The Genetics of Usher Syndrome

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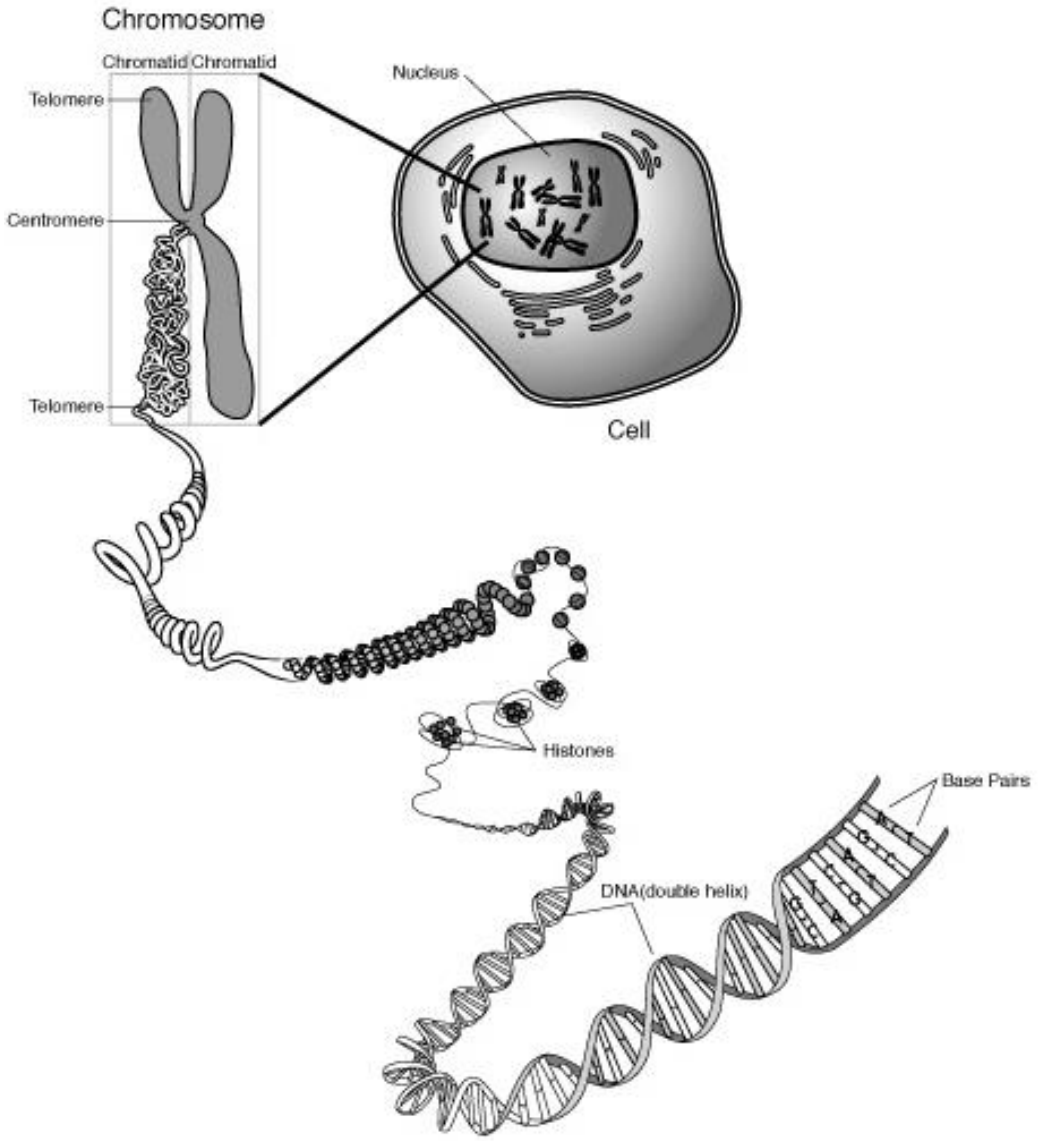
DNA is Highly Compacted into Chromosomes

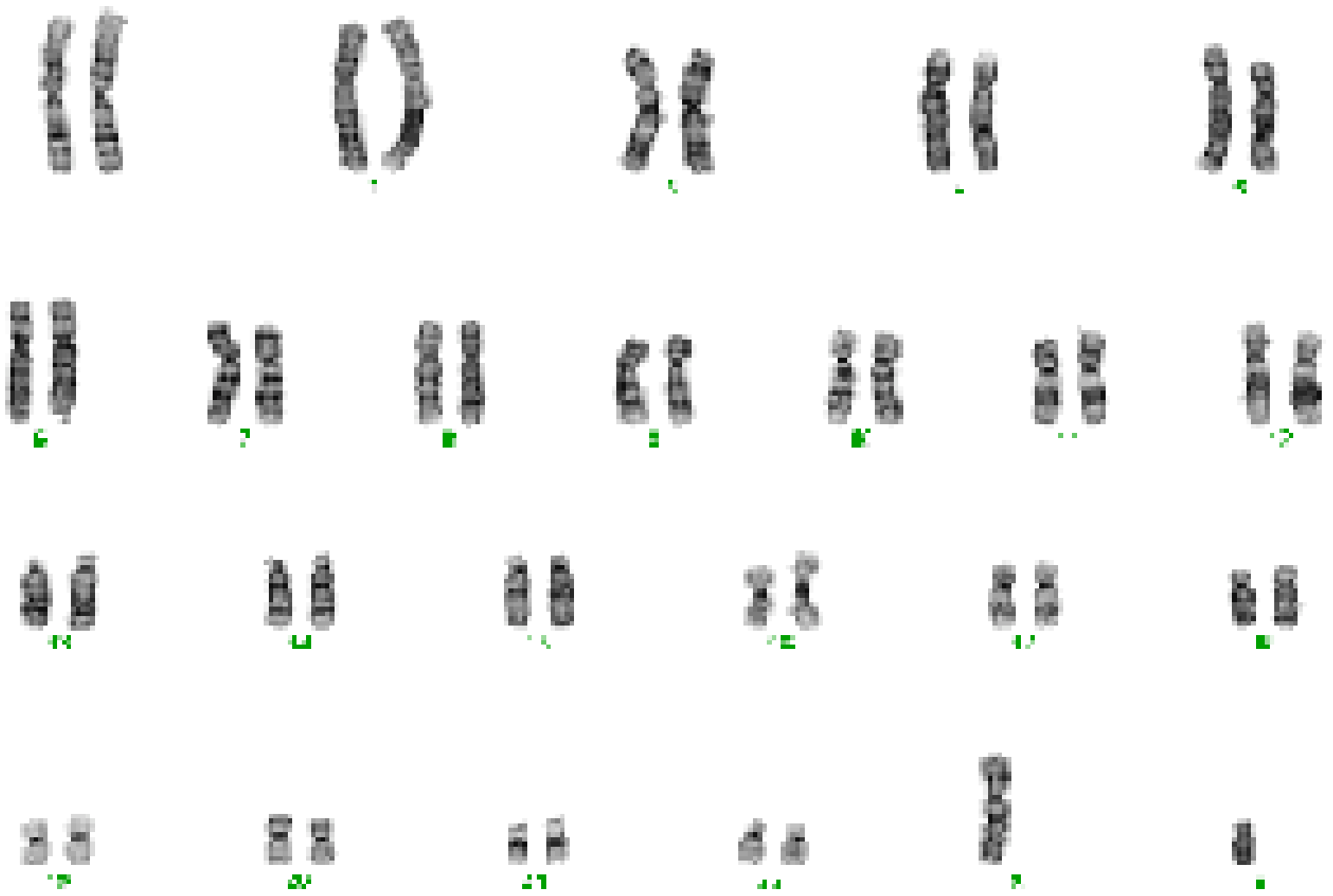


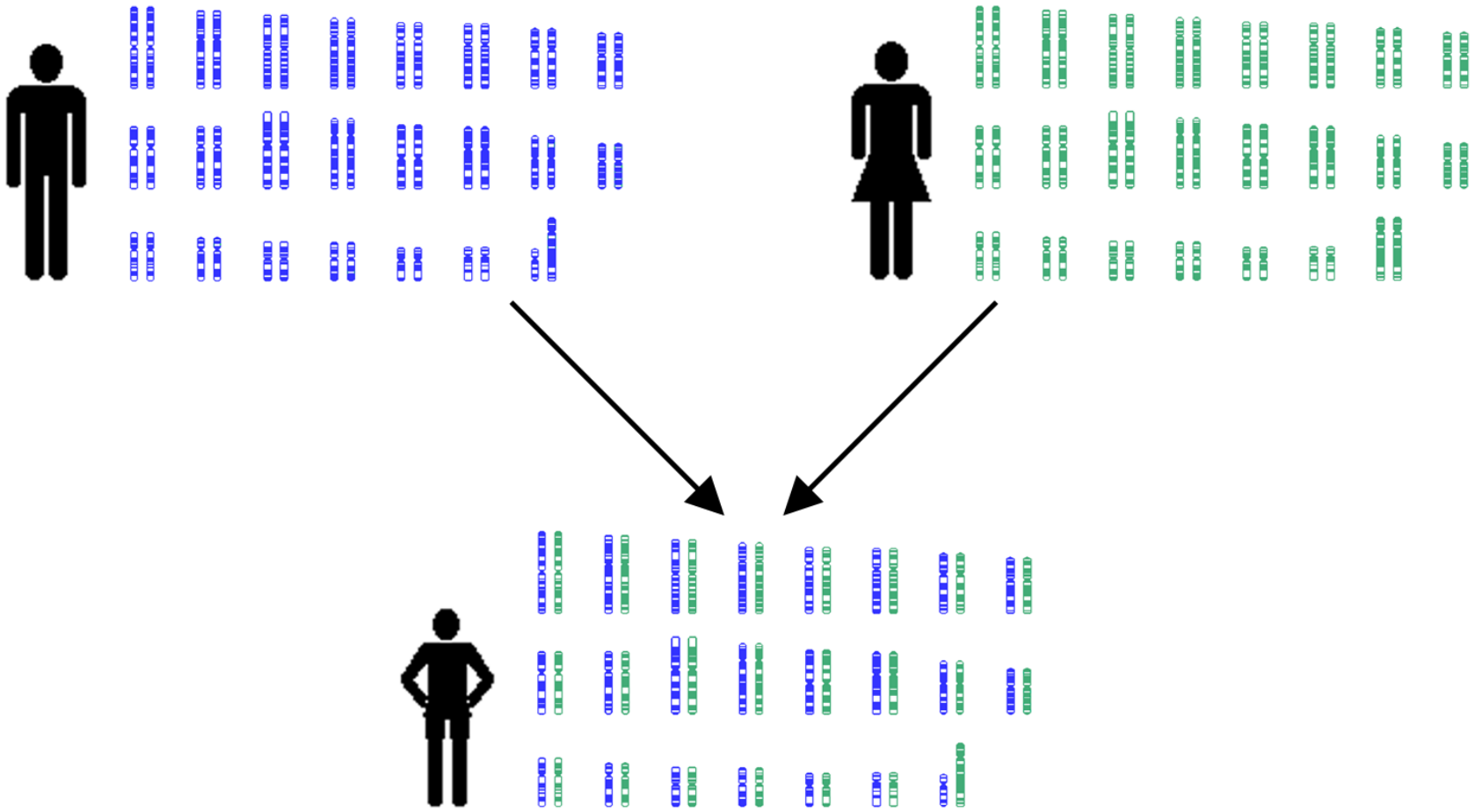
The DNA from one cell stretches 7.5 feet.

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

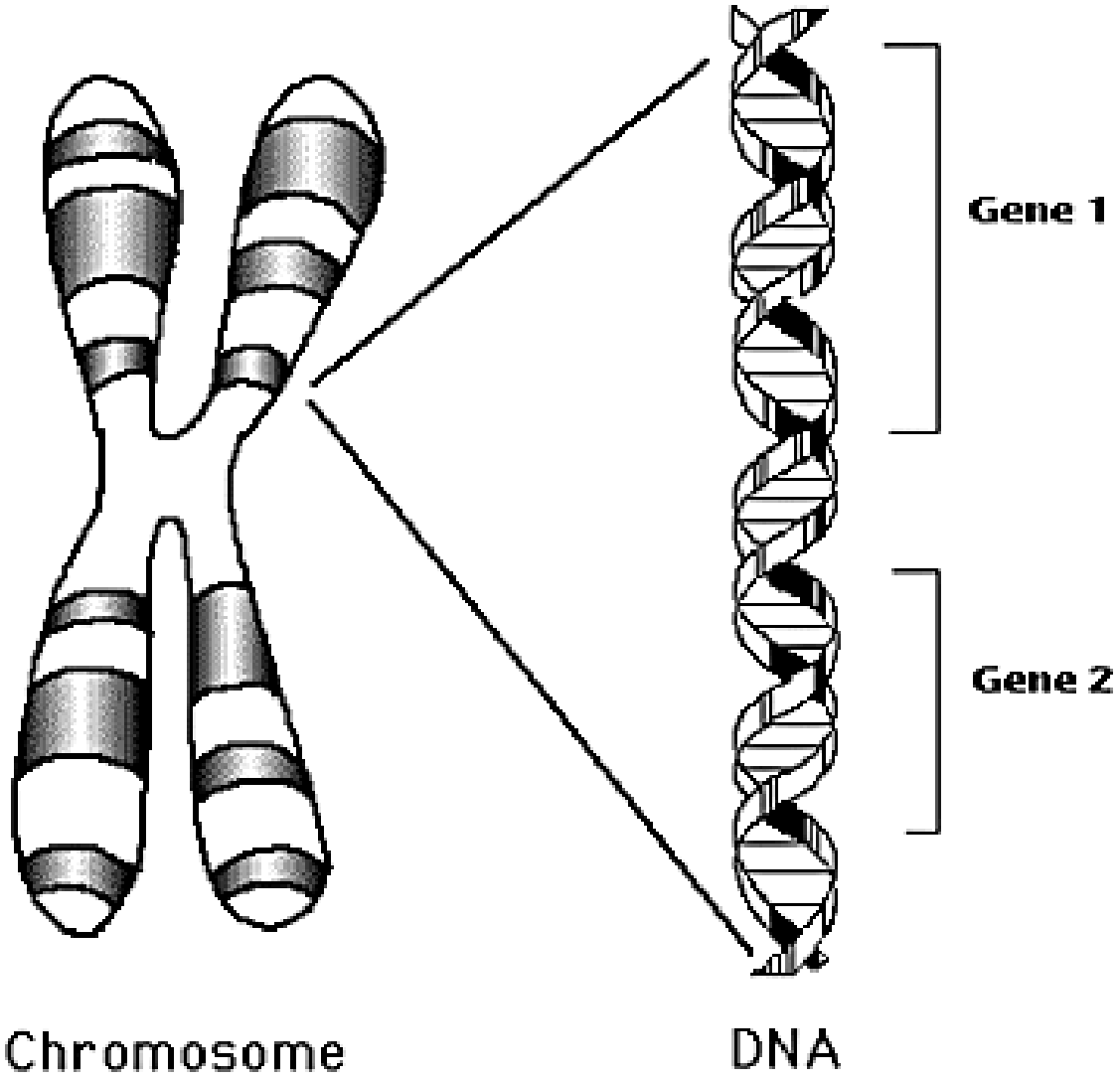
http://www.accessexcellence.org/AB/GG/

Human Karyotype





We inherit two copies of each chromosome (and each gene), one from each parent.



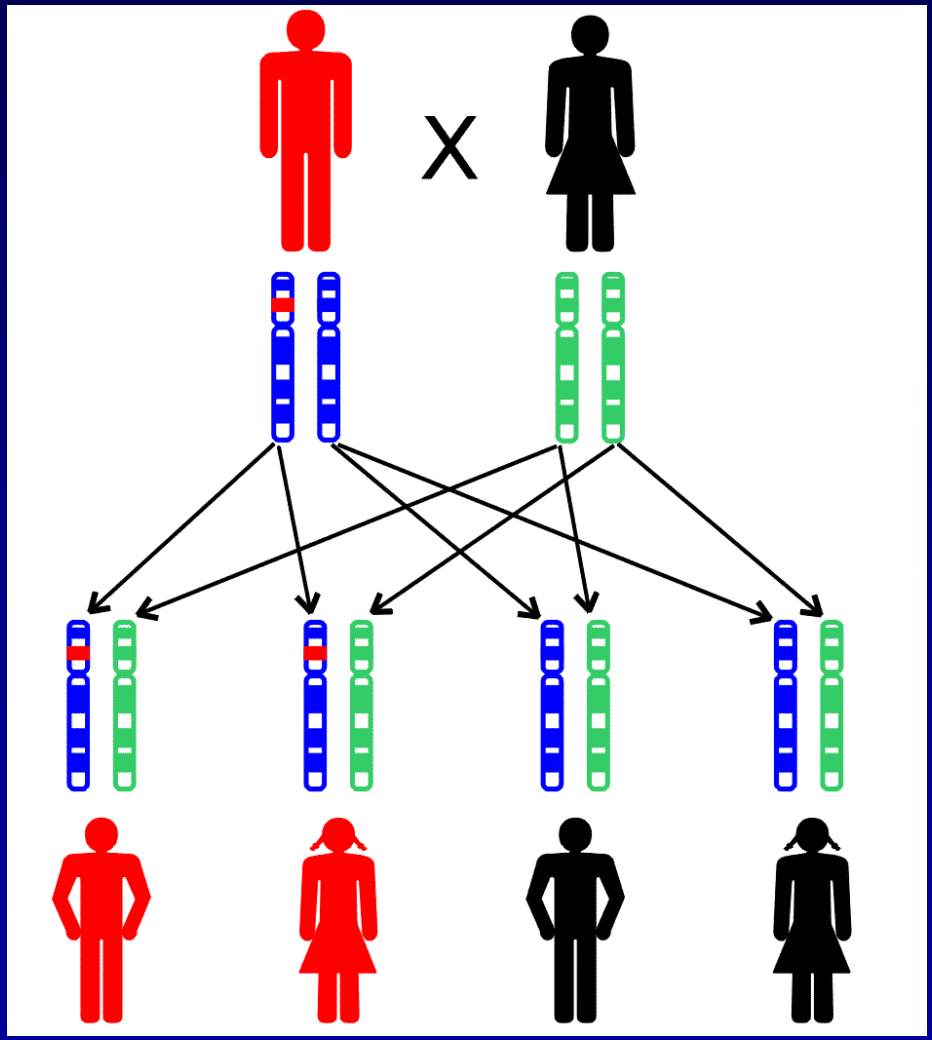
Genes

http://www.accessexcellence.org/AB/GG/

How are genetic disorders inherited?

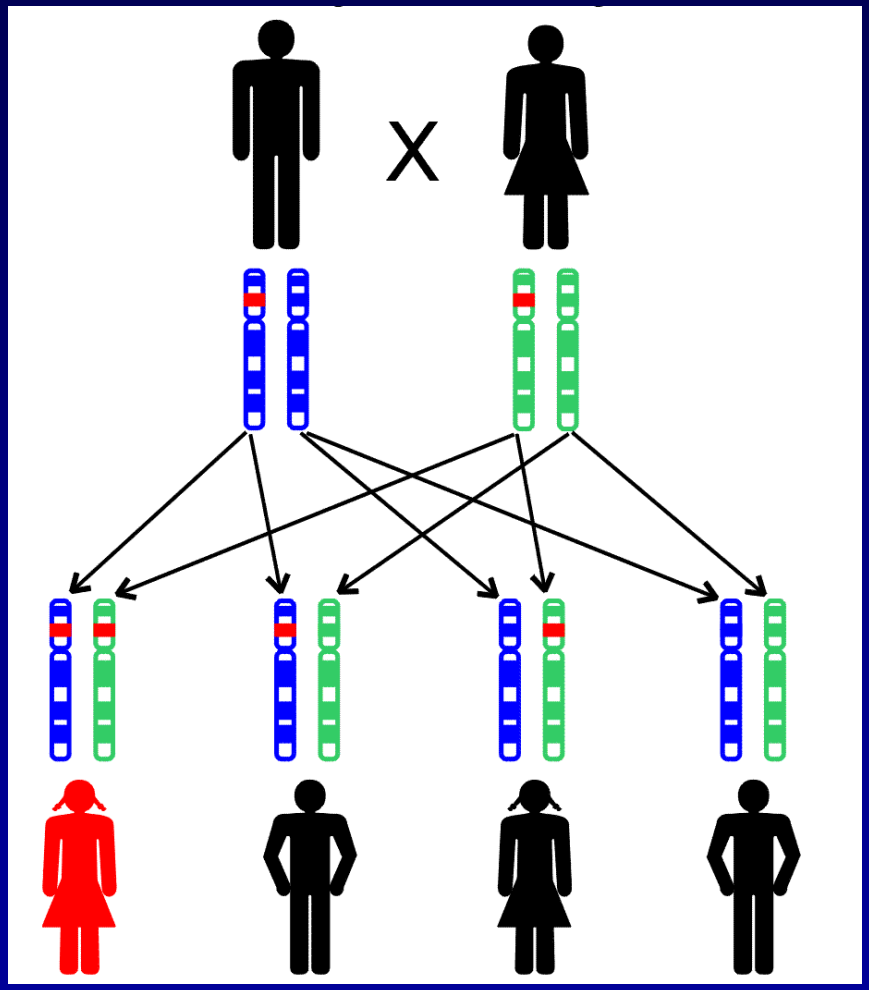
Autosomal Dominant Mutations

- 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

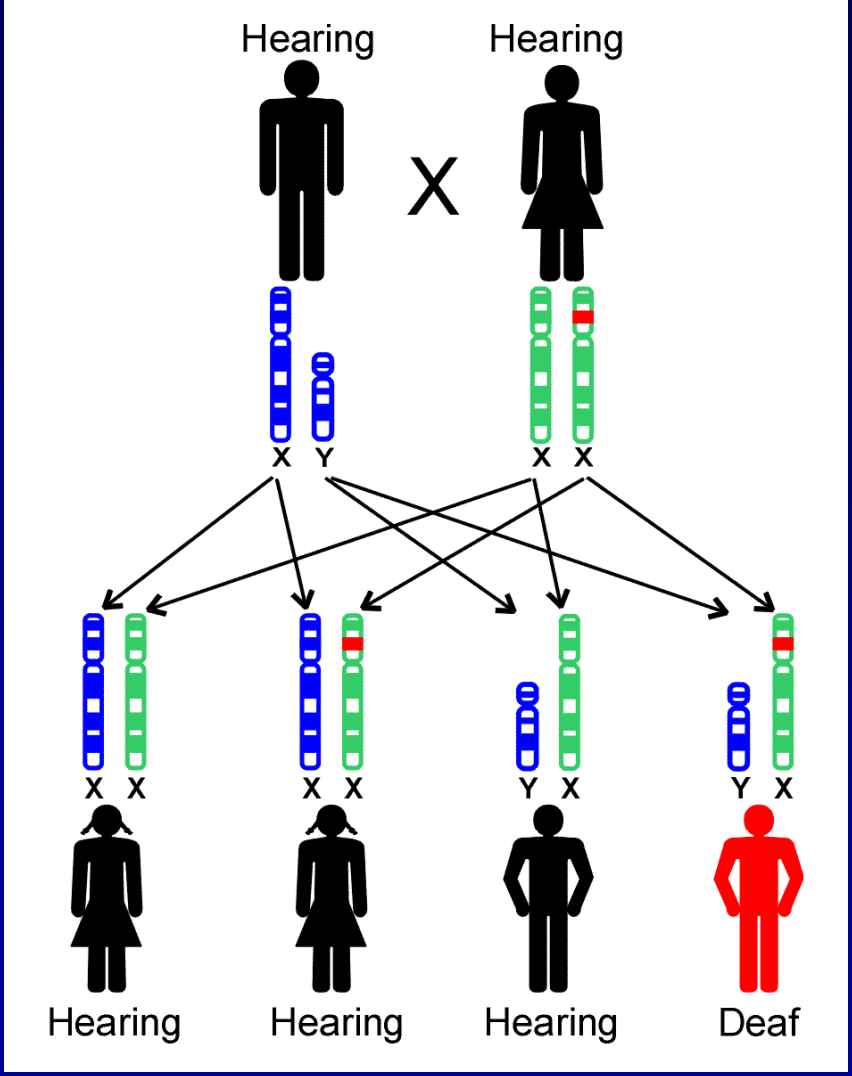
- For some diseases, both copies of a gene must be mutated to get the disease.
- Often, there is no family history of the disease.
- Each child will have a 25% chance of getting the disease.



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

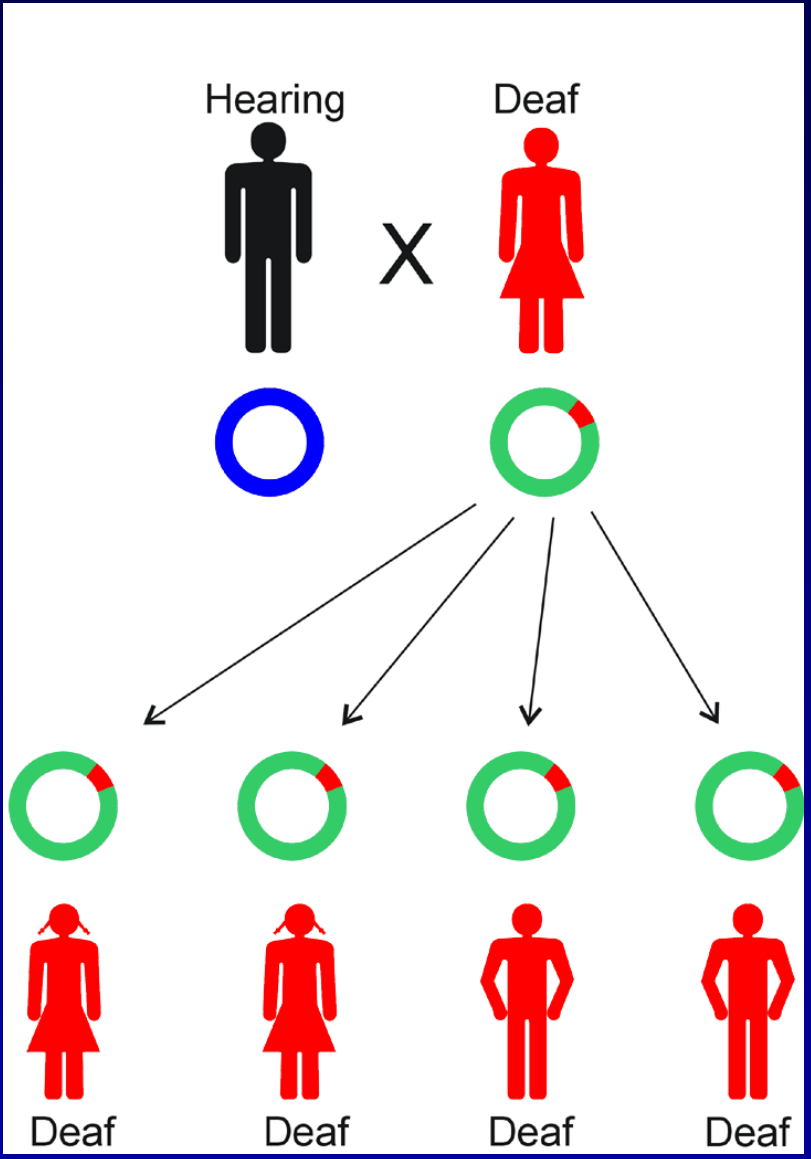
X-Linked Recessive Mutations

- 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.



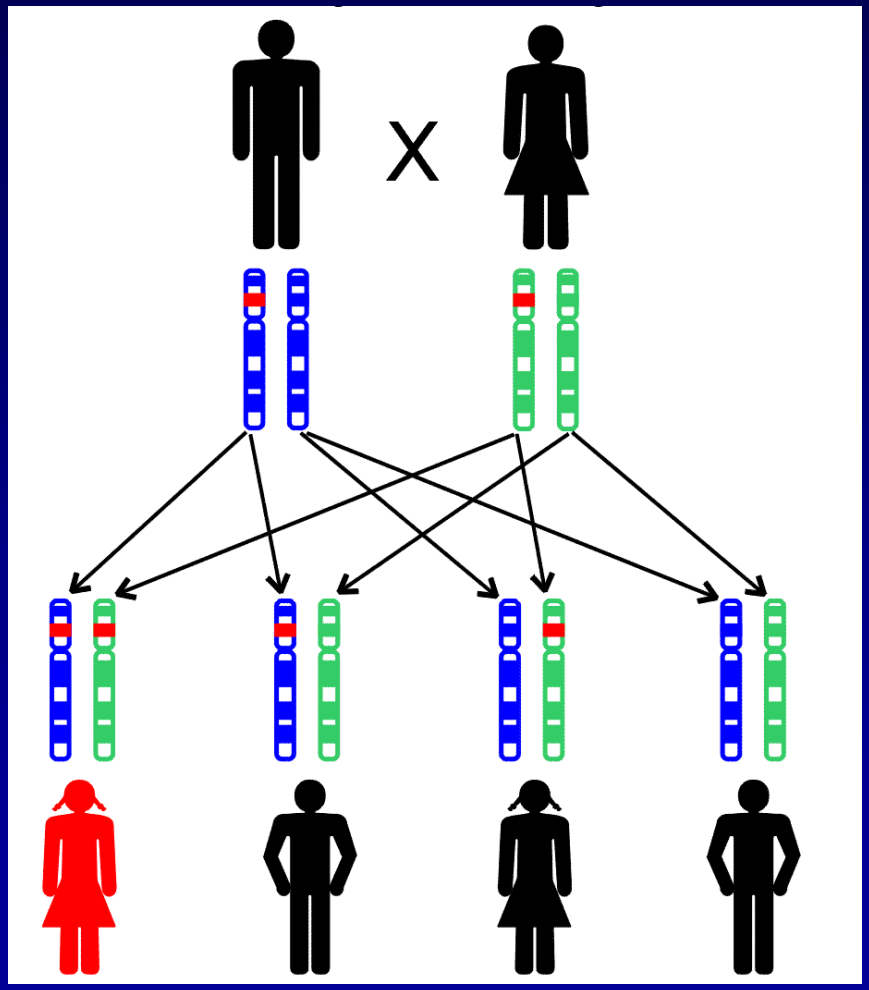
Mitochondrial Mutations

- 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.

Genetic Testing

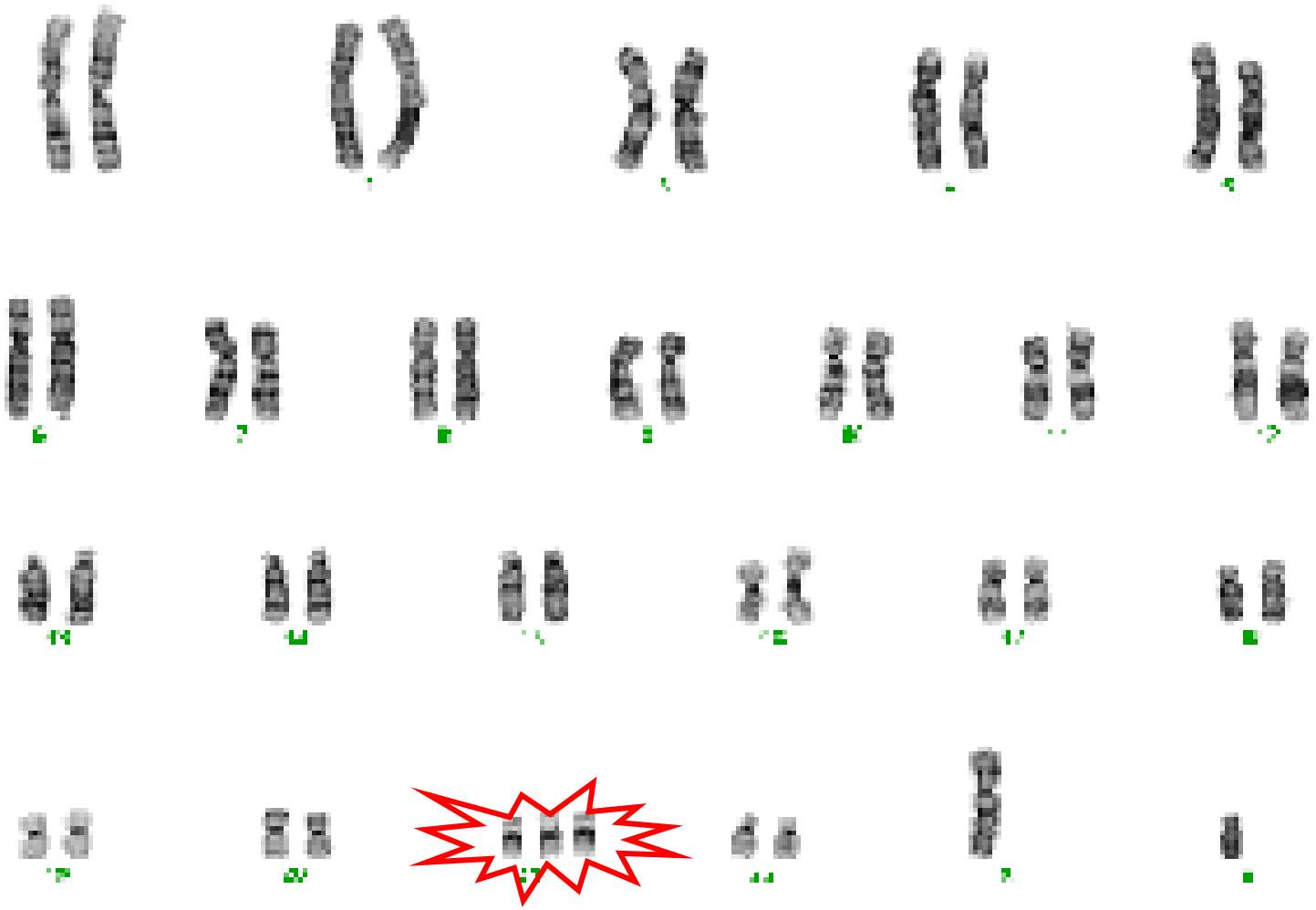
What is it?

Determine whether you have a variant in a gene which can result in a disease

What can be tested?

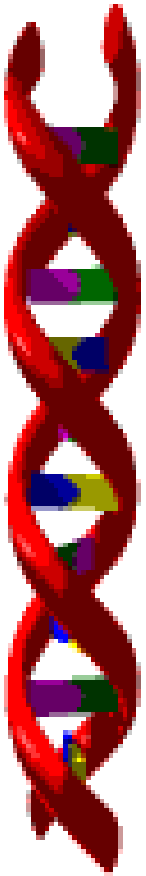
- Metabolic substances (newborn screening – e.g. PKU)
- Proteins (IRT for CF screening)
- Chromosomes (Down's Syndrome)
- DNA (Connexin 26)

Chromosome Abnormalities



Trisomy 21 (Down's Syndrome)

DNA Testing



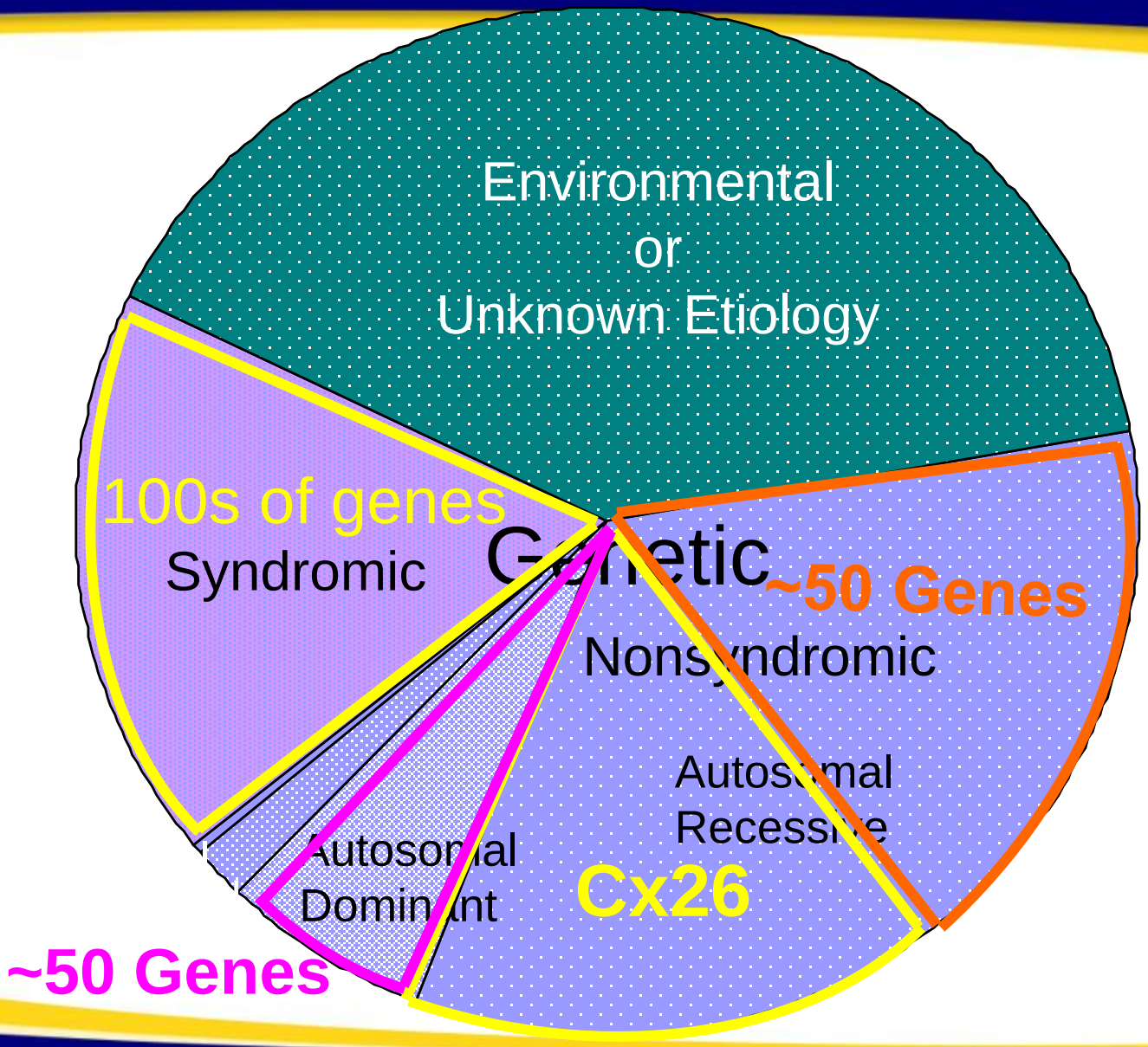
Normal Sequence

ATG GTG CCT CAG GAT

Mutated Sequence

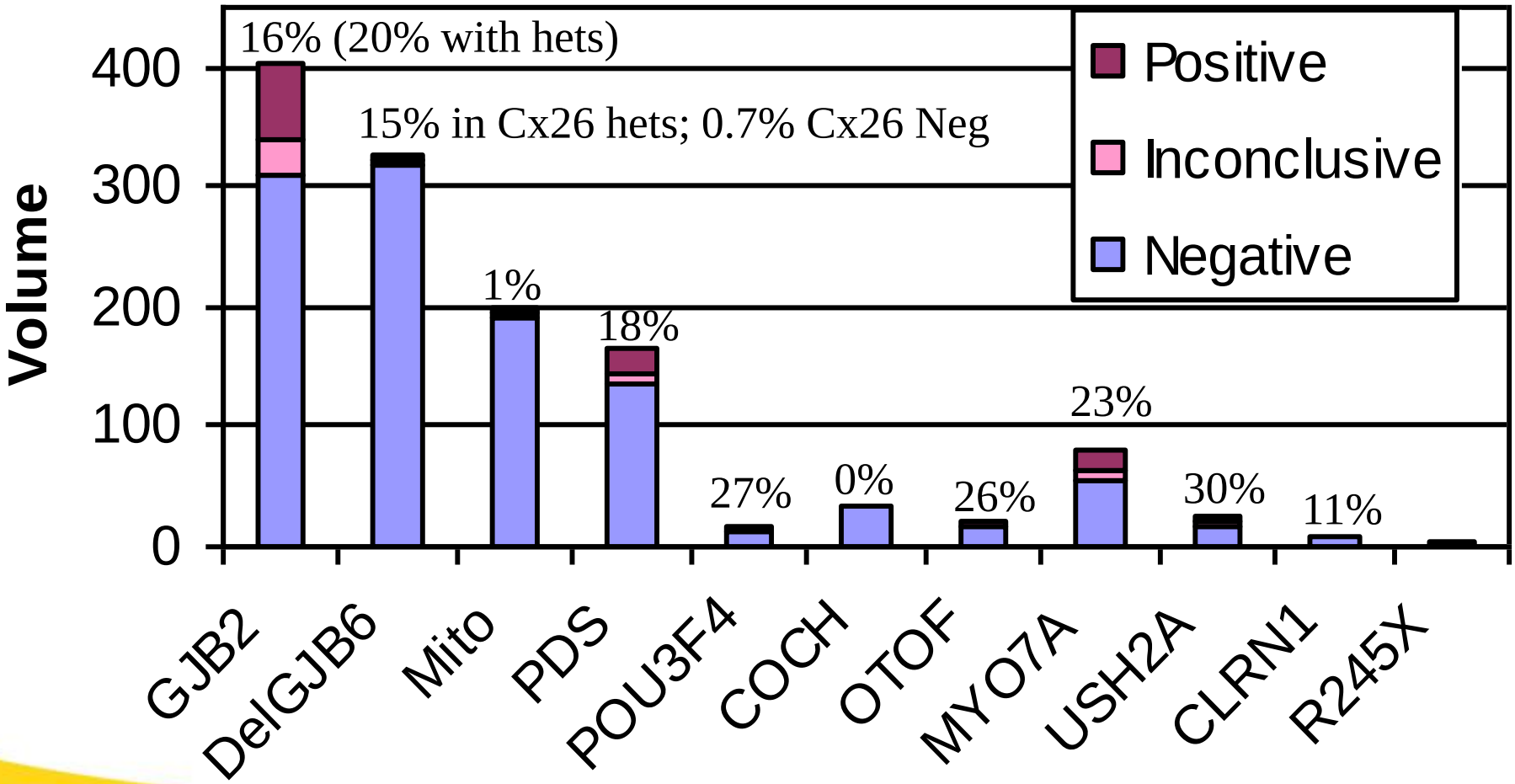
ATG GTG CCT TAG GAT

Causes of Childhood Hearing Loss



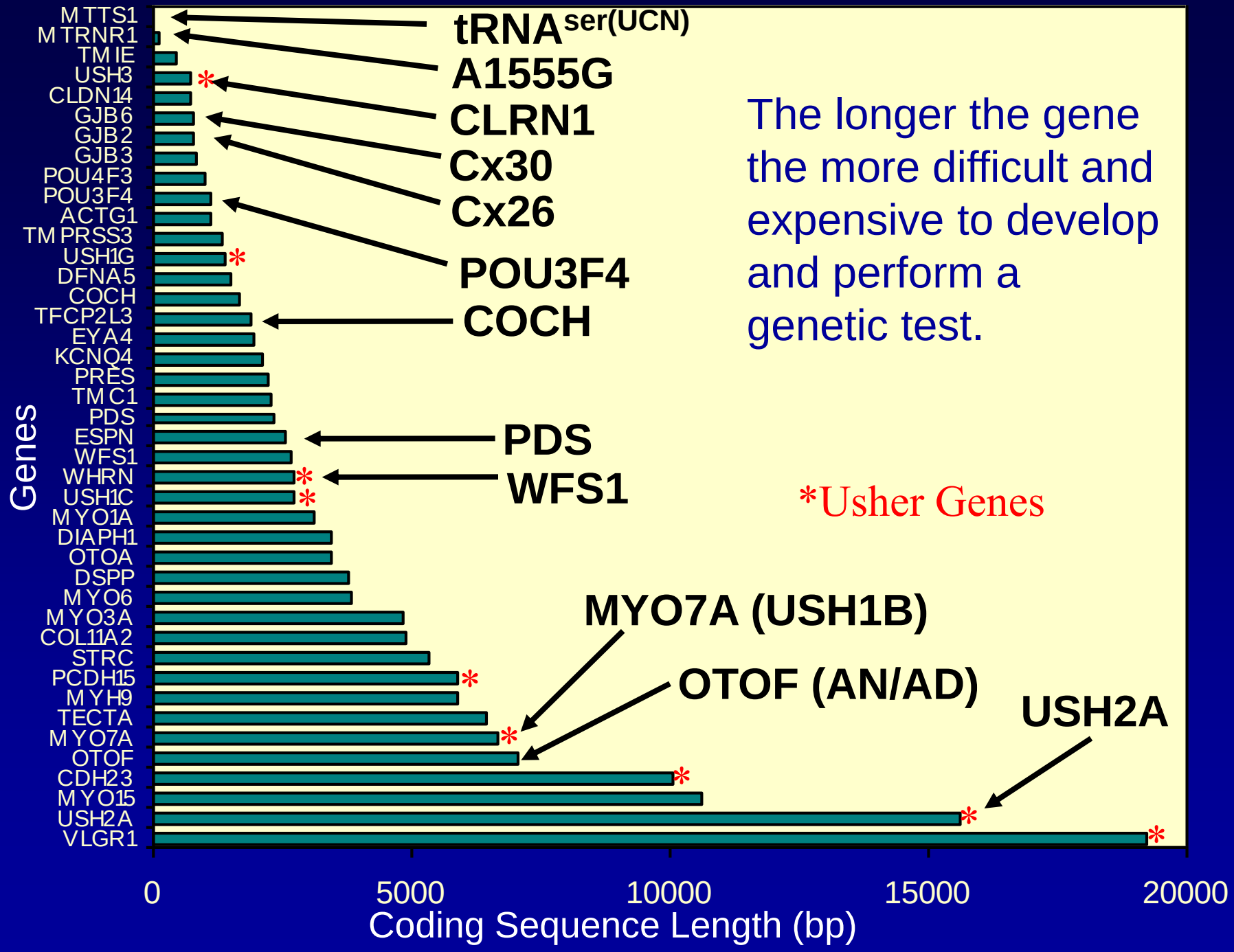
LMM Hearing Loss Test Volume and Yield

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



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

*Relative incidences from Usher I/II GeneReviews


MTTS1
MTRNR1
TMIE
USH3
CLDN14
GJB6
GJB2
GJB3
POU4F3
POU3F4
ACTG1
TMPRSS3
USH1G
DFNA5
COCH
TFCP2L3
EYA4
KCNQ4
PRES
TMC1
PDS
ESPN
WFS1
WHRN
USH1C
MYO1A
DIAPH1
OTOA
DSPP
MYO6
MYO3A
COL11A2
STRC
PCDH15
MYH9
TECTA
MYO7A
OTOF
CDH23
MYO15
USH2A
VLGR1
Genes
0
5000
10000
15000
20000
Coding Sequence Length (bp)
tRNA ser(UCN)
A1555G
CLRN1
Cx30
Cx26
POU3F4
COCH
PDS
WFS1
MYO7A (USH1B)
OTOF (AN/AD)
USH2A
The longer the gene the more difficult and expensive to develop and perform a genetic test.
*Usher Genes

OtoChip for Hearing Loss and Usher Syndrome

Nonsyndromic

Cx26 Testing

Aminoglycosides

Mitochondrial
- 12S rRNA
- tRNA ser

X-linked
- POU3F4

Family Hx

Dominant
- MYO6
- ACTG1
- DSPP
- TECTA
- EYA4
- MYO7A
- COL11A2
- POU4F3
- TMC1
- MYO1A
- DIAPH1
- WFS1
- GJB3
- KCNQ4
- GJB6
- DFNA5
- MYH9
- COCH
- TFCP2L3

Recessive
- MYO7A
- MYO15
- PDS
- OTOF
- TMPRSS3
- TECTA
- GJB6
- CLDN14
- TMC1
- STRC
- TMIE
- CDH23
- USH1C
- OTOA
- MYO3A
- PCDH15
- WHRN
- ESPN
- MYO6
- PRES

No Family Hx

Syndromic

Appropriate Gene(s)

Usher Syndrome
- MYO7A
- SANS
- USH1C
- USH2A
- CDH23
- VLGR1
- PCDH15
- USH3

JLNS
- KCNE1
- KCNQ1

19 genes - 430 amplicons
~70,000 bases

Usher Syndrome Early Diagnosis

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

OtoChip Test Cost Comparison

GJB2/GJB6del	$400
Mito Panel	$350
SLC26A4	$1,100
OTOF	$1,500
MYO6	$1,500
TMC1	$1,100
TMIE	$700
TMPRSS3	$925
MYO7A	$1,500
USH1C	$1,500
CDH23	$2,100
PCDH15	$1,500
USH1G	$700
USH2A	$1,700
GPR98*	$925
DFNB31	$1,100
CLRN1	$650
Total	**$19,250**

Usher	
MYO7A	$1,500
USH1C	$1,500
CDH23	$2,100
PCDH15	$1,500
USH1G	$700
USH2A	$1,700
GPR98*	$925
DFNB31	$1,100
CLRN1	$650
Total	**$11,675**

GJB2/GJB6del	$400
OtoChip	$3,800
Total	**$4,200**

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_4339delCT in *USH2A*)	$400 Imp-USH1C-km; Imp-USH2A-km
___ ☐ Finnish Common Mutation for Usher Syndrome (Y176X in *CLRN1*)	$400 Imp-CLRN1-km

FAMILIAL MUTATION TESTING $400

___ ☐ **Familial Mutation Test** (Indicate gene, mutation, and proband information (1st person tested) below)

Gene_____ Mutation_____ Proband (1st tested)_____

LMM Accession #: PM-_____ Relationship to proband _____

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

___ ☐ *+ *MYO7A* (*USH1B*) Gene Sequencing Test (39-55%)	Usher Type 1	$1,500 Imp-MYO7A-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%)	Usher Type 2	$1,700 Imp-USH2A-a
___ ☐ **GPR98* (*VLGR1/USH2C*) Gene Sequencing Test (15%)		$2,700 Imp-GRP98-a
___ ☐ *+ *DFNB31* (*WHRN/USH2D*) Gene Sequencing Test (5%)		$1,100 Imp-DFNB31-a
___ ☐ **CLRN1* (*USH3A*) Gene Sequencing Test (100%)	Usher Type 3	$650 Imp-CLRN1-a

Comparison to Common Mutation Panels

Dx	Allele 1	Allele 2
NSNHL	E166fs-*MYO7A*	H1109fs-*MYO7A*
NSNHL	C652fs-*MYO7A*	C652fs-*MYO7A*
NSNHL	R1746Q-*CDH23*	D2148N-*CDH23*
NSNHL	C1447fs-*USH2A*	P2811T -*USH2A*
NSNHL	E767fs-*USH2A*	Not detected
Usher	S211G-*MYO7A*	Q1178P-*MYO7A*
Usher	R147H-*MYO7A*	A1540V-*MYO7A*
Usher	R1232fs-*MYO7A*	R1232fs-*MYO7A*
Usher	Q1798X-*MYO7A*	G519fs-*MYO7A*
Usher	R1861fs-*MYO7A*	Q234fs-*MYO7A*
Usher	Q2138fs-*CDH23*	Deletion
Usher	E767fs-*USH2A*	3158-6A>G-*USH2A*
Usher	W2994X-*USH2A*	W2133X-*USH2A*

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

4/13 (31%) hets by Carver test

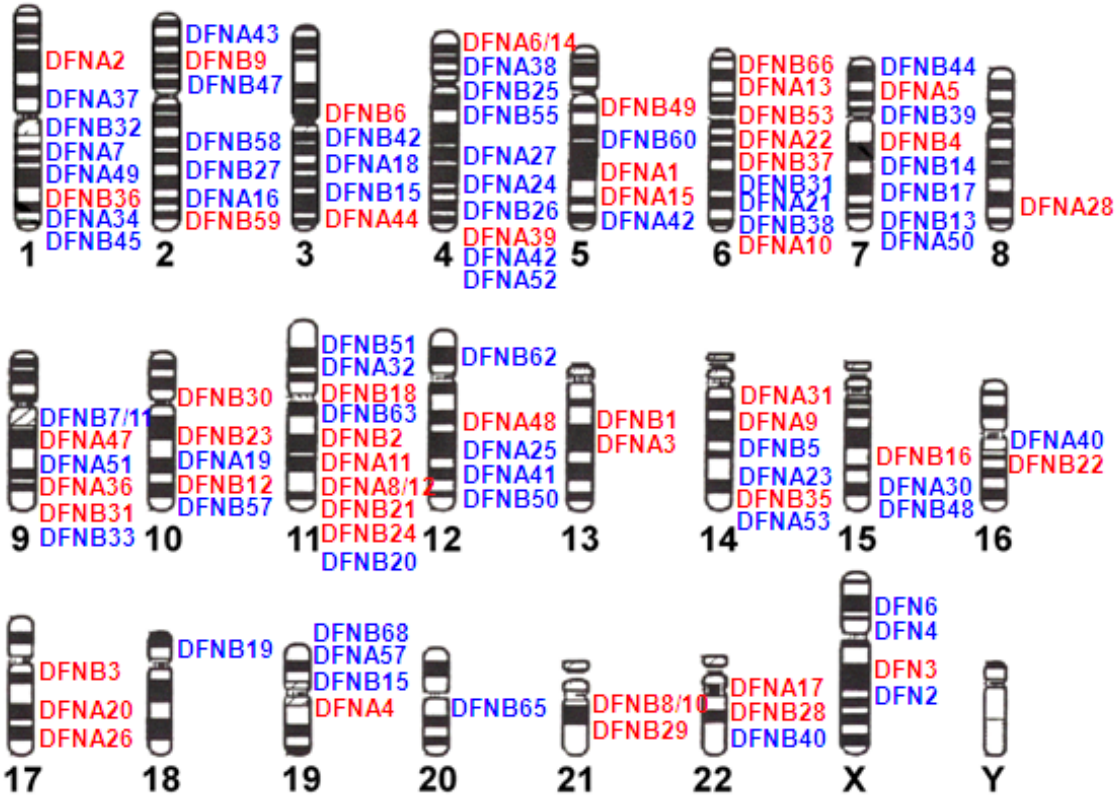
Given 50% detection of OtoChip, implies overall detection of Carver and Asper arrays = 15-19%

Not on Asper or Carver
Not on Carver

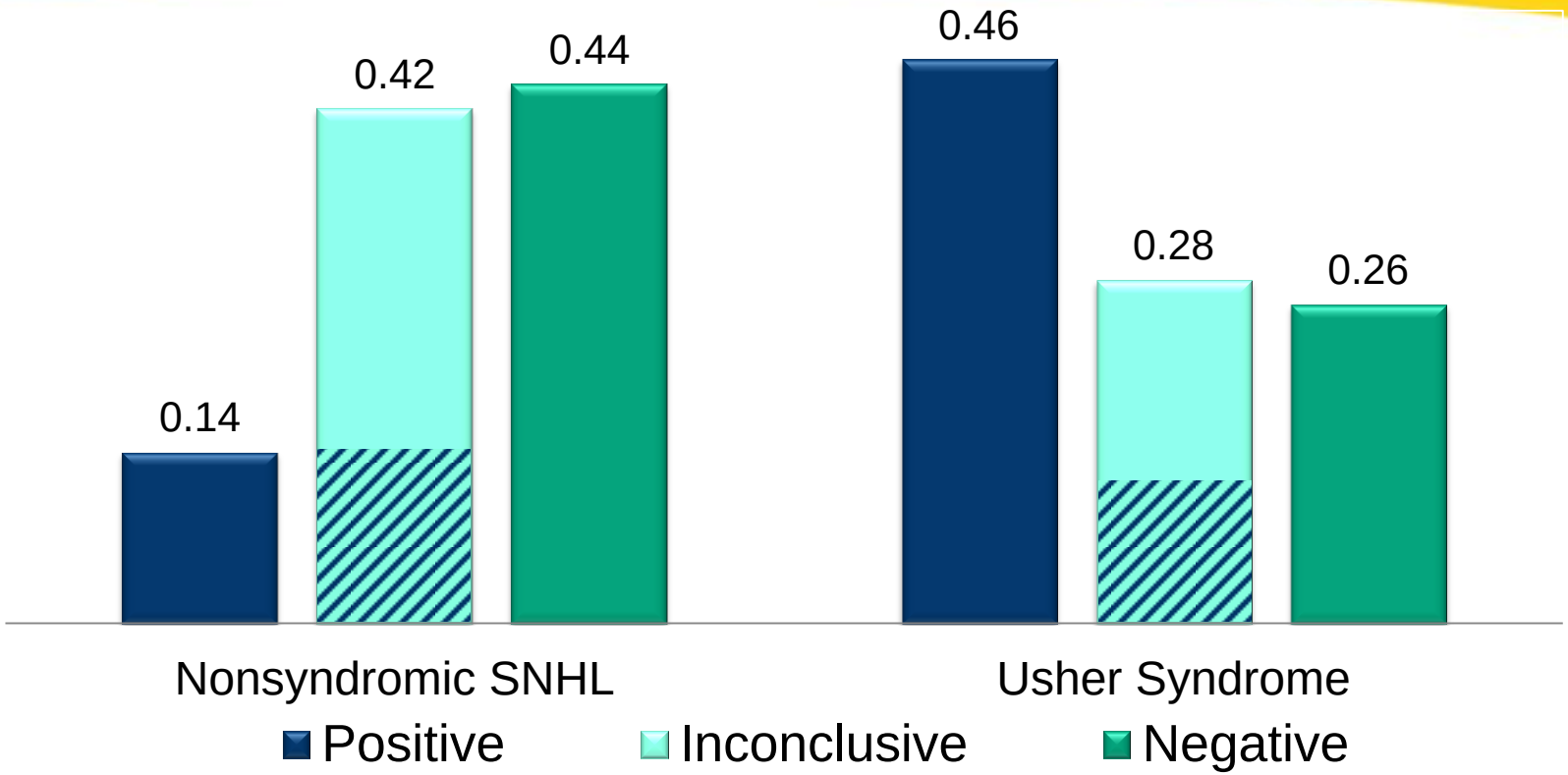
OtoGenome Test

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.

Rate of predicted RP development in NSNHL

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

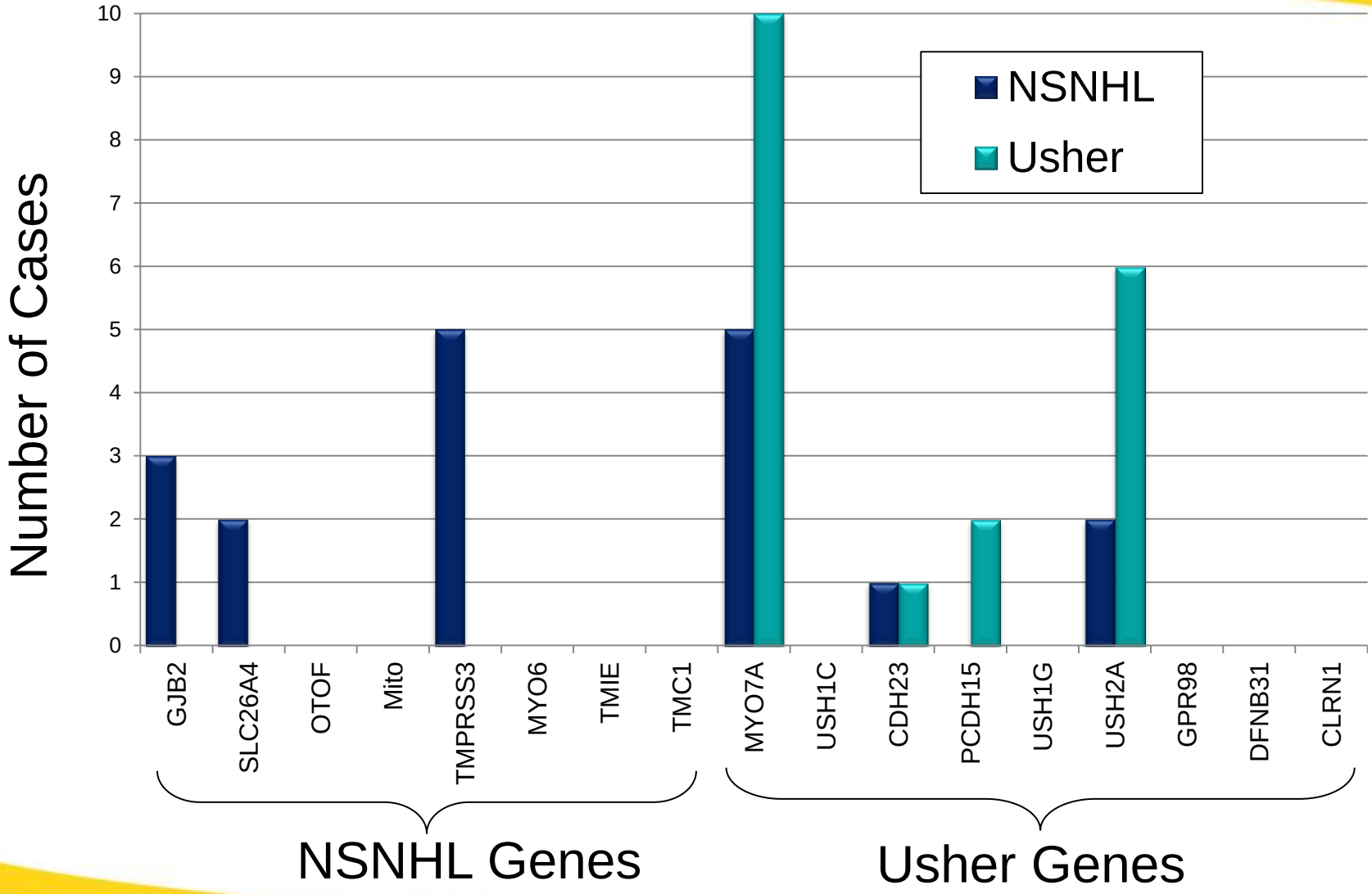
However, not all cases sent with a possible Usher phenotype probably had Usher so final number is somewhere in between:

7-14% of Cx26 negative cases have Usher

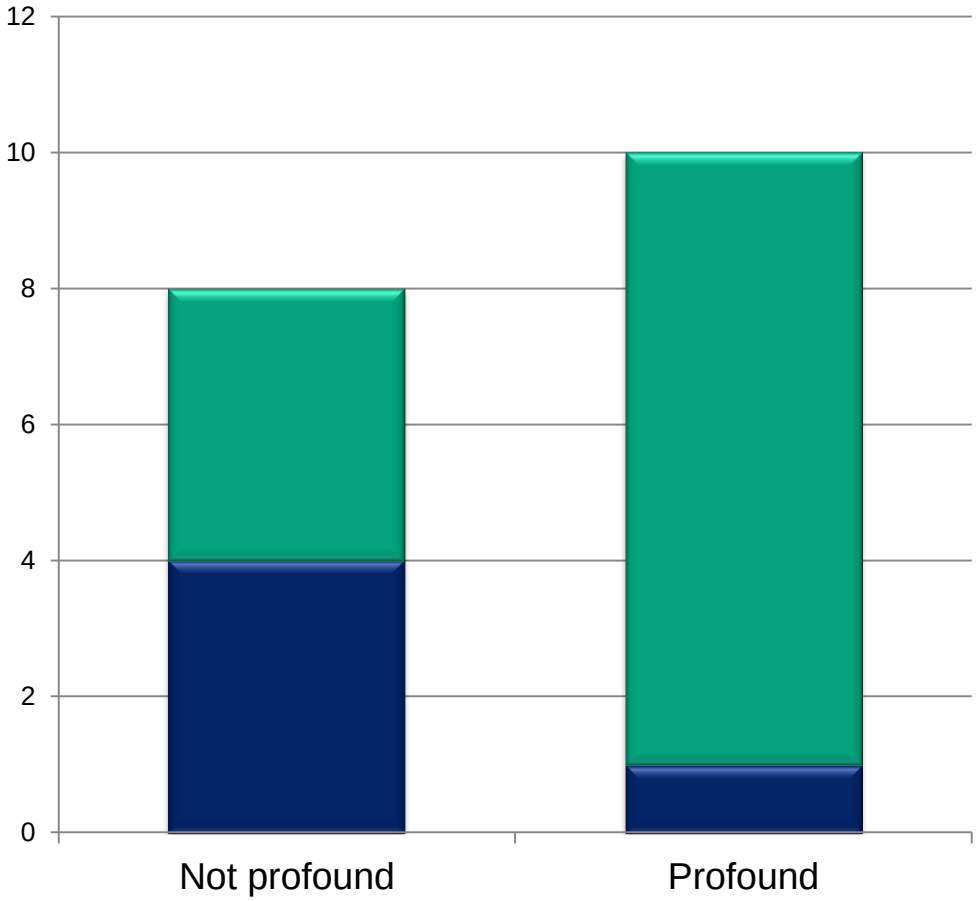
OR

6-11% of all early SNHL cases have Usher

Gene Distribution of Positive OtoChip Cases



Hearing Loss Severity with USH1 Gene Mutations



Mutations in Usher Type 1 genes may not cause an Usher Type 1 phenotype

Usher Syndrome

	Hearing Loss	Vestibular System	Retinitis Pigmentosa
Type I	Congenital profound	Congenital balance problems	Onset pre-puberty
Type II	Congenital mild-severe sloping	Normal	Onset in teens-20s
Type III	Progressive later onset	Progressive balance problems	Variable onset

Nonsyndromic Hearing Loss or RP due to Usher Gene Mutations

Usher Type	Gene	Nonsyndromic Form
USH1B	*MYO7A*	DFNA11, DFNB2 (rare)
USH1C	*USH1C*	DFNB18 (mild mutations)
USH1D	*CDH23*	DFNB12 (mild mutations)
USH1F	*PCDH15*	DFNB23 (mild mutations)
USH1G	*USH1G (SANS)*	Not reported
USH2A	*USH2A*	Autosomal recessive RP (12% of arRP)
USH2C	*VLGR1*	Not reported
USH2D	*DFNB31 (WHRN)*	DFNB31 (short isoform mutations)
USH3A	*CLRN1*	Not reported

Why are there different clinical presentations for certain Usher genes?

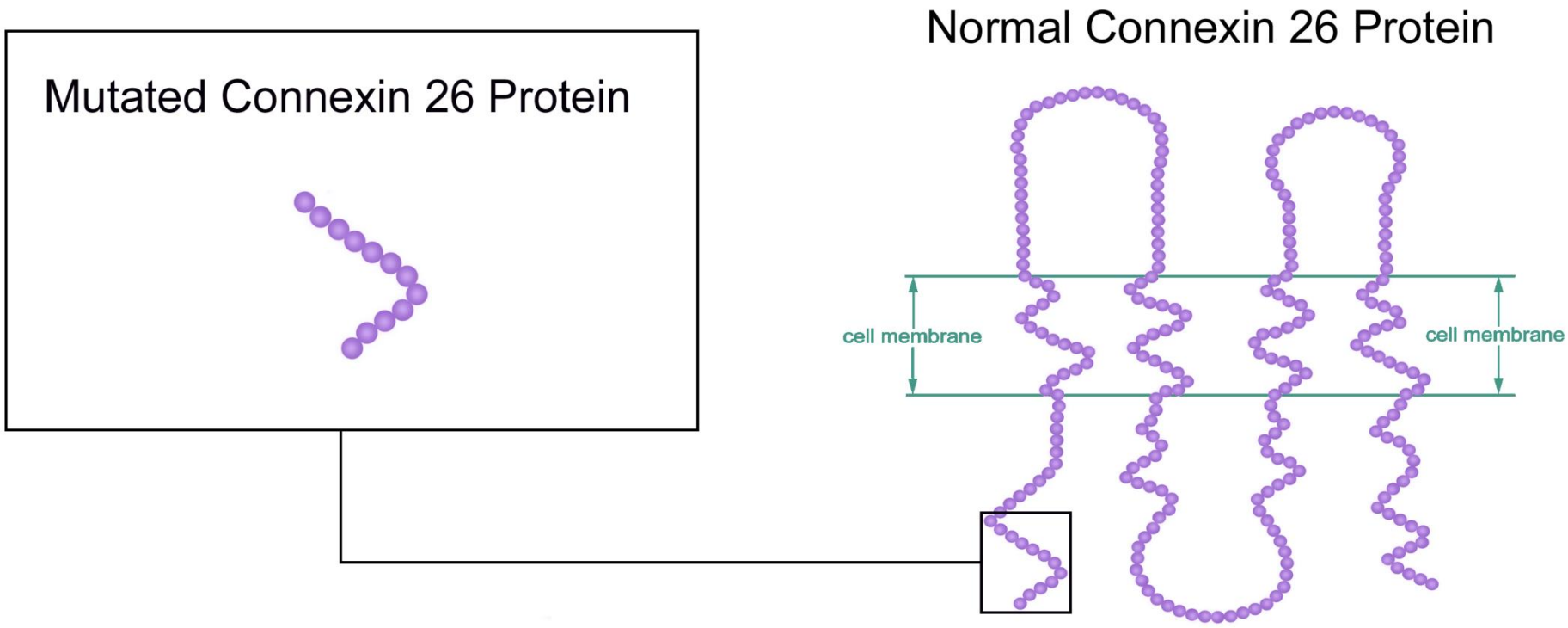
Some variants are milder than others.

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).

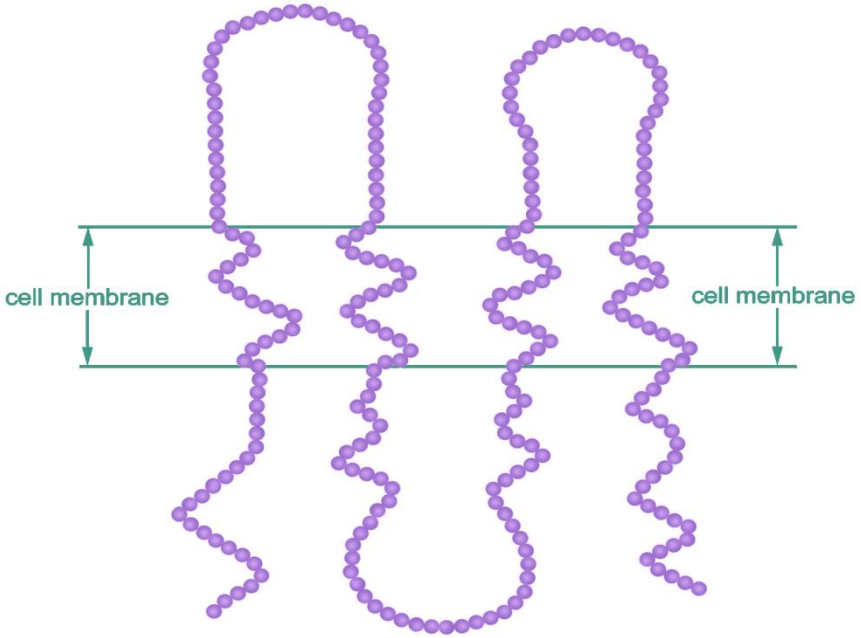
Other variants may leave the protein intact but modify it slightly (e.g. certain missense variants) – it is these variants that can lead to nonsyndromic presentations with Usher syndrome gene variants.

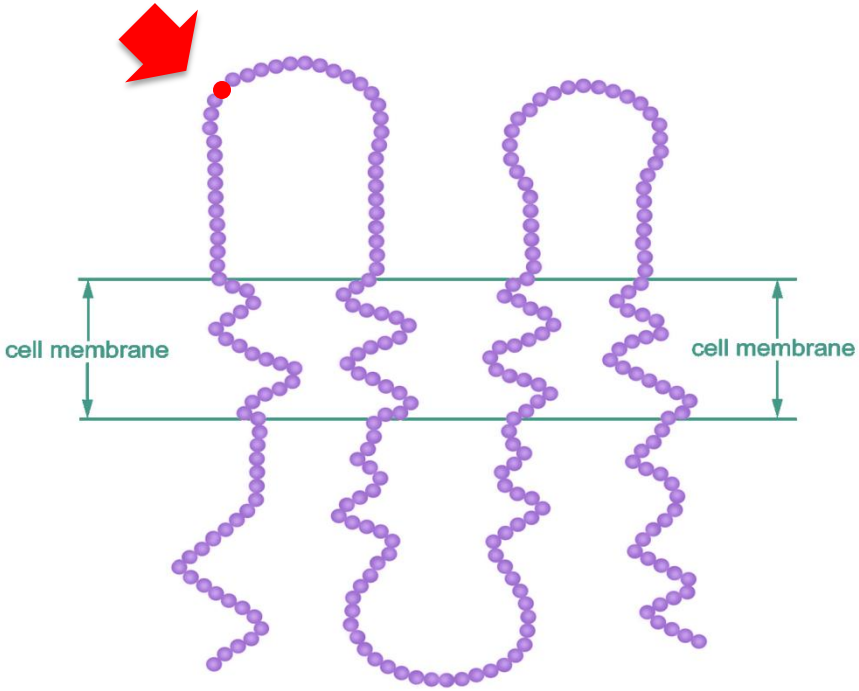
Sometimes clinical presentation is affected by modifiers. Modifiers can be genetic (variants in other genes) or environmental (exposures, lifestyle, etc).

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.

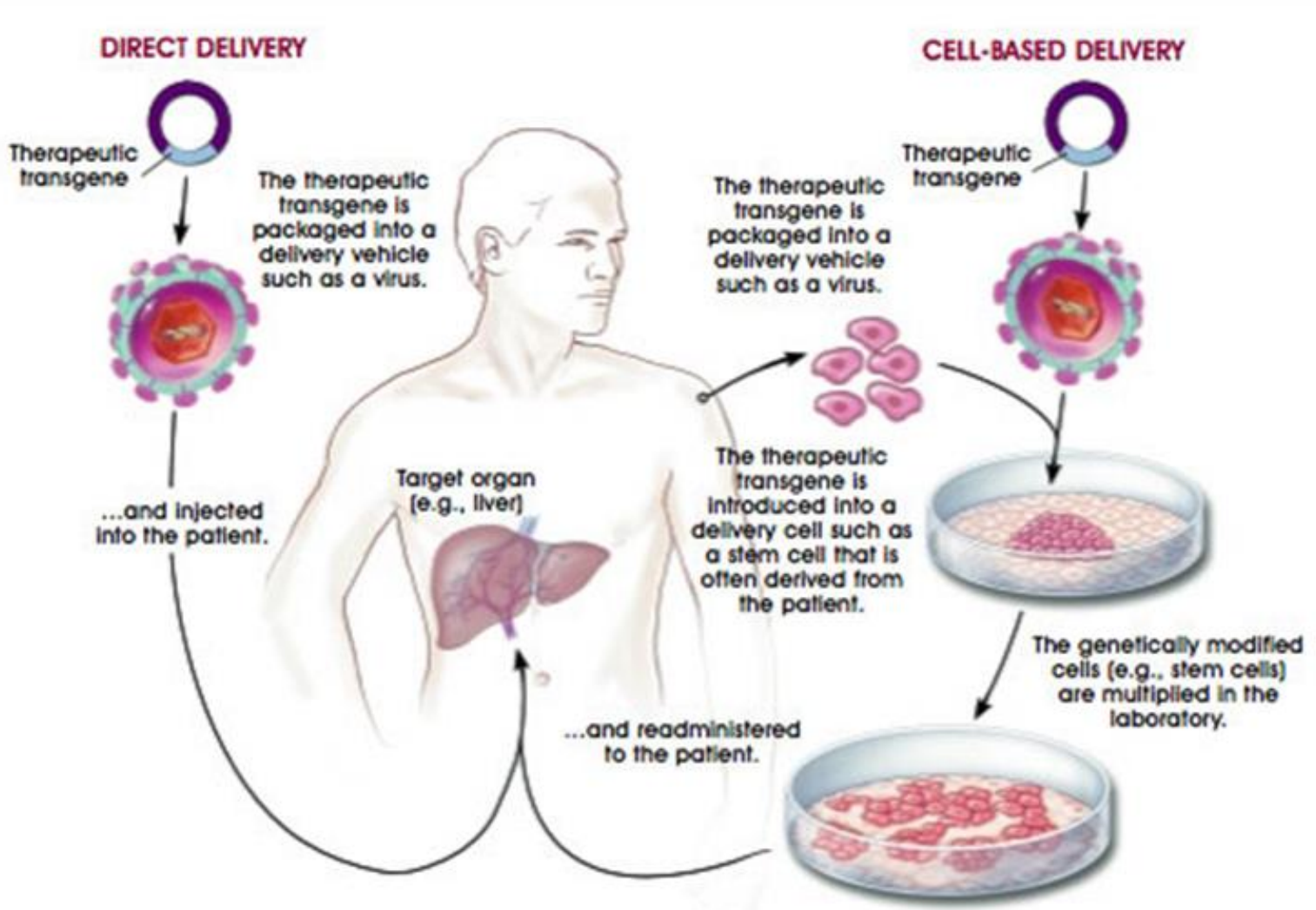
Clinical trials may require genetic test confirmation or knowledge of specific gene involved.

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)

Acknowledgments

OtoChip

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