The Genetics of Usher Syndrome

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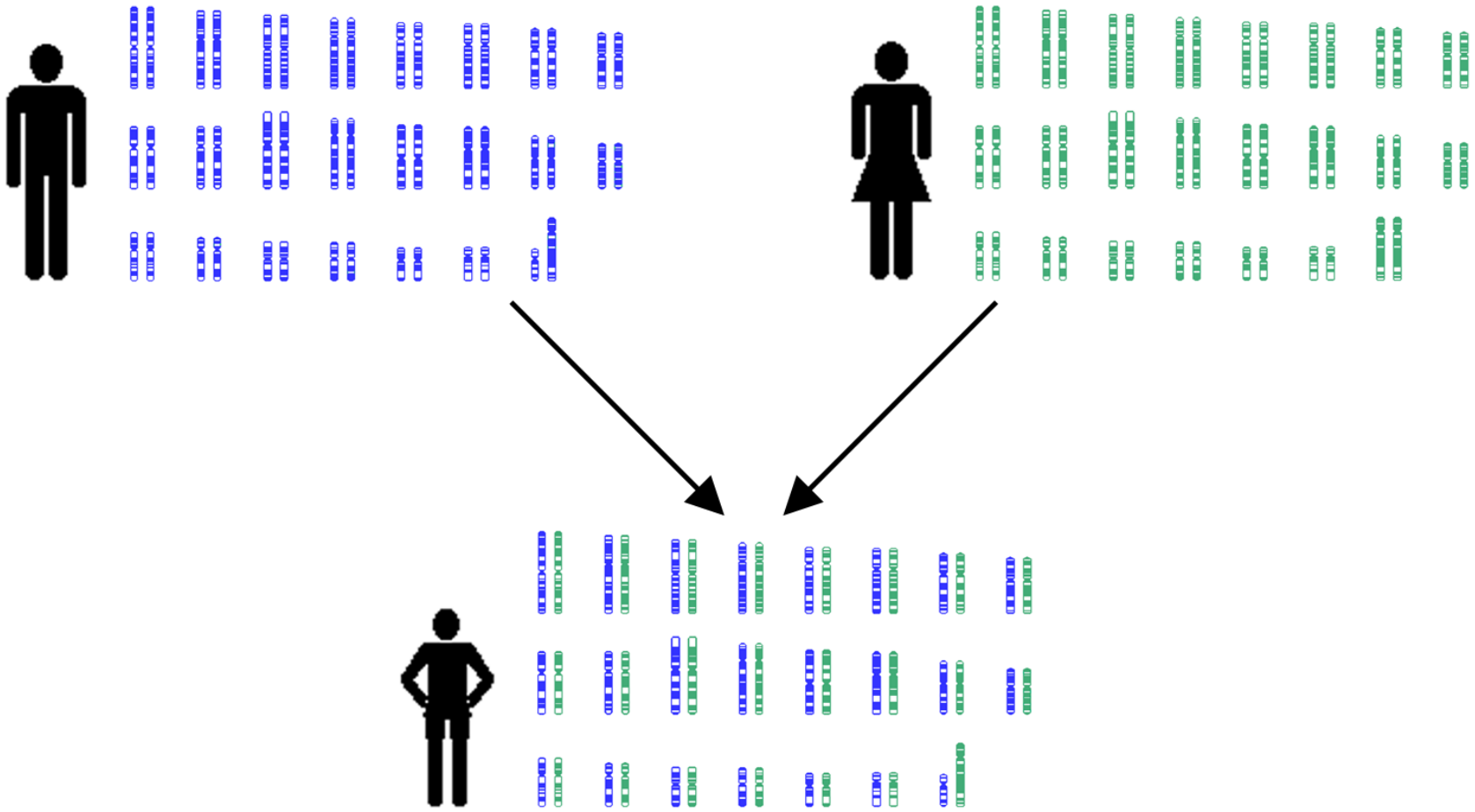
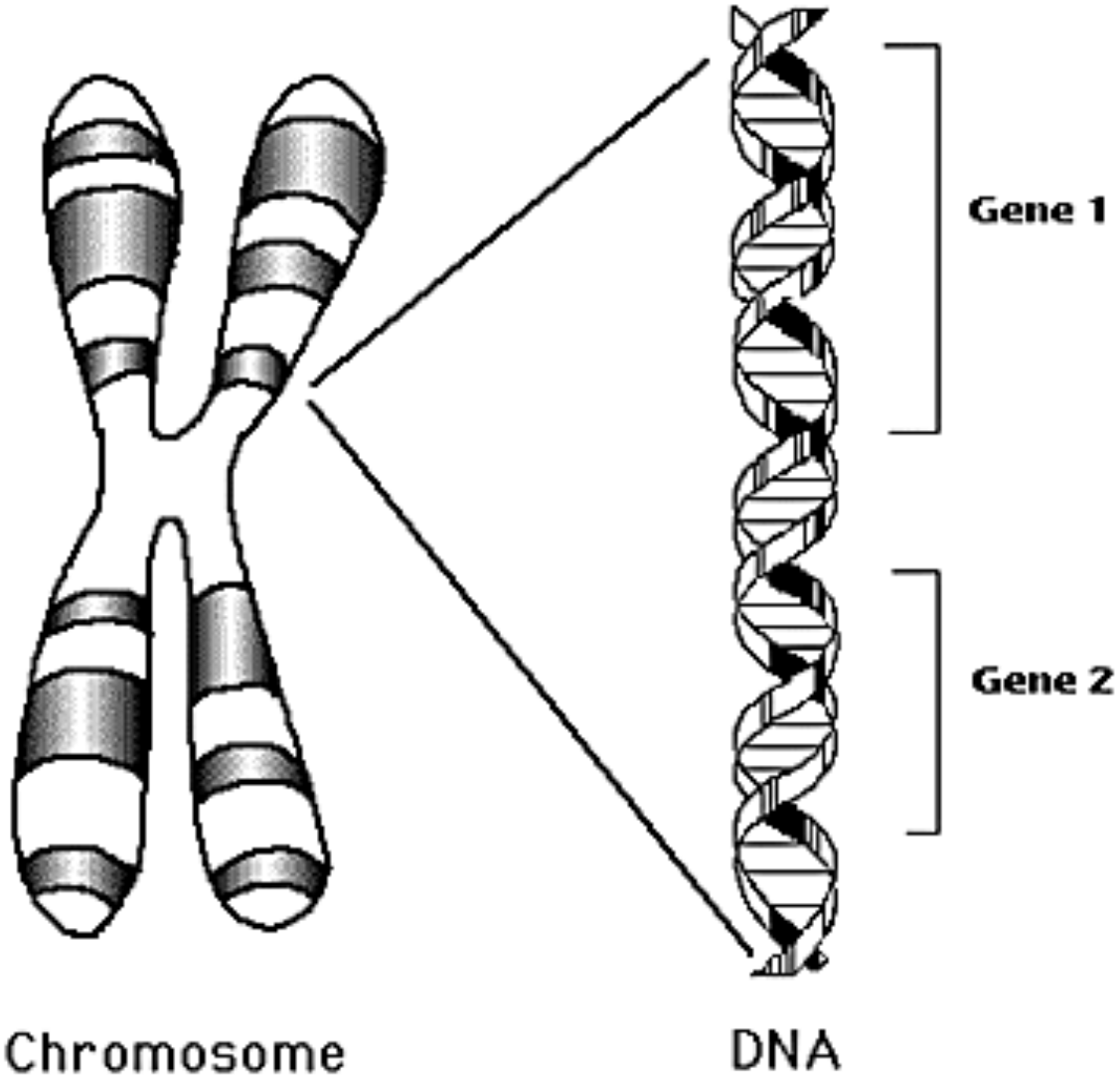
HARVARD MEDICAL SCHOOL



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



Genes

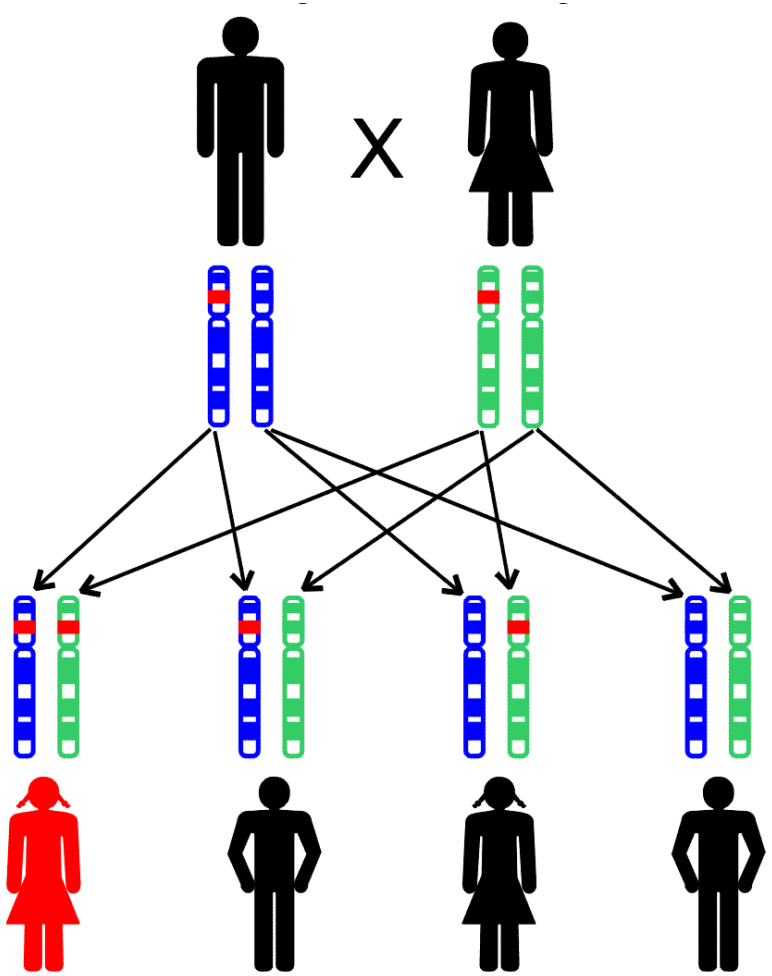
http://www.accessexcellence.org/AB/GG/

Autosomal Recessive Inheritance

For Usher syndrome, both copies of a gene must be mutated to get the disease.

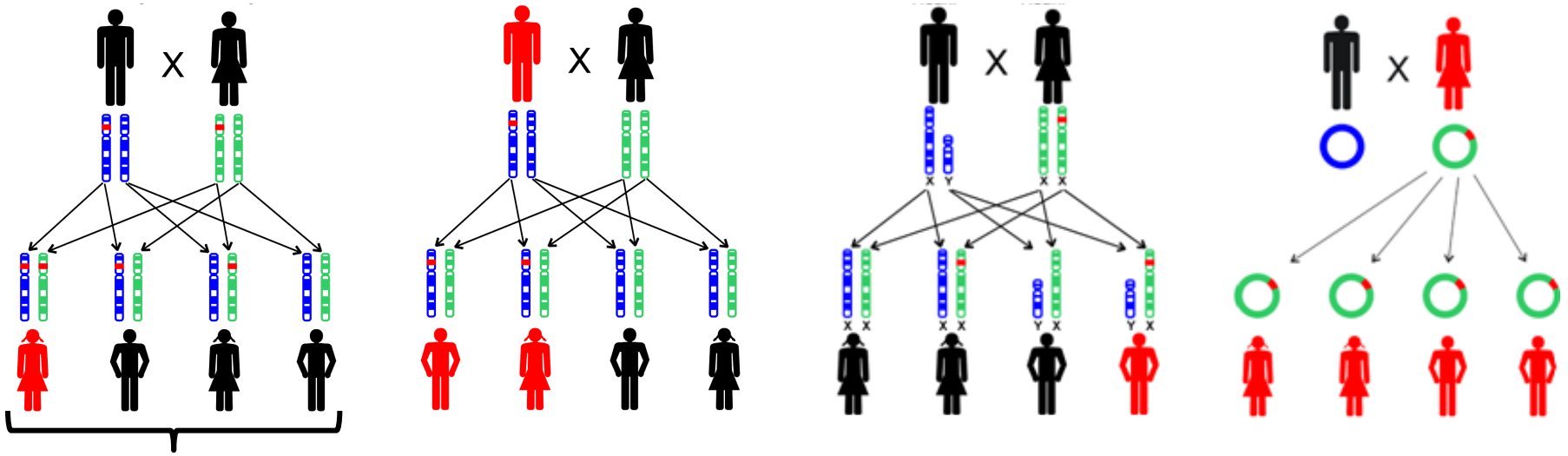
Often, there is no family history of Usher syndrome.

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



A carrier is a person who has one copy of a recessive mutation, but is not affected.

Inheritance Patterns Observed with Usher and RP



Usher syndrome
is recessive

Retinitis pigmentosa can be recessive, dominant, X-linked or mitochondrial

Genetic Testing

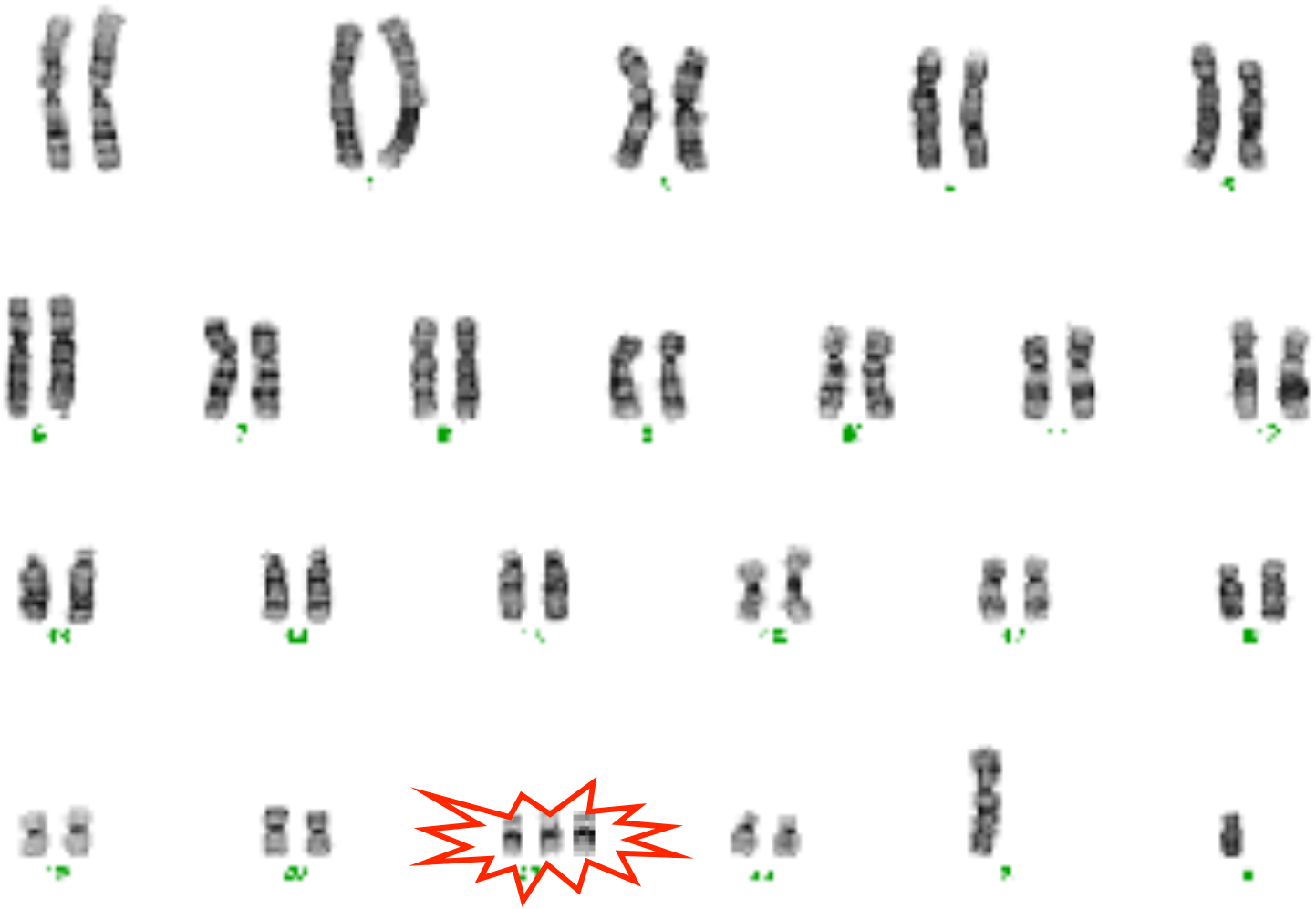
What is it?

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

Usher genetic testing must be done by DNA analysis.

Normal Sequence

ATG GTG CCT CAG GAT

Mutated Sequence

ATG GTG CCT TAG GAT

Usher Syndrome Early Diagnosis

ERG and other ophthalmological exams – may not be positive initially. Often requires sedation for infants and very young children.

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)

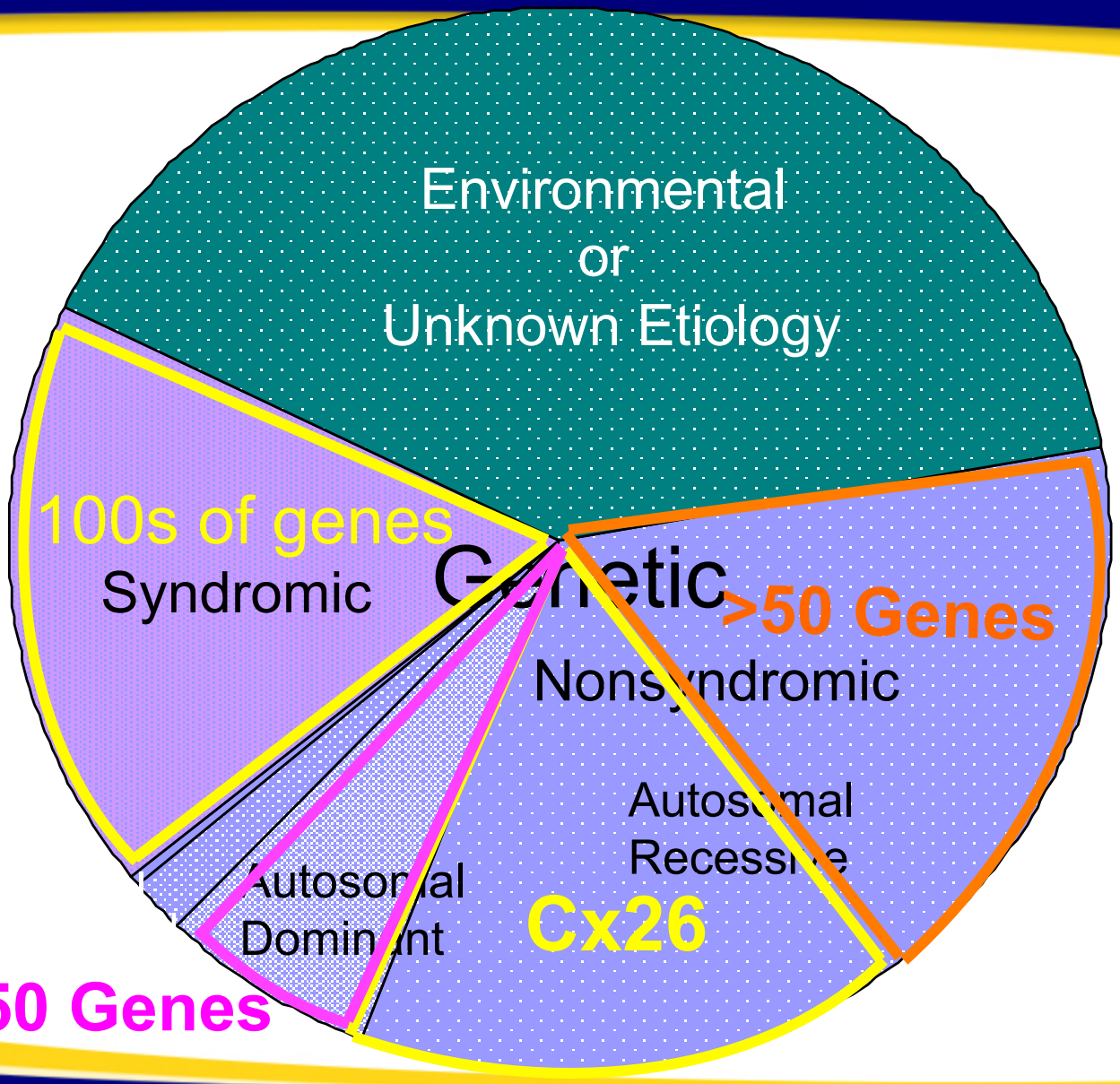
- Teschner 2007: 16.2% of deaf children had absent vestibular responses from a new "minimized rotation" test and 50% of them had abnormal ERGs

Genetic testing: Can be performed at any age with a blood sample. May not find the cause.

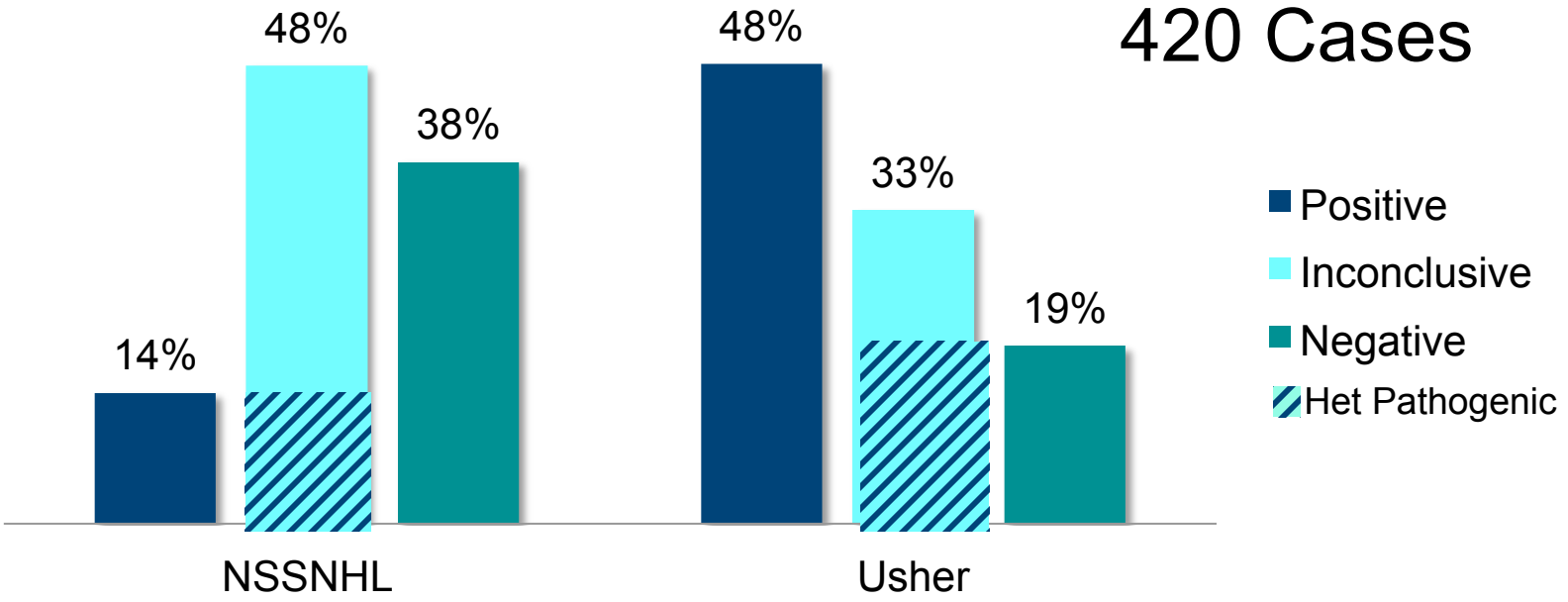
Usher Type	Locus	Gene	Relative Incidence*
USH1A	Retracted (6/9 families have MYO7A mutations)		
USH1B	11q13.5	*MYO7A*	39-55%
USH1C	11p15.1	*USH1C*	6-7%
USH1D	10q	*CDH23*	19-35%
USH1E	21q	unknown	Rare
USH1F	10q21.1	*PCDH15*	10-20%
USH1G	17q24-25	*SANS*	7%
USH2A	1q41	*USH2A*	80%
USH2B	Retracted		
USH2C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

*Relative incidences from Usher I/II GeneReviews

Causes of Childhood Hearing Loss



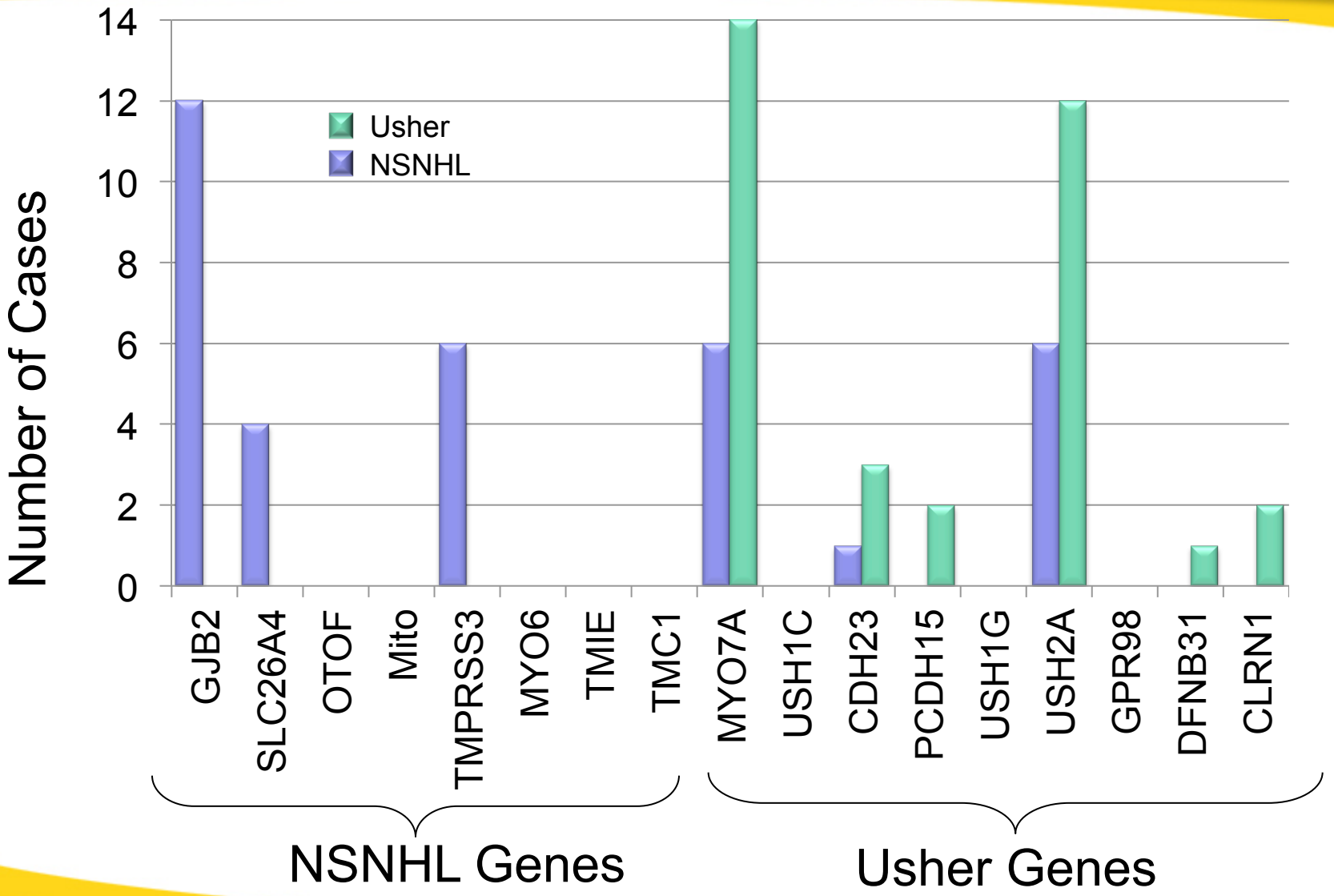
OtoChip Results – 19 Genes for HL and Usher



The OtoChip detected a clear or likely etiology in 28% of hearing loss cases and 68% of possible Usher syndrome cases.

23/241 (9%) of early childhood (≤10yr) HL cases tested positive for an Usher gene mutation

Gene Distribution of Positive OtoChip Cases



September 2012

Laboratory for Molecular Medicine

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OtoGenome Test for Hearing Loss

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LMM

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OtoGenome Test for Hearing Loss

We are pleased to announce the launch of the new ***OtoGenome Test***™ for hearing loss and related syndromes, performed using next-generation sequencing. The comprehensive approach of the OtoGenome Test™ now makes it possible to sequence 71 genes known to cause nonsyndromic hearing loss and syndromes that can present as nonsyndromic such as Usher, Pendred, Jervell and Lange-Nielsen (JLNS), and Branchio-Oto-Renal syndrome (BOR).

For a lower cost, patients can also order the ***Usher Syndrome Panel*** examining only the 10 genes associated with Usher syndrome. This test panel is recommended for individuals with a clinical diagnosis of Usher syndrome. If this test is negative patients can reflex to the remaining 61 genes of the OtoGenome Test for a discounted price.

OtoGenome Test™: ACTG1, ATP6V1B1, BSND, CCDC50, CDH23, CLDN14, CLRN1, COCH, COL11A2, CRYM, DFNA5, DFNB31, DFNB59, DIAPH1, ESPN, ESRRB, EYA1, EYA4, GIPC3, GJB2, GJB3, GJB6, GPR98, GPSM2, GRHL2, GRXCR1, HGF, ILDR1, KCNE1, KCNQ1, KCNQ4, LHFPL5, LOXHD1, LRTOMT, MARVELD2, MIR183, MIR96, MSRB3, MTRNR1 (12S rRNA), MTTS1 (tRNAser(UCN)), MYH14, MYH9, MYO15A, MYO1A, MYO3A, MYO6, MYO7A, OTOA, OTOF, PCDH15, PDZD7, POU3F4, POU4F3, PRPS1, RDX, SERPINB6, SLC17A8, SLC26A4 (PDS), SLC26A5, TECTA, TIMM8A, TJP2, TMC1, TMIE, TMPRSS3, TPRN, TRIOBP, USH1C, USH1G, USH2A, WFS1

Usher Syndrome Panel: CDH23, CLRN1, DFNB31, GPR98, MYO7A, PCDH15, PDZD7, USH1C, USH1G, USH2A

Test Pricing

OtoGenome Test for Hearing Loss (71 genes)	$3600
Usher Syndrome Panel (10 genes)	$2500
Reflex from Usher Syndrome Panel to full OtoGenome Test	$1800

Turn-Around-Time: 8-12 weeks

Why does genetic testing take so long?

The Usher Gene Panel covers 9 genes broken into 208 pieces (exons) and spanning 44,607 bases of DNA sequencing.

The test is first run using a technology called next generation sequencing. Then we fill in the missing holes and confirm all of the variants we find using a higher quality technology called Sanger sequencing.

Then we interpret all of the DNA variants we find (typically 50-100 per patient).

PIPELINE OUTPUT

List of exons that need sanger sequencing

Failed Regions (7 entries, 9 amplicons)

Gene	% uncallab	Uncallable/	Coordinate	NGS ROI i	Sanger Co	Mapping Message
CTF1	100	55/55	16:309079	CTF1_EXON_01		
CTF1	48	237/492	16:309133	CTF1_EXON_03		
DES	2	14/608	2:2202831	DES_EXON_01		
DSC2	9	50/527	18:286818	DSC2_EXON_01		
DSG2	15	Nov-75	18:290782	DSG2_EXON_01		
RBM20	98	217/221	10:112404	RBM20_EXON_01		
TPM1	73	119/162	15:633407	TPM1_EXON_02A_(1)		

Uncallable bases

Gene	Exon	cDNA Posi	Reference	Variant bas	Coverage	A;B breakd	B frequenc	Category	Classificati	Predicted 2	Predicted \	Predicted /	Strand bias	Sanger Co	Coordinate	Mapping M
DSP	Exon 24	c.8466T	T	.	0		0	Uncallable					0	Uncallable	6:7585961-7585961	
DSP	Exon 24	c.8470G	G	.	0		0	Uncallable					0	Uncallable	6:7585965-7585965	
DSP	Exon 24	c.8471G	G	.	0		0	Uncallable					0	Uncallable	6:7585966-7585966	
LDB3	Exon 12	c.1294T	T	.	0		0	Uncallable					0	Uncallable	10:88476146-8847614	
LMNA	Exon 10	c.1706G	G	.	19	19;0		0 Uncallable					-10.01	Uncallable	1:156107542-1561075	
LMNA	Exon 10	c.1707T	T	.	17	17;0		0 Uncallable					-10.01	Uncallable	1:156107543-1561075	
TNNT2	Intron 03	c.53-8C	G	.	0		0	0 Uncallable					0	Uncallable	1:201341177-2013411	
TNNT2	Intron 03	c.53-7T	A	.	0		0	0 Uncallable					0	Uncallable	1:201341176-2013411	
TTN	Intron 45	c.10361-6T	A	.	0		0	0 Uncallable					0	Uncallable	2:179616772-1796167	
TTN	Intron 45	c.10361-5T	A	.	0		0	0 Uncallable					0	Uncallable	2:179616771-1796167	

SANGER SEQUENCE

Follow-up (16 entries, 16 amplicons)

Gene	Exon	cDNA Posi	Reference	Variant bas	Coverage	A;B breakd	B frequenc	Category	Classificati	Predicted 2	Predicted \	Predicted /	Strand bias	Sanger Co	Coordinate	Mapping M
DSC2	Intron 07	c.942+12_9	T	TTAA	524	74;450	0.86	Variant		(Het)	c.942+12_942+13insT		Unknown	Variant	18:28666526-2866652	
LAMA4	Exon 08	c.827C	G	T	573	0;573	1	Variant		(Hom)	c.827C>A	p.Ala276G	-7610.62	Variant	6:112508770-1125087	
LDB3	Intron 07	c.756-12_7	TTC	T	294	133;161	0.55	Variant		(Het)	c.756-12_756-11delTC		Unknown	Variant	10:88458996-8845899	
LMNA	Exon 10	c.*4C	C	G	35	11;24	0.69	Variant		(Het)	c.*4C>G		-10.01	Variant	1:156107559-1561075	
MYH6	Exon 07	c.622G	C	T	809	389;420	0.52	Variant	(Unclassifi	(Het)	c.622G>A	p.Asp208A	-6747.06	Variant	14:23873940-2387394	
PKP2	Exon 09	c.1955_19	G	GCTTC	822	577;245	0.3	Variant		(Het)	c.1955_19	p.Ser652A	Unknown	Variant	12:32975416-3297541	
PRKAG2	Exon 05	c.700_701i	G	GC	74	66;8	0.11	Variant		(Het)	c.700_701i	p.Ala234G	Unknown	Variant	7:151329208-1513292	
RBM20	Exon 09	c.2303G	G	C	430	0;430	1	Variant	(Unclassifi	(Hom)	c.2303G>C	p.Trp768S	-7751.62	Variant	10:112572458-112572	
RBM20	Intron 12	c.3452-9G	G	C	561	0;561	1	Variant	(Unclassifi	(Hom)	c.3452-9G>C		-7959.5	Variant	10:112590810-112590	
RYR2	Intron 15	c.1477-11_	A	AT	358	134;224	0.63	Variant		(Het)	c.1477-11_1477-10ins		Unknown	Variant	1:237619875-2376198	
RYR2	Intron 29	c.3599-9de	AT	A	707	393;314	0.44	Variant	(Unclassifi	(Het)	c.3599-9delT		Unknown	Variant	1:237753074-2377530	
RYR2	Intron 97	c.14091-11	A	AT	417	291;126	0.3	Variant		(Het)	c.14091-11_14091-10i		Unknown	Variant	1:237965133-2379651	
TTN	Exon 43	c.10049C	G	A	628	308;320	0.51	Variant		(Het)	c.10049C>	p.Pro3350l	-3786.36	Variant	2:179628969-1796289	
TTN	Exon 44B	c.10213G	C	T	365	0;365	1	Variant	(Unclassifi	(Hom)	c.10213G>	p.Ala3405T	-3228.31	Variant	2:179621477-1796214	
TTN	Intron 45	c.10361-5d	GA	G	349	244;105	0.3	Variant		(Het)	c.10361-5delT		Unknown	Variant	2:179616770-1796167	
TTN	Exon 275	c.77167C	G	A	846	407;439	0								425988-1794259	

FILTER OUT

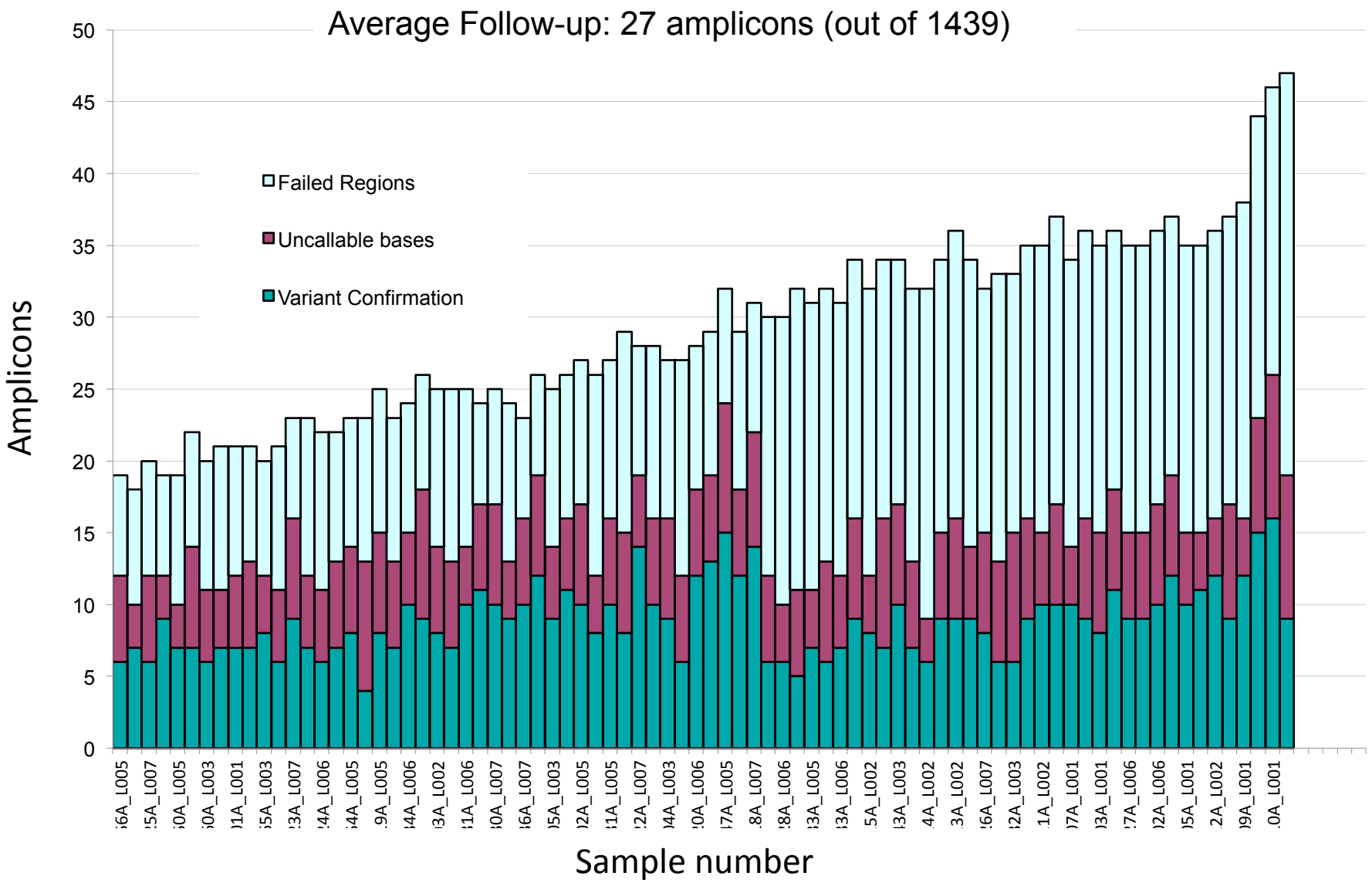
- No Sanger
- No further variant assessment
- Not included on patient report

Common SNPs (71 entries, 66 amplicons)

Gene	Exon	cDNA Posi	Reference	Variant bas	Coverage	A;B breakd	B frequ								dinate	Mapping M
CASQ2	Intron 03	c.420+6T	A	G	626	0;626									283343-1162833	
CASQ2	Exon 11	c.1185C	G	A	597	299;298									243877-1162438	
DSC2	Exon 15A	c.2326A	T	C	784	361;423	0								649042-2864904	
DSC2	Exon 15A	c.2393G	C	T	713	393;320	0								648975-2864897	
DSG2	Exon 08	c.861C	C	T	797	431;366	0								104698-2910469	
DSG2	Exon 14	c.2318G	G	A	449	256;193	0								122799-2912279	
DSG2	Exon 15	c.3321T	T	C	693	368;325	0.47	Variant	Benign	(Het)	c.3321T>C	p.Val1107\	-3592.72	Variant	18:29126670-2912667	
DSP	Exon 24	c.7122C	C	T	1000	520;480	0.48	Variant	Benign	(Het)	c.7122C>T	p.Thr2374	-7131.27	Variant	6:7584617-7584617	
DSP	Exon 24	c.8175C	C	A	591	290;301	0.51	Variant	Benign	(Het)	c.8175C>A	p.Arg2725/	-3177.6	Variant	6:7585670-7585670	

Courtesy of Birgit Funke

Sanger Sequencing Follow-Up for OtoGenome



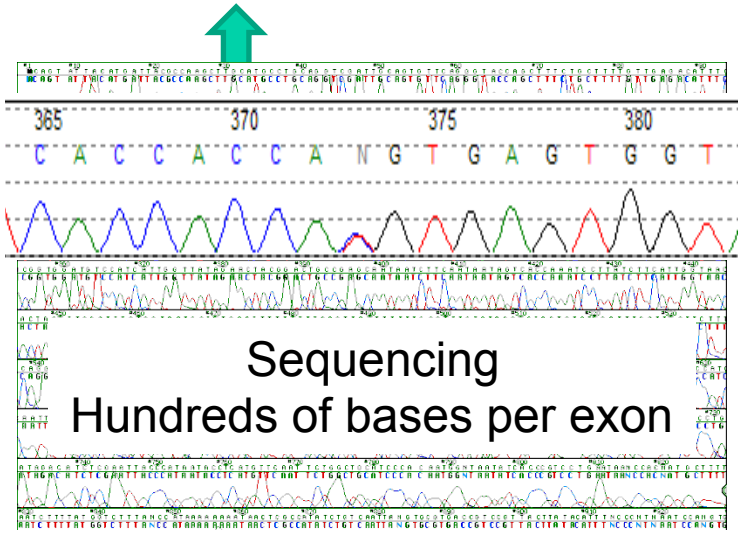
A Genetic Sequencing Test is Not One Test

One Usher Test is actually 44,607 tests with an infinite number of possible results. After the NGS process, Sanger follow-up begins:

DNA

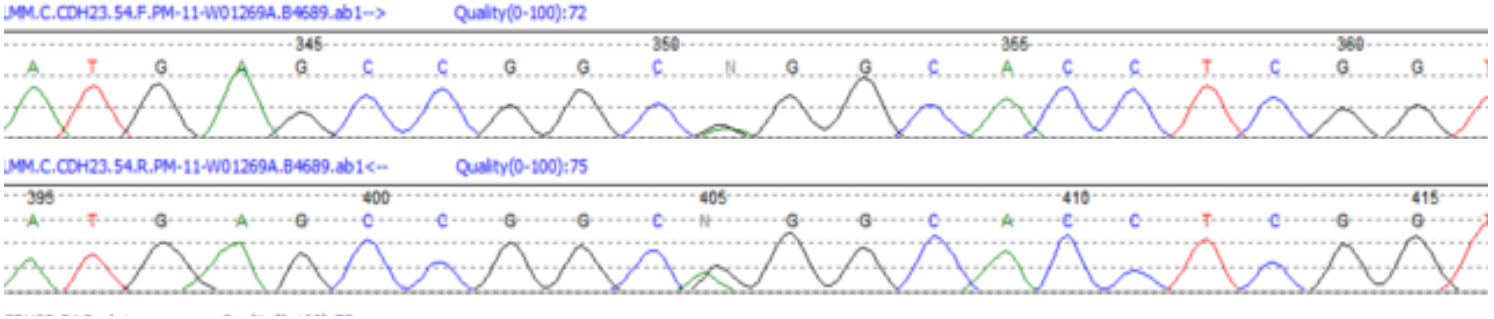
PCR
Many assays per sample

Failed exons/bases

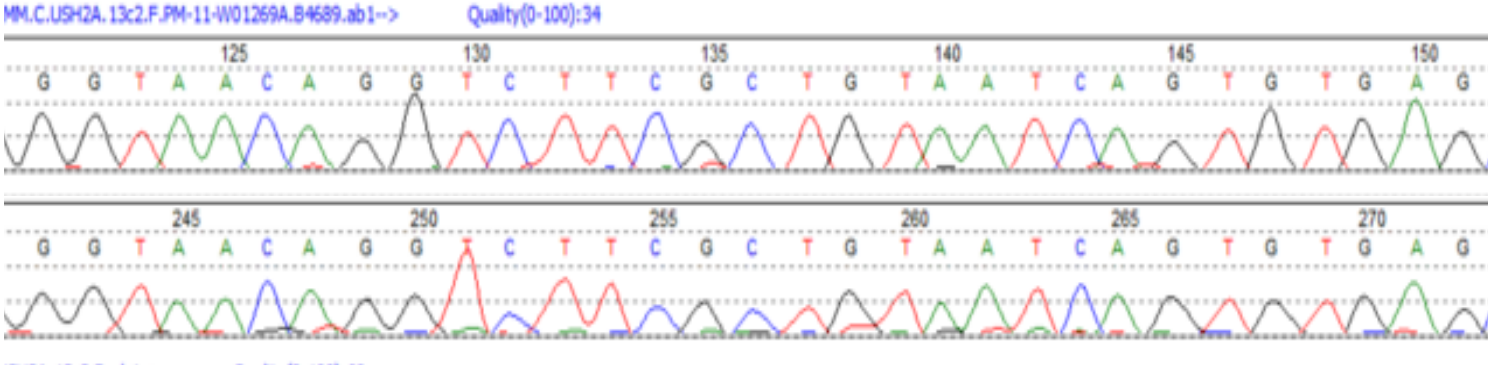


Sanger Sequencing Follow-up

Good Sequence with Mutation



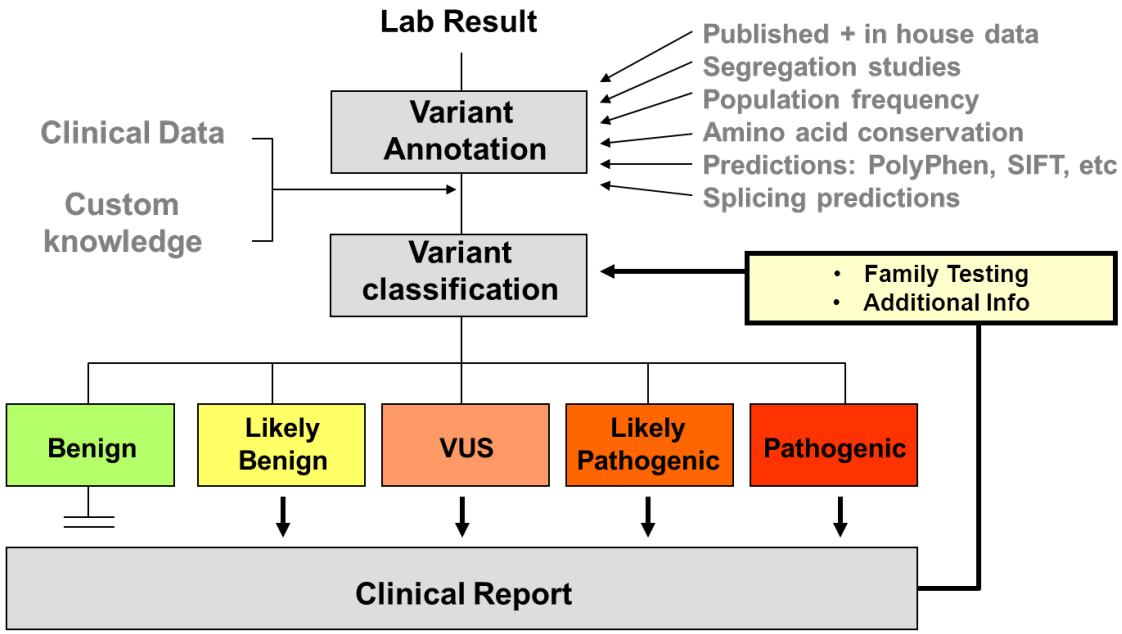
Failed Sequence



Each round of Sanger sequencing takes ~ 1 week

Any failed sequence must be repeated. We often repeat certain exons up to 6-8 times before success

Variant Assessment and Reporting



Variant Assessment Type	Average
Variant with no data	22 min
Variant with dbSNP/ESP data only	25 min
Varant with publications	120 min

~15,000 variants interpreted in patient reports to date

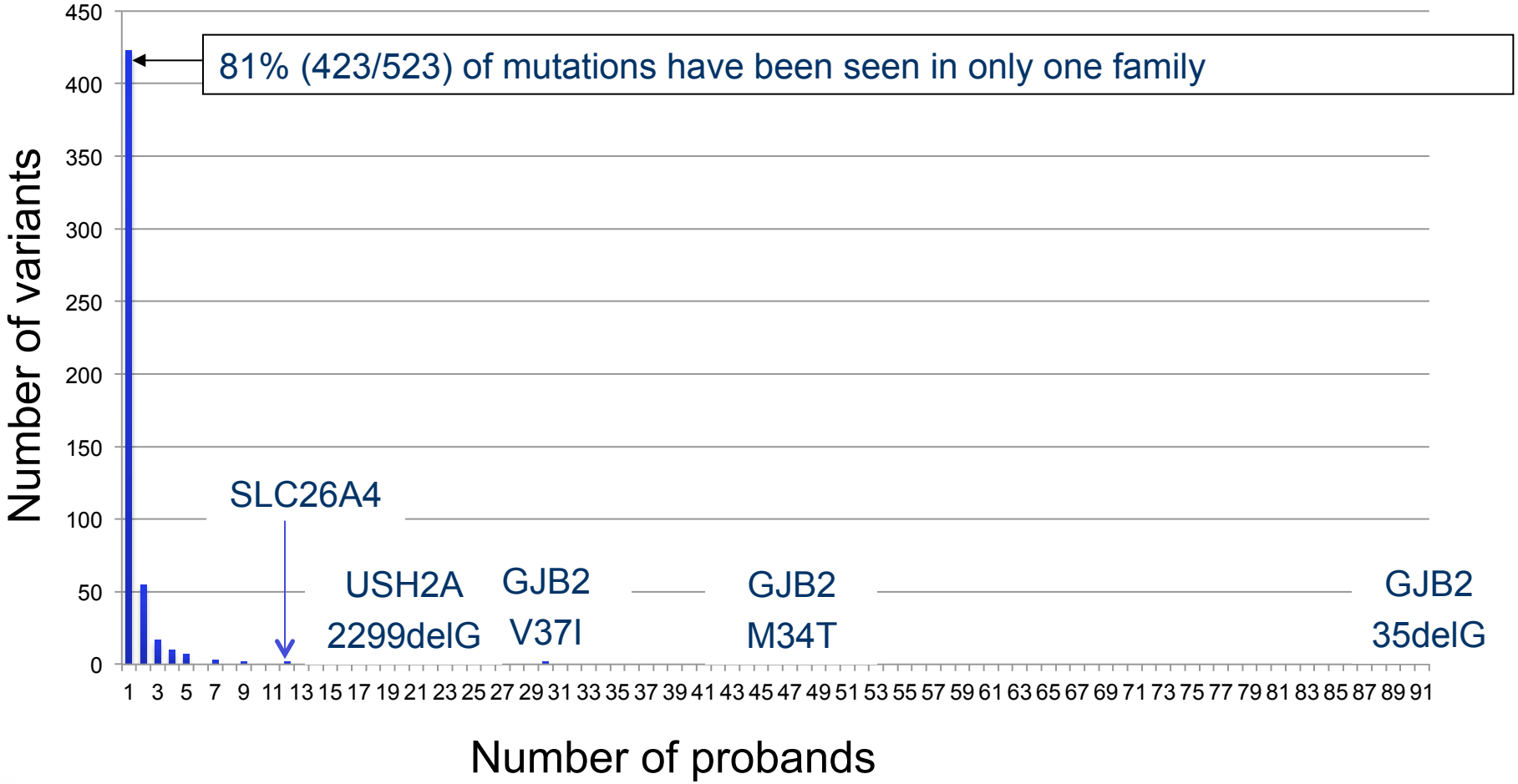
NVAs
Fellows/
Residents

Draft Reports
Genetic
Counselors

Signout
Geneticists

We typically find 50-100 variants per patient in an Usher test.

Hearing Loss Gene Mutations – 2000 Cases Tested



Usher Common Mutation Testing Panel

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*

Blue mutations not on common mutation panel test

Only 1/13 of the initial positive OtoChip cases would have been positive by a common mutation test

Overall <2% of Usher patients would test positive by a common mutation panel

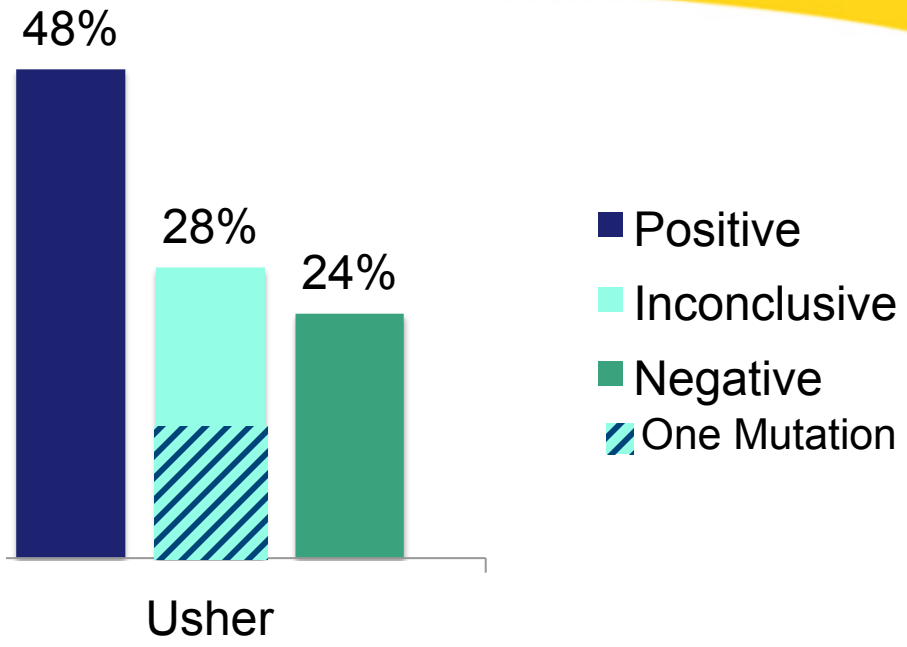
Usher Syndrome Gene Deletions

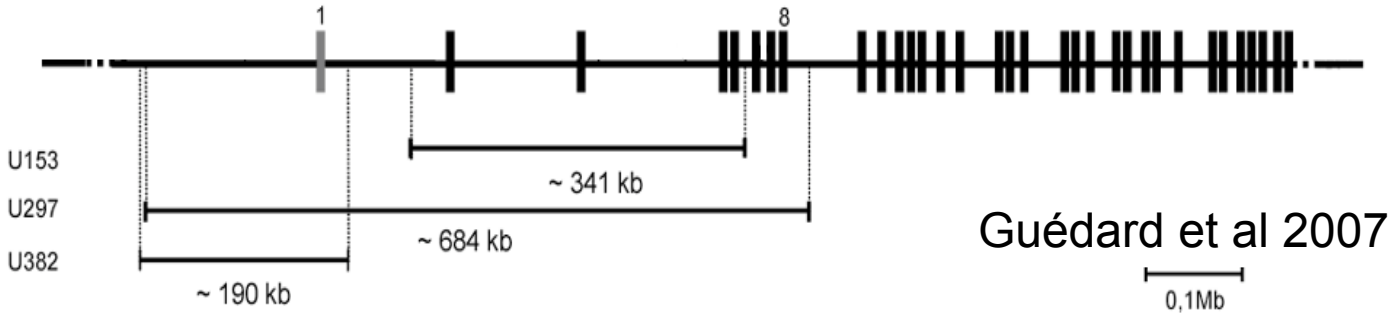
Many patients are found with only one mutation by sequencing

Some of these patients have deletions

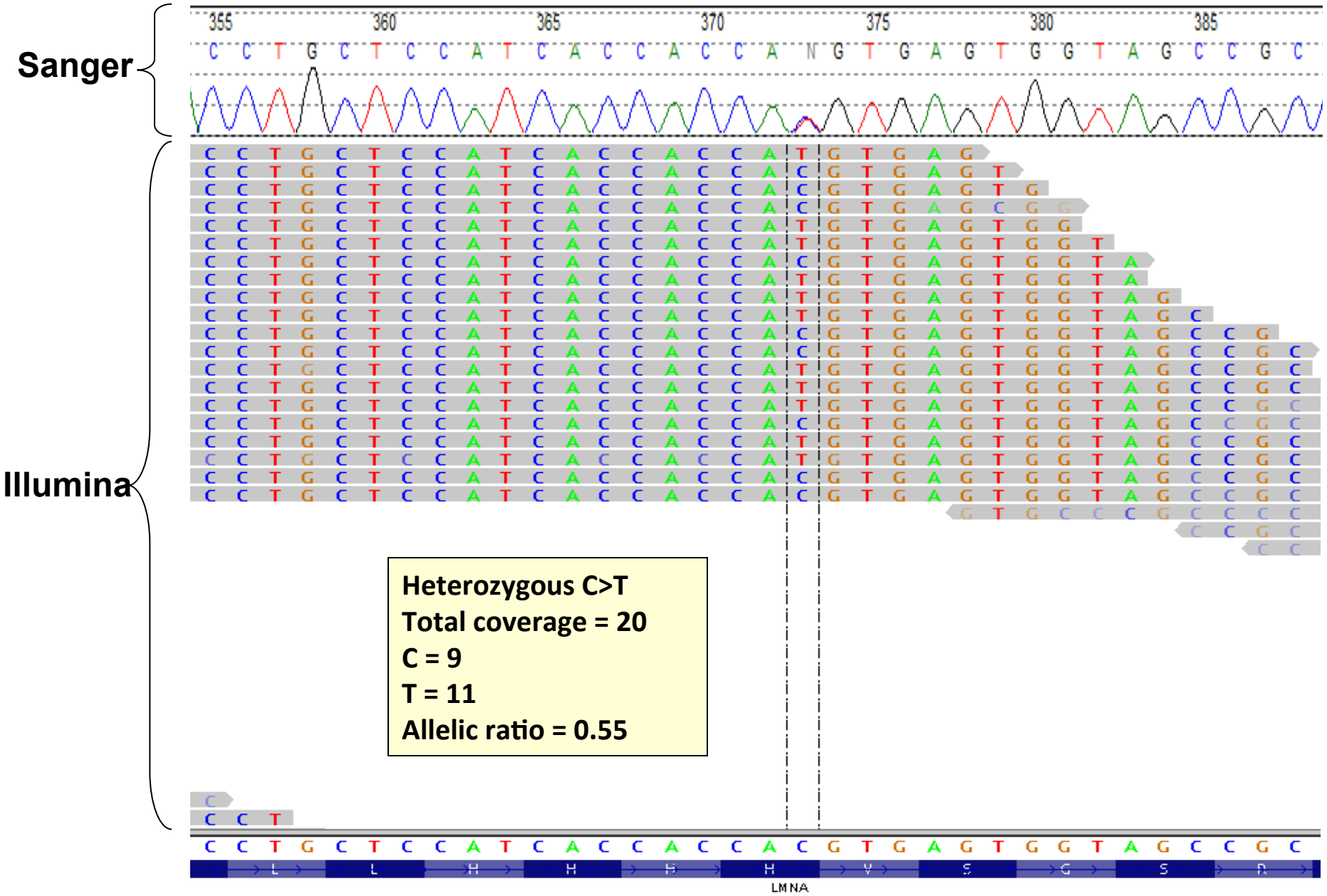
Faugere et al 2010: 8% of Usher cases have larger dels/dups

- 5 in MYO7A
- 1 in CDH23
- 6 in PCDH15
- 10 in USH2A



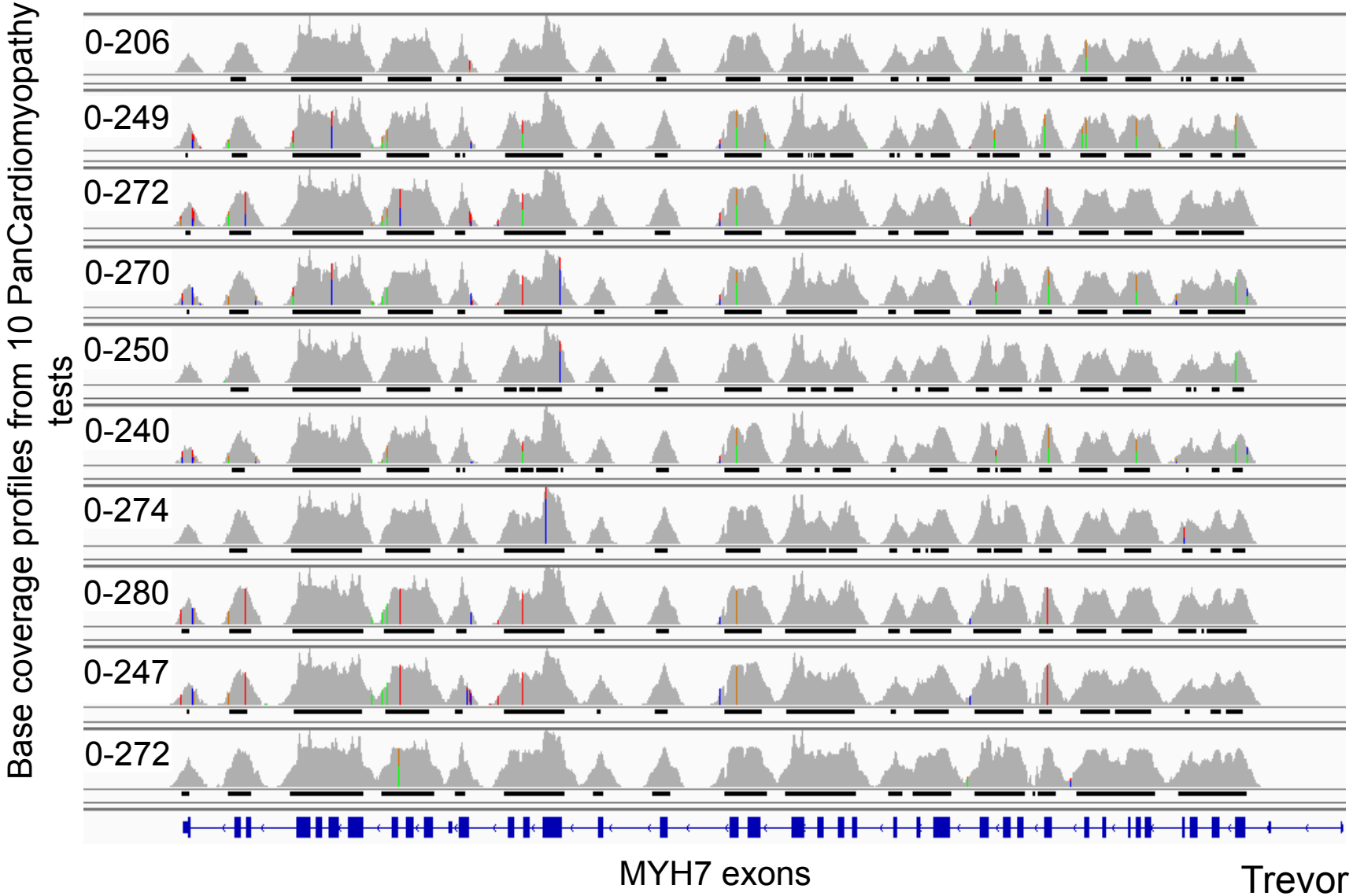


VARIANT DETECTION USING SANGER & ILLUMINA SEQUENCING



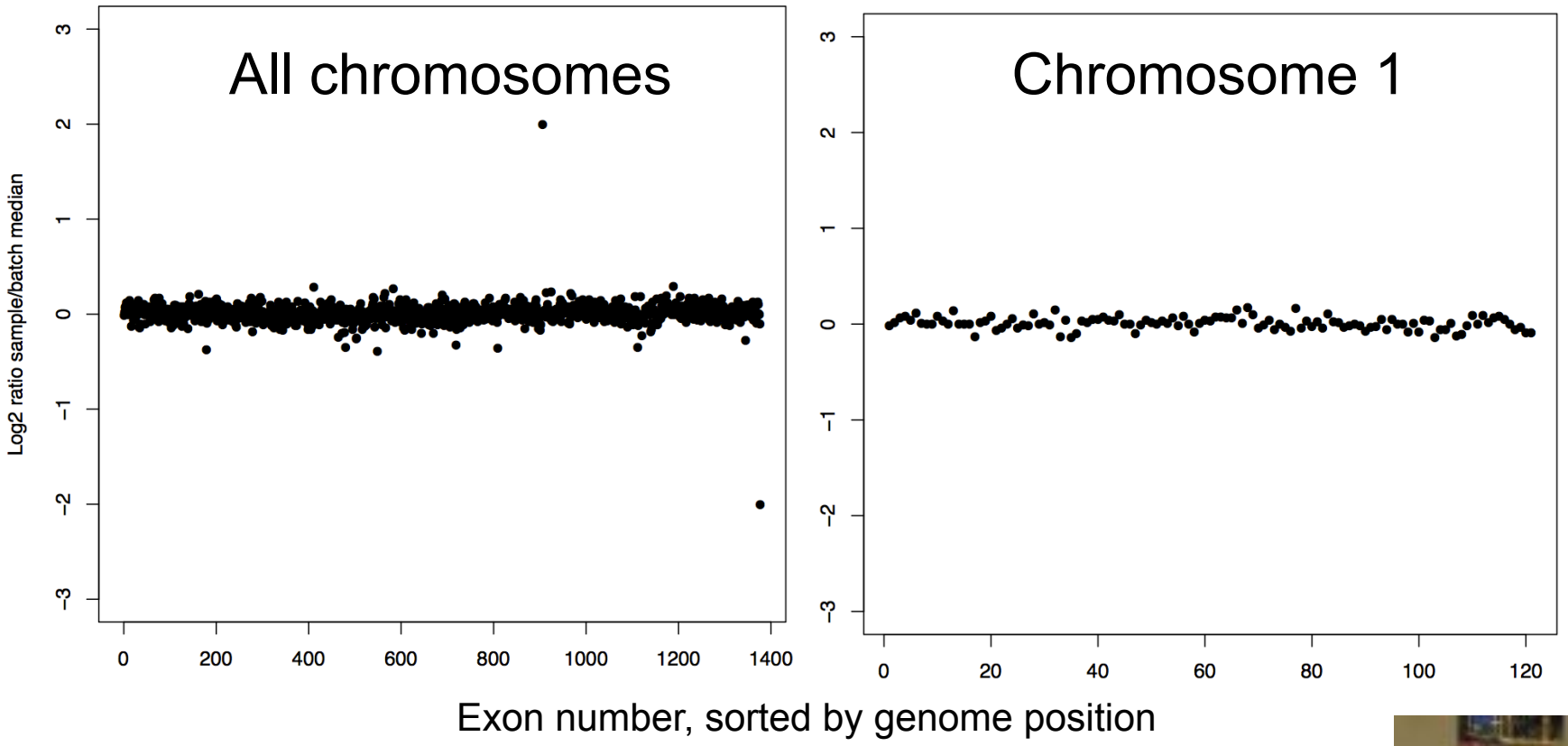
Courtesy of Trevor Pugh

Relative sequence coverage is reproducible and comparable across exons and samples



Trevor Pugh

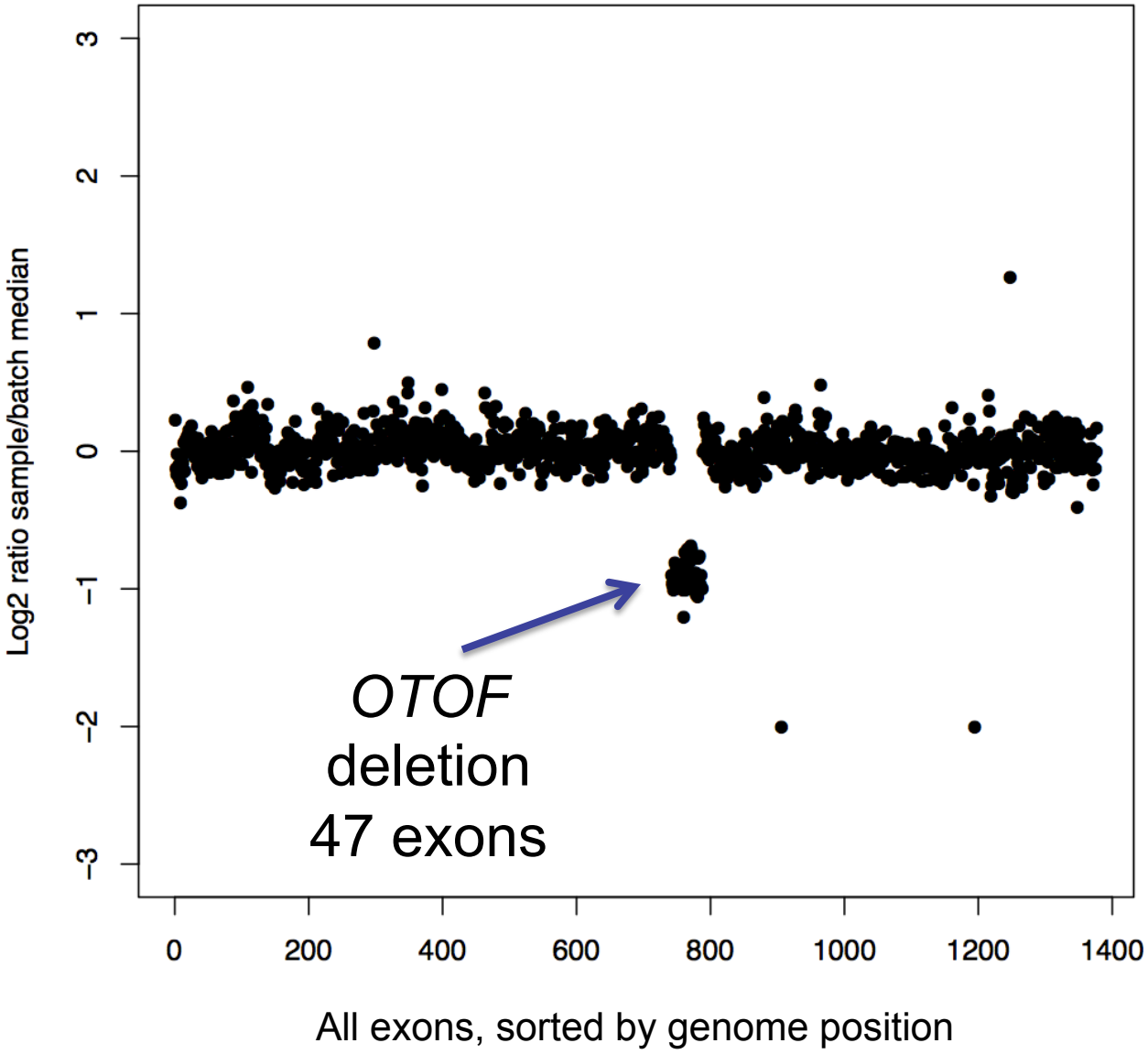
VisCap: An in-house CNV detection tool



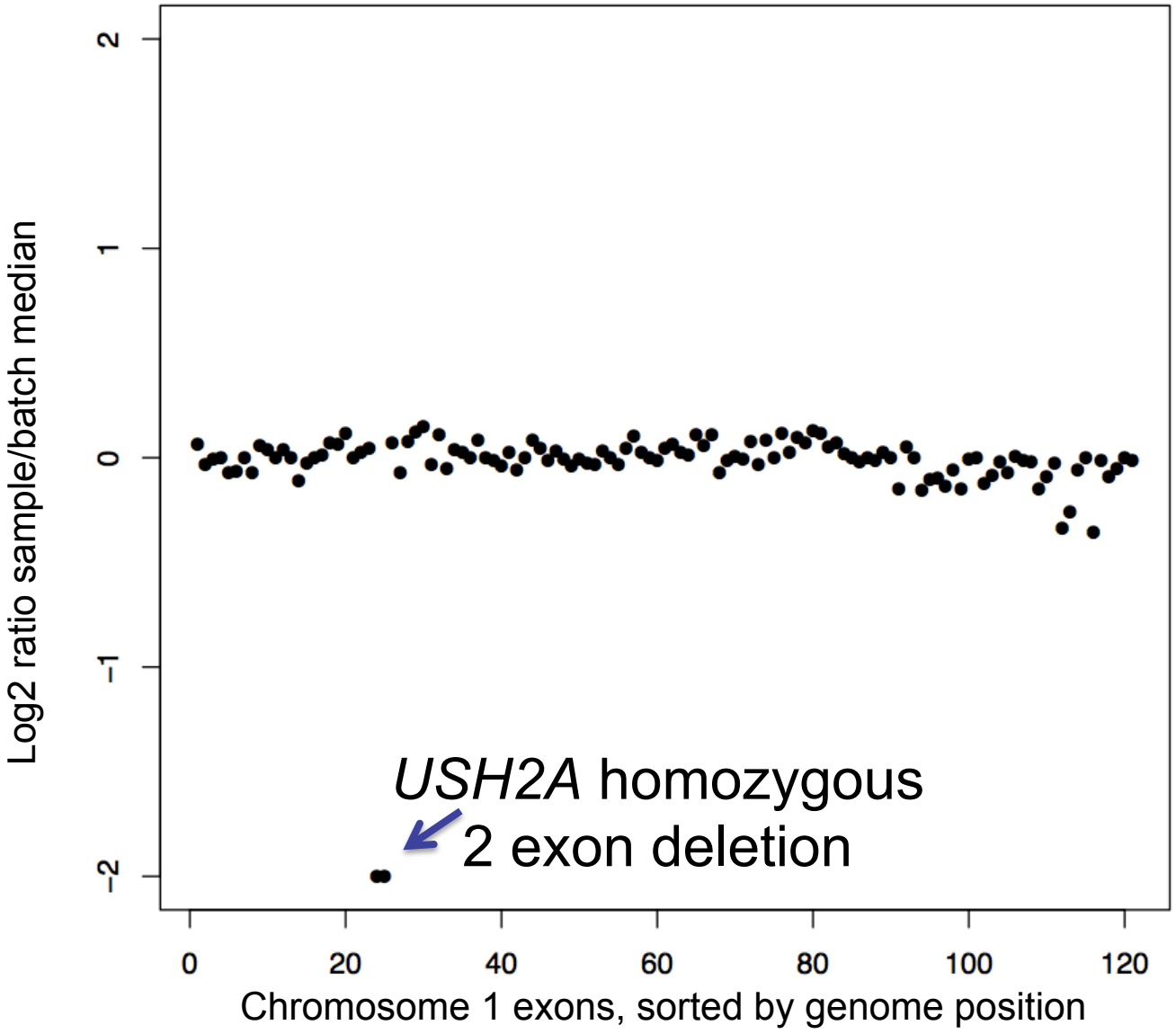
Calculates fraction of total coverage assigned to each exon
Compares fractional coverage against <u>median for the batch</u> ($\log_2$ ratio)

Trevor Pugh

Single-copy losses evident at $\log_2$ ratio ≅ -1



Homozygous deletion results in ~0 coverage



Case 1

40 yr old female

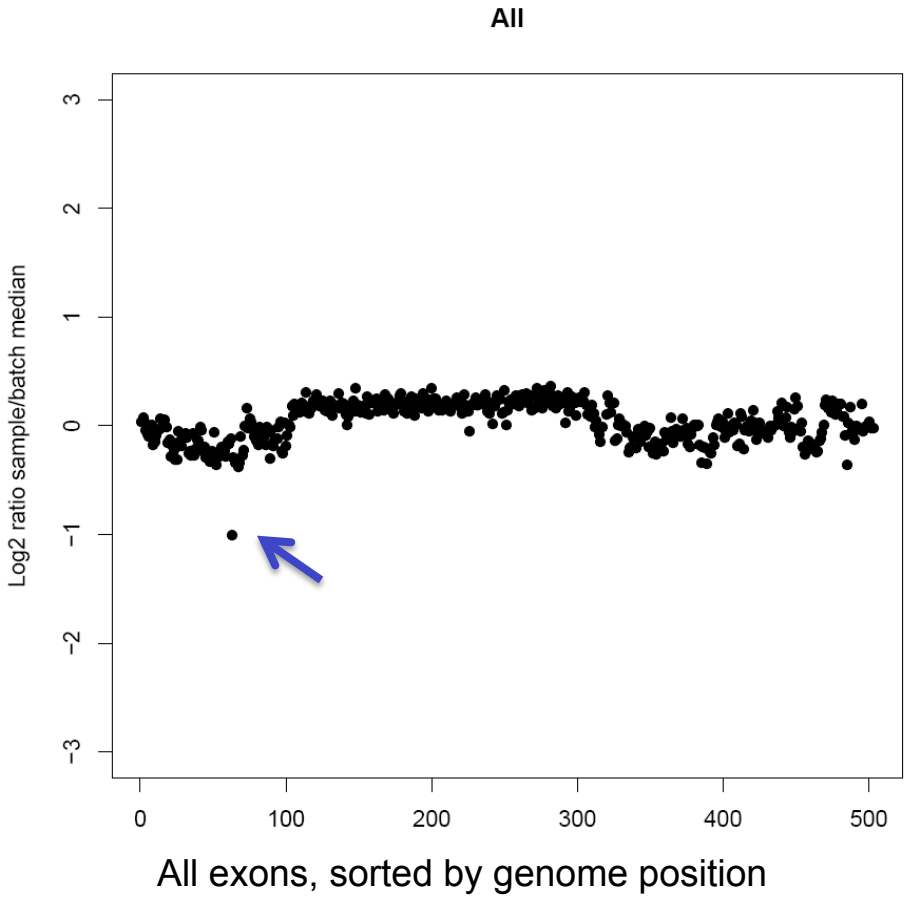
Progressive hearing loss with retinitis pigmentosa

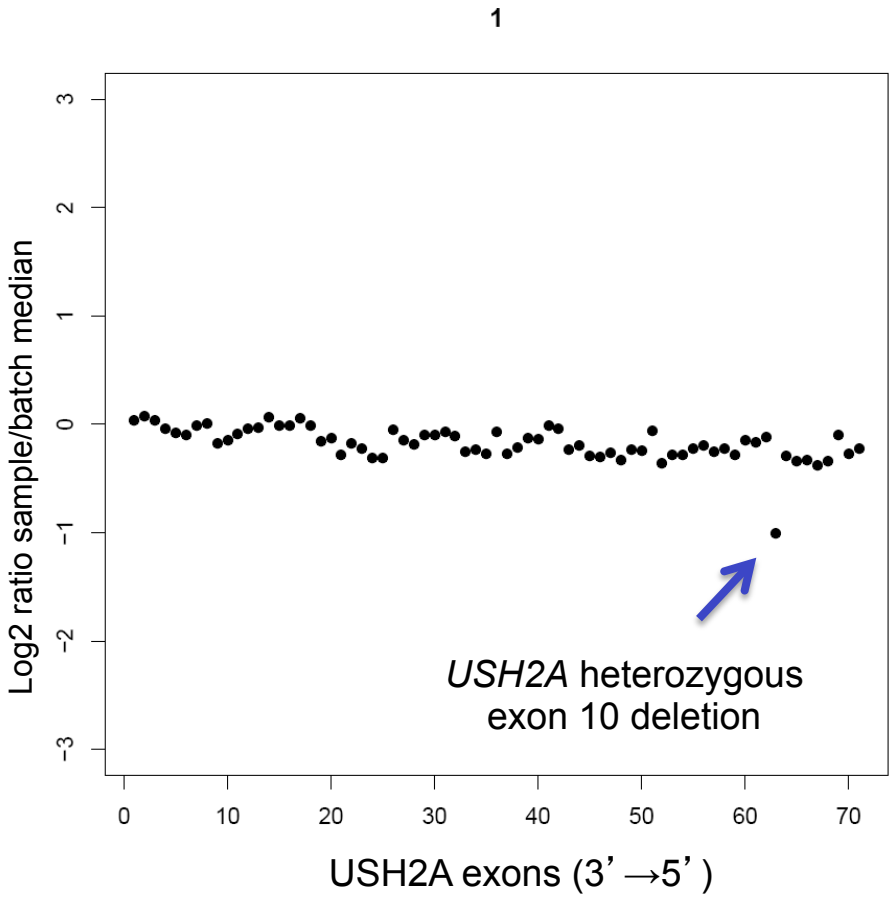
Genetic testing:

Heterozygous c.3309C>A (p.Tyr1103X), USH2A, Pathogenic

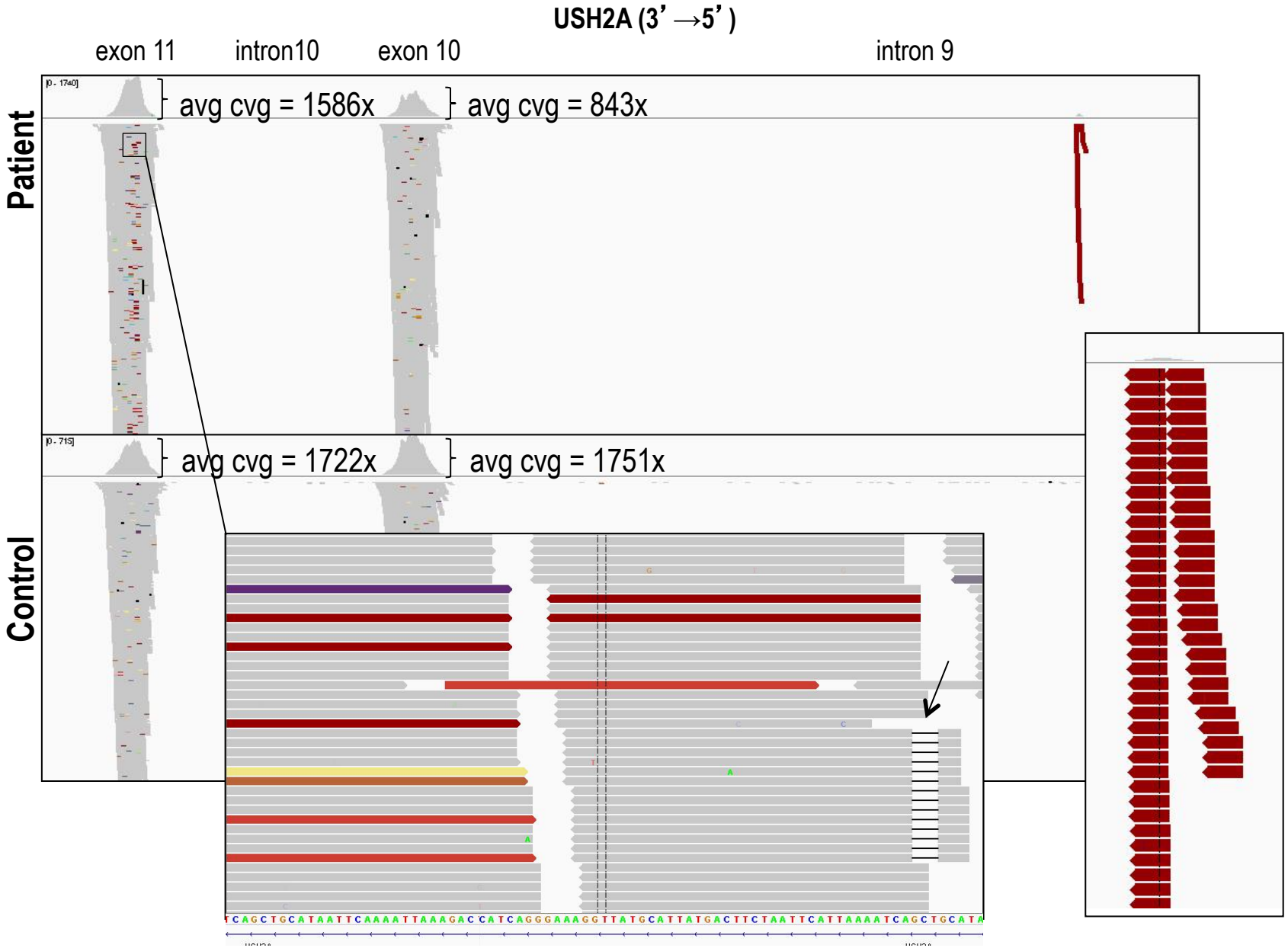
No mutation found on second copy of the gene

Single heterozygous exons deletion





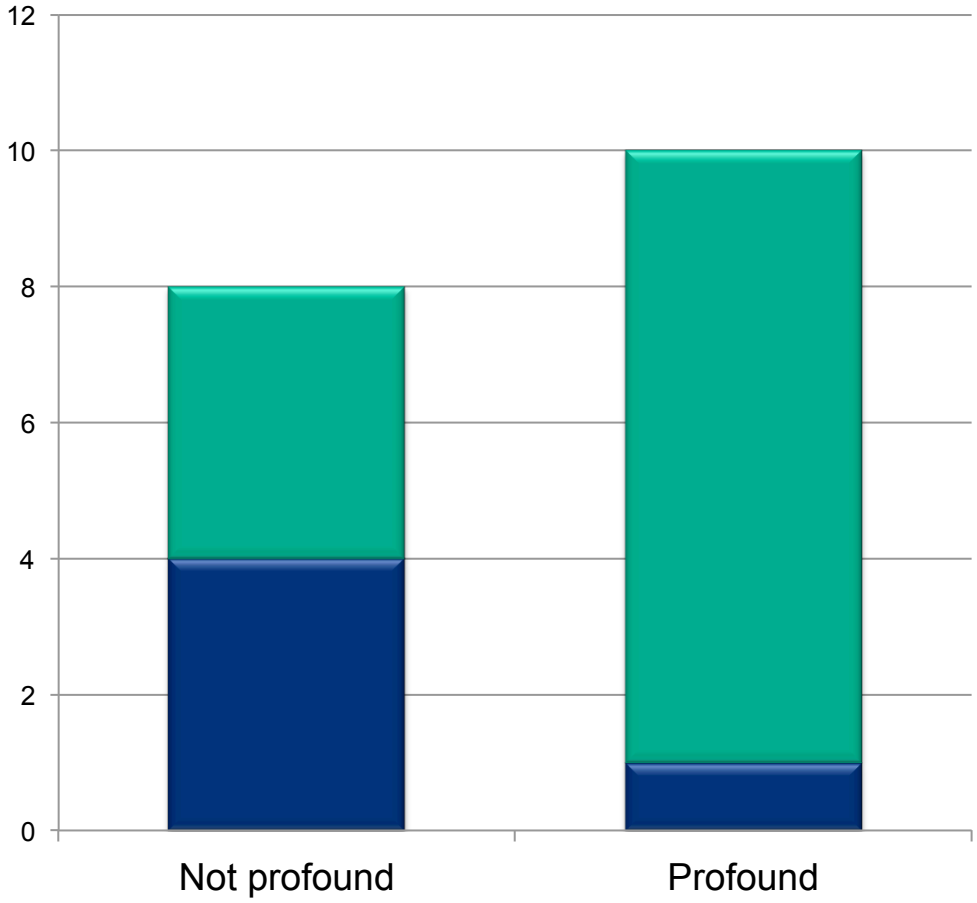
Detection of Copy Number Variants by Paired-End Read Mapping



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

Hearing Loss Severity with USH1 Gene Mutations



Usher 3 yr - 48 yr

NSNHL 4 mo - 5 yr

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

Nonsyndromic Hearing Loss or RP due to Usher Gene Mutations

Usher Type	Gene	Nonsyndromic Form
USH1B	*MYO7A*	DFNA11, DFNB2 (rare)
USH1C	*USH1C*	DFNB18 (mutations in a certain region of gene)
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 genes?

Some variants are milder than others.

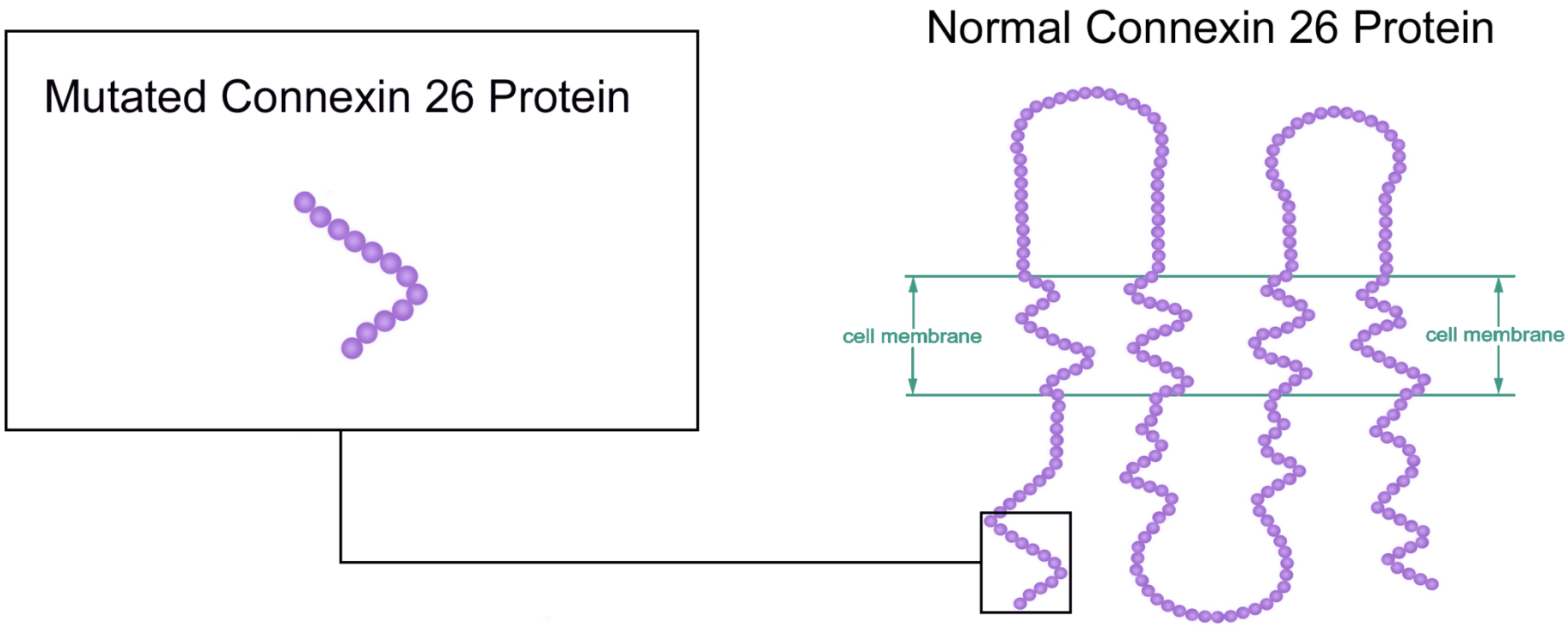
Some variants lead to full loss of the protein (e.g. full or partial gene deletions, nonsense, frameshift and splice variants as well as some missense variants (due to protein misfolding or mislocalization).

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

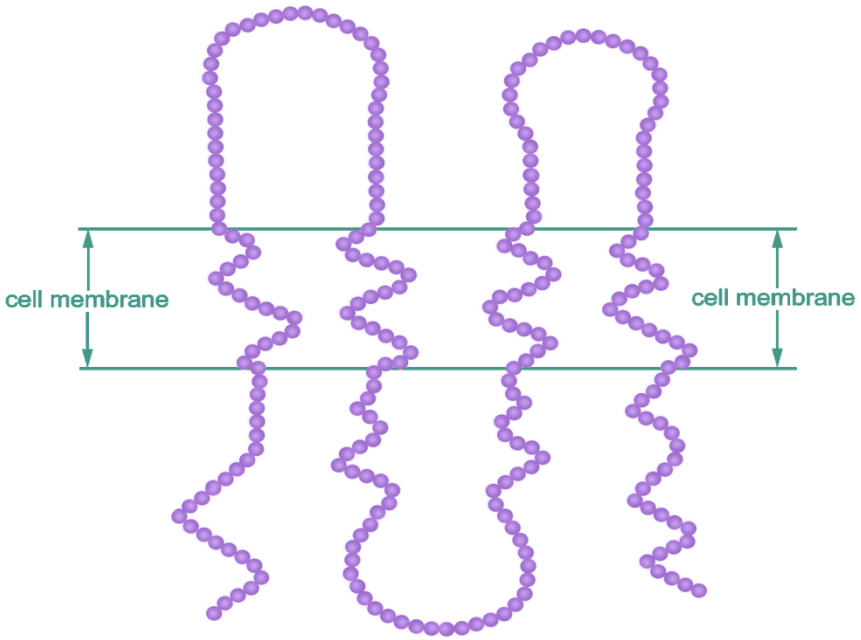
In some cases (USH1C) different parts of the gene create different proteins in eye versus ear only cause hearing loss

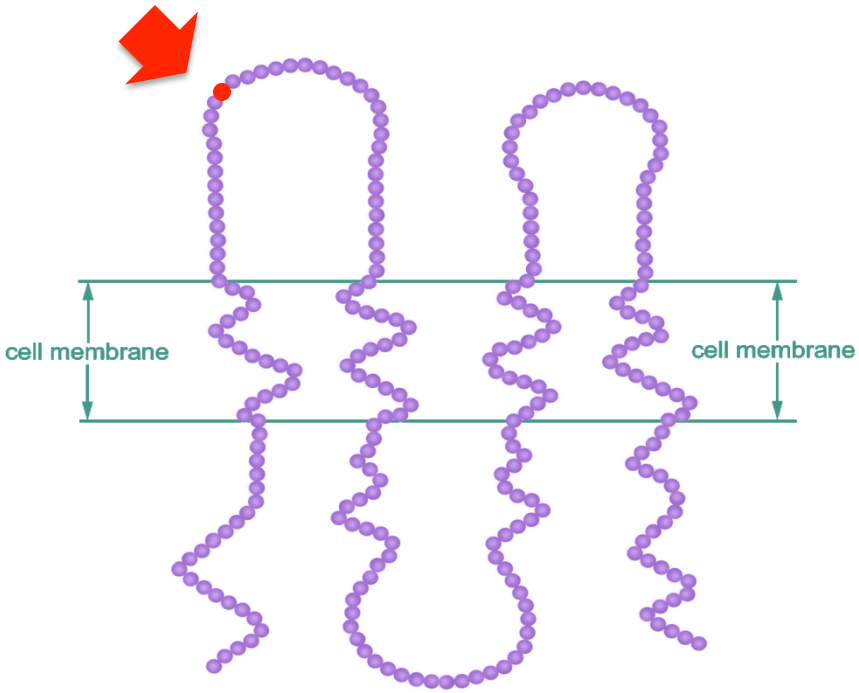
Sometimes clinical presentation is affected by modifiers. Modifiers can be genetic (variants in other genes) or environmental (exposures, lifestyle, etc).

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





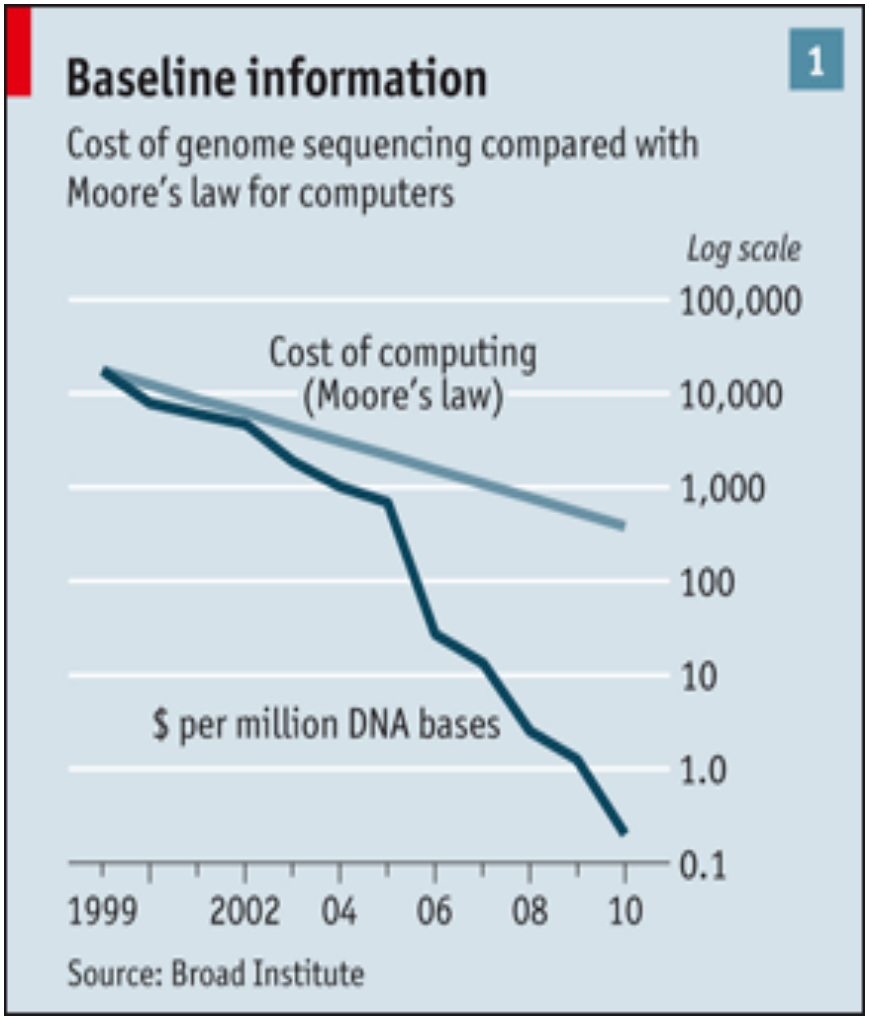
Whole Exome and Genome Sequencing

Sequencing the genome could increase disease detection rates to 100%.

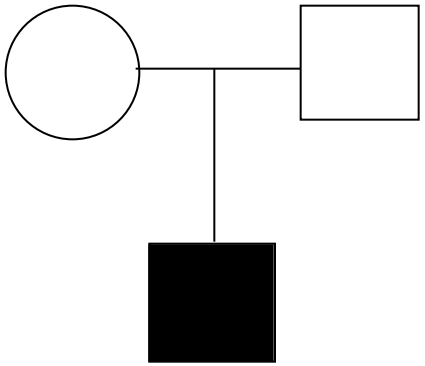
Sequencing costs are dropping rapidly and soon a whole genome will cost the same as disease-targeted tests.

Cost of the first human genome:
$2.7 billion

Cost of a genome today: $9000



Case #1: Whole Exome Sequencing

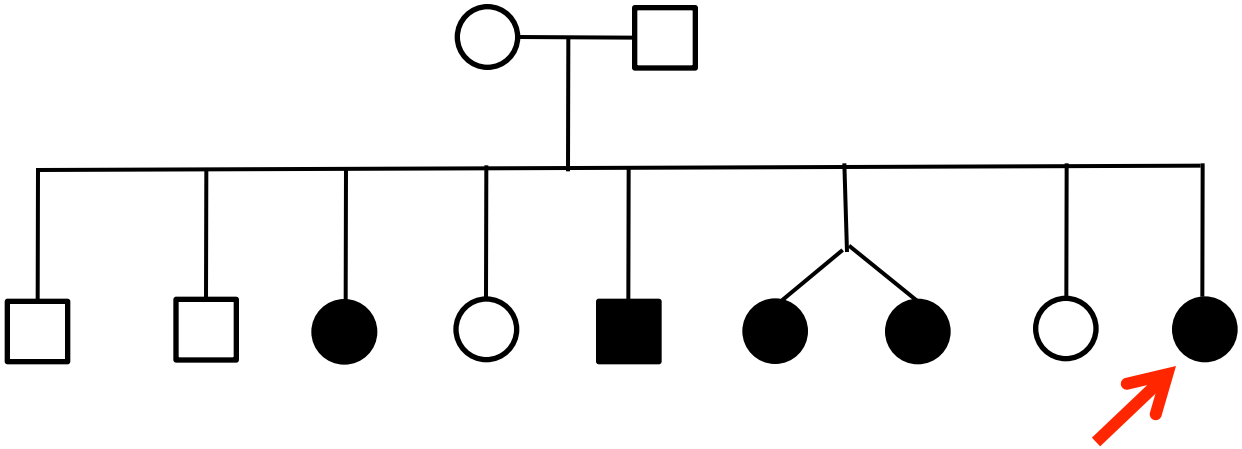


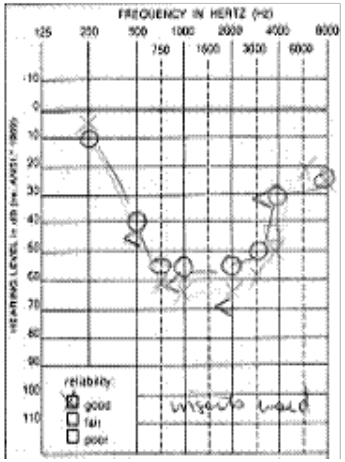
Autistic
Moderate hearing loss

Child had whole exome sequencing through an autism research study

Incidental finding:
USH2A Tyr4238X/Trp2075X
Causative for Usher syndrome

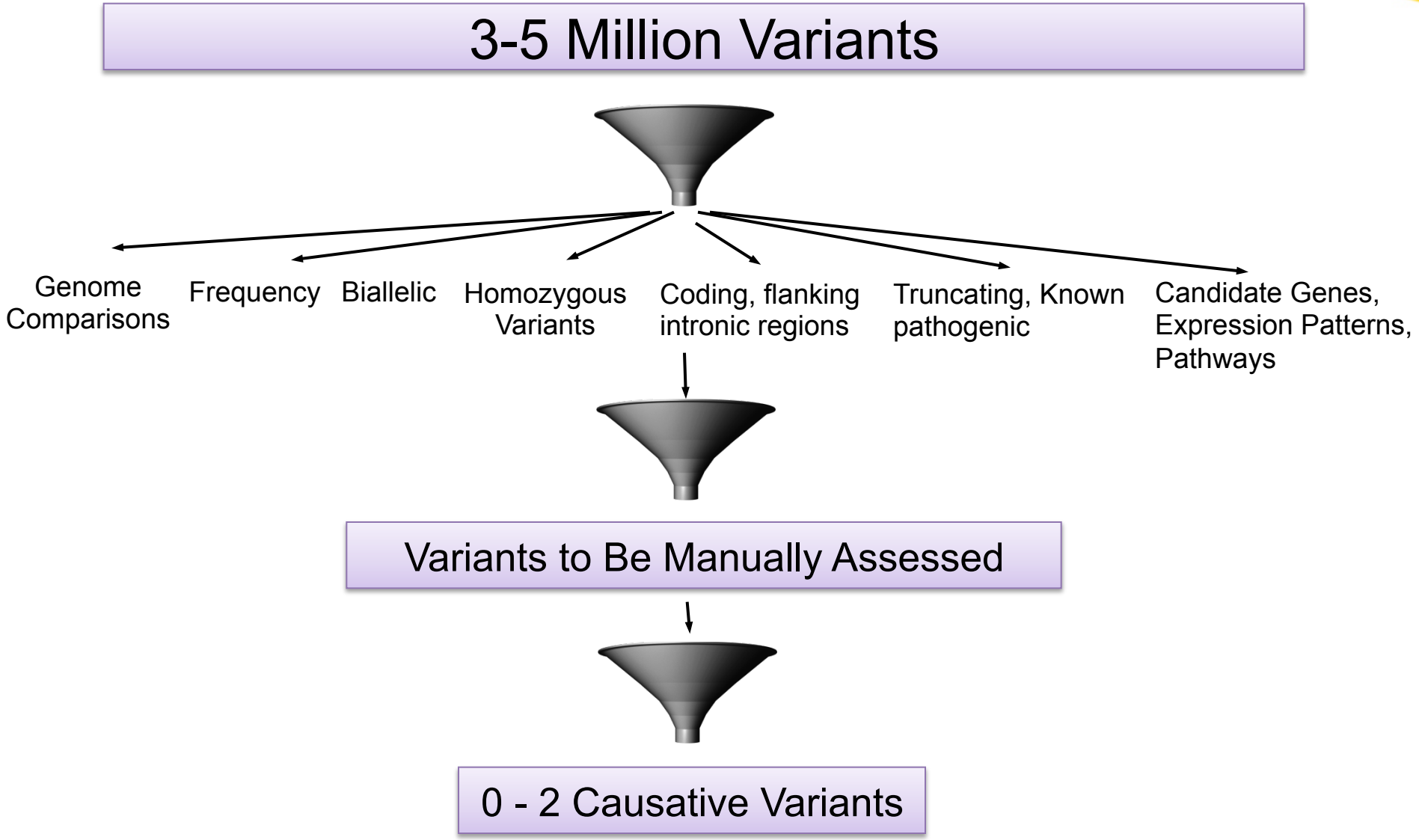
Case 2: Nonsyndromic Hearing Loss





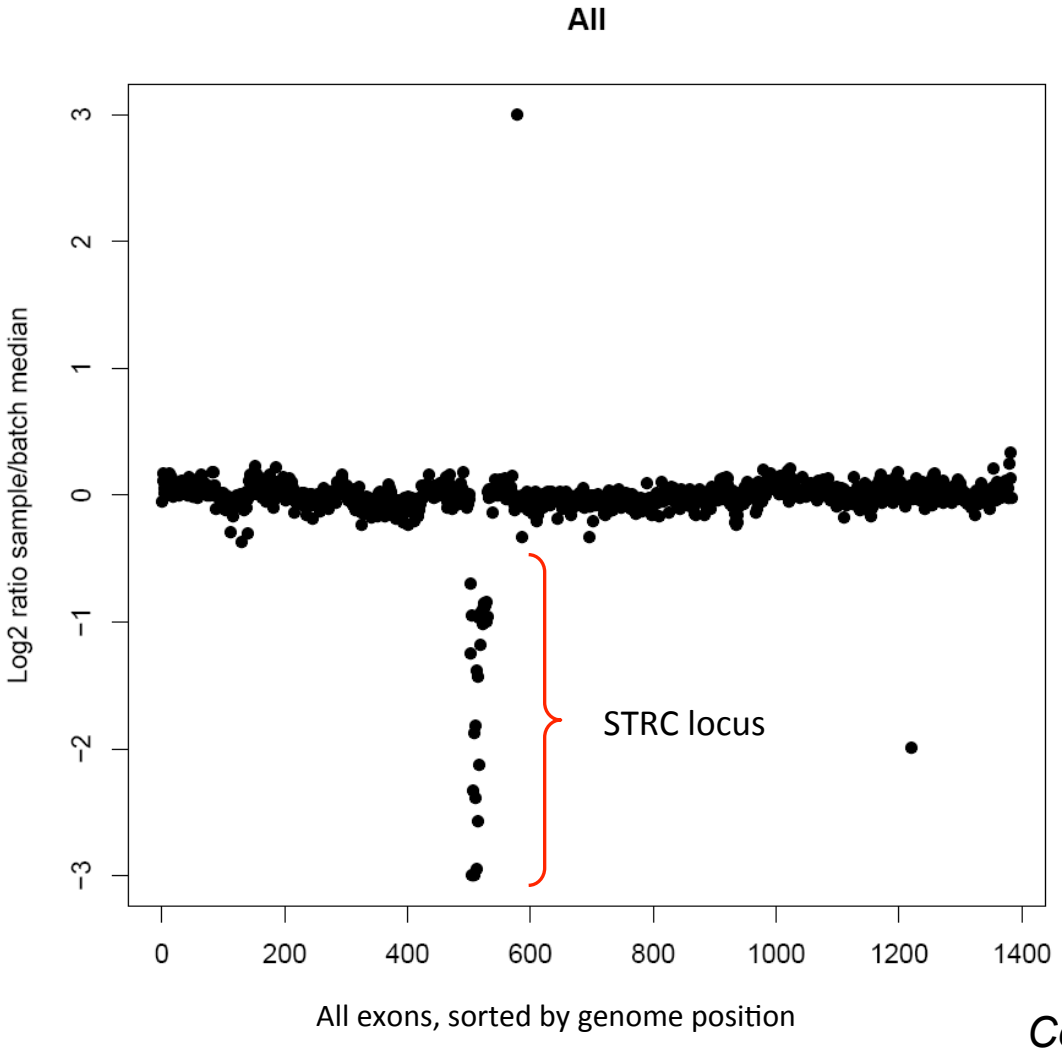
- Sept 2010: 2 yr old girl born presents to Genetics
- History of congenital bilateral sensorineural hearing loss
- Mild-moderate “cookie-bite” shaped audiogram
- No other complaints

Whole Genome Sequencing Data Analysis



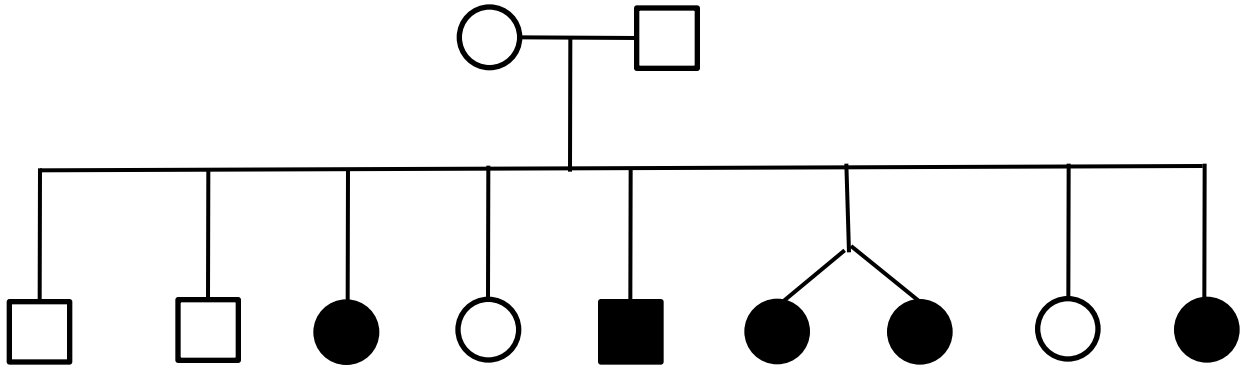
Analyzed WGS Case by OtoGenome

Included VisCap Analysis for CNVs



Courtesy of Trevor Pugh

Case 2: Nonsyndromic Hearing Loss



Affected children have a 100,000 bp deletion involving 4 genes.

STRC deletion causes hearing loss
CATSPER deletion causes male infertility

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.

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Trevor Pugh – geneticist

Jun Shen - fellow

LMM Staff

Patients, Families and Physicians