Foundation Fighting Blindness

Usher Syndrome 1B Landscape

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Selected Reference Abstracts

Table of Contents

Genes and Variants

Jouret G, Poirsier C, Spodenkiewicz M, et al. Genetics of Usher syndrome: new insights from a meta-analysis. *Otol Neurotol*. 2019;40(1):121-129.

Objective: To describe the genetic and phenotypic spectrum of Usher syndrome after 6 years of studies by next-generation sequencing, and propose an up-to-date classification of Usher genes in patients with both visual and hearing impairments suggesting Usher syndrome, and in patients with seemingly isolated deafness. Study design: The systematic review and meta-analysis protocol was based on Cochrane and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We performed 1) a meta-analysis of data from 11 next-generation sequencing studies in 684 patients with Usher syndrome; 2) a meta-analysis of data from 21 next-generation studies in 2,476 patients with seemingly isolated deafness, to assess the involvement of Usher genes in seemingly nonsyndromic hearing loss, and thus the proportion of patients at high risk of subsequent retinitis pigmentosa (RP); 3) a statistical analysis of differences between parts 1) and 2). Results: In patients with both visual and hearing impairments, the biallelic disease-causing mutation rate was assessed for each Usher gene to propose a classification by frequency: USH2A: 50% (341/684) of patients, MYO7A: 21% (144/684), CDH23: 6% (39/684), ADGRV1: 5% (35/684), PCDH15: 3% (21/684), USH1C: 2% (17/684), CLRN1: 2% (14/684), USH1G: 1% (9/684), WHRN: 0.4% (3/684), PDZD7 0.1% (1/684), CIB2 (0/684). In patients with seemingly isolated sensorineural deafness, 7.5% had disease-causing mutations in Usher genes, and are therefore at high risk of developing RP. These new findings provide evidence that usherome dysfunction is the second cause of genetic sensorineural hearing loss after connexin dysfunction. Conclusion: These results promote generalization of early molecular screening for Usher syndrome in deaf children.

Kuppa A, Sergeev YV. Homology modeling and global computational mutagenesis of human myosin VIIa. *J Anal Pharm Res*. 2021;10(1):41-48.

PMID: 33889793

Usher syndrome type 1B (USH1B) is a genetic disorder caused by mutations in the unconventional Myosin VIIa (MYO7A) protein. USH1B is characterized by hearing loss due to abnormalities in the inner ear and vision loss due to retinitis pigmentosa. Here, we present the model of human MYO7A homodimer, built using homology modeling, and refined using 5 ns molecular dynamics in water. Global computational mutagenesis was applied to evaluate the effect of missense mutations that are critical for maintaining protein structure and stability of MYO7A in inherited eye disease. We found that 43.26% (77 out of 178 in HGMD) and 41.9% (221 out of 528 in ClinVar) of the disease-related missense mutations were associated with higher protein structure destabilizing effects. Overall, most mutations destabilizing the MYO7A protein were found to associate with USH1 and USH1B. Particularly, motor domain and MyTH4 domains were found to be most susceptible to mutations causing the USH1B phenotype. Our work contributes to the understanding of inherited disease from the atomic level of protein structure and analysis of the impact of genetic mutations on protein stability and genotype-to-phenotype relationships in human disease.

Lu Y, Zhou D, King R, et al. The genetic dissection of *Myo7a* gene expression in the retinas of BXD mice. *Mol Vis*. 2018;24:115-126.
PMID: 29430167
Purpose: Usher syndrome (US) is characterized by a loss of vision due to retinitis pigmentosa (RP) and deafness. US has three clinical subtypes, but even within each subtype, the severity varies. Myosin VIIA, coded by *Myo7a*, has been identified as one of the causal genes of US. This study aims to identify pathways and other genes through which *Myo7a* interacts to affect the presentation of US symptoms. Methods: In this study, we used the retinal tissue of BXD recombinant inbred (RI) mice to examine the expression of *Myo7a* and perform genetic mapping. Expression quantitative trait locus (eQTL), single nucleotide polymorphism (SNP), and gene correlation analysis were performed using GeneNetwork. Gene set enrichment analysis was performed using WebGestalt, and gene network construction was performed using the Gene Cohesion Analysis Tool. Results: We found *Myo7a* to be *cis*-regulated, with varied levels of

expression across BXD strains. Here, we propose a genetic network with 40 genes whose expression is highly correlated with *Myo7a*. Among these genes, six have been linked to retinal diseases, three to deafness, and five share a transcription factor with *Myo7a.* Gene ontology and pathway analysis revealed a strong connection among ion channel activity, *Myo7a*, and US. Conclusions: Although *Myo7a* is a causal gene of US type I, this gene works with many other genes and pathways to affect the severity of US. Many of the genes found in the genetic network, pathways, and gene ontology categories of *Myo7a* are related to either deafness or blindness. Further investigation is needed to examine the specific relationships between these genes, which may assist in the treatment of US.

Pathology

Gibbs D, Kitamoto J, Williams DS. Abnormal phagocytosis by retinal pigmented epithelium that lacks myosin VIIa, the Usher syndrome 1B protein. *Proc Natl Acad Sci U S A*. 2003;100(11):6481-6486.

PMID: 12743369

Mutations in the myosin VIIa gene (MYO7A) cause Usher syndrome type 1B (USH1B), a major type of the deaf-blind disorder, Usher syndrome. We have studied mutant phenotypes in the retinas of Myo7a mutant mice (shaker1), with the aim of elucidating the role(s) of myosin VIIa in the retina and what might underlie photoreceptor degeneration in USH1B patients. A photoreceptor defect has been described. Here, we report that the phagocytosis of photoreceptor outer segment disks by the retinal pigment epithelium (RPE) is abnormal in Myo7a null mice. Both in vivo and in primary cultures of RPE cells, the transport of ingested disks out of the apical region is inhibited in the absence of Myo7a. The results with the cultured RPE cells were the same, irrespective of whether the disks came from wild-type or mutant mice, thus demonstrating that the RPE is the source of this defect. The inhibited transport seems to delay phagosome-lysosomal fusion, as the degradation of ingested disks was slower in mutant RPE. Moreover, fewer packets of disk membranes were ingested in vivo, possibly because retarded removal of phagosomes from the apical processes inhibited the ingestion of additional disk membranes. We

conclude that Myo7a is required for the normal processing of ingested disk membranes in the RPE, primarily in the basal transport of phagosomes into the cell body where they then fuse with lysosomes. Because the phagocytosis of photoreceptor disks by the RPE has been shown to be critical for photoreceptor cell viability, this defect likely contributes to the progressive blindness in USH1B.

Hasson T, Heintzelman MB, Santos-Sacchi J, Corey DP, Mooseker MS. Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type 1B. *Proc Natl Acad Sci U S A*. 1995;92(21):9815-9819.

PMID: 7568224

Myosin VIIa is a newly identified member of the myosin superfamily of actin-based motors. Recently, the myosin VIIa gene was identified as the gene defective in shaker-1, a recessive deafness in mice [Gibson, F., Walsh, J., Mburu, P., Varela, A., Brown, K.A., Antonio, M., Beisel, K.W., Steel, K.P. & Brown, S.D.M. (1995) Nature (London) 374, 62-64], and in human Usher syndrome type 1B, an inherited disease characterized by congenital deafness, vestibular dysfunction, and retinitis pigmentosa [Weil, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., Mburu, P., Varela, A., Levilliers, J., Weston, M.D., Kelley, P.M., Kimberling, W.J., Wagenaar, M., Levi-Acobas, F., Larget-Piet, D., Munnich, A., Steel, K.P., Brown, S.D.M. & Petit, C. (1995) Nature (London) 374, 60-61]. To understand the normal function of myosin VIIa and how it could cause these disease phenotypes when defective, we generated antibodies specific to the tail portion of this unconventional myosin. We found that myosin VIIa was expressed in cochlea, retina, testis, lung, and kidney. In cochlea, myosin VIIa expression was restricted to the inner and outer hair cells, where it was found in the apical stereocilia as well as the cytoplasm. In the eye, myosin VIIa was expressed by the retinal pigmented epithelial cells, where it was enriched within the apical actin-rich domain of this cell type. The cell-specific localization of myosin VIIa suggests that the blindness and deafness associated with Usher syndrome is due to lack of proper myosin VIIa function within the cochlear hair cells and the retinal pigmented epithelial cells

Li J, Chen Y, Deng Y, et al. Ca^{2+}-induced rigidity change of the myosin VIIa IQ motif-single α helix lever arm extension. *Structure*. 2017;25(4):579-591.e4.
PMID: 28262393
Several unconventional myosins contain a highly charged single α helix (SAH) immediately following the calmodulin (CaM) binding IQ motifs, functioning to extend lever arms of these myosins. How such SAH is connected to the IQ motifs and whether the conformation of the IQ motifs-SAH segments are regulated by Ca^{2+} fluctuations are not known. Here, we demonstrate by solving its crystal structure that the predicted SAH of myosin VIIa (Myo7a) forms a stable SAH. The structure of Myo7a IQ5-SAH segment in complex with apo-CaM reveals that the SAH sequence can extend the length of the Myo7a lever arm. Although Ca^{2+}-CaM remains bound to IQ5-SAH, the Ca^{2+}-induced CaM binding mode change softens the conformation of the IQ5-SAH junction, revealing a Ca^{2+}-induced lever arm flexibility change for Myo7a. We further demonstrate that the last IQ motif of several other myosins also binds to both apo- and Ca^{2+}-CaM, suggesting a common Ca^{2+}-induced conformational regulation mechanism.

Liu X, Vansant G, Udovichenko IP, Wolfrum U, Williams DS. Myosin VIIa, the product of the Usher 1B syndrome gene, is concentrated in the connecting cilia of photoreceptor cells. *Cell Motil Cytoskeleton*. 1997;37(3):240-252.
PMID: 9227854
Usher syndrome is the most common form of combined deafness and blindness. The gene that is defective in Usher syndrome 1B (USH1B) encodes for an unconventional myosin, myosin VIIa. To understand the cellular function of myosin VIIa and why defects in it lead to USH1B, it is essential to determine the precise cellular and subcellular localization of the protein. We investigated the distribution of myosin VIIa in human and rodent photoreceptor cells and retinal pigment epithelium (RPE), primarily by immunoelectron microscopy, using antibodies generated against two different domains of the protein. In both human and rodent retinae, myosin VIIa was detected in the apical processes of the RPE and in the cilium of

rod and cone photoreceptor cells. Immunogold label was most concentrated in the connecting cilium. Here, myosin VIIa appeared to be distributed outside the ring of doublet microtubules near the ciliary plasma membrane. These observations indicate that a major role of myosin VIIa in the retina is in the photoreceptor cilium, perhaps in such a function as trafficking newly synthesized phototransductive membrane or maintaining the diffusion barrier between the inner and outer segments. Our results support the notion that defective ciliary function is the underlying cellular abnormality that leads to cellular degeneration in Usher syndrome.

Liu X, Udovichenko IP, Brown SD, Steel KP, Williams DS. Myosin VIIa participates in opsin transport through the photoreceptor cilium. *J Neurosci*. 1999;19(15):6267-6274.

PMID: 10414956

Two types of Usher syndrome, a blindness-deafness disorder, result from mutations in the myosin VIIa gene. As for most other unconventional myosins, little is known about the function or functions of myosin VIIa. Here, we studied the photoreceptor cells of mice with mutant myosin VIIa by electron immunomicroscopy and microscopic autoradiography. We found evidence that myosin VIIa functions in the connecting cilium of each photoreceptor cell and participates in the transport of opsin through this structure. These findings provide the first direct evidence that opsin travels along the connecting cilium en route to the outer segment. They demonstrate that a myosin may function in a cilium and suggest that abnormal opsin transport might contribute to blindness in Usher syndrome.

Lopes VS, Ramalho JS, Owen DM, Karl MO, Strauss O, Futter CE, Seabra MC. The ternary Rab27a-Myrip-Myosin VIIa complex regulates melanosome motility in the retinal pigment epithelium. *Traffic*. 2007;8(5):486-499.

PMID: 17451552

The retinal pigment epithelium (RPE) contains melanosomes similar to those found in the skin melanocytes, which undergo dramatic light-dependent movements in fish and amphibians. In mammals, those movements are more subtle and appear to be regulated by the Rab27a

GTPase and the unconventional myosin, Myosin VIIa (MyoVIIa). Here we address the hypothesis that a recently identified Rab27a- and MyoVIIa-interacting protein, Myrip, promotes the formation of a functional tripartite complex. In heterologous cultured cells, all three proteins co-immunoprecipitated following overexpression. Rab27a and Myrip localize to the peripheral membrane of RPE melanosomes as observed by immunofluorescence and immunoelectron microscopy. Melanosome dynamics were studied using live-cell imaging of mouse RPE primary cultures. Wild-type RPE melanosomes exhibited either stationary or slow movement interrupted by bursts of fast movement, with a peripheral directionality trend. Nocodazole treatment led to melanosome paralysis, suggesting that movement requires microtubule motors. Significant and similar alterations in melanosome dynamics were observed when any one of the three components of the complex was missing, as studied in ashen- (Rab27a defective) and shaker-1 (MyoVIIa mutant)-derived RPE cells, and in wild-type RPE cells transduced with adenovirus carrying specific sequences to knockdown Myrip expression. We observed a significant increase in the number of motile melanosomes, exhibiting more frequent and prolonged bursts of fast movement, and inversion of directionality. Similar alterations were observed upon cytochalasin D treatment, suggesting that the Rab27a-Myrip-MyoVIIa complex regulates tethering of melanosomes onto actin filaments, a process that ensures melanosome movement towards the cell periphery

Sahly I, Dufour E, Schietroma C, et al. Localization of Usher 1 proteins to the photoreceptor calyceal processes, which are absent from mice. *J Cell Biol*. 2012;199(2):381-399.

PMID: 23045546

The mechanisms underlying retinal dystrophy in Usher syndrome type I (USH1) remain unknown because mutant mice lacking any of the USH1 proteins-myosin VIIa, harmonin, cadherin-23, protocadherin-15, sans-do not display retinal degeneration. We found here that, in macaque photoreceptor cells, all USH1 proteins colocalized at membrane interfaces (i) between the inner and outer segments in rods and (ii) between the microvillus-like calyceal processes and the outer segment basolateral

region in rods and cones. This pattern, conserved in humans and frogs, was mediated by the formation of an USH1 protein network, which was associated with the calyceal processes from the early embryonic stages of outer segment growth onwards. By contrast, mouse photoreceptors lacked calyceal processes and had no USH1 proteins at the inner-outer segment interface. We suggest that USH1 proteins form an adhesion belt around the basolateral region of the photoreceptor outer segment in humans, and that defects in this structure cause the retinal degeneration in USH1 patients.

Sakai T, Jung HS, Sato O, et al. Structure and regulation of the movement of human myosin VIIA. *J Biol Chem*. 2015;290(28):17587-17598.

PMID: 26001786

Human myosin VIIA (HM7A) is responsible for human Usher syndrome type 1B, which causes hearing and visual loss in humans. Here we studied the regulation of HM7A. The actin-activated ATPase activity of full-length HM7A (HM7AFull) was lower than that of tail-truncated HM7A (HM7AΔTail). Deletion of the C-terminal 40 amino acids and mutation of the basic residues in this region (R2176A or K2179A) abolished the inhibition. Electron microscopy revealed that HM7AFull is a monomer in which the tail domain bends back toward the head-neck domain to form a compact structure. This compact structure is extended at high ionic strength or in the presence of Ca(2+). Although myosin VIIA has five isoleucine-glutamine (IQ) motifs, the neck length seems to be shorter than the expected length of five bound calmodulins. Supporting this observation, the IQ domain bound only three calmodulins in Ca(2+), and the first IQ motif failed to bind calmodulin in EGTA. These results suggest that the unique IQ domain of HM7A is important for the tail-neck interaction and, therefore, regulation. Cellular studies revealed that dimer formation of HM7A is critical for its translocation to filopodial tips and that the tail domain (HM7ATail) markedly reduced the filopodial tip localization of the HM7AΔTail dimer, suggesting that the tail-inhibition mechanism is operating in vivo. The translocation of the HM7AFull dimer was significantly less than that of the HM7AΔTail dimer, and R2176A/R2179A mutation rescued the filopodial tip translocation. These results suggest that HM7A

can transport its cargo molecules, such as USH1 proteins, upon release of the tail-dependent inhibition.

Disease Models

Calabro KR, Boye SL, Choudhury S, et al. A novel mouse model of *MYO7A* USH1B reveals auditory and visual system haploinsufficiencies. *Front Neurosci*. 2019;13:1255.

PMID: 31824252

Usher's syndrome is the most common combined blindness-deafness disorder with USH1B, caused by mutations in *MYO7A*, resulting in the most severe phenotype. The existence of numerous, naturally occurring *shaker1* mice harboring variable *MYO7A* mutations on different genetic backgrounds has complicated the characterization of MYO7A knockout (KO) and heterozygote mice. We generated a novel MYO7A KO mouse (*Myo7a* $^{-/-}$) that is easily genotyped, maintained, and confirmed to be null for MYO7A in both the eye and inner ear. Like USH1B patients, *Myo7a* $^{-/-}$ mice are profoundly deaf, and display near complete loss of inner and outer cochlear hair cells (HCs). No gross structural changes were observed in vestibular HCs. *Myo7a* $^{-/-}$ mice exhibited modest declines in retinal function but, unlike patients, no loss of retinal structure. We attribute the latter to differential expression of MYO7A in mouse vs. primate retina. Interestingly, heterozygous *Myo7a* $^{+/-}$ mice had reduced numbers of cochlear HCs and concomitant reductions in auditory function relative to *Myo7a* $^{+/+}$ controls. Notably, this is the first report that loss of a single *Myo7a* allele significantly alters auditory structure and function and suggests that audiological characterization of USH1B carriers is warranted. Maintenance of vestibular HCs in *Myo7a* $^{-/-}$ mice suggests that gene replacement could be used to correct the vestibular dysfunction in USH1B patients. While *Myo7a* $^{-/-}$ mice do not exhibit sufficiently robust retinal phenotypes to be used as a therapeutic outcome measure, they can be used to assess expression of vectored *MYO7A* on a null background and generate valuable pre-clinical data toward the treatment of USH1B.

Colella P, Sommella A, Marrocco E, et al. Myosin7a deficiency results in reduced retinal activity which is improved by gene therapy. *PLoS One*. 2013;8(8):e72027.

PMID: 23991031

Mutations in MYO7A cause autosomal recessive Usher syndrome type IB (USH1B), one of the most frequent conditions that combine severe congenital hearing impairment and retinitis pigmentosa. A promising therapeutic strategy for retinitis pigmentosa is gene therapy, however its pre-clinical development is limited by the mild retinal phenotype of the shaker1 (sh1(-/-)) murine model of USH1B which lacks both retinal functional abnormalities and degeneration. Here we report a significant, early-onset delay of sh1(-/-) photoreceptor ability to recover from light desensitization as well as a progressive reduction of both b-wave electroretinogram amplitude and light sensitivity, in the absence of significant loss of photoreceptors up to 12 months of age. We additionally show that subretinal delivery to the sh1(-/-) retina of AAV vectors encoding the large MYO7A protein results in significant improvement of sh1(-/-) photoreceptor and retinal pigment epithelium ultrastructural anomalies which is associated with improvement of recovery from light desensitization. These findings provide new tools to evaluate the efficacy of experimental therapies for USH1B. In addition, although AAV vectors expressing large genes might have limited clinical applications due to their genome heterogeneity, our data show that AAV-mediated MYO7A gene transfer to the sh1(-/-) retina is effective.

Derks MFL, Megens HJ, Giacomini WL, Groenen MAM, Lopes MS. A natural knockout of the MYO7A gene leads to pre-weaning mortality in pigs. *Anim Genet*. 2021;52(4):514-517.

PMID: 33955556

The pig breeding system provides a unique framework to study recessive defects and the consequence on the phenotype. We examined a commercial synthetic Duroc population for recessive defects and identified a haplotype on chromosome 9 significantly affecting pre-weaning mortality. To identify the causal variant underlying the mortality, we examined sequence data of four carrier animals and 21 non-carrier animals from the

same population. The results yield a strong candidate causal stop-gained variant (NM_001099928.1:c.541C>T) affecting the MYO7A gene in complete linkage disequilibrium with the lethal haplotype. The variant leads to an impaired (p.Gln181*) MYO7A protein that truncates 2032 amino acids from the protein. We examined a litter from a carrier sow inseminated by a carrier boar. From the resulting piglets, two confirmed homozygous piglets suffered from severe balance difficulties and the inability to walk properly. The variant segregates at a carrier frequency of 8.2% in the evaluated population and will be gradually purged from the population, improving animal welfare. Finally, this 'natural knockout' will increase our understanding of the functioning of the MYO7A gene and provides a potential model for Usher syndrome in humans.

Peng YW, Zallocchi M, Wang WM, Delimont D, Cosgrove D. Moderate light-induced degeneration of rod photoreceptors with delayed transducin translocation in shaker1 mice. *Invest Ophthalmol Vis Sci*. 2011;52(9):6421-6427.

PMID: 21447681

PURPOSE. Usher syndrome is characterized by congenital deafness associated with retinitis pigmentosa (RP). Mutations in the myosin VIIa gene (MYO7A) cause a common and severe subtype of Usher syndrome (USH1B). Shaker1 mice have mutant MYO7A. They are deaf and have vestibular dysfunction but do not develop photoreceptor degeneration. The goal of this study was to investigate abnormalities of photoreceptors in shaker1 mice. METHODS. Immunocytochemistry and hydroethidine-based detection of intracellular superoxide production were used. Photoreceptor cell densities under various conditions of light/dark exposures were evaluated. RESULTS. In shaker1 mice, the rod transducin translocation is delayed because of a shift of its light activation threshold to a higher level. Even moderate light exposure can induce oxidative damage and significant rod degeneration in shaker1 mice. Shaker1 mice reared under a moderate light/dark cycle develop severe retinal degeneration in less than 6 months. CONCLUSIONS. These findings show that, contrary to earlier studies, shaker1 mice possess a robust retinal phenotype that may link to defective rod protein translocation. Importantly, USH1B animal models are likely

vulnerable to light-induced photoreceptor damage, even under moderate light.

Wasfy MM, Matsui JI, Miller J, Dowling JE, Perkins BD. myosin 7aa(-/-) mutant zebrafish show mild photoreceptor degeneration and reduced electroretinographic responses. *Exp Eye Res*. 2014;122:65-76.
PMID: 24698764
Mutations in myosin VIIa (MYO7A) cause Usher Syndrome 1B (USH1B), a disease characterized by the combination of sensorineural hearing loss and visual impairment termed retinitis pigmentosa (RP). Although the shaker-1 mouse model of USH1B exists, only minor defects in the retina have been observed during its lifespan. Previous studies of the zebrafish mariner mutant, which also carries a mutation in myo7aa, revealed balance and hearing defects in the mutants but the retinal phenotype has not been described. We found elevated cell death in the outer nuclear layer (ONL) of myo7aa(-/-) mutants. While myo7aa(-/-) mutants retained visual behaviors in the optokinetic reflex (OKR) assay, electroretinogram (ERG) recordings revealed a significant decrease in both a- and b-wave amplitudes in mutant animals, but not a change in ERG threshold sensitivity. Immunohistochemistry showed mislocalization of rod and blue cone opsins and reduced expression of rod-specific markers in the myo7aa(-/-) ONL, providing further evidence that the photoreceptor degeneration observed represents the initial stages of the RP. Further, constant light exposure resulted in widespread photoreceptor degeneration and the appearance of large holes in the retinal pigment epithelium (RPE). No differences were observed in the retinomotor movements of the photoreceptors or in melanosome migration within the RPE, suggesting that myo7aa(-/-) does not function in these processes in teleosts. These results indicate that the zebrafish myo7aa(-/-) mutant is a useful animal model for the RP seen in humans with USH1B.

Epidemiology

Kimberling WJ, Hildebrand MS, Shearer AE, et al. Frequency of Usher syndrome in two pediatric populations: Implications for genetic

screening of deaf and hard of hearing children. *Genet Med*. 2010;12(8):512-516.
PMID: 20613545
Purpose: Usher syndrome is a major cause of genetic deafness and blindness. The hearing loss is usually congenital and the retinitis pigmentosa is progressive and first noticed in early childhood to the middle teenage years. Its frequency may be underestimated. Newly developed molecular technologies can detect the underlying gene mutation of this disorder early in life providing estimation of its prevalence in at risk pediatric populations and laying a foundation for its incorporation as an adjunct to newborn hearing screening programs. Methods: A total of 133 children from two deaf and hard of hearing pediatric populations were genotyped first for GJB2/6 and, if negative, then for Usher syndrome. Children were scored as positive if the test revealed > or =1 pathogenic mutations in any Usher gene. Results: Fifteen children carried pathogenic mutations in one of the Usher genes; the number of deaf and hard of hearing children carrying Usher syndrome mutations was 15/133 (11.3%). The population prevalence was estimated to be 1/6000. Conclusion: Usher syndrome is more prevalent than has been reported before the genome project era. Early diagnosis of Usher syndrome has important positive implications for childhood safety, educational planning, genetic counseling, and treatment. The results demonstrate that DNA testing for Usher syndrome is feasible and may be a useful addition to newborn hearing screening programs.

Stephenson K, Dockery A, Wynne NC, et al. The phenotype & genotype of Usher syndrome in Ireland. Presented at: The Association for Research in Vision Ophthalmology; April 28-May 2, 2019; Vancouver, Canada.
Purpose : Usher Syndrome (USH) is the most common syndromic retinitis pigmentsoa (RP) manifesting with dual sensory impairments (deafness, blindness). Three subtypes are described by timing, severity and progression of hearing loss and presence or absence of vestibular function. Fourteen genes have been implicated (USH1:9, USH2:3, USH3:2). Herein, we describe the first comprehensive phenotype & genotype analysis of

USH in Ireland. Methods : Irish patients with inherited retinal degenerations (IRDs) were invited to participate in the Irish national IRD population study (Target 5000). Thorough clinical assessment clarified the nature of visual, hearing and vestibular symptoms. USH subtype was determined by clinical and genetic characteristics. Genotype was assessed via a panel-based NGS approach of 256 IRD-implicated genes. Clinical and genetic features were analysed between the groups. Results : Thirty-nine patients with an USH phenotype from 36 pedigrees were recruited. Mean age: 42.65y; 58.97% male. Overall prevalence of USH: 1.53:100 000. Subtype breakdown: USH1: 33%, USH2: 67%, no cases of USH3. Where possible, these outcomes were validated against genotype, a genetic diagnosis being confirmed in 89.7% of cases. Genotype was confirmed in 84.6% of USH1 (76.9% MYO7A) and 92.3% of USH2 (69.2% USH2A). Novel variants were detected including the largest USH1C homozygous deletion yet reported. Deafness was congenital in 61%, <5y in 33% and 6–10y in 6%. Vestibular imbalance was poorly reported. Mean visual acuity was 0.49(R) and 0.48(L) LogMAR. Cystoid macular oedema was present in 22% of eyes. Lens status: 33.3% pseudophakic, 41.6% non-visually significant posterior subcapsular cataract, and 25% no clinically detectable cataract. Conclusions : The Irish diaspora has far-reaching influence on the genetic landscape of the western world. >10% of the American, Canadian, New Zealand, Australian and UK populations claim Irish heritage. Determining the genetic aetiology of inherited retinal degenerations in Ireland is highly relevant, both for Irish patients and global IRD statistics. Absence of USH3 in this cohort is consistent with data from the UK; clusters of USH3 exist in geographically isolated areas (e.g. rural Scandanavia). Usher Syndrome is a devastating diagnosis with dual sensory impairments, thus confirming genotype early on confers useful prognostic data and allows assessment of novel therapeutic avenues (e.g. MYO7A gene therapy).

Clinical Manifestations

Jacobson SG, Aleman TS, Sumaroka A, et al. Disease boundaries in the retina of patients with Usher syndrome caused by MYO7A gene mutations. *Invest Ophthalmol Vis Sci*. 2009;50(4):1886-1894.

PMID: 19074810

Purpose: To study retinal microstructure in Usher Syndrome type 1B (USH1B) caused by MYO7A mutations as a prelude to treatment initiatives. Methods: Patients with MYO7A-USH1B (n=17; ages 5-61) were studied with optical coherence tomography. Retinal laminae across horizontal and vertical meridians were measured. Colocalized visual sensitivity was measured with automated perimetry to enable comparisons of function and structure in the transition zones. Results: Laminar architecture of the central retina in MYO7A-USH1B ranged from normal to severely abnormal. Within the transition zone between normal and abnormal retina, the first detectable abnormality was an increase in prominence of the OLM (outer limiting membrane). Declining ONL thickness was accompanied by increased thickness of the OPL and normal or increased INL. Undetectable ONL and OPL and hyperthick INL were features of severe laminopathy at further eccentricities into the transition zone. Visual sensitivity in the transition zone declined with the decrease in ONL thickness. Conclusions: Patients with MYO7A-USH1B can have regions of structurally and functionally normal retina with definable transitions to severe laminopathy and visual loss. The earliest detectable structural markers of disease may represent Müller glial cell response to photoreceptor stress and apoptosis. Visual losses were predictably related to a decline in ONL thickness. The prospect of focal treatment of MYO7A-USH1B, such as subretinal gene therapy, prompts the need to identify retinal locations that warrant consideration for treatment in early phase trials. The transition zones are candidate sites for treatment, and laminar architecture and visual sensitivity are possible outcomes to assess safety and efficacy.

Jacobson SG, Cideciyan AV, et al. Usher syndromes due to MYO7A, PCDH15, USH2A or GPR98 mutations share retinal disease mechanism. Hum Mol Genet. 2008;17(15):2405-2415.

PMID: 19324852

PURPOSE—Usher syndrome (USH) is a genetically heterogeneous disease with autosomal recessive deafness and blindness. Gene therapy is under development for use in the most common genetic variant of USH1, USH1B, which is caused by mutations in the MYO7A gene. This study was undertaken to identify an imaging method for noninvasively monitoring the RPE component of the USH1B disease. METHODS—NIR-autofluorescence (NIR-AF) was examined in USH1B patients with scanning laser ophthalmoscopy, and retinal thickness with spectral-domain optical coherence tomography. Myo7a-null mouse retinas and purified RPE melanosomes were analyzed by spectral deconvolution confocal microscopy. RESULTS—In USH1B patients, NIR-AF was normal in regions of retained photoreceptors and abnormal in regions lacking photoreceptors. Subtle changes in NIR-AF were associated with intermediate photoreceptor loss. In ex vivo mouse retinas, the NIR-AF source was traced to the melanosomes in the RPE and choroid. Purified RPE melanosomes emitted the same signal. Fluorophores, excited by long-wavelength light, were evident throughout the apical RPE of WT mouse eyecups. In Myo7a-null eyecups, these fluorophores had a more restricted distribution. They were absent from the apical processes of the RPE, thus correlating with the melanosome localization defects described previously by conventional microscopy. CONCLUSIONS—The data indicate that melanosomes in the RPE and choroid are the dominant source of NIR-AF from the posterior region of the eye. NIR-AF is a novel tool that provides sensitive and label-free imaging of the retina and RPE and is currently the only melanosome-specific, noninvasive technique for monitoring RPE disease in new therapeutic initiatives for retinal degenerations.

Subirà O, Català-Mora J, Díaz-Cascajosa J, et al. Retinal findings in pediatric patients with Usher syndrome Type 1 due to mutations in MYO7A gene. *Eye (Lond)*. 2020;34(3):499-506.

PMID: 31320737

Purpose: To describe retinal alterations detected by swept-source optical coherence tomography (SS-OCT) in paediatric patients with Usher syndrome type 1 (USH1) and to compare these findings to previously published reports. Methods: Thirty-two eyes from 16 patients (11 males

and 5 females) with a genetic diagnosis of USH1 because of MYO7A mutations underwent SS-OCT. Patients ranged in age from 4 to 17 years (mean, 11,13 ± 4,29). The subfoveal and macular area were analysed with SS-OCT at 1050 nm using 12 radial scans of 12.0 mm. Structural abnormalities were evaluated and correlated with best-corrected visual acuity (BCVA). Results: The most common qualitative retinal abnormality was external layer damage in macular area. Specific alterations included external limiting membrane loss/disruption (27 eyes; 84.4%), disruption of the Myoid zone (27 eyes; 84.4%); Ellipsoid zone disruption (28 eyes; 87.5%), and loss of the outer segments (29 eyes; 90.6%). The damage of the retinal pigment epithelium was divided according to the loss of the different layers: phagosome zone (30 eyes; 93.8%), melanosome zone (29 eyes; 90.6%) and mitochondria zone (0 eyes; 0%). The presence of cystoid macular oedema (CMO) was significantly correlated with alterations in photoreceptors. Disruption or absence of the myoid and ellipsoid zones of the photoreceptors were the only variables independently associated with decreased BCVA. Conclusions: The findings of this study suggest that the physiopathologic basis of early-stage Usher syndrome (USH) may be changes in the outer retinal layer, particularly the photoreceptors, which in turn may cause alterations-such as CMO-in the inner retinal layers. Accordingly, monitoring the condition of photoreceptors during follow-up may be advisable for the early detection of pathologic changes.

Natural History

Jacobson SG, Cideciyan AV, Gibbs D, et al. Retinal disease course in Usher syndrome 1B due to MYO7A mutations. *Invest Ophthalmol Vis Sci*. 2011;52(11):7924-7936.

PMID: 21873662

PURPOSE. To determine the disease course in Usher syndrome type IB (USH1B) caused by myosin 7A (MYO7A) gene mutations. METHODS. USH1B patients (n = 33, ages 2-61) representing 25 different families were studied by ocular examination, kinetic and chromatic static perimetry, dark adaptometry, and optical coherence tomography (OCT). Consequences of the mutant alleles were predicted. RESULTS. All MYO7A patients had

severely abnormal ERGs, but kinetic fields revealed regional patterns of visual loss that suggested a disease sequence. Rod-mediated vision could be lost to different degrees in the first decades of life. Cone vision followed a more predictable and slower decline. Central vision ranged from normal to reduced in the first four decades of life and thereafter was severely abnormal. Dark adaptation kinetics was normal. Photoreceptor layer thickness in a wide region of central retina could differ dramatically between patients of comparable ages; and there were examples of severe losses in childhood as well as relative preservation in patients in the third decade of life. Comparisons were made between the mutant alleles in mild versus more severe phenotypes. CONCLUSIONS. A disease sequence in USH1B leads from generally full but impaired visual fields to residual small central islands. At most disease stages, there was preserved temporal peripheral field, a potential target for early phase clinical trials of gene therapy. From data comparing patients' rod disease in this cohort, the authors speculate that null MYO7A alleles could be associated with milder dysfunction and fewer photoreceptor structural losses at ages when other genotypes show more severe phenotypes.

Lenassi E, Saihan Z, Cipriani V, et al. Natural history and retinal structure in patients with Usher syndrome type 1 owing to MYO7A mutation. *Ophthalmology*. 2014;121(2):580-587.
PMID: 24199935
Purpose: To evaluate the phenotypic variability and natural history of ocular disease in a cohort of 28 individuals with MYO7A-related disease. Mutations in the MYO7A gene are the most common cause of Usher syndrome type 1, characterized by profound congenital deafness, vestibular arreflexia, and progressive retinal degeneration.
Design: Retrospective case series. Participants: Twenty-eight patients from 26 families (age range, 3-65 years; median, 32) with 2 likely disease-causing variants in MYO7A. Methods: Clinical investigations included fundus photography, optical coherence tomography, fundus autofluorescence (FAF) imaging, and audiologic and vestibular assessments. Longitudinal visual acuity and FAF data (over a 3-year period) were available for 20 and 10 study subjects, respectively. Main

outcome measures: Clinical, structural, and functional characteristics. Results: All patients with MYO7A mutations presented with features consistent with Usher type 1. The median visual acuity for the cohort was 0.39 logarithm of the minimum angle of resolution (logMAR; range, 0.0-2.7) and visual acuity in logMAR correlated with age (Spearman's rank correlation coefficient, r = 0.71; P<0.0001). Survival analysis revealed that acuity ≤ 0.22 logMAR was maintained in 50% of studied subjects until age 33.9; legal blindness based on loss of acuity (≥ 1.00 logMAR) or loss of field (≤ 20°) was reached at a median age of 40.6 years. Three distinct patterns were observed on FAF imaging: 13 of 22 patients tested had relatively preserved foveal autofluorescence surrounded by a ring of high density, 4 of 22 had increased signal in the fovea with no obvious hyperautofluorescent ring, and 5 of 22 had widespread hypoautofluorescence corresponding to retinal pigment epithelial atrophy. Despite a number of cases presenting with a milder phenotype, there seemed to be no obvious genotype-phenotype correlation.
Conclusions: MYO7A-related ocular disease is variable. Central vision typically remains preserved at least until the third decade of life, with 50% of affected individuals reaching legal blindness by 40 years of age. Distinct phenotypic subsets were identified on FAF imaging. A specific allele, previously reported in nonsyndromic deafness, may be associated with a mild retinopathy.

Sumaroka A, Matsui R, Cideciyan AV, et al. Outer retinal changes including the ellipsoid zone band in Usher syndrome 1B due to MYO7A mutations. *Invest Ophthalmol Vis Sci*. 2016;57(9):OCT253-61.
PMID: 27409480
Purpose: To study transition zones from normal to abnormal retina in Usher syndrome IB (USH1B) caused by myosin 7A (MYO7A) mutations.
Methods: Optical coherence tomography (OCT) scattering layers in outer retina were segmented in patients (n = 16, ages 2-42; eight patients had serial data, average interval 4.5 years) to quantify outer nuclear layer (ONL) and outer segments (OS) as well as the locus of EZ (ellipsoid zone) edge and its extent from the fovea. Static perimetry was measured under dark-adapted (DA) and light-adapted (LA) conditions. Results: Ellipsoid

zone edge in USH1B-MYO7A could be located up to 23° from the fovea. Ellipsoid zone extent constricted at a rate of 0.51°/year with slower rates at smaller eccentricities. A well-defined EZ line could be associated with normal or abnormal ONL and/or OS thickness; detectable ONL extended well beyond EZ edge. At the EZ edge, the local slope of LA sensitivity loss was 2.6 (±1.7) dB/deg for central transition zones. At greater eccentricities, the local slope of cone sensitivity loss was shallower (1.1 ± 0.4 dB/deg for LA) than that of rod sensitivity loss (2.8 ± 1.2 dB/deg for DA). Conclusions: In USH1B-MYO7A, constriction rate of EZ extent depends on the initial eccentricity of the transition. Ellipsoid zone edges in the macula correspond to large local changes in cone vision, but extramacular EZ edges show more pronounced losses on rod-based vision tests. It is advisable to use not only the EZ line but also other structural and functional parameters for estimating natural history of disease and possible therapeutic effects in future clinical trials of USH1B-MYO7A.

Testa F, Melillo P, Bonnet C, et al. Clinical presentation and disease course of Usher syndrome because of mutations in MYO7A or USH2A. *Retina*. 2017;37(8):1581-1590.

PMID: 27828912

Purpose: To evaluate differences in the visual phenotype and natural history of Usher syndrome caused by mutations in MYO7A or USH2A, the most commonly affected genes of Usher syndrome Type I (USH1) and Type II (USH2), respectively. Methods: Eighty-eight patients with a clinical diagnosis of USH1 (26 patients) or USH2 (62 patients) were retrospectively evaluated. Of these, 48 patients had 2 disease-causing mutations in MYO7A (10 USH1 patients), USH2A (33 USH2 patients), and other USH (5 patients) genes. Clinical investigation included best-corrected visual acuity, Goldmann visual field, fundus photography, electroretinography, and audiologic and vestibular assessments. Longitudinal analysis was performed over a median follow-up time of 3.5 years. Results: Patients carrying mutations in MYO7A had a younger age of onset of hearing and visual impairments than those carrying mutations in USH2A, leading to an earlier diagnosis of the disease in the former patients. Longitudinal analysis showed that visual acuity and visual field decreased more rapidly in

subjects carrying MYO7A mutations than in those carrying USH2A mutations (mean annual exponential rates of decline of 3.92 vs. 3.44% and of 8.52 vs. 4.97%, respectively), and the former patients reached legal blindness on average 15 years earlier than the latter. Conclusion: The current study confirmed a more severe progression of the retinal disease in USH1 patients rather than in USH2 patients. Furthermore, most visual symptoms (i.e., night blindness, visual acuity worsening) occurred at an earlier age in USH1 patients carrying mutations in MYO7A.

Characterized Clinical Cohorts

Galbis-Martínez L, Blanco-Kelly F, García-García G, et al. Genotype-phenotype correlation in patients with Usher syndrome and pathogenic variants in MYO7A: implications for future clinical trials. *Acta Ophthalmol*. 2021 Feb 11. doi: 10.1111/aos.14795. Epub ahead of print.

PMID: 33576163

Purpose: We aimed to establish correlations between the clinical features of a cohort of Usher syndrome (USH) patients with pathogenic variants in MYO7A, type of pathogenic variant, and location on the protein domain. Methods: Sixty-two USH patients from 46 families with biallelic variants in MYO7A were examined for visual and audiological features. Participants were evaluated based on self-reported ophthalmological history and ophthalmological investigations (computerized visual field testing, best-corrected visual acuity, and ophthalmoscopic and electrophysiological examination). Optical coherence tomography and fundus autofluorescence imaging were performed when possible. Auditory and vestibular functions were evaluated. Patients were classified according to the type of variant and the protein domain where the variants were located. Results: Most patients displayed a typical USH1 phenotype, that is, prelingual severe-profound sensorineural hearing loss, prepubertal retinitis pigmentosa (RP) and vestibular dysfunction. No statistically significant differences were observed for the variables analysed except for the onset of hearing loss due to the existence of two USH2 cases, defined as postlingual sensorineural hearing loss, postpubertal onset of RP, and absence of

vestibular dysfunction, and one atypical case of USH. Conclusion: We were unable to find a correlation between genotype and phenotype for MYO7A. However, our findings could prove useful for the assessment of efficacy in clinical trials, since the type of MYO7A variant does not seem to change the onset, severity or course of visual disease.

Guzmán HO, Palacios AM, De Almada MI, Utrera RA. A novel homozygous MYO7A mutation involved in a Venezuelan population with high frequency of USHER1B. *Ophthalmic Genet*. 2016;37(3):328-330.
PMID: 26864046
Background: Macanao's population in Venezuela has perhaps the greatest incidence of USH1B known in Latin America (79 cases per 100,000 population); however, until now no mutation in the MYO7A gene had been reported for this population. Materials and methods: This study aimed to evaluate the entire coding region of the MYO7A gene by direct sequencing of PCR products obtained from patients clinically diagnosed with USH1B. Results: A novel mutation named c.6079_6081del was detected on exon 45 of the MYO7A gene, causing the loss of a single histidine amino acid at codon 2027 (p.H2027del) located within the second FERM domain of the human protein myosin VIIA. Three patients with clinical diagnosis of USH1B were detected positive in homozygosis for the c.6079_6081del mutation; whereas six people from the same affected family were heterozygotes and three other family members were negative. Conclusion: We suggest that this new mutation named c.6079_6081del (p.H2027del) is the main cause of deaf-blindness found in this family clinically diagnosed as USH1B. Additional studies should be performed on this population to determine whether the c.6079_6081del mutation is the main cause of USH1B for the rest of the population. [*Note: this abstract misquotes the prevalence.*]

Jaijo T, Aller E, Oltra S, et al. Mutation profile of the MYO7A gene in Spanish patients with Usher syndrome type I. *Hum Mutat*. 2006;27(3):290-291.
PMID: 16470552

Usher syndrome type I is the most severe form of Usher syndrome. It is an autosomal recessive disorder characterized by profound congenital sensorineural deafness, retinitis pigmentosa, and vestibular abnormalities. Mutations in the myosin VIIA gene (MYO7A) are responsible for Usher syndrome type 1B (USH1B). This gene is thought to bear greatest responsibility for USH1 and, depending on the study, has been reported to account for between 24% and 59% of USH1 cases. In this report a mutation screening of the MYO7A gene was carried out in a series of 48 unrelated USH1 families using single strand conformation polymorphism analysis (SSCP) and direct sequencing of those fragments showed an abnormal electrophoretic pattern. Twenty-five mutations were identified in 23 out of the 48 families studied (47.9%). Twelve of these mutations were novel, including five missense mutations, three premature stop codons, three frameshift, and one putative splice-site mutation. Based on our results we can conclude there is an absence of hot spot mutations in the MYO7A gene and that this gene plays a major role in Usher syndrome.

Keogh IJ, Godinho RN, Wu TP, et al. Clinical and genetic linkage analysis of a large Venezuelan kindred with Usher syndrome. *Int J Pediatr Otorhinolaryngol*. 2004;68(8):1063-1068.
PMID: 15236894
Objective: To undertake a comprehensive investigation into the very high incidence of congenital deafness on the Macano peninsula of Margarita Island, Venezuela.
Methods: Numerous visits were made to the isolated island community over a 4-year-period. During these visits, it became apparent that a significant number of individuals complained of problems with hearing and vision. Socioeconomic assessments, family pedigrees and clinical histories were recorded on standard questionnaires. All individuals underwent thorough otolaryngologic and ophthalmologic examinations. Twenty milliliters of peripheral venous blood was obtained from each participant. A genome-wide linkage analysis study was performed. Polymorphic microsatellite markers were amplified by polymerase chain reaction and separated on polyacrylamide gels. An ABI 377XL sequencer was used to separate fragments and LOD scores were calculated by using published

software. Results: Twenty-four families were identified, comprising 329 individuals, age range 1-80 years, including 184 children. All families were categorized in the lower two (least affluent) socioeconomic categories. A high incidence of consanguinity was detected. Fifteen individuals (11 adults, 4 children) had profound congenital sensorineural hearing loss, vestibular areflexia and retinitis pigmentosa. A maximum LOD score of 6.76 (Linkage >3.0), between markers D11s4186 and D11s911, confirmed linkage to chromosome 11q13.5. The gene myosin VIIA (MYO7A) was confirmed in the interval. Clinical and genetic findings are consistent with a diagnosis of Usher syndrome 1B for those with hearing and vision problems. Conclusions: We report 15 Usher syndrome 1B individuals from a newly detected Latin American socio-demographic origin, with a very high prevalence of 76 per 100,000 population. [*Note: this abstract misquotes the prevalence.*]

Khateb S, Mohand-Saïd S, Nassisi M, et al. Phenotypic characteristics of rod-cone dystrophy associated with MYO7A mutations in a large French cohort. *Retina*. 2020;40(8):1603-1615.
PMID: 31479088
Purpose: To document the rod-cone dystrophy phenotype of patients with Usher syndrome type 1 (USH1) harboring MYO7A mutations.
Methods: Retrospective cohort study of 53 patients (42 families) with biallelic MYO7A mutations who underwent comprehensive examination, including functional visual tests and multimodal retinal imaging. Genetic analysis was performed either using a multiplex amplicon panel or through direct sequencing. Data were analyzed with IBM SPSS Statistics software v. 21.0. Results: Fifty different genetic variations including 4 novel were identified. Most patients showed a typical rod-cone dystrophy phenotype, with best-corrected visual acuity and central visual field deteriorating linearly with age. At age 29, binocular visual field demonstrated an average preservation of 50 central degrees, constricting by 50% within 5 years. Structural changes based on spectral domain optical coherence tomography, short wavelength autofluorescence, and near-infrared autofluorescence measurements did not however correlate with age. Our study revealed a higher percentage of epiretinal membranes and cystoid

macular edema in patients with MYO7A mutations compared with rod-cone dystrophy patients with other mutations. Subgroup analyses did not reveal substantial genotype-phenotype correlations. Conclusion: To the best of our knowledge, this is the largest French cohort of patients with MYO7A mutations reported to date. Functional visual characteristics of this subset of patients followed a linear decline as in other typical rod-cone dystrophy, but structural changes were variable indicating the need for a case-by-case evaluation for prognostic prediction and choice of potential therapies.

Therapeutic Strategies

Bigot K, Gondouin P, Bénard R, et al. Transferrin non-viral gene therapy for treatment of retinal degeneration. *Pharmaceutics*. 2020;12(9):836.

PMID: 32882879

Dysregulation of iron metabolism is observed in animal models of retinitis pigmentosa (RP) and in patients with age-related macular degeneration (AMD), possibly contributing to oxidative damage of the retina. Transferrin (TF), an endogenous iron chelator, was proposed as a therapeutic candidate. Here, the efficacy of TF non-viral gene therapy based on the electrotransfection of pEYS611, a plasmid encoding human TF, into the ciliary muscle was evaluated in several rat models of retinal degeneration. pEYS611 administration allowed for the sustained intraocular production of TF for at least 3 and 6 months in rats and rabbits, respectively. In the photo-oxidative damage model, pEYS611 protected both retinal structure and function more efficiently than carnosic acid, a natural antioxidant, reduced microglial infiltration in the outer retina and preserved the integrity of the outer retinal barrier. pEYS611 also protected photoreceptors from N-methyl-N-nitrosourea-induced apoptosis. Finally, pEYS611 delayed structural and functional degeneration in the RCS rat model of RP while malondialdehyde (MDA) ocular content, a biomarker of oxidative stress, was decreased. The neuroprotective benefits of TF non-viral gene delivery in retinal degenerative disease models further validates iron overload as a therapeutic target and supports the continued development of pEYS611 for treatment of RP and dry AMD.

Campochiaro PA, Iftikhar M, Hafiz G, et al. Oral N-acetylcysteine improves cone function in retinitis pigmentosa patients in phase I trial. *J Clin Invest*. 2020;130(3):1527-1541.

PMID: 31805012

BACKGROUND: In retinitis pigmentosa (RP), rod photoreceptors degenerate from 1 of many mutations, after which cones are compromised by oxidative stress. N-acetylcysteine (NAC) reduces oxidative damage and increases cone function/survival in RP models. We tested the safety, tolerability, and visual function effects of oral NAC in RP patients. METHODS: Subjects (n = 10 per cohort) received 600 mg (cohort 1), 1200 mg (cohort 2), or 1800 mg (cohort 3) NAC bid for 12 weeks and then tid for 12 weeks. Best-corrected visual acuity (BCVA), macular sensitivity, ellipsoid zone (EZ) width, and aqueous NAC were measured. Linear mixed-effects models were used to estimate the rates of changes during the treatment period. RESULTS: There were 9 drug-related gastrointestinal adverse events that resolved spontaneously or with dose reduction (maximum tolerated dose 1800 mg bid). During the 24-week treatment period, mean BCVA significantly improved at 0.4 (95% CI: 0.2-0.6, $P < 0.001$), 0.5 (95% CI: 0.3-0.7, $P < 0.001$), and 0.2 (95% CI: 0.02-0.4, $P = 0.03$) letters/month in cohorts 1, 2, and 3, respectively. There was no significant improvement in mean sensitivity over time in cohorts 1 and 2, but there was in cohort 3 (0.15 dB/month, 95% CI: 0.04-0.26). There was no significant change in mean EZ width in any cohort. CONCLUSION: Oral NAC is safe and well tolerated in patients with moderately advanced RP and may improve suboptimally functioning macular cones. A randomized, placebo-controlled trial is needed to determine if oral NAC can provide long-term stabilization and/or improvement in visual function in patients with RP.

Ferla R, Dell'Aquila F, Doria M, et al. Towards a clinical trial of gene therapy for retinitis pigmentosa associated with Usher syndrome type IB. Presented at: 24th Annual meeting of the American Society of Gene & Cell Therapy; May 11-14, 2021; Virtual.

Usher syndrome type IB (USHIB) due to biallelic mutations in the *MYO7A* gene, is the most severe form of Usher syndrome, which is the most common form of retinitis pigmentosa associated with hearing loss.To transfer the large MYO7A gene to the retina, we have developed dual hybrid adeno-associated viral vector serotype 8 (dual AAV8.h*MYO7A*) which reconstitute full-length MYO7A protein to therapeutic levels in the Shaker-1 (sh1) mouse model of USHIB. In view of a gene therapy clinical trial for USHIB retinitis pigmentosa, we performed a GLP-compliant non-clinical study to assess the safety of dual AAV8.h*MYO7A.*A 13-weeks study was conducted in non-human primates (NHPs) using a GMP-like lot of dual AAV8.h*MYO7A*. NHPs received a single sub-retinal injection in the right eye of either the formulation buffer as control (CTR group) or the dual AAV8.h*MYO7A* at one of the following doses: a low dose of 1.37x1012 (LD group) and a high dose of 3.75x1012 total genome copies (GC)/eye (HD group), which correspond to 1.6X and 4.3X the highest dose proposed for the clinical trial, respectively. Sub-retinal injection of formulation buffer was well-tolerated. Eyes treated with dual AAV8.h*MYO7A* showed microscopic alterations that were dose-dependent in terms of severity, and improved at the end of the study compared to the interim evaluation (6-9 weeks post-injection), suggesting recovery over time. Optical coherence tomography (OCT) supported the histopathological findings with areas of retinal degeneration and presence of hyperreflective sub-retinal material away from the injection site, as well as alteration of the IS/OS line in the macula, exclusively in the HD group. Consistent with this, electroretinography (ERG) showed a dose-dependent reduction of retinal function, which improved over time with some eyes in the LD-group falling in the range of normality at the end of the study.In conclusion, our data showed that sub-retinal administration of dual AAV8.h*MYO7A* at the low dose results in a mild toxicity which improved over time. Importantly, some of the observed alterations were similar to those previously reported for AAV8 administered in NHPs at similar doses and then safely used in humans. Based on this, we believe that this safety study, once completed by biodistribution, expression and immunology data, will pave the way for a gene therapy clinical trial for USHIB retinitis pigmentosa.

Weiss JN, Levy S. Stem Cell Ophthalmology Treatment Study (SCOTS): bone marrow derived stem cells in the treatment of Usher syndrome. *Stem Cell Investig*. 2019;6:31.

PMID: 31620478

Background: Usher syndrome is the most common form of syndromic retinitis pigmentosa and includes types I, II, and III with varying degrees of hearing loss. We present results of 10 eyes with Usher syndrome treated with autologous bone marrow derived stem cells (BMSC) within the Stem Cell Ophthalmology Treatment Study (SCOTS). Methods: Preoperative Snellen visual acuities ranged from 20/30-1 to 20/400 with the average pre-operative Snellen acuity approximately 20/85 and the average logarithm of the minimum angle of resolution (LogMAR) acuity 0.635. All eyes had significantly impaired visual fields and patients reported hearing loss as part of this syndromic retinitis pigmentosa. Treatment using the protocols of the SCOTS study using BMSC provided by retrobulbar, subtenons, intravitreal and intravenous injections. Results: Following treatment, 80% of the Usher eyes showed an improvement in visual acuity. Of the eyes that improved the average increase in visual acuity was 36.4% on LogMAR with improvements ranging from 23% to 94%. The average post-operative change in all treated eyes was a gain of 0.18 LogMAR and an increase in visual acuity of 28.3% on LogMAR. The results showed high statistical significance with $P<0.001$. Visual fields generally improved. No patient experienced a loss of vision. One patient underwent preoperative and 4-month post-operative audiometry testing which demonstrated improvement. The procedures were performed safely and without complications. Conclusions: Findings confirm meaningful improvement in visual acuity is possible in Usher syndrome using BMSC protocols developed in the SCOTS study. Statistical significance and safety were established.

Zallocchi M, Binley K, Lad Y, et al. EIAV-based retinal gene therapy in the shaker1 mouse model for usher syndrome type 1B: development of UshStat. *PLoS One*. 2014;9(4):e94272.

PMID: 24705452

Usher syndrome type 1B is a combined deaf-blindness condition caused by mutations in the MYO7A gene. Loss of functional myosin VIIa in the retinal

pigment epithelia (RPE) and/or photoreceptors leads to blindness. We evaluated the impact of subretinally delivered UshStat, a recombinant EIAV-based lentiviral vector expressing human MYO7A, on photoreceptor function in the shaker1 mouse model for Usher type 1B that lacks a functional Myo7A gene. Subretinal injections of EIAV-CMV-GFP, EIAV-RK-GFP (photoreceptor specific), EIAV-CMV-MYO7A (UshStat) or EIAV-CMV-Null (control) vectors were performed in shaker1 mice. GFP and myosin VIIa expression was evaluated histologically. Photoreceptor function in EIAV-CMV-MYO7A treated eyes was determined by evaluating α-transducin translocation in photoreceptors in response to low light intensity levels, and protection from light induced photoreceptor degeneration was measured. The safety and tolerability of subretinally delivered UshStat was evaluated in macaques. Expression of GFP and myosin VIIa was confirmed in the RPE and photoreceptors in shaker1 mice following subretinal delivery of the EIAV-CMV-GFP/MYO7A vectors. The EIAV-CMV-MYO7A vector protected the shaker1 mouse photoreceptors from acute and chronic intensity light damage, indicated by a significant reduction in photoreceptor cell loss, and restoration of the α-transducin translocation threshold in the photoreceptors. Safety studies in the macaques demonstrated that subretinal delivery of UshStat is safe and well-tolerated. Subretinal delivery of EIAV-CMV-MYO7A (UshStat) rescues photoreceptor phenotypes in the shaker1 mouse. In addition, subretinally delivered UshStat is safe and well-tolerated in macaque safety studies These data support the clinical development of UshStat to treat Usher type 1B syndrome.