Protocadherin-15 and its implication in disease

This presentation investigates the structure of Protocadherin-15 (PCDH15) and its implication in disease, specifically Usher syndrome. Other proteins involved in Usher syndrome will also be presented.
The goal is to determine which mutations in the PCDH15 gene are pathogenic.

First, the structure of stereocilia will be presented with the two tethers. Second, PCDH15 and CDH23 will be shown histologically. Next, the structure of stereocilia with and without the PCDH15/CDH23 tip-link, plus the dimeric structure versus heterotetramic structure under increasing tension. Followed by a general presentation of two diseases that occur as a result of PCDH15 mutations. Finally, investigations reviewing a small sample size of a specific population are presented to give a better idea of the mutations identified in Usher syndrome.

Gene	Location	Protein
MYO7A	11q13.5	Myosin 7
USH1C	11p15.1	Harmonin
PCDH23	10q11.2-q21	Protocadherin-15
CDH15	10q22.1	Cadherin-23
DFNB31	9q32-34	Whirlin

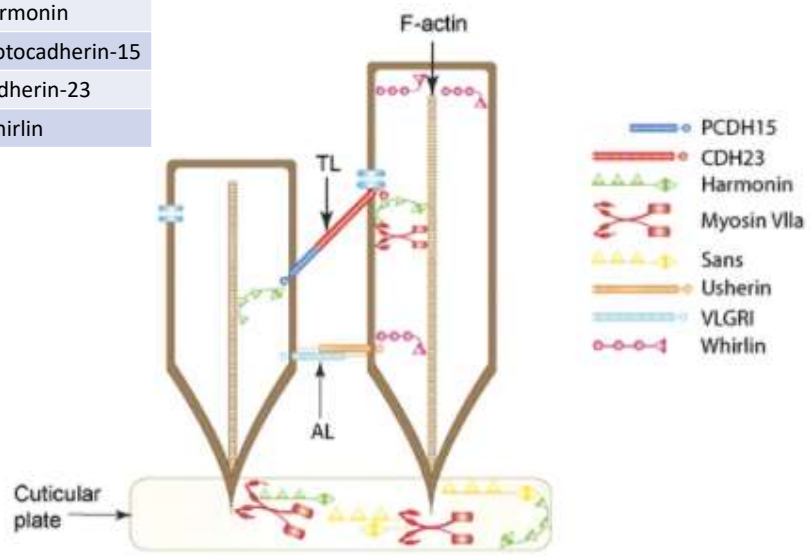


Image: https://www.nature.com/articles/jhg201029

Description (same source): The diagram shows the localization of CDH23 and PCDH15 at tip links. The binding of harmonin B to CDH23, PCDH15 and F-actin could anchor the interstereocilia links to the stereocilia actin core. Myo7a is believed to use long filaments of actin as tracks along which to transport other USH complex molecules. Sans located below the cuticular plate may have a role in trafficking molecules of the USH complex. Both Usherin and VLGR1 are members of the ankle links (AL) that are tethered to the actin stereocilia core through the scaffold proteins whirlin and possibly harmonin B.

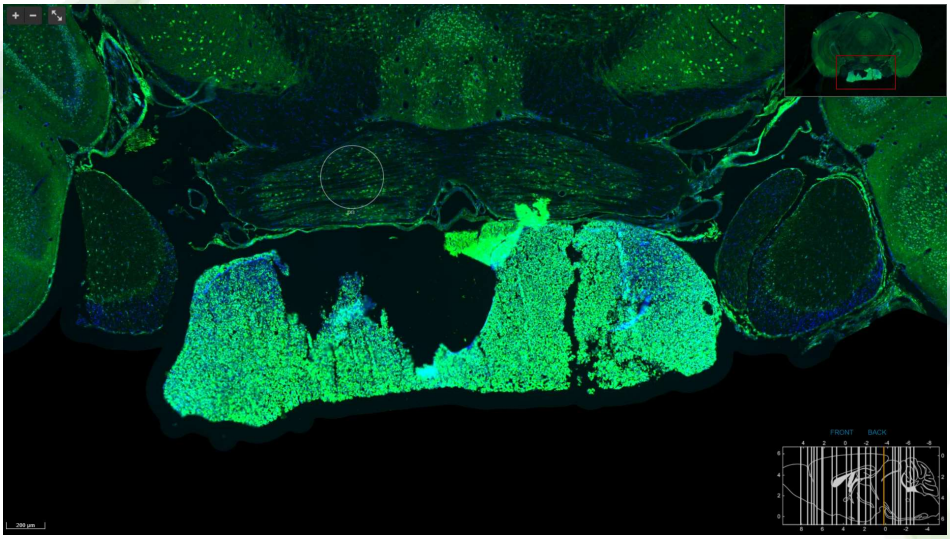
https://www.genecards.org/cgi-bin/carddisp.pl?gene=MYO7A&keywords=myo7A
https://www.genecards.org/cgi-bin/carddisp.pl?gene=USH1C&keywords=ush1c

Protocadherin-15 (PCDH15) and Cadherin-23 (CDH23)

We will take a look at both PCDH15 and CDH23 as these proteins are integral for the tip-link that aids in hearing via mechanotransduction.

Other proteins are also involved in this mechanotransduction and integrity of the stereocilia, i.e. whirlin and harmonin. We will only focus on the tip-link region, and more specifically PCDH15.

Protocadherin-15

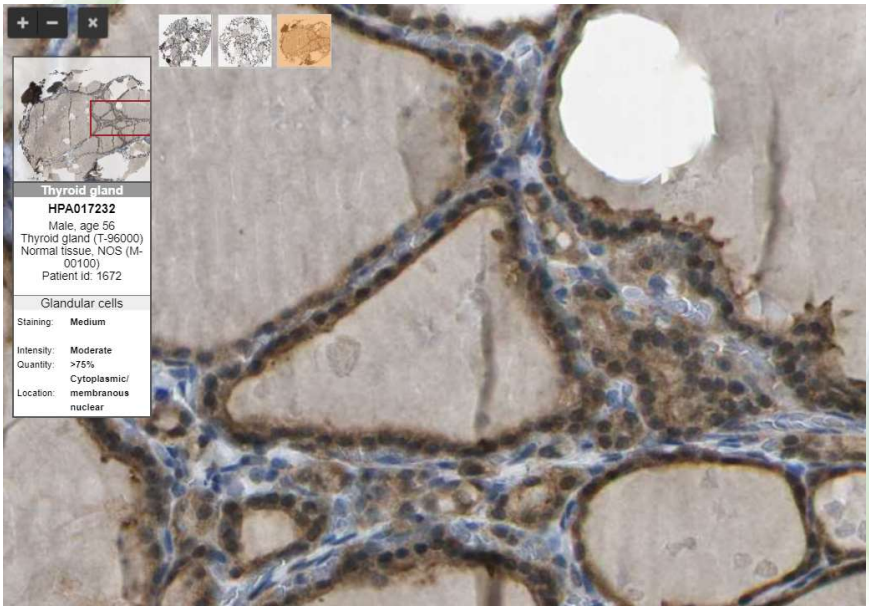


UniProt: Calcium-dependent cell-adhesion protein. Essential for maintenance of normal retinal and cochlear function.

Entrez: This gene is a member of the cadherin superfamily. Family members encode integral membrane proteins that mediate calcium-dependent cell-cell adhesion. It plays an essential role in maintenance of normal retinal and cochlear function. Mutations in this gene result in hearing loss and Usher Syndrome Type IF (USH1F). Extensive alternative splicing resulting in multiple isoforms has been observed in the mouse ortholog. Similar alternatively spliced transcripts are inferred to occur in human, and additional variants are likely to occur. [provided by RefSeq, Dec 2008]

Protocadherin-15 stains strongly in the median eminence (?) as well as other placed within the brain. Although it stains in this region of the brain, the region we want to focus on is in the ears.

Cadherin-23



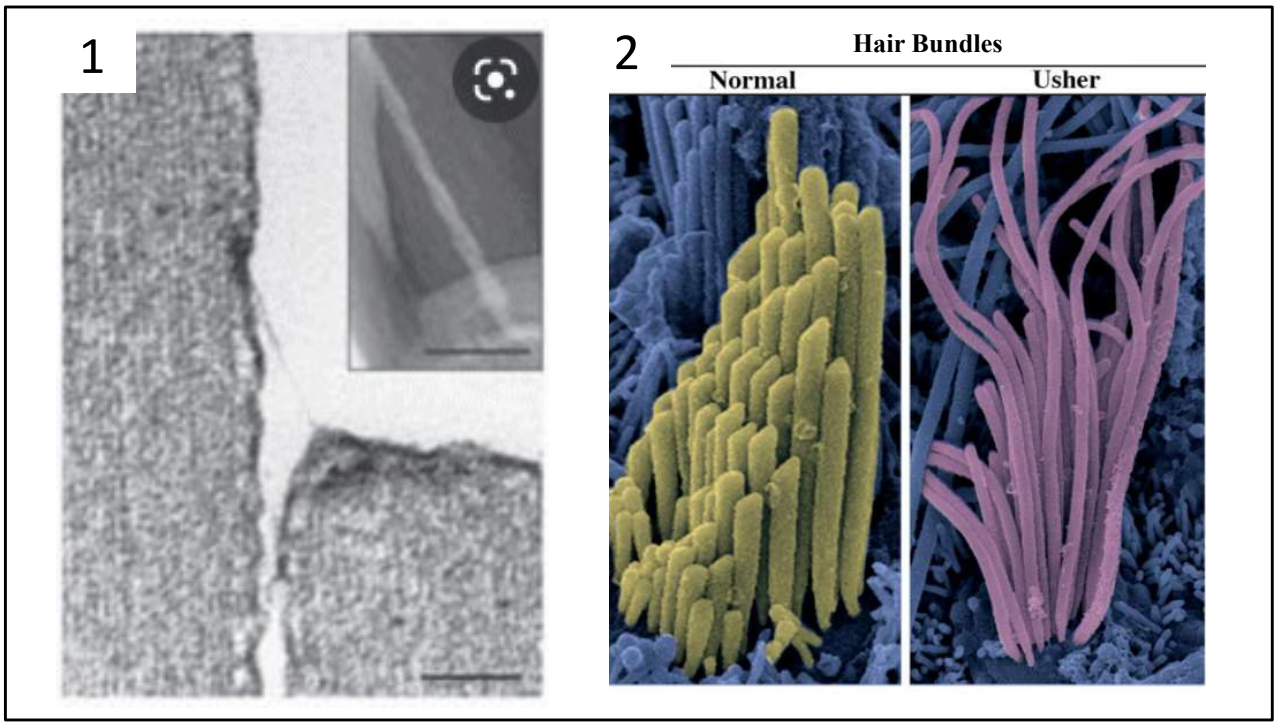
UniProt: Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells. CDH23 is required for establishing and/or maintaining the proper organization of the stereocilia bundle of hair cells in the cochlea and the vestibule during late embryonic/early postnatal development. It is part of the functional network formed by USH1C, USH1G, CDH23 and MYO7A that mediates mechanotransduction in cochlear hair cells. Required for normal hearing. (I WONDER WHY PCDH15 IS NOT MENTIONED HERE)

Entrez: This gene is a member of the cadherin superfamily, whose genes encode calcium dependent cell-cell adhesion glycoproteins. The encoded protein is thought to be involved in stereocilia organization and hair bundle formation. The gene is located in a region containing the human deafness loci DFNB12 and USH1D. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of this cadherin-like gene. Upregulation of this gene may also be associated with breast cancer. Alternative splice variants encoding different isoforms have been described. [provided by RefSeq, May 2013]

Stains moderately in the thyroid gland. Again, our focus is the ears, and there are no preparations uploaded to this site to demonstrate presence of CDH23 in the

ears.

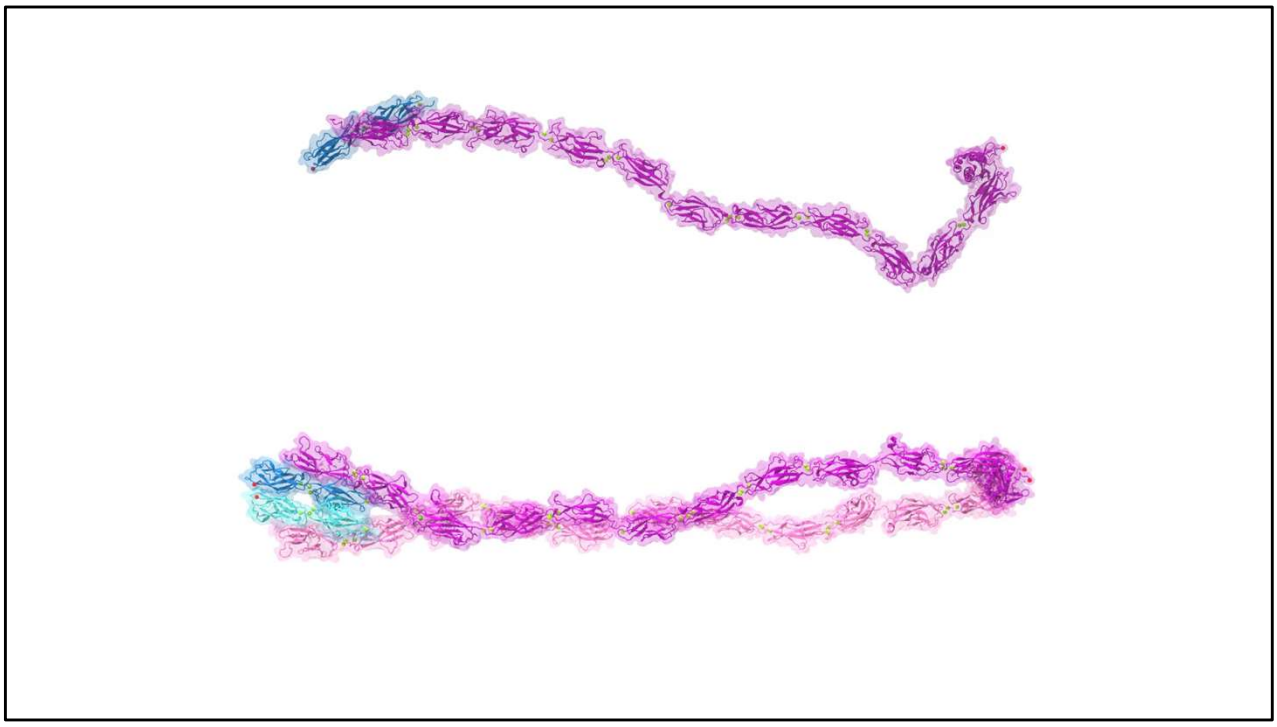
Structure



The images above highlight the tip-link structure and its importance in the auditory system. Image 1 shows the fine detail of the tip-link. Image two shows the difference between hair bundles with an in-tact tip-link and hair bundles without an intact tip-link. Mechanotransduction of sound relies on the precise movement of these hair bundles.

The tip-link is seemingly very delicate. As you can see from the comparison in image 2, the PCDH15-CDH23 protein complex may also play a role in stereocilia organization. This is difficult to determine from the image, because there are other proteins that are also involved in the organization of the stereocilia which are more basal.

Image 1: (2007). *Stringing the Fiddle: the Inner Ear's Two-Part Invention*, *10*, 1232–1233. https://doi.org/https://doi.org/10.1038/nn1007-1232
https://www.nature.com/articles/nn1007-1232#citeas
Image 2: https://www.eurekalert.org/news-releases/708128



TIP: when in presentation mode, from slide 7 to 8, hit the arrow (or enter/space, whatever advances you to the next slide), then hit that same key twice quickly so the videos play simultaneously (or close to).

These images show the difference between the heterotetramer of PCDH15 x2 and CDH23 x2 which make up one tip-link and the dimer PCDH15-CDH23. The importance of this slide is to demonstrate how stability increases in the heterotetramer form compared to the dimer.

The PCDH15 + CDH23 interaction with focus on the salt bridge (p.R113:p.E77) is important for the maintenance of the salt bridge, which seems to be the linkage that keeps PCDH15 and CDH23 linked unless under high amount of pressure.

In the setting of the ear, the sensitivity to disruptions in the hairs needs to be significant, in order to help maintain balance, hear a variety of sounds and help determine location of sound.

Disease

Usher Syndrome

Within Usher syndrome, there are a couple proteins that can be implicated. Referring back to slide 3, we are interested in the apical, diagonal one, made up of protocadherin-15 and cadherin-23.

Mutations in the tether towards the basal side of the stereocilia also result in Usher syndrome. In total, mutations in genes

https://www.mdhearingaid.com/en/product/mdhearingaid-hearing-aid-volt-pair?hea_variant=right&campaign_phone=&gclid=Cj0KCQjwnJaKBhDgARIsAHmvz6ewL0hJfzaQ3K73fs4lxKhRHTkk1vmgZwaurg3qY4bCITnjHAN2xvIaAlUTEALw_wcB
https://www.shooos.com/18131ff-chpo-noway-blue-light-glasses

Noise-induced hearing loss

Table 1. Distributions of *PCDH15* Alleles and Genotypes in the Case and Control Subjects

SNPs	Minor/major Allele (A1/A2)	Location	Minor Allele Frequency			*P* (H-W)[a]	A1A1/A1A2/A2A2		*P*[b]
			HapMap-CHB	Case	Control		Case	Control	
rs10825112	C/A	3'UTR	0.057	0.071	0.078	0.693	0/49/295	3/48/293	0.537
rs10825113	A/G	intron32	0.146	0.214	0.201	0.116	17/113/212	19/100/225	0.593
rs1900443	T/C	intron27	0.306	0.259	0.230	1.000	22/134/185	18/122/204	0.251
rs12258253	C/T	intron25	0.278	0.243	0.219	0.509	22/123/197	14/123/204	0.479
rs2135720	G/A	exon20	0.427	0.507	0.480	0.792	89/171/82	78/174/89	0.335
rs11004085	C/T	intron16	0.023	0.061	0.030	0.129	0/21/323	1/8/333	**0.039**
rs11004142	C/A	intron9	0.159	0.156	0.153	0.504	9/89/246	10/85/249	0.957
rs996320	A/G	intron9	0.159	0.177	0.154	0.328	14/94/236	11/84/247	0.364
rs7081730	T/C	intron8	0.232	0.195	0.170	0.085	18/98/228	15/87/242	0.312
rs978842	C/T	intron7	0.222	0.193	0.235	0.550	15/103/225	18/126/200	0.118

file:///C:/Users/finnokelly/Downloads/The+Effect+of+PCDH15+Gene+Variations+on+the+Risk+of+Noise-induced+Hearing+Loss+in+a+Chinese+Population.pdf

The Effect of PCDH15 Gene Variations on the Risk of Noise-induced Hearing Loss in a Chinese population, by XU Xiang Rong et.al

"The results shows that compared with the TT genotype of rs11004085, CT/CC genotypes were associated with an increased risk of NIHL. Additionally, significant interactions between the noise exposure were observed in the high-level exposure groups. Furthermore, the risk haplotype TAGCC was observed when combined with higher levels of noise exposure."

Genetic predisposition in addition to environmental stressors result in NIHL.

"Thus, this SNP genotype (CT/CC on rs11004085) was identified as a risk factor associated with NIHL. Although this paper does not focus on Usher syndrome, or the other tether proteins of the stereocilia, SNPs have been identified as disease-causing.

Of note, PCDH15 exons 2 and 10 have been implicated in Usher syndrome. In this paper focusing on NIHL, rs11004085 is on intron 16.


Genetics

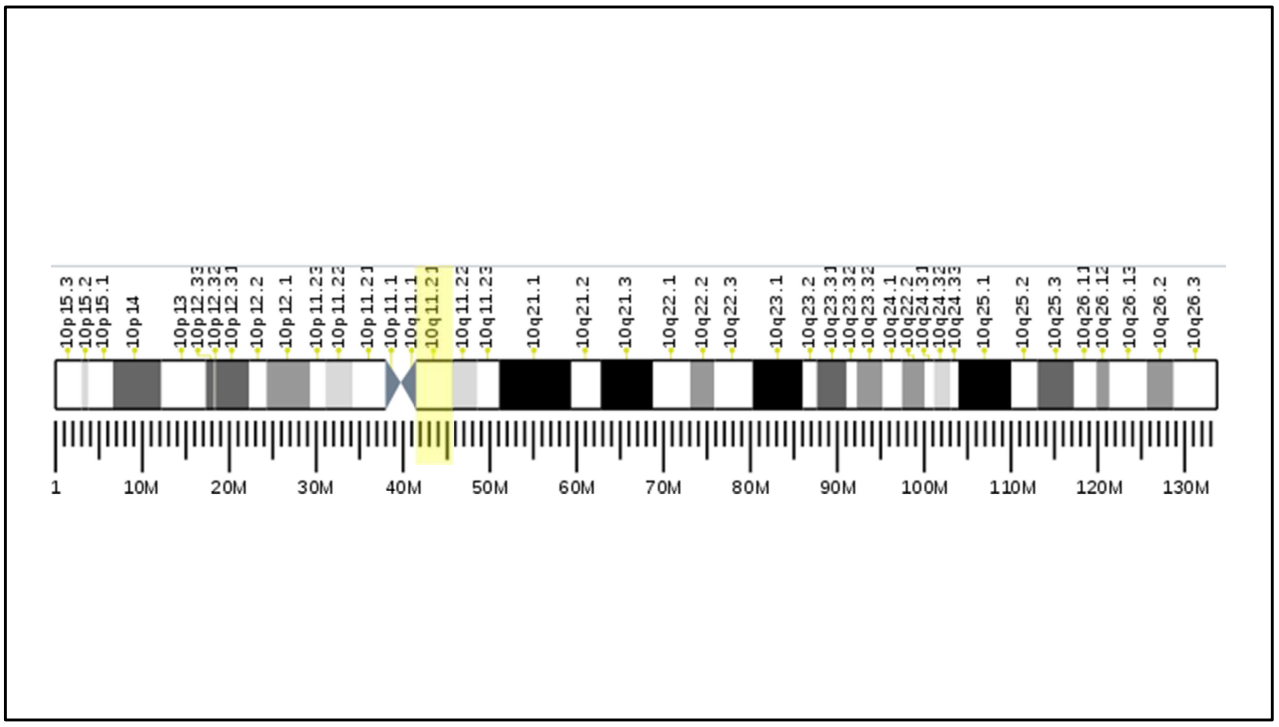
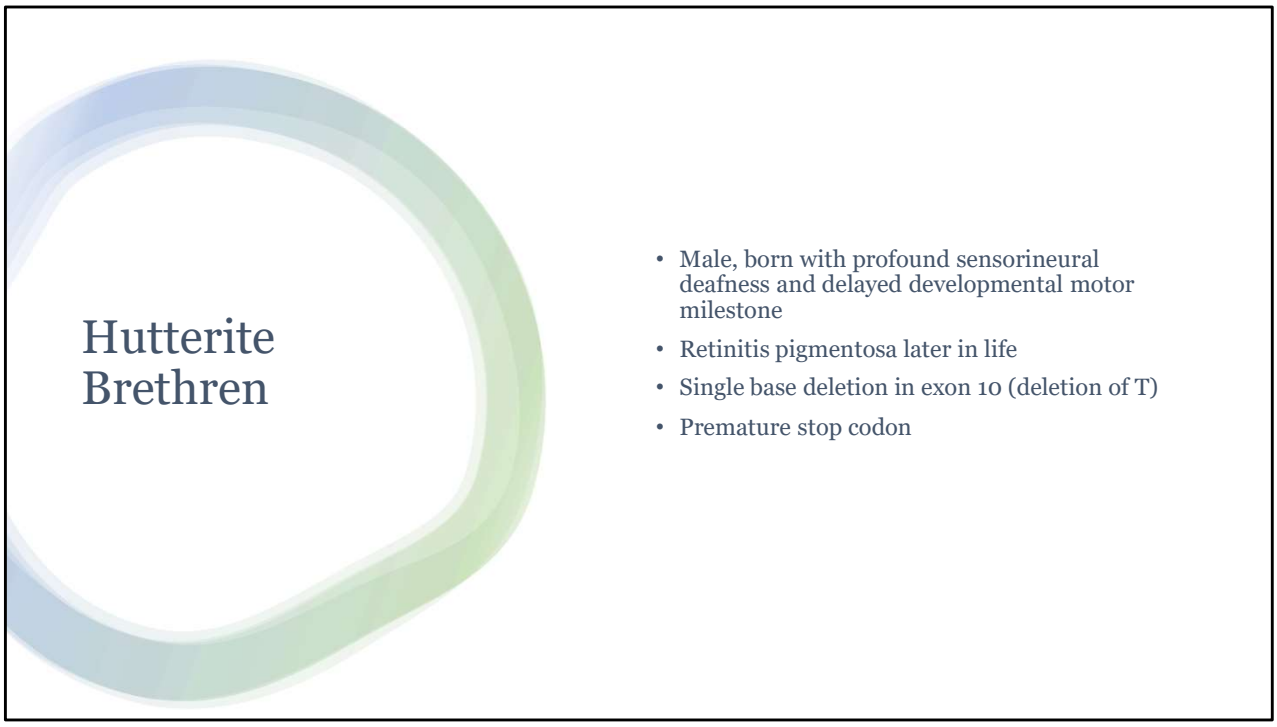


Image: https://en.wikipedia.org/wiki/Chromosome_10

Our focus will be on the protocadherin-15 protein which is found at locus 10q11.2-q21



The paper by Alagramam et. al. investigates two families with Usher syndrome type I (USH1F). One family is from the Hutterite Brethren ethnic group, and the other is from a consanguineous Indian family.

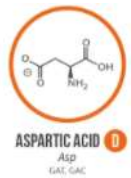
IMPORTANT: 15cM region on chromosome 10 flanked by markers D10S199 and D10S596. LOD of this interval in both cases was greater than 3, indicating linked genes.

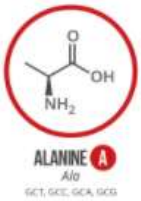
Consanguineous Indian

- Male, born with profound sensorineural deafness and delayed developmental motor milestone
- Retinitis pigmentosa later in life
- Nonsense mutation in exon 2
- Premature stop codon

Consanguineous Pakistani

- Linkage to chromosome 10q21.1
- Three different point mutations identified, one of which was found to be segregating with the disease phenotype
- c.1304A>C missense mutation
- Amino acid aspartic acid > alanine at residue 435





https://pubmed-ncbi-nlm-nih-gov.gcsom.idm.oclc.org/27275418/
In silico analysis of a disease-causing mutation in PCDH15 gene in a consanguineous Pakistani family with Usher phenotype by S. Saleha et. al.

Note that this paper identified residue 435 as being pathogenic, but in the paper by Alagramam, 419 was identified as an important residue in disease pathogenesis.
Aspartic acid is a negatively charged amino group, with carboxyl group.
Alanine is a non-polar amino acid

The codes for aspartic acid: GAT, GAC
The codes for alanine: GCT, GCC, GCA, GCG

Since the mutation was identified as an A>C transversion, it would be either GAT or GAC that have been converted to GCT or GCC which would result in the change in amino acid.

Images: https://i0.wp.com/www.compoundchem.com/wp-content/uploads/2014/09/20-Common-Amino-Acids-v3.png?ssl=1

Spanish patients

- In most patients, detected mutations led to a truncated protein, reinforcing the hypothesis that severe mutations cause the Usher I phenotype and that missense variants are mainly responsible for non-syndromic hearing impairment

Table 1

Probable pathogenic point mutations identified in Spanish Usher I patients in the *PCDH15* gene.

Nucleotide change	Amino acid change	Exon/ intron	Number patients/ alleles	SNP	Domain	References
Missense						
c.401G>A	p.R134Q	5	1/1	—	EC1	[22]
Frameshift						
c.1304_1305insC	**p.T436YfsX12**	11	1/1	—	EC4	This study
Splice-site						
c.2868+5G>A	—	21	1/1	—	EC9	This study
c.3717+2dupT	—	27	1/2	—	EC11	This study
Nonsense						
c.733C>T	p.R245X	8	1/1	rs111033260	EC2	[24]
c.1737C>G	**p.Y579X**	14	1/1	—	EC5	This study
c.2782A>T	**p.K928X**	21	1/2	—	—	This study

In bold: Variants not previously reported.

https://www-ncbi-nlm-nih-gov.gcsom.idm.oclc.org/pmc/articles/PMC3398493/

Mutation screening of the *PCDH15* gene in Spanish patients with Usher syndrome type I by T. Jaijo et al.