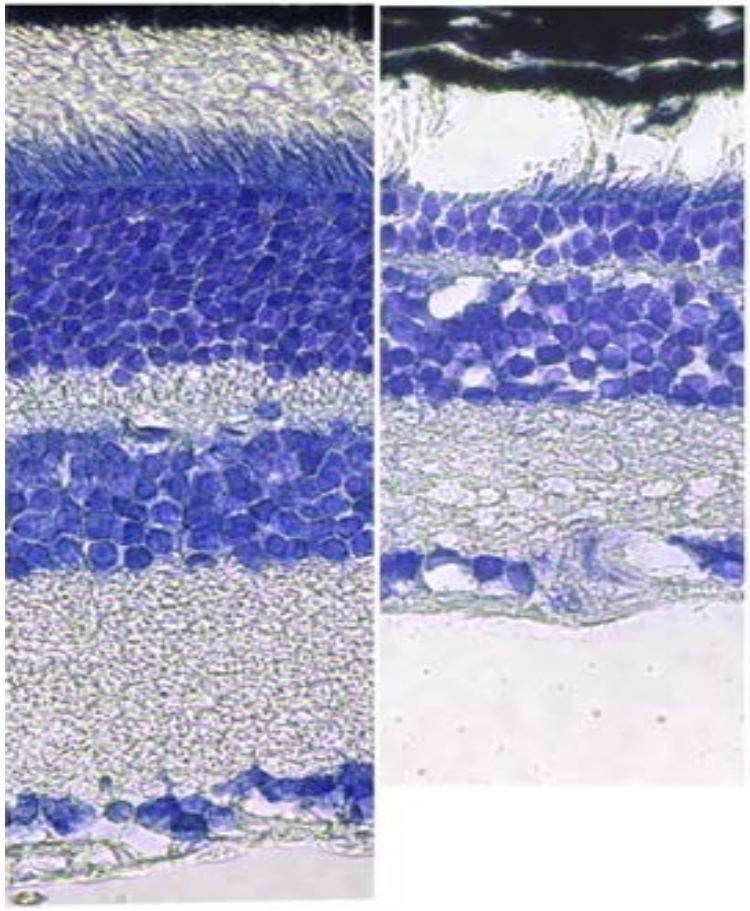


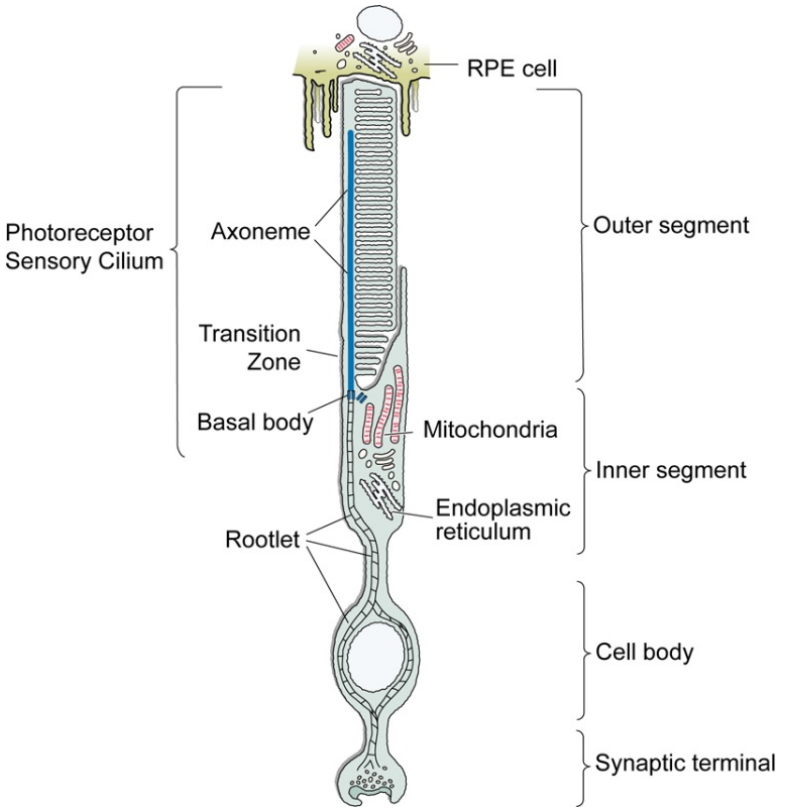
Outline

- Genetic diagnostic testing service of the Ocular Genomics Institute
  - *G*enetic *E*ye *Di*sease (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



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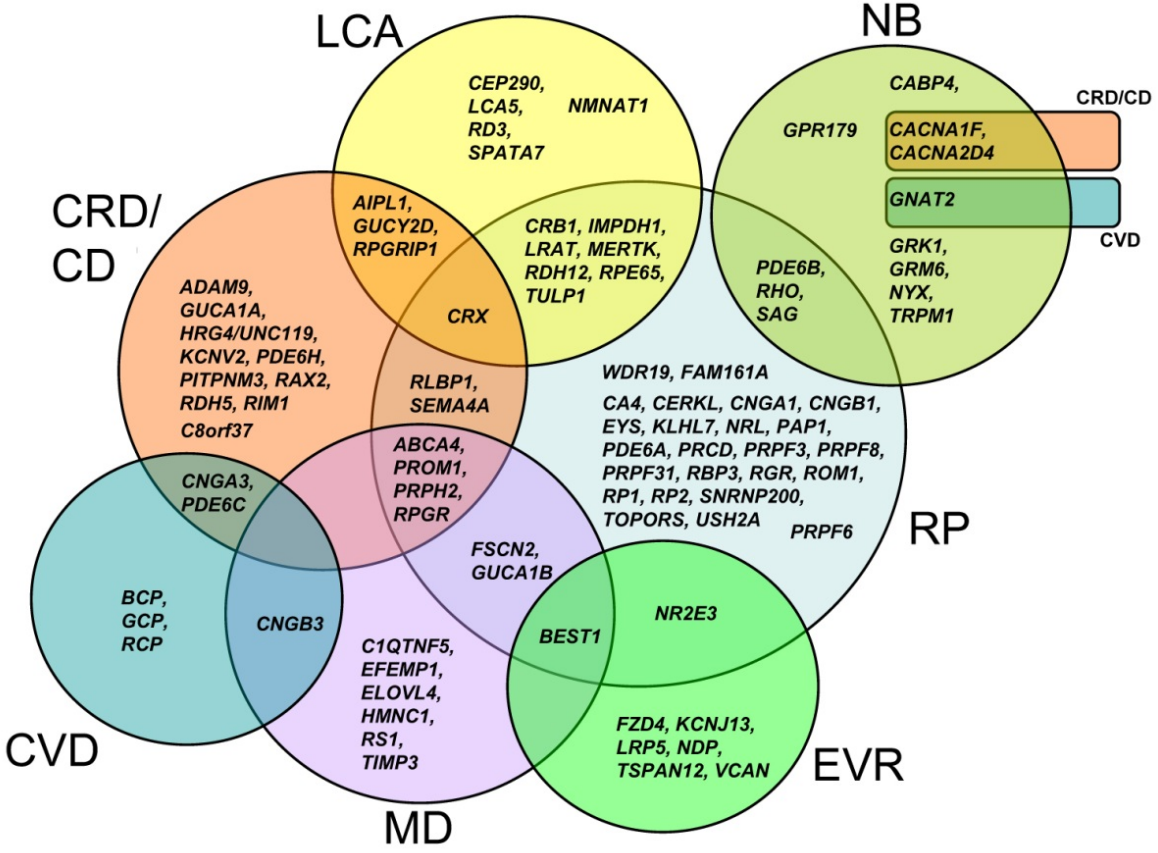
IRDs: Genetically Diverse

- 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)

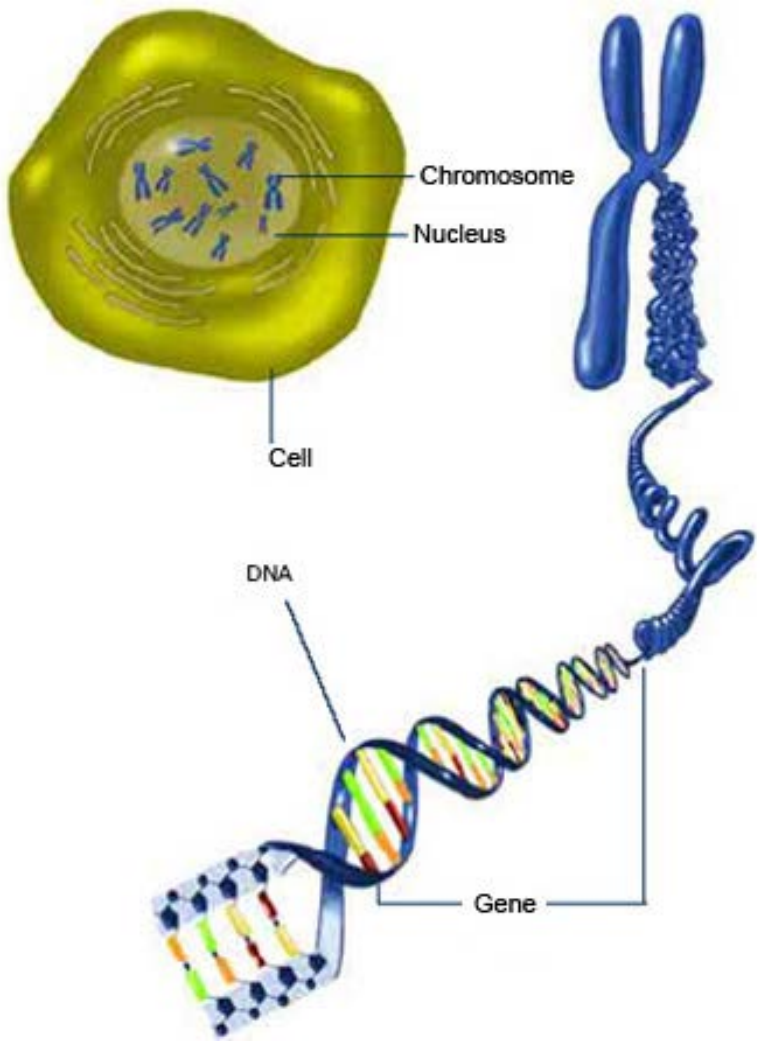
Usher Syndrome

Type	Gene	Protein	Function	Non syndromic form
Usher 1	*MYO7A*	Myosin VIIa	Actin-based motor protein	DFNB2
				DFNA11
	USH1C	Harmonin	Scaffold protein	DFNB18
	CDH23	Cadherin 23	Cell-cell adhesion	DFNB12
	PCDH15	Protocadherin 15	Cell-cell adhesion	DFNB23
	USH1G	SANS	Scaffold protein	--
	CIB2	Calcium and integrin binding family member 2	Calcium homeostasis	DFNB48
Usher 2	*USH2A*	Usherin	Matrix	RP39
			Cell adhesion	
	GPR98	G protein-coupled receptor 98	Cell adhesion	reflex-seizure
	WHRN	Whirlin	Stereocilia elongation	DFNB31
Usher 3	*CLRN1*	Clarin 1	Cell adhesion	RP

Genes and Mutations

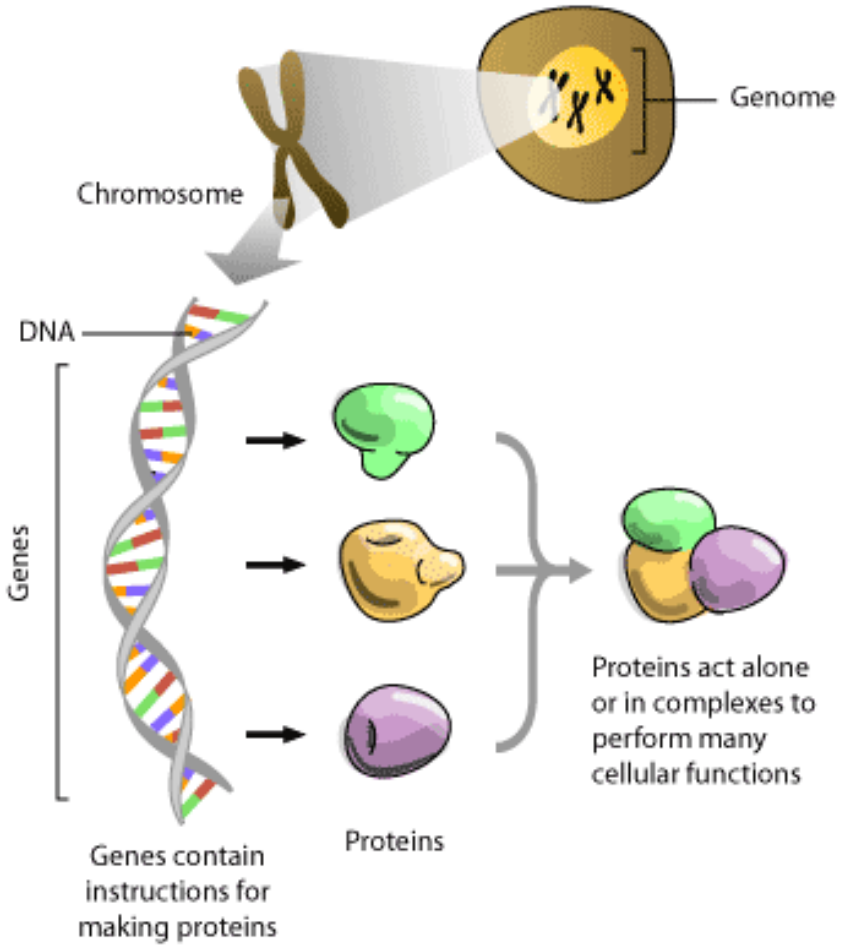
- Genes = genetic information
  - Stored in "library" – nucleus of cells
  - 25,000 genes; think 25,000 books in library

    But, this library has two copies of each book



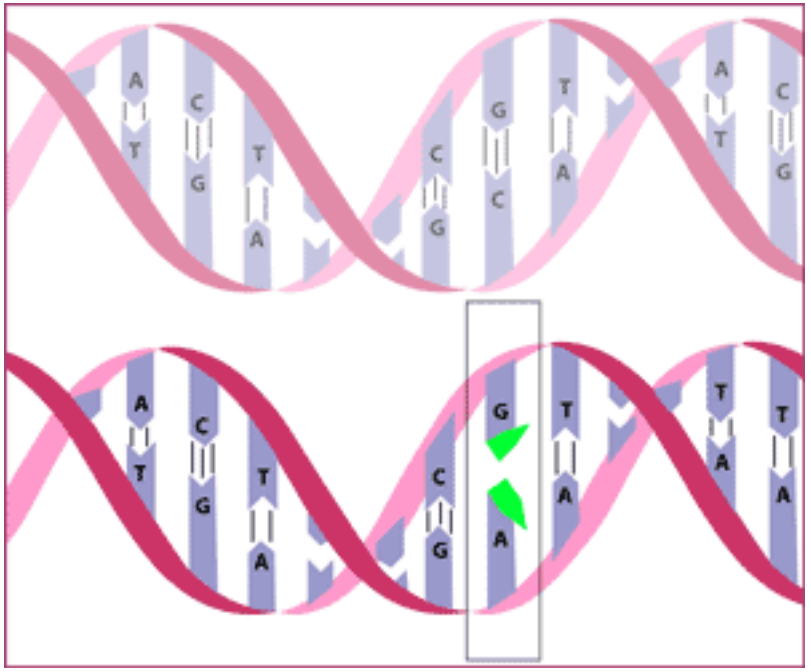
Genes and Mutations

- 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 <u>can</u> 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

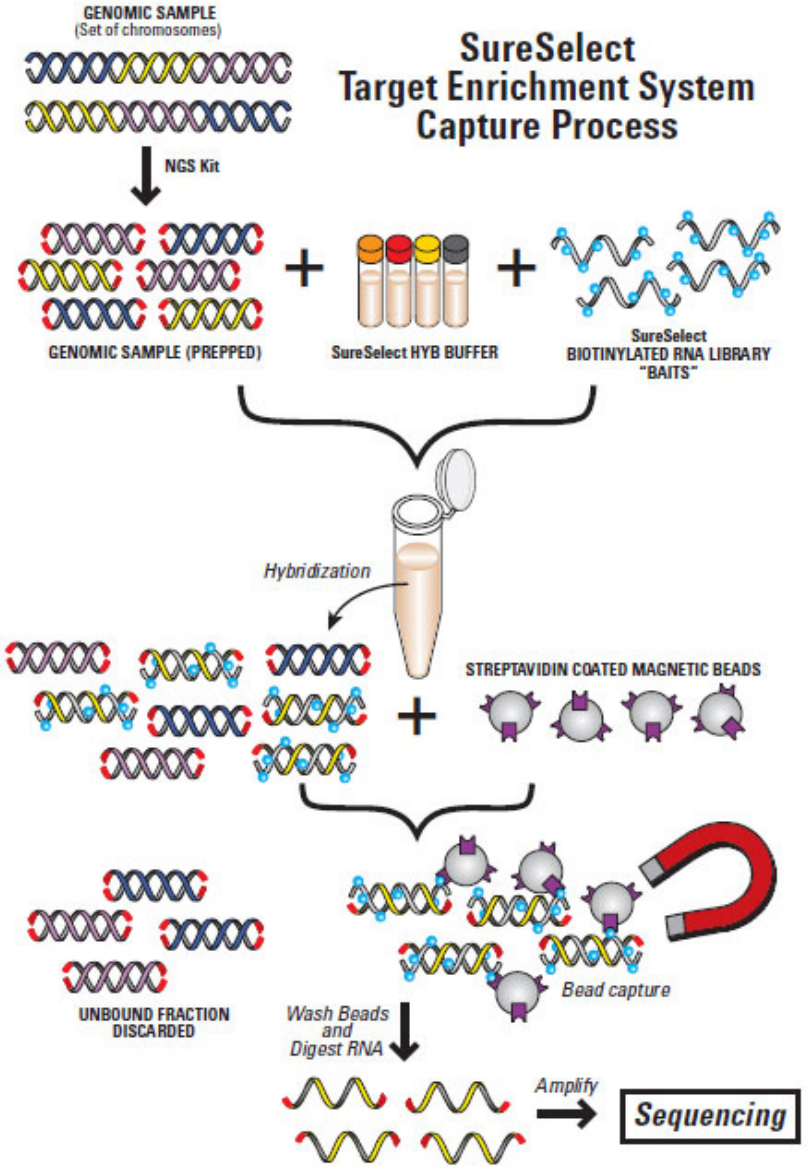
- Traditional Sequencing
  - One gene at a time
  - Read two hundred books in the library, one at a time (but both copies…)
- Next Generation Sequencing
  - Hundreds to thousands of genes at once
  - Read both copies of two hundred books in the library all at once, automatically
  - Or, read 25,000 books (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

- *G*enetic *E*ye *Di*sease 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

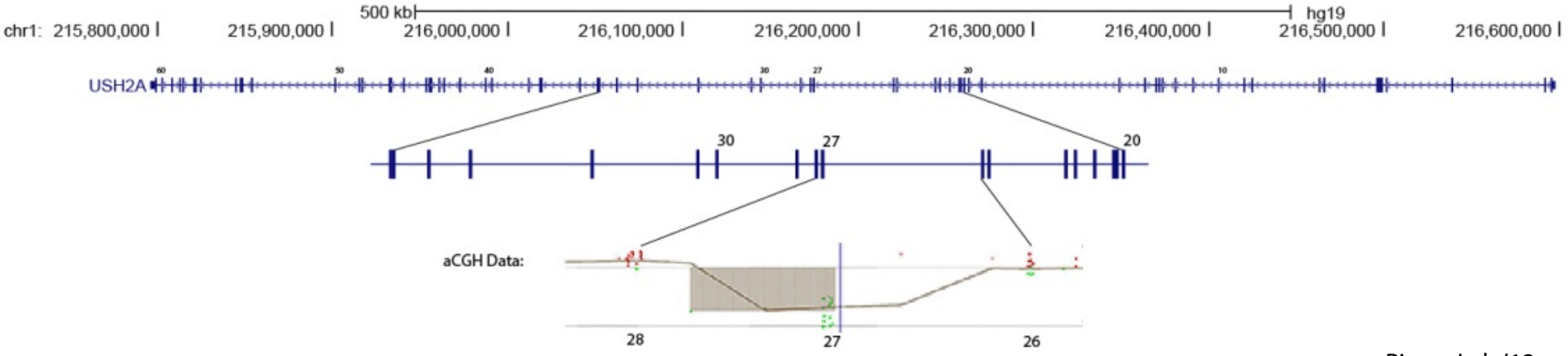
CNVs as Pathogenic Alleles

- 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*

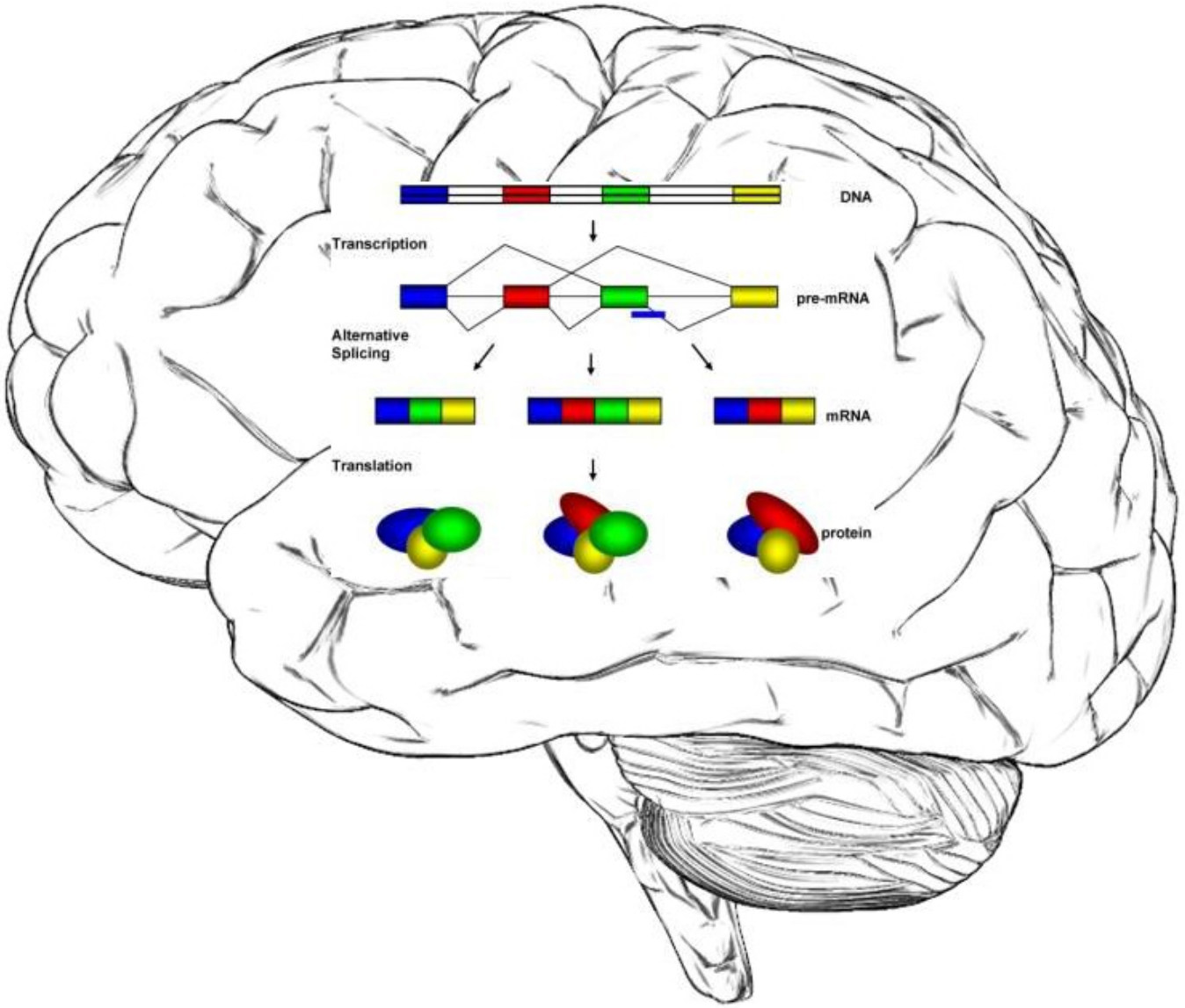
    Causes frameshift, null allele



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



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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

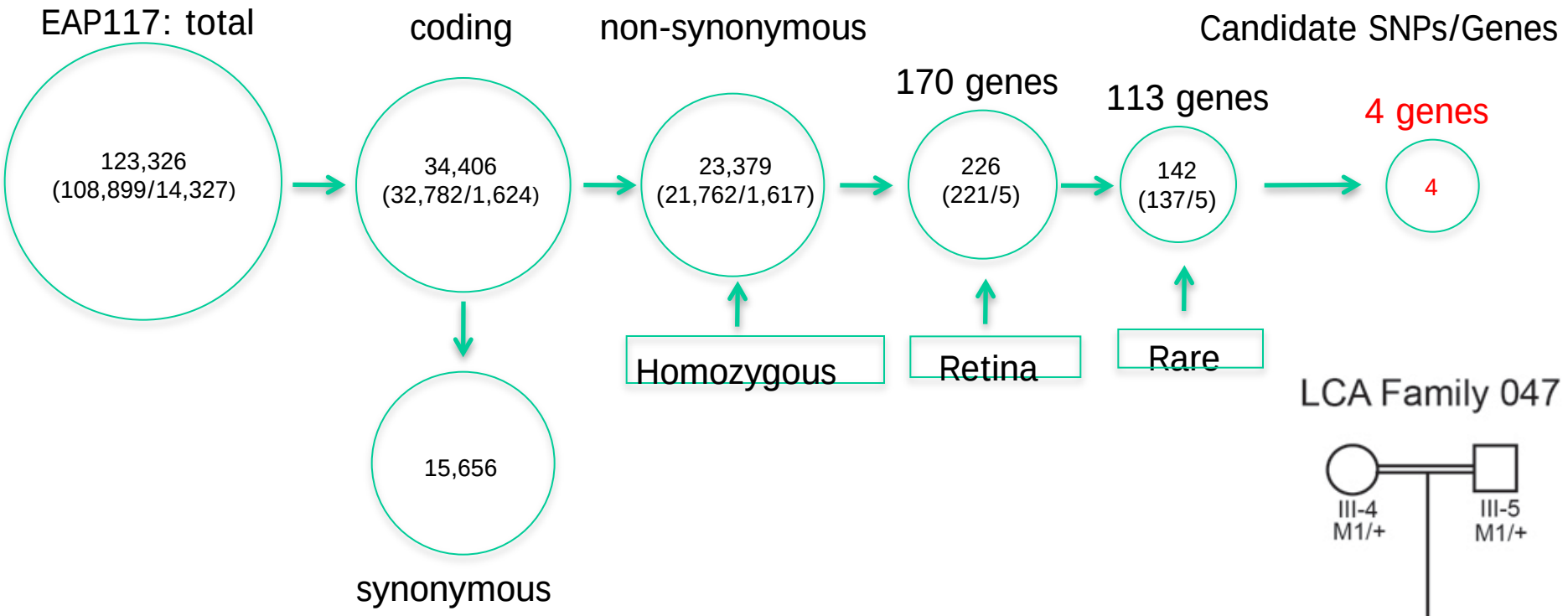
Usher Genome Sequencing

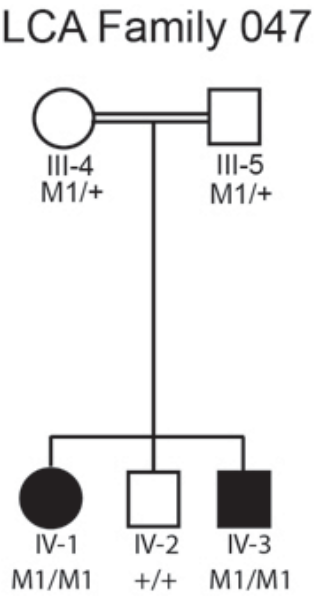
- Develop and test selective capture and Illumina sequencing of entire genomic regions of 10 Usher syndrome genes
  - Size: 3.6 Mb
  - Pros: High likelihood of identifying non-coding mutations in Usher genes
  - Cons: Need to develop a new test

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



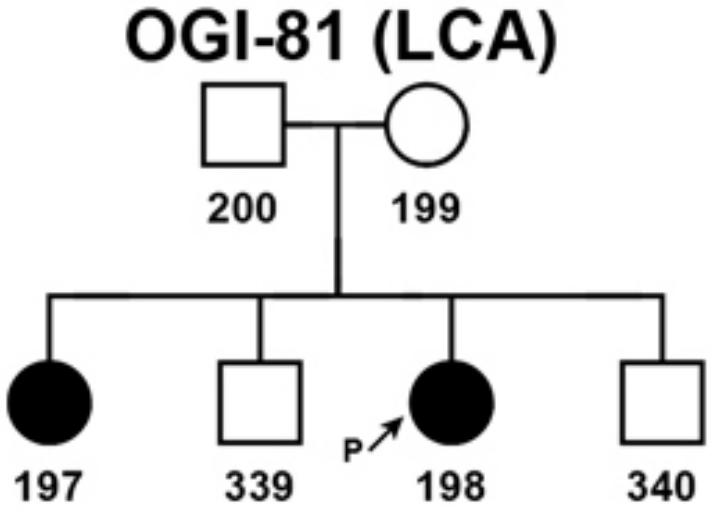


- Variants in 1 of 4 genes predicted to be pathogenic
- Variants in the *NMNAT1* gene segregate with disease

(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



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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:
  - The14kb 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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