Genetic Testing and Gene Therapy

Dr. Rachelle Lin, OD, MS, FAAO

Disclosures

- Employed at MBKU SCCO
  - Ketchum Health- University Eye Center
  - Low Vision Department
    - Genetic Testing
- Consultant
  - Applied Genetic Technologies Corporation (AGTC)
  - Spark Therapeutics- ID YOUR IRD Speakers' Bureau

CE content for educational purposes only

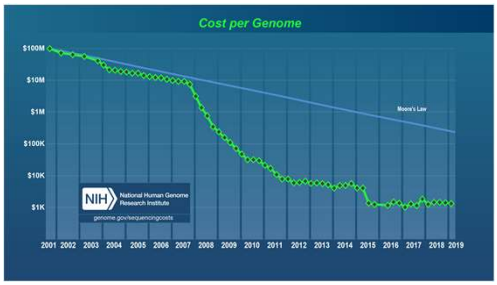
Genetic Testing and Gene Therapy

- Pertinent and happening now
  - Increase in direct-to-consumer genetic testing
  - Growth in clinical genetic testing
  - Gene therapies approved since 2017 and more on the way

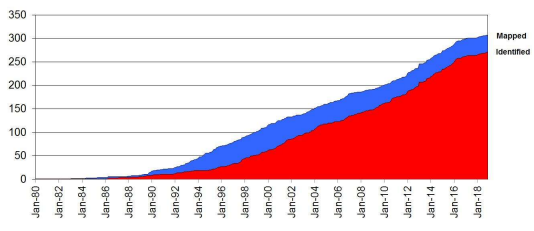
Growth is Fast

1859	• *On the Origin of Species* published
1865	• Gregor Mendel experiments published
Late 1880s	• "Chromosome" discovered
1953	• Structure of DNA published
1956	• Correct number of human chromosomes
1950s	• Genetic tests for Down syndrome
1990	• BRCA1/ BRCA2 mutations linked to breast cancer risk
1990-2003	• Human Genome Project
2017	• First FDA approved *in vivo* gene therapy

Decrease in Cost



Improved Testing



Mapped and Identified Retinal Disease Genes 1980 - 2019

https://sph.uth.edu/retnet/sum-dis.htm

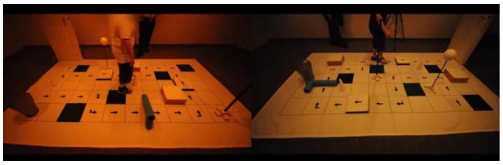
What's New?

- 40+ Clinical Trials
- In development…
  - More gene replacement therapies
  - CRISPR gene editing
  - Antisense oligonucleotide based therapies
  - Optogenetics

Importance of Genetic Testing & Gene Therapy

Gene Therapy Outcomes

- *voretigene neparvovec-rzyl* result
  - Improvement in multi-luminance mobility testing (MLMT) score
  - [Luxturna video from Spark Therapeutics]



https://luxturnahcp.com/efficacy/clinical-trial-results/

voretigene neparvovec-rzyl

- FDA approved 2017
- Only for patients with RPE65 gene mutations (small percentage of patients with RP and LCA)

Gene Therapy Outcomes

- Improvement in functional vision
- Improvement in mobility under different luminance settings
- Improvement in light sensitivity
- Improvement in VF
- Non-statistical improvement in VA

Testing Recommendations

- **"Recommendations on Clinical Assessment of Patients with Inherited Retinal Degenerations – 2016"**

- Numbers refer to clinical phenotypes:
  - 1. Rod-cone degenerations
  - 2. Cone-rod degenerations
  - 3. Chorioretinal degenerations
  - 4. Inherited dystrophies that involve the macula

Clinical Evaluation: Inherited Retinal Degenerative Diseases

Assessment	Initial Visit	Follow Up Visit Every 1-2 Years
History • Ocular (including current needs) • Medical (including current medications and history of retinotoxic medication use)	1-4[a]	1-4
Pedigree (family history)	1-4	1-4
Clinical eye examination • Best corrected visual acuity: ETDRS (or equivalent) • Slit-lamp biomicroscopy • Intraocular pressure • Indirect Ophthalmoscopy	1-4	1-4
Imaging • Color fundus photos • Spectral Domain Optical Coherence Tomography • Fundus autofluorescence: Short wavelength with reduced illumination when possible • Infrared autofluorescence (when available)	1-4 1-4 1-4[b] 1, 3, 4	 1-4 1-4[b] 1, 3, 4
Visual fields • Kinetic • Static • Microperimetry (when available)	1-4[c] 1-3[d] 1-4	1-4[c] 1-3[d] 1-4
Electroretinography • Full-field ERG[e] (when appropriate) • Multifocal ERG[f] (when appropriate)	1-4 2,4	1-3 2,4
Genetic Diagnostic Testing	1-4	

AAOphthalmology Clinical Statement

- "Genetic testing plays an important role in improving the accuracy of diagnosis and prognosis, providing patients and families with specific inheritance risks, and guiding treatment decisions."

AAOphthalmology Clinical Statement

- "…a causative mutation can be identified in up to 60-80% of patients with inherited retinal disorders"

GENETIC TESTING

Types of Genetic Testing

- Direct-to-consumer
  - Mainly provide info on ancestry/ genealogy services, traits, genetic predisposition to common conditions, carrier status
- Health care provider ordered

Differences Between Direct-to-Consumer and Heath Care Provider Ordered Tests

- Regulation
- Analytical validity, clinical validity, clinical utility
- Diagnostic ability
- Guidance and counseling access
- Data privacy
- Relevance to patients with inherited retinal dystrophies

Ethical & Other Considerations

- Data privacy
- Does the patient want to know? Do the family members want to know?
- What will be done with the information?
- Is it ethical to test children?
- Who can give consent for testing?

AAOphthalmology Current Recommendations

- "Recommendations for Genetic Testing of Inherited Eye Diseases- Report of the American Academy of Ophthalmology Task Force on Genetic Testing"

5 Steps

- 1) clinical exam – presence of genetic eye disease
- 2) molecular investigation of genomic samples
- 3) analysis of data
- 4) interpretation of data in context of clinical findings
- 5) patient counseling

"Recommendations for Genetic Testing of Inherited Eye Diseases- Report of the American Academy of Ophthalmology Task Force on Genetic Testing"

Additional Specific Recommendations

- Use of CLIA- approved laboratories
- Avoid direct-to-consumer tests
- Avoid unnecessary parallel testing
- Avoid genetic testing for genetically complex disorders
- Avoid testing asymptomatic minors for untreatable disorders

"Recommendations for Genetic Testing of Inherited Eye Diseases- Report of the American Academy of Ophthalmology Task Force on Genetic Testing"

Current Genetic Testing Relating to Eye Care

- **Multiple single gene and panel tests available**, including for:
  - Inherited retinal dystrophies
  - Age-related macular degeneration risk
  - Keratoconus risk
  - Corneal dystrophies
  - Glaucoma risk
  - Mitochondrial conditions
  - And more…
- **Consider the pros, cons, and limitations**

TIMELINE OF SIGNIFICANT GENETICS DISCOVERIES AND BREAKTHROUGHS

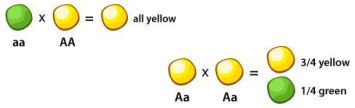
1859	*On the Origin of Species* published
1865	Gregor Mendel experiments published
Late 1880s	"Chromosome" discovered
1953	Structure of DNA published
1956	Correct number of human chromosomes
1950s	Genetic tests for Down syndrome
1990	BRCA1/ BRCA2 mutations linked to breast cancer risk
1990-2003	Human Genome Project
2017	First FDA approved *in vivo* gene therapy

1859

- Charles Darwin published, *On the Origin of Species by Means of Natural Selection*

1865

- Gregor Mendel first publishes on his plant hybridization experiments
- Mendelian inheritance

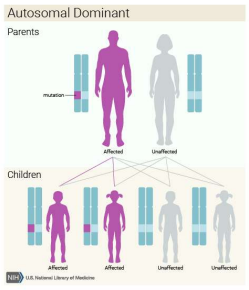


Genotypes vs Phenotypes

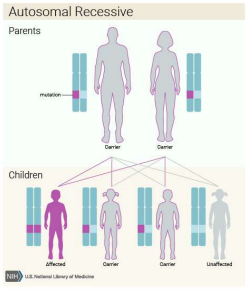
- 2 copies of each gene; one from each parent
- Alleles
  - Dominant
  - Recessive
- Genotype- the genes
- Phenotype- how they are expressed as characteristics

Inheritance Patterns

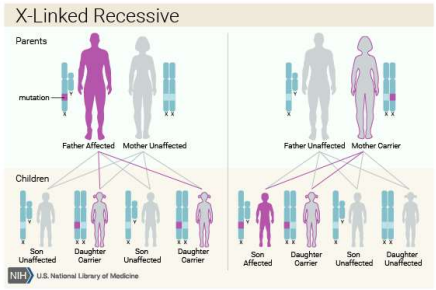
- Autosomal dominant
  - One mutated copy of the gene is sufficient for a person to be affected
- Autosomal recessive
  - Both copies of the gene must be mutated for a person to be affected
- X-linked
  - Caused by mutations in genes on the X chromosome
  - Females have XX; Males have XY



Source: U.S. National Library of Medicine



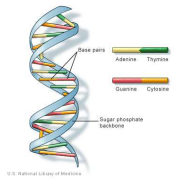
Source: U.S. National Library of Medicine



Source: U.S. National Library of Medicine

1953

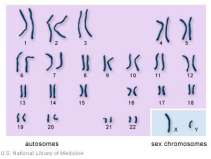
- Watson & Crick published structure of DNA



Source: U.S. National Library of Medicine

1956

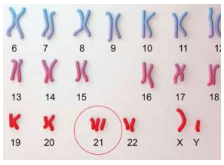
- Correct number of human chromosomes reported as 46



Credit: U.S. National Library of Medicine

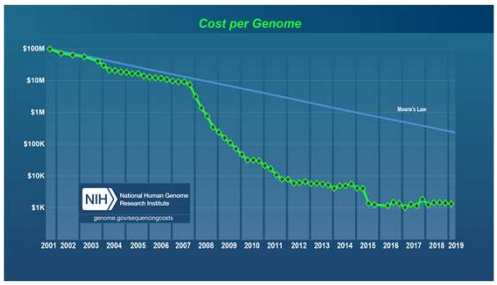
1950s

- Genetic tests for Down syndrome and more



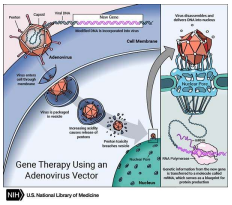
1990-2003

- Human Genome Project launched and officially completed
- Improvements in sequencing ability and processing speed



2017

- First FDA approved *in vivo* gene therapy

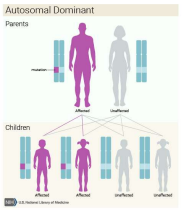


Credit: U.S. National Library of Medicine

WHAT WE KNOW NOW…GROUPED BY INHERITANCE PATTERNS

Autosomal Dominant Inheritance

- Ex. Best Vitelliform Macular Dystrophy



Source: U.S. National Library of Medicine

Best Vitelliform Macular Dystrophy

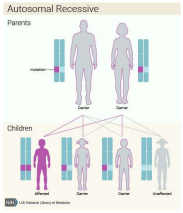
- Autosomal dominant BEST1
- Early-onset vitelliform macular dystrophy
- Yolk-like macular lesion
- Decreased central VA and metamorphopsia

Best Vitelliform Macular Dystrophy

- *Clinical implications:*
  - Patients will have family members with the condition
  - Straightforward to do genetic testing for this condition

Autosomal Recessive Inheritance

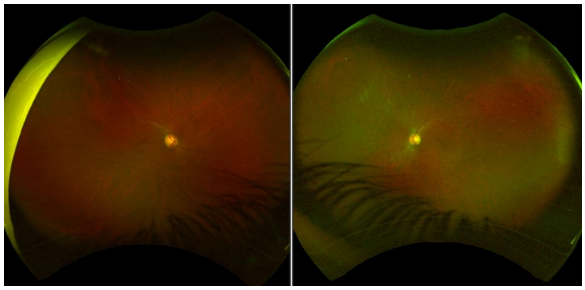
- Ex. Achromatopsia, Oculocutaneous albinism

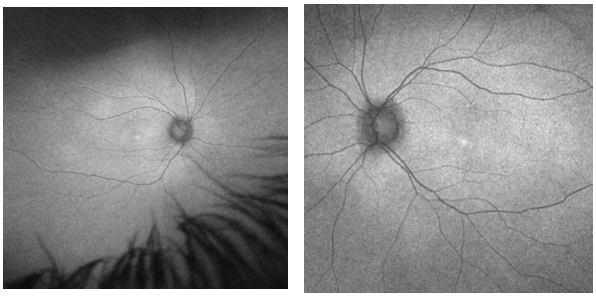


Source: U.S. National Library of Medicine

Achromatopsia

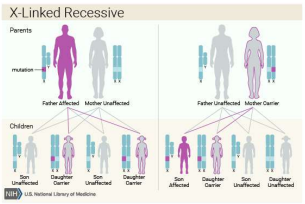
- Autosomal Recessive
- CNGA3, CNGB3, GNAT2, PDE6C, PDE6H
  - CNGA3, CNGB3 account for ~75%
- *Clinical implications:*
  - Genetic causes of this condition have been well identified
  - Genetic testing will most likely give a positive result
  - Genetic testing, though unlikely to change clinical diagnosis easily determined through clinical testing, is needed for eligibility for current clinical trials





X-Linked Recessive Inheritance

- Ex. Choroideremia, X-Linked Juvenile Retinoschisis, X-Linked RP



Source: U.S. National Library of Medicine

Choroideremia

- 1 in 50,000-100,000
- Progressive vision loss- childhood night vision impairment, peripheral vision loss, later decreased VA

Choroideremia

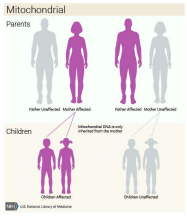
- X-Linked Recessive
- CHM
  - Rab escort protein-1 (REP-1) → loss of retina, choroid, RPE
- Genetic testing very effective
- *Clinical implications:*
  - More likely to affect males than females
  - Females likely to be carriers

X-Linked Juvenile Retinoschisis

- 1 in 5,000-25,000 men
- RS1 gene
  - Retinoschisin protein, likely involved in intercellular adhesion & retinal cell organization
- Macular involvement in 1st decade

Mitochondrial Inheritance

- Ex. Leber Hereditary Optic Neuropathy



Mitochondrial Inheritance

- Mitochondrial DNA (mtDNA) mutation
- Maternal inheritance
  - Males: none of their children will inherit the mtDNA mutation
  - Females: all children will inherit the mtDNA mutation
- Ex. Leber hereditary optic neuropathy

GENETICS & GENOMICS CHALLENGES

Our genes aren't everything

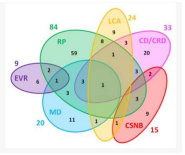
- CONs
  - Depending on the condition and the genetic test, genetic testing by itself may not tell us the whole clinical picture.
- PROs
  - Lifestyle and environmental changes can make a difference in some cases.

Genotypic & Phenotypic Heterogeneity

- Patients may have similar phenotype but have different affected genes.
- Patients may have same affected gene and have different phenotypes
  - Even within the same family!

Genotypic Heterogeneity

- Some IRDs are associated with many different genes
- Some genes are associated with many different IRDs



Cremers FPM, Boon CJF, Bujakowska K, Zeitz C. Special Issue Introduction: Inherited Retinal Disease: Novel Candidate Genes, Genotype–Phenotype Correlations, and Inheritance Models. *Genes*. 2018; 9(4):215. https://doi.org/10.3390/genes9040215

Ex. Retinitis Pigmentosa

- >100 identified genes, >60 genes associated with non-syndromic RP
- **AD** (RHO,…), **AR** (USH2A…), **X-linked recessive** (RPGR, RP2,…), **de novo**

Ex. Retinitis Pigmentosa

- <u>Highly varied RP fundus appearances, age of onset, speed of progression, VA and VF loss</u>
  - Ex. X-linked RP associated with earlier onset and faster progression
- **Understanding genetics can provide better understanding of condition and prognoses.**

Ex. Retinitis Pigmentosa

- <u>20-30% have syndromic RP</u>
  - Ex. Usher syndrome
  - Ex. Bardet-Biedl syndrome
- **Early identification can lead to better management of systemic condition.**

Leber Congenital Amaurosis (LCA)

- 2-3 / 100,000 newborns
- Mutations in 14-22 (?) different genes
- Most autosomal recessive
- Most common mutations: CEP290, CRB1, GUCY2D, RPE65
- Some autosomal dominant: CRX, IMPDH1
- 30% unknown cause

Stargardt Macular Degeneration

- 1 / 8,000-10,000
- Most common- Autosomal recessive (ABCA4)
- Autosomal dominant (ELOVL4)

Conditions affected by one gene vs multiple genes

- Added complexity when multiple genes are involved.
  - Ex. Risk of keratoconus affected by multiple genes

Complex Multifactorial Disorders

- AAOphthalmology Recommendation: Avoid genetic testing for genetically complex disorder.
  - Ex. AMD
    - Other risk factors: age, smoking

"Nature vs. Nurture"

- Both genetics and environment play a role.
  - Some conditions are more affected by genetics.
  - Some conditions are more affected by environment.

Epigenomics

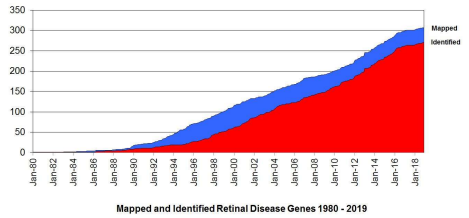
- Not all genes are active.
- External epigenetic factors can affect gene activity, determining when genes are "turned off" or "turned on".

Credit: Darryl Leja, NHGRI

Not always as simple as Punnett Squares…

- Reduced penetrance
  - Ex. Not all people with the mutation will develop the condition
- Variable expressivity
  - Ex. Not all people with the condition exhibit the same severity

Unidentified Genes



Mapped and Identified Retinal Disease Genes 1980 - 2019

https://sph.uth.edu/retnet/sum-dis.htm

Result Interpretation

- Not all genes are tested. Not all tests will deliver satisfactory results.
- **Positive result-** found pathogenic mutation in the gene(s) tested
- **Negative result-** did not find mutation in the gene(s) tested
- **Inconclusive results**

Variant Classification

- Pathogenic
- Likely Pathogenic
- Uncertain Significance
- Likely Benign
- Benign

Result Interpretation

- Variants of Uncertain Significance
  - May be reclassified in the future
- Pathogenic (low penetrance)

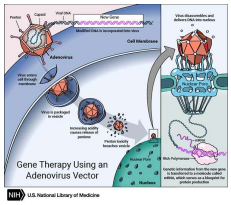
Genetic Testing + Clinical Findings

- IMPORTANT: Consider the genetic testing results within the context of clinical findings
  - Ocular findings
  - Systemic findings
  - Family history

GENE THERAPY - CURRENT

2017

- First FDA approved *in vivo* gene therapy



Credit: U.S. National Library of Medicine

Why gene therapy for inherited retinal dystrophies?

- Pros
  - Effective treatment
  - Eye is immune privileged
  - Physiology of the eye
- Cons
  - Limitations of treatment
  - Number and rarity of genetic mutations
  - Cost

Current FDA Approved Gene Therapies

- Luxturna (*voretigene neparvovec-rzyl)*- Spark Therapeutics
- Approved for biallelic RPE65-mutation associated retinitis pigmentosa & Leber congenital amaurosis

voretigene neparvovec-rzyl

- Single subretinal injection
- Copy of the RPE65 gene is delivered via a viral vector
  - Adeno-associated viral (AAV) vector

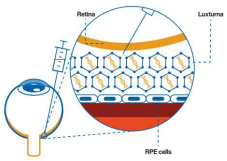


image: novartis.gcs-web.com

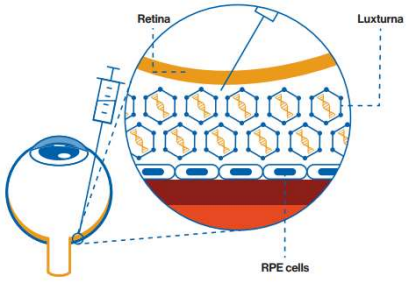


image: novartis.gcs-web.com

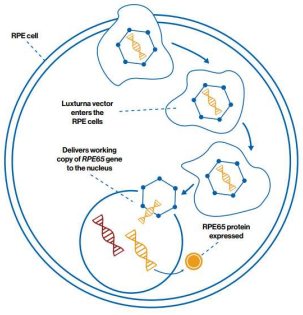
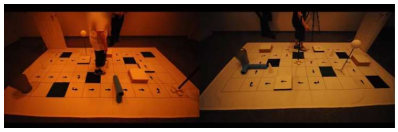


image: novartis.gcs-web.com

voretigene neparvovec-rzyl

- Result
  - Improvement in multi-luminance mobility testing (MLMT) score



voretigene neparvovec-rzyl

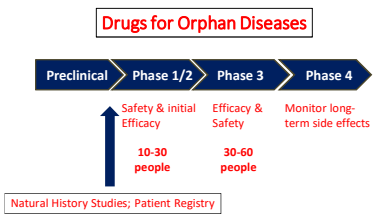
- Adverse Reactions
  - Conjunctival hyperemia 22%
  - Cataract 20%
  - Increased IOP 15%
  - Retinal tear 10%
  - Dellen 7%
  - Macular hole 7%
  - Subretinal deposits 7%
- Cost $$$

GENE THERAPY – CLINICAL TRIALS & FUTURE

Achromatopsia videos

- https://www.youtube.com/watch?v=7MLSxOz4HnE
- https://www.youtube.com/watch?v=OaugUyMDkdk

Phases of Clinical Development



Do Patients Need to Have Genetic Testing Before Enrolling in a Clinical Trial?

- Many clinical trials underway are for genetic therapies and will require that patients have a confirmed genetic diagnosis.
- There are several emerging cross-cutting therapies – for example, stem cells, oral drugs, and optogenetic approaches – that may not require a genetic diagnosis.

Time Commitment to Participate in a Clinical Trial?

- Depends on the clinical trial. Time commitment can be extensive.
  - Most trials for inherited retinal disorders (IRDs) require the visits to occur at the clinical trial site. About 12 visits year 1, visits 1-2x a year for 4 more years.
  - Consider time commitment for both patient and travel companion.
- Consider number and duration of visits.

Cost of Participation in a Clinical Trial?

- Depends on the clinical trial.
- All sponsors will cover the medical costs associated with a clinical trial
- Not all sponsors cover the travel and miscellaneous costs (food, lodging).
- Be wary of clinical trials that require out-of-pocket costs to participate or receive therapy.

Side effects/ risks of IRD gene therapy?

- Depends on route of administration and therapy (subretinal, intravitreal, suprachoroidal).
- The risks will be covered with the patient.
- Consider contraindications of steroids.
  - Oral, topical, and subtenon steroids may be administered before, during & after the procedure

Options for Patients Who May Not Qualify for a Current Clinical Trial

Patient Registries:

- Enrolling in the registry enables patients to get on the radar screen of clinical trial sponsors and investigators. Free genetic testing is often available through the registry.
- Personal information is never shared with third parties. Only de-identified information can be shared.

Natural History Studies

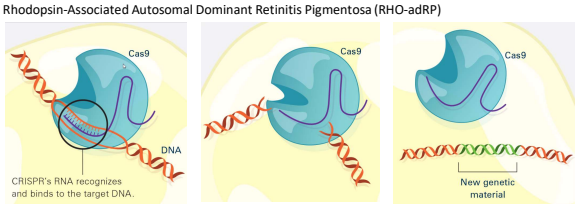
- A natural history study collects information about the natural history of a disease in the absence of an intervention, from the disease's onset until either its resolution or the individual's death. Although knowledge of a disease's natural history can benefit drug development for many disorders and conditions, natural history information is usually not available or is incomplete for most rare diseases; therefore, natural history information is particularly needed for these diseases.

Ongoing and Future Gene Therapy Research & Clinical Trials

- Achromatopsia (CNGA3, CNGB3) – AGTC, MeiraGTx/Janssen
- Batten Disease (CLN5) – Neurogene
- Choroideremia (REP1) – 4DMT
- LCA (GUCY2D) - Atsena
- RP (PDE6B) - Coave
- RP (RLBP1) - Novartis
- RP (NR2E3, RHO) - Ocugen
- X-Linked Retinoschisis – NEI
- X-Linked RP (RPGR) – AGTC, MeiraGTx/Janssen

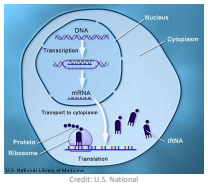
CRISPR/Cas9 Gene Editing

- Editas Medicine/ Allergan
  - Leber Congenital Amaurosis 10 (CEP290)
  - Usher Syndrome (USH2A)
  - Rhodopsin-Associated Autosomal Dominant Retinitis Pigmentosa (RHO-adRP)



Antisense Oligonucleotide-Based Therapy

- Targets mRNA
  - Small fragments form complementary pairs with target mRNA - Inhibit mRNA translation, regulate gene expression
- ProQR
  - LCA (LCA10)
  - USH2A Exon 13
  - Autosomal dominant RP (RHO-P23H)



Credit: U.S. National Library of Medicine

Optogenetics

- Targets ganglion cells
- Vision enhanced when using goggles
- Limited improvement in vision
- Clinical trial: patients with advanced RP

Credit: Sahel, et al.; Nature Medicine

More…

- Gensight Biologics
  - LHON (mitochondrial gene ND4)
- jCyte
  - Neurotrophic factors for RP
- Alkeus
  - Modified vitamin A for Stargardts

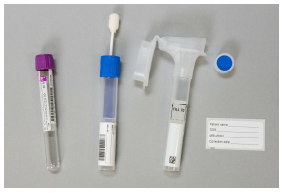
Clinical Application of Genetic Testing, Gene Therapy, and Clinical Trial Referrals

Genetic Testing Referrals

- Search provider lists
  - ID Your IRD
- Complete Clinical Exam & Genetic Test

In-Office Testing

- Test panels
  - Can test just one or many (300+) genes in one test panel
  - **Need to select appropriate panel**
- Collection kit
  - Saliva-sample
  - Blood-sample
  - Buccal swab



Sample Test Panels

- Blueprint Genetics
  - https://blueprintgenetics.com/tests/panels/ophthalmology/retinal-dystrophy-panel/
  - 351 genes (including RPGR)
- Invitae
  - https://www.invitae.com/en/inherited-retinal-disorders-panel/
  - Recently updated to 330 genes (including RPGR)
- Alternative labs

Conditions Tested Can Include…

- Achromatopsia
- Best disease
- Choroidal dystrophies
- Choroideremia
- Cone dystrophy
- Cone monochromacy
- Cone-rod dystrophy
- Congenital stationary night blindness
- Fundus albipunctatus
- Fundus flavimaculatus
- Gyrate atrophy
- Leber congenital amaurosis
- Retinitis pigmentosa
- Retinoschisis (juvenile X-linked retinoschisis)
- Rod-cone dystrophy
- Rod dystrophy
- Rod monochromacy
- Stargardt disease
- Usher syndrome
- *…and more…*

Example Panel

Panel Content

Genes in the Retinal Dystrophy Panel and their clinical significance

Gene	Associated phenotypes	Inheritance	ClinVar	HGMD
ABCA4	Stargardt disease, Retinitis pigmentosa, Cone rod dystrophy, Retinal dystrophy, early-onset severe, Fundus flavimaculatus	AR	308	1231
ABCC6*	Pseudoxanthoma elasticum	AR	352	377
ABHD12	Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract	AR	16	20
ACO2	Optic atrophy, Infantile cerebellar-retinal degeneration	AR	16	15
ADAM9	Cone rod dystrophy	AR	6	10

Example Panel

Order test

Primary panel (293 genes)

ABCA4	ABCC6	ABHD12	ACBD5	ACO2	ADAM9	ADAMTS18	ADGRA3
ADGRV1	ADIPOR1	AGBL5	AHI1	AHR	AIPL1	ALMS1	ARHGEF18
ARL13B	ARL2BP	ARL3	ARL6	ARMC9	ARSG	ASRGL1	ATF6
ATOH7	B9D1	BBIP1	BBS1	BBS10	BBS12	BBS2	BBS4
BBS5	BBS7	BBS9	BEST1	C1QTNF5	C8orf37	CA4	CABP4
CACNA1F	CACNA2D4	CAPN5	CC2D2A	CCT2	CDH23	CDH3	CDHR1
CEP164	CEP19	CEP250	CEP290	CEP41	CEP78	CEP83	CERKL
CFAP410	CHM	CIB2	CISD2	CLCC1	CLN2 (TPP1)	CLN3	CLRN1
CLUAP1	CNGA1	CNGA3	CNGB1	CNGB3	CNNM4	COL11A1	COL11A2
COL18A1	COL2A1	COL9A1	COL9A2	COL9A3	CPLANE1	CRB1	CRX
CSPP1	CTNNA1	CWC27	CYP4V2	DHDDS	DHX38	DRAM2	DTHD1
EFEMP1	ELOVL4	EMC1	EXOSC2	EYS	FAM161A	FLVCR1	FRMD7

Ex. Lab Selection Process

- Your patient has a clinical diagnosis of retinitis pigmentosa. He has a relatively early onset and rapidly progressive field loss.
- After completing a pedigree, you suspect an x-linked inheritance pattern.

Ex. Lab Selection Process

- You search for information about retinitis pigmentosa inheritance patterns and associated genes on MedlinePlus.
- "Together, mutations in the RPGR and RP2 genes account for most cases of X-linked retinitis pigmentosa."
- https://medlineplus.gov/genetics/condition/retinitis-pigmentosa/#synonyms

Ex. Lab Selection Process

- You look through both test panels from the labs (Ex. Blueprint Genetics & Invitae)
- You find that both Invitae and Blueprint's panels currently test RPGR!
- The Blueprint panel testing is being sponsored by Foundation Fighting Blindness's My Retina Tracker Program and Invitae panel is being sponsored by Spark Therapeutics, so now you check that your patient meets their eligibility requirements.

Ex. Alternate Scenario – past testing

- Patient has clinical diagnosis of RP with possible x-linked pattern.
- Genetic testing > 5 years ago with Invitae lab panel was negative
  - (This panel did NOT include RPGR 5 years ago)
- Consider retesting!

INVITAE

ID YOUR IRD

ORDER ID
For Invitae internal use only

Requisition Form
ID YOUR ID® TRF948-2

This requisition form can be used to submit a specimen for the **ID YOUR IRD® program**, a sponsored testing program brought to you by **Spark Therapeutics** and **Invitae Corporation**. Please confirm that the patient meets the eligibility requirements for the program, including provision of written patient consent. Submit the sample according to the kit instructions with the below **Test Requisition Form (TRF, pages 1-3)** and **Patient Authorization forms (pages 4-7) completed and signed**.

INSTRUCTIONS: Review the ordering options and then complete all sections of this form. Your ordering option will be indicated in the test selection section.

ORDERING OPTIONS

1. ID YOUR IRD® PROGRAM
For individuals that meet the eligibility criteria below and wish to receive the program specific genetic testing panels.
REQUIRED: You must select the appropriate eligibility criteria for this patient below.

This program is available to patients suspected of having an inherited retinal disease AND who have experienced one or more of the following (select all that apply, patients must have at least one to qualify):
Note: This program does not test for genes associated with age-related macular degeneration or ocular/oculocutaneous albinism.

- ○ Nyctalopia
- ○ Peripheral field loss
- ○ Central vision loss
- ○ Deterioration of color vision
- ○ Photophobia
- ○ Clinical or suspected diagnosis of an inherited retinal disease

2. GENE-SPECIFIC FAMILY FOLLOW-UP TESTING
For relatives of program participants who received a Pathogenic/Likely Pathogenic result or approved VUS who want to receive gene specific family follow-up testing at no additional cost. Relatives do not need to meet the eligibility criteria listed above. Learn more at www.invitae.com/family.

PATIENT INFORMATION

First name | MI | Last name

Date of birth (MM/DD/YYYY) | Biological sex ○ M ○ F | MRN (medical record number)

Ancestry ○ Asian ○ Black/African American ○ White/Caucasian ○ Ashkenazi Jewish ○ Hispanic ○ Native American ○ Pacific Islander ○ French Canadian ○ Sephardic Jewish ○ Mediterranean ○ Other:

Phone | Email address (report access after clinician releases)

Address | City

State/Prov | ZIP/Postal code | Country

Ship a saliva kit to this patient (to submit, fax this form and Patient Authorization form to Client Services at 415-276-4164)
○ Ship kit to address above
○ Ship kit to alternate address:

SPECIMEN INFORMATION

Specimen type: Blood (3-mL purple EDTA) -OR- Saliva (Oragene™) -OR- Assisted Saliva
We are unable to accept blood/saliva from patients with:
- *Allogeneic bone marrow transplants*
- *Blood transfusion <2 weeks prior to specimen collection*

Specimen collection date (MM/DD/YYYY): *If not provided, the day before specimen receipt will be used*

Special cases: ○ History of/current hematologic malignancy in patient

INVITAE PARTNER CODE SPARK

CLINICIAN INFORMATION

Organization name

Phone | Fax

Address | City

State/Prov | ZIP/Postal code | Country

Primary clinical contact name (if different from ordering provider) | NPI

Primary clinical contact email address (for report access)

Ordering provider (select one ordering provider by marking the checkbox before the name)

	Name	NPI	Email address (for report access)
○			
○			
○			
○			
○			

Additional clinical or laboratory contacts (optional, to share access to order online)
○ **Share this order with the primary clinical contact's default clinical team, manage at** invitae.com

Name | Email address (for report access)

Name | Email address (for report access)

CLINICAL HISTORY

FAMILY HISTORY

Is there a family history of disease for which the patient is being tested? ○ Yes ○ No If yes, describe below and attach pedigree and/or clinical notes.

Relative's relationship to this patient	Maternal or paternal	Diagnosed condition	Age at diagnosis	Relative's relationship to this patient	Maternal or paternal	Diagnosed condition	Age at diagnosis

PERSONAL HISTORY

Is/was this patient affected or symptomatic?† ○ Yes ○ No
Provide details in the required clinical history questions (if applicable).

† Symptomatic means this patient has features or signs known or suspected to be related to the genetic testing being ordered and could include findings on physical examination, laboratory tests, or imaging.

REQUIRED CLINICAL HISTORY

Age of onset: _____

Clinical diagnosis, if known:

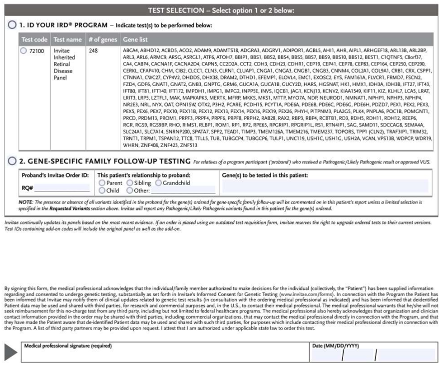
Ocular features	Y	N	UNKNOWN
Bone spicules/pigment clumping	○	○	○
Optic nerve atrophy/optic disc pallor	○	○	○
Abnormal fundus appearance	○	○	○
Abnormal ERG results	○	○	○
Is disease progressive in this individual?	○	○	○

Other ocular features and/or detailed test results (e.g., ERG):

Extraocular features	Y	N	UNKNOWN
Developmental delay	○	○	○
Intellectual disability	○	○	○
Sensorineural hearing loss	○	○	○
Skeletal abnormalities	○	○	○
Renal disease	○	○	○
Genital abnormalities	○	○	○
Ataxia	○	○	○
Organ laterality defect (e.g., situs inversus)	○	○	○
Molar tooth sign/vermian hypoplasia	○	○	○

Other extraocular features:

Y = test performed and/or medical history taken AND material finding reported; N = test performed and/or medical history taken AND no material finding; Unknown = not in medical record and/or test not performed



TEST SELECTION – Select option 1 or 2 below:

1. ID YOUR IRD® PROGRAM – Indicate test(s) to be performed below:

Test code	Test name	# of genes	Gene list
72100	Invitae Inherited Retinal Disease Panel	248	ABCA4, ABHD12, ACBD5, ACO2, ADAM9, ADAMTS18, ADGRA3, ADGRV1, ADIPOR1, AGBL5, AHI1, AHR, AIPL1, ARHGEF18, ARL13B, ARL2BP, ARL3, ARL6, ARMC9, ARSG, ASRGL1, ATF6, ATOH7, BBIP1, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, BEST1, C1QTNF5, C8orf37, CA4, CABP4, CACNA1F, CACNA2D4, CAPN5, CC2D2A, CCT2, CDH3, CDH23, CDHR1, CEP19, CEP41, CEP78, CEP83, CEP164, CEP250, CEP290, CERKL, CFAP410, CHM, CIB2, CLCC1, CLN3, CLRN1, CLUAP1, CNGA1, CNGA3, CNGB1, CNGB3, CNNM4, COL2A1, COL9A1, CRB1, CRX, CSPP1, CTNNA1, CWC27, CYP4V2, DHDDS, DHX38, DRAM2, DTHD1, EFEMP1, ELOVL4, EMC1, EXOSC2, EYS, FAM161A, FLVCR1, FRMD7, FSCN2, FZD4, GDF6, GNAT1, GNAT2, GNB3, GNPTG, GRM6, GUCA1A, GUCA1B, GUCY2D, HARS, HGSNAT, HK1, HMX1, IDH3A, IDH3B, IFT27, IFT43, IFT80, IFT81, IFT140, IFT172, IMPDH1, IMPG1, IMPG2, INPP5E, INVS, IQCB1, JAG1, KCNJ13, KCNV2, KIAA1549, KIF11, KIZ, KLHL7, LCA5, LRAT, LRIT3, LRP5, LZTFL1, MAK, MAPKAPK3, MERTK, MFRP, MKKS, MKS1, MTTP, MYO7A, NDP, NEUROD1, NMNAT1, NPHP1, NPHP3, NPHP4, NR2E3, NRL, NYX, OAT, OPN1SW, OTX2, P3H2, PCARE, PCDH15, PCYT1A, PDE6A, PDE6B, PDE6C, PDE6G, PDE6H, PDZD7, PEX1, PEX2, PEX3, PEX5, PEX6, PEX7, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26, PHYH, PITPNM3, PLA2G5, PLK4, PNPLA6, POC1B, POMGNT1, PRCD, PRDM13, PROM1, PRPF3, PRPF4, PRPF6, PRPF8, PRPH2, RAB28, RAX2, RBP3, RBP4, RCBTB1, RD3, RDH5, RDH11, RDH12, REEP6, RGR, RGS9, RGS9BP, RHO, RIMS1, RLBP1, ROM1, RP1, RP2, RPE65, RPGRIP1, RPGRIP1L, RS1, RTN4IP1, SAG, SAMD11, SDCCAG8, SEMA4A, SLC24A1, SLC7A14, SNRNP200, SPATA7, SPP2, TEAD1, TIMP3, TMEM126A, TMEM216, TMEM237, TOPORS, TPP1 (CLN2), TRAF3IP1, TRIM32, TRNT1, TRPM1, TSPAN12, TTC8, TTLL5, TUB, TUBGCP4, TUBGCP6, TULP1, UNC119, USH1C, USH1G, USH2A, VCAN, VPS13B, WDPCP, WDR19, WHRN, ZNF408, ZNF423, ZNF513

2. GENE-SPECIFIC FAMILY FOLLOW-UP TESTING *For relatives of a program participant ('proband') who received a Pathogenic/Likely Pathogenic result or approved VUS.*

Proband's Invitae Order ID:	This patient's relationship to proband:	Gene(s) to be tested in this patient:
RQ#	Parent / Sibling / Grandchild / Child / Other:	

NOTE: The presence or absence of all variants identified in the proband for the gene(s) ordered for gene-specific family follow-up will be commented on in this patient's report unless a limited selection is specified in the ***Requested Variants*** *section above. Invitae will report any Pathogenic/Likely Pathogenic variants found in this patient for the gene(s) ordered.*

Invitae continually updates its panels based on the most recent evidence. If an order is placed using an outdated test requisition form, Invitae reserves the right to upgrade ordered tests to their current versions. Test IDs containing add-on codes will include the original panel as well as the add-on.

By signing this form, the medical professional acknowledges that the individual/family member authorized to make decisions for the individual (collectively, the "Patient") has been supplied information regarding and consented to undergo genetic testing, substantially as set forth in Invitae's Informed Consent for Genetic Testing (www.invitae.com/forms). In connection with the Program the Patient has been informed that Invitae may notify them of clinical updates related to genetic test results (in consultation with the ordering medical professional as indicated) and has been informed that deidentified Patient data may be used and shared with third parties, for research and commercial purposes and, in the U.S., to contact their medical professional. The medical professional warrants that he/she will not seek reimbursement for this no-charge test from any third party, including but not limited to federal healthcare programs. The medical professional also hereby acknowledges that organization and clinician contact information provided in the order may be shared with third parties, including commercial organizations, that may contact the medical professional directly in connection with the Program, and that they have made the Patient aware that de-identified Patient data may be used and shared with such third parties, for purposes which include contacting their medical professional directly in connection with the Program. A list of third party partners may be provided upon request. I attest that I am authorized under applicable state law to order this test.

Medical professional signature (required)	Date (MM/DD/YYYY)
	/ /

Result Interpretation

- Not all genes are tested. Not all tests will deliver satisfactory results.
- **Positive result-** found pathogenic mutation in the gene(s) tested
- **Negative result-** did not find mutation in the gene(s) tested
- **Inconclusive results**
  - Variants of Uncertain Significance

AAOphthalmology Clinical Statement

- "…a causative mutation can be identified in up to **60-80%** of patients with inherited retinal disorders"

Results Interpretation

- Gene with mutation may be associated with several different eye conditions
- Other factors must still be taken into account
  - Patient ocular findings
  - Patient medical history
  - Patient family history
- <u>Is the variant pathogenic? Does it cause the clinical condition?</u>
- <u>Is it a secondary finding?</u>

COMMONLY ASKED PATIENT QUESTIONS – Q&A

Genetic Testing Q&A

- How much does genetic testing cost?
  - The lab testing might be free if the patient meets the eligibility requirements.
  - There may be cost associated with office visits.
- How long will it take to get the results?
  - Could be as short as 2 weeks; could be longer depending on the lab and testing.
- Do you need a blood sample?
  - Saliva sample is sufficient in most cases.

Genetic Testing Q&A

- Will this tell me what eye condition I have?
  - Not always. Genetic testing does not replace a clinical exam. Depending on the eye condition, genetic testing panel, and current knowledge on the condition, the possibility of positive or negative genetic test results varies.
- I did genetic testing a long time ago. Is this the same?
  - Consider retesting if genetic test was inconclusive in the distant past.
- Can children be tested?
  - Yes. However, there may be ethical considerations.

Gene Therapy Q&A

- Will I qualify for gene therapy?
  - Most likely not right now since gene therapy is only available for RPE65 mutation, but if you have RP or LCA there is a small possibility.
- Will I qualify for a gene therapy clinical trial?
  - Possibly. Can determine after receiving the genetic testing results.

Patient Recommendations

- **Genetic Counseling** is recommended
  - Family counseling
  - Inheritance patterns
- Additional family member testing is indicated in some cases

Eligibility for Gene Therapy & Clinical Trials

- Determine eligibility and referral for gene therapy
- Determine eligibility and referral for clinical trial
  - ClinicalTrials.gov
- Continuation of ocular health management
- Continuation of low vision rehabilitation

Next Steps

- Consider joining a registry
  - Ex. My Retina Tracker
- Consider joining a natural history study

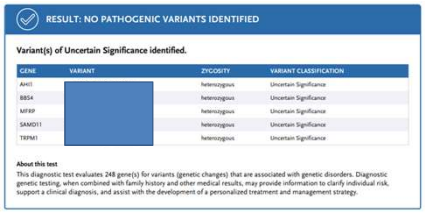
Continued Management

- Continuation of ocular health management
- Continuation of low vision rehabilitation

GENETIC TESTING RESULT CASE EXAMPLES

Result- VUS Only

- Retest in future



RESULT: NO PATHOGENIC VARIANTS IDENTIFIED

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
ANO1		heterozygous	Uncertain Significance
BBS4		heterozygous	Uncertain Significance
MFRP		heterozygous	Uncertain Significance
SAMD11		heterozygous	Uncertain Significance
TRPM1		heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 248 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

Result- Positive

- Patient with achromatopsia, interested in clinical trial

Genetic Testing Summary

Positive Results

Syndrome	Test Type	Genes Tested/ Panel Name	Result (Positive Gene, Mutation, Lab)
Inherited retinal dystrophies	panel testing	*Retinal D… Panel*	CNGA3 (pathogenic) Lab: Blueprint homozygous

Variants of Uncertain Significance Identified

Syndrome	Test Type	Test	Results
Inherited retinal dystrophies	panel testing	*Retinal Dystrophies Panel*	see details in table below Lab: Blueprint

Gene	Specific Variant	Zygosity
PCARE		*heterozygous*
TTLL5		*heterozygous*

Find a study

Status

Recruiting and not yet recruiting studies

All studies

Condition or disease (For example: breast cancer)

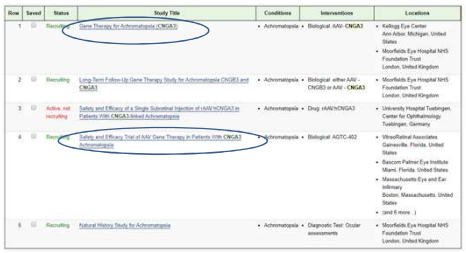
Other terms (For example: NCT number, drug name, investigator name)

CNGA3

Country

Search Advanced Search

Referral for Clinical Trial

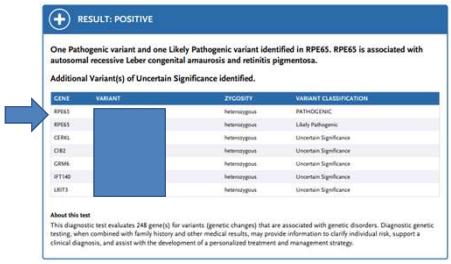


Row	Saved	Status	Study Title	Conditions	Interventions	Locations
1		Recruiting	Gene Therapy for Achromatopsia (CNGA3)	• Achromatopsia	• Biological: rAAV- CNGA3	• Kellogg Eye Center Ann Arbor, Michigan, United States • Moorfields Eye Hospital NHS Foundation Trust London, United Kingdom
2		Recruiting	Long-Term Follow-Up Gene Therapy Study for Achromatopsia CNGB3 and CNGA3	• Achromatopsia	• Biological: either rAAV-CNGB3 or rAAV - CNGA3	• Moorfields Eye Hospital NHS Foundation Trust London, United Kingdom
3		Active, not recruiting	Safety and Efficacy of a Single Subretinal Injection of rAAV.hCNGA3 in Patients With CNGA3-linked Achromatopsia	• Achromatopsia	• Drug: rAAV.hCNGA3	• University Hospital Tuebingen, Center for Ophthalmology Tuebingen, Germany
4		Recruiting	Safety and Efficacy Trial of AAV Gene Therapy in Patients With CNGA3 Achromatopsia	• Achromatopsia	• Biological: AGTC-402	• Vitreoretinal Associates Gainesville, Florida, United States • Bascom Palmer Eye Institute Miami, Florida, United States • Massachusetts Eye and Ear Infirmary Boston, Massachusetts, United States • (and 6 more…)
5		Recruiting	Natural History Study for Achromatopsia	• Achromatopsia	• Diagnostic Test: Ocular assessments	• Moorfields Eye Hospital NHS Foundation Trust London, United Kingdom

Result- Positive

- 2 Brothers
  - 20 years old
  - 12 years old
- Retinitis Pigmentosa

Result- Positive



RESULT: POSITIVE

One Pathogenic variant and one Likely Pathogenic variant identified in RPE65. RPE65 is associated with autosomal recessive Leber congenital amaurosis and retinitis pigmentosa.

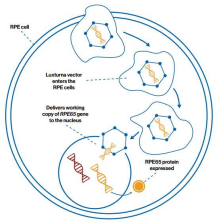
Additional Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
RPE65	[redacted]	heterozygous	PATHOGENIC
RPE65		heterozygous	Likely Pathogenic
CERKL		heterozygous	Uncertain Significance
CIB2		heterozygous	Uncertain Significance
GRM6		heterozygous	Uncertain Significance
IFT140		heterozygous	Uncertain Significance
LRIT3		heterozygous	Uncertain Significance

About this test

This diagnostic test evaluates 248 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

Referral for Gene Therapy



RESOURCES

Resources

- Genetic Testing Resources
  - eyewant2know.com/IDYOURIRD/
  - Foundation Fighting Blindness
    - Fightblindness.org
    - myretinatracker.org
      - Online registry
- National Institutes of Health (NIH) ~~Genetics Home Reference~~ → MedlinePlus Genetics
  - ~~ghr.nlm.nih.gov/primer/testing/genetictesting~~
  - https://medlineplus.gov/genetics/

Resources

- Search for gene specific clinical trials/ Determine eligibility for clinical trials
  - ClinicalTrials.gov

Benefits of genetic testing

- Improve accuracy and specificity of diagnosis
- Improve accuracy of prognosis
- Improve accuracy of genetic counseling, family counseling
- Mutation/ mechanism-specific care
  - Gene therapy & clinical trials

Conclusions

- Increase public and patient awareness
- Increase access to genetic testing and gene therapy
  - Early testing and referrals

Q&A

Rachelle Lin
rlin@ketchum.edu