The Genetics of Usher Syndrome

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DNA is Highly Compacted into Chromosomes

The DNA from one cell stretches 7.5 feet.

All of the DNA in your body would stretch from here to the moon 300,000 times.

http://www.accessexcellence.org/AB/GG/
Human Karyotype
We inherit two copies of each chromosome (and each gene), one from each parent.
How are genetic disorders inherited?
Autosomal Dominant Mutations

• Some diseases can be caused by only one copy of a mutated gene

• These diseases are seen in every generation

• If a parent has a dominant mutation, each child has a 50% chance of inheriting it.
Autosomal Recessive Mutations

- For some diseases, both copies of a gene must be mutated to get the disease.
- Often, there is no family history of the disease.
- Each child will have a 25% chance of getting the disease.

A carrier is a person who carries one copy of a recessive mutation, but does not have the disease.
X-Linked Recessive Mutations

- Only males are affected.
- Each son will have a 50% chance of getting the disease.
- Each daughter has a 50% chance of being a carrier.
Mitochondrial Mutations

• Only the mother passes mitochondria to her children.

• All children will inherit a mitochondrial mutation from their mother.

• Mitochondrial mutations are often variable in their expression of the disease.
Usher Syndrome Shows Autosomal Recessive Inheritance

- Both copies of the gene must be mutated.

- Often, there is no family history of Usher Syndrome.

- Each child will have a 25% chance of getting Usher Syndrome.

A carrier “carries” one copy of the recessive mutation, but does not have Usher Syndrome.
What is it?

Determine whether you have a variant in a gene which can result in a disease.
What can be tested?

- Metabolic substances (newborn screening – e.g. PKU)
- Proteins (IRT for CF screening)
- Chromosomes (Down’s Syndrome)
- DNA (Connexin 26)
Chromosome Abnormalities

Trisomy 21 (Down’s Syndrome)
DNA Testing

Normal Sequence

ATG GTG CCT CAG GAT

Mutated Sequence

ATG GTG CCT TAG GAT
Causes of Childhood Hearing Loss

- Environmental or Unknown Etiology
- Genetic
  - Syndromic
  - Nonsyndromic
    - Autosomal Recessive
    - Autosomal Dominant
    - Mitochondrial
  - ~50 Genes
  - Cx26
  - ~50 Genes
  - 100s of genes

~50 Genes
LMM Hearing Loss Test Volume and Yield

679 probands, 1289 tests, 19% positive, 8% inconclusive (mostly hets)

16% (20% with hets)

15% in Cx26 hets; 0.7% Cx26 Neg

Volume

Positive
Inconclusive
Negative

GJB2
DelGJB6
Mito
PDS
POU3F4
COCH
OTOF
MYO7A
USH2A
CLRN1
R245X

27%
0%
26%
23%
30%
11%

20% with hets

15% in Cx26 hets; 0.7% Cx26 Neg
<table>
<thead>
<tr>
<th>Usher Type</th>
<th>Locus</th>
<th>Gene</th>
<th>Relative Incidence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>USH1A</td>
<td>Retracted (6/9 families have MYO7A mutations)</td>
<td>MYO7A</td>
<td>39-55%</td>
</tr>
<tr>
<td>USH1B</td>
<td>11q13.5</td>
<td>MYO7A</td>
<td>39-55%</td>
</tr>
<tr>
<td>USH1C</td>
<td>11p15.1</td>
<td>USH1C</td>
<td>6-7%</td>
</tr>
<tr>
<td>USH1D</td>
<td>10q</td>
<td>CDH23</td>
<td>19-35%</td>
</tr>
<tr>
<td>USH1E</td>
<td>21q</td>
<td>unknown</td>
<td>Rare</td>
</tr>
<tr>
<td>USH1F</td>
<td>10q21.1</td>
<td>PCDH15</td>
<td>10-20%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(R245X in AJ)</td>
</tr>
<tr>
<td>USH1G</td>
<td>17q24-25</td>
<td>SANS</td>
<td>7%</td>
</tr>
<tr>
<td>USH2A</td>
<td>1q41</td>
<td>USH2A</td>
<td>80%</td>
</tr>
<tr>
<td>USH2B</td>
<td>Retracted</td>
<td>USH2A</td>
<td>80%</td>
</tr>
<tr>
<td>USH2C</td>
<td>5q14.3-q21.3</td>
<td>VLGR1</td>
<td>15%</td>
</tr>
<tr>
<td>USH2D</td>
<td>9q32</td>
<td>WHRN</td>
<td>5%</td>
</tr>
<tr>
<td>USH3</td>
<td>3q21-q25</td>
<td>USH3</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Relative incidences from Usher I/II GeneReviews
The longer the gene the more difficult and expensive to develop and perform a genetic test.
OtoChip for Hearing Loss and Usher Syndrome

Nonsyndromic

Syndromic

Cx26 Testing

Appropriate Gene(s)

Aminoglycosides

Family Hx

No Family Hx

Mitochondrial

X-linked

Dominant

Recessive

Mitochondrial

12S rRNA
tRNA ser

X-linked

POU3F4

POU4F3

MYO6

ACTG1

DSPP

TECTA

EYA4

MYO7A

COL11A2

POU4F3

TMC1

MYO1A

WFS1

GJB3

KCNQ4

GJB6

DFNA5

MYH9

COCH

TFCP2L3

19 genes - 430 amplicons

~70,000 bases

Usher Syndrome

MYO7A

SANS

USH1C

USH2A

CDH23

VLGR1

PCDH15

USH3

MYO7A

MYO15

PDS

OTOF

TMPRSS3

TECTA

GJB6

CLDN14

TMC1

MYO1A

DIAPH1

WFS1

GJB3

KCNQ4

GJB6

DFNA5

MYH9

COCH

TFCP2L3

TMIE

CDH23

USH1C

OTOA

MYO3A

PCDH15

WHRN

ESPN

MYO6

PRES

JLNS

KCNE1

KCNQ1
ERG and other ophthalmological exams – may not be positive until adolescence

Vestibular assessment (delayed motor milestones, VEMP, minimized rotation testing, caloric, rotary chair) – test methods are age dependent and not diagnostic for USH1 (not useful for USH2)

- Teschner 2007: 16.2% of deaf children had absent vestibular responses from a new “minimized rotation” test and 50% of them had abnormal ERGs

Genetic testing: not age dependent

- In some cases, may not have conclusive distinction between syndromic vs nonsyndromic prediction if performed early
<table>
<thead>
<tr>
<th>Test Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJB2/GJB6del</td>
<td>$400</td>
</tr>
<tr>
<td>Mito Panel</td>
<td>$350</td>
</tr>
<tr>
<td>SLC26A4</td>
<td>$1,100</td>
</tr>
<tr>
<td>OTOF</td>
<td>$1,500</td>
</tr>
<tr>
<td>MYO6</td>
<td>$1,500</td>
</tr>
<tr>
<td>TMC1</td>
<td>$1,100</td>
</tr>
<tr>
<td>TMIE</td>
<td>$700</td>
</tr>
<tr>
<td>TMPRSS3</td>
<td>$925</td>
</tr>
<tr>
<td>MYO7A</td>
<td>$1,500</td>
</tr>
<tr>
<td>USH1C</td>
<td>$1,500</td>
</tr>
<tr>
<td>USH1G</td>
<td>$1,500</td>
</tr>
<tr>
<td>CDH23</td>
<td>$2,100</td>
</tr>
<tr>
<td>PCDH15</td>
<td>$1,500</td>
</tr>
<tr>
<td>USH2A</td>
<td>$1,700</td>
</tr>
<tr>
<td>GPR98*</td>
<td>$925</td>
</tr>
<tr>
<td>DFNB31</td>
<td>$1,100</td>
</tr>
<tr>
<td>CLRN1</td>
<td>$650</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$11,675</strong></td>
</tr>
</tbody>
</table>

**Total for OtoChip**

- GJB2/GJB6del: $400
- OtoChip: $3,800

**Total Cost**: $19,250
Clinical Usher Tests at the LMM

**USHER SYNDROME**

- **OtoChip™ Test for Hearing Loss and Usher Syndrome (19 Genes Sequenced)**
  - $3,800 Imp-OtoA

**ETHNICITY BASED TESTING**

- **Ashkenazi Jewish Panel for Hearing Loss and Usher Syndrome**
  - (167delT & 35delG in GJB2, GJB6-D13S1830 Deletion, R245X in PCDH15, N48K in CLRN1)
  - $600 Imp-AJHLAv2-a

- **Acadian/French Canadian Usher Panel (216G->A in USH1C and 4338_4339delICT in USH2A)**
  - $400 Imp-USH2A-km

- **Finnish Common Mutation for Usher Syndrome (Y176X in CLRN1)**
  - $400 Imp-CLRN1-km

**FAMILIAR MUTATION TESTING**

- **Familial Mutation Test** (Indicate gene, mutation, and proband information (1st person tested) below)
  - Gene_______________ Mutation___________________________ Proband (1st tested)___________________________
  - LMM Accession #: PM___________________________ Relationship to proband___________________________
  - $400

**USHER SYNDROME** (Relative Contribution Per Type) (* Also Associated with Nonsyndromic HL)

- **MYOT A (USH1B) Gene Sequencing Test (39-55%)**
  - $1,500 Imp-MYOTA-a

- **USH1C Gene Sequencing Test (6-7%)**
  - $1,500 Imp-USH1C-a

- **CDH23 (USH1D) Gene Sequencing Test (19-35%)**
  - $2,100 Imp-CDH23-a

- **PCDH15 (USH1F) Gene Sequencing Test (10-20%)**
  - $1,500 Imp-PCDH15-a

- **USH1G (SANS) Gene Sequencing Test (7%)**
  - $700 Imp-USH1G-a

- **USH2A Gene Sequencing Test (80%)**
  - $1,700 Imp-USH2A-a

- **GPR98 (VLGR1/USH2C) Gene Sequencing Test (15%)**
  - $2,700 Imp-GPR98-a

- **DFNB31 (WHRN/USH2D) Gene Sequencing Test (5%)**
  - $1,100 Imp-DFNB31-a

- **CLRN1 (USH3A) Gene Sequencing Test (100%)**
  - $650 Imp-CLRN1-a
Comparison to Common Mutation Panels

<table>
<thead>
<tr>
<th>Dx</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSNHL</td>
<td>E166fs-MYO7A</td>
<td>H1109fs-MYO7A</td>
</tr>
<tr>
<td>NSNHL</td>
<td>C652fs-MYO7A</td>
<td>C652fs-MYO7A</td>
</tr>
<tr>
<td>NSNHL</td>
<td>R1746Q-CDH23</td>
<td>D2148N-CDH23</td>
</tr>
<tr>
<td>NSNHL</td>
<td>C1447fs-USH2A</td>
<td>P2811T-USH2A</td>
</tr>
<tr>
<td>NSNHL</td>
<td>E767fs-USH2A</td>
<td>Not detected</td>
</tr>
<tr>
<td>Usher</td>
<td>S211G-MYO7A</td>
<td>Q1178P-MYO7A</td>
</tr>
<tr>
<td>Usher</td>
<td>R147H-MYO7A</td>
<td>A1540V-MYO7A</td>
</tr>
<tr>
<td>Usher</td>
<td>R1232fs-MYO7A</td>
<td>R1232fs-MYO7A</td>
</tr>
<tr>
<td>Usher</td>
<td>Q1798X-MYO7A</td>
<td>G519fs-MYO7A</td>
</tr>
<tr>
<td>Usher</td>
<td>R1861fs-MYO7A</td>
<td>Q234fs-MYO7A</td>
</tr>
<tr>
<td>Usher</td>
<td>Q2138fs-CDH23</td>
<td>Deletion</td>
</tr>
<tr>
<td>Usher</td>
<td>E767fs-USH2A</td>
<td>3158-6A&gt;G-USH2A</td>
</tr>
<tr>
<td>Usher</td>
<td>W2994X-USH2A</td>
<td>W2133X-USH2A</td>
</tr>
</tbody>
</table>

5/13 (38%) Usher gene cases would be hets by Asper array

4/13 (31%) hets by Carver test

Given 50% detection of OtoChip, implies overall detection of Carver and Asper arrays = 15-19%

Not on Asper or Carver
Not on Carver
OtoGenome Test

75 genes for nonsyndromic hearing loss, Usher syndrome and a few other syndromic genes that can mimic NSNHL early on

Testing using next generation sequencing (Illumina HiSeq)
OtoChip Results – 175 Cases Tested

The OtoChip detects a clear etiology in 14% of nonsyndromic SNHL cases and 46% of possible Usher syndrome cases.
9/132 (7%) of early childhood NSNHL cases, negative for Cx26, tested positive for an Usher gene mutation.

With a 46% detection rate for Usher, we would predict 14% will develop RP.

However, not all cases sent with a possible Usher phenotype probably had Usher so final number is somewhere in between:

- 7-14% of Cx26 negative cases have Usher
- OR
- 6-11% of all early SNHL cases have Usher
Gene Distribution of Positive OtoChip Cases

Number of Cases

GJB2  SLC26A4  OTOF  Mito  TMPRSS3  MYO6  TMIE  TMC1  MYO7A  USH1C  CDH23  PCDH15  USH1G  USH2A  GPR98  DFNB31  CLRN1

NSNHL Genes  Usher Genes
Mutations in Usher Type 1 genes may not cause an Usher Type 1 phenotype.
# Usher Syndrome

<table>
<thead>
<tr>
<th>Type</th>
<th>Hearing Loss</th>
<th>Vestibular System</th>
<th>Retinitis Pigmentosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>Congenital profound</td>
<td>Congenital balance problems</td>
<td>Onset pre-puberty</td>
</tr>
<tr>
<td>Type II</td>
<td>Congenital mild-severe sloping</td>
<td>Normal</td>
<td>Onset in teens-20s</td>
</tr>
<tr>
<td>Type III</td>
<td>Progressive later onset</td>
<td>Progressive balance problems</td>
<td>Variable onset</td>
</tr>
</tbody>
</table>
## Nonsyndromic Hearing Loss or RP due to Usher Gene Mutations

<table>
<thead>
<tr>
<th>Usher Type</th>
<th>Gene</th>
<th>Nonsyndromic Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>USH1B</td>
<td>MYO7A</td>
<td>DFNA11, DFNB2 (rare)</td>
</tr>
<tr>
<td>USH1C</td>
<td>USH1C</td>
<td>DFNB18 (mild mutations)</td>
</tr>
<tr>
<td>USH1D</td>
<td>CDH23</td>
<td>DFNB12 (mild mutations)</td>
</tr>
<tr>
<td>USH1F</td>
<td>PCDH15</td>
<td>DFNB23 (mild mutations)</td>
</tr>
<tr>
<td>USH1G</td>
<td>USH1G (SANS)</td>
<td>Not reported</td>
</tr>
<tr>
<td>USH2A</td>
<td>USH2A</td>
<td>Autosomal recessive RP (12% of arRP)</td>
</tr>
<tr>
<td>USH2C</td>
<td>VLGR1</td>
<td>Not reported</td>
</tr>
<tr>
<td>USH2D</td>
<td>DFNB31 (WHRN)</td>
<td>DFNB31 (short isoform mutations)</td>
</tr>
<tr>
<td>USH3A</td>
<td>CLRN1</td>
<td>Not reported</td>
</tr>
</tbody>
</table>
Why are there different clinical presentations for certain Usher genes?

Some variants are milder than others.

Some variants lead to full loss of the protein (e.g. full or partial gene deletions, nonsense, frameshift and splice variants as well as some missense variants (due to protein misfolding or mislocalization).

Other variants may leave the protein intact but modify it slightly (e.g. certain missense variants) – it is these variants that can lead to nonsyndromic presentations with Usher syndrome gene variants.

Sometimes clinical presentation is affected by modifiers. Modifiers can be genetic (variants in other genes) or environmental (exposures, lifestyle, etc).
Truncating Mutations such as Nonsense, Frameshift, Splicing and Large deletions Usually Lead to Complete Loss of a Protein
Missense Mutations Change Only One Amino Acid
Why is genetic testing useful?

It can detect Usher syndrome before eye disease is apparent.

It can clarify a diagnosis (not all hearing loss with retinal disease is Usher).

The type of mutation may predict disease severity.

Clinical trials may require genetic test confirmation or knowledge of specific gene involved.

Certain therapies may only work on certain types of mutations. Read-through therapies (e.g. PTC124) only work for nonsense mutations.

It can enable family member testing for carrier status or prenatal/preimplantation testing.
Methods of Gene Therapy

Regenerative Medicine. DHHS. Aug 2006./info/scireport/2006report.htm
Gene Therapy Factors to Consider

Method to get gene to cells in need
   Viral targeting vs. local injection to organ
   Easier to reach eye but injection could risk damage to retina

Timing of therapy (is disease congenital vs delayed onset) – easier to address eye disease in Usher than hearing loss

Size of gene (vectors can only hold so much DNA)
   Usher genes are very big!

Cells with foreign DNA may be targeted by the immune system for destruction (the viral vectors that carry the replacement gene encode other proteins to aid in cell entry and gene transfer)
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pcpgm.partners.org/lmm