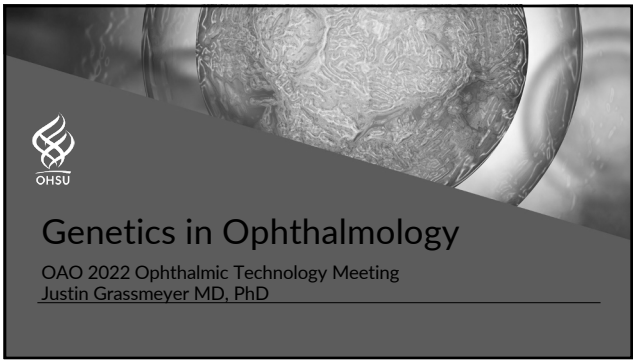

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Learning objectives:

- 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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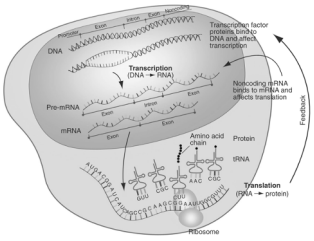
Genetic eye disease



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

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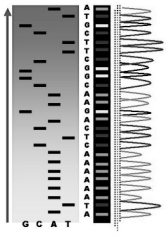
Genetic eye disease

- 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 not disease-causing and are more common



By Abizar at English Wikipedia, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=18698825

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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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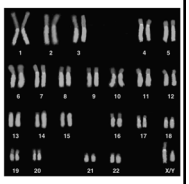
Genetic eye disease

- Inherited diseases can follow many patterns of inheritance
  - Dominant (1 gene mutation causes disease)
  - Recessive (2 gene mutations required to cause disease)
  - Autosomal (22 pairs of chromosomes)
  - X-linked (X chromosome, males always affected)
  - 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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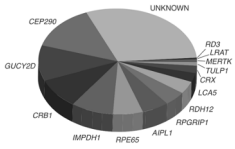
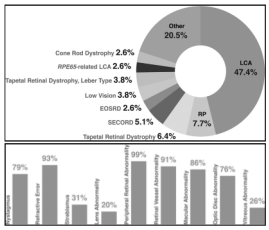
The challenge of ophthalmic genetics

- 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 can 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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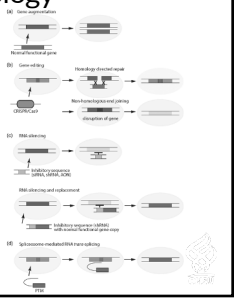
Gene therapy in ophthalmology

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

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



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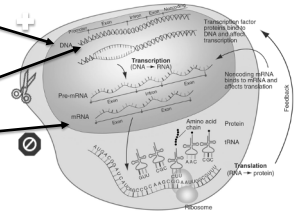
Gene therapy in ophthalmology

- **Gene augmentation**; add a non-mutant gene to produce normal protein
- **Gene editing (CRISPR)**; fix a mutation
- **RNA modulation**; block mRNA from making an abnormal protein
- **"Biofactory"** approach; add a gene to make cells produce a biologic therapy



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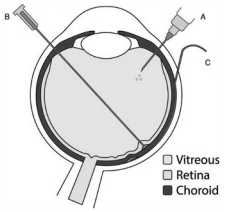
Gene therapy in ophthalmology

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



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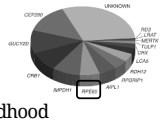
Gene therapy in ophthalmology



- **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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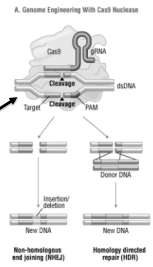
Gene therapy in ophthalmology: CRISPR

- 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

- **Leber's Congenital Amaurosis (LCA)**
  - Severe vision loss at birth or in early childhood
  - subset of patients have *CEP290* mutations
- CRISPR clinical trials: editing mutant copy of *CEP290*

THE ENDPOINT NEWSLETTER

A Gene-Editing Experiment Let These Patients With Vision Loss See Color Again

September 28, 2021 - 8:00 AM ET
Heard on All Things Considered

"I've always loved colors. Since I was a kid it's one of those things I could enjoy with just a small amount of vision. But now I realize how much brighter they were as a kid because I can see them a lot more brilliantly now," she says. "It's just amazing."

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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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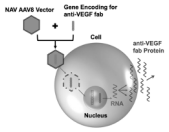
Gene therapy in ophthalmology

- "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. 2015

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

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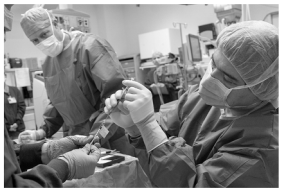
Gene therapy at Casey Eye Institute



Casey Eye Institute operating room team prepares to deliver first involvo gene editing treatment using CRISPR/Cas9 - of Kristyna Wentz-Graff.

Pioneering the first-ever CRISPR gene editing in vivo

- Dedicated space in a new 60,000-square-foot building for the Paul H. Casey Ophthalmic Genetics Division, with imaging technology integrated into exam spaces and a mobility maze
- Intraoperative OCT
- Experienced surgical and OR team, which has performed more than 150 ocular gene therapy procedures
- Four ophthalmic geneticists
- One genetic counselor
- Eight clinical trial coordinators
- 14 clinical trials investigating new genetic treatments for ophthalmic conditions
- 50 vision-related clinical trials overall

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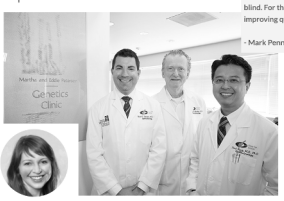
Gene therapy at Casey Eye Institute

Ophthalmic Genetics

"We are entering an era of new therapy for those who otherwise would become irreversibly blind. For the first time, we are offering hope for these patients and their families and improving quality of life."

- Mark Pennesi, M.D., Ph.D., Associate Professor, Chief, Ophthalmic Genetics Service



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 4428-301 Emixustat 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

A leading center on the west coast for inherited eye diseases

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Sources:

- Vikram S. Brar, M. (2021). *2021-2022 Basic and Clinical Science Course, Section 2: Fundamentals and Principles of Ophthalmology*. American Academy of Ophthalmology.
- Chung, D. C., Bertelsen, M., … Reape, K. Z. (2019). The Natural History of Inherited Retinal Dystrophy Due to Biallelic Mutations in the RPE65 Gene. *American Journal of Ophthalmology*, *199*, 58–70.
- Ku, C. A., & Pennesi, M. E. (2020). The new landscape of retinal gene therapy. *American journal of medical genetics. Part C, Seminars in medical genetics*, *184*(3), 846–859.
- Xu, D., Khan, M. A., & Ho, A. C. (2021). Creating an Ocular Biofactory: Surgical Approaches in Gene Therapy for Acquired Retinal Diseases. *Asia-Pacific journal of ophthalmology (Philadelphia, Pa.)*, *10*(1), 5–11.
- https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
- https://www.npr.org/sections/health-shots/2021/09/29/1040879179/vision-loss-crispr-treatment
- Heier, J., et al. (2018) "Six Month Results of the Phase I Study to Evaluate Safety & Tolerability of RGX-314 Gene Therapy in nAMD Subjects." https://www.regenxbio.com/wp-content/uploads/2019/03/RGX-314-AAO-Late-Breaker-2018-FINAL.pdf

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OHSU

Thank you

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