Genetic screening of Usher syndrome and Other hereditary forms of hearing loss

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Universal Genetic Screening

- Established under Title XXVI of the Children's Health Care Act of 2000 "Screening for Heritable Disorders"

- Advisory Committee to the Secretary
  - Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children.
  - http://mchb.hrsa.gov/programs/genetics/committee/default.htm

- Legislation:
  - **"… enhance, improve or expand the ability of State and local public health agencies to provide screening, counseling or health care services to newborns and children having or at risk for heritable disorders…"**

The goal of genetic screening

Ensure that children with heritable disorders and their families have access to quality care and appropriate genetic expertise and information in the context of a medical home that provides accessible, family centered, continuous, comprehensive, coordinated, compassionate, and culturally effective care.

Universal Genetic Screening is Expanding Rapidly

9 Core Disorders

e.g. hearing loss, hemoglobinopathies,

5 Fatty Acid Disorders (8)

e.g. carnitine uptake defect.

9 Organic Acid Disorders (6)

e.g. glutaric acidemia

6 Amino Acid Disorders (8)

e.g. phenylketonuria

There are 29 core disorders in the Universal newborn screening panel and another 20 in the supplemental panel

Types of Genetic Screening Tests

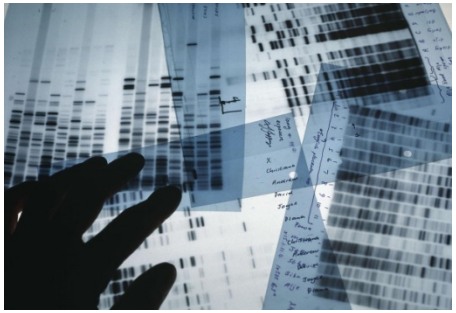
Routine

Metabolic

Functional

Developing

DNA

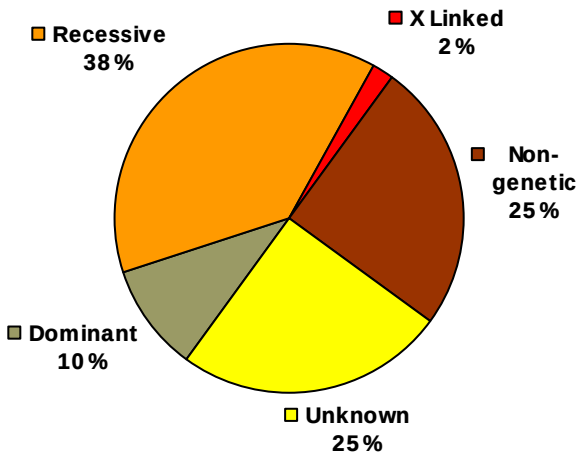


Newborn Hearing Screening is a genetic test!

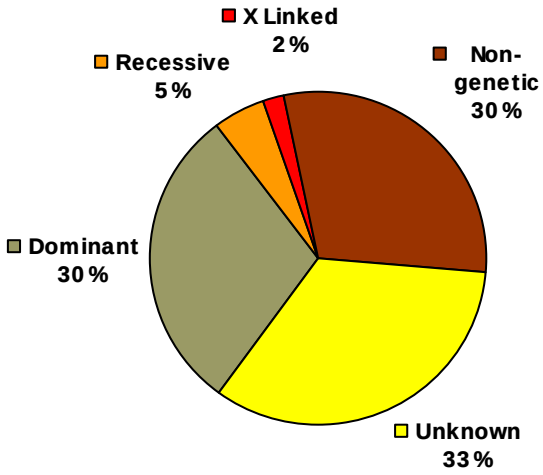
Families need to have access to the same set of medical alternatives as provided for the other genetics disorders for which screening has been provided.

Causes of Hearing Loss
Averaged from the literature

Childhood



Adult



How many genes involved in hearing loss have been discovered?

- 27 non-syndromic recessive genes.
- 22 non-syndromic dominant genes.
- 1 x-linked gene.
- 13 mitochondrial mutations.
- 9 significant syndromic genes.

That's not all, folks!

Hereditary Hearing Loss Home Page
webh01.ua.ac.be/hhh/

Important Hearing loss genes

- GJB2/6 aka Cx26
  - 10 to 40% of all congenital deafness.
- OTOF
  - Major cause of auditory neuropathy.
- SLC26A4
  - Pendred Syndrome
- The 9 Usher genes

What we don’t know about genes and hearing loss

- A Lot!
- What are their relative frequencies?
  - Are there ethnic and population differences?
  - How do the frequencies change with age?
- Do phenotypes overlap?
  - Does phenotype expansion extend into new syndromes?
  - Are there high risk groups?
  - Are there any other special risks?
  - What are the major medical issues beyond hearing?
- What determines severity?

Is DNA screening for hearing loss feasible?

!YES!

As many as 50% of congenitally deaf children can be identified as having a heritable hearing loss using relatively inexpensive genetic testing.

WHERE SHOULD WE START DNA SCREENING FOR HEARING LOSS DISORDERS?

1. GJB2 detects a common cause of childhood hearing loss and is easy to test.

2. The Usher genes are associated with a progressive loss of vision and are the second most common type of genetic hearing loss

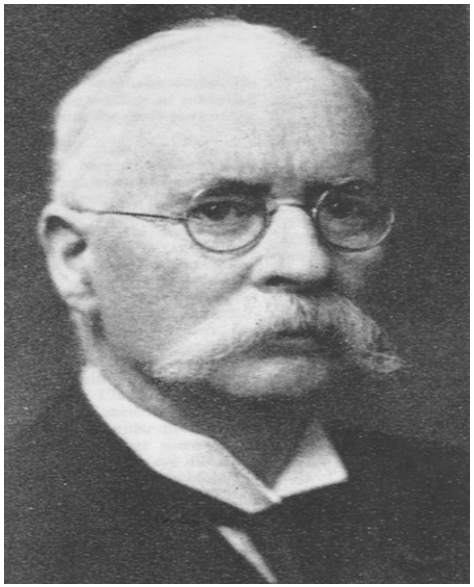
Children with hearing loss

- 50 % of children (7years) with HL have visual impairment.
  - (normal hearing- 20%)
- More than ½ of these require more than refraction correction.
- The majority are believed to have Usher syndrome

What is Usher Syndrome?

Usher syndrome
Definition

- Defined as hearing loss with retinitis pigmentosa in the absence of other significant symptoms.
- It is inherited as an autosomal recessive.
- There are three clinical types.
- There are at least twelve genes involved



Dr. Charles H. Usher

Most deaf/blind syndromes have retinal degeneration

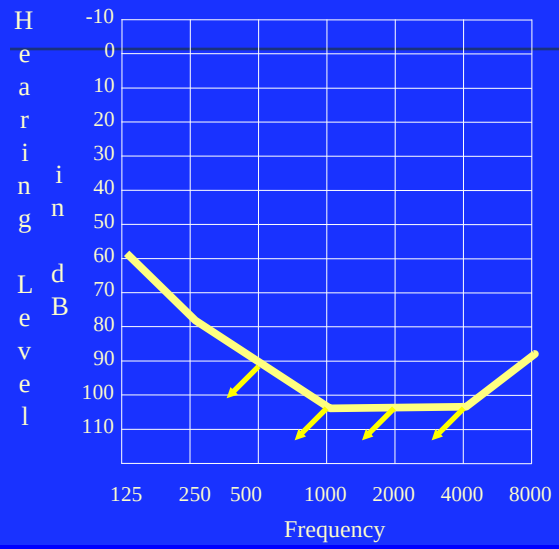
Clinical Types

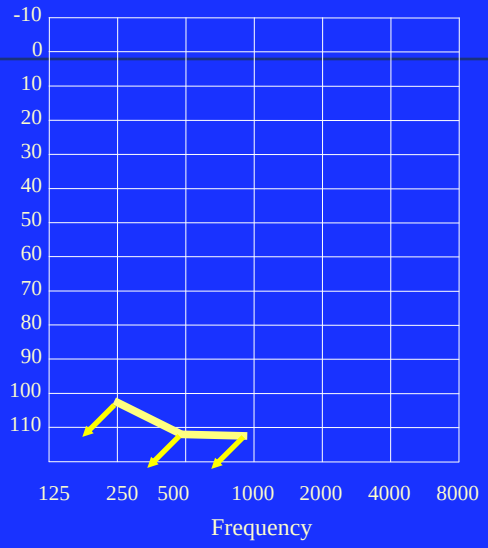
- Type 1:
  - Profound hearing loss (deaf).
  - Early onset RP.
  - Balance problems.
- Type 2:
  - Moderate to sever hearing loss (hard of hearing)
  - RP evident in their teens
  - No balance problems
- Type 3:
  - Progressive hearing loss.
  - Looks like type 2 as children.
  - Looks like type 1 as older adults.
- Atypical
  - Doesn’t fit any of the first three categories.

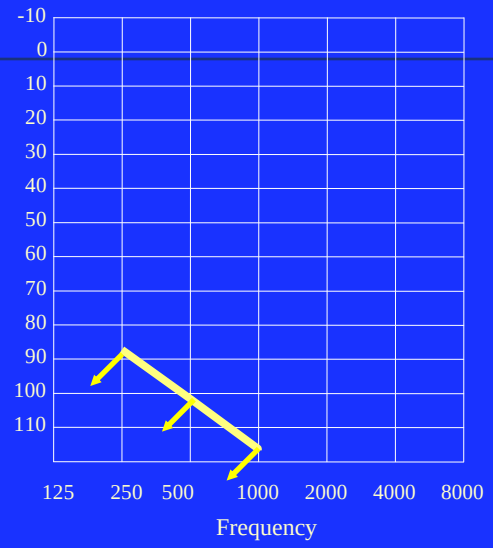
Usher Syndrome Audiograms

Type I Vs Type II

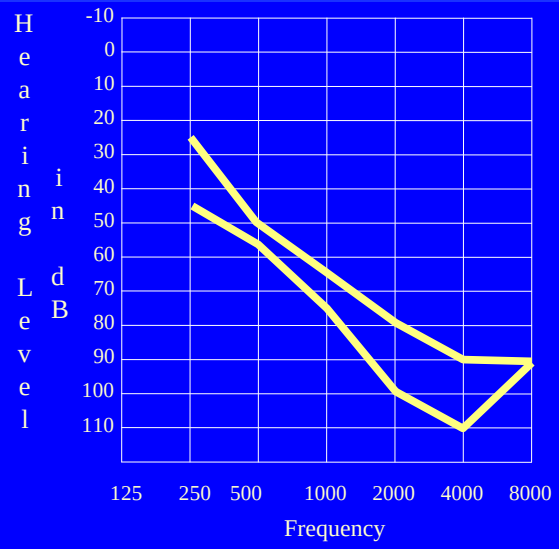
TYPE I

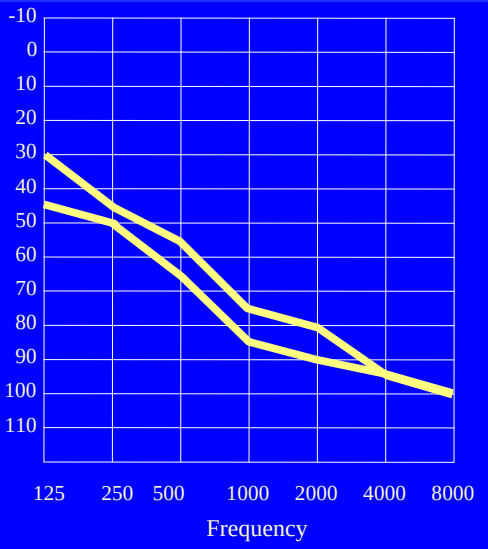


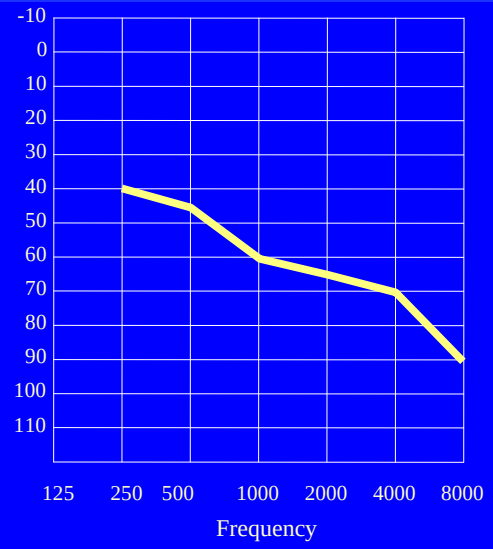




TYPE II

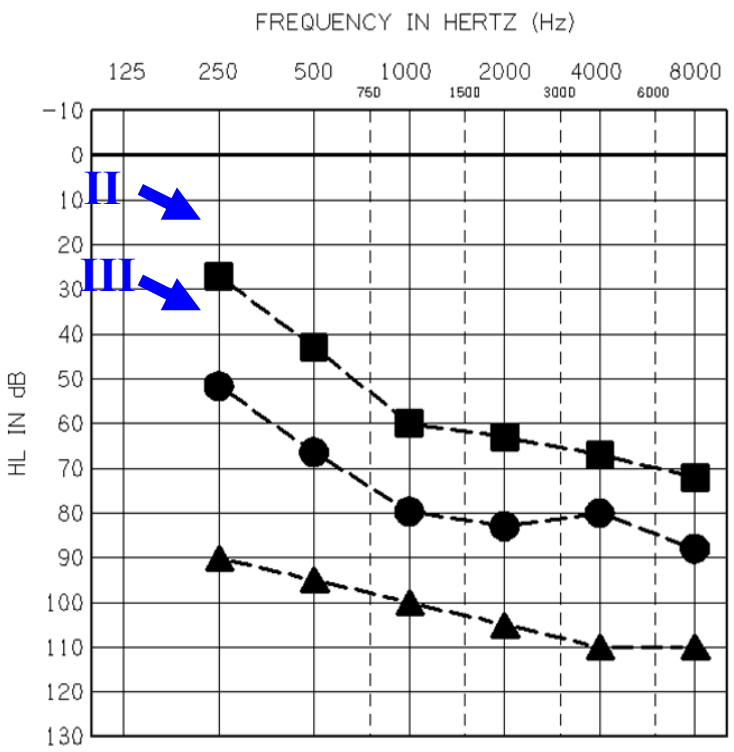






Comparison of hearing loss between Usher type I, II and III

4-9y



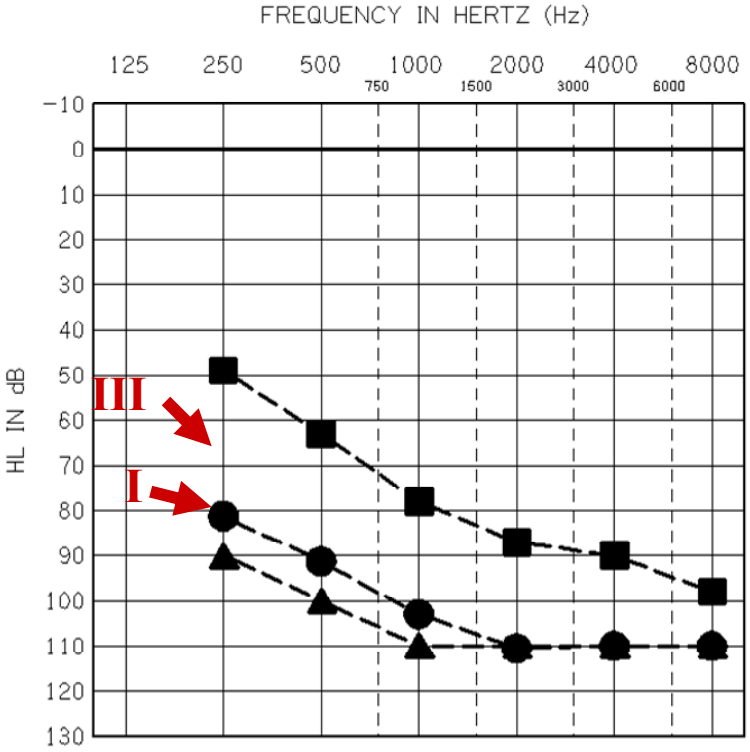
–■– Usher type II

–●– Usher type III (USH3)

–▲– Usher type I

Type III can wrongly be diagnosed as type II

40-49y



–■– Usher type II

–●– Usher type III (USH3)

–▲– Usher type I

Type III can wrongly be diagnosed as type I

Hearing is important in the differential diagnosis of retinitis pigmentosa

- Audiologic evaluation
  - Audiogram shape is helpful
  - R/O tests: tympanometry; OAE, CT scan
- Degree of hearing loss and history of progression will channel molecular testing into one of three different strategies.
- Usher 2 patients may not have ‘deaf speech’
- Eye guys: Usher patients often require vision to communicate – talk to them directly.
- Ear guys: Usher patients, especially children, may not do well under condition of dim light

Balance can be useful in discriminating between Usher clinical types

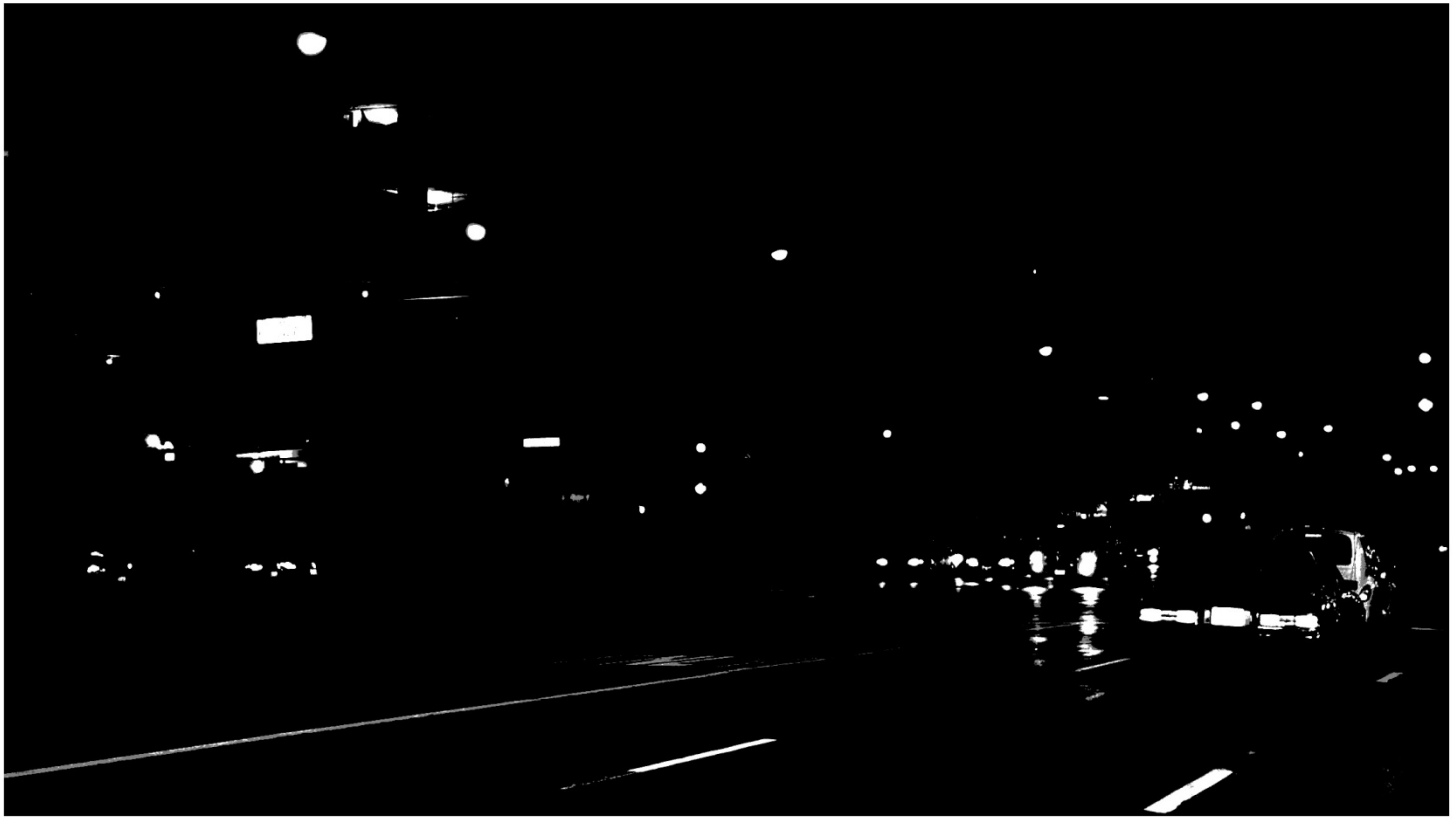
- Usher 1 poor balance
- Usher 2 good balance
- Usher 3, like Usher 2 in childhood developing into an Usher 1 phenotype as adults
- Simple indicators
  - age when walking (US1 > 18 mo.)
  - Not dizzy on carnival rides.

Vision

- Visual Acuity
- Nightblindness
- Peripheral vision
- Treatable Complications
  - Cataract
  - Macular edema

What we see

What an Usher person sees



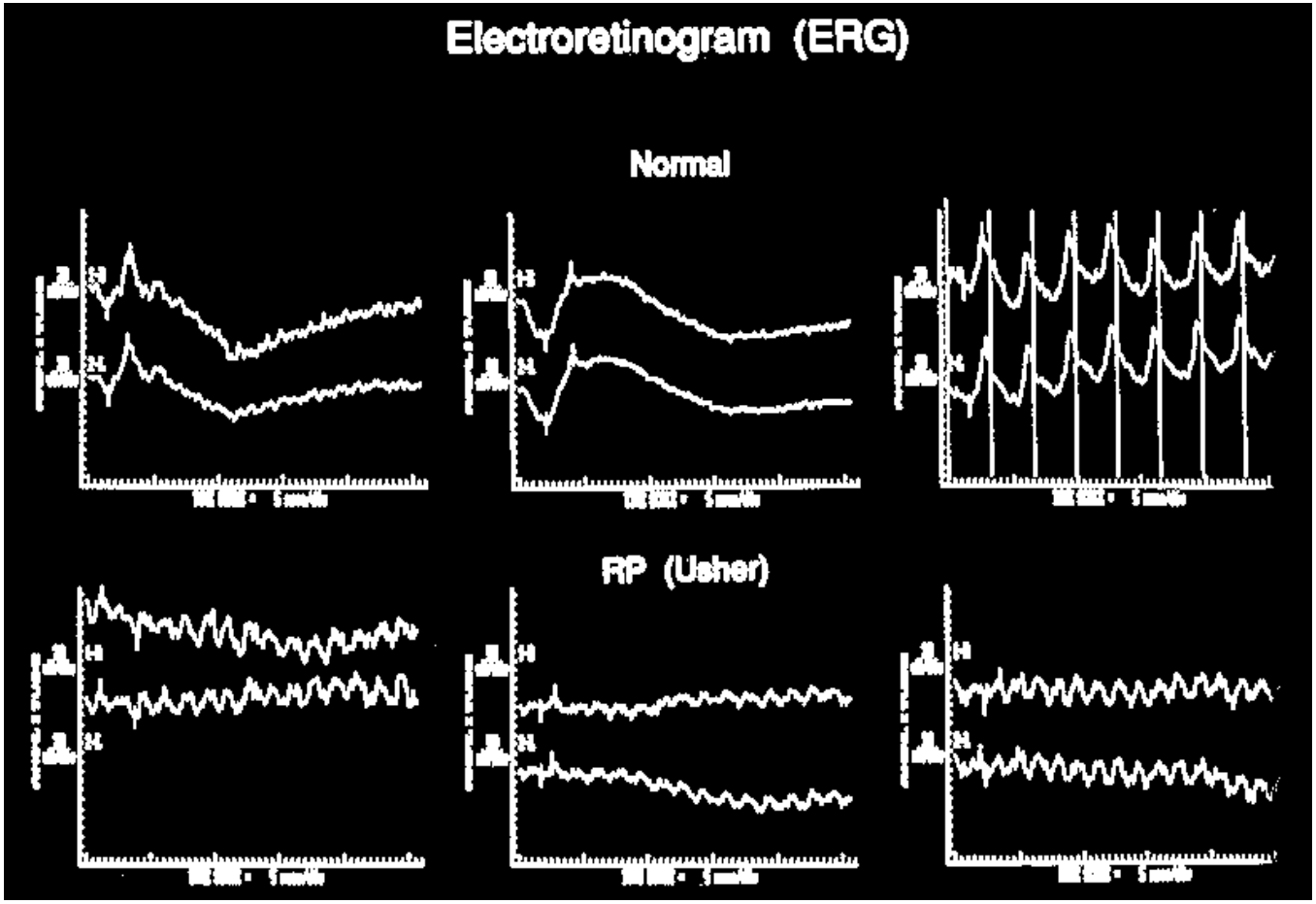
The way most of us see it.
The old Iowa Capitol building

Usher at 2-8 years of age

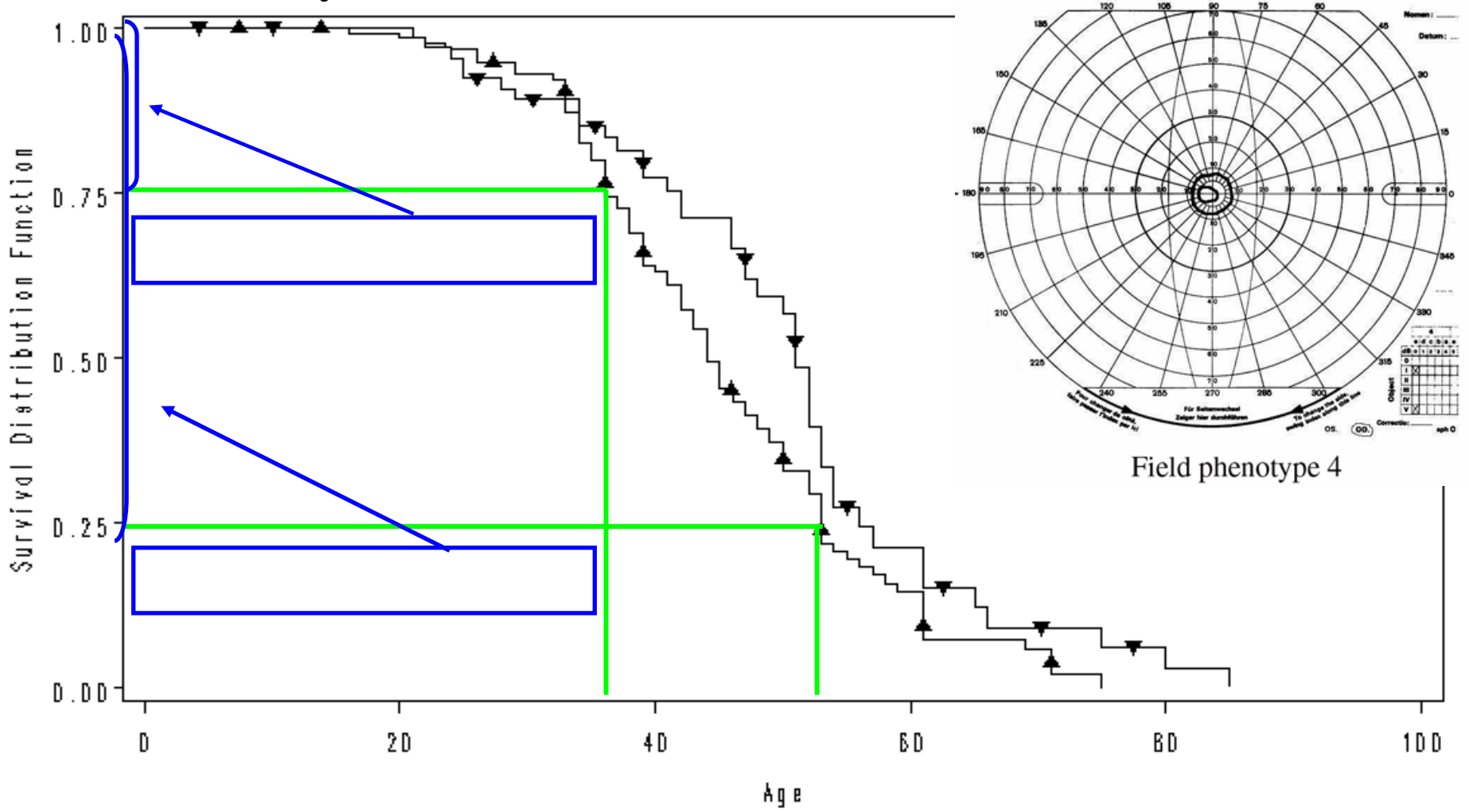
Usher at 5 to 15 years

Usher at 18 to 30 years

Electroretinogram From a Patient With Usher Syndrome Type I



Survival analysis- visual field –The likelihood of having <5 deg



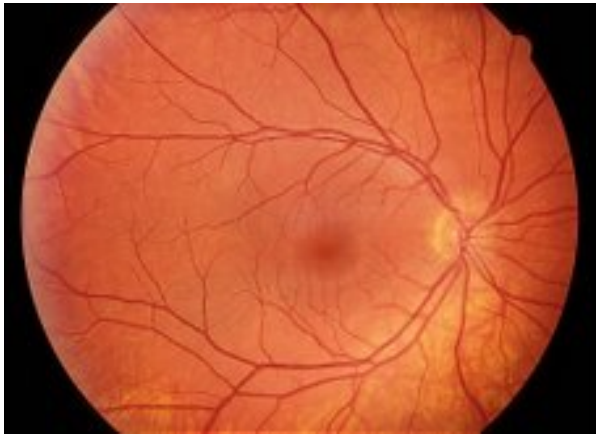
Field phenotype 4

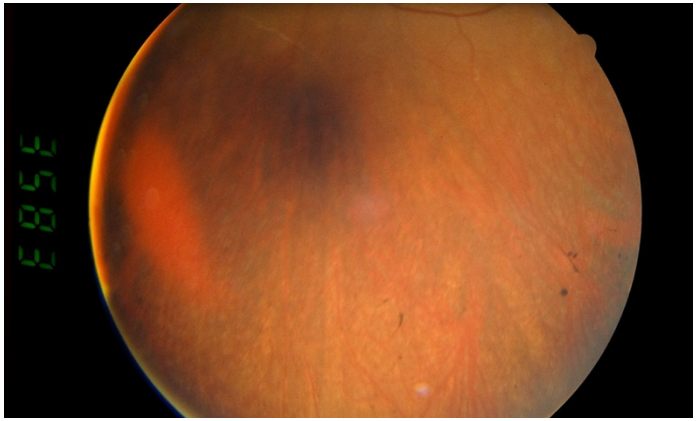
Usher type I

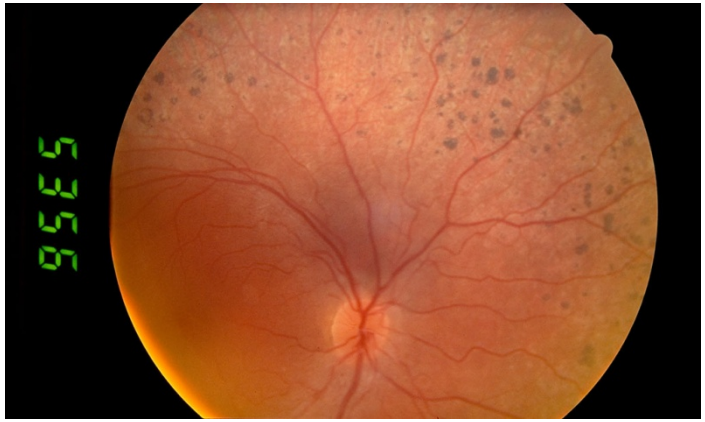
Usher type II

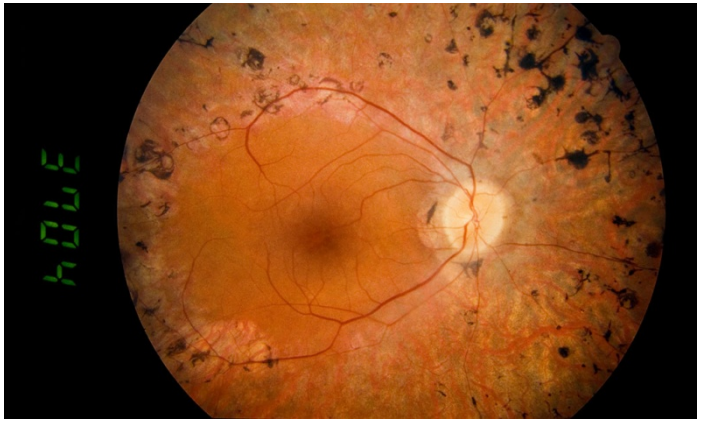
($p<0.05$)

Usher Retinitis Pigmentosa Stages



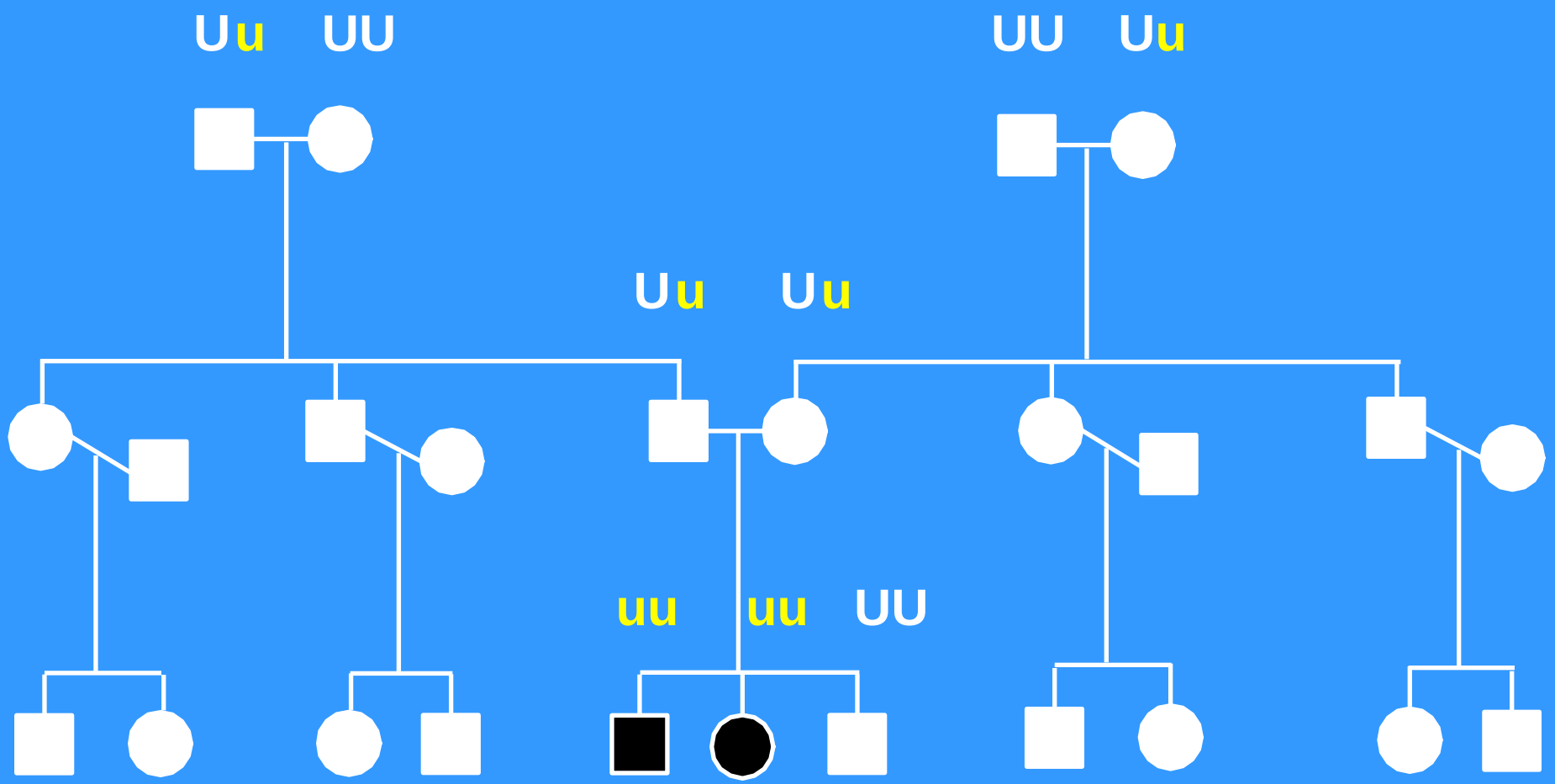




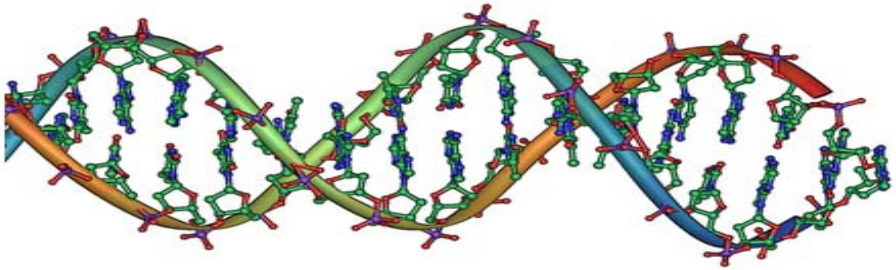


Usher syndrome is **defined** as an autosomal recessive disorder

Demonstration Pedigree Showing Recessive Inheritance for Usher syndrome



Why has it been important to find and characterize the Usher genes?



1. This is fundamental to understanding the basic biological processes underlying vision and hearing, **in everyone**.
2. Nine or more genes discovered.
3. **Diagnosis is vastly improved** and accurate diagnoses are key to treatment.

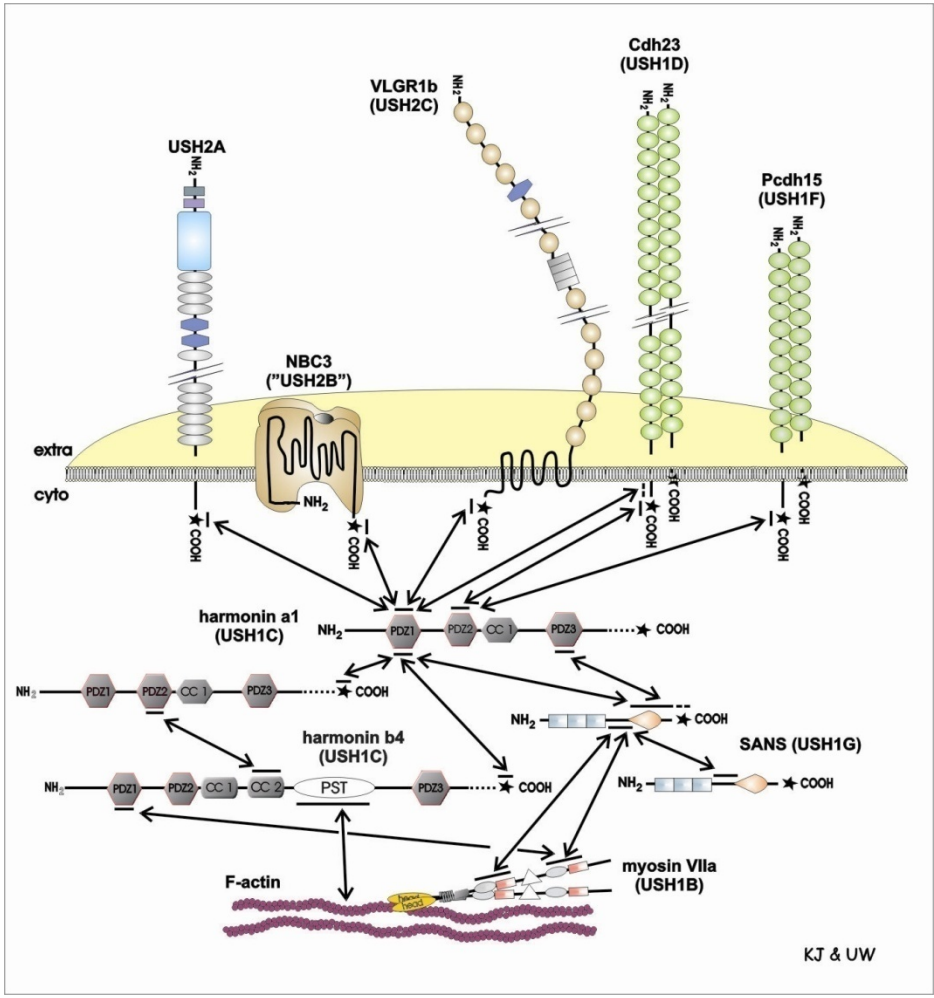
There are >11 Genes involved

- Usher Type I
  - Usher 1b: MYO7A
  - Usher 1c: Harmonin
  - Usher 1d: CDH23
  - Usher 1f: PCDH15
  - Usher 1g: Sans
- Usher type II and III
  - Usher 2a: Usherin
  - Usher 2c: VLGR1
  - Usher 2d: Whirlin
  - Usher 3a: Clarin-1

There are two others recognized through linkage (Usher Ie and 1h)

Usher syndrome interactome as proposed by Uwe Wolfrum

- The function of the interactome is unclear.
- Structural in the cochlea?
  - Tip and ankle links
- Cargo transport in the retinal?



The observations that the Usher genes interact with each other is appealing in the sense that it ties them all to the common phenotype of cochlear and retinal dystrophy

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But, an hypothesis of two different mechanisms, one in the cochlea and another in the retina seems counter intuitive.

How frequent is Usher syndrome?

- 4.4 per 100,000 (Boughman, 1983)
  - USA
- 3.6 per 100,000 (Groendahl and Mjoen, 1983)
  - Scandinavia
- >50 studies in the 20th century of schools for the deaf indicate about 1/20 with retinitis pigmentosa
  - Throughout the world
- **All previous studies focused on the deaf (i.e. profound hearing loss) and used data from schools for the deaf and were phenotypically driven.**

How frequent is Usher syndrome?

- Study #1
- High School
- 78 children, 16 to 19 years
  - Seven positive
    - 1 Usher 1B
    - 3 Usher 1D
    - 3 Usher 2A
- **8.9% rate**

- Study #2
- Cochlear Implant
- Pre-screened for GJB2
- 55 children, <5 years
  - Seven positive
    - 2 Usher 1B
    - 1 Usher 1C
    - 3 Usher 1D
    - 1 Usher 2A
- **8.2% adjusted rate**

ASPER chip with <50% detection rate

The prevalence of Usher syndrome is 3 to 4 times greater than previously reported!

- Since the sensitivity is <50%, the prevalence in a pediatric D/HOH population for children carrying at least one Usher gene mutation is estimated at **~17%**.
- The prevalence of Usher syndrome is estimated at **~1 of 6700** births
- Implies ~45,000 Americans affected.

Can Usher syndrome be treated?

- **Safety and geographic orientation**
- **Life style changes**
  - **Smoking cessation**
  - **Sunglasses**
  - **Diet**
- **Vitamin supplementation and antioxidants**
- Prosthetics
  - **Cochlear Implants**
  - Retinal Implants
- CNTF and other growth factors
- Gene therapy

Vitamin A supplementation may slow the progression of RP

- Harvard medical school.
- First study reported Vitamin A slowed the progression of RP.
- **Later study showed a greater effect when in combination with diet (fish important).**
- BUT, it remains controversial.

+

Anti-oxidants may slow progression of RP?

- The RP retina has high concentrations of oxygen.
- Animal models are suggestive.
- Shown helpful in macular degeneration.
- Vitamin E, Vitamin C, alpha-lipoic acid, TUDCA, …

Prosthetic devices

- Cochlear implant

- Retinal implant

Therapies for Usher syndrome
CNTF

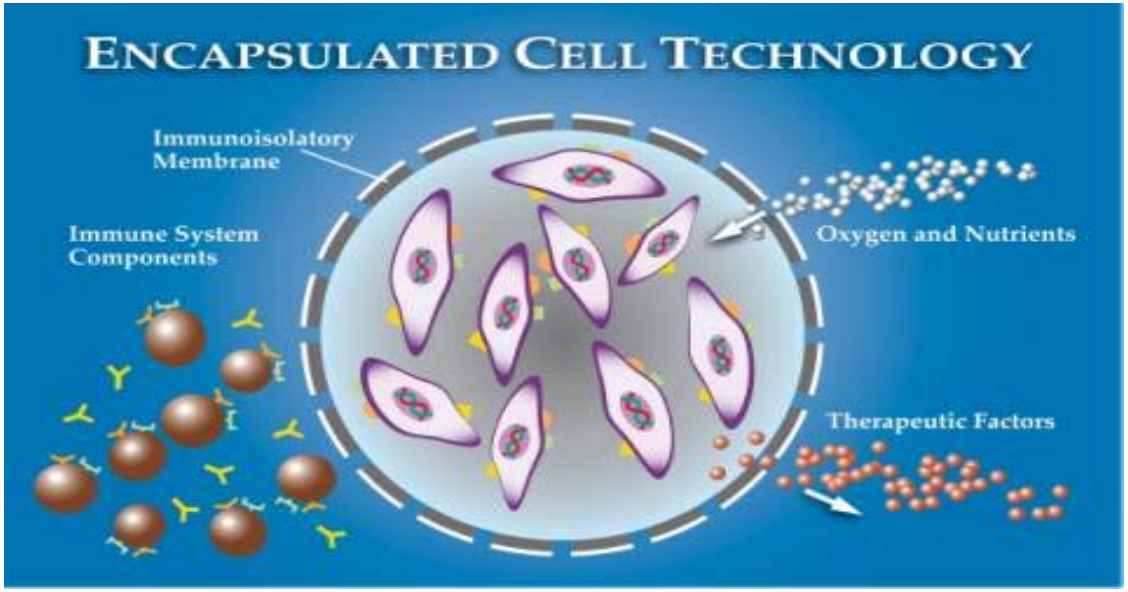
- CNTF = Ciliary neurotropic factor
  - **Neuroprotective**
- **Novel delivery**
  - **Encapsulated cells genetically programmed to make CNTF**
  - **Inserted within the vitreous**
- Phase I clinical trial completed.
  - Sieving et al., (2006) **PNAS** *Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants.*
  - Safety demonstrated
  - Some indication that vision improved.
- Phase II clinical trials underway and **will soon be reported**.

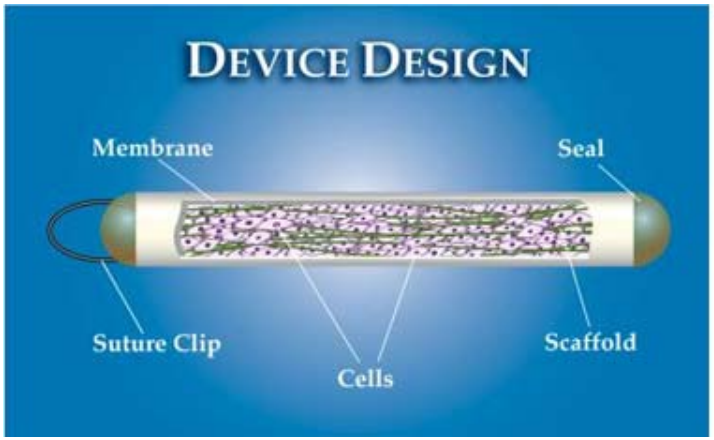
Emerging Treatment Stabilizes Vision in People with Dry AMD
Owings Mills, MD - March 26, 2009

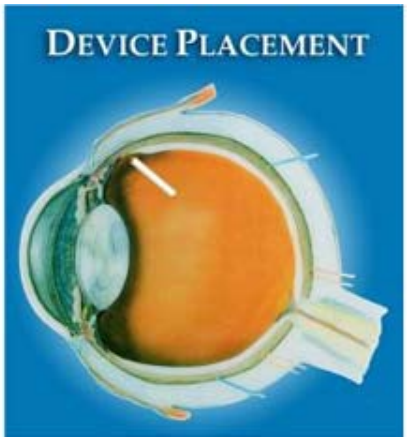
An innovative technology, employing a tiny capsule implanted in the eye, is stabilizing vision in people suffering from dry age-related macular degeneration (AMD). Encapsulated Cell Technology (ECT), developed by Rhode Island-based Neurotech, preserved vision in a majority of the 51 people who participated in a Phase II clinical trial.

News from the Foundation Fighting Blindness

How does CNTF delivery work?







Gene therapy will be the gold standard or treatment

- Genes are information.
- When a gene is ‘knocked out’, the information is cell needs is missing.
- To correct the problem, the functional gene must be delivered to the cells that need to use that information

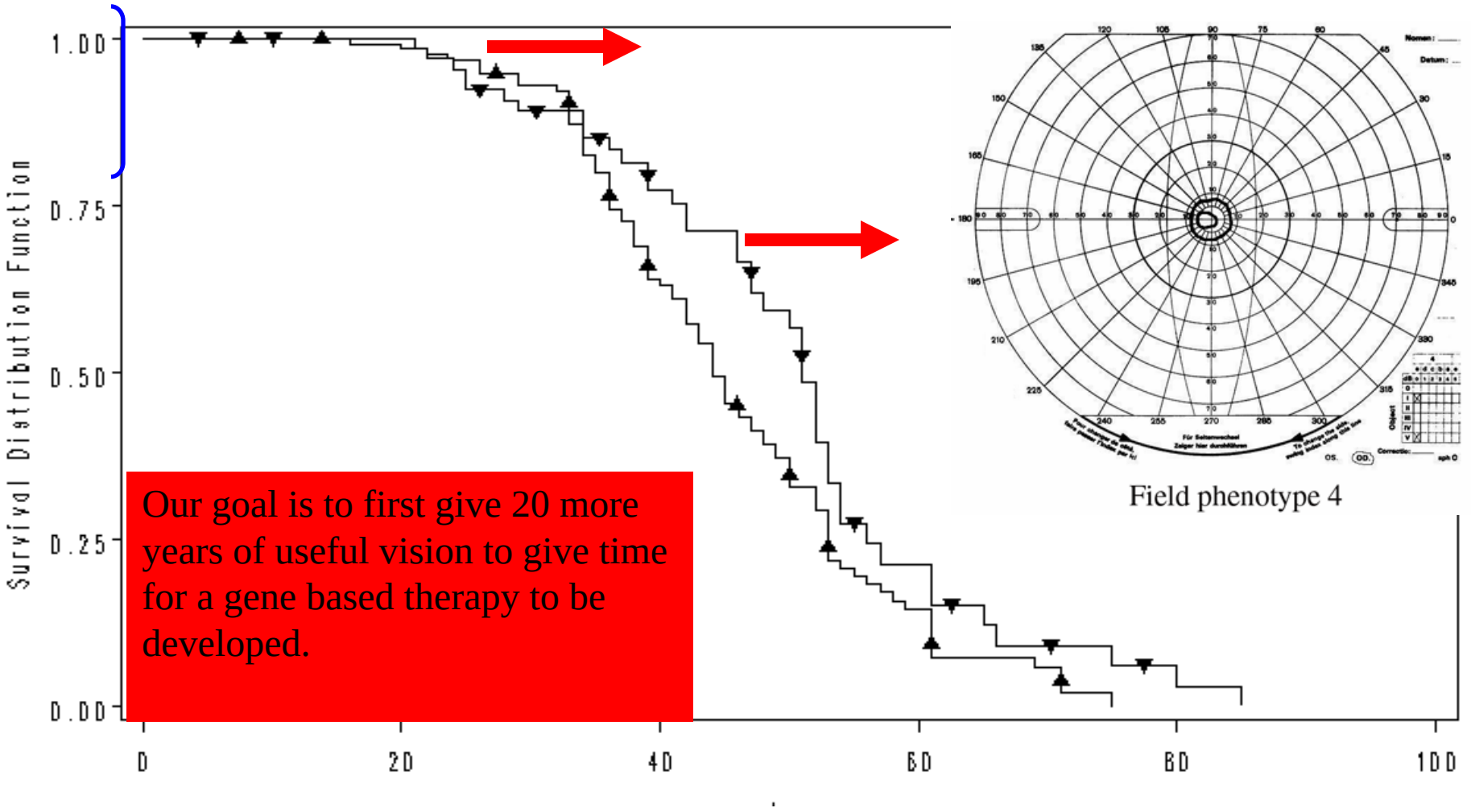
Gene therapy and the future

- Lancelot, the Briard Dog
  - Leber's congenital amaurosis, a severe juvenile RP.
- Gene (RPE65) inserted via a viral vector in one eye.
  - Acland, et al., (2005) **Mol. Ther.** *Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness.*
- **Gene therapy programs are now underway for Usher 1b, 2a, and 3a**
- **Phase 1, safety is completed: phase 2 underway and includes on 5 year old child.**

We are committed to developing research programs that will tell us how to delay the progression of the retinitis pigmentosa.

1. **Give Young adults with Usher syndrome 20 more years of useful vision using diet, life style, and traditional therapeutics AND**
2. **Cure the vision and hearing problems with gene therapy.**

Survival analysis- visual field –The likelihood of having <5 deg



Usher type I

Usher type II

($p<0.05$)

The problem

- Few clinical trials have been attempted involving Usher syndrome. None have targeted children.
- A major problem with clinical trials for orphan disorders like Usher is the availability of suitable subjects.
- An inexpensive and reliable screening method needs to be developed.

SNPlex (ABI)

- Allele specific ligation assay using fluorescent detection.
- 24 multiplexed alleles can be tested for ~$5.
- ~10% of mutations are incompatible.
- Very high throughput.

- We have developed an ***USHplex*** test that currently tests for 94 of the most common Usher mutations.

Preliminary estimate of performance of the USHplex assay

Test set	Number of individuals	Number of Positives	Per cent
1A	301	40	13.3
1B	403	100	24.8
2A	411	11	2.7
2B	434	23	5.3
3A	318	25	7.9
3B	274	24	8.8
Total Positive Hits		183	62.7%
Adjusted Sensitivity			43.5%

Sample is a mix of Usher, Hearing loss, and RP subjects

USHplex Specificity

- ~5% false positives are predicted, due to possibility of heterozygotes.
- Requires follow-up to identify second mutation.

USHplex is dynamic

- New sets added as more mutations are observed.
- Improved sensitivity with time.
- Each set adds $5 to cost.

Who should be screened?

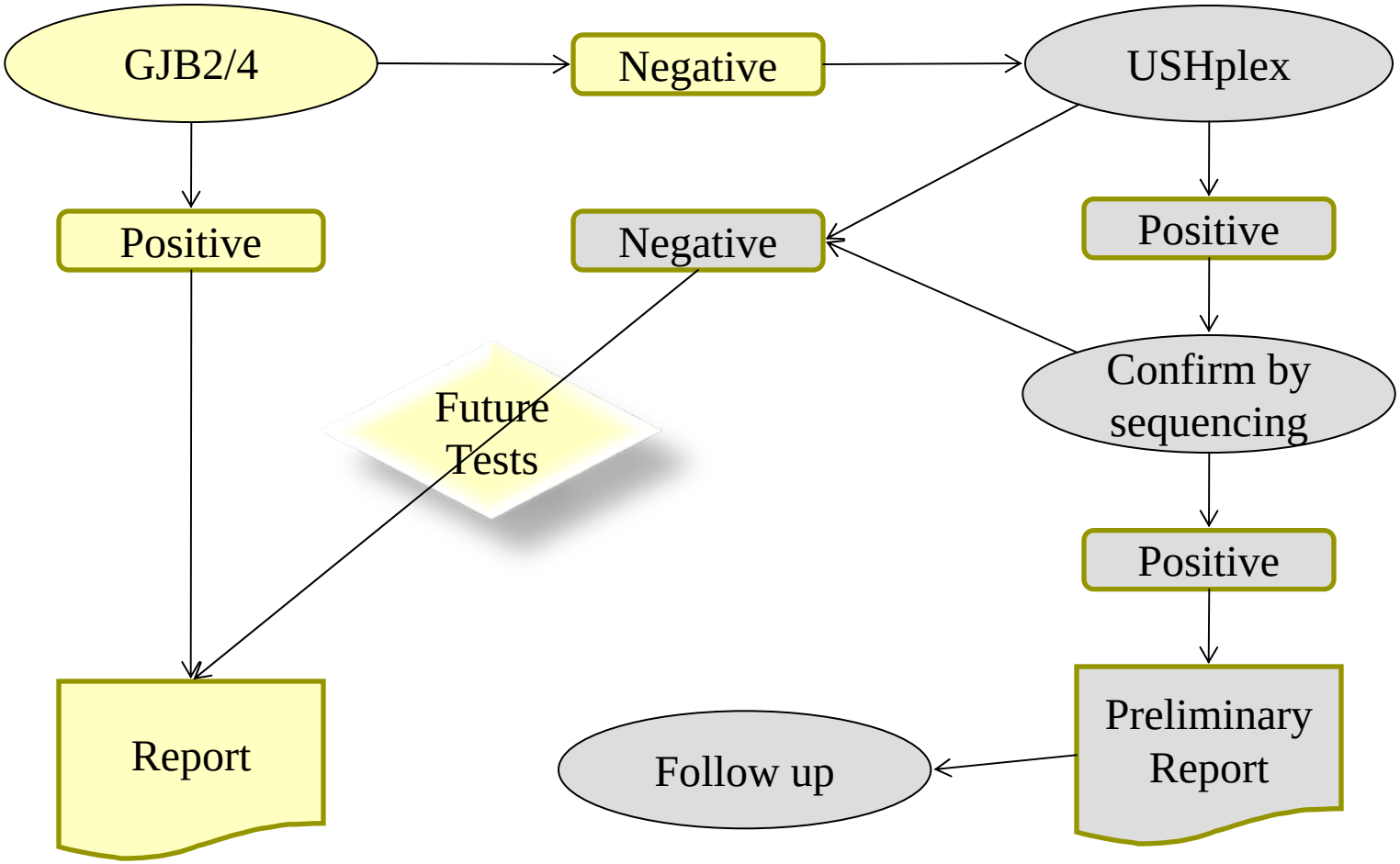
(hearing loss is an indicator of risk)

- Deaf and hard of hearing children in special education classes (ages 5 to 18).
- Children undergoing cochlear implantation.
- Children who ultimately fail newborn hearing screening.

What should be screened?

- First, GJB2 will rule out 40% from having Usher syndrome.
- Second, those negative for GJB2 will be screened for the most common Usher mutations.
- Other deafness genes can be integrated with time.

Flow of screening for D/HOH children



Usher syndrome screening in the USA

- Out of 4,000,000 births each year.
  - 4,000 fail the Newborn hearing screen.
  - 600 will have Usher syndrome.
- The annual cost of rehabilitation, lost wages/taxes, etc. has been estimated to be $50,000 per year per deaf/blind person.
- Assuming 20 year extension of visual health. The national savings is about ½ billion for each year screened.

Carver Lab

Welcome

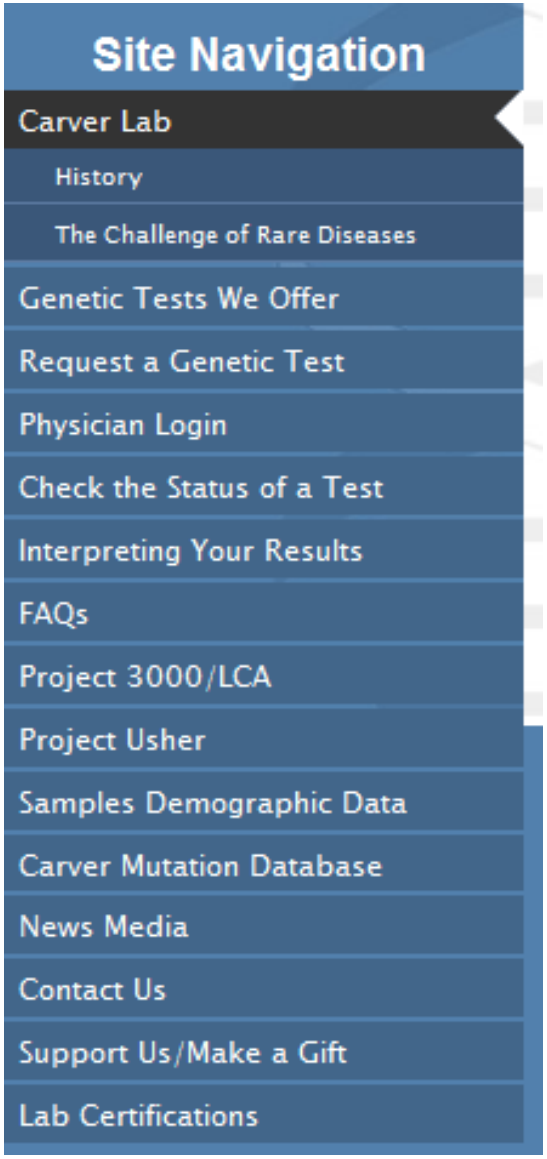
The John and Marcia Carver Nonprofit Genetic Testing Laboratory is dedicated to providing non-profit genetic testing for rare eye diseases to meet a societal need. Most of the diseases that we study are so rare that commercial tests would be unlikely to be viable for the long term. As a result, many individuals affected with these diseases and their families would have little access to molecular information. Genes that are available for screening by the Carver Laboratory have previously been extensively studied in the research laboratories of Dr. Stone and Dr. Sheffield. By incorporating this research information into the design of the tests, the laboratory is able to offer genetic tests that provide the most clinically relevant information to patients and their families while keeping the tests affordable.

Now Available! *Online Ordering and Tracking System*

The Carver Lab is pleased to announce the beginning of a new era in terms of our ability to offer affordable molecular genetic testing for inherited eye conditions to patients and their families. A two-year, multi-phase project has resulted in a sophisticated, yet user-friendly approach to applying for a genetic test. And we couldn't be more excited!

This ***Online Ordering and Tracking System*** is specifically designed to enhance communication between a patient, his or her physician and the Carver Lab. Key to this new process is an Online Test Requisition form. While any and all tests must be ordered through a referring physician, the patient will be able to, for the first time, walk away from an appointment with a unique, confidential "Identifier" – a computer generated number assigned which will allow the patient, and his or her physician, to keep close track of the status of a test. From the day a blood sample arrives in the Lab, to the day a test result report is forwarded to the referring physician, the status of a genetic test in progress will now be available online.

This new system is a work in progress. Your comments and suggestions are welcome and much appreciated.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The Challenge of Rare Diseases
Genetic Tests We Offer
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Physician Login
Check the Status of a Test
Interpreting Your Results
FAQs
Project 3000/LCA
Project Usher
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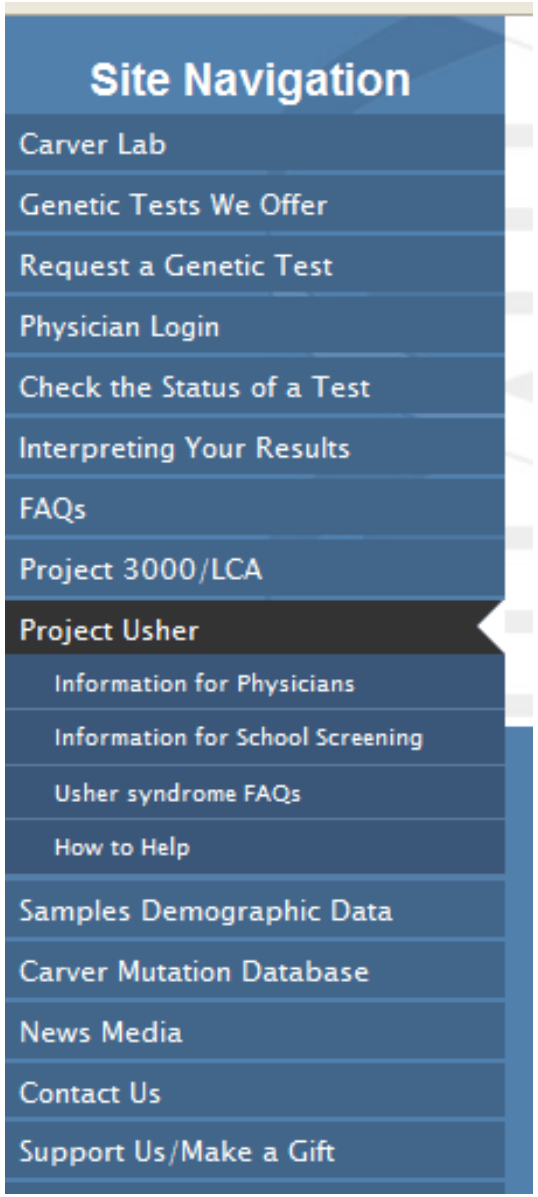
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Check the Status of a Test
Interpreting Your Results
FAQs
Project 3000/LCA
Project Usher
Information for Physicians
Information for School Screening
Usher syndrome FAQs
How to Help
Samples Demographic Data
Carver Mutation Database
News Media
Contact Us
Support Us/Make a Gift

What is needed to progress with Usher research.

- Interactive research programs between different NIH Institutes that would help support the development of multidisciplinary programs involving sight, hearing, and genetics
- Improved molecular diagnostics.
  - Reduce cost even further
  - More inclusive
- Epidemiologic studies.
  - What works and what doesn’t
- Natural history studies.
  - When do we treat
- Clinical trials

Support From:

- National Institute for Deafness and Communication Disorders
- Foundation Fighting Blindness.
- National Eye Institute.
- National Institute for Child Health and Development.
- Hear See Hope Foundation.
- Howard Hughes Medical Institute.
- And all the Usher people and their families.

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Thanks for lettin’ me bend yur ear, podner

Wild Bill