# Genetics in Ophthalmology

OAO 2023 Ophthalmic Technology Meeting
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## Learning objectives:

- Understand basic principles of genetic eye disease
- Understand principles of gene editing technology
- Understand how gene therapy can be delivered to the retina
- Understand current approaches for treating eye diseases using gene therapy

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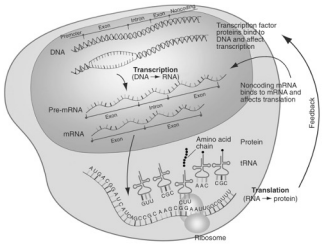
## Genetic eye disease




- Central dogma of genetics:
  - DNA is transcribed to mRNA
  - mRNA is translated to protein

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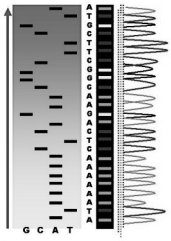
## Genetic eye disease

- Mutations in the DNA sequence cause production of proteins that:
  - Do not get produced
  - Function abnormally (poorly, too well)
  - Do not function at all
- DNA *mutations* cause disease
- DNA *polymorphisms* are DNA variations that are more common and do not cause disease



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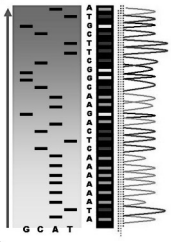
## Genetic eye disease

- Genotype: DNA profile
  - Mutations or polymorphisms?
- Phenotype: the outcome of DNA expression
  - Normal? Disease? At risk?



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## Genetic eye disease

- Genetic eye diseases may or may not be hereditary
  - Inherited: passed down from generation to generation (e.g., inherited retinal dystrophies)
  - Non-inherited: new mutations that have arisen *de novo* (e.g., ocular melanoma)
- Developmental abnormalities do not imply a genetic cause

> The most valuable tool in clinical genetics is the question: "Does anyone else in the family have . . . ?"

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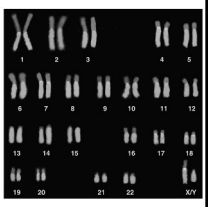
# Genetic eye disease

- Inherited diseases can follow many patterns of inheritance
  - Autosomal (22 pairs of chromosomes)
  - X-linked (X chromosome, males always affected)
  - Dominant (1 gene mutation causes disease)
  - Recessive (2 gene mutations required to cause disease)
  - Mitochondrial (maternal inheritance)
- New mutations can arise sporadically and be passed on to subsequent generations

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# The challenge of ophthalmic genetics

- All eye structures are subject to genetic diseases
- Manifestations may be present at birth (*congenital*) or arise later in life
- Eye disorders may be syndromic or isolated
- A particular genetic mutation can have variable manifestations in different individuals (*expressivity*)
  - Phenotypes are multifactorial

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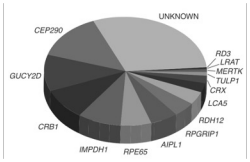
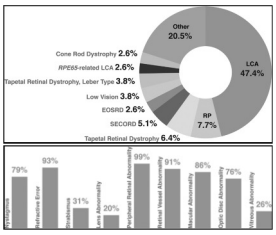
# The challenge of ophthalmic genetics

- Leber's congenital amaurosis (LCA): one disease, many causative genes:




- *RPE65* mutations: one gene, diverse manifestations:

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## Gene therapy in ophthalmology

- Many genes, variable phenotypes...how do we diagnose and treat?

**Clinical evaluation** (examination, testing, imaging)

**Targeted genetic evaluation** (DNA sequencing, chromosomal analysis)

**Treatment** (gene therapy or other)

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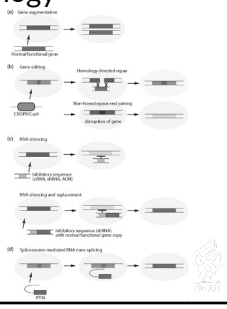
## Gene therapy in ophthalmology

- **Gene augmentation**: add a non-mutant gene to produce normal protein
- **Gene editing (e.g., CRISPR)**: fix a mutation
- **RNA modulation**: block abnormal protein from being made
- **"Biofactory" approach**: add a gene so cells produce a biologic therapy

- Vectors for delivering therapeutics
  - Viral: most common (AAV)
  - Lipid nanoparticles (e.g., COVID mRNA vaccines)

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## Gene therapy in ophthalmology

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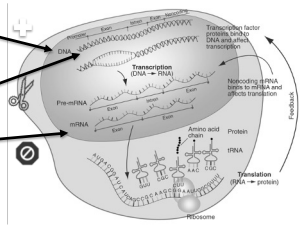


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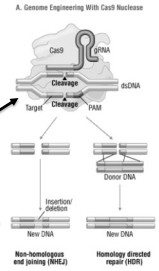
## Gene therapy in ophthalmology: CRISPR

- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology: gene editing
  - Guide RNA (gRNA) recognizes a cell's specific DNA sequence and an enzyme cuts open DNA
    - DNA is disrupted or a new sequence is introduced

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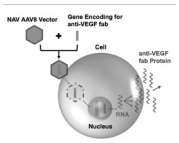
## Gene therapy in ophthalmology

- "Biofactory" approach: a type of gene augmentation
  - Add a gene to make cells produce a biologic therapy
  - Macular degeneration, diabetic retinopathy, others
    - anti-VEGF, complement inhibition




Heier, et al., 2018.

- Gene encoding anti-VEGF protein is delivered to retinal cells
- Therapeutic protein is produced in the retina instead of being injected into the vitreous

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## Gene therapy targets in ophthalmology

- Glaucoma: proteins that promote ganglion cell survival
- Wet AMD, diabetic retinopathy: anti-VEGF
- Dry AMD: complement factors
- Stargardt's disease: *ABCA4*
- Achromatopsia: *CNGA3, CNGB3*
- Retinitis pigmentosa: multiple genes
- X-linked retinitis pigmentosa: *RPGR*
- Choroideremia: *CHM*
- Leber's congenital amaurosis: *RPE65, CEP290*
- X-linked retinoschisis: *XLRS1*
- Leber's hereditary optic neuropathy: mtDNA

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## Gene therapy in ophthalmology

- **Gene augmentation** in Leber's Congenital Amaurosis
  - Luxturna: delivery of non-mutant *RPE65* gene to retina
- **Gene editing** in Leber's Congenital Amaurosis
  - CRISPR: editing of disease-causing mutated *CEP290* gene
- **RNA interference**
  - Ongoing clinical trials: LCA, RP, Usher syndrome
- **"Biofactory"** approach
  - Macular degeneration, diabetic retinopathy, others

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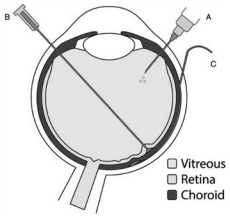
## Gene therapy in ophthalmology

- Sites of delivery:
  - Retina:
    - A: intravitreal
    - B: subretinal
    - C: suprachoroidal
  - Anterior segment:
    - Anterior chamber

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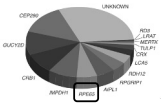
## Gene therapy in ophthalmology




## Gene therapy in ophthalmology

- **Leber's Congenital Amaurosis (LCA)**
  - Severe vision loss at birth or in early childhood
  - subset of patients have 2 mutated copies of *RPE65* (autosomal recessive)
- Luxterna (voretigene neparvovec-rzyl)
  - First FDA-approved gene therapy
  - Delivers a functional copy of *RPE65*: gene augmentation
  - Effective and safe over 5+ years

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## Gene therapy at Casey Eye Institute

**Open genetics clinical trials at CEI:**

- Achromatopsia (CNGB3) Gene Therapy Trial
- Achromatopsia (CNGA3) Gene Therapy Trial
- Choroideremia: Retinal Gene Therapy for Choroideremia
- Leber Congenital Amaurosis: Allergan Leber Congenital Amaurosis (CEP290) Gene Therapy Trial
- Leber Congenital Amaurosis (CEP290) Natural History Study
- Leber Congenital Amaurosis: ProQR Leber Congenital Amaurosis (CEP290) RNA Therapy Trial
- Retinitis Pigmentosa: ProQR RNA Therapy Trial for Patients with Autosomal Dominant Retinitis Pigmentosa
- Stargardt Disease: Acucela 4429-301 Emixust for Stargardt Disease
- Usher Syndrome Type 2A (USH2A) Natural History Study
- X-linked Retinitis Pigmentosa: AGTC X-linked Retinitis Pigmentosa (RPGR) Natural History Study
- X-Linked Retinitis Pigmentosa (RPGR) Gene Therapy Trial
- X-Linked Retinitis Pigmentosa (RPGR) Gene Therapy Trial
- X-linked Retinitis Pigmentosa: Nightstar X-Linked Retinitis Pigmentosa (RPGR) Natural History Study

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## Thank you!

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