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Tissue-specific genotype-phenotype correlations among USH2A-related disorders in the RUSH2A study

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Abstract (200) words max / current 172)

We assessed genotype-phenotype correlations among the visual, auditory, and olfactory phenotypes of 127 participants with Usher syndrome (USH2) (n=80) or nonsyndromic autosomal recessive retinitis pigmentosa (ARRP) (n=47) due to *USH2A* variants, using clinical data and molecular diagnostics from the Rate of Progression in *USH2A* Related Retinal Degeneration (RUSH2A) study. *USH2A* truncating alleles were associated with USH2 and had a dose-dependent effect on hearing loss severity with no effect on visual loss severity within the USH2 subgroup. A group of missense alleles in an inter-fibronectin domain appeared to be hypomorphic in ARRP. These alleles were associated with later age of onset, larger visual field area, better sensitivity thresholds, and better electroretinographic responses. No effect of genotype on the severity of olfactory deficits was observed. This study unveils a unique, tissue-specific *USH2A* allelic hierarchy with important prognostic implications for patient counseling and treatment trial endpoints. These findings may inform clinical care or research approaches in others with allelic disorders or pleiotropic phenotypes.

Keywords: *USH2A*, hearing loss, photoreceptor degeneration, genotype, Usher syndrome, retinitis pigmentosa.

INTRODUCTION

Retinitis pigmentosa (RP; MIM# 268000) is a form of retinal degeneration characterized by early loss of rod photoreceptor function, manifesting as nyctalopia, peripheral field loss, and diminished dark-adapted electroretinographic (ERG) recordings. The later stages include cone dysfunction, including constricted visual fields, loss of central vision, and reduced light-adapted ERG responses. RP has extreme locus heterogeneity, with >90 genes associated with the nonsyndromic form, and is associated with hundreds of syndromic disorders, including ciliopathies, peroxisomal disorders, and multiple (>500) malformation syndromes (Hartong et al., 2006; Schneider et al., 2021; Verbakel et al., 2018). Recently, an FDA-approved gene-directed therapy, the first in its class, has emerged for early-onset retinal degeneration caused by variants in the *RPE65* gene (MIM# 180069). However, there are no effective treatments for the vast majority of patients with RP. Defining genotype-phenotype correlations may allow for better selection of outcome measures for future clinical trials.

Usher syndrome (Usher syndrome, MIM# 276900) comprises a group of autosomal recessive disorders characterized by congenital, childhood-onset, or progressive post-lingual hearing loss and retinal degeneration. Genes associated with various forms of Usher syndrome encode proteins that localize mainly to the stereocilia and synaptic regions of inner ear hair cells and the connecting of cilium of retinal photoreceptors. Variants in the *USH2A* gene (MIM# 608400) are the leading cause of Usher syndrome type 2 (USH2) (USH2A; MIM# 276901). Notably, patients with USH2 have congenital hearing loss with progressive vision loss, providing a window of opportunity for intervention as the hearing loss is often diagnosed early in life and

genetic testing often reveals the potential for subsequent retinal degeneration before vision loss actually begins. *USH2A* mutations can also cause nonsyndromic autosomal recessive RP (ARRP, isolated RP with normal hearing at birth) (RP39; MIM# 613809). In many populations, the most common pathogenic variants are located in exon 13 of the *USH2A* gene, in particular NM_206933.4:c.2299delG p.(Glu767Ser*fs*Ter21), which accounts for as high as ~16% of disease alleles (Lenassi et al., 2015; Pierrache et al., 2016). As such, *USH2A* exon 13 variants are the current targets for allele-directed therapy (NCT03780257).

Optimal design of gene therapy trials relies on natural history studies and deep clinical phenotyping to select reliable outcomes of treatment response. However, phenotypic correlates are poorly understood for many Mendelian conditions, and as a result the interplay between genotype and treatment response is largely overlooked. With over a thousand variants reported in the literature, *USH2A* offers a valuable opportunity for elucidating treatment-informing genotype-phenotype correlations.

Presumed truncating alleles, including nonsense, frameshift, and canonical splice variants, have been more frequently associated with hearing loss and, therefore, syndromic disease. Biallelic truncating variants are associated with more severe hearing loss (Hartel et al., 2016; Inaba et al., 2020; Meng et al., 2020; Pierrache et al., 2016). Notably, while earlier onset of visual impairment was noted in patients with USH2, the role of truncating variants has not been clearly established as a risk factor for severe visual impairment. Intriguingly, a subset of missense alleles is enriched in patients without hearing loss and ARRP (Lenassi et al., 2015; Molina-Ramirez et al., 2020).

Overall, there appears to be a genotype-diagnosis correlation for *USH2A* truncating and specific missense variants for USH2 and ARRP, respectively.

The Rate of Progression in *USH2A*-related Retinal Degeneration (RUSH2A) natural history study includes 127 international participants with USH2 and ARRP related to variants in *USH2A*. Recently, RUSH2A baseline visual field data was reported, indicating that USH2 participants have more severe visual field loss than those with ARRP after adjusting for duration of disease and age of enrollment (Duncan et al., 2020).

Given the known association between diagnosis and genotype, we hypothesized that genotype influences audiometric and visual outcomes independent of the clinical diagnosis (USH2 versus ARRP). Here, we performed a deep analysis of *USH2A* genotypes to investigate whether the allelic hierarchy for hearing impairment applied to both severity of hearing loss and retinal degeneration. Through standardized variant classification and case-control analyses to ascertain pathogenic genotypes enriched in USH2 and ARRP subgroups, we ascertained genotype-phenotype correlations that are both tissue-specific and independent of clinical diagnosis. This work demonstrates the importance of genotype analysis in natural history studies and treatment trials for rare disorders.

PATIENTS AND METHODS

This multicenter, longitudinal, international natural history study enrolled participants with bi-allelic *USH2A* variants at 16 clinical sites in Canada, France, Germany, the Netherlands, the United Kingdom, and the United States (US). The

protocol and informed consent process adhered to the tenets of the Declaration of Helsinki and were approved by the ethics boards associated with each participating site, including compliance with the associated federal regulations. Informed consent was obtained from all participants prior to enrollment. The RUSH2A protocol is listed on www.clinicaltrials.gov (NCT03146078), with registration completed prior to enrolling the first participant. Inclusion criteria stated that participants were required to have a clinical diagnosis of USH2 or ARRP and two pathogenic or likely pathogenic variants in *USH2A* from a certified testing lab obtained prior to study enrollment. Variants were demonstrated to be *in trans* for individuals with ARRP.

Variant analysis and interpretation

USH2A variant analysis was performed by two reviewers independently who used a five-tier classification system recommended by the 2015 American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines and each variant was classified as benign, likely benign, variant of unknown significance (VUS), likely pathogenic, or pathogenic.(Richards et al., 2015) Discordant results were resolved by an independent adjudicator. Variant analysis of the entire cohort was performed following the initial review, to standardize evidence used for recurrent variants. Healthy population frequency data were obtained from gnomAD (v2.1.1 accessed on Oct. 30, 2018, https://gnomad.broadinstitute.org/).(Karczewski et al., 2019) A consensus verdict for *in-silico* pathogenicity predictions for missense variants was acquired from Varsome (https://varsome.com/) and Franklin (https://franklin.genoox.com/clinical-db/home) webtools. Individual in silico predictions

were acquired from Variant Effect Predictor (VEP; http://grch37.ensembl.org/Homo_sapiens/Tools/VEP) **(Supp. Table S1)**.

Statistics

Statistical analysis was performed using the R system (v. 3.5.1) and SAS software (v. 9.4) for statistical computing. Statistical tests employed are listed in the text and figure legends. All t-tests assume two tails and unequal variance.

RESULTS

Of the 127 participants enrolled in RUSH2A, 80 were clinically diagnosed as USH2 and 47 as ARRP. Across the cohort, 140 unique variants comprising 128 single-nucleotide variants (SNVs) or small indels and 12 exonic deletions were determined to be disease-associated by variant analysis. Variants considered benign were excluded from analysis.

To assess genotype-phenotype correlation in the RUSH2A cohort, we first established disease-association of each variant by (i) standardized clinical variant interpretation using 2015 ACMG/AMP criteria **(Supp. Table S1)** and (ii) case-control comparison of *USH2A* allele frequencies (AF) in the RUSH2A cohort compared to a general subpopulation (gnomAD database v2.1.1).

USH2A variants in ClinVar and gnomAD

The *USH2A* canonical transcript, NM_206933.4, encodes for a large 6002 amino acid protein, Usherin. The *USH2A* transcript in the human population is highly variable, including many rare missense (gnomAD missense constraint Z-score = -2.5) and

truncating variations (low probability of being loss-of-function [LoF] intolerant; gnomAD LoF score = 0). The variations observed in gnomAD appear to be randomly distributed throughout the coding region **(Supp. Figure S1A)**. To determine whether disease-associated variants are distributed non-randomly, we then examined the distribution of *USH2A* coding variants present in the ClinVar database **(Supp. Figure S1B)**. While ClinVar may have submission or population bias, we observed no apparent spatially restricted clusters of pathogenic or likely-pathogenic variants. However, exon 13 harbors the most frequently submitted variants, c.2276G>T p.(Cys759Phe) and c.2299delG. Among the pathogenic or likely-pathogenic variants in ClinVar, c.2276G>T p.(Cys759Phe) has the highest gnomAD AF of 0.0010. The c.2299delG p.(Glu767Ser*fs*Ter21) variant is the most frequent LoF variant (AF_{gnomAD} = 0.0007) in the *USH2A* gene in the gnomAD dataset. It is noteworthy that 94% of the LoF variants were classified as pathogenic or likely-pathogenic in ClinVar. However, only 12% of missense or in-frame-indel variants with gnomAD AF less than 0.001 were classified as pathogenic or likely-pathogenic, and 68% such rare variants were classified as a VUS **(Supp. Figure S1C)**. This represents a major challenge for definitive classification of rare missense variants as pathogenic or benign.

USH2A variant enrichment in the RUSH2A cohort

We next applied a similar analysis to the RUSH2A cohort. Similar to ClinVar, there is no hotspot for disease associated *USH2A* variation **(Figure 1A**). The c.2299delG, p.(Glu767Ser*fs*Ter21) (AF_{RUSH2A} = 0.138) and c.2276G>T p.(Cys759Phe) (AF_{RUSH2A} = 0.083) variants in exon 13 are the most frequent in this cohort **(Figure 1A)**, and these variants demonstrate clear enrichment of AF_{RUSH2A} compared to AF_{gnomAD}

(Fig. 1B-C). To establish which *USH2A* alleles are significantly associated with disease status, allele frequencies were compared between the RUSH2A and gnomAD cohorts. Among *USH2A* variants present in the RUSH2A cohort, 58% (74/128) SNVs or indels were also present in the general population (gnomAD) **(Figure 1B)**. We applied Fisher's exact test to determine which variants in the RUSH2A cohort were enriched as compared to the gnomAD database **(Figure 1C)**. A Bonferroni-corrected *P*-value of 0.00039 (=0.05/128 variants) was used as the cut-off to determine significant enrichment. Of the 128 variants, 23% (30/128) were statistically enriched in the RUSH2A cohort. An additional 9% (12/128) of *USH2A* variants were reclassified after application of the 2015 ACMG guidelines to determine pathogenicity level PS4, which is based on enrichment of variants in the affected population compared to controls (further description in **Supplemental Methods and Results** and **Supp. Figure S2**).

Association of clinical diagnosis and hearing loss severity with truncating variants

Following the establishment of individual variant disease-association, we sought to investigate phenotype associations using the power of this cohort. Typically, truncating alleles represent total loss of function and may be more likely to correlate with phenotypic severity. We grouped exonic deletions, nonsense, frameshift, canonical (+/-2) splicing site, and non-canonical splicing variants that were supported by RNA or minigene-based evidence as truncating variants. Consistent with previous studies, the predicted LoF variants or exonic deletions in the RUSH2A cohort were detected more frequently in participants with USH2 than ARRP **(Figure 2A)**.(Iannaccone et al., 2021)

Next, we sought to determine if the number of truncating variants was associated with clinical diagnosis. In the RUSH2A cohort, the majority (50%) of participants had 1 truncating variant, followed by those with 2 truncating alleles (33%) and 0 truncating variants (17%). The number of truncating variants in each patient was significantly associated with the clinical diagnosis (χ^2 = 36.9, ***P*** <0.001) **(Figure 2B)**. All 42 participants with two truncating variants were in the USH2 group and constituted 53% of all USH2 participants.

Given the association between truncating variants and clinical diagnosis of USH2, we hypothesized that the number of truncating variants also correlates with a greater degree of hearing loss.(Hartel et al., 2016) The number of truncating variants in each participant correlated positively with hearing sensitivity represented by a 4 frequency (.5/1/2/4 kHz) pure tone average in the entire cohort **(Supp. Figure S3A)** and the USH2 group **(Figure 2C, Supp. Figure S3B)**. No such correlation was observed in the ARRP subgroup (data not shown). Notably, more severe hearing loss was associated with the presence of 2 truncating variants than 0 or 1, as shown by the Tukey multiple comparisons of means analysis (adjusted *P*-value for pair-wise comparisons < 0.03) **(Figure 2C, Supp. Figure S3B)**.

Association of vision loss onset age and visual function with truncating variants

Participants with ARRP self-reported a later age of vision loss onset than those with USH2 (mean vision loss onset age in ARRP vs USH2: 31.8 vs 18.4, ***P*** < 0.001) **(Supp. Figure S4A)**. While the presence of two truncating variants was associated with earlier vision loss onset across all study participants (Tukey multiple comparisons of means, 1-0, ***P*** = 0.39; 2-0, ***P*** = 0.001; 2-1, ***P*** = 0.004) **(Supp. Figure S4B)**, there was no

association between vision loss onset and the number of truncating variants within either the USH2 or ARRP subgroups **(Supp. Figure S4C)**. In addition, USH2 participants had lower static perimetry full field hill of vision (mean V_{TOT} in ARRP vs USH2: 37.1 vs 22.7 decibel-steradian (dB-sr), ***P*** = 0.001) and lower kinetic perimetry V4e seeing area (mean in ARRP vs USH2: 9878 vs 6477 deg^2, ***P*** < 0.001) compared to ARRP participants **(Supp. Figure S4D-E)**. We find similar results when adjusting for disease of duration and age (**Supp. Table S2A**). Similarly, these differences in hill of vision and kinetic perimetry characteristics were not associated with the number of truncating variants in either the entire cohort or the USH2 or ARRP subgroups when adjusting for disease duration and age (adjusted ***P*** = 0.67 and ***P*** = 0.26, respectively; **Supp. Figure S4D-E; Supp. Table S2A-B**). Therefore, unlike hearing loss, the earlier and more severe vision loss observed in USH2 compared to ARRP may not be dependent on the number of truncating variants, suggesting that a different genotype association determines variability among retinal phenotypes.

Missense alleles cluster in ARRP

To determine whether other variant classes determine clinical endpoints in the RUSH2A cohort and USH2 and ARRP subgroups, we compared the variant landscape between these clinical diagnoses. The most frequently observed variants in both groups were in exon 13, c.2299delG p.(Glu767Ser*fs*Ter21) and c.2276G>T p.(Cys759Phe). However, the AF of c.2276G>T was greater in the ARRP subgroup, while c.2299delG was greater in the USH2 group **(Figure 3A-C and Supp. Table S3)**. Further, missense or in-frame-indel variants were more frequent in the ARRP group **(Figure 2A, 3B-C)**. Previous studies indicated that specific *USH2A* missense variants are associated with a

clinical diagnosis of ARRP.(Lenassi et al., 2015) Comparisons of allele frequencies of individual variants between the ARRP and USH2 groups revealed a group of missense alleles with enriched AF in the ARRP group (**Figure 3C**). Fisher's exact test showed five alleles statistically associated with the ARRP group (***P*** < 0.05): p.Cys759Phe (***P*** < 0.001), p.Cys3358Tyr (***P*** < 0.001), p.Cys3294Trp (***P*** = 0.02), p.Arg4192His (***P*** = 0.05), and *cis* variants p.Cys2040Gly (***P*** = 0.05) and p.Ser2492Leu (***P*** = 0.05) **(Figure 3C, Table 1 and Supp. Table S2)**. Three of these variants, p.Cys759Phe, p.Cys3358Tyr, and p.Arg4192His, were previously reported to be enriched in patients with ARRP.(Lenassi et al., 2015) Thus, this comparison of allelic diagnoses confirms and expands the known hierarchy of missense variants in disorders.

ARRP-associated missense variants are hypomorphic

Because patients with ARRP have later vision loss onset and better retained visual function compared to USH2, we next sought to understand if these ARRP-associated missense variants have hypomorphic effects on retinal photoreceptors and, therefore, patient phenotypic outcomes, when compared to other missense variants. Since the diseases are inherited in an autosomal recessive manner, it has been challenging to perform in-depth genotype-phenotype association studies. We postulated this could be studied by examining the missense variants *in trans* to the truncating alleles among the 1-truncating variant group. Among these 62 participants, there were 63 missense variants (including 3 pairs of *cis*-variants) known or presumed to be *in trans* to a truncating variant in 60 participants **(Figure 3D and Supp. Table S4)**. Of the five participants with known or predicted pairs of missense variants *in cis*, each had at

least one pathogenic or likely pathogenic variant. Thus, we only included the likely pathogenic or pathogenic missense variant of these pairs for further analysis.

To compare clinical correlates with missense genotypes, we evaluated the subgroup of participants with one missense variant and one truncating variant. Of this subgroup, we postulated that ARRP-enriched missense variants would have milder retinal manifestations than USH2. As described above, 62 participants harbored 1 truncating variant and at least one pathogenic or likely pathogenic missense. By comparing the disease phenotypes to Usherin protein location of the missense variants, we noted that missense variants in the N-terminus including the laminin N-terminal domain and the C-terminus including the fibronectin type-III domain, appear to be associated with the USH2 in this 1-truncating group **(Figure 3D)**, which was observed previously.(Pierrache et al., 2016)

The ARRP-enriched missense variants represented multiple times among those with 1-truncating variant were cysteine substitutions, p.Cys759Phe, p.Cys3294Trp, and p.Cys3358Tyr **(Figure 3D and Supp. Table S4)**. These three variants, defined as "ARRP-enriched" in the subsequent analyses, had significantly higher AF in the ARRP group as compared to the USH2 group both in the whole RUSH2A cohort **(Table 1 and Supp. Table S2)** and in the 62 participants with compound heterozygous truncating and missense variants. We then evaluated clinical characteristics among patients harboring one of these ARRP-enriched missense variants. Patients with ARRP-enriched missense alleles in the 1-truncating subgroup had later vision loss onset regardless of clinical diagnosis (32.9+/-12.8 years ARRP-enriched vs 20.8+/-10.1 years Other; $P < 0.001$) **(Figure 4A and Supp. Table S5).** V_{TOT} and III4e isopter visual field areas were also

increased in these participants (***P*** < 0.001 for both), indicating larger visual fields at their initial study visit **(Figure 4B-C and Supp. Table S5)**. ERG measures including cone 30-Hz flicker response, which corresponds to the function of cone photoreceptors, were also increased in those with ARRP-enriched missense alleles (***P*** = 0.04) **(Figure 4D and Supp. Table S5)**.

To further investigate functional vision mediated by photoreceptor subtypes, full-field stimulus testing (FST), which evaluates rod and cone-mediated function sensitivity responses, was examined using white, blue, and red wavelengths.(Birch et al., 2020) Notably, FST stimulus testing enables determination of the type of photoreceptor mediating sensitivity; white FST thresholds < -30 dB indicate preserved rod photoreceptor function.(Birch et al., 2020) Patients with ARRP-enriched missense alleles had lower FST thresholds for white (-40.0+/-12.6dB ARRP-enriched vs -29.8+/-11.7dB Other; ***P*** = 0.007). The difference in sensitivity to blue relative to red is also an index of rod-mediated sensitivity. Patients with ARRP-enriched missense alleles had greater blue-red differences (-19.6 +/-7.8dB ARRP-enriched vs -9.3+/-9.0dB Other; ***P*** < 0.001), indicating better preserved rod function in those with ARRP-enriched missense variants **(Figure 4E-F and Supp. Table S5)**. Thus, ARRP-enriched alleles appear hypomorphic on multimodal retinal assessments including psychometric and electrophysiologic measures.

To determine whether ARRP-enriched alleles exhibit hypomorphic properties independent of clinical diagnosis, we repeated this in only those with ARRP. Remarkably, all above measures (with the exception of vision loss onset age; ***P*** = 0.10) indicated better visual function in ARRP participants with ARRP-enriched missense

variants in conjunction with a truncating allele **(Supp. Figure S5A-F and Supp. Table S5)**. We also eliminated the possibility of younger age as a confounding variable, as participants with ARRP-enriched missense alleles were, on average, older in the 1-truncating group (47.9+/-15.1 years vs 38.9+/-12.29 years; ***P*** = 0.017) and of the same age in the ARRP subgroup (***P*** = 0.05). Additionally, ARRP-enriched missense alleles in the ARRP 1-truncating group appeared to have no effect on hearing among patients with Usher syndrome (***P*** = 0.61) and olfaction measures (***P*** = 0.23). These missense alleles have a milder effect on retinal dysfunction and degeneration, yet no effect on auditory or olfactory outcomes. This indicates a tissue-specific genotype-phenotype correlation, where retinopathy onset and progression are influenced by a subset of hypomorphic missense alleles, and hearing by the number of truncating alleles.

Variants in exon 13 are not significantly different from other regions

Finally, we investigated the effect of the most common individual variants, c.2299delG p.(Glu767Ser*fs*Ter21)and c.2276G>T p.(Cys759Phe) in exon 13, which is the target of a current gene therapy clinical trial (NCT03780257). We found no differences in measures of auditory or visual function with 0, 1, or 2 copies of c.2299delG p.(Glu767Ser*fs*Ter21) in the 2-truncating genotype subgroup **(Supp. Figure S6** and **data not shown)**. We also observed no differences among patients with and without p.Cys759Phe in the 1-truncating subgroup, or among those with 0 or 1 copy of p.Cys759Phe in the 2-missense genotype subgroup (**Supp. Figure S6** and **data not shown**). Therefore, the observations in the RUSH2A cohort of the influence of truncating variants on hearing loss endpoints, and missense variants for retinopathy endpoints, are not primarily driven by these commonly observed exon 13 variants.

DISCUSSION

RUSH2A is a natural history study of visual phenotypes and a cross sectional study of hearing and olfactory phenotypes among patients with *USH2A*-related disease, with the goal of identifying reliable clinical endpoints in the assessment of progression or therapeutic outcomes as well as identifying subpopulations most likely to benefit from treatment.(Birch et al., 2020; Duncan et al., 2020; Iannaccone et al., 2021) Here, we analyze the effect of genotype on clinical measures to better understand whether genotype determines clinical diagnosis, and whether variant effects are global or tissue-specific.

First, we standardized clinical variant interpretation at the cohort level using a case:control analysis and reclassified 2.4% of VUSs as likely pathogenic or benign, and 7.8% of likely pathogenic variants as pathogenic. Such classifications are tantamount to standardizing clinical variant interpretations for gene therapy trials, and for public repositories such as ClinVar, LOVD, and ClinGen.(Richards et al., 2015) The advantage of this study cohort is the large number of cases (127) which allowed us to both calculate disease-specific allele frequencies as critical evidence for pathogenicity ascertainment and separately analyze the USH2 and ARRP subgroups to explore genotype effects independent of clinical diagnosis, which has not been achieved previously.

Next, we demonstrated several important genotype-phenotype correlations at the tissue- and diagnosis-levels. First, USH2 is associated with truncating alleles, where

biallelic truncating alleles almost always cause USH2.(Lenassi et al., 2015; Pierrache et al., 2016) Second, in the RUSH2A cohort, hearing loss severity in USH2 is directly related to the number of truncating alleles, as similarly noted by Hartel et al. and Molina-Ramirez et al, as well as the RUSH2A study.(Hartel et al., 2016; Iannaccone et al., 2021; Molina-Ramirez et al., 2020) Third, truncating alleles are also associated with vision loss in USH2 patients, with earlier onset of and more severe retinal degeneration compared to ARRP.(Inaba et al., 2020; Meng et al., 2020; Pierrache et al., 2016) However, we found that the impact of truncating alleles on retinal degeneration may be dependent on clinical diagnosis, as we found no differences in visual symptom onset or severity in those with and without truncating variants in the USH2 and ARRP subgroups.

Furthermore, we confirmed and expanded the list of ARRP-associated missense alleles, adding p.Cys3294Trp and *cis* variants p.Cys2040Gly and p.Ser2492Leu through the RUSH2A study. Intriguingly, several of the hypomorphic missense alleles are located in the inter-fibronectin domain p.Cys3358Tyr, p.Cys3294Trp, and p.Glu3448Lys. Additionally, p.Arg4192His is in a fibronectin-3 repeat domain. Usherin interacts with fibronectin in retinal basement membranes, and is disrupted with certain mutations found in *USH2A*-related disorders.(Bhattacharya & Cosgrove, 2005) Further, human disease-associated variants in fibronectin-3 domains in usherin appear to be located within a “hotspot” for pathogenic missense variation.(Baux et al., 2014)

Analysis of both the entire cohort and the ARRP subgroup indicated that ARRP-enriched missense alleles among patients with 1-truncating allele have a later age of onset and better-preserved cone and rod photoreceptor function as measured by

psychometric and electrophysiological testing. Thus, the effect of ARRP-specific missense alleles on visual phenotypes and truncating alleles on the auditory phenotype are independent of the phenotypic differences observed between USH2 and ARRP. Further, we did not observe differences in hearing loss in individuals with ARRP-enriched missense alleles, nor did we observe differences in vision loss with different numbers of truncating alleles in the USH2 or ARRP groups. This implies these variant classes may have mutually exclusive effects, with less severe photoreceptor degeneration occurring with retinal-specific hypomorphic missense variants, and cochlear hair cells being more sensitive to truncating alleles.

Multiple studies from different countries have recognized an *USH2A* allelic hierarchy, where truncating alleles are associated with the clinical diagnosis of USH2 and hearing loss, and several missense alleles are associated with clinical diagnosis of ARRP.(Gao et al., 2021; Hartel et al., 2016; Inaba et al., 2020; Lenassi et al., 2015; Meng et al., 2020; Molina-Ramirez et al., 2020; Pierrache et al., 2016) The presence of specific missense alleles enriched in ARRP is associated with differences in age of onset and severity of retinal degeneration. Previously, Lenassi et al. described six variants, five missense and one intronic variant, that were found more frequently in ARRP than USH2, indicating that a different mutational spectrum exists between these two clinical diagnoses, which goes beyond the association of truncating variants with syndromic disease.(Lenassi et al., 2015) Here, we establish that the ARRP-enriched missense alleles are hypomorphic, in multiple tests of cone and rod photoreceptor function, and that these effects are independent of clinical diagnosis, even when adjusted for age of onset and disease duration.

Despite being the most expansive *USH2A* genotype-phenotype study to date, there are several limitations. First, we controlled for retinal dysfunction attributed to individual missense alleles by selecting patients with one truncating and one missense variant. As we and others have demonstrated, truncating variants predispose to Usher syndrome, which is an independent risk factor for more severe retinal degeneration. However, it is likely that the milder effects of ARRP-associated missense alleles are underestimated by this analysis design. Patients with homozygous or compound heterozygous missense alleles were not frequent in this population and would provide a better comparison.

Prospective longitudinal studies in cohorts such as these will be critical to determine if these effects indeed alter disease progression in addition to the onset and measures of phenotype severity performed here. Larger studies would also permit analysis of variant-specific effects. However, in our analysis, we did not find that the most common truncating variant c.2299delG p.(Glu767Ser*fs*Ter21) had different effects on visual and auditory endophenotypes from other truncating alleles, and patients with the most common missense variant c.2276G>T p.(Cys759Phe) did not have milder disease course than those with other missense alleles. This is likely because the other hypomorphic *USH2A* alleles were included in the control group of this analysis.

In conclusion, we demonstrated correlations of *USH2A* truncating variants with the presence and severity of hearing loss and of hypomorphic missense variants with the onset and severity of retinal degeneration (**Supplemental Graphic**). Importantly, these effects are independent of clinical diagnosis, and will allow for further subgrouping of patients to provide prognostic information and clinical endpoints for gene therapy trials.

As such, these findings highlight the importance of considering the effect of genotype on outcome measures for clinical trials. A deep understanding of genotype-phenotype correlations is critical in this era of gene augmentation therapy. Understanding the mechanism of disease, improving clinical molecular diagnostics for eligibility, and providing prognostic information for disease onset and progression are essential for determining the efficacy of new therapies.

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Conflict of Interests Statement

J. Duncan is a consultant for ConeSight Theraputics, DTx Pharma, Inc., Editas Theraputics, Eyevensys Theraputics, Nacuity, PYC Therapeutics, Spark Therapeutics, and Vedere Bio, Astellas; she receives financial support for clinical trials from Acucela, Abbvie/Allergan, AGTC Theraputics, Biogen/Nightstarx Theraputics, Inc., ProQR Therapeutics, Second Sight Medical Products, Inc and Neurotech USA, Inc., ;and she

serves as a clinical advisory board member for SparingVision, Gyroscope Therapeutics, AGTC Therapeutics, Spark Therapeutics, ProQR Therapeutics, Nacuity, RD fund, and Foundation Fighting Blindness; Spouse: stock in RxSight.

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K. Branham is a consultant/advisor for ProQR, Biogen, and Janssen

I. Audo is a consultant/advisor for Novartis, Sparing Vision, Janssen, Roche

C. Kay is a consultant for AGTC, Spark Therapeutics, Novartis, Astena Therapeutics; and receives clinical trial funding/investigator for AGTC, Foundation Fighting Blindness, Alkeus, Gyroscope, Regenx Bio, Nightstar Therapeutics/Biogen, Iveric Bio, ProQR Therapeutics, MeiraGTx/Janssen, and Kodiak; and receives equity from Astena Therapeutics

A. Iannaccone is a consultant for ClearView Healthcare Partners, Teladoc Health, GLG Group, Guidepoint, Astellas Institute for Regenerative Medicine, Roivant Pharma, Editas, Rhythm Pharmaceuticals, IQVIA, Gyroscope, Ocugen, and is a board member

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Ethics Approval Statement: Jaeb Center for Health Research IRB is the overseeing IRB and approved this study. There is not a reference number or ID. This investigation adhered to the tenets of the Declaration of Helsinki and was approved by the

institutional review boards (IRBs), or ethics boards associated with each participating site.

Data Sharing and Data Accessibility Statement:

A deidentified database is available upon request through the public domain on the FFB/Jaeb public website.

Contributorship Statement

All authors contributed equally to the data collection, drafting, review, and finalization of manuscript. Robert Hufnagel takes responsibility for the data and analysis in the manuscript.

Web Resources:

ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/

gnomAD: https://gnomad.broadinstitute.org/

Varsome: https://varsome.com/

Franklin: https://franklin.genoox.com/clinical-db/home

Variant Effect Predictor: http://grch37.ensembl.org/Homo_sapiens/Tools/VEP

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FIGURE LEGENDS

Figure 1. Variant enrichment in the RUSH2A cohort. **A**. *USH2A* variant allele frequency in the RUSH2A cohort by cDNA position. **B-C**. *USH2A* variant allele frequency in the RUSH2A cohort vs allele frequency in gnomAD. Only variants present in both RUSH2A and gnomAD are shown. **B.** Clinical significance was obtained from ClinVar. **C.** Variants statistically (Fisher's exact test) enriched in the RUSH2A cohort as compared to gnomAD are shown in orange. Dotted lines in **A** represent exon 13 boundary; LoF, predicted loss of function variants; Variants labeled are those with allele frequency over 0.015.

Figure 2. Truncating alleles correlate with USH2 and degree of hearing loss. **A**. *USH2A* variant types in USH2 and ARRP. **B**. Bar chart showing patient diagnosis and number of truncating alleles. **C**. Box and dot plot showing 4 frequency (.5/1.2/4 kHz) pure tone average (4F PTA) in dB HLby number of truncating alleles in the USH2 group, adjusted for sex and age according to International Organization for Standardization (ISO) standards (ISO 7029: 2017; ANOVA, ***P*** = 0.0001). Larger numbers mean worse hearing. Adjusted *P*-values in the Tukey multiple comparisons of means between truncating allele groups in **C**. 1-0, ***P*** = 0.10; 2-0, ***P*** < 0.001; 2-1, ***P*** = 0.01.

Figure 3. *USH2A* variants enriched in patients with USH2 and ARRP. **A-B**. *USH2A* variant allele frequency in USH2 (**A**) or ARRP (**B**) by cDNA position. Variants labeled are those with allele frequency in patient subgroup over 0.015. Dotted lines, exon 13 boundary. **C**. *USH2A* variant allele frequency comparison by diagnosis. Variants labeled in **C** are those with *P*-value (Fisher's exact test) less than 0.05 (blue) or c.2299delG p.(Glu767Ser*fs*Ter21) (red, ***P*** = 0.09). LoF, predicted loss of function variants. **D**.

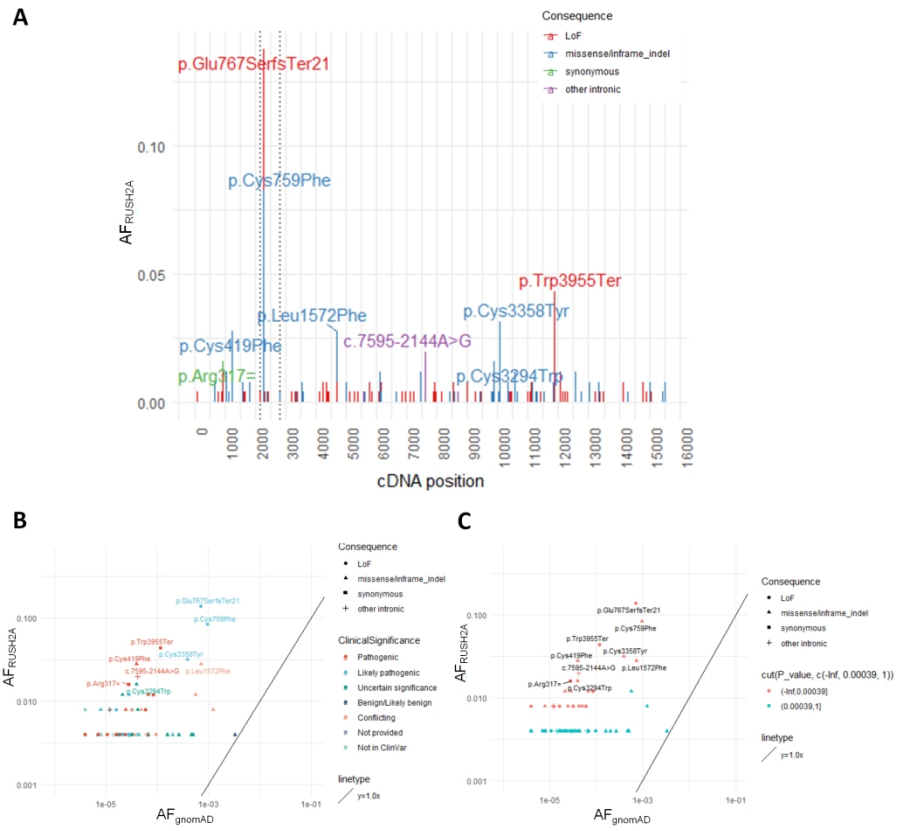
Histogram of missense variants within the 1-truncating variant subgroup by protein position.

Figure 4. Retinal phenotypic differences due to RP-enriched *USH2A* missense variants. **A-E**. Box and dot plot comparing RP-enriched and Other missense variants in the 1-truncating group, for age of vision loss onset (**A**; Welch's t-test; ***P*** < 0.001), full-field hill of vision (**B**; ***P*** < 0.001), iii4E seeing area (**C**; ***P*** < 0.001), cone flicker amplitude (**D**; ***P*** = 0.04), and full-field stimulus thresholds for White (**E**; ***P*** = 0.007) and threshold differences Blue-Red (**F**; ***P*** < 0.001). Circles = females, triangles = males, red = ARRP, blue = USH2. Full field hill of vision units as V_{TOT}, decibel-steradian (dB-sr).

TABLES

Table 1. *USH2A* variants enriched in patients with Usher syndrome type 2 (USH2) or nonsyndromic retinitis pigmentosa (ARRP).

cDNA	Protein	AF_ARRP	AF_USH2	Odds Ratio	95% Confidence Interval	*P*-value
c.2276G>T	p.Cys759Phe	0.181	0.025	8.54	2.66;36.04	<0.001
c.10073G>A	p.Cys3358Tyr	0.085	0	Inf	3.07;Inf	<0.001
c.9882C>G	p.Cys3294Trp	0.043	0	Inf	1.14;Inf	0.02
c.12575G>A	p.Arg4192His	0.032	0	Inf	0.71;Inf	0.05
c.6118T>G	p.Cys2040Gly	0.032	0	Inf	0.71;Inf	0.05
c.7475C>T	p.Ser2492Leu	0.032	0	Inf	0.71;Inf	0.05
c.2299del	p.Glu767SerfsTer21	0.085	0.169	0.46	0.17;1.1	0.09
c.7595-2144A>G	p.?	0	0.031	0	0;1.84	0.16
Exon deletion	p.?	0.032	0.075	0.41	0.07;1.57	0.18



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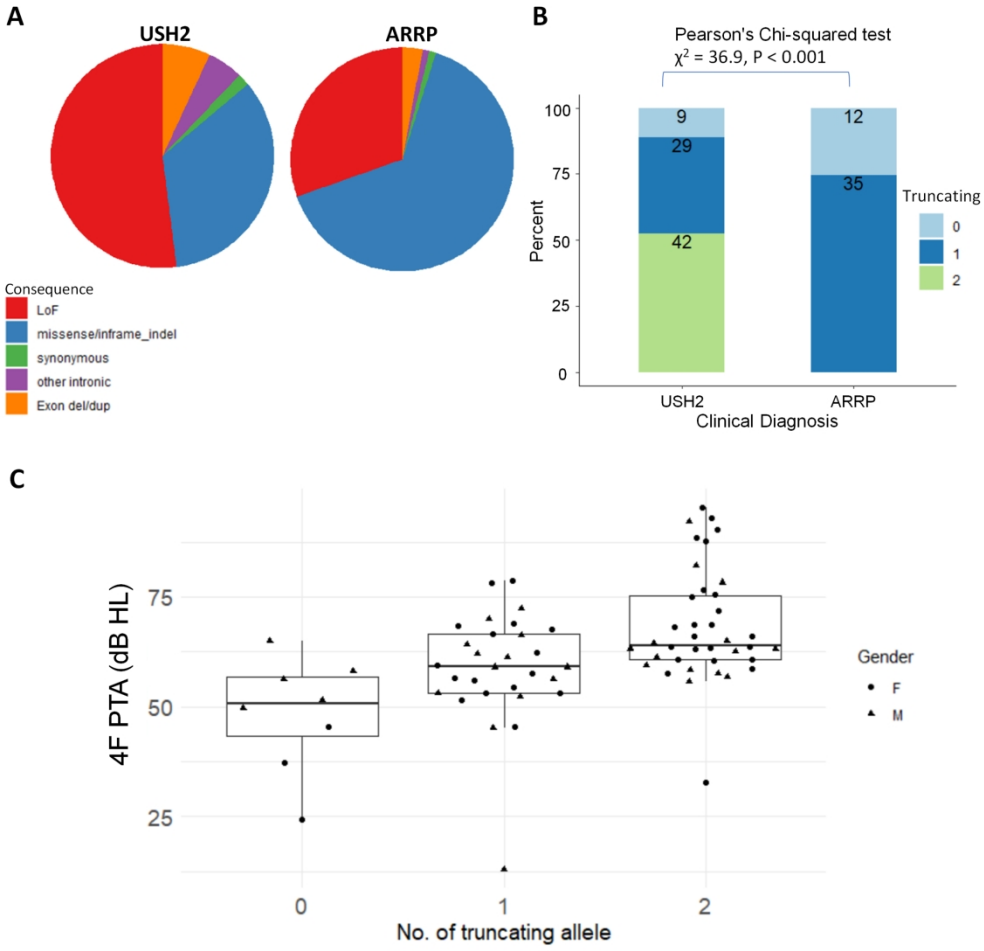

Figure 2. Truncating alleles correlate with USH2 and degree of hearing loss. A. USH2A variant types in USH2 and ARRP. B. Bar chart showing patient diagnosis and number of truncating alleles. C. Box and dot plot showing 4 frequency (.5/1.2/4 kHz) pure tone average (4F PTA) in dB HLby number of truncating alleles in the USH2 group, adjusted for sex and age according to International Organization for Standardization (ISO) standards (ISO 7029: 2017; ANOVA, P = 0.0001). Larger numbers mean worse hearing. Adjusted P-values in the Tukey multiple comparisons of means between truncating allele groups in C. 1-0, P = 0.10; 2-0, P < 0.001; 2-1, P = 0.01.

215x215mm (300 x 300 DPI)

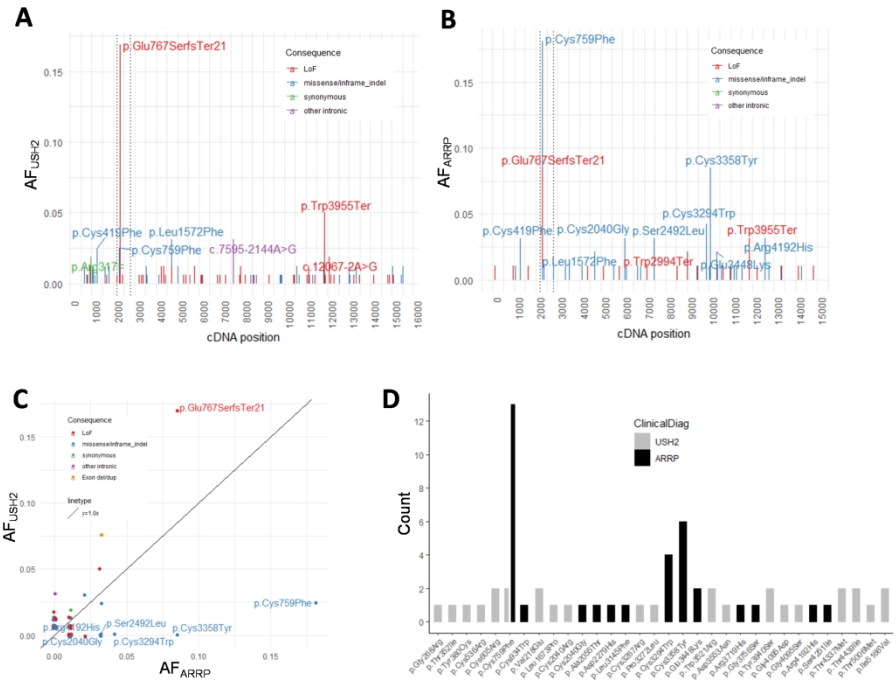

Figure 3. USH2A variants enriched in patients with USH2 and ARRP. A-B. USH2A variant allele frequency in USH2 (A) or ARRP (B) by cDNA position. Variants labeled are those with allele frequency in patient subgroup over 0.015. Dotted lines, exon 13 boundary. C. USH2A variant allele frequency comparison by diagnosis. Variants labeled in C are those with P-value (Fisher's exact test) less than 0.05 (blue) or c.2299delG p.(Glu767SerfsTer21) (red, P = 0.09). LoF, predicted loss of function variants. D. Histogram of missense variants within the 1-truncating variant subgroup by protein position.

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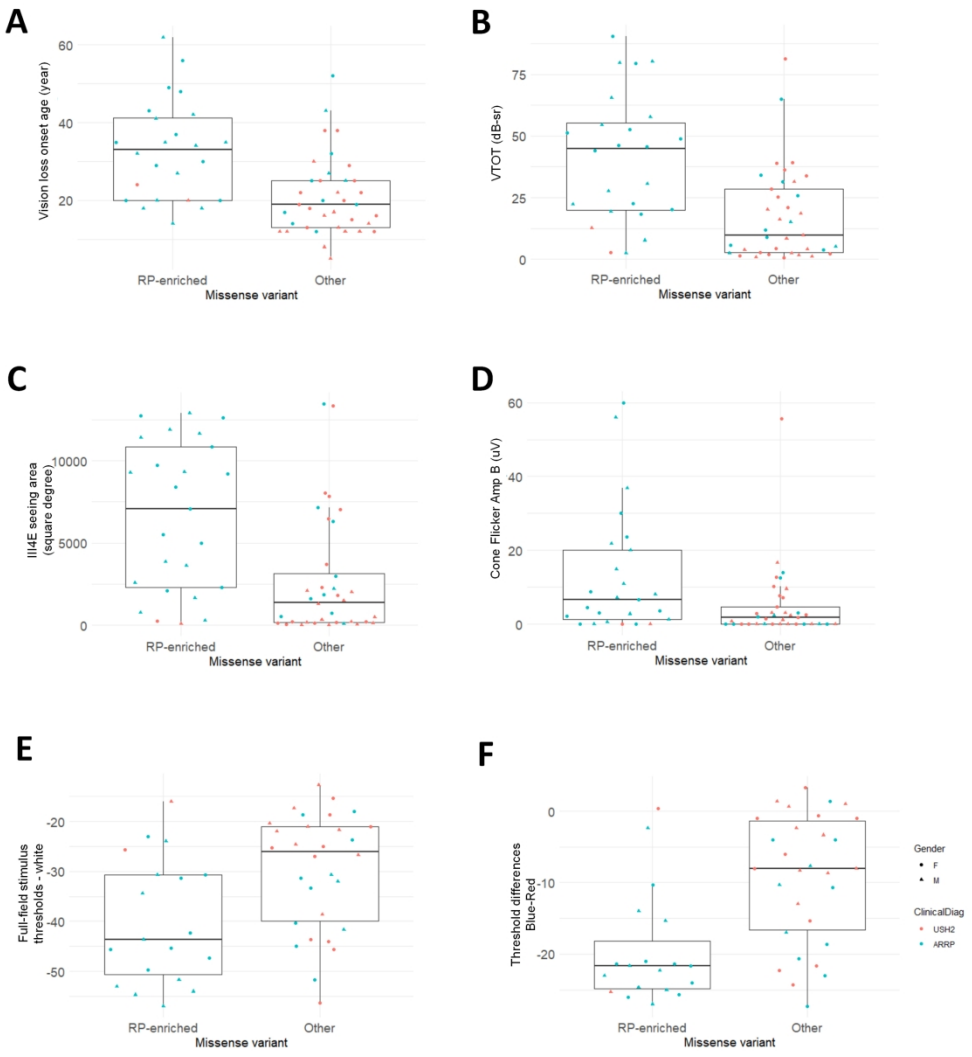

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215x228mm (300 x 300 DPI)

Supplemental Methods

Variant analysis and interpretation

In order to invoke a strong criteria of pathogenicity PS4 (i.e. “The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls”), the number of reports in the literature was used and termed rPS4. PS4 was independently assessed based on statistical enrichment of a variant in the RUSH2A cohort as compared to the healthy population (gnomAD browser) calculated by Fisher’s Exact test with Bonferroni correction, termed fPS4. Variant classification by rPS4fPS4 was then compared to evaluate the clinical utility of either one, and this criterion was only applied once per variant. The variant classification in this study was compared with the classification reported in ClinVar database downloaded on Oct. 21, 2019.(Landrum et al., 2018) Variant classification in case of conflicting evidence was determined with the use of a Bayesian classification framework.(Tavtigian et al., 2018)

For splice-altering intronic variants, we systematically analyzed whether RUSH2A variants could affect splicing by SpliceAI, a tool recently developed that had been shown to outperform other popular splicing prediction tools **(Supp. Table S1)**.(Jaganathan et al., 2019; Wai et al., 2020) The potential splicing effects were then evaluated to determine whether a variant will lead to out-of-frame or in-frame alterations based on SpliceAI predictions. Variants within the canonical splicing sites (+/-2) were given a very strong pathogenicity (PVS1) or strong pathogenicity (PS) according to ClinGen recommendations.(Abou Tayoun et al., 2018) For non-canonical splicing variants, we used high-recall delta score of 0.2 as the cutoff and found eleven variants that could affect splicing. Four of these variants had previously been shown to cause

splicing defects using an RNA analysis or a minigene assay and we applied a strong evidence for pathogenicity for three, c.949C>A p.(Arg317=), c.7595-2144A>G, c.7595-3C>G, and moderate evidence for pathogenicity for one, c.5573-834A>G. We then used the recommended SpliceAI delta score of 0.5 as cutoff for considering applying PP3 supporting evidence **(Supp. Table S1)**.(Jaganathan et al., 2019) One variant, c.10387+5C>G, is expected to enhance splicing of the original canonical site and was found in cis with another nonsense variant in the patient, thus we classified it as likely-benign. Of note, the c.6163G>A p.(Ala2055Thr) variant was identified in a nonsyndromic RP patient and *in trans* to a loss-of-function (LoF; includes nonsense, frameshift, or splice-altering) variant. The c.6163G>A variant is located at the terminal exonic nucleotide at the intron-exon junction, which typically affects splicing, so with the strong splice-altering prediction (delta score > 0.8, PP3) we applied an additional supporting evidence (PPx, terminal Guanine in an exon). In total, we applied splicing-deduced PP3 to six non-canonical splicing variants including three with RNA and/or minigene data.

Supplemental Results

Cohort-level variant classification

Subsequently, 2015 ACMG/AMP clinical variant interpretation criteria were applied to standardize variant interpretation using cohort-level information. Predicted LoF variants comprised 47% (120/254) of total alleles and received PVS1 criteria, as loss-of-function is a known mechanism for USH2A-related disorders. A detailed description of splice-altering intronic variants is provided in the methods. Variants determined to be enriched in the RUSH2A cohort compared to gnomAD was applied as PS4 criteria. This study classified ~51% variants as pathogenic, ~27 % as likely pathogenic, ~20% as variants of uncertain significance, ~2% as likely benign **(Supp. Figure S2A)**. Notably, the single likely-benign allele was *in cis* with a pathogenic allele, and this complex allele was *in trans* with another pathogenic allele. No patients were excluded from the study on the basis of genetic testing interpretation.

Of the 128 SNVs and small Indels, ~35% (45/128) were either not present (44)or the classification was not provided in the ClinVar database (1). This analysis provided clinical interpretation for ~11% as pathogenic (14/45), 14% likely pathogenic (18/45), and ~9% VOUS (12/45) **(Supp. Figure S2B)**, including one variant in ClinVar without interpretation, which was classified as likely pathogenic in this study.

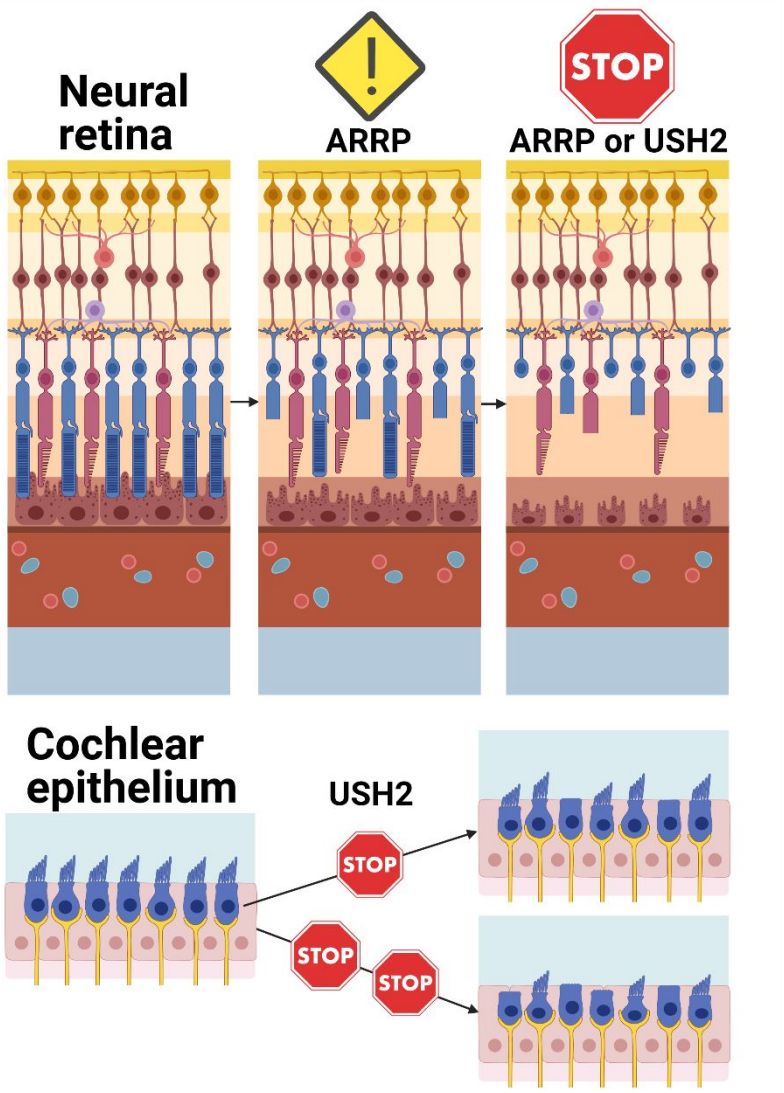
Clinical interpretation in this study disagreed with ClinVar for 13 variants. Seven variants for which ClinVar interpretations were determined “conflicting,” were classified as pathogenic (2), likely pathogenic (3), and likely benign (2) in this analysis **(Supp. Figure S2B**). Five variants listed as “uncertain” in ClinVar were classified as pathogenic

(3),likely pathogenic (1) and likely benign (1). One variant was listed as likely benign in ClinVar which was classified as a variant of uncertain significance in this study. Thus, classification was clarified for nearly 10% of variants through standardized variant analysis.

Next, we evaluated the effect of disease-enrichment (PS4) criteria on variant interpretation whether through multiple independent literature reports (rPS4), significant RUSH2A cohort enrichment (fPS4), or both. In total, 41% (53/128) variants were determined to be enriched in disease by rPS4 (14/128), fPS4 (18/128), or both (21/128). Furthermore, ~9 % variants (12/128) were reclassified, including 2 variants that were reclassified from VOUS to likely pathogenic and 10 that were reclassified from likely pathogenic to pathogenic by application of PS4 **(Supp. Figure S2C)**. Notably, previously unreported missense variant c.6118T>G (p.Cys2040Gly) was statistically enriched in this cohort and was able to be classified as pathogenic through cohort-level assessment.

Supplemental Graphic

The variants/genotypes and their effects are independent of clinical diagnosis. For the retina, the yellow sign with exclamation point indicates a nonsyndromic RP-associated missense allele, with intermediate degeneration compared to the degeneration due to biallelic loss of function variation (stop sign). In the inner ear, the number of loss of function variants correlates with onset and severity of hearing loss.



Supplemental Tables Legend

Supp. Table S1. Annotations and allele frequencies for *USH2A* variants observed in the RUSH2A cohorts

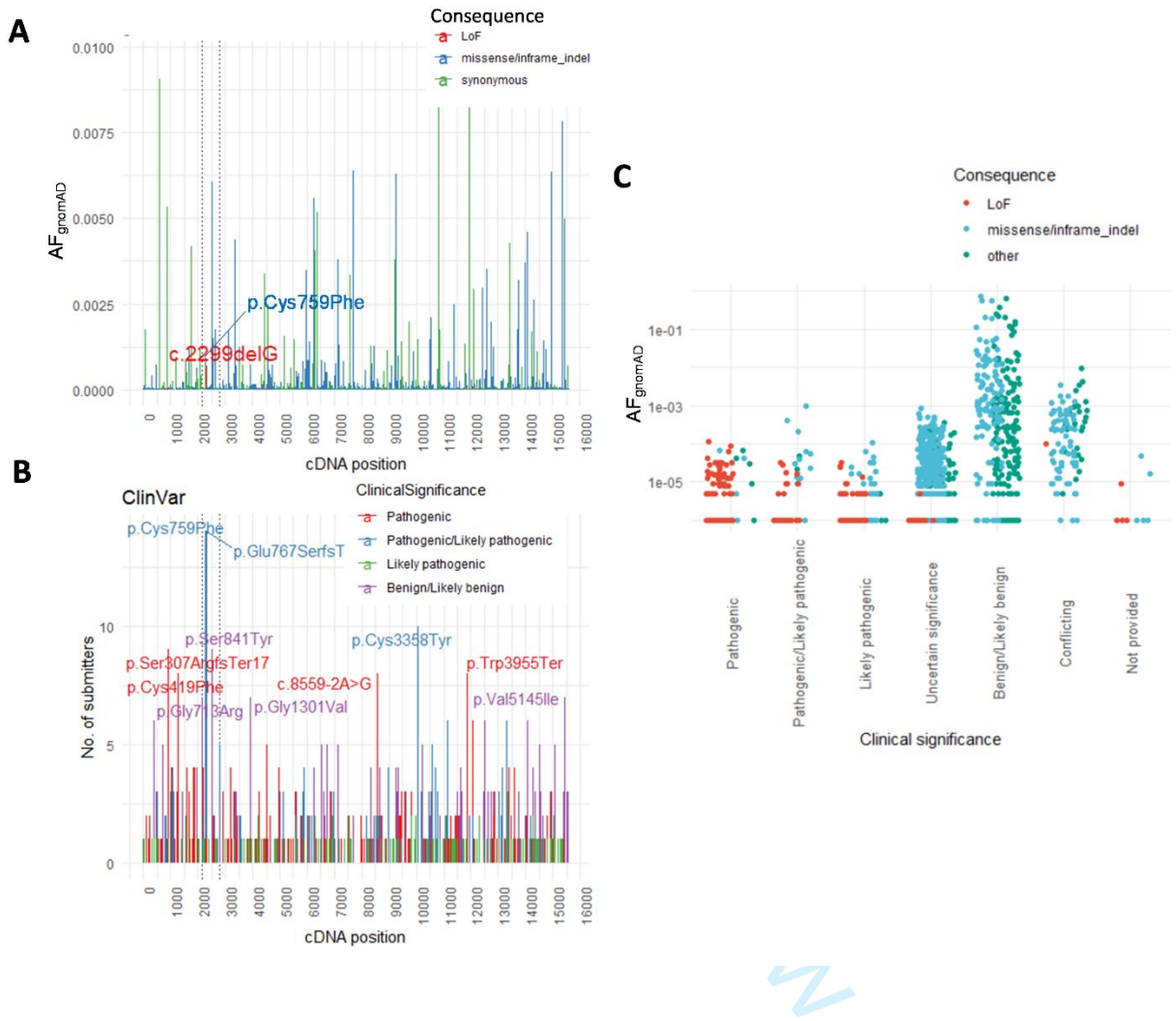
Supp. Table S2. V_{TOT} comparison adjusted for age of onset and disease duration

Supp. Table S3. Comparison of missense variant frequencies in the USH2 and ARRP subgroups

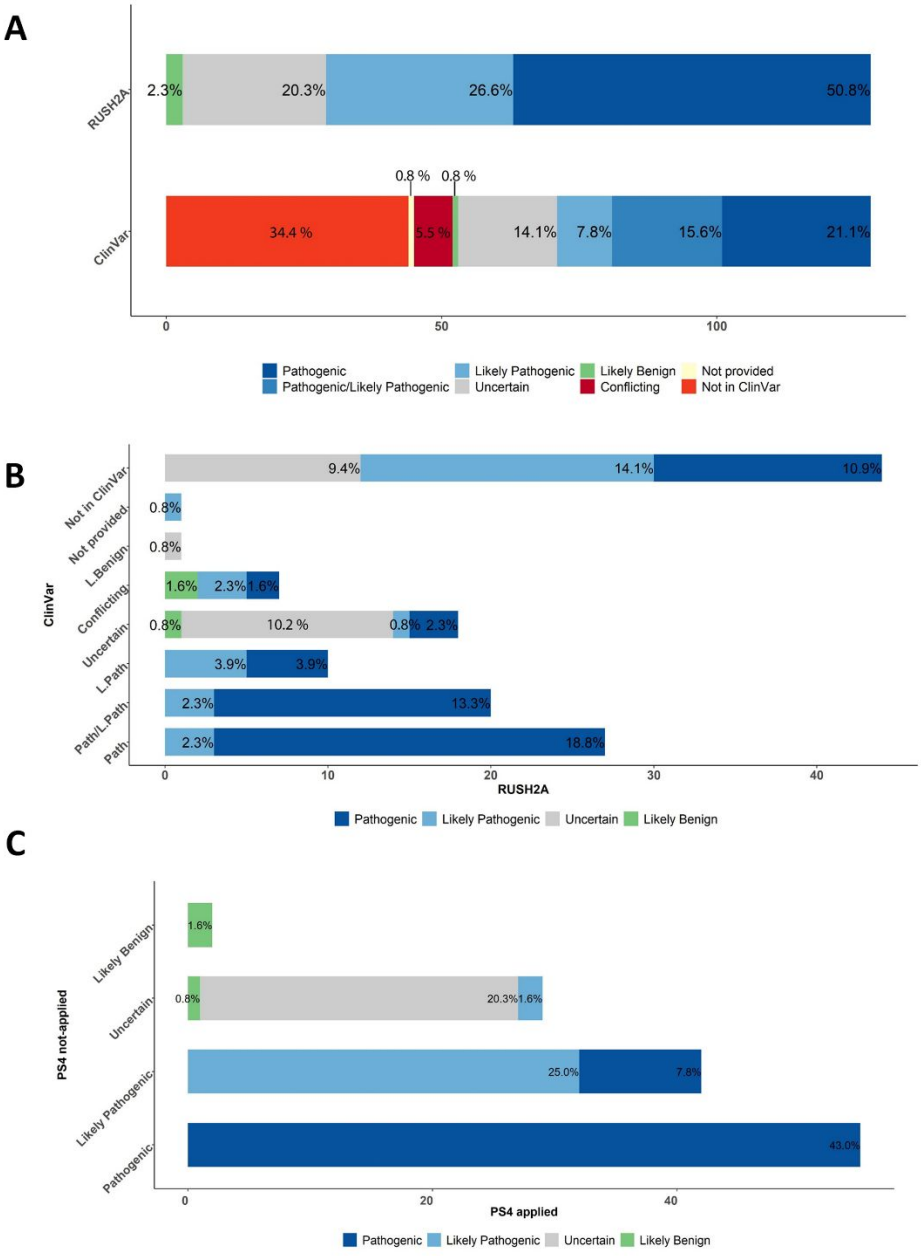
Supp. Table S4. Comparison of missense variant frequencies among patients with 1 truncating variant in the USH2 and ARRP subgroups

Supp. Table S5. Phenotype:genotype correlations and comparisons between ARRP and USH2 subgroups

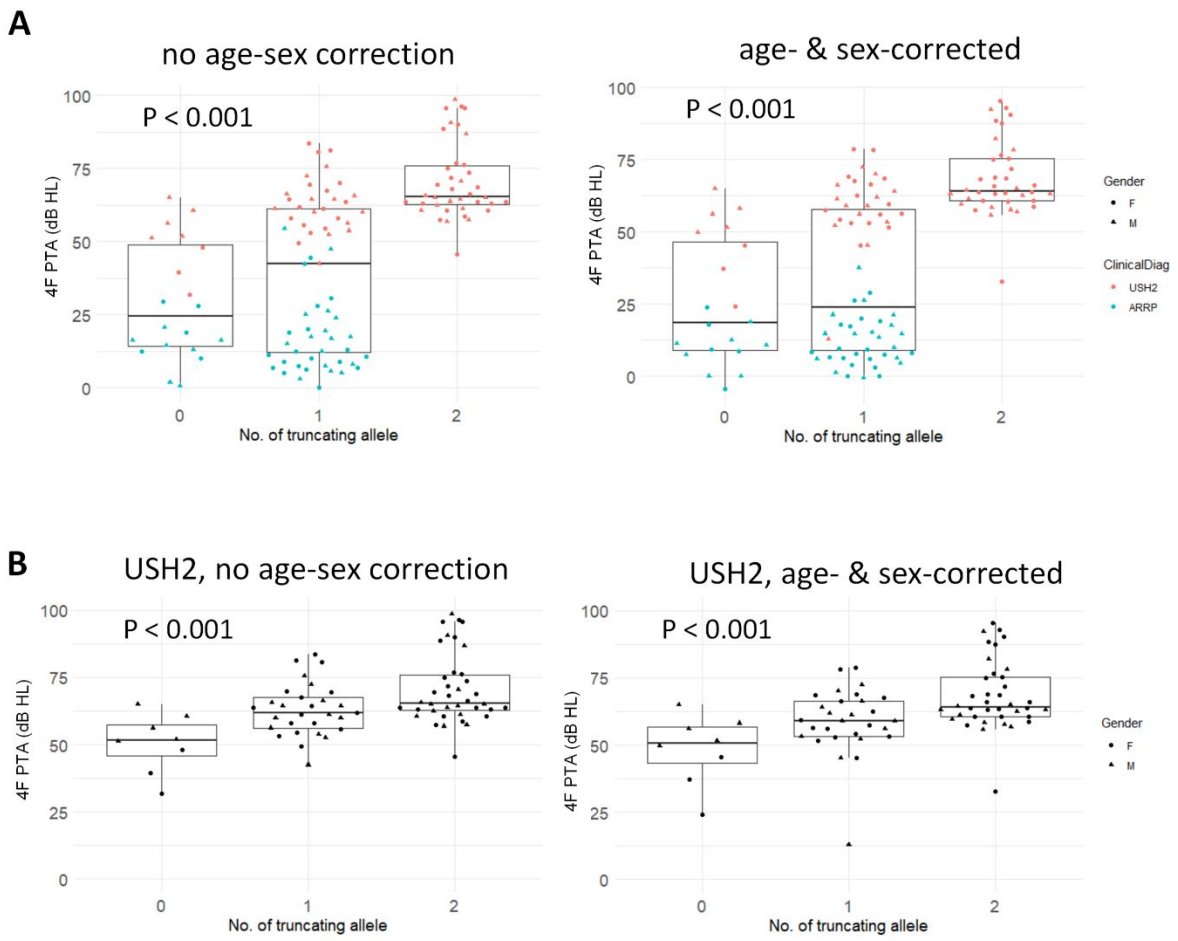
Supplemental Figures and Legends



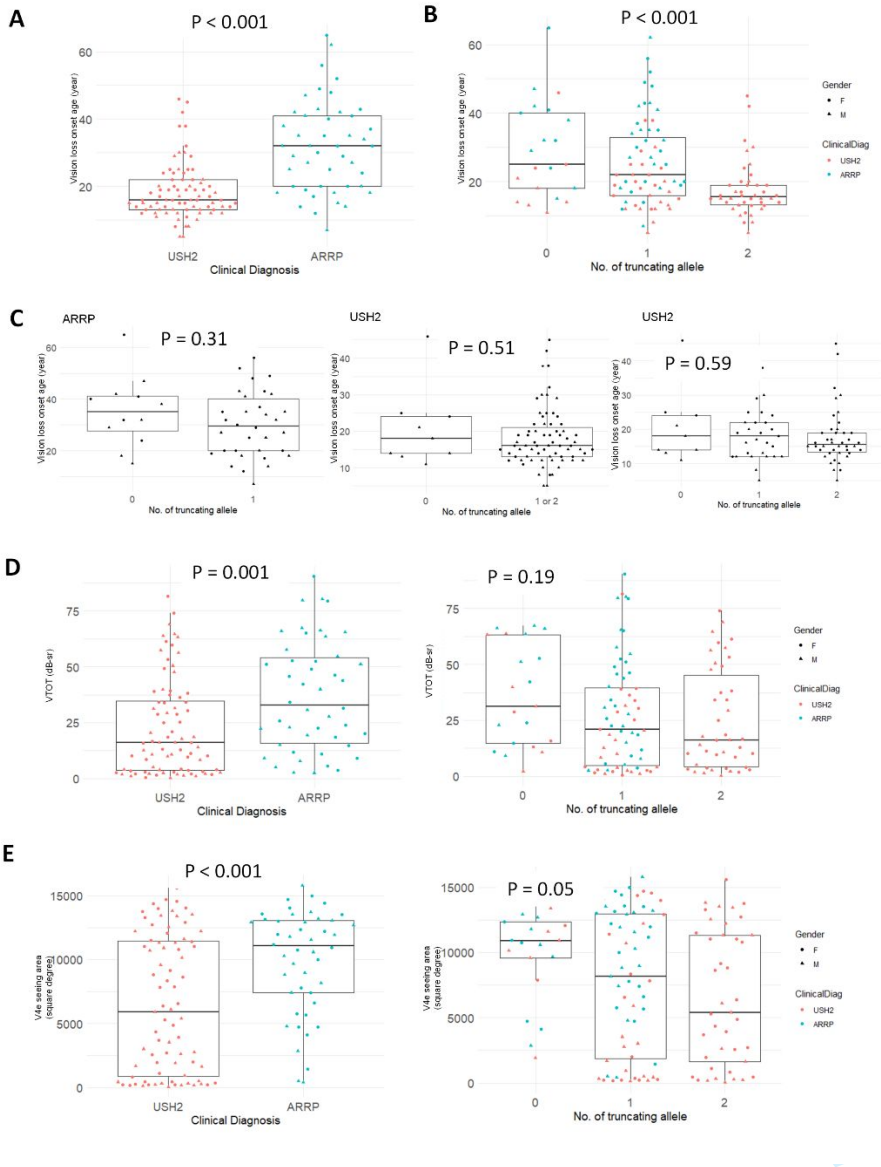
Supp. Figure S1. *USH2A* variants in gnomAD and ClinVar. **A**. Allele frequencies of rare *USH2A* variant in gnomAD by cDNA position. Only variants with AF less than 0.01 are shown. **B**. Number of submitters for *USH2A* variants present in ClinVar by cDNA position. Only variants classified as pathogenic, likely pathogenic, likely benign, or benign are shown. **C**. gnomAD allele frequencies of ClinVar *USH2A* variants. AF, allele frequency; Dotted lines, exon 13 boundary; LoF, predicted loss of function variants, including frameshift, nonsense, and canonical splicing variants.



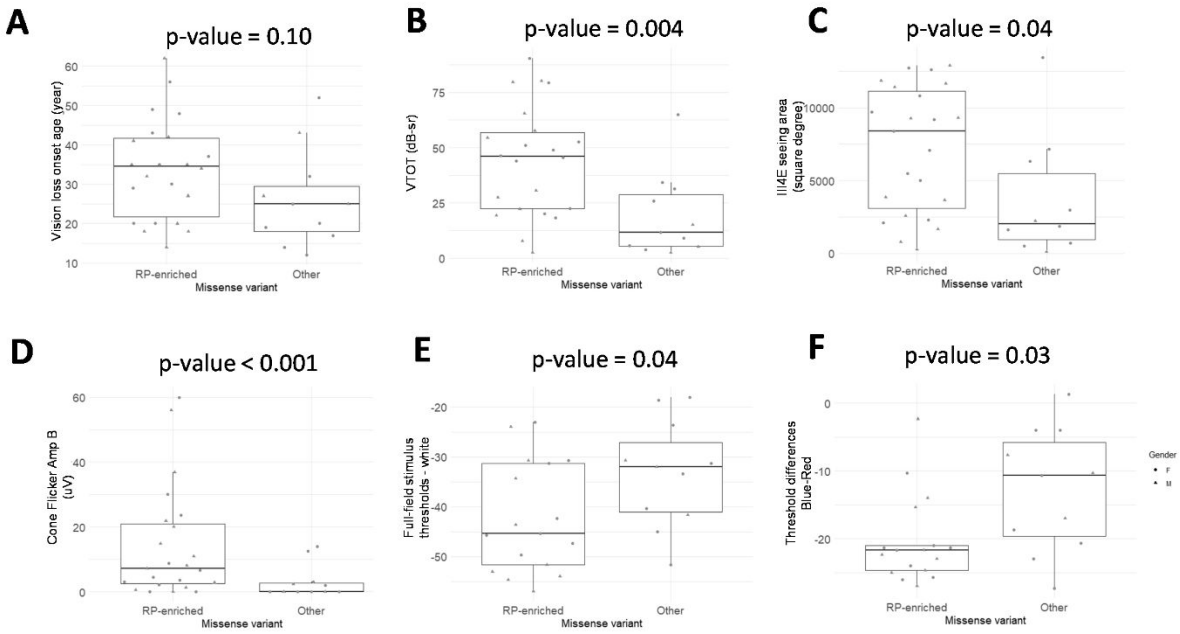
Supp. Figure S2. Variant interpretation comparison with ClinVar and effect by PS4. **A**. Variant interpretation in current study as compared to ClinVar. **B**. Variant interpretation and ClinVar concordance. **C**. Variant interpretation with and without application of PS4.



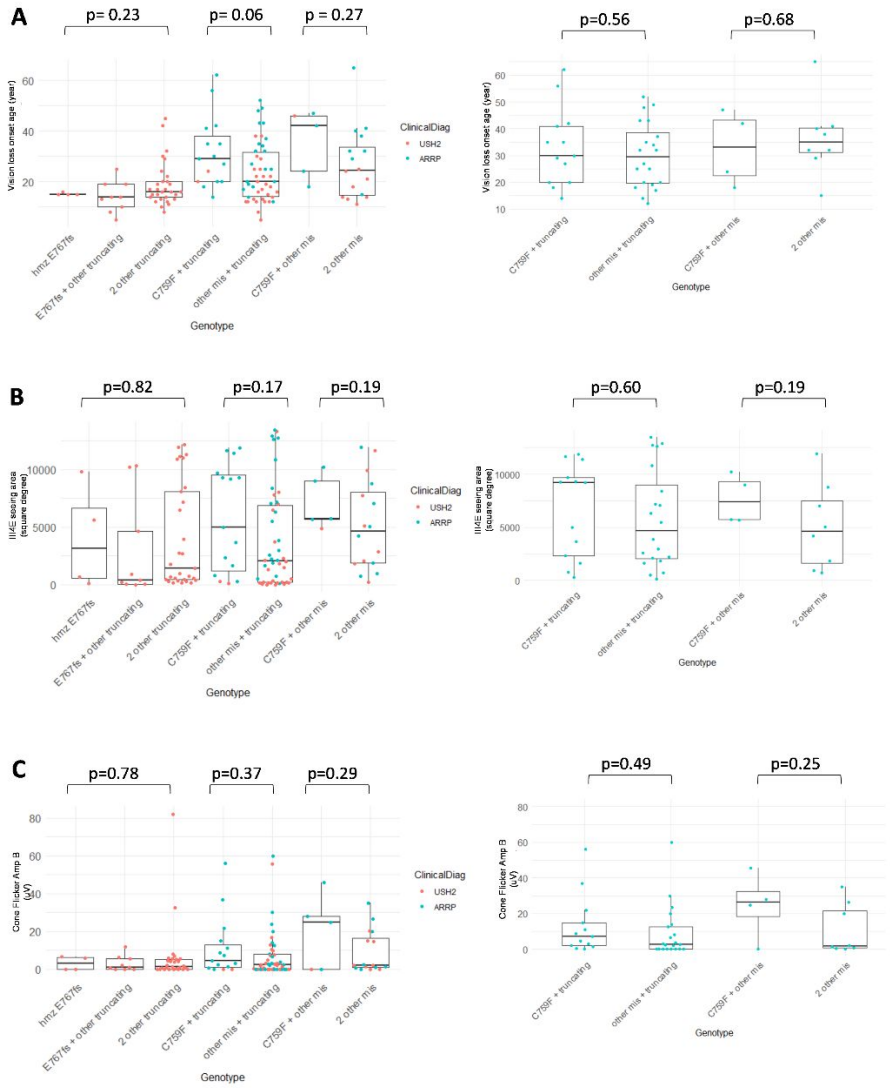
Supp. Figure S3. Truncating alleles correlate with hearing loss severity in USH2A-related disorders. Box and dot plot showing audiologic 4F PTA Score (ac_4f_pta) by number of truncating alleles for the entire RUSH2A cohort (**A**), and USH2 group (**B**). ANOVA or t-test *P*-values are noted on the plots. Left column is unadjusted for age and sex, right column is adjusted for age and sex. 4F PTA score< 20 db implies normal hearing.



Supp. Figure S4. Correlation of visual function with diagnosis and number of truncating alleles. **A & B**. Box and dot plot showing age of vision loss onset by diagnosis (**A**) and by number of truncating alleles (**B**). **C**. Box and dot plot showing age of vision loss onset by truncating number in the ARRP (left) or USH2(middle, right) groups. **D**. Box and dot plot showing full field hill of vision by diagnosis (left) and by truncating group (right). **E**. Box and dot plot showing V4e seeing area by diagnosis (left) and by truncating group (right). ANOVA or t-test *P*-values are noted on the plots. V_{TOT}, decibel-steradian (dB-sr).



Supp. Figure S5. Retinal phenotypic differences due to RP-enriched *USH2A* missense variants in the ARRP subgroup. **A-E**. Box and dot plot comparing RP-enriched and Other missense variants in the 1-truncating group, for age of vision loss onset (**A**), full-field hill of vision (**B**), iii4E seeing area (**C**), cone flicker amplitude (**D**), and full-field stimulus thresholds for White (**E**) and Blue-Red (**F**) stimulus. Circles = females, triangles = males. V_{TOT}, decibel-steradian (dB-sr). Welch's t-test *P*-values are noted on the plots.



Supp. Figure S6. Analysis of variant-specific effects of c.2299delG and c.2276G>T p.(Cys759Phe). **A-C**. Box and dot plots comparing genotypes with c.2299delG and other truncating alleles in the entire RUSH2A cohort (left column), or c.2276G>T p.(Cys759Phe) with other missense in combination with truncating or other missense alleles in the entire RUSH2A cohort (left column) or ARRP subgroup (right column), for vision loss onset (**A**), iii4E seeing area (**B**), or cone flicker amplitudes (**C**). ANOVA or t-test *P*-values are noted on the plots.

Supplemental References

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0.003937008	Likely Pathogenic
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0.003937008	Pathogenic
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0.003937008	Uncertain
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0.007874016	Likely Pathogenic

0.007874016	Pathogenic
0.007874016	Pathogenic
0.003937008	Pathogenic
0.003937008	Pathogenic
0.007874016	Pathogenic
0.007874016	Pathogenic
0.007874016	Pathogenic
0.011811024	Pathogenic
0.007874016	Pathogenic
0.043307087	Pathogenic
0.003937008	Pathogenic
0.003937008	Likely Pathogenic
0.003937008	Likely Pathogenic
0.003937008	Pathogenic
0.003937008	Likely Pathogenic
0.003937008	Likely Pathogenic
0.003937008	Uncertain
0.007874016	Likely Pathogenic
0.003937008	Uncertain
0.007874016	Likely Pathogenic
0.003937008	Likely Pathogenic
0.003937008	Likely Pathogenic
0.003937008	Uncertain
0.059055118	NA

ACMG Criteria
1xPM:PM2(rare for recessive in ExAC, in cis with truncating, not applied), BP2 (in cis with truncating), BP4 (spli
1XPS, 1xPM: PVS1_S(Exon 56 skipping, inframe 36 a.a. del), PM2 (absent in ExAC), PP5
2 x PS, 1xPM, 1xPP: PVS1_S (Exon 62 del, inframe 76 a.a. del), PM2 (rare for recessive in ExAC), PS4 (Commonl
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPVS, 1xPM: PVS1 (cryptic acceptor site, out-of-frame), PM2 (rare for recessive in ExAC)
PMx3, PPx1: PMx.PS3 (downgraded functional study), PM2 (absent ExAC/gnomAD), PM3 (in trans with truncat
1xPM, 2xPS: PVS1_S (Exon 28 skipping, inframe 68 a.a. del), PM2 (rare for recessive in ExAC), PS4 (Commonly
1x PS, 2xPM: PVS1_S (Exon 29 skipping, Inframe 27 a.a. del), PM2 (absent ExAC), PM3 (in trans in this patient)
PSx2, PMx1, PPx2: PS3 (functional data), PS4 (commonly reported) & PS4f, PM2 (absent from EXac), PP3 (Splic
2XPS, 1PM, 2PP: PS3 (functional data), PS4 (commonly reported) & PS4f, PM2 (absent EXAC), PP3 (SpliceAI = 0
PSx1, PMx2, PPx2: PS4 (multiple reports), PM2 (low freq in exac for recessive dz), PM3 (in trans with path varia
1xPVS, 2xPM: PVS1 (truncating), PM2 (absent from ExAC), PM3 (in trans in this patient)
2xPM, 2xPP: PM2 (low freq in exac), PM3 (in trans with recessive), PP3 (SpliceAI predicts donor loss (score 0.83
1xPVS, 2xPM, 1xPP: PVS1 (truncating), PMX.PS4 (multiple reports), PM2 (rare for recessive in ExAC), PP5 (ClinV
2xPM, 1PP: PM2 (rare for recessive in EXAC), PM3 (detected in trans with recessive mutation), PP3 (computati
2xPS, 1xPM,2xPP: PS3 (Functional studies),PS4 (commonly reported) & PS4f, PM2 (rare for recessive), PP3 (Spl
1x PVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPVS, 1x PS, 1xPM, 1xPP: PVS1 (truncating), PS4 (multiple reports), PM2 (rare for recessive in ExAC), PP5 (Clin
1xPS, 2xPM, 2xPP; PS4f & PMx.PS4 (reported 4x), PM3 (In trans with recessive pathogenic variant), PM2 (rare f
1xPS,2xPM,2xPP: PS4 (commonly reported), PM2 (absent from Exac), PM5 (Previous pathogenic change at san
2xPS, 1xPM: PS3 (functional studies), PS4(multiple publications), PM2 (low freq in exac)
1xPVS, 1XPS, 1xPM; PVS1 (truncating), PM2 (rare for recessive in EXAC), PS4 (multiple reports) & PS4f
1x PVS, 2xPM: PVS1 (truncating), PM2 (absent ExAC), PM3 (in trans with Path)
1xPVS, 1xPM. 1xPP; PVS1 (truncating), PM2 (rare for recessive in exac), PP5 (clinvar)
1xPS,2xPM,2xPP: PS4 (commonly reported) & PS4f, PM2 (absent from Exac), PM3 (in trans with Path, this case
1xPVS1, 2XPM: PVS1 (truncating), PM2 (rare for recessive), PM3 (in trans with path)
2xPM, 1PP: PM2(Absent from ExAC), PM3 (in trans with pathogenic variant), PP3 (in silico analysis)
PMx2, PPx1: PM2 (low freq in exac for recessive dz), PM3 (in trans with Path; this case), PP3 (computational ar
2xPM, 1xPP: PMx (Downgraded 2 publications), PM2 (Rare for recessive), PP3 (computational support)
1x PVS, 1xPM, 1xPS, 2xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PS4 (Commonly reported) & PS4
2xPM, 1xPP: PM2(low freq in exac for recessive dz),PM5 (p.Cys2040Gly is Path), PP3 (computational analysis)
1xPS, 2xPM, 2xPP: PS4f, PM2 (low freq in exac for recessive dz), PM3(detected in trans with pathogenic varian
1xPVS, 1xPM, 1 PP; PVS1 (truncating), PM2 (rare for recessive in Exac), PP5 (clinvar)
2xPM, 3xPP: PM2 (absent ExAC), PMx (multiple reports, downgraded PS4), PP3 (in silico), PP5 (ClinVar), PPx (o
1xPM, 1 xPP: PM2 (rare for recessive), PP3 (Computational support). There are two reports, but one report ca
1xPS, 2xPM, 1xPP; PS4f & PMx (two publications, downgraded PS4), PM2 (low frequency in EXAC), PM3 (in tra
1xPM, 1xPP: PM2 (low freq in EXAC), PP3 (In Silico)
1xPS, 2xPM, 2xPP: PS4 (multiple reports) & PS4f, PM2 (rare for recessive in ExAC), PM3 (in trans with path), PP
1xPM, 2xPP: PM2 (absent from EXAC), PP1 (brother affected), PP3 (In silico analysis)
1xPS, 2 xPM, 3xPP: PS4 (5+ reports) & PS4f, PM2 (rare for recessive), PM3 (in trans with Path), PP1 (cosegregat
2xPM, 1xPP: PM2 (rare for recessive), PM5 (path p.Cys419Phe), PP3 (in silico)
1xPS, 1xPM, 2xPP: PS4 (commonly reported) & PS4f, PM2 (rare for recessive in ExAC), PP3 (in silico), PP5 (ClinV
1xPS, 2xPM, 2xPP: PS4f, PM2 (rare for recessive in EXAC), PM3 (in trans with Path), PP1 (co-segregation), PP3 (
1xPS, 2xPM, 2xPP: PS4 (commonly reported) & PS4f, PM2 (rare for recessive in ExAC), PM3 (in trans with Path,
3xPM, 1xPP: PM2 (absent from EXAC), PMx.PS4 (3 reports), PM3 (in trans with p.Cys759Phe), PP3 (computatio
PMx2, PPx2: PM2 (low freq in exac for recessive dz), PM3 (in trans with path variant), PP3 (computational anal
1xPS, 2xPM, 2xPP: PS4 (multiple publications), PM2 (low freq in exac for recessive dz), PM3 (detected in trans
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
1xPVS1, 1xPS, 2XPM, : PVS1 (truncating), PM2 (rare for recessive in exac); PMx (multiple reports, downgraded
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
PMX1, BP x1: PM2 (absent in EXAC), BP4 (in silico)

1xPVS, 1xPS, 1xPM: PVS1 (truncating), PMx.PS4 (2 reports), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
1x PVS, 2xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC), PM3 (in trans in this patient)
1xPVS, 2xPM: PVS1 (truncating), PM2 (absent from ExAC), PM3 (in trans in this patient)
1xPVS, 1xPM. 1xPP; PVS1 (truncating), PM2 (rare for recessive in exac), PP5 (clinvar)
1xPS, 2xPM: PS4f & PMx (multiple reports, downgraded PS4), PM2 (rare for recessive in ExAC), PM3 (in trans v
1xPVS, 1xPM: PVS1 (truncating), PM2 (absent from ExAC)
2xPM: PM2 (rare for recessive in ExAC), PMx (multiple reports, downgraded PS4)
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
1xPVS, 2xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC), PMx (downgrade PS4 - 2 publications)
3xPM,1xPP: PM2 (rare for recessive in ExAC), PMX (reported 3X), PM4 (inframe) , PP5 (Clinvar)
1xPVS, 2xPM: PVS1 (truncating), PM2 (absent in EXAC), PM3 (in trans in this patient)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1x PVS1, 2xPS, 1xPM, 1xPP: PVS1 (truncating), PS3 (additionally affects splicing), PS4 (commonly reported) & P
PMx1, PPx1: PM2 (low freq in exac for recessive dz), PP3 (computational analysis)
1xPS, 2xPM, 2xPP: PS4(multiple reports), PM2 (low frequency for recessive disease in exac), PP3 (computation
1XPVS, 1xPS, 1xPM, 1xPP: PVS1 (truncating), PS4 (Multiple Publications), PM2 (rare for recessive in Exac), PP5
1x PVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
2xPM. 1xPP: PM2 (rare for recessive in exac), PM3 (detected in trans with pathogenic variant), PP3 (in silico)
PMx2, PPx2: PM2 (low freq in exac for recessive dz), PMx (downgrade PS4 - 2 publications), PP3 (computationa
PM x1, PPx2: PM2 (absent in EXAC), PP3 (in silico), PP (downgraded PM5, previous L-Path at same AA)
PMx1, PPx1: PM2 (low freq in exac for recessive dz), PP3 (computational analysis)
1xPVS, 2xPM: PVS1 (truncating), PM2 (rare for recessive in EXAC), PM3 (in trans in this patient)
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (absent from ExAC); PP1 (multiple affected family members)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPS, 2xPM: PS4f & PMx.PS4 (two reports), PM2 (rare for recessive)
1xPP, 2xBP: PS4 (commonly reported) & PS4f (not applied for in cis with c.2299delG), PM2 (rare for recessive i
1xPVS, 1xPS, 1xPM: PVS1 (truncation), PM2 (Absent from EXAC), PMX (2 reports) & PS4f
1xPS, 2xPM, 2xPP: PMX(3 reports) & PS4f, PM2 (absent from exac), PM3 (in trans in this patient), PP3 (compu
PMx1, PPx2: PM2(low freq in exac for recessive dz), PP3 (computational analysis), PPx.PM3 (in trans with L-pat
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPVS, 1xPM: PVS1 (truncating), PM2 (absent from ExAC)
1xPVS, 2xPM: PVS1 (truncating), PM2 (absent from ExAC), PM3 (in trans with path; this case)
1xPM, 2xPP: PM2 (absent from EXAC), PP1 (brother affected), PP3 (In silico analysis)
1xBS, 1xBP, BS2 (Observed in homozygous state in healthy), BP5 (Variant found in case with alternate cause fo
PPx1; PP3 (Computational evidence)
1xPS, 2xPM, 1xPP: PS4 (reported several times), PM2 (rare for recessive in EXAC), PM3 (Detected in trans with
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (absent from ExAC), PP5(Clinvar)
2xPM, 1xPP: PM2 (Rare for recessive), PM3 (in trans with pathogenic), PP3 (Computational Support)
1x PVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
2xPM, 1xPP: PM2 (rare in ExAC), PM3 (in trans with path, this case, but also in cis with path), PPx (reported, or
1x PVS, 1xPM, 1xPS: PVS1 (truncating), PM2 (rare for recessive in ExAC), PS4 (Commonly reported)
1xPVS, 1xPM. 1xPP; PVS1 (truncating), PM2 (rare for recessive in exac), PP5 (clinvar)
1xPS, 2xPM, 1xPP: PS4f, PM2(low freq in exac for recessive dz), PM3(detected in trans with pathogenic variant
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1x PVS, 1xPS, 2xPM, 1xPP: PVS1 (truncating), PS4 (commonly reported) & PS4f, PM2 (rare for recessive in ExAC
1xPVS, 2xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC), PMx.PS4 (multiple reports)
PMx2, PPx1: PM2 (rare in ExAC), PM3 (in trans with path, this case), PP3 (computational analysis)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
PSx1, PMx2, PPx2: PS4 (multiple publications) & PS4f, PM2 (low freq in exac for recessive dz), PM3 (detected i
1x PVS, 2xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC), PM3 (in trans)
1 PM , 1PP: PM2 (absent from Exac), PP3 (insilico)
1xPS, 1xPM, 1xPP: PS4 (commonly reported) & PS4f, PM2 (rare for recessive in ExAC), PP3 (in silico)

1xPS, 2xPM, 2xPP: PS4 (multiple publications) & PS4f, PM2 (low freq in exac), PM3 (in trans with path; PMID 2
1xPS, 2xPM, 2xPP: PS4f & PMx(downgraded for 2 publications), PM2 (rare for recessive), PM3 (in trans with pa
1xPVS, 2xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC), PM3 (in trans with Path; this case)
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
1xPVS, 1xPS, 1xPM, 1x PP: PVS1 (truncating), PS4f, PM2 (rare for recessive in ExAC), PPx (2 reports different SN
1xPVS, 1xPS, 1xPM, 1xPP: PVS1 (truncating), PS4f, PM2 (absent from exac), PP5 (Clinvar)
1xPVS, 1xPS, 1xPM, 1xPP: PVS1 (truncating), PS4f & PMx.PS4 (multiple reports), PM2 (rare for recessive in ExA
1xPS, 2xPM, 2xPP: PS4f & PMx (multiple reports, downgraded PS4), PM2 (rare for recessive in ExAC), PM3 (in t
1xPVS, 1xPS, 1xPM: PVS1 (truncating), PS4f, PM2 (absent from ExAC)
1x PVS, 1x PS, 1xPM, 1xPP: PVS1 (truncating), PS4 (mutliple reports) & PS4f, PM2 (rare for recessive in ExAC), P
1xPVS, 1xPM, 1xPP: PVS1 (truncating), PM2 (rare for recessive in ExAC), PP5 (ClinVar)
PMx3, PPx1: PMx.PS4 (multiple publications), PM2 (low freq in exac for recessive dz), PM3 (detected in trans v
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPVS, 2xPM: PVS1 (truncating), PM2 (absent from ExAC), PM3 (in trans in this patient)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
1xPVS, 1xPM2: PVS1 (truncating), PM2 (absent from ExAC)
PMx2, PPx1: PM2 (rare in ExAC), PM3 (in trans with path, this case), PP3 (computational analysis)
1xPS, 1xPM, 1xPP: PS4f, PM2 (low freq in exac for recessive dz), PP3 (computational analysis)
1xPM, 1xPP: PM2 (rare in ExAC), PP3 (SpliceAI = 0.63, new acceptor gain, out-of-frame)
1xPS, 1xPM, 1xPP: PS4r (commonly reported) & PS4f, PM2 (rare for recessive in ExAC), PP3 (in silico)
1xPVS, 1xPM: PVS1 (truncating), PM2 (rare for recessive in ExAC)
PMx2, PPx2: PM2 (low freq in exac for recessive dz), PM3 (in trans with path; this case), PP1 (familial segregati
1xPP; PP3 (in silico)
NA

PS4_report	PS4_fisher	ClinVar_Clinical_Signifi	Location.hg19	Allele
-	-	Uncertain significance	1:215960007-215960007	C
-	-	Pathogenic	1:215940022-215940022	T
PS4r	PS4f	Pathogenic	1:215853720-215853720	C
-	-	Pathogenic/Likely path	1:215848960-215848960	C
-	-	Not in ClinVar	1:216424244-216424244	T
-	-	Pathogenic/Likely path	1:216420570-216420570	C
-	-	Not in ClinVar	1:216247476-216247476	C
PS4r	PS4f	Pathogenic	1:216246438-216246438	T
-	-	Conflicting	1:216246229-216246229	G
PS4r	PS4f	Pathogenic	1:216064540-216064540	C
PS4r	PS4f	Pathogenic/Likely path	1:216062399-216062399	C
PS4r	-	Pathogenic	1:216040521-216040521	C
-	-	Not in ClinVar	1:216363567-216363567	CTGCTAAA(
-	-	Not in ClinVar	1:216221876-216221876	T
PMx.PS4	-	Pathogenic/Likely path	1:216138812-216138812	A
-	-	Uncertain significance	1:216051205-216051205	T
PS4r	PS4f	Pathogenic	1:216498841-216498841	T
-	-	Pathogenic/Likely path	1:215956215-215956215	A
PS4r	-	Pathogenic	1:216595579-216595579	A
PMx.PS4	PS4f	Pathogenic/Likely path	1:215933077-215933077	T
PS4r	-	Conflicting	1:215848679-215848679	A
PS4r	-	Conflicting	1:215848678-215848678	T
PS4r	PS4f	Pathogenic	1:215814065-215814065	A
-	-	Not in ClinVar	1:216370011-216370011	GA
-	-	Pathogenic	1:216062040-216062040	G
PS4r	PS4f	Pathogenic	1:216498754-216498754	G
-	-	Likely pathogenic	1:216256817-216256818	-
-	-	Not in ClinVar	1:216144089-216144089	G
-	-	Uncertain significance	1:215955467-215955467	T
PMx.PS4	-	Uncertain significance	1:216373196-216373196	A
PS4r	PS4f	Not in ClinVar	1:216363621-216363623	-
-	-	Not in ClinVar	1:216221921-216221921	G
-	PS4f	Uncertain significance	1:216221921-216221921	C
-	-	Pathogenic	1:216011434-216011434	T
PMx.PS4	-	Pathogenic	1:215972408-215972408	G
-	-	Uncertain significance	1:215972365-215972365	A
PMx.PS4	PS4f	Uncertain significance	1:215972325-215972325	C
-	-	Uncertain significance	1:215963573-215963573	A
PS4r	PS4f	Pathogenic/Likely path	1:215963510-215963510	T
-	-	Uncertain significance	1:215940074-215940074	C
PS4r	PS4f	Pathogenic	1:216497582-216497582	A
-	-	Not in ClinVar	1:216497582-216497582	T
PS4r	PS4f	Pathogenic	1:216495263-216495263	G
-	PS4f	Uncertain significance	1:216465544-216465544	G
PS4r	PS4f	Pathogenic/Likely path	1:216420460-216420460	A
PMx.PS4	-	Not in ClinVar	1:216420440-216420440	G
-	-	Likely pathogenic	1:216420352-216420352	T
PS4r	-	Pathogenic/Likely path	1:216419934-216419934	C
-	-	Pathogenic	1:216380742-216380744	-
PMx.PS4	PS4f	Pathogenic	1:216369924-216369924	A
-	-	Pathogenic	1:215990440-215990440	A
-	-	Not in ClinVar	1:215916551-215916551	C

	PMx.PS4	-	Pathogenic	1:215901561-215901563	-
	-	PS4f	Pathogenic	1:215844316-215844316	A
	-	-	Not in ClinVar	1:216495251-216495251	A
	-	PS4f	Pathogenic/Likely path	1:216221879-216221880	-
	PMx.PS4	PS4f	Conflicting	1:215960057-215960057	T
	-	-	Likely pathogenic	1:215916662-215916664	AAA
	PMx.PS4	-	Uncertain significance	1:215901623-215901623	T
	-	-	Pathogenic	1:215853632-215853632	AA
	PMx.PS4	-	Likely pathogenic	1:215853553-215853553	A
	PMx.PS4	-	Likely pathogenic	1:215847905-215847918	CAAG
	-	-	Not in ClinVar	1:215847786-215847786	C
	-	-	Not in ClinVar	1:215813982-215813982	T
	PS4r	PS4f	Pathogenic/Likely path	1:216420436-216420437	-
	-	-	Uncertain significance	1:216166497-216166497	A
	PS4r	-	Pathogenic/Likely path	1:216500979-216500979	T
	PS4r	-	Pathogenic	1:215990485-215990485	A
	-	-	Not in ClinVar	1:215955488-215955488	A
	-	-	Not in ClinVar	1:215932060-215932060	T
	PMx.PS4	-	Likely pathogenic	1:215853501-215853501	T
	-	-	Not in ClinVar	1:215853502-215853502	T
	-	-	Not in ClinVar	1:215848235-215848235	G
	-	-	Pathogenic	1:215848044-215848046	-
	-	-	Not in ClinVar	1:216373232-216373234	-
	-	-	Not in ClinVar	1:216144077-216144077	GATT
	PMx.PS4	PS4f	Uncertain significance	1:215802179-215802179	C
	PS4r - not applied	PS4f	Conflicting	1:216270469-216270469	A
	PMx.PS4	PS4f	Not in ClinVar	1:216270468-216270469	-
	PMx.PS4	PS4f	Likely pathogenic	1:216258189-216258189	G
	-	-	Not in ClinVar	1:215990476-215990476	A
	-	-	Not in ClinVar	1:215847897-215847898	-
	-	-	Not in ClinVar	1:216420424-216420426	G
	-	-	Not in ClinVar	1:216420305-216420305	A
	-	-	Uncertain significance	1:216246612-216246612	C
	-	-	Conflicting	1:216373248-216373248	C
	-	-	Uncertain significance	1:216052233-216052233	T
	PS4r	-	Likely pathogenic	1:215972392-215972392	A
	-	-	Pathogenic	1:215916655-215916656	-
	-	-	Not in ClinVar	1:215824005-215824005	A
	-	-	Pathogenic	1:216465677-216465678	-
	-	-	Uncertain significance	1:216370040-216370040	A
	PS4r	-	Not in ClinVar	1:216348781-216348783	-
	-	-	Pathogenic	1:216108014-216108014	C
	-	PS4f	Uncertain significance	1:216073536-216073536	A
	-	-	Not in ClinVar	1:216538302-216538304	-
	-	-	Not in ClinVar	1:216062107-216062107	G
	PS4r	PS4f	Pathogenic	1:216498869-216498869	TGGC
	PMx.PS4	-	Not in ClinVar	1:216498872-216498872	CAGC
	-	-	Not in ClinVar	1:215848501-215848501	A
	-	-	Not in ClinVar	1:216373398-216373399	-
	PS4r	PS4f	Pathogenic/Likely path	1:216498735-216498735	A
	-	-	Likely pathogenic	1:215940095-215940095	TA
	-	-	Not in ClinVar	1:215932027-215932027	A
	PS4r	PS4f	Pathogenic/Likely path	1:215848243-215848243	A

PS4r	PS4f	Pathogenic/Likely path	1:215847937-215847937	A
PMx.PS4	PS4f	Likely pathogenic	1:215812532-215812532	A
-	-	Not in ClinVar	1:215808016-215808035	GC
-	-	Pathogenic/Likely path	1:216258089-216258089	T
-	PS4f	Not in ClinVar	1:216062060-216062060	T
-	PS4f	Pathogenic/Likely path	1:216052142-216052142	T
PMx.PS4	PS4f	Pathogenic/Likely path	1:216019240-216019240	T
PMx.PS4	PS4f	Pathogenic/Likely path	1:215956104-215956104	G
NA	PS4f	Pathogenic/Likely path	1:215933128-215933128	T
PS4r	PS4f	Pathogenic	1:215901574-215901574	T
-	-	Pathogenic	1:216380622-216380622	T
PMx.PS4	-	Not provided	1:216373412-216373412	C
-	-	Not in ClinVar	1:216251618-216251618	T
-	-	Not in ClinVar	1:216221955-216221955	T
-	-	Not in ClinVar	1:216108124-216108126	-
-	-	Not in ClinVar	1:215956258-215956258	T
-	-	Uncertain significance	1:216498651-216498651	C
-	PS4f	Not in ClinVar	1:215901619-215901619	G
-	-	Not in ClinVar	1:216370038-216370038	G
PS4r	PS4f	Conflicting	1:216538426-216538426	T
-	-	Not in ClinVar	1:216061847-216061848	-
-	-	Not in ClinVar	1:215848684-215848684	G
-	-	Benign/Likely benign	1:215802242-215802242	T
-	-	NA	NA	NA

Consequen	EXON	INTRON	SpliceAI	spliceai_ma
other intro	-	52/71	C|USH2A|0.00|0.00|	0.63
LoF	-	56/71	T|USH2A|0.00|0.00|	0.76
LoF	-	61/71	C|USH2A|0.00|0.97|	0.97
LoF	-	62/71	C|USH2A|0.08|0.90|	0.9
LoF	-	Dec-71	T|USH2A|0.00|0.00|	0.91
LoF	-	Dec-71	C|USH2A|0.77|0.82|	0.82
other intro	-	27/71	C|USH2A|0.00|0.00|	0.27
LoF	-	28/71	T|USH2A|0.00|0.00|	0.99
LoF	-	29/71	G|USH2A|0.00|0.00	0.88
other intro	-	40/71	C|USH2A|0.02|0.00|	0.69
other intro	-	40/71	C|USH2A|0.63|0.89|	0.89
other intro	-	43/71	C|USH2A|0.95|0.81|	0.95
LoF	20/72	-	GCTGCTAAAGTTTT|U	0.05
missense/in	31/72	-	T|USH2A|0.00|0.00|	0.83
LoF	37/72	-	A|USH2A|0.00|0.03|	0.03
missense/in	43/72	-	T|USH2A|0.00|0.02|	0.02
synonymou	Jun-72	-	T|USH2A|0.00|0.00|	0.73
LoF	53/72	-	A|USH2A|0.00|0.00|	0.16
LoF	Feb-72	-	GA|USH2A|0.01|0.0	0.01
missense/in	57/72	-	T|USH2A|0.00|0.00|	0
missense/in	63/72	-	A|USH2A|0.00|0.00|	0
missense/in	63/72	-	T|USH2A|0.00|0.00|	0
LoF	68/72	-	A|USH2A|0.03|0.00|	0.03
LoF	19/72	-	TGA|USH2A|0.01|0.	0.17
LoF	41/72	-	TG|USH2A|0.00|0.0	0
missense/in	Jun-72	-	G|USH2A|0.00|0.00	0.03
LoF	26/72	-	T|USH2A|0.00|0.00|	0.08
missense/in	36/72	-	G|USH2A|0.02|0.00	0.02
missense/in	54/72	-	T|USH2A|0.01|0.00|	0.01
missense/in	17/72	-	A|USH2A|0.00|0.00|	0
LoF	20/72	-	C|USH2A|0.00|0.01|	0.01
missense/in	31/72	-	G|USH2A|0.00|0.00	0.05
missense/in	31/72	-	C|USH2A|0.00|0.00|	0.03
LoF	47/72	-	T|USH2A|0.00|0.00|	0
missense/in	50/72	-	G|USH2A|0.00|0.00	0
missense/in	50/72	-	A|USH2A|0.00|0.00|	0
missense/in	50/72	-	C|USH2A|0.00|0.00|	0
missense/in	51/72	-	A|USH2A|0.00|0.00|	0
missense/in	51/72	-	T|USH2A|0.00|0.00|	0
missense/in	56/72	-	C|USH2A|0.00|0.00|	0
missense/in	Jul-72	-	A|USH2A|0.00|0.00|	0
missense/in	Jul-72	-	T|USH2A|0.00|0.00|	0
missense/in	Sep-72	-	G|USH2A|0.00|0.00	0.02
missense/in	Oct-72	-	G|USH2A|0.00|0.00	0
missense/in	13/72	-	A|USH2A|0.00|0.00|	0.01
missense/in	13/72	-	G|USH2A|0.00|0.00	0
missense/in	13/72	-	T|USH2A|0.00|0.00|	0
missense/in	13/72	-	C|USH2A|0.00|0.00|	0.01
LoF	16/72	-	T|USH2A|0.00|0.04|	0.04
LoF	19/72	-	A|USH2A|0.00|0.00|	0.45
LoF	48/72	-	A|USH2A|0.00|0.00|	0.03
missense/in	59/72	-	C|USH2A|0.00|0.00|	0.37

LoF	61/72	-	T|USH2A|0.00|0.00|	0
LoF	64/72	-	A|USH2A|0.00|0.00|	0.34
LoF	Sep-72	-	A|USH2A|0.00|0.00|	0.04
LoF	31/72	-	C|USH2A|0.00|0.00|	7.00E-02
missense/ir	52/72	-	T|USH2A|0.00|0.00|	0.09
LoF	59/72	-	TAAA|USH2A|.|.|.|	0
missense/ir	61/72	-	T|USH2A|0.00|0.00|	0
LoF	62/72	-	TAA|USH2A|0.00|0.(	0
LoF	62/72	-	A|USH2A|0.00|0.00|	0
missense/ir	63/72	-	GCAAG|USH2A|.|.|.	0
LoF	63/72	-	GC|USH2A|0.00|0.0(	0.01
LoF	68/72	-	CT|USH2A|0.00|0.0(	0.08
LoF	13/72	-	T|USH2A|0.00|0.01|	0.06
missense/ir	35/72	-	A|USH2A|0.00|0.00|	0
missense/ir	May-72	-	T|USH2A|0.00|0.00|	0
LoF	48/72	-	A|USH2A|0.00|0.00|	0.01
LoF	54/72	-	A|USH2A|0.00|0.00|	0
missense/ir	58/72	-	T|USH2A|0.00|0.20|	0.2
missense/ir	62/72	-	T|USH2A|0.00|0.00|	0
missense/ir	62/72	-	T|USH2A|0.00|0.00|	0.01
missense/ir	63/72	-	G|USH2A|0.01|0.00	0.01
LoF	63/72	-	G|USH2A|0.00|0.00	0
LoF	17/72	-	A|USH2A|0.00|0.00|	0
LoF	36/72	-	GGATT|USH2A|0.14|	0.14
missense/ir	71/72	-	C|USH2A|0.00|0.00|	0
missense/ir	22/72	-	A|USH2A|0.00|0.17|	0.17
LoF	22/72	-	A|USH2A|0.00|0.24|	0.24
missense/ir	25/72	-	G|USH2A|0.02|0.00	0.02
missense/ir	48/72	-	A|USH2A|0.00|0.00|	0.01
LoF	63/72	-	C|USH2A|0.00|0.00|	0
LoF	13/72	-	TG|USH2A|.|.|.|.|.|	0
LoF	13/72	-	A|USH2A|0.00|0.00|	0
missense/ir	28/72	-	C|USH2A|0.00|0.00|	0
missense/ir	17/72	-	C|USH2A|0.00|0.00|	0
missense/ir	42/72	-	T|USH2A|0.02|0.00|	0.02
missense/ir	50/72	-	A|USH2A|0.01|0.00|	0.01
LoF	59/72	-	A|USH2A|0.00|0.04|	0.04
missense/ir	65/72	-	A|USH2A|0.00|0.00|	0
LoF	Oct-72	-	A|USH2A|0.00|0.01|	0.01
missense/ir	19/72	-	A|USH2A|0.11|0.31|	0.31
LoF	21/72	-	G|USH2A|0.30|0.05	0.3
LoF	38/72	-	C|USH2A|0.00|0.00|	0
missense/ir	40/72	-	A|USH2A|0.00|0.03|	0.06
LoF	Apr-72	-	A|USH2A|0.00|0.00|	0
LoF	41/72	-	TG|USH2A|0.00|0.0(	0
LoF	Jun-72	-	GTGGC|USH2A|0.00	0
LoF	Jun-72	-	GCAGC|USH2A|0.00	0.02
missense/ir	63/72	-	A|USH2A|0.00|0.00|	0
LoF	17/72	-	T|USH2A|0.00|0.00|	0
missense/ir	Jun-72	-	A|USH2A|0.00|0.00|	0.13
LoF	56/72	-	GTA|USH2A|0.00|0.(	0.01
missense/ir	58/72	-	A|USH2A|0.00|0.00|	0
missense/ir	63/72	-	A|USH2A|0.00|0.04|	0.04

missense/ir	63/72	-	A|USH2A|0.00|0.00|	0	
missense/ir	69/72	-	A|USH2A|0.00|0.05|	0.05	
LoF	70/72	-	TGC|USH2A|.|.|.|.	0	
LoF	25/72	-	T|USH2A|0.00|0.00|	0	
LoF	41/72	-	T|USH2A|0.02|0.00|	0.02	
LoF	42/72	-	T|USH2A|0.11|0.02|	0.11	
LoF	45/72	-	T|USH2A|0.00|0.10|	0.1	
missense/ir	53/72	-	G|USH2A|0.00|0.00	0.04	
LoF	57/72	-	T|USH2A|0.00|0.01|	0.01	
LoF	61/72	-	T|USH2A|0.00|0.00|	0	
LoF	16/72	-	T|USH2A|0.00|0.00|	0.04	
missense/ir	17/72	-	C|USH2A|0.00|0.00|	0	
LoF	27/72	-	T|USH2A|0.00|0.00|	0	
LoF	31/72	-	T|USH2A|0.00|0.07|	7.00E-02	
LoF	38/72	-	G|USH2A|0.01|0.01	0.01	
LoF	53/72	-	T|USH2A|0.00|0.00|	0	
missense/ir	Jun-72	-	C|USH2A|0.00|0.01|	0.12	
missense/ir	61/72	-	G|USH2A|0.00|0.00	0	
missense/ir	19/72	-	G|USH2A|0.63|0.41	0.63	
missense/ir	Apr-72	-	T|USH2A|0.00|0.00|	0	
LoF	41/72	-	A|USH2A|0.00|0.00|	0	
missense/ir	63/72	-	G|USH2A|0.00|0.00	0	
missense/ir	71/72	-	T|USH2A|0.09|0.03|	0.09	
NA	NA	NA	NA	NA	

reference_evidence_SpliceAI	Allele.Coun	Allele.Numl
not reported, BP4 (enhance canonical site)	7	251102
PVS1_S (Exon 56 skipping, inframe 36 a.a. del)	1	31400
PVS1_S (Exon 62 skipping, inframe 76 a.a. del)	21	249584
PVS1 (Exon 63 skipping, out-of-frame)	4	250288
PVS1 (Exon 12 skipping, out-of-frame)	0	250000
PVS1 (cryptic acceptor site, out-of-frame)	0	250000
26629787 minigene-incomplete, PM	2	143314
PVS1_S (Exon 28 skipping, inframe 68 a.a. del)	3	250072
PVS1_S (Exon 29 skipping, Inframe 27 a.a. del)	3	31394
22009552 RNA & minigene complete, PP3, PS3	6	143330
PubMed 20052763 minigene, PP3, PS3	3	248586
PubMed 23591405, PP3 (SpliceAI score 0.95, out-of-frame)	18	281218
NA	0	250000
PP3 (SpliceAI predicts donor loss (delta score 0.83), inframe deletion	2	251384
NA	2	250964
NA	19	282564
20513143 - RNA, PP3 (SpliceAI score 0.73), PS3	8	282184
NA	7	250938
NA	2	281782
NA	17	282700
NA	10	280880
NA	159	280866
NA	3	251364
NA	0	250000
NA	0	250000
NA	19	282376
NA	6	282130
NA	1	250976
NA	0	250000
NA	1	31406
NA	1	250692
NA	0	250000
NA	0	250000
NA	1	251288
NA	2	282444
NA	2	282516
NA	10	251252
NA	5	282256
NA	111	282496
NA	1	251436
NA	10	250184
NA	0	250000
NA	7	282382
NA	0	250000
NA	273	282114
NA	10	250826
NA	0	250000
NA	57	282482
NA	1	31396
nonsense	15	250944
NA	4	250496
no splicing PP3 (SpliceAI score 0.37, out-of-frame)	0	250000

NA	2	282616
nonsense	1	249154
NA	0	250000
NA	8	251396
NA	14	282704
NA	0	250000
NA	131	281496
NA	3	282422
NA	1	251188
NA	0	250000
NA	0	250000
NA	0	250000
NA	198	282180
NA	142	282444
NA	6	282212
NA	6	250190
NA	0	250000
30924848, no splicing PP3 (Exon 58 skipping, out-of-frame)	0	250000
NA	4	250940
NA	1	31386
NA	0	250000
NA	5	250850
NA	1	251000
NA	0	250000
NA	12	282882
NA	206	282678
frameshift	0	250000
NA	1	31402
NA	2	250240
NA	0	250000
NA	0	250000
NA	0	250000
NA	0	250000
NA	350	282412
NA	75	282668
NA	11	251004
NA	0	250000
NA	0	250000
NA	6	251252
no splicing PP3 (Possible 9 a.a. inframe del, not applied), 4 reports	45	281972
frameshift	0	250000
NA	0	250000
NA	6	281976
NA	0	250000
NA	0	250000
NA	0	250000
NA	0	250000
NA	0	250000
NA	0	250000
NA	2	250992
NA	0	250000
NA	0	250000
NA	0	250000

NA		3	250792
NA		2	251404
NA		0	250000
NA		0	250000
NA		0	250000
NA		0	250000
NA		4	251094
NA		8	282530
NA		0	250000
NA		33	282556
NA		0	250000
NA		4	250852
NA		0	250000
NA		0	250000
NA		0	250000
NA		0	250000
NA		1	250440
NA		0	250000
28838317, PP3 (SpliceAI = 0.63, new acceptor gain, out-of-frame)		0	250000
NA		8	249288
NA		0	250000
NA		0	250000
NA		933	282856
NA	NA	NA	

Allele.Frequency.gn	OR_RUSH2At	95CI	Pvalue	CADD_phre	MetaLR_pr	MetaLR_sc	MetaSVM_
2.79E-05	141.62	3.13;1125.3	8.06E-03	-	-	-	-
3.18E-05	123.85	1.57;8821.6	1.60E-02	-	-	-	-
8.41E-05	141.89	26.93;475.4	0	-	-	-	-
1.60E-05	247.19	5;2380.19	5.06E-03	-	-	-	-
0	Inf	25.26;Inf	1.01E-03	-	-	-	-
0	Inf	25.26;Inf	1.01E-03	-	-	-	-
1.40E-05	283.64	4.78;6220.4	5.30E-03	-	-	-	-
1.20E-05	653.97	55.04;8149	1.00E-05	-	-	-	-
9.56E-05	41.31	0.78;521.59	3.17E-02	-	-	-	-
4.19E-05	482.29	114.71;183	0	-	-	-	-
1.21E-05	649.93	54.54;8099	1.00E-05	-	-	-	-
6.40E-05	61.74	1.48;391.54	1.70E-02	-	-	-	-
0	Inf	25.26;Inf	1.01E-03	-	-	-	-
7.96E-06	493.41	8.39;8192	3.03E-03	36	T	0.2055	T
7.97E-06	492.66	8.37;8192	3.03E-03	45	-	-	-
6.72E-05	58.77	1.41;370.62	1.78E-02	25.5	T	0.3326	T
2.84E-05	555.52	123.22;236	0	-	-	-	-
2.79E-05	141.52	3.13;1124.9	8.06E-03	46	-	-	-
7.10E-06	547.87	9.4;16384	2.70E-03	-	-	-	-
6.01E-05	131.4	14.71;554.1	1.40E-04	26.8	T	0.3049	T
3.56E-05	111.02	2.55;784.81	9.89E-03	29.8	D	0.7031	D
5.66E-04	21.1	4.28;63.49	4.60E-04	22.6	T	0.355	T
1.19E-05	657.49	55.32;8192	1.00E-05	41	-	-	-
0	Inf	25.26;Inf	1.01E-03	-	-	-	-
0	Inf	25.26;Inf	1.01E-03	-	-	-	-
6.73E-05	177.71	33.47;606.3	0	25.9	D	0.6819	D
2.13E-05	186.25	4.03;1564	6.28E-03	-	-	-	-
3.98E-06	962.85	12.59;4503	2.02E-03	25.2	T	0.3389	T
0	Inf	25.26;Inf	1.01E-03	31	T	0.3589	T
3.18E-05	123.87	1.58;8823.1	1.60E-02	22	T	0.2883	T
3.99E-06	2077.92	103.31;450	0	-	-	-	-
0	Inf	25.26;Inf	1.01E-03	27.4	T	0.379	T
0	Inf	406.53;Inf	0	26.8	T	0.3874	T
3.98E-06	962.85	12.6;45035	2.02E-03	39	-	-	-
7.08E-06	549.2	9.42;16384	2.69E-03	29.6	T	0.4755	D
7.08E-06	549.34	9.43;16384	2.69E-03	24.6	T	0.291	T
3.98E-05	398.91	91.15;1421	0	32	T	0.4771	D
1.77E-05	221.91	4.7;1911.85	5.38E-03	31	T	0.2451	T
3.93E-04	82.87	34.47;171.0	0	29.8	T	0.3094	T
3.98E-06	962.85	12.61;4503	2.02E-03	25.5	T	0.4116	T
4.00E-05	702.61	225.56;192	0	28.3	D	0.5539	D
0	Inf	25.26;Inf	1.01E-03	27.7	D	0.5539	D
2.48E-05	319.74	32.26;1595	3.00E-05	24.5	D	0.8946	D
0	Inf	185.05;Inf	0	25	D	0.9348	D
9.68E-04	92.96	55.71;148.7	0	28.9	D	0.9471	D
3.99E-05	98.99	2.28;692.19	1.11E-02	26.2	D	0.9378	D
0	Inf	25.26;Inf	1.01E-03	25.2	D	0.9497	D
2.02E-04	19.58	0.49;114.67	5.08E-02	25.5	D	0.9294	D
3.19E-05	123.83	1.57;8820.6	1.60E-02	-	-	-	-
5.98E-05	133.18	14.65;568.0	1.40E-04	35	-	-	-
1.60E-05	247.39	5.01;2381.2	5.05E-03	37	-	-	-
0	Inf	25.26;Inf	1.01E-03	21.7	T	0.127	T

7.08E-06		549.54	9.43;16384	2.69E-03	-		-	
4.01E-06		2071.22	102.06;450	0	58	-	-	
0	Inf		25.26;Inf	1.01E-03	35	-	-	
3.18E-05		249.26	25.65;1216	5.00E-05	-		-	
4.95E-05		160.58	17.58;714.9	1.00E-04	29.1	T	8.10E-02	T
0	Inf		25.26;Inf	1.01E-03	-		-	
4.65E-04		8.49	0.21;48.58	0.11226	16.77	T	0.1257	T
1.06E-05		370.24	7.06;5876.9	3.59E-03	-		-	
3.98E-06		962.85	12.6;45035	2.02E-03	46	-	-	
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	-		-	
7.02E-04		227.52	150.01;338	0	-		-	
5.03E-04		7.86	0.2;44.87	0.12065	24.3	T	0.3488	T
2.13E-05		186.31	4.03;1564.(	6.28E-03	29	D	0.8398	D
2.40E-05		165.02	3.57;1315.5	7.08E-03	46	-	-	
0	Inf		25.26;Inf	1.01E-03	38	-	-	
0	Inf		25.26;Inf	1.01E-03	32	D	0.5491	D
1.59E-05		247.83	5.02;2383.5	5.05E-03	29.2	D	0.5508	D
3.19E-05		123.79	1.57;8818.1	1.60E-02	31	D	0.5489	D
0	Inf		25.26;Inf	1.01E-03	29.1	D	0.5941	D
1.99E-05		199.16	4.18;1820.8	6.05E-03	-		-	
3.98E-06		962.85	12.59;4503	2.02E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	-		-	
4.24E-05		187.56	20.21;842.3	7.00E-05	14.73	T	7.77E-02	T
7.29E-04		38.81	15.27;82.53	0	24.9	D	0.7669	D
0	Inf		185.05;Inf	0	-		-	
3.18E-05		248.68	12.9;12864	1.90E-04	26.6	D	0.6446	D
7.99E-06		491.36	8.35;8192	3.04E-03	23.7	T	0.2098	T
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	25.4	-	-	
0	Inf		25.26;Inf	1.01E-03	27.2	D	0.6127	D
1.24E-03		6.4	0.77;23.52	4.05E-02	24.1	T	0.3916	T
2.65E-04		14.89	0.37;86.41	6.60E-02	23.3	T	0.3013	T
4.38E-05		90.17	2.09;625.8(	1.21E-02	31	T	0.1269	T
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	24.8	D	0.5186	D
2.39E-05		165.59	3.58;1321.1	7.05E-03	-		-	
1.60E-04		24.74	0.61;145.85	4.06E-02	16.1	T	0.1095	T
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	37	-	-	
2.13E-05		552.8	90.1;2628.1	0	22.7	T	0.1844	T
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		406.53;Inf	0	-		-	
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	25	D	0.8664	D
0	Inf		25.26;Inf	1.01E-03	-		-	
7.97E-06		1022.96	71.84;1638	1.00E-05	24	D	0.892	D
0	Inf		25.26;Inf	1.01E-03	-		-	
0	Inf		25.26;Inf	1.01E-03	22.8	T	0.3317	T
0	Inf		185.05;Inf	0	27.9	T	0.4987	D

		1.20E-05		655.93	55.2;8174.3	1.00E-05		25.4	D		0.5059	D
		7.96E-06		1022.97	71.96;1638	1.00E-05		24.5	T		9.09E-02	T
		0	Inf		25.26;Inf	1.01E-03	-	-		-		-
		0	Inf		25.26;Inf	1.01E-03		40	-	-		-
		0	Inf		185.05;Inf	0		43	-	-		-
		0	Inf		185.05;Inf	0		50	-	-		-
		1.59E-05		494.68	44.86;3345	2.00E-05		35	-	-		-
		2.83E-05		423.63	71.64;1833	0		24.5	T		0.4214	T
		0	Inf		185.05;Inf	0		53	-	-		-
		1.17E-04		383.59	173.62;799	0		48	-	-		-
		0	Inf		25.26;Inf	1.01E-03		35	-	-		-
		1.59E-05		247.74	5.01;2383.1	5.05E-03		23.8	D		0.7708	D
		0	Inf		25.26;Inf	1.01E-03		32	-	-		-
		0	Inf		25.26;Inf	1.01E-03		35	-	-		-
		0	Inf		25.26;Inf	1.01E-03	-	-		-		-
		0	Inf		25.26;Inf	1.01E-03		38	-	-		-
		3.99E-06		962.85	12.56;4503	2.03E-03		29.9	D		0.6983	D
		0	Inf		185.05;Inf	0		24	T		0.3438	T
		0	Inf		25.26;Inf	1.01E-03		15.75	T		0.1687	T
		3.21E-05		247.17	25.44;1210	5.00E-05		30	D		0.5683	D
		0	Inf		25.26;Inf	1.01E-03	-	-		-		-
		0	Inf		25.26;Inf	1.01E-03		25.3	T		0.4736	D
		3.30E-03		1.19	0.03;6.73	0.56817		17.73	T		0.1042	T
NA		NA		NA		NA		NA		NA		NA

MetaSVM_	MutPred_T	MutPred_s	MutationAs	MutationAs	MutationTa	MutationTa	PROVEAN_	PROVEAN_s
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-0.9052	Gain_of_gly	0.212	L	1.175	D,D	0.999977,0	N	-2.12
-	-	-	-	-	A,A	1,1	-	-
-0.3513	Loss_of_Mo	0.694	M	2.915	D,D	0.958713,0	N	-2.26
-	-	-	-	-	-	-	-	-
-	-	-	-	-	A,A	1,1	-	-
-	-	-	-	-	-	-	-	-
-0.4872	Loss_of_cat	0.502	H	3.58	D,D	0.999877,0	D	-3.51
0.4337	Loss_of_Mo	0.69	M	3.045	D,D	0.992115,0	D	-4.05
-0.7842	-	-	L	1.655	N,N	0.835074,0	N	-1.15
-	-	-	-	-	A,A	1,1	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
0.4899	-	-	M,M	3.2,3.2	D,D,D	1,1,1	D,D	-2.98,-3.42
-	-	-	-	-	-	-	-	-
-0.3656	Loss_of_sta	0.489	M	2.81	D,D	0.999955,0	D	-3.59
-0.3399	Loss_of_ub	0.278	M	3.35	D,D	0.999999,0	D	-3.44
-0.7568	Loss_of_cat	0.402	M,M	2.855,2.855	N,N,N	0.999977,0	D,D	-3.57,-3.82
-	-	-	-	-	-	-	-	-
-0.3951	Gain_of_dis	0.352	H	3.54	D,D	1,1	D	-6.94
-0.3583	-	-	H	3.54	D,D	0.999989,0	D	-6.4
-	-	-	-	-	A,A	1,1	-	-
3.76E-02	Gain_of_dis	0.784	M	2.83	D,D	1,1	D	-9
-0.293	Loss_of_dis	0.623	M	2.83	D,D	0.999996,0	D	-8.11
4.75E-02	-	-	M	2.83	D,D	1,1	D	-8.26
-0.6155	-	-	M	2.83	D,D	1,1	D	-7.5
-0.4394	-	-	M	2.83	D,D	1,1	D	-7.88
-0.2113	Gain_of_dis	0.491	M	2.505	D,D	0.999986,0	D	-8.32
0.296	-	-	H,H	3.75,3.75	A,A,A	1,1,1	D,D	-7.6,-8.47
0.296	Gain_of_sh	0.987	H,H	3.75,3.75	D,D,D	1,1,1	D,D	-7.5,-8.26
0.9813	Gain_of_dis	0.965	H,H	4.185,4.185	D,D,D	1,1,1	D,D	-7.66,-9.09
1.0877	Gain_of_dis	0.883	H,H	4.83,4.83	D,D,D	1,1,1	D,D	-10.13,-11.2
1.0888	-	-	H,H	4.58,4.58	A,A,A	1,1,1	D,D	-9.39,-10.66
1.0383	-	-	H,H	4.585,4.585	D,D,D	1,1,1	D,D	-10.24,-11.8
1.0851	Loss_of_dis	0.899	H,H	4.7,4.7	D,D,D	1,1,1	D,D	-9.39,-10.52
0.9587	-	-	H,H	4.34,4.34	D,D,D	1,1,1	D,D	-9.09,-10.03
-	-	-	-	-	-	-	-	-
-	-	-	.,.	.,.	A,A,A	1,1,1	.,.	.,.
-	-	-	-	-	A,A	1,1	-	-
-0.9998	Gain_of_M	0.503	L	1.195	N,N	0.999051,0	N	-1.22

-	-	-	-	-	-	-	-	-			
-	-	-	-	-	A,A	1,1	-	-			
-	-	-	.,.	.,.	A,A,A	1,1,1	.,.	.,.			
-	-	-	-	-	-	-	-	-			
-1.0939	-	-	M	2.7	D,D	0.999988,0	N		-1.88		
-	-	-	-	-	-	-	-	-			
-1.0139	-	-	M	2.65	D,D	0.994903,0	N		-2.25		
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	A,A	1,1	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-0.3106	-	-	M	2.945	D,D	0.999627,0	D		-5.46		
0.859	Gain_of_M	0.835	M,M	2.925,2.925	D,D,D	0.999999,0	D,D	-5.2,-5.68			
-	-	-	-	-	A,A	1,1	-	-			
-	-	-	-	-	A,A	1,1	-	-			
0.1773	Gain_of_ph	0.409	H	3.785	D,D	0.999995,0	D		-4.04		
0.3065	Loss_of_cat	0.626	H	3.84	D,D	1,1	D		-5.04		
0.2737	Loss_of_cat	0.5	M	3.495	D,D	1,1	D		-4.19		
0.4092	Gain_of_ph	0.863	H	3.715	D,D	1,1	D		-5.76		
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-1.0336	-	-	M	2.175	N,N	0.999791,0	N		-0.21		
0.6255	-	-	M	2.625	D,D	0.979443,0	N		-2.39		
-	-	-	-	-	-	-	-	-			
0.3423	Loss_of_sta	0.744	M	2.475	D,D	1,1	D		-3.75		
-0.8636	-	-	L	1.87	N,N	0.996026,0	N		-2.15		
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	.,.	.,.	A,A,A	1,1,1	.,.	.,.			
0.504	Gain_of_m	0.67	M	2.825	D,D	0.579154,0	D		-2.87		
-0.3041	Loss_of_me	0.531	M,M	2.75,2.75	D,D,D	0.999995,0	D,D	-3.96,-4.63			
-0.724	Gain_of_gly	0.301	M	2.385	D,D	0.999995,0	D		-3.74		
-0.9793	Loss_of_dis	0.539	M	2.785	D,D	1,1	D		-7.36		
-	-	-	-	-	-	-	-	-			
0.1071	Loss_of_me	0.485	H	3.545	D,D	1,1	D		-5.03		
-	-	-	-	-	-	-	-	-			
-0.996	-	-	L,L	1.485,1.485	N,N,N	1,1,1	N,N	-1.8,-1.83			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	A,A	1,1	-	-			
-0.7258	-	-	L	1.46	D,D	0.852109,0	D		-2.74		
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
-	-	-	-	-	-	-	-	-			
0.8288	Loss_of_cat	0.706	M	3.46	D,D	1,1	D		-4.06		
-	-	-	-	-	-	-	-	-			
0.9806	Gain_of_sh	0.9	M,M	3.07,3.07	D,D,D	1,1,1	D,D	-4.37,-5.13			
-	-	-	-	-	-	-	-	-			
-0.2245	Gain_of_dis	0.429	M	3.495	D,D	0.963425,0	N		-2.25		
0.2102	Loss_of_gly	0.486	H	3.575	D,D	0.999992,0	D		-4.03		

0.1459	Loss_of_gly	0.515	H	3.875	D,D	1,1	D	-3.72	
-1.1096	Loss_of_me	0.413	M	2.395	D,D	0.986891,0	N	-2.46	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	-	-	A,A	1,1	-	-	
-6.12E-02	-	-	H	3.63	D,D	0.999447,0	D	-9.88	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	.,.	.,.	A,A,A	1,1,1	.,.	.,.	
0.6662	Loss_of_sta	0.625	H,H	3.57,3.57	D,D,D	0.999958,0	D,D	-6.3,-6.74	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	-	-	A,A	1,1	-	-	
-	-	-	-	-	-	-	-	-	
-	-	-	-	-	A,A	1,1	-	-	
0.5615	Loss_of_cat	0.845	M,M	3.28,3.28	D,D,D	1,1,1	D,D	-6.35,-6.75	
-0.334	Gain_of_dis	0.719	M	3.285	D,D	0.999987,0	D	-6.68	
-0.8317	Gain_of_loc	0.63	M,M	3.005,3.005	D,D,D	0.979984,0	N,N	-1.77,-1.87	
0.2206	-	-	M,M	2.875,2.875	D,D,D	0.99998,0.9	D,D	-3.82,-4.23	
-	-	-	-	-	-	-	-	-	
0.3695	Gain_of_ca	0.647	M	3.185	D,D	0.731402,0	D	-2.61	
-0.8164	-	-	M	2.325	D,D	0.840491,0	N	-0.55	
NA	NA	NA	NA	NA	NA	NA	NA	NA	

Polyphen2_	Polyphen2_	Polyphen2_	Polyphen2_	PrimateAI_	PrimateAI_	REVEL_scor	SIFT_pred	SIFT_score
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D	0.988	P	0.761	T	0.479633	0.236	T	7.70E-02
-	-	-	-	-	-	-	-	-
D	0.997	P	0.896	T	0.293477	0.348	T	7.00E-02
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D	1	D	0.925	T	0.377846	0.334	D	0.01
D	0.999	P	0.877	T	0.232624	0.599	D	5.00E-03
B	1.30E-02	B	1.10E-02	T	0.21378	0.119	T	0.148
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D,D	1.0,0.998	D,P	0.999,0.90	T	0.479651	0.737	D,D	0.0,0.003
-	-	-	-	-	-	-	-	-
D	1	D	0.972	T	0.387636	0.305	D	1.60E-02
D	0.999	D	0.914	T	0.568325	0.278	D	0
D,D	0.999,0.99	D,P	0.977,0.90	T	0.435425	0.271	T,T	0.198,0.26
-	-	-	-	-	-	-	-	-
D	1	D	0.999	T	0.589947	0.459	D	1.30E-02
D	1	D	0.999	T	0.476748	0.47	D	2.50E-02
-	-	-	-	-	-	-	-	-
D	1	D	0.998	T	0.699792	0.781	D	0
P	0.89	B	0.286	T	0.611313	0.454	D	0
D	1	D	0.987	T	0.686669	0.569	D	0
D	1	D	0.998	T	0.616676	0.489	D	3.00E-03
D	1	D	0.998	T	0.733102	0.588	D	0
D	1	D	0.999	T	0.445792	0.412	D	1.40E-02
D,D	1.0,0.999	D,D	1.0,0.98	T	0.575381	0.608	D,D	0.0,0.0
D,D	1.0,1.0	D,D	1.0,0.986	T	0.649322	0.62	D,D	0.0,0.0
D,P	1.0,0.745	D,P	0.999,0.79	T	0.645519	0.873	D,D	0.0,0.005
D,D	1.0,1.0	D,D	0.999,1.0	T	0.703189	0.971	D,D	0.0,0.0
D,D	1.0,1.0	D,D	0.999,0.99	T	0.539825	0.902	D,D	0.0,0.0
D,D	1.0,1.0	D,D	0.996,1.0	T	0.681494	0.985	D,D	0.0,0.0
D,D	1.0,1.0	D,D	0.999,1.0	T	0.680256	0.917	D,D	0.0,0.0
D,D	1.0,1.0	D,D	0.999,1.0	T	0.701898	0.849	D,D	0.0,0.0
-	-	-	-	-	-	-	-	-
.,.	.,.	.,.	.,.	-	-	-	.,.	.,.
-	-	-	-	-	-	-	-	-
B	1.20E-02	B	1.80E-02	T	0.317526	0.166	T	0.47

-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
.,.	.,.	.,.	.,.	-	-	-	.,.	.,.
-	-	-	-	-	-	-	-	-
D	0.999	P	0.833	T	0.425593	0.23	T	0.124
-	-	-	-	-	-	-	-	-
B	0.445	B	7.40E-02	T	0.421465	0.15	T	0.153
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D	1	D	1	T	0.486896	0.415	D	4.00E-03
D,D	1.0,1.0	D,D	1.0,1.0	T	0.652135	0.902	D,D	0.0,0.0
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D	1	D	0.999	T	0.431446	0.464	D	0
D	1	D	0.999	T	0.594752	0.716	D	1.10E-02
D	1	D	0.999	T	0.504093	0.496	D	1.50E-02
D	1	D	1	T	0.492463	0.7	D	0
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
B	0.01	B	0.01	T	0.326803	1.60E-02	T	0.269
D	0.978	P	0.885	T	0.353551	0.655	D	1.10E-02
-	-	-	-	-	-	-	-	-
D	0.983	P	0.899	T	0.591164	0.808	D	2.00E-03
P	0.883	P	0.459	T	0.272653	0.263	D	2.10E-02
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
.,.	.,.	.,.	.,.	-	-	-	.,.	.,.
D	0.999	D	0.947	T	0.523945	0.686	D	6.00E-03
D,D	0.999,1.0	D,D	0.953,0.98	T	0.416215	0.394	D,T	0.042,0.107
D	1	D	0.984	T	0.5552	0.329	T	0.309
D	1	D	0.999	T	0.563603	0.5	D	0
-	-	-	-	-	-	-	-	-
D	1	D	0.999	T	0.413277	0.482	D	0
-	-	-	-	-	-	-	-	-
B,B	0.035,0.061	B,B	0.038,0.022	T	0.370376	0.231	T,T	0.503,0.262
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
B	0.06	B	2.30E-02	T	0.389904	0.102	T	0.117
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D	1	D	0.999	T	0.41368	0.658	D	0
-	-	-	-	-	-	-	-	-
D,D	1.0,1.0	D,D	0.999,0.98	T	0.569895	0.779	D,D	0.003,0.002
-	-	-	-	-	-	-	-	-
D	0.976	P	0.53	T	0.378411	0.291	D	0.05
D	1	D	0.986	T	0.389547	0.522	D	2.00E-03

D	1	D	0.997	T	0.484738	0.564	D	1.10E-02
D	1	D	0.981	T	0.47839	0.603	D	7.00E-03
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D	0.998	D	0.909	T	0.605682	0.654	D	0
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
.,.	.,.	.,.	.,.	-	-	-	.,.	.,.
D,D	1.0,1.0	D,D	0.998,1.0	T	0.553375	0.665	D,D	0.001,0.0
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
D,D	1.0,1.0	D,D	0.989,0.994	T	0.67523	0.89	D,D	0.0,0.001
D	0.999	D	0.96	T	0.487403	0.494	D	2.00E-03
P,P	0.775,0.859	B,B	0.306,0.334	T	0.457287	0.279	T,T	0.131,0.116
D,D	1.0,1.0	D,D	0.999,0.994	T	0.603996	0.858	D,D	0.001,0.001
-	-	-	-	-	-	-	-	-
D	0.99	P	0.814	T	0.417244	0.595	D	4.00E-03
P	0.553	B	4.70E-02	T	0.301491	0.126	D	0.03
NA	NA	NA	NA	NA	NA	NA	NA	NA

3

7

2

2

Models	N=126[a]	Mean Vtot (SD) – decibel steradians	Adjusted Mean Vtot (95% CI)- decibel steradians[b]	Difference from Reference Group (95% CI)	P-value[c]
Clinical Diagnosis					0.007
USH2	80	22.5 (21.5)	22.6 (17.6, 27.6)	Reference	
ARRP	46	37.1 (24.7)	35.7 (28.6, 42.8)	13.2 (3.6, 22.7)	
Duration of disease, yrs[d]					0.04
<10	36	40.5 (22.6)	40.1 (32.8, 47.3)	Reference	
[10,20)	46	28.5 (21.9)	28.9 (22.6, 35.2)	-11.2 (-20.3, -2.0)	
>=20	43	15.0 (18.2)	24.9 (16.0, 33.6)	-15.2 (-27.1, -3.3)	
Age of enrollment, yrs[e]					0.03
<35	44	35.7 (23.0)	38.0 (29.3, 46.8)	Reference	
35-45	43	23.9 (22.4)	28.9 (22.3, 35.4)	-9.2 (-20.1, 1.7)	
>=45	39	23.1 (24.2)	26.9 (19.9, 33.9)	-11.1 (-23.0, 0.7)	
Truncating group					0.67
0	21	36.2 (22.9)	36.4 (27.2, 45.6)	Reference	
1	63	27.0 (24.2)	27.2 (22.0, 32.4)	-9.2 (-19.6, 1.28)	
2	42	24.9 (22.9)	30.2 (22.4, 37.9)	-6.2 (-18.5, 6.1)	

[a]Static perimetry results were graded by a reading center. Results are based on the average of 3 f
[b]Simultaneous adjustment for duration of disease, clinical diagnosis, and age of enrollment, trunca
[c]Factors are presented categorically to show the data but were analyzed using continuous version
[d]1 participant in the ARRP group was missing age of onset (a participant-reported field based on th
[e]28 participants were not permitted to report date of birth due to regulatory restrictions. Therefore,

fields when 3 tests were performed (primary cohort); otherwise they based on just the 1 test performed (
ating group
of the factor in the model.
heir awareness of visual symptoms) and duration of disease (computed based on age of onset and date
only year of birth and categorical age was reported. For those participants, July 1st with the reported bir

(secondary cohort). Static perimetry data is not included for 1 participant in the ARRP group (participant

e of enrollment)
rth year was imputed as birth date to calculate continuous age

was not tested).

Models	N=125[a]	Mean V4e seeing area (SD) – decibel steradians	Adjusted Mean V4e seeing area (95% CI)- decibel steradians[b]
Clinical Diagnosis			
USH2	79	6476.8 (5320.4)	6845.9 (5791.3, 7900.6)
ARRP	46	9877.8 (4088.1)	9129.2 (7639.7, 10619.0)
Duration of disease, yrs[d]			
<10	37	10726 (3686.3)	10682.0 (9166.9, 12198.0)
[10,20)	44	8288.2 (4743.1)	8212.8 (6848.8, 9576.8)
>=20	43	4421.0 (4826.9)	6467.5 (4593.8, 8341.3)
Age of enrollment, yrs[e]			
<35	43	9532.2 (4544.1)	9898.0 (8032.4, 11764.0)
35-45	43	7284.9 (5343.8)	8267.9 (6870.8, 9664.9)
>=45	39	6228.4 (5113.2)	7196.8 (5703.1, 8690.4)
Truncating group			
0	21	9932.3 (3599.0)	9834.6 (7882.7, 11786.0)
1	63	7739.4 (5400.8)	7895.9 (6791.8, 9000.1)
2	41	6582.5 (5179.1)	7632.2 (5957.4, 9306.9)

[a]Kinetic perimetry results were graded by a reading center. Seeing area was calculated as isopter
[b]Simultaneous adjustment for duration of disease, clinical diagnosis, and age of enrollment, trunca
[c]Factors are presented categorically to show the data but were analyzed using continuous version
[d]1 participant in the ARRP group was missing age of onset (a participant-reported field based on th
[e]28 participants were not permitted to report date of birth due to regulatory restrictions. Therefore,

Difference from Reference Group (95% CI)	P-value[c]
	<0.001
Reference	
2283.2 (274.8, 4291.7)	
	<0.001
Reference	
-2469.6 (-4433.5, -505.6)	
-4214.8 (-6736.8, -1692.9)	
	0.16
Reference	
-1630.2 (-3950.8, 690.5)	
-2701.2 (-5236.8, -165.8)	
	0.26
Reference	
-1938.6 (-4163.6, 286.4)	
-2202.4 (-4834.3, 429.6)	

area minus scotoma. Scotoma not tested/measured was treated as 0 in the calculation. Twenty-eight participant
ating group
of the factor in the model.
heir awareness of visual symptoms) and duration of disease (computed based on age of onset and date of enrol
only year of birth and categorical age was reported. For those participants, July 1st with the reported birth year v

ts in USH2 group and 14 participants in ARRP group have V4e scotomas not tested/measured and treat

llment)
was imputed as birth date to calculate continuous age

ted as 0 (2 subjects were excluded for procedure issues)

HGVSc.vep	HGVSp.vep	patientAC.RP	patient_freq.RI	patientAC.Usher	patient_freq.Ush
c.2276G>T	p.Cys759Phe	17	0.180851064	4	0.025
c.10073G>A	p.Cys3358Tyr	8	0.085106383	0	0
c.9882C>G	p.Cys3294Trp	4	0.042553191	0	0
c.12575G>A	p.Arg4192His	3	0.031914894	0	0
c.6118T>G	p.Cys2040Gly	3	0.031914894	0	0
c.7475C>T	p.Ser2492Leu	3	0.031914894	0	0
c.10342G>A	p.Glu3448Lys	2	0.021276596	0	0
c.8981G>A	p.Trp2994Ter	2	0.021276596	0	0
c.10010G>T	p.Cys3337Phe	1	0.010638298	0	0
c.10387+5C>G	c.10387+5C>G	1	0.010638298	0	0
c.10636G>T	p.Gly3546Ter	1	0.010638298	0	0
c.10974_10975i	p.Thr3659Ter	1	0.010638298	0	0
c.10996T>G	p.Cys3666Gly	1	0.010638298	0	0
c.11266G>A	p.Gly3756Ser	1	0.010638298	0	0
c.11516A>G	p.Gln3839Arg	1	0.010638298	0	0
c.12232G>T	p.Glu4078Ter	1	0.010638298	0	0
c.12574C>T	p.Arg4192Cys	1	0.010638298	0	0
c.12752G>T	p.Ser4251Ile	1	0.010638298	0	0
c.13335_13347c	p.Glu4445_Ser444	1	0.010638298	0	0
c.13355del	p.Leu4452CysfsTe	1	0.010638298	0	0
c.14272C>T	p.Pro4758Ser	1	0.010638298	0	0
c.1618C>T	p.Gln540Ter	1	0.010638298	0	0
c.2296T>C	p.Cys766Arg	1	0.010638298	0	0
c.2384G>A	p.Cys795Tyr	1	0.010638298	0	0
c.2802T>G	p.Cys934Trp	1	0.010638298	0	0
c.3368A>G	p.Tyr1123Cys	1	0.010638298	0	0
c.3547_3548del	p.Ile1183PhefsTer	1	0.010638298	0	0
c.4106C>T	p.Ser1369Leu	1	0.010638298	0	0
c.4393_4394ins/	p.Ala1465GlufsTer	1	0.010638298	0	0
c.5118G>A	p.Trp1706Ter	1	0.010638298	0	0
c.5603T>G	p.Phe1868Cys	1	0.010638298	0	0
c.5857+2T>C	c.5857+2T>C	1	0.010638298	0	0
c.6163G>A	p.Ala2055Thr	1	0.010638298	0	0
c.6670G>T	p.Gly2224Cys	1	0.010638298	0	0
c.6835G>C	p.Asp2279His	1	0.010638298	0	0
c.7132_7133del	p.Tyr2378HisfsTer	1	0.010638298	0	0
c.9424G>T	p.Gly3142Ter	1	0.010638298	0	0
c.9433C>T	p.Leu3145Phe	1	0.010638298	0	0
c.9469C>T	p.Gln3157Ter	1	0.010638298	0	0
c.99_100insT	p.Arg34SerfsTer41	1	0.010638298	0	0
c.1256G>T	p.Cys419Phe	3	0.031914894	4	0.025
c.11156G>A	p.Arg3719His	1	0.010638298	1	0.00625
c.14131C>T	p.Gln4711Ter	1	0.010638298	1	0.00625
c.14803C>T	p.Arg4935Ter	1	0.010638298	1	0.00625
c.6159del	p.Glu2054LysfsTer	1	0.010638298	1	0.00625
c.8522G>A	p.Trp2841Ter	1	0.010638298	1	0.00625
c.2299del	p.Glu767SerfsTer2	8	0.085106383	27	0.16875
c.7595-2144A>G	c.7595-2144A>G	0	0	5	0.03125
Exon del/dup	Exon del/dup	3	0.031914894	12	0.075
c.12067-2A>G	c.12067-2A>G	0	0	3	0.01875
c.1055C>T	p.Thr352Ile	0	0	2	0.0125
c.11105G>A	p.Trp3702Ter	0	0	2	0.0125

c.11819A>C	p.Tyr3940Ser	0	0	2	0.0125
c.13010C>T	p.Thr4337Met	0	0	2	0.0125
c.13316C>T	p.Thr4439Ile	0	0	2	0.0125
c.15017C>T	p.Thr5006Met	0	0	2	0.0125
c.15496A>G	p.Ile5166Val	0	0	2	0.0125
c.1606T>C	p.Cys536Arg	0	0	2	0.0125
c.1813T>C	p.Cys605Arg	0	0	2	0.0125
c.3532C>G	p.Pro1178Ala	0	0	2	0.0125
c.4222C>T	p.Gln1408Ter	0	0	2	0.0125
c.4338_4339del	p.Cys1447GlnfsTe	0	0	2	0.0125
c.4714del	p.Leu1572PhefsTe	0	0	2	0.0125
c.5018T>C	p.Leu1673Pro	0	0	2	0.0125
c.5776+1G>A	c.5776+1G>A	0	0	2	0.0125
c.653T>A	p.Val218Glu	0	0	2	0.0125
c.7595-3C>G	c.7595-3C>G	0	0	2	0.0125
c.7931G>A	p.Trp2644Ter	0	0	2	0.0125
c.11864G>A	p.Trp3955Ter	3	0.031914894	8	0.05
c.1036A>C	p.Asn346His	1	0.010638298	2	0.0125
c.10561T>C	p.Trp3521Arg	1	0.010638298	2	0.0125
c.920_921insGC	p.Ser307ArgfsTer1	1	0.010638298	2	0.0125
c.4714C>T	p.Leu1572Phe	2	0.021276596	5	0.03125
c.949C>A	p.Arg317=	1	0.010638298	3	0.01875
c.10407C>A	p.Tyr3469Ter	0	0	1	0.00625
c.10450C>T	p.Arg3484Ter	0	0	1	0.00625
c.10657G>A	p.Asp3553Asn	0	0	1	0.00625
c.11047+1G>A	c.11047+1G>A	0	0	1	0.00625
c.11299A>T	p.Thr3767Ser	0	0	1	0.00625
c.1139A>G	p.Tyr380Cys	0	0	1	0.00625
c.11403_11404c	p.Glu3802LeufsTe	0	0	1	0.00625
c.11411del	p.Pro3804LeufsTe	0	0	1	0.00625
c.11815G>A	p.Glu3939Lys	0	0	1	0.00625
c.11875_11876c	p.Gln3959AsnfsTe	0	0	1	0.00625
c.12152_12153i	p.Glu4051AspfsTe	0	0	1	0.00625
c.12283G>A	p.Gly4095Ser	0	0	1	0.00625
c.12284G>A	p.Gly4095Asp	0	0	1	0.00625
c.12295-2A>G	c.12295-2A>G	0	0	1	0.00625
c.12569T>C	p.Val4190Ala	0	0	1	0.00625
c.1256G>A	p.Cys419Tyr	0	0	1	0.00625
c.13018G>C	p.Gly4340Arg	0	0	1	0.00625
c.13207_13208c	p.Gly4403ProfsTe	0	0	1	0.00625
c.13466dup	p.Glu4491GlyfsTe	0	0	1	0.00625
c.14885dup	p.Glu4963GlyfsTe	0	0	1	0.00625
c.15063_15081c	p.Thr5022GlnfsTe	0	0	1	0.00625
c.15433G>A	p.Val5145Ile	0	0	1	0.00625
c.1679del	p.Pro560LeufsTer3	0	0	1	0.00625
c.2167+1G>A	c.2167+1G>A	0	0	1	0.00625
c.2168-2A>G	c.2168-2A>G	0	0	1	0.00625
c.2310_2311del	p.Lys770AsnfsTer1	0	0	1	0.00625
c.2431A>T	p.Lys811Ter	0	0	1	0.00625
c.3187_3188del	p.Gln1063SerfsTe	0	0	1	0.00625
c.3309C>A	p.Tyr1103Ter	0	0	1	0.00625
c.3381del	p.Thr1128ProfsTe	0	0	1	0.00625
c.3584G>T	p.Cys1195Phe	0	0	1	0.00625

c.4108G>C	p.Val1370Leu	0	0	1	0.00625
c.4133_4134dup	p.Asn1379SerfsTe	0	0	1	0.00625
c.4438_4439del	p.Ser1480HisfsTer	0	0	1	0.00625
c.5278del	p.Asp1760MetfsTe	0	0	1	0.00625
c.5385T>A	p.Tyr1795Ter	0	0	1	0.00625
c.5573-834A>G	c.5573-834A>G	0	0	1	0.00625
c.6084T>A	p.Tyr2028Ter	0	0	1	0.00625
c.6118T>C	p.Cys2040Arg	0	0	1	0.00625
c.6847_6848ins	p.Ile2283AsnfsTer	0	0	1	0.00625
c.6967C>T	p.Arg2323Ter	0	0	1	0.00625
c.7244C>G	p.Ser2415Ter	0	0	1	0.00625
c.775_776del	p.Ser259PhefsTer	0	0	1	0.00625
c.7883dup	p.Ser2629LysfsTer	0	0	1	0.00625
c.7950dup	p.Asn2651GlnfsTe	0	0	1	0.00625
c.802G>A	p.Gly268Arg	0	0	1	0.00625
c.8143del	p.Val2715Ter	0	0	1	0.00625
c.8431C>A	p.Pro2811Thr	0	0	1	0.00625
c.8576G>A	p.Arg2859His	0	0	1	0.00625
c.8682-9A>G	c.8682-9A>G	0	0	1	0.00625
c.917_918insGC	p.Ser307LeufsTer1	0	0	1	0.00625
c.9270C>A	p.Cys3090Ter	0	0	1	0.00625
c.9799T>C	p.Cys3267Arg	0	0	1	0.00625
c.9815C>T	p.Pro3272Leu	0	0	1	0.00625
c.9842G>T	p.Cys3281Phe	0	0	1	0.00625

OR_RPtoUsl	95CI	Pvalue	Location.hg	Allele	Consequen	EXON	INTRON
8.54	2.66;36.04	2.00E-05	1:2164204(	A	missense/in	13/72	-
Inf	3.07;Inf	2.90E-04	1:2159635:	T	missense/in	51/72	-
Inf	1.14;Inf	0.01801	1:2159723:	C	missense/in	50/72	-
Inf	0.71;Inf	0.04966	1:2158486:	T	missense/in	63/72	-
Inf	0.71;Inf	0.04966	1:2162219:	C	missense/in	31/72	-
Inf	0.71;Inf	0.04966	1:2160735:	A	missense/in	40/72	-
Inf	0.32;Inf	0.13604	1:2159600!	T	missense/in	52/72	-
Inf	0.32;Inf	0.13604	1:2160192-	T	LoF	45/72	-
Inf	0.04;Inf	0.37008	1:2159635:	A	missense/in	51/72	-
Inf	0.04;Inf	0.37008	1:2159600(	C	other intro	-	52/71
Inf	0.04;Inf	0.37008	1:2159554!	A	LoF	54/72	-
Inf	0.04;Inf	0.37008	1:2159400!	TA	LoF	56/72	-
Inf	0.04;Inf	0.37008	1:2159400:	C	missense/in	56/72	-
Inf	0.04;Inf	0.37008	1:2159320(	T	missense/in	58/72	-
Inf	0.04;Inf	0.37008	1:2159165!	C	missense/in	59/72	-
Inf	0.04;Inf	0.37008	1:2158535!	A	LoF	62/72	-
Inf	0.04;Inf	0.37008	1:2158486:	A	missense/in	63/72	-
Inf	0.04;Inf	0.37008	1:2158485(	A	missense/in	63/72	-
Inf	0.04;Inf	0.37008	1:2158479(	CAAG	missense/in	63/72	-
Inf	0.04;Inf	0.37008	1:2158478!	-	LoF	63/72	-
Inf	0.04;Inf	0.37008	1:2158240(	A	missense/in	65/72	-
Inf	0.04;Inf	0.37008	1:2164952!	A	LoF	9/72	-
Inf	0.04;Inf	0.37008	1:2164204-	G	missense/in	13/72	-
Inf	0.04;Inf	0.37008	1:2164203!	T	missense/in	13/72	-
Inf	0.04;Inf	0.37008	1:2164199:	C	missense/in	13/72	-
Inf	0.04;Inf	0.37008	1:2163734:	C	missense/in	17/72	-
Inf	0.04;Inf	0.37008	1:2163732:	-	LoF	17/72	-
Inf	0.04;Inf	0.37008	1:2163700-	A	missense/in	19/72	-
Inf	0.04;Inf	0.37008	1:2163635(	CTGCTAAA(	LoF	20/72	-
Inf	0.04;Inf	0.37008	1:2162580!	T	LoF	25/72	-
Inf	0.04;Inf	0.37008	1:2162466:	C	missense/in	28/72	-
Inf	0.04;Inf	0.37008	1:2162462:	G	LoF	-	29/71
Inf	0.04;Inf	0.37008	1:2162218:	T	missense/in	31/72	-
Inf	0.04;Inf	0.37008	1:2161664!	A	missense/in	35/72	-
Inf	0.04;Inf	0.37008	1:2161440!	G	missense/in	36/72	-
Inf	0.04;Inf	0.37008	1:2161081:	-	LoF	38/72	-
Inf	0.04;Inf	0.37008	1:2159904!	A	LoF	48/72	-
Inf	0.04;Inf	0.37008	1:2159904:	A	missense/in	48/72	-
Inf	0.04;Inf	0.37008	1:2159904-	A	LoF	48/72	-
Inf	0.04;Inf	0.37008	1:2165955:	A	LoF	2/72	-
1.28	0.18;7.77	0.71199	1:2164975!	A	missense/in	7/72	-
1.71	0.02;134.9	1	1:2159330:	T	missense/in	57/72	-
1.71	0.02;134.9	1	1:2158443:	A	LoF	64/72	-
1.71	0.02;134.9	1	1:2158140(	A	LoF	68/72	-
1.71	0.02;134.9	1	1:2162218:	-	LoF	31/72	-
1.71	0.02;134.9	1	1:2160521-	T	LoF	42/72	-
0.46	0.17;1.1	0.0885	1:2164204!	-	LoF	13/72	-
0	0;1.84	0.16098	1:2160645-	C	other intro	-	40/71
0.41	0.07;1.57	0.18163	NA	NA	Exon del/d	NA	NA
0	0;4.12	0.29787	1:2158537:	C	LoF	-	61/71
0	0;9.07	0.53192	1:2164987!	A	missense/in	6/72	-
0	0;9.07	0.53192	1:2159331:	T	LoF	57/72	-

	0	0;9.07	0.53192	1:2159016	G	missense/in	61/72	-
	0	0;9.07	0.53192	1:2158482	A	missense/in	63/72	-
	0	0;9.07	0.53192	1:2158479	A	missense/in	63/72	-
	0	0;9.07	0.53192	1:2158125	A	missense/in	69/72	-
	0	0;9.07	0.53192	1:2158021	C	missense/in	71/72	-
	0	0;9.07	0.53192	1:2164952	G	missense/in	9/72	-
	0	0;9.07	0.53192	1:2164655	G	missense/in	10/72	-
	0	0;9.07	0.53192	1:2163732	C	missense/in	17/72	-
	0	0;9.07	0.53192	1:2163699	A	LoF	19/72	-
	0	0;9.07	0.53192	1:2163636	-	LoF	20/72	-
	0	0;9.07	0.53192	1:2162704	-	LoF	22/72	-
	0	0;9.07	0.53192	1:2162581	G	missense/in	25/72	-
	0	0;9.07	0.53192	1:2162464	T	LoF	-	28/71
	0	0;9.07	0.53192	1:2165384	T	missense/in	4/72	-
	0	0;9.07	0.53192	1:2160623	C	other intro	-	40/71
	0	0;9.07	0.53192	1:2160620	T	LoF	41/72	-
	0.63	0.1;2.7	0.751	1:2159015	T	LoF	61/72	-
	0.85	0.01;16.54	1	1:2164987	G	missense/in	6/72	-
	0.85	0.01;16.54	1	1:2159561	G	missense/in	53/72	-
	0.85	0.01;16.54	1	1:2164988	TGGC	LoF	6/72	-
	0.67	0.06;4.22	1	1:2162704	A	missense/in	22/72	-
	0.56	0.01;7.14	1	1:2164988	T	synonymou	6/72	-
	0	0;66.32	1	1:2159562	T	LoF	53/72	-
	0	0;66.32	1	1:2159562	A	LoF	53/72	-
	0	0;66.32	1	1:2159554	T	missense/in	54/72	-
	0	0;66.32	1	1:2159400	T	LoF	-	56/71
	0	0;66.32	1	1:2159320	A	missense/in	58/72	-
	0	0;66.32	1	1:2164986	C	missense/in	6/72	-
	0	0;66.32	1	1:2159166	AAA	LoF	59/72	-
	0	0;66.32	1	1:2159166	-	LoF	59/72	-
	0	0;66.32	1	1:2159016	T	missense/in	61/72	-
	0	0;66.32	1	1:2159015	-	LoF	61/72	-
	0	0;66.32	1	1:2158536	AA	LoF	62/72	-
	0	0;66.32	1	1:2158535	T	missense/in	62/72	-
	0	0;66.32	1	1:2158535	T	missense/in	62/72	-
	0	0;66.32	1	1:2158489	C	LoF	-	62/71
	0	0;66.32	1	1:2158486	G	missense/in	63/72	-
	0	0;66.32	1	1:2164975	T	missense/in	7/72	-
	0	0;66.32	1	1:2158482	G	missense/in	63/72	-
	0	0;66.32	1	1:2158480	-	LoF	63/72	-
	0	0;66.32	1	1:2158477	C	LoF	63/72	-
	0	0;66.32	1	1:2158139	T	LoF	68/72	-
	0	0;66.32	1	1:2158080	GC	LoF	70/72	-
	0	0;66.32	1	1:2158022	T	missense/in	71/72	-
	0	0;66.32	1	1:2164656	-	LoF	10/72	-
	0	0;66.32	1	1:2164242	T	LoF	-	12/71
	0	0;66.32	1	1:2164205	C	LoF	-	12/71
	0	0;66.32	1	1:2164204	G	LoF	13/72	-
	0	0;66.32	1	1:2164203	A	LoF	13/72	-
	0	0;66.32	1	1:2163807	-	LoF	16/72	-
	0	0;66.32	1	1:2163806	T	LoF	16/72	-
	0	0;66.32	1	1:2163733	-	LoF	17/72	-
	0	0;66.32	1	1:2163731	A	missense/in	17/72	-

0	0;66.32	1	1:2163700	G	missense/i	19/72	-
0	0;66.32	1	1:2163700	GA	LoF	19/72	-
0	0;66.32	1	1:2163487	-	LoF	21/72	-
0	0;66.32	1	1:2162568	-	LoF	26/72	-
0	0;66.32	1	1:2162516	T	LoF	27/72	-
0	0;66.32	1	1:2162474	C	other intro	-	27/71
0	0;66.32	1	1:2162219	T	LoF	31/72	-
0	0;66.32	1	1:2162219	G	missense/i	31/72	-
0	0;66.32	1	1:2161440	GATT	LoF	36/72	-
0	0;66.32	1	1:2161388	A	LoF	37/72	-
0	0;66.32	1	1:2161080	C	LoF	38/72	-
0	0;66.32	1	1:2165383	-	LoF	4/72	-
0	0;66.32	1	1:2160621	G	LoF	41/72	-
0	0;66.32	1	1:2160620	G	LoF	41/72	-
0	0;66.32	1	1:2165009	T	missense/i	5/72	-
0	0;66.32	1	1:2160618	-	LoF	41/72	-
0	0;66.32	1	1:2160522	T	missense/i	42/72	-
0	0;66.32	1	1:2160512	T	missense/i	43/72	-
0	0;66.32	1	1:2160405	C	other intro	-	43/71
0	0;66.32	1	1:2164988	CAGC	LoF	6/72	-
0	0;66.32	1	1:2160114	T	LoF	47/72	-
0	0;66.32	1	1:2159724	G	missense/i	50/72	-
0	0;66.32	1	1:2159723	A	missense/i	50/72	-
0	0;66.32	1	1:2159723	A	missense/i	50/72	-

HGVSc.vep	HGVSp.vep	patientAC.RP	patient_fre	patientAC.Usl	patient_fre	OR	
c.2276G>T	p.Cys759Phe	13	0.185714	2	0.034483		6.31
c.10073G>A	p.Cys3358Tyr	6	0.085714	0	0	Inf	
c.9882C>G	p.Cys3294Trp	4	0.057143	0	0	Inf	
c.10342G>A	p.Glu3448Lys	2	0.028571	0	0	Inf	
c.11156G>A	p.Arg3719His	1	0.014286	0	0	Inf	
c.11266G>A	p.Gly3756Ser	1	0.014286	0	0	Inf	
c.12575G>A	p.Arg4192His	1	0.014286	0	0	Inf	
c.12752G>T	p.Ser4251Ile	1	0.014286	0	0	Inf	
c.13335_13347de	p.Glu4445_Ser4	1	0.014286	0	0	Inf	
c.2802T>G	p.Cys934Trp	1	0.014286	0	0	Inf	
c.6118T>G	p.Cys2040Gly	1	0.014286	0	0	Inf	
c.6163G>A	p.Ala2055Thr	1	0.014286	0	0	Inf	
c.6670G>T	p.Gly2224Cys	1	0.014286	0	0	Inf	
c.6835G>C	p.Asp2279His	1	0.014286	0	0	Inf	
c.7475C>T	p.Ser2492Leu	1	0.014286	0	0	Inf	
c.9433C>T	p.Leu3145Phe	1	0.014286	0	0	Inf	
c.10561T>C	p.Trp3521Arg	0	0	2	0.034483		0
c.11819A>C	p.Tyr3940Ser	0	0	2	0.034483		0
c.13010C>T	p.Thr4337Met	0	0	2	0.034483		0
c.13316C>T	p.Thr4439Ile	0	0	2	0.034483		0
c.15496A>G	p.Ile5166Val	0	0	2	0.034483		0
c.1813T>C	p.Cys605Arg	0	0	2	0.034483		0
c.653T>A	p.Val218Glu	0	0	2	0.034483		0
c.1055C>T	p.Thr352Ile	0	0	1	0.017241		0
c.10657G>A	p.Asp3553Asn	0	0	1	0.017241		0
c.1139A>G	p.Tyr380Cys	0	0	1	0.017241		0
c.11815G>A	p.Glu3939Lys	0	0	1	0.017241		0
c.12283G>A	p.Gly4095Ser	0	0	1	0.017241		0
c.12284G>A	p.Gly4095Asp	0	0	1	0.017241		0
c.15017C>T	p.Thr5006Met	0	0	1	0.017241		0
c.1606T>C	p.Cys536Arg	0	0	1	0.017241		0
c.5018T>C	p.Leu1673Pro	0	0	1	0.017241		0
c.6118T>C	p.Cys2040Arg	0	0	1	0.017241		0
c.802G>A	p.Gly268Arg	0	0	1	0.017241		0
c.8431C>A	p.Pro2811Thr	0	0	1	0.017241		0
c.8576G>A	p.Arg2859His	0	0	1	0.017241		0
c.8682-9A>G	c.8682-9A>G	0	0	1	0.017241		0
c.9799T>C	p.Cys3267Arg	0	0	1	0.017241		0
c.9815C>T	p.Pro3272Leu	0	0	1	0.017241		0

95CI_RPtoUsher	Pvalue	Location.hg19	Allele	Consequence	EXON
1.34;60.17	0.01117	1:216420460-216420460	A	missense/inframe_indel	13/72
1.01;Inf	0.03164	1:215963510-215963510	T	missense/inframe_indel	51/72
0.56;Inf	0.12572	1:215972325-215972325	C	missense/inframe_indel	50/72
0.16;Inf	0.50049	1:215960057-215960057	T	missense/inframe_indel	52/72
0.02;Inf	1	1:215933077-215933077	T	missense/inframe_indel	57/72
0.02;Inf	1	1:215932060-215932060	T	missense/inframe_indel	58/72
0.02;Inf	1	1:215848678-215848678	T	missense/inframe_indel	63/72
0.02;Inf	1	1:215848501-215848501	A	missense/inframe_indel	63/72
0.02;Inf	1	1:215847905-215847918	CAAG	missense/inframe_indel	63/72
0.02;Inf	1	1:216419934-216419934	C	missense/inframe_indel	13/72
0.02;Inf	1	1:216221921-216221921	C	missense/inframe_indel	31/72
0.02;Inf	1	1:216221876-216221876	T	missense/inframe_indel	31/72
0.02;Inf	1	1:216166497-216166497	A	missense/inframe_indel	35/72
0.02;Inf	1	1:216144089-216144089	G	missense/inframe_indel	36/72
0.02;Inf	1	1:216073536-216073536	A	missense/inframe_indel	40/72
0.02;Inf	1	1:215990476-215990476	A	missense/inframe_indel	48/72
0;4.39	0.20337	1:215956104-215956104	G	missense/inframe_indel	53/72
0;4.39	0.20337	1:215901619-215901619	G	missense/inframe_indel	61/72
0;4.39	0.20337	1:215848243-215848243	A	missense/inframe_indel	63/72
0;4.39	0.20337	1:215847937-215847937	A	missense/inframe_indel	63/72
0;4.39	0.20337	1:215802179-215802179	C	missense/inframe_indel	71/72
0;4.39	0.20337	1:216465544-216465544	G	missense/inframe_indel	10/72
0;4.39	0.20337	1:216538426-216538426	T	missense/inframe_indel	4/72
0;32.31	0.45312	1:216498735-216498735	A	missense/inframe_indel	6/72
0;32.31	0.45312	1:215955467-215955467	T	missense/inframe_indel	54/72
0;32.31	0.45312	1:216498651-216498651	C	missense/inframe_indel	6/72
0;32.31	0.45312	1:215901623-215901623	T	missense/inframe_indel	61/72
0;32.31	0.45312	1:215853502-215853502	T	missense/inframe_indel	62/72
0;32.31	0.45312	1:215853501-215853501	T	missense/inframe_indel	62/72
0;32.31	0.45312	1:215812532-215812532	A	missense/inframe_indel	69/72
0;32.31	0.45312	1:216495263-216495263	G	missense/inframe_indel	9/72
0;32.31	0.45312	1:216258189-216258189	G	missense/inframe_indel	25/72
0;32.31	0.45312	1:216221921-216221921	G	missense/inframe_indel	31/72
0;32.31	0.45312	1:216500979-216500979	T	missense/inframe_indel	5/72
0;32.31	0.45312	1:216052233-216052233	T	missense/inframe_indel	42/72
0;32.31	0.45312	1:216051205-216051205	T	missense/inframe_indel	43/72
0;32.31	0.45312	1:216040521-216040521	C	other intronic	-
0;32.31	0.45312	1:215972408-215972408	G	missense/inframe_indel	50/72
0;32.31	0.45312	1:215972392-215972392	A	missense/inframe_indel	50/72

INTRON	HGVSc	HGVSp	SpliceAI	spliceai_ma	reference_evidence.SpliceAI
-	NM_20693	NP_99681€	A|USH2A|(	0.01	NA
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	C|USH2A|(	0	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.09	NA
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.2	30924848, no splicing PP3 (Exon 58 skipping,
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	A|USH2A|(	0	NA
-	NM_20693	NP_99681€	GCAAG|US	0	NA
-	NM_20693	NP_99681€	C|USH2A|(	0.01	NA
-	NM_20693	NP_99681€	C|USH2A|(	0.03	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.83	PP3 (SpliceAI predicts donor loss (delta score
-	NM_20693	NP_99681€	A|USH2A|(	0	NA
-	NM_20693	NP_99681€	G|USH2A|(	0.02	NA
-	NM_20693	NP_99681€	A|USH2A|(	0.06	NA
-	NM_20693	NP_99681€	A|USH2A|(	0.01	NA
-	NM_20693	NP_99681€	G|USH2A|(	0.04	NA
-	NM_20693	NP_99681€	G|USH2A|(	0	NA
-	NM_20693	NP_99681€	A|USH2A|(	0.04	NA
-	NM_20693	NP_99681€	A|USH2A|(	0	NA
-	NM_20693	NP_99681€	C|USH2A|(	0	NA
-	NM_20693	NP_99681€	G|USH2A|(	0	NA
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	A|USH2A|(	0.13	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.01	NA
-	NM_20693	NP_99681€	C|USH2A|(	0.12	NA
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.01	NA
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	A|USH2A|(	0.05	NA
-	NM_20693	NP_99681€	G|USH2A|(	0.02	NA
-	NM_20693	NP_99681€	G|USH2A|(	0.02	NA
-	NM_20693	NP_99681€	G|USH2A|(	0.05	NA
-	NM_20693	NP_99681€	T|USH2A|(	0	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.02	NA
-	NM_20693	NP_99681€	T|USH2A|(	0.02	NA
43/71	NM_20693	-	C|USH2A|(	0.95	PubMed 23591405, PP3 (SpliceAI score 0.95,
-	NM_20693	NP_99681€	G|USH2A|(	0	NA
-	NM_20693	NP_99681€	A|USH2A|(	0.01	NA

out-of-frame)

0.83), inframe deletion of 38 a.a.), PPx (terminal Guanine nucleotide in exon)

out-of-frame)

Measurement	Unit	ARRP & Usher patients			
		Clinical Diagn	n	mean	sd
ac_4f_pta	dB HL	ARRP	47	16.6502026	12.1670349
ac_4f_pta	dB HL	Usher	75	65.522619	13.1285182
ac_4f_pta_adj	dB HL	ARRP	47	11.6987635	8.73255835
ac_4f_pta_adj	dB HL	Usher	75	62.5839582	14.1245358
ERG ConeFlickerAmpB	uV	ARRP	47	11.5234043	15.4679081
ERG ConeFlickerAmpB	uV	Usher	79	5.25443038	11.8736489
i4e_seeingArea	squared degre	ARRP	47	1633.14255	2549.25884
i4e_seeingArea	squared degre	Usher	80	404.98	1009.20637
iII4e_seeingArea	squared degre	ARRP	46	6151.22174	4243.13085
iII4e_seeingArea	squared degre	Usher	80	3370.94625	4064.23728
V4e_seeingArea	squared degre	ARRP	46	9877.76957	4088.12825
V4e_seeingArea	squared degre	Usher	79	6476.78228	5320.37477
V30	dB-sr	ARRP	46	10.0467971	5.86497517
V31	dB-sr	Usher	75	8.40912889	5.94384116
Vtot	dB-sr	ARRP	46	37.1198551	24.7020822
Vtot	dB-sr	Usher	80	22.4597542	21.4992548
SP Mean Sensitivity	dB	ARRP	46	11.874058	6.03281403
SP Mean Sensitivity	dB	Usher	80	9.28607583	6.02515057
EZArea	mm^2	ARRP	46	4.32738261	5.5878147
EZArea	mm^2	Usher	80	3.14050125	5.65799294
VA ETSRS score		ARRP	47	80.3191489	10.1897356
VA ETSRS score		Usher	80	76.475	12.4076079
Central subfield thickness	um	ARRP	47	263.617021	32.9126335
Central subfield thickness	um	Usher	79	253.139241	57.4703344
Age	yr	ARRP	47	44.2978723	13.2055899
Age	yr	Usher	80	37.25	13.841325
VisionLossOnsetAge	yr	ARRP	46	31.7608696	13.5370845
VisionLossOnsetAge	yr	Usher	80	18.4125	8.33582136
Duration of disease	yr	ARRP	46	13.5027081	8.50271198
Duration of disease	yr	Usher	80	19.2428046	12.8244394
MP mean sensitivity	dB	ARRP	37	6.73378378	5.08859092
MP mean sensitivity	dB	Usher	55	5.43090909	4.88965737
FST blue stimulus	dB	ARRP	37	-45.144144	13.7046763
FST blue stimulus	dB	Usher	56	-30.511905	11.2411517
FST red stimulus	dB	ARRP	37	-27.846847	7.66610086
FST red stimulus	dB	Usher	56	-22.970238	5.68474009
FST (Blue-Red)	dB	ARRP	37	-17.297297	8.41213988
FST (Blue-Red)	dB	Usher	56	-7.5416667	8.56409288
FST white stimulus	dB	ARRP	37	-39.324324	12.8609579
FST white stimulus	dB	Usher	56	-26.339286	9.97987694
UPSIT score		ARRP	47	34.2765957	3.63379145
UPSIT score		Usher	80	34.65	3.22215432
SITPerc		ARRP	47	0.38297872	0.30173351
SITPerc		Usher	80	0.33675	0.26430599
SITZscore		ARRP	47	-0.3808705	1.07110978
SITZscore		Usher	80	-0.5373213	0.86791673

	Usher patients					
p-value	No. of truncat n		mean	sd	p-value	No. of truncat
< 2.2e-16	0	8	50.546875	10.8533112	0.002632	0
	1 or 2	67	67.3107676	12.2607508		1
< 2.2e-16	0	8	48.4149407	12.8862601	0.009719	0
	1 or 2	67	64.2757811	13.3728216		1
0.01921	0	9	6.07777778	8.03006503	0.7657	0
	1 or 2	70	5.14857143	12.3205819		1
0.002569	0	9	903.622222	1837.81949	0.3906	0
	1 or 2	71	341.771831	852.887515		1
0.0005262	0	9	5147.58889	3903.22494	0.1787	0
	1 or 2	71	3145.73803	4055.03825		1
0.0001116	0	9	10160.4	3635.13075	0.009603	0
	1 or 2	70	6003.17429	5335.6365		1
0.1412	0	8	10.79425	6.83450753	0.3192	0
	1 or 2	67	8.12433831	5.82112972		1
0.001178	0	9	29.8473704	22.3313242	0.3148	0
	1 or 2	71	21.5232958	21.3716296		1
0.02254	0	9	11.0836667	6.61019245	0.4025	0
	1 or 2	71	9.05821221	5.95886747		1
0.2561	0	9	3.67737778	4.51184956	0.7212	0
	1 or 2	71	3.07244648	5.81038552		1
0.06125	0	9	79	6.2249498	0.2849	0
	1 or 2	71	76.1549296	12.9765149		1
0.1957	0	8	266	53.540372	0.4956	0
	1 or 2	71	251.690141	58.0742361		1
0.005271	0	9	36.4444444	12.6007055	0.8446	0
	1 or 2	71	37.3521127	14.0703321		1
7.64E-08	0	9	20.6666667	10.7238053	0.5094	0
	1 or 2	71	18.1267606	8.0337317		1
0.003141	0	9	16.1379573	8.41163621	0.2955	0
	1 or 2	71	19.6363768	13.2713601		1
0.2251	0	7	5.49285714	6.489763	0.9785	0
	1 or 2	48	5.421875	4.70024791		1
9.48E-07	0	7	-31.666667	9.12465119	0.738	0
	1 or 2	49	-30.346939	11.5828256		1
0.001544	0	7	-23.333333	4.35464843	0.8271	0
	1 or 2	49	-22.918367	5.88529658		1
6.04E-07	0	7	-8.3333333	10.2071144	0.8291	0
	1 or 2	49	-7.4285714	8.42092851		1
2.30E-06	0	7	-26.714286	7.03806732	0.8909	0
	1 or 2	49	-26.285714	10.387849		1
0.5616	0	9	32	5.67890835	0.1563	0
	1 or 2	71	34.9859155	2.6484117		1
0.3856	0	9	0.24555556	0.25652052	0.285	0
	1 or 2	71	0.34830986	0.26479095		1
0.3975	0	9	-0.8534924	0.8744923	0.2756	0
	1 or 2	71	-0.4972433	0.86501413		1

ARRP patients				ARRP & Usher patients in the 1-		
n	mean	sd	p-value	Group	n	mean
12	15.2380952	8.61446963	0.5751	RP-enriched	25	23.1416667
35	17.1343537	13.241265		Other	37	47.6785714
12	9.78545817	8.18176614	0.3699	RP-enriched	25	16.2663348
35	12.3547539	8.93193878		Other	37	44.6741327
12	15.4166667	16.4207094	0.3448	RP-enriched	25	12.908
35	10.1885714	15.1419707		Other	37	4.8027027
12	1403.10833	2128.83028	0.6902	RP-enriched	25	2259
35	1712.01143	2702.05448		Other	37	327.189189
12	5933.025	3639.2111	0.8226	RP-enriched	25	6601.888
34	6228.23235	4484.76135		Other	36	2685.74722
12	9761.15833	3723.62303	0.9045	RP-enriched	25	10096.124
34	9918.92647	4261.6537		Other	36	5885.11667
12	9.80847222	5.54176113	0.8674	RP-enriched	24	10.878375
34	10.1309118	6.05320499		Other	37	6.60957658
12	40.8883056	23.1858442	0.5301	RP-enriched	24	40.9396806
34	35.7898137	25.4145633		Other	37	17.4749189
12	11.9154167	5.74871389	0.9776	RP-enriched	24	12.8039861
34	11.8594608	6.21390287		Other	37	7.53365225
11	4.2663	6.10154566	0.9694	RP-enriched	25	4.77576
35	4.34658	5.51127923		Other	37	3.44081622
12	80.5	13.5344678	0.9546	RP-enriched	25	80.32
35	80.2571429	9.01091775		Other	37	75.5945946
12	268.583333	25.1593407	0.4843	RP-enriched	25	263.48
35	261.914286	35.3423354		Other	37	247.540541
12	46.5833333	11.9427295	0.4677	RP-enriched	25	47.88
35	43.5142857	13.6863423		Other	37	38.8648649
12	35.25	13.5721975	0.3125	RP-enriched	24	32.875
34	30.5294118	13.5092231		Other	37	20.7837838
12	11.9087953	7.11634342	0.409	RP-enriched	24	16.4470397
34	14.0652655	8.96966692		Other	37	18.5555802
9	6.43333333	5.29073483	0.8465	RP-enriched	20	7.7975
28	6.83035714	5.11774942		Other	25	4.67
8	-46.75	18.6758056	0.7763	RP-enriched	19	-47.508772
29	-44.701149	12.3832147		Other	30	-33.333333
8	-29.875	11.6509248	0.5622	RP-enriched	19	-27.947368
29	-27.287356	6.33441353		Other	30	-24.011111
8	-16.875	9.88976945	0.8904	RP-enriched	19	-19.561404
29	-17.413793	8.15263867		Other	30	-9.3222222
8	-41.416667	17.9503283	0.6996	RP-enriched	19	-40
29	-38.747126	11.4242346		Other	30	-29.777778
12	32.5833333	3.96480731	0.09355	RP-enriched	25	34.8
35	34.8571429	3.37937392		Other	37	35.0540541
12	0.25166667	0.25337121	0.06146	RP-enriched	25	0.4708
35	0.428	0.30697576		Other	37	0.37189189
12	-0.7722978	0.91201762	0.1166	RP-enriched	25	-0.0915333
35	-0.2466669	1.1002167		Other	37	-0.4348046

-truncating group		ARRP patients in the 1-truncating group				
sd	p-value	Group	n	mean	sd	p-value
17.789532	3.95E-05	RP-enriched	23	20.2626812	14.8234051	0.01534
25.7354187		Other	11	10.7873377	6.7405473	
14.2032185	5.07E-07	RP-enriched	23	14.1454255	9.5348655	0.05365
25.2525614		Other	11	8.39001458	6.7812563	
16.946212	0.03762	RP-enriched	23	14.0304348	17.2283708	0.009107
9.75868521		Other	11	3.08181818	5.16407168	
3027.41316	0.004351	RP-enriched	23	2453.1913	3083.11548	0.003228
775.989581		Other	11	299.509091	474.700951	
4556.05434	0.0008568	RP-enriched	23	7161.64348	4306.90683	0.04371
3637.95159		Other	10	3695.93	4171.62135	
4422.81948	0.001583	RP-enriched	23	10830.9826	3756.71041	0.08412
5465.40192		Other	10	7620.16	4862.95857	
6.71757183	0.01132	RP-enriched	22	11.6468182	6.48010853	0.01652
5.10118348		Other	11	6.95878788	4.04612215	
26.0602139	0.0004789	RP-enriched	22	43.9648939	25.0291136	0.003775
18.7114875		Other	11	19.0630909	18.9579476	
6.65978113	0.00234	RP-enriched	22	13.6201061	6.34038185	0.009932
5.43714241		Other	11	8.22109091	4.63179518	
6.11546822	0.4331	RP-enriched	23	5.10287391	6.27411681	0.2111
7.1018607		Other	11	2.93687273	3.5794245	
10.0859638	0.1297	RP-enriched	23	80.3913043	10.3999088	0.5717
14.1214169		Other	11	78.9090909	4.72132493	
34.2796344	0.1362	RP-enriched	23	266.73913	32.6597019	0.2776
48.7907064		Other	11	250.818182	41.2184866	
15.1831047	0.01739	RP-enriched	23	46.8695652	14.1398995	0.05156
12.2976108		Other	11	38.0909091	10.3966778	
12.7767197	0.0003403	RP-enriched	22	33.8636364	12.8888858	0.103
10.1328266		Other	11	26	12.2882057	
13.0328124	0.5158	RP-enriched	22	14.4981333	9.4994052	0.5943
11.0143954		Other	11	12.7418331	8.43364406	
5.87144013	0.06115	RP-enriched	18	8.58888889	5.64440263	0.002419
4.73033209		Other	9	3.79444444	1.01194258	
11.7847822	0.0002865	RP-enriched	17	-49.098039	11.1085332	0.02464
12.7900824		Other	11	-38	12.2292909	
7.60544246	0.05769	RP-enriched	17	-28.705882	7.47856414	0.1035
5.25698465		Other	11	-25.090909	3.75970126	
7.83869212	0.0001338	RP-enriched	17	-20.392157	6.44769047	0.02981
9.00765249		Other	11	-12.909091	9.05917694	
12.560471	0.007121	RP-enriched	17	-42.254902	11.1015073	0.04465
11.6725218		Other	11	-33.30303	10.7107989	
3.27871926	0.7569	RP-enriched	23	34.9565217	3.36395683	0.6489
2.95283241		Other	11	34.3636364	3.55732279	
0.31989477	0.2159	RP-enriched	23	0.46956522	0.30596313	0.1693
0.28035531		Other	11	0.31363636	0.29489906	
1.18695679	0.2272	RP-enriched	23	-0.0875408	1.14023789	0.1437
0.90413577		Other	11	-0.6575196	0.96825544	

Usher patients in the 1-truncating group					ARRP & Usl
Group	**n**	**mean**	**sd**	**p-value**	**Group**
RP-enriched	2	56.25	19.445436	0.6988	C759F + truncating
Other	26	63.2864	9.131411		other mis + truncating
RP-enriched	2	40.7	39.3	0.6127	C759F + truncating
Other	26	60	8.69		other mis + truncating
					C759F + truncating
					other mis + truncating
					C759F + truncating
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					other mis + truncating
					C759F + truncating
					other mis + truncating

her patients in the 1-truncating group				ARRP & Usher patients in the		
n	mean	sd	p-value	Group	n	mean
15	28.8333333	18.7378929	0.06743	C759F + other	5	18.375
47	40.641464	27.154865		2 other mis	15	33.0238095
15	20.6731829	15.6788426	0.005405	C759F + other	5	14.1488145
47	37.2234795	26.8984447		2 other mis	15	28.9333968
15	11.2	16.0119152	0.3736	C759F + other	5	19.7
47	7.07234043	12.7838162		2 other mis	16	8.825
15	1878.57333	2799.64712	0.2058	C759F + other	5	2285.52
47	859.625532	1954.82916		2 other mis	16	846.39375
15	5761.47333	4687.58144	0.1676	C759F + other	5	7112.48
46	3811.13043	4312.80693		2 other mis	16	5122.6375
15	8719.85333	4844.94753	0.3358	C759F + other	5	11938.16
46	7249.33696	5624.31725		2 other mis	16	9305.41875
15	10.0646	6.85898207	0.2447	C759F + other	5	11.2242667
46	7.71013768	5.80922109		2 other mis	15	9.86228889
15	37.0506	26.9621526	0.0911	C759F + other	5	47.1643333
46	23.334029	23.0622505		2 other mis	16	32.7165208
15	11.9125556	6.73787963	0.1343	C759F + other	5	13.8667333
46	8.85548841	6.23148757		2 other mis	16	10.8377708
15	5.33356	6.61533356	0.3737	C759F + other	4	3.0481
47	3.54682553	6.74197707		2 other mis	16	4.23958125
15	80.2666667	11.4046774	0.3085	C759F + other	5	86.2
47	76.6170213	13.1720277		2 other mis	16	77.875
15	264.8	34.5112818	0.2106	C759F + other	4	284.75
47	250.510638	46.3201864		2 other mis	16	263.25
15	50.2	15.5893737	0.03296	C759F + other	5	45.4
47	40.0425532	12.8654533		2 other mis	16	41.25
15	31.5333333	13.9533236	0.0599	C759F + other	5	35.4
46	23.5869565	11.6801372		2 other mis	16	27
15	19.1320557	14.9485247	0.6599	C759F + other	5	10.5581109
46	17.267491	10.7196947		2 other mis	16	14.7097878
11	6.98181818	5.5254535	0.5318	C759F + other	5	6.07
34	5.76176471	5.45791139		2 other mis	11	6
14	-48.52381	12.7967105	0.002655	C759F + other	5	-53.066667
35	-34.952381	12.8460321		2 other mis	10	-33.033333
14	-29.833333	6.64194133	0.007102	C759F + other	5	-32.333333
35	-23.819048	5.65589683		2 other mis	10	-24.066667
14	-18.690476	8.73196729	0.01313	C759F + other	5	-20.733333
35	-11.133333	9.57365667		2 other mis	10	-8.9666667
14	-41.190476	13.26116	0.01761	C759F + other	5	-46.133333
35	-30.761905	11.6729875		2 other mis	10	-28.766667
15	34.6	2.69390847	0.5839	C759F + other	5	29.8
47	35.0638298	3.19241121		2 other mis	16	33.125
15	0.458	0.33483898	0.5327	C759F + other	5	0.152
47	0.39702128	0.28815692		2 other mis	16	0.279375
15	-0.1341845	1.21375789	0.5412	C759F + other	5	-1.2354438
47	-0.3481561	0.97639433		2 other mis	16	-0.6732366

missense group	
sd	p-value
8.50780891	0.04439
21.5983355	
7.15533366	0.04792
23.9597828	
19.6706634	0.2938
11.3494493	
2990.10237	0.3512
1516.62178	
2346.18255	0.1928
3946.59712	
867.563616	0.022
3912.68431	
6.71396131	0.6992
5.86877254	
21.3424472	0.2348
22.9675855	
6.41409804	0.3821
5.87154906	
3.04437659	0.5842
5.79949536	
5.11859356	0.03739
11.4535293	
24.5814971	0.2131
39.8070346	
7.95612971	0.4252
14.2571619	
13.4461891	0.2682
14.2548237	
6.65943776	0.2812
8.05001009	
7.37788926	0.9853
5.12220656	
11.6437871	0.01636
14.5690777	
8.91627725	0.1265
8.67919323	
6.14365074	0.01722
10.3797643	
12.7488562	0.0394
13.760787	
6.05805249	0.3006
4.03112887	
0.19175505	0.2671
0.26126535	
0.71717822	0.1872
0.89591129	

Target Journal: Human Mutation

Word count (3,500 max): current 4,145

Table/Figure limits (5 tables/illustrations) max): current 4 figures, 1 Table = 5 display items

Title (#### characters max / current 112 with spaces): Tissue-specific genotype-phenotype correlations among USH2A-related disorders in the RUSH2A study

Running Head (maximum of **#### characters**): Allelic hierarchy predicts phenotype in *USH2A*-related retinal degeneration

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Abstract (200) words max / current 172)

We assessed genotype-phenotype correlations among the visual, auditory, and olfactory phenotypes of 127 participants with Usher syndrome (USH2) (n=80) or nonsyndromic autosomal recessive retinitis pigmentosa (ARRP) (n=47) due to *USH2A* variants, using clinical data and molecular diagnostics from the Rate of Progression in *USH2A* Related Retinal Degeneration (RUSH2A) study. *USH2A* truncating alleles were associated with USH2 and had a dose-dependent effect on hearing loss severity with no effect on visual loss severity within the USH2 subgroup. A group of missense alleles in an inter-fibronectin domain appeared to be hypomorphic in ARRP. These alleles were associated with later age of onset, larger visual field area, better sensitivity thresholds, and better electroretinographic responses. No effect of genotype on the severity of olfactory deficits was observed. This study unveils a unique, tissue-specific *USH2A* allelic hierarchy with important prognostic implications for patient counseling and treatment trial endpoints. These findings may inform clinical care or research approaches in others with allelic disorders or pleiotropic phenotypes.

Keywords: *USH2A*, hearing loss, photoreceptor degeneration, genotype, Usher syndrome, retinitis pigmentosa.

INTRODUCTION

Retinitis pigmentosa (RP; MIM# 268000) is a form of retinal degeneration characterized by early loss of rod photoreceptor function, manifesting as nyctalopia, peripheral field loss, and diminished dark-adapted electroretinographic (ERG) recordings. The later stages include cone dysfunction, including constricted visual fields, loss of central vision, and reduced light-adapted ERG responses. RP has extreme locus heterogeneity, with >90 genes associated with the nonsyndromic form, and is associated with hundreds of syndromic disorders, including ciliopathies, peroxisomal disorders, and multiple (>500) malformation syndromes (Hartong et al., 2006; Schneider et al., 2021; Verbakel et al., 2018). Recently, an FDA-approved gene-directed therapy, the first in its class, has emerged for early-onset retinal degeneration caused by variants in the *RPE65* gene (MIM# 180069). However, there are no effective treatments for the vast majority of patients with RP. Defining genotype-phenotype correlations may allow for better selection of outcome measures for future clinical trials.

Usher syndrome (Usher syndrome, MIM# 276900) comprises a group of autosomal recessive disorders characterized by congenital, childhood-onset, or progressive post-lingual hearing loss and retinal degeneration. Genes associated with various forms of Usher syndrome encode proteins that localize mainly to the stereocilia and synaptic regions of inner ear hair cells and the connecting of cilium of retinal photoreceptors. Variants in the *USH2A* gene (MIM# 608400) are the leading cause of Usher syndrome type 2 (USH2) (USH2A; MIM# 276901). Notably, patients with USH2 have congenital hearing loss with progressive vision loss, providing a window of opportunity for intervention as the hearing loss is often diagnosed early in life and

genetic testing often reveals the potential for subsequent retinal degeneration before vision loss actually begins. *USH2A* mutations can also cause nonsyndromic autosomal recessive RP (ARRP, isolated RP with normal hearing at birth) (RP39; MIM# 613809). In many populations, the most common pathogenic variants are located in exon 13 of the *USH2A* gene, in particular NM_206933.4:c.2299delG p.(Glu767Ser*fs*Ter21), which accounts for as high as ~16% of disease alleles.(Lenassi et al., 2015; Pierrache et al., 2016) As such, *USH2A* exon 13 variants are the current targets for allele-directed therapy (NCT03780257).

Optimal design of gene therapy trials relies on natural history studies and deep clinical phenotyping to select reliable outcomes of treatment response. However, phenotypic correlates are poorly understood for many Mendelian conditions, and as a result the interplay between genotype and treatment response is largely overlooked. With over a thousand variants reported in the literature, *USH2A* offers a valuable opportunity for elucidating treatment-informing genotype-phenotype correlations.

Presumed truncating alleles, including nonsense, frameshift, and canonical splice variants, have been more frequently associated with hearing loss and, therefore, syndromic disease. Biallelic truncating variants are associated with more severe hearing loss.(Hartel et al., 2016; Inaba et al., 2020; Meng et al., 2020; Pierrache et al., 2016) Notably, while earlier onset of visual impairment was noted in patients with USH2, the role of truncating variants has not been clearly established as a risk factor for severe visual impairment. Intriguingly, a subset of missense alleles is enriched in patients without hearing loss and ARRP.(Lenassi et al., 2015; Molina-Ramirez et al., 2020)

Overall, there appears to be a genotype-diagnosis correlation for *USH2A* truncating and specific missense variants for USH2 and ARRP, respectively.

The Rate of Progression in *USH2A*-related Retinal Degeneration (RUSH2A) natural history study includes 127 international participants with USH2 and ARRP related to variants in *USH2A*. Recently, RUSH2A baseline visual field data was reported, indicating that USH2 participants have more severe visual field loss than those with ARRP after adjusting for duration of disease and age of enrollment (Duncan et al., 2020).

Given the known association between diagnosis and genotype, we hypothesized that genotype influences audiometric and visual outcomes independent of the clinical diagnosis (USH2 versus ARRP). Here, we performed a deep analysis of *USH2A* genotypes to investigate whether the allelic hierarchy for hearing impairment applied to both severity of hearing loss and retinal degeneration. Through standardized variant classification and case-control analyses to ascertain pathogenic genotypes enriched in USH2 and ARRP subgroups, we ascertained genotype-phenotype correlations that are both tissue-specific and independent of clinical diagnosis. This work demonstrates the importance of genotype analysis in natural history studies and treatment trials for rare disorders.

PATIENTS AND METHODS

This multicenter, longitudinal, international natural history study enrolled participants with bi-allelic *USH2A* variants at 16 clinical sites in Canada, France, Germany, the Netherlands, the United Kingdom, and the United States (US). The

protocol and informed consent process adhered to the tenets of the Declaration of Helsinki and were approved by the ethics boards associated with each participating site, including compliance with the associated federal regulations. Informed consent was obtained from all participants prior to enrollment. The RUSH2A protocol is listed on www.clinicaltrials.gov (NCT03146078), with registration completed prior to enrolling the first participant. Inclusion criteria stated that participants were required to have a clinical diagnosis of USH2 or ARRP and two pathogenic or likely pathogenic variants in *USH2A* from a certified testing lab obtained prior to study enrollment. Variants were demonstrated to be *in trans* for individuals with ARRP.

Variant analysis and interpretation

USH2A variant analysis was performed by two reviewers independently who used a five-tier classification system recommended by the 2015 American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines and each variant was classified as benign, likely benign, variant of unknown significance (VUS), likely pathogenic, or pathogenic.(Richards et al., 2015) Discordant results were resolved by an independent adjudicator. Variant analysis of the entire cohort was performed following the initial review, to standardize evidence used for recurrent variants. Healthy population frequency data were obtained from gnomAD (v2.1.1 accessed on Oct. 30, 2018, https://gnomad.broadinstitute.org/).(Karczewski et al., 2019) A consensus verdict for *in-silico* pathogenicity predictions for missense variants was acquired from Varsome (https://varsome.com/) and Franklin (https://franklin.genoox.com/clinical-db/home) webtools. Individual in silico predictions

were acquired from Variant Effect Predictor (VEP; http://grch37.ensembl.org/Homo_sapiens/Tools/VEP) **(Supp. Table S1).**

Statistics

Statistical analysis was performed using the R system (v. 3.5.1) and SAS software (v. 9.4) for statistical computing. Statistical tests employed are listed in the text and figure legends. All t-tests assume two tails and unequal variance.

RESULTS

Of the 127 participants enrolled in RUSH2A, 80 were clinically diagnosed as USH2 and 47 as ARRP. Across the cohort, 140 unique variants comprising 128 single-nucleotide variants (SNVs) or small indels and 12 exonic deletions were determined to be disease-associated by variant analysis. Variants considered benign were excluded from analysis.

To assess genotype-phenotype correlation in the RUSH2A cohort, we first established disease-association of each variant by (i) standardized clinical variant interpretation using 2015 ACMG/AMP criteria **(Supp. Table S1)** and (ii) case-control comparison of *USH2A* allele frequencies (AF) in the RUSH2A cohort compared to a general subpopulation (gnomAD database v2.1.1).

USH2A variants in ClinVar and gnomAD

The *USH2A* canonical transcript, NM_206933.4, encodes for a large 6002 amino acid protein, Usherin. The *USH2A* transcript in the human population is highly variable,

including many rare missense (gnomAD missense constraint Z-score = -2.5) and truncating variations (low probability of being loss-of-function [LoF] intolerant; gnomAD LoF score = 0). The variations observed in gnomAD appear to be randomly distributed throughout the coding region **(Supp. Figure S1A)**. To determine whether disease-associated variants are distributed non-randomly, we then examined the distribution of *USH2A* coding variants present in the ClinVar database **(Supp. Figure S1B)**. While ClinVar may have submission or population bias, we observed no apparent spatially restricted clusters of pathogenic or likely-pathogenic variants. However, exon 13 harbors the most frequently submitted variants, c.2276G>T p.(Cys759Phe) and c.2299delG. Among the pathogenic or likely-pathogenic variants in ClinVar, c.2276G>T p.(Cys759Phe) has the highest gnomAD AF of 0.0010. The c.2299delG p.(Glu767Ser*fs*Ter21) variant is the most frequent LoF variant (AF_{gnomAD} = 0.0007) in the *USH2A* gene in the gnomAD dataset. It is noteworthy that 94% of the LoF variants were classified as pathogenic or likely-pathogenic in ClinVar. However, only 12% of missense or in-frame-indel variants with gnomAD AF less than 0.001 were classified as pathogenic or likely-pathogenic, and 68% such rare variants were classified as a VUS **(Supp. Figure S1C)**. This represents a major challenge for definitive classification of rare missense variants as pathogenic or benign.

USH2A variant enrichment in the RUSH2A cohort

We next applied a similar analysis to the RUSH2A cohort. Similar to ClinVar, there is no hotspot for disease associated *USH2A* variation **(Figure 1A)**. The c.2299delG, p.(Glu767Ser*fs*Ter21) (AF_{RUSH2A} = 0.138) and c.2276G>T p.(Cys759Phe) (AF_{RUSH2A} = 0.083) variants in exon 13 are the most frequent in this cohort **(Figure 1A)**,

and these variants demonstrate clear enrichment of AF_{RUSH2A} compared to AF_{gnomAD} (Fig. 1B-C). To establish which *USH2A* alleles are significantly associated with disease status, allele frequencies were compared between the RUSH2A and gnomAD cohorts. Among *USH2A* variants present in the RUSH2A cohort, 58% (74/128) SNVs or indels were also present in the general population (gnomAD) **(Figure 1B)**. We applied Fisher's exact test to determine which variants in the RUSH2A cohort were enriched as compared to the gnomAD database **(Figure 1C)**. A Bonferroni-corrected *P*-value of 0.00039 (=0.05/128 variants) was used as the cut-off to determine significant enrichment. Of the 128 variants, 23% (30/128) were statistically enriched in the RUSH2A cohort. An additional 9% (12/128) of *USH2A* variants were reclassified after application of the 2015 ACMG guidelines to determine pathogenicity level PS4, which is based on enrichment of variants in the affected population compared to controls (further description in **Supplemental Methods and Results** and **Supp. Figure S2**).

Association of clinical diagnosis and hearing loss severity with truncating variants

Following the establishment of individual variant disease-association, we sought to investigate phenotype associations using the power of this cohort. Typically, truncating alleles represent total loss of function and may be more likely to correlate with phenotypic severity. We grouped exonic deletions, nonsense, frameshift, canonical (+/-2) splicing site, and non-canonical splicing variants that were supported by RNA or minigene-based evidence as truncating variants. Consistent with previous studies, the predicted LoF variants or exonic deletions in the RUSH2A cohort were detected more frequently in participants with USH2 than ARRP **(Figure 2A)**.(Iannaccone et al., 2021)

Next, we sought to determine if the number of truncating variants was associated with clinical diagnosis. In the RUSH2A cohort, the majority (50%) of participants had 1 truncating variant, followed by those with 2 truncating alleles (33%) and 0 truncating variants (17%). The number of truncating variants in each patient was significantly associated with the clinical diagnosis (χ^2 = 36.9, ***P*** <0.001) **(Figure 2B)**. All 42 participants with two truncating variants were in the USH2 group and constituted 53% of all USH2 participants.

Given the association between truncating variants and clinical diagnosis of USH2, we hypothesized that the number of truncating variants also correlates with a greater degree of hearing loss.(Hartel et al., 2016) The number of truncating variants in each participant correlated positively with hearing sensitivity represented by a 4 frequency (.5/1/2/4 kHz) pure tone average in the entire cohort **(Supp. Figure S3A)** and the USH2 group **(Figure 2C, Supp. Figure S3B)**. No such correlation was observed in the ARRP subgroup (data not shown). Notably, more severe hearing loss was associated with the presence of 2 truncating variants than 0 or 1, as shown by the Tukey multiple comparisons of means analysis (adjusted *P*-value for pair-wise comparisons < 0.03) **(Figure 2C, Supp. Figure S3B)**.

Association of vision loss onset age and visual function with truncating variants

Participants with ARRP self-reported a later age of vision loss onset than those with USH2 (mean vision loss onset age in ARRP vs USH2: 31.8 vs 18.4, ***P*** < 0.001) **(Supp. Figure S4A)**. While the presence of two truncating variants was associated with earlier vision loss onset across all study participants (Tukey multiple comparisons of means, 1-0, ***P*** = 0.39; 2-0, ***P*** = 0.001; 2-1, ***P*** = 0.004) **(Supp. Figure S4B)**, there was no

association between vision loss onset and the number of truncating variants within either the USH2 or ARRP subgroups **(Supp. Figure S4C)**. In addition, USH2 participants had lower static perimetry full field hill of vision (mean V_{TOT} in ARRP vs USH2: 37.1 vs 22.7 decibel-steradian (dB-sr), ***P*** = 0.001) and lower kinetic perimetry V4e seeing area (mean in ARRP vs USH2: 9878 vs 6477 deg^2, ***P*** < 0.001) compared to ARRP participants **(Supp. Figure S4D-E)**. We find similar results when adjusting for disease of duration and age (**Supp. Table S2A**). Similarly, these differences in hill of vision and kinetic perimetry characteristics were not associated with the number of truncating variants in either the entire cohort or the USH2 or ARRP subgroups when adjusting for disease duration and age (adjusted ***P*** = 0.67 and ***P*** = 0.26, respectively; **Supp. Figure S4D-E; Supp. Table S2A-B**). Therefore, unlike hearing loss, the earlier and more severe vision loss observed in USH2 compared to ARRP may not be dependent on the number of truncating variants, suggesting that a different genotype association determines variability among retinal phenotypes.

Missense alleles cluster in ARRP

To determine whether other variant classes determine clinical endpoints in the RUSH2A cohort and USH2 and ARRP subgroups, we compared the variant landscape between these clinical diagnoses. The most frequently observed variants in both groups were in exon 13, c.2299delG p.(Glu767Ser*fs*Ter21) and c.2276G>T p.(Cys759Phe). However, the AF of c.2276G>T was greater in the ARRP subgroup, while c.2299delG was greater in the USH2 group **(Figure 3A-C and Supp. Table S3)**. Further, missense or in-frame-indel variants were more frequent in the ARRP group **(Figure 2A, 3B-C)**. Previous studies indicated that specific *USH2A* missense variants are associated with a

clinical diagnosis of ARRP.(Lenassi et al., 2015) Comparisons of allele frequencies of individual variants between the ARRP and USH2 groups revealed a group of missense alleles with enriched AF in the ARRP group (**Figure 3C**). Fisher's exact test showed five alleles statistically associated with the ARRP group (***P*** < 0.05): p.Cys759Phe (***P*** < 0.001), p.Cys3358Tyr (***P*** < 0.001), p.Cys3294Trp (***P*** = 0.02), p.Arg4192His (***P*** = 0.05), and *cis* variants p.Cys2040Gly (***P*** = 0.05) and p.Ser2492Leu (***P*** = 0.05) **(Figure 3C, Table 1 and Supp. Table S2)**. Three of these variants, p.Cys759Phe, p.Cys3358Tyr, and p.Arg4192His, were previously reported to be enriched in patients with ARRP.(Lenassi et al., 2015) Thus, this comparison of allelic diagnoses confirms and expands the known hierarchy of missense variants in disorders.

ARRP-associated missense variants are hypomorphic

Because patients with ARRP have later vision loss onset and better retained visual function compared to USH2, we next sought to understand if these ARRP-associated missense variants have hypomorphic effects on retinal photoreceptors and, therefore, patient phenotypic outcomes, when compared to other missense variants. Since the diseases are inherited in an autosomal recessive manner, it has been challenging to perform in-depth genotype-phenotype association studies. We postulated this could be studied by examining the missense variants *in trans* to the truncating alleles among the 1-truncating variant group. Among these 62 participants, there were 63 missense variants (including 3 pairs of *cis*-variants) known or presumed to be *in trans* to a truncating variant in 60 participants **(Figure 3D and Supp. Table S4)**. Of the five participants with known or predicted pairs of missense variants *in cis*, each had at

least one pathogenic or likely pathogenic variant. Thus, we only included the likely pathogenic or pathogenic missense variant of these pairs for further analysis.

To compare clinical correlates with missense genotypes, we evaluated the subgroup of participants with one missense variant and one truncating variant. Of this subgroup, we postulated that ARRP-enriched missense variants would have milder retinal manifestations than USH2. As described above, 62 participants harbored 1 truncating variant and at least one pathogenic or likely pathogenic missense. By comparing the disease phenotypes to Usherin protein location of the missense variants, we noted that missense variants in the N-terminus including the laminin N-terminal domain and the C-terminus including the fibronectin type-III domain, appear to be associated with the USH2 in this 1-truncating group **(Figure 3D)**, which was observed previously.(Pierrache et al., 2016)

The ARRP-enriched missense variants represented multiple times among those with 1-truncating variant were cysteine substitutions, p.Cys759Phe, p.Cys3294Trp, and p.Cys3358Tyr **(Figure 3D and Supp. Table S4)**. These three variants, defined as "ARRP-enriched" in the subsequent analyses, had significantly higher AF in the ARRP group as compared to the USH2 group both in the whole RUSH2A cohort **(Table 1 and Supp. Table S2)** and in the 62 participants with compound heterozygous truncating and missense variants. We then evaluated clinical characteristics among patients harboring one of these ARRP-enriched missense variants. Patients with ARRP-enriched missense alleles in the 1-truncating subgroup had later vision loss onset regardless of clinical diagnosis (32.9+/-12.8 years ARRP-enriched vs 20.8+/-10.1 years Other; $P < 0.001$) **(Figure 4A and Supp. Table S5).** V_{TOT} and III4e isopter visual field areas were also

increased in these participants (***P*** < 0.001 for both), indicating larger visual fields at their initial study visit **(Figure 4B-C and Supp. Table S5)**. ERG measures including cone 30-Hz flicker response, which corresponds to the function of cone photoreceptors, were also increased in those with ARRP-enriched missense alleles (***P*** = 0.04) **(Figure 4D and Supp. Table S5)**.

To further investigate functional vision mediated by photoreceptor subtypes, full-field stimulus testing (FST), which evaluates rod and cone-mediated function sensitivity responses, was examined using white, blue, and red wavelengths.(Birch et al., 2020) Notably, FST stimulus testing enables determination of the type of photoreceptor mediating sensitivity; white FST thresholds < -30 dB indicate preserved rod photoreceptor function.(Birch et al., 2020) Patients with ARRP-enriched missense alleles had lower FST thresholds for white (-40.0+/-12.6dB ARRP-enriched vs -29.8+/-11.7dB Other; ***P*** = 0.007). The difference in sensitivity to blue relative to red is also an index of rod-mediated sensitivity. Patients with ARRP-enriched missense alleles had greater blue-red differences (-19.6 +/-7.8dB ARRP-enriched vs -9.3+/-9.0dB Other; ***P*** < 0.001), indicating better preserved rod function in those with ARRP-enriched missense variants **(Figure 4E-F and Supp. Table S5)**. Thus, ARRP-enriched alleles appear hypomorphic on multimodal retinal assessments including psychometric and electrophysiologic measures.

To determine whether ARRP-enriched alleles exhibit hypomorphic properties independent of clinical diagnosis, we repeated this in only those with ARRP. Remarkably, all above measures (with the exception of vision loss onset age; ***P*** = 0.10) indicated better visual function in ARRP participants with ARRP-enriched missense

variants in conjunction with a truncating allele **(Supp. Figure S5A-F and Supp. Table S5)**. We also eliminated the possibility of younger age as a confounding variable, as participants with ARRP-enriched missense alleles were, on average, older in the 1-truncating group (47.9+/-15.1 years vs 38.9+/-12.29 years; ***P*** = 0.017) and of the same age in the ARRP subgroup (***P*** = 0.05). Additionally, ARRP-enriched missense alleles in the ARRP 1-truncating group appeared to have no effect on hearing among patients with Usher syndrome (***P*** = 0.61) and olfaction measures (***P*** = 0.23). These missense alleles have a milder effect on retinal dysfunction and degeneration, yet no effect on auditory or olfactory outcomes. This indicates a tissue-specific genotype-phenotype correlation, where retinopathy onset and progression are influenced by a subset of hypomorphic missense alleles, and hearing by the number of truncating alleles.

Variants in exon 13 are not significantly different from other regions

Finally, we investigated the effect of the most common individual variants, c.2299delG p.(Glu767Ser*fs*Ter21)and c.2276G>T p.(Cys759Phe) in exon 13, which is the target of a current gene therapy clinical trial (NCT03780257). We found no differences in measures of auditory or visual function with 0, 1, or 2 copies of c.2299delG p.(Glu767Ser*fs*Ter21) in the 2-truncating genotype subgroup **(Supp. Figure S6** and **data not shown)**. We also observed no differences among patients with and without p.Cys759Phe in the 1-truncating subgroup, or among those with 0 or 1 copy of p.Cys759Phe in the 2-missense genotype subgroup (**Supp. Figure S6** and **data not shown**). Therefore, the observations in the RUSH2A cohort of the influence of truncating variants on hearing loss endpoints, and missense variants for retinopathy endpoints, are not primarily driven by these commonly observed exon 13 variants.

DISCUSSION

RUSH2A is a natural history study of visual phenotypes and a cross sectional study of hearing and olfactory phenotypes among patients with *USH2A*-related disease, with the goal of identifying reliable clinical endpoints in the assessment of progression or therapeutic outcomes as well as identifying subpopulations most likely to benefit from treatment.(Birch et al., 2020; Duncan et al., 2020; Iannaccone et al., 2021) Here, we analyze the effect of genotype on clinical measures to better understand whether genotype determines clinical diagnosis, and whether variant effects are global or tissue-specific.

First, we standardized clinical variant interpretation at the cohort level using a case:control analysis and reclassified 2.4% of VUSs as likely pathogenic or benign, and 7.8% of likely pathogenic variants as pathogenic. Such classifications are tantamount to standardizing clinical variant interpretations for gene therapy trials, and for public repositories such as ClinVar, LOVD, and ClinGen.(Richards et al., 2015) The advantage of this study cohort is the large number of cases (127) which allowed us to both calculate disease-specific allele frequencies as critical evidence for pathogenicity ascertainment and separately analyze the USH2 and ARRP subgroups to explore genotype effects independent of clinical diagnosis, which has not been achieved previously.

Next, we demonstrated several important genotype-phenotype correlations at the tissue- and diagnosis-levels. First, USH2 is associated with truncating alleles, where

biallelic truncating alleles almost always cause USH2.(Lenassi et al., 2015; Pierrache et al., 2016) Second, in the RUSH2A cohort, hearing loss severity in USH2 is directly related to the number of truncating alleles, as similarly noted by Hartel et al. and Molina-Ramirez et al, as well as the RUSH2A study.(Hartel et al., 2016; Iannaccone et al., 2021; Molina-Ramirez et al., 2020) Third, truncating alleles are also associated with vision loss in USH2 patients, with earlier onset of and more severe retinal degeneration compared to ARRP.(Inaba et al., 2020; Meng et al., 2020; Pierrache et al., 2016) However, we found that the impact of truncating alleles on retinal degeneration may be dependent on clinical diagnosis, as we found no differences in visual symptom onset or severity in those with and without truncating variants in the USH2 and ARRP subgroups.

Furthermore, we confirmed and expanded the list of ARRP-associated missense alleles, adding p.Cys3294Trp and *cis* variants p.Cys2040Gly and p.Ser2492Leu through the RUSH2A study. Intriguingly, several of the hypomorphic missense alleles are located in the inter-fibronectin domain p.Cys3358Tyr, p.Cys3294Trp, and p.Glu3448Lys. Additionally, p.Arg4192His is in a fibronectin-3 repeat domain. Usherin interacts with fibronectin in retinal basement membranes, and is disrupted with certain mutations found in *USH2A*-related disorders.(Bhattacharya & Cosgrove, 2005) Further, human disease-associated variants in fibronectin-3 domains in usherin appear to be located within a “hotspot” for pathogenic missense variation.(Baux et al., 2014)

Analysis of both the entire cohort and the ARRP subgroup indicated that ARRP-enriched missense alleles among patients with 1-truncating allele have a later age of onset and better-preserved cone and rod photoreceptor function as measured by

psychometric and electrophysiological testing. Thus, the effect of ARRP-specific missense alleles on visual phenotypes and truncating alleles on the auditory phenotype are independent of the phenotypic differences observed between USH2 and ARRP. Further, we did not observe differences in hearing loss in individuals with ARRP-enriched missense alleles, nor did we observe differences in vision loss with different numbers of truncating alleles in the USH2 or ARRP groups. This implies these variant classes may have mutually exclusive effects, with less severe photoreceptor degeneration occurring with retinal-specific hypomorphic missense variants, and cochlear hair cells being more sensitive to truncating alleles.

Multiple studies from different countries have recognized an *USH2A* allelic hierarchy, where truncating alleles are associated with the clinical diagnosis of USH2 and hearing loss, and several missense alleles are associated with clinical diagnosis of ARRP.(Gao et al., 2021; Hartel et al., 2016; Inaba et al., 2020; Lenassi et al., 2015; Meng et al., 2020; Molina-Ramirez et al., 2020; Pierrache et al., 2016) The presence of specific missense alleles enriched in ARRP is associated with differences in age of onset and severity of retinal degeneration. Previously, Lenassi et al. described six variants, five missense and one intronic variant, that were found more frequently in ARRP than USH2, indicating that a different mutational spectrum exists between these two clinical diagnoses, which goes beyond the association of truncating variants with syndromic disease.(Lenassi et al., 2015) Here, we establish that the ARRP-enriched missense alleles are hypomorphic, in multiple tests of cone and rod photoreceptor function, and that these effects are independent of clinical diagnosis, even when adjusted for age of onset and disease duration.

Despite being the most expansive *USH2A* genotype-phenotype study to date, there are several limitations. First, we controlled for retinal dysfunction attributed to individual missense alleles by selecting patients with one truncating and one missense variant. As we and others have demonstrated, truncating variants predispose to Usher syndrome, which is an independent risk factor for more severe retinal degeneration. However, it is likely that the milder effects of ARRP-associated missense alleles are underestimated by this analysis design. Patients with homozygous or compound heterozygous missense alleles were not frequent in this population and would provide a better comparison.

Prospective longitudinal studies in cohorts such as these will be critical to determine if these effects indeed alter disease progression in addition to the onset and measures of phenotype severity performed here. Larger studies would also permit analysis of variant-specific effects. However, in our analysis, we did not find that the most common truncating variant c.2299delG p.(Glu767Ser*fs*Ter21) had different effects on visual and auditory endophenotypes from other truncating alleles, and patients with the most common missense variant c.2276G>T p.(Cys759Phe) did not have milder disease course than those with other missense alleles. This is likely because the other hypomorphic *USH2A* alleles were included in the control group of this analysis.

In conclusion, we demonstrated correlations of *USH2A* truncating variants with the presence and severity of hearing loss and of hypomorphic missense variants with the onset and severity of retinal degeneration (**Supplemental Graphic**). Importantly, these effects are independent of clinical diagnosis, and will allow for further subgrouping of patients to provide prognostic information and clinical endpoints for gene therapy trials.

As such, these findings highlight the importance of considering the effect of genotype on outcome measures for clinical trials. A deep understanding of genotype-phenotype correlations is critical in this era of gene augmentation therapy. Understanding the mechanism of disease, improving clinical molecular diagnostics for eligibility, and providing prognostic information for disease onset and progression are essential for determining the efficacy of new therapies.

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Conflict of Interests Statement

J. Duncan is a consultant for ConeSight Theraputics, DTx Pharma, Inc., Editas Theraputics, Eyevensys Theraputics, Nacuity, PYC Therapeutics, Spark Therapeutics, and Vedere Bio, Astellas; she receives financial support for clinical trials from Acucela, Abbvie/Allergan, AGTC Theraputics, Biogen/Nightstarx Theraputics, Inc., ProQR Therapeutics, Second Sight Medical Products, Inc and Neurotech USA, Inc., ;and she serves as a clinical advisory board member for SparingVision, Gyroscope Therapeutics, AGTC Therapeutics, Spark Therapeutics, ProQR Therapeutics, Nacuity, RD fund, and Foundation Fighting Blindness; Spouse: stock in RxSight.

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Ethics Approval Statement: Jaeb Center for Health Research IRB is the overseeing IRB and approved this study. There is not a reference number or ID. This investigation adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review boards (IRBs), or ethics boards associated with each participating site.

Data Sharing and Data Accessibility Statement:

A deidentified database is available upon request through the public domain on the FFB/Jaeb public website.

Contributorship Statement

All authors contributed equally to the data collection, drafting, review, and finalization of manuscript. Robert Hufnagel takes responsibility for the data and analysis in the manuscript.

Web Resources:

ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/

gnomAD: https://gnomad.broadinstitute.org/

Varsome: https://varsome.com/

Franklin: https://franklin.genoox.com/clinical-db/home

Variant Effect Predictor: http://grch37.ensembl.org/Homo_sapiens/Tools/VEP

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FIGURE LEGENDS

Figure 1. Variant enrichment in the RUSH2A cohort. **A**. *USH2A* variant allele frequency in the RUSH2A cohort by cDNA position. **B-C**. *USH2A* variant allele frequency in the RUSH2A cohort vs allele frequency in gnomAD. Only variants present in both RUSH2A and gnomAD are shown. **B.** Clinical significance was obtained from ClinVar. **C.** Variants statistically (Fisher's exact test) enriched in the RUSH2A cohort as compared to gnomAD are shown in orange. Dotted lines in **A** represent exon 13 boundary; LoF, predicted loss of function variants; Variants labeled are those with allele frequency over 0.015.

Figure 2. Truncating alleles correlate with USH2 and degree of hearing loss. **A**. *USH2A* variant types in USH2 and ARRP. **B**. Bar chart showing patient diagnosis and number of truncating alleles. **C**. Box and dot plot showing 4 frequency (.5/1.2/4 kHz) pure tone average (4F PTA) in dB HLby number of truncating alleles in the USH2 group, adjusted for sex and age according to International Organization for Standardization (ISO) standards (ISO 7029: 2017; ANOVA, ***P*** = 0.0001). Larger numbers mean worse hearing. Adjusted *P*-values in the Tukey multiple comparisons of means between truncating allele groups in **C**. 1-0, ***P*** = 0.10; 2-0, ***P*** < 0.001; 2-1, ***P*** = 0.01.

Figure 3. *USH2A* variants enriched in patients with USH2 and ARRP. **A-B**. *USH2A* variant allele frequency in USH2 (**A**) or ARRP (**B**) by cDNA position. Variants labeled are those with allele frequency in patient subgroup over 0.015. Dotted lines, exon 13 boundary. **C**. *USH2A* variant allele frequency comparison by diagnosis. Variants labeled in **C** are those with *P*-value (Fisher's exact test) less than 0.05 (blue) or c.2299delG p.(Glu767Ser*fs*Ter21) (red, ***P*** = 0.09). LoF, predicted loss of function variants. **D**. Histogram of missense variants within the 1-truncating variant subgroup by protein position.

Figure 4. Retinal phenotypic differences due to RP-enriched *USH2A* missense variants. **A-E**. Box and dot plot comparing RP-enriched and Other missense variants in the 1-truncating group, for age of vision loss onset (**A**; Welch's t-test; ***P*** < 0.001), full-field hill of vision (**B**; ***P*** < 0.001), iii4E seeing area (**C**; ***P*** < 0.001), cone flicker amplitude (**D**; ***P*** = 0.04), and full-field stimulus thresholds for White (**E**; ***P*** = 0.007) and threshold differences Blue-Red (**F**; ***P*** < 0.001). Circles = females, triangles = males, red = ARRP, blue = USH2. Full field hill of vision units as V_{TOT}, decibel-steradian (dB-sr).

REFEREE COMMENTS (responses in blue text)
Referee: 1

Comments to the Author
The author presented phenotype-genotype study of USH2A based on clinical and molecular diagnostics from the RUSH2A study, which includes 127 patients with either USH2 or ARRP phenotype due to mutations in USH2A. Several interesting observations are reported. For example, dosage-dependent on the truncation allele in USH2A is observed for the severity in hearing loss both across all patients and also within USH2 group. In addition, truncate alleles are enriched in USH2 group while missense mutations is enriched in ARRP group. Several missense mutations, including the common Cys759Phe allele in exon 13, are found enriched in ARRP cohort as they are likely to be hypomorphic. Overall it is a well written manuscript and information rich which will be useful for guiding disease prognosis based on molecular diagnosis. My specific comments are the following:

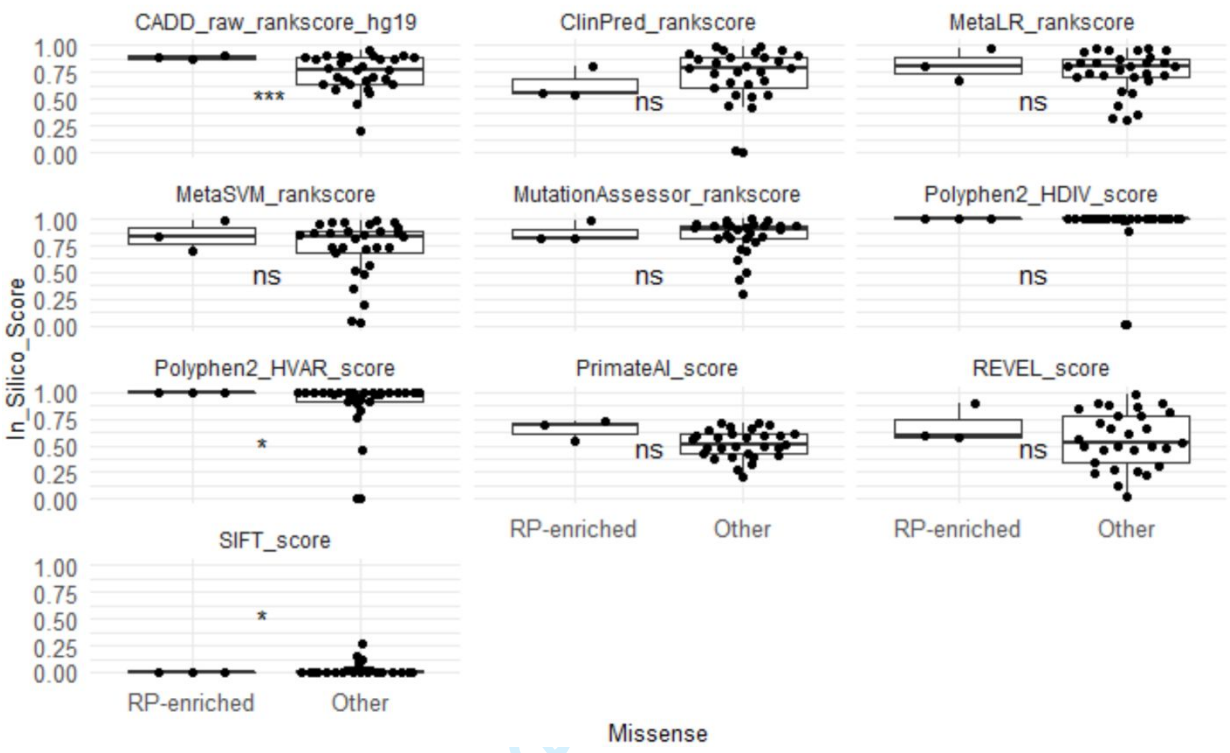
We are thankful to the reviewer for reviewing this manuscript, and for sharing compliments as well as identifying areas of improvement.

1. In the abstract, the author states that the dosage of USH2A truncating alleles has no effect on visual loss severity. This statement is misleading since patient with two USH2A truncating alleles has more server visual phenotype than ones with 1 or 0 truncating alleles in general without divide patients into subclinical groups first. The author means is that within USH2 or ARRP patient group, the number of truncating allele seems no associated with visual defect severity. This is interesting observation but probably need to be clarified more clearly.

To avoid any confusion on this point, which is elaborated upon later in the paper, we changed the sentence to read on lines 52-54 in the marked up version "USH2A truncating alleles were associated with USH2 and had a dose-dependent effect on hearing loss severity with no effect on visual loss severity within the USH2 subgroup."

2. It seems overall the trend is that weaker alleles lead to weaker phenotype. I am wondering, based the known alleles and corresponding clinical data, if a functional score can be assigned or learnt for each allele? If the functional score can be determined for the patient based on their genotype, it would be more quantitative and useful. For missense alleles, do weak allele also has lower in silico prediction score?

Generating functional scores based on clinical data is a great idea, which could be possibly obtained through machine learning. However, this would be out of the scope of the current study, and we suspect that the patient numbers in the RUSH2A study may be too low to produce meaningful scores. We plotted several in silico scores comparing the scores among missense variants. It appears that majority of the in silico predictors showed non-significant differences between the "RP-enriched" and "Other" groups by t-test. However, it is interesting to note that the scores of the RP-enriched variants showed narrow variations, possibly because they all affect Cysteine residues. We prefer not to present this data because of the uncertainty on the meaning of this result.



3. I am wondering if genetic background plays any role in the phenotype severity. Has the patient ethnic background been taken into consideration in the analysis?

This is an interesting question and indeed an area of consideration. We couldn't include the ethnic background into analysis with the available data as the cohort is predominantly White. Race/ethnicity data was reported in the RUSH2A baseline perimetry paper Duncan, J. L., Liang, W., Maguire, M. G., Audo, I., Ayala, A. R., Birch, D. G., . . . Sahel, J. A. (2020). Baseline Visual Field Findings in the RUSH2A Study: Associated Factors and Correlation With Other Measures of Disease Severity. Am J Ophthalmol, 219, 87-100. https://doi.org/10.1016/j.ajo.2020.05.024 (PMID: 32446738). No significant differences were observed for race/ethnicity and clinical diagnosis.

4. Given no ARRP patients carry two truncating mutations, it is clear that LOF will lead defect in both vision and hearing. In contrast, some of the hypomorphic allele leads to vision defect only. I am wondering if this observation suggests that hearing is more tolerate to partial loss of function of USH2A or these hypomophic allele affect USH2A function domain in the retina specifically. Are there reported alleles in USH2A lead to hearing loss only? It seems plausible since KO Ush2A in mice only lead to hearing loss without obvious retinal phenotype.

Thank you for bringing up this intriguing area of discussion. The two mechanisms raised by the reviewer are perhaps the best explanation for the observation. The first mechanism in which hearing is more tolerate to partial loss of function of USH2A seems to be more likely. However, more studies are needed to understand the mechanism. Hearing loss may precede the onset of RP in patients. For example, Vona et al. (PMID: 24875298) reported an one-year-old patient with two truncating alleles, in whom Vona et al. noted that the patient was younger than the age of onset for RP. We were not able to find adult patients with two truncating alleles and with hearing loss only in the literature.

Please also note that Ush2a knockout mice have been reported with progressive retinal degeneration as well as non-progressive hearing loss (Adato et al., 2005, PMID 16301217; Liu et al., 2007; PMID 17360538).

5. Given missense mutation in exon 13 lead to RP, I am wondering what is the implication on the exon13 skip therapy.

Since exon 13 is a common site of pathogenic variants the premise is that skipping that exon could result in production of a slightly shortened usherin protein. Antisense oligonucleotide therapy is being investigated in clinical trials of patients with USH2A-related retinal degeneration associated with variants in exon 13. Preliminary results indicate this approach is safe and clinical trials are enrolling patients with USH2A-related retinal degeneration and earlier stage disease (NCT05176717) and those with more severe vision loss (NCT051582963).

EDITORIAL BOARD'S COMMENTS

Communicating Editor
Comments to the Author:
This is an interesting and well conducted study, presenting a phenotype-specific allelic hierarchy of the USH2A gene, which is a target for gene therapy. It has potential impact on prognosis/genetic counseling and on treatment trial endpoints.
There are several referee's comments and editorial comments that should be addressed.

Specific editorial comments:

1/ The title refers to 'A tissue-specific allelic hierarchy'. Although the concept is interesting, the term 'tissue-specific allelic hierarchy' may be somewhat misleading, however. Tissue-specificity on itself has not been proven, but it is rather '(sub)phenotype-specific allelic hierarchy' that has been demonstrated in this study. The term 'tissue-specific genotype-phenotype correlation' that has been used later on in the study is probably more accurate.

Thank you for suggesting this, we are happy to revise the title of the manuscript as "Tissue-specific genotype-phenotype correlations among USH2A-related disorders in the RUSH2A study"

2/ Introduction: 'RP has extreme locus heterogeneity, with >90 genes associated with the nonsyndromic form, and is associated with hundreds of syndromic disorders, including ciliopathies, peroxisomal disorders, and multiple malformation syndromes.(Hartong, Berson, & Dryja, 2006)' -> many more RP genes have been identified since 2006, so (a) more recent reference(s) is (are) recommended. For instance: PMID: 29597005 (non-sydnromic RP) and PMID: 34839010 (general overview of IRD).

Thank you, we have added these references to the manuscript as suggested on lines 73 to 74 in the marked up version of the manuscript.

3/ Variants were demonstrated to be in trans for individuals with ARRP due to extensive locus heterogeneity of this clinical diagnosis: it not entirely clear why this has not been assessed for individuals with USH2A, even if the locus heterogeneity is much smaller.

Thank you for this comment. If resources were unlimited, we agree that segregation studies in all participants would have been ideal. However, the clinical phenotype of patients with USH2A-related Usher syndrome type 2 is relatively specific with mild to moderate congenital hearing loss and retinal degeneration beginning in childhood or adolescence. Among patients with this phenotype, 57-79% of cases are attributed to pathogenic variants in USH2A (PMID: 20301515). As pointed out, the locus heterogeneity in Usher syndrome type 2 is quite low with only 3 genes (USH2A, ADGRV1 and WHRN) associated with Usher syndrome type 2 compared to over 80 genes associated with nonsyndromic ARRP. Many but not all patients had broad sequencing panels performed, further reducing the chance of another causal gene for Usher syndrome in those patients. To conduct the study as efficiently as possible with limited resources we elected to require segregation studies only for patients with ARRP associated with USH2A variants.

4/ A consensus verdict for in silico pathogenicity predictions for missense variants was acquired from Varsome (https://varsome.com/) and Franklin (https://franklin.genoox.com/clinical-db/home) webtools. The Varsome as well as the Genoox tools are commercial prediction webtools. Could the individual predictions behind the consensus verdict for the missense variants assessed be provided in Table S1, allowing a more independent inspection.

Thank you for this excellent suggestion. We agree that it is valuable to know the individual in silico predictions for the variants. It is more unbiased approach, therefore, we have added in silico predictions of 12 different tools from the variant effect predictor (VEP) in Table S1 (Columns X – AS) and added the use of VEP in the text as well "Individual in silico predictions were acquired from Variant Effect Predictor (VEP; http://grch37.ensembl.org/Homo_sapiens/Tools/VEP) (Supp. Table S1" on lines 153 to 154 in the marked up version .Varsome, Franklin and VEP use almost the same in silico prediction tools, we simply used VEP for this as it allows for batch queries and is a free tool.

5/ p.9: examination of the distribution of USH2A coding variants present in ClinVar may be biased, as there may be a submission or a population bias.

We have added this point to the text, lines 181 -185 in the marked version of the manuscript, "While ClinVar may have submission or population bias, we observed no apparent spatially restricted clusters of pathogenic or likely-pathogenic variants. However, exon 13 harbors the most frequently submitted variants, c.2276G>T p.(Cys759Phe) and c.2299delG."

6/ p.10: 'We grouped exonic deletions, nonsense, frameshift, canonical (+/-2) splicing site, and non-canonical splicing variants that were supported by RNA or minigene-based evidence as truncating variants.' -> As to the canonical splicing sites: has it been assessed if they are predicted to lead to a truncating variant? Exon 13 (ENSE00001336973) for instance is a multiple of three.

Yes – we assessed this notion and added evidence wherever applicable in Supp. Table S1, column Q. For example, four splice variants (NM_206933.2:c.11047+1G>A, NM_206933.2:c.12067-2A>G, NM_206933.2:c.5776+1G>A, NM_206933.2:c.5857+2T>C) cause inframe exon skipping. We assigned them a downgraded PVS1 criteria (i.e. the criteria was downgraded from "Very Strong" to "Strong" criteria of pathogenicity) according to ClinGen recommendations for PVS1 (PMID: 30192042).

MANAGING EDITOR COMMENTS:

Please respond to the Managing Editor's comments beneath your responses to the reviewers and the editorial board; otherwise the final decision could be delayed.

1) Please include the OMIM accession numbers using this format, e.g.:
"(RP; MIM# 268000)" with these characters and this spacing. You must the same format regardless of whether the MIM# relates to a locus or a phenotype. Visit http://www.omim.org which has the current OMIM version.

We reviewed the manuscript and made changes wherever applicable as per above guidelines.

a-Please ensure that you use HUGO HGNC-approved gene symbols. Common gene symbol aliases may also be used at first mention (Title, Abstract and main text) but the approved symbol MUST be used also in Title, Abstract and main text. Verify gene symbols at http://www.genenames.org/

We reviewed the manuscript and made sure to use HUGO HGNC-approved gene symbols.

b-Human gene symbols must be in all caps italics and protein symbols in all caps Roman.

We reviewed the manuscript and made sure to follow the suggested formatting.

2) Regarding any in silico prediction methods and your current use of them in the paper: please see our Author guidelines on this topic (under "Editorial Policies and Ethical Considerations" https://tinyurl.com/yd26wb2y) and confirm that your paper conforms with them.
If no prediction methods used, respond "none".

We confirm that our manuscript conforms with the suggested guidelines.

Otherwise, provide additional information in appropriate table or a new supplementary table, or in the text (actual numeric output data, ranges/cut-offs, websites, software versions, etc.) as noted in the Vihinen (2013) article indicated in our guidelines – refer to the final section of the article and to Box 2: https://onlinelibrary.wiley.com/doi/full/10.1002/humu.22253

We have added two additional columns in Supp. Table S1, with Varsome and Franklin in silico predictions.

3) VERIFYING NOMENCLATURE OF DNA VARIANTS AND SHARING VARIANT DATA Documenting variation in our genomes is an important undertaking for human research and clinical care. Accuracy in the notation of DNA variants is essential for the success of this endeavor. Because of the importance of the issue and the overall consensus on the rules, Journal is adopting an editorial policy that requires compliance with the recommendations to describe sequence variants before manuscripts can be accepted and published.

**Furthermore, variants reported in manuscripts must be submitted to a public database (e.g. ClinVar https://www.ncbi.nlm.nih.gov/clinvar/ or Global Variome shared LOVD http://www.lovd.nl) prior to publication.

Variant submission to ClinVar is in progress and is expected to be completed ahead of publication of this manuscript. We will be happy to provide updates/confirmation of this process.

Variant descriptions should follow current recommendations of the Human Genome Variation Society (HGVS) (https://onlinelibrary.wiley.com/doi/full/10.1002/humu.22981). Please visit https:/varnomen.hgvs.org/ for the latest nomenclature updates, for examples of acceptable nomenclature, guidance concerning reference sequences, or if you have further questions. Compliance with HGVS nomenclature must be verified using tools such as the Mutalyzer program (https://mutalyzer.nl/ ; instructions: https://github.com/mutalyzer/mutalyzer/wiki/Mutalyzer_explain.pdf) or VariantValidator (https://variantvalidator.org/ ; instructions: https://variantvalidator.org/batch_instructions/). The file resulting from this check containing each variant noted in your manuscript must be included in your submission (as a supplementary file for review but not publication). These tools are freely available on the web.

We followed HGVSc nomenclature while reporting DNA sequence variants in this manuscript. The result file has been submitted.

Important considerations include:
Variants should be described in the text and tables using both DNA and protein designations whenever appropriate.

We followed this guideline in our manuscript.

- Reference sequences defined in the HGVS nomenclature guidelines (http://varnomen.hgvs.org/bg-material/refseq/) must be used for reporting sequence variants. Authors should always include the Accession Number of the relevant reference sequence(s), with version number where applicable (e.g.: RefSeq NM_003002.3, LRG_9t1 or GenBank NC_000011.10), in the Materials and Methods section and as a footnote in any tables listing variants. Please note, RefSeq and Ensembl transcript reference sequences that have been denoted as the default reporting references through the Matched Annotation from the NCBI and EBI (MANE) project) may be used once approved by the HGVS variant nomenclature working group.

We followed HGVSc nomenclature as well as guidelines mentioned above, while reporting DNA sequence variants in this manuscript.

- If alternative nomenclature schemes are commonly found in the literature, they may also be used in addition to approved nomenclature, but they must be defined clearly (e.g. CFTR p.Phe508del and deltaF508).

We did not use any alternative nomenclature other than HGVS nomenclature, while reporting DNA sequence variants in this manuscript.

- Standard HGVS nomenclature using g. annotation and identifying the genome build must be used for non-coding variation, including those variants identified in GWAS studies (e.g., NC_000017.11:g.50201450C>T). Variants may also be described using dbSNP genomic location identifiers, in addition to approved nomenclature, if the specific nucleotide change is also included. Acceptance and/or publication may be delayed if authors do not follow these guidelines.

We followed HGVSc nomenclature as well as guidelines mentioned above, while reporting DNA sequence variants in this manuscript. The g. information has been added to column J in Supp. Table S1.

- Protein-level variants are to be described using the 3-letter aa code, as is used in ClinVar and LOVD. The only exception: single-letter aa code may be used in figures, in keeping with formatting and image constraints.

We followed HGVSc nomenclature as well as guidelines mentioned above, while reporting DNA sequence variants in this manuscript.

--FIGURES which use non-HGVS traditional nomenclature (e.g. D104G instead of p.D104G, may retain the non-HGVS nomenclature, but use HGVS nomenclature in the figure legends.

Our manuscript conforms this guideline.

--DATA AVAILABILITY STATEMENT
Provide a brief Data Availability Statement at the end of your main text near the Acknowledgments. Include a statement such as was provided in the submission form on availability.
Also include the URL(s) of the database you submitted to and links to any accession numbers (if the number of such accession numbers is reasonable).

Data availability statement is available in the marked up version in the manuscript on lines 523 -525.

--WEB RESOURCES: A list of web resources and URLs used by the author should be included at the end of the main text, along with other author declarations.

Web resources section has been added to the manuscript.

4) On resubmission:

We reviewed the manuscript to follow the guidelines mentioned below to make sure that the manuscript conforms and made necessary changes wherever it was needed.

a-The authors should be listed with names and surnames per journal style (e.g. Maria A.M. Smith, David Jones).
--Please double check the author names and affiliations carefully. These are often a source of typographical errors. Do not include academic degrees.

b-After making edits to the manuscript, please provide a HIGHLIGHTED VERSION of your revised manuscript by highlighting the revised text (preferably in red ink), or any other changes done to the manuscript. Kindly avoid strike-through text or comments and avoid submitting a document with tracked changes. Please ensure to upload a CLEAN VERSION (no highlighted sentences, strike-through words, or comments in margins) along with the HIGHLIGHTED VERSION of your manuscript. In this way, the reviewers/editors can easily see your edits in the highlighted versions and the final paper in the clean versions.
For Supporting Information files only, please upload DOC files with highlighted and accepted changes, PLUS upload one PDF file with changes accepted.

Tables in Excel do not require tracked changes. Contact the Editorial Office if you need assistance.

c-Title Page (with grant numbers and corresponding author contact information), Abstract (180-200 words max), Key Words, Main Text, References, and Figure Legends should be combined into one file for the manuscript and submitted as a *.doc file.

Manuscript abides by the above.

--The Abstract must be unstructured, written as one paragraph and with no subheadings.

We have corrected the abstract to be unstructured and written as one paragraph with no subheadings.

--Please list the specific Web Resources used at the end of the main text. They may also be added in the main text at first mention.

Web resources have been added on lines 531 to 536 in the marked-up version in the manuscript text.

--The text should be made 12 point double-spaced (not 1.5 lines) throughout.

Text is 12 points doubled spaced throughout.

--We do not publish Abbreviations lists, abbreviations should be defined in the main text at first mention.
Abbreviations are defined at first mention.

d-Figures for main article must be submitted as separate files with high resolution (at least 300-600 dpi) as *.tif or *.eps format only.

All the figures have been prepared in tif format at 300 dpi.

e-Tables must be submitted individually as separate *.doc files (with their titles and legends included). Please use the MS Word table format if possible. Excel (*.xls or .xlsx) files can be submitted for very large tables-check with the editorial office humu@wiley.com .

Supplemental tables have been included in separate tabs of an excel file.

f-Any Supporting Tables or Figures should be named and cited from the text as follows: 'Supp. Table S1' and 'Supp. Figure S1' (see below).

All supplemental tables and figures have been cited in the text.

g-Supporting Figures and Tables (unless they require to be submitted as Excel files) should be prepared in a single MS Word *.doc file labeled 'Supp_Mat', with Figures preceding Tables, with changes highlighted, and one PDF file, with all changes accepted. Each table/figure should be accompanied with its legend.

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5) Please confirm that all tables, figures, supplementary tables, and supplementary figures are cited in numerical order. This is the most common reason why accepted papers are returned to authors for edits prior to typesetting, so please check your paper carefully.

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NG_009497.2:g.752831A>G                 NP_996816.3:p.? NC_000001.10:g.215848960T>C
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NG_009497.2:g.355562T>C NP_996816.3:p.? NC_000001.10:g.216246229A>G
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(USH2A), transcript variant 2, mRNA
NM_206933.4:c.7595-2144A>G MANE NM_206933.4:c.7595-2144A>G
NC_000001.10(NM_206933.4):c.7595-2144A>G
NG_009497.2(NM_206933.4):c.7595-2144A>G NG_009497.2:g.537251A>G
NP_996816.3:p.? NC_000001.10:g.216064540T>C NC_000001.11:g.215891198T>C 1
216064540 . T C 1 215891198 . T C
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NM_206933.4:c.7595-3C>G MANE NM_206933.4:c.7595-3C>G
NC_000001.10(NM_206933.4):c.7595-3C>G NG_009497.2(NM_206933.4):c.7595-3C>G
NG_009497.2:g.539392C>G NP_996816.3:p.? NC_000001.10:g.216062399G>C
NC_000001.11:g.215889057G>C 1 216062399 . G C 1
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NG_009497.2:g.561270A>G NP_996816.3:p.? NC_000001.10:g.216040521T>C
NC_000001.11:g.215867179T>C 1 216040521 . T C 1
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NM_206933.4:c.4393_4394insAAAACTTTAGCAG MANE
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NG_009497.2:g.238223_238224insAAAACTTTAGCAG
NP_996816.3:p.(Ala1465GlufsTer16)
NC_000001.10:g.216363579_216363580insTCTGCTAAAGTTT
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G GCTGCTAAAGTTTT 1 216190225 . G GCTGCTAAAGTTTT
USH2A HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA

NM_206933.4:c.6163G>A MANE NM_206933.4:c.6163G>A
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NC_000001.10:g.216221876C>T NC_000001.11:g.216048534C>T 1 216221876
. C T 1 216048534 . C T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NG_009497.2:g.462979C>T                 NP_996816.3:p.(Arg2323Ter)
NC_000001.10:g.216138812G>A     NC_000001.11:g.215965470G>A     1       216138812
.       G       A       1       215965470       .       G       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.8576G>A           MANE    NM_206933.4:c.8576G>A
NG_009497.2:g.550586G>A                 NP_996816.3:p.(Arg2859His)
NC_000001.10:g.216051205C>T     NC_000001.11:g.215877863C>T     1       216051205
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NC_000001.10:g.216498841G>T     NC_000001.11:g.216325499G>T     1       216498841
.       G       T       1       216325499       .       G       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.10450C>T          MANE    NM_206933.4:c.10450C>T
NG_009497.2:g.645576C>T                 NP_996816.3:p.(Arg3484Ter)
NC_000001.10:g.215956215G>A     NC_000001.11:g.215782873G>A     1       215956215
.       G       A       1       215782873       .       G       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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        NG_009497.2:g.6211_6212insT
NP_996816.3:p.(Arg34SerfsTer41) NC_000001.10:g.216595579_216595580insA
NC_000001.11:g.216422237_216422238insA  1       216595579       .       G       GA
1       216422237       .       G       GA      USH2A   HGNC:12601      Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11156G>A          MANE    NM_206933.4:c.11156G>A
NG_009497.2:g.668714G>A                 NP_996816.3:p.(Arg3719His)
NC_000001.10:g.215933077C>T     NC_000001.11:g.215759735C>T     1       215933077
.       C       T       1       215759735       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12574C>T          MANE    NM_206933.4:c.12574C>T
NG_009497.2:g.753112C>T                 NP_996816.3:p.(Arg4192Cys)
NC_000001.10:g.215848679G>A     NC_000001.11:g.215675337G>A     1       215848679
.       G       A       1       215675337       .       G       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12575G>A          MANE    NM_206933.4:c.12575G>A
NG_009497.2:g.753113G>A                 NP_996816.3:p.(Arg4192His)
NC_000001.10:g.215848678C>T     NC_000001.11:g.215675336C>T     1       215848678
.       C       T       1       215675336       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.14803C>T          MANE    NM_206933.4:c.14803C>T
NG_009497.2:g.787726C>T                 NP_996816.3:p.(Arg4935Ter)
NC_000001.10:g.215814065G>A     NC_000001.11:g.215640723G>A     1       215814065
.       G       A       1       215640723       .       G       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4133_4134dup              MANE    NM_206933.4:c.4133_4134dup
        NG_009497.2:g.231778_231779dup
NP_996816.3:p.(Asn1379SerfsTer54)       NC_000001.10:g.216370017_216370018dup
NC_000001.11:g.216196675_216196676dup   1       216370011       .       T       TGA
1       216196669       .       T       TGA     USH2A   HGNC:12601      Homo sapiens
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usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.7950dup MANE NM_206933.4:c.7950dup
NG_009497.2:g.539750dup NP_996816.3:p.(Asn2651GlnfsTer10)
NC_000001.10:g.216062044dup NC_000001.11:g.215888702dup 1 216062040
. T TG 1 215888698 . T TG USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.1036A>C MANE NM_206933.4:c.1036A>C
NG_009497.2:g.103037A>C NP_996816.3:p.(Asn346His)
NC_000001.10:g.216498754T>G NC_000001.11:g.216325412T>G 1 216498754
. T G 1 216325412 . T G USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.5278del MANE NM_206933.4:c.5278del
NG_009497.2:g.344973del NP_996816.3:p.(Asp1760MetfsTer10)
NC_000001.10:g.216256818del NC_000001.11:g.216083476del 1 216256817
. TC T 1 216083475 . TC T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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. C T 1 215782125 . C T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3584G>T MANE NM_206933.4:c.3584G>T
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NC_000001.10:g.216373196C>A NC_000001.11:g.216199854C>A 1 216373196
. C A 1 216199854 . C A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4338_4339del MANE NM_206933.4:c.4338_4339del
NG_009497.2:g.238168_238169del
NP_996816.3:p.(Cys1447GlnfsTer29) NC_000001.10:g.216363626_216363627del
NC_000001.11:g.216190284_216190285del 1 216363621 . CAG C
1 216190279 . CAG C USH2A HGNC:12601 Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
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NG_009497.2:g.379870T>C NP_996816.3:p.(Cys2040Arg)
NC_000001.10:g.216221921A>G NC_000001.11:g.216048579A>G 1 216221921
. A G 1 216048579 . A G USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NC_000001.10:g.216221921A>C NC_000001.11:g.216048579A>C 1 216221921
. A C 1 216048579 . A C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NC_000001.10:g.216011434G>T NC_000001.11:g.215838092G>T 1 216011434

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NC_000001.10:g.215972408A>G     NC_000001.11:g.215799066A>G     1       215972408
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.9842G>T           MANE    NM_206933.4:c.9842G>T
NG_009497.2:g.629426G>T                 NP_996816.3:p.(Cys3281Phe)
NC_000001.10:g.215972365C>A     NC_000001.11:g.215799023C>A     1       215972365
.       C       A       1       215799023       .       C       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NC_000001.10:g.215972325G>C     NC_000001.11:g.215798983G>C     1       215972325
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NG_009497.2:g.638218G>T                 NP_996816.3:p.(Cys3337Phe)
NC_000001.10:g.215963573C>A     NC_000001.11:g.215790231C>A     1       215963573
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NG_009497.2:g.638281G>A                 NP_996816.3:p.(Cys3358Tyr)
NC_000001.10:g.215963510C>T     NC_000001.11:g.215790168C>T     1       215963510
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NC_000001.10:g.215940074A>C     NC_000001.11:g.215766732A>C     1       215940074
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NG_009497.2:g.104209G>T                 NP_996816.3:p.(Cys419Phe)
NC_000001.10:g.216497582C>A     NC_000001.11:g.216324240C>A     1       216497582
.       C       A       1       216324240       .       C       A       USH2A
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NG_009497.2:g.104209G>A                 NP_996816.3:p.(Cys419Tyr)
NC_000001.10:g.216497582C>T     NC_000001.11:g.216324240C>T     1       216497582
.       C       T       1       216324240       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.1606T>C           MANE    NM_206933.4:c.1606T>C
NG_009497.2:g.106528T>C                 NP_996816.3:p.(Cys536Arg)
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
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NC_000001.10:g.216420460C>A	NC_000001.11:g.216247118C>A	1	216420460
.	C	A	1	216247118	.	C	A	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.2296T>C	MANE	NM_206933.4:c.2296T>C
NG_009497.2:g.181351T>C		NP_996816.3:p.(Cys766Arg)
NC_000001.10:g.216420440A>G	NC_000001.11:g.216247098A>G	1	216420440
.	A	G	1	216247098	.	A	G	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.2384G>A	MANE	NM_206933.4:c.2384G>A
NG_009497.2:g.181439G>A		NP_996816.3:p.(Cys795Tyr)
NC_000001.10:g.216420352C>T	NC_000001.11:g.216247010C>T	1	216420352
.	C	T	1	216247010	.	C	T	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.2802T>G	MANE	NM_206933.4:c.2802T>G
NG_009497.2:g.181857T>G		NP_996816.3:p.(Cys934Trp)
NC_000001.10:g.216419934A>C	NC_000001.11:g.216246592A>C	1	216419934
.	A	C	1	216246592	.	A	C	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3187_3188del		MANE	NM_206933.4:c.3187_3188del
	NG_009497.2:g.221047_221048del
NP_996816.3:p.(Gln1063SerfsTer15)	NC_000001.10:g.216380744_216380745del
NC_000001.11:g.216207402_216207403del	1	216380742	.	TTG	T
1	216207400	.	TTG	T	USH2A	HGNC:12601	Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4222C>T	MANE	NM_206933.4:c.4222C>T
NG_009497.2:g.231867C>T		NP_996816.3:p.(Gln1408Ter)
NC_000001.10:g.216369924G>A	NC_000001.11:g.216196582G>A	1	216369924
.	G	A	1	216196582	.	G	A	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.9469C>T	MANE	NM_206933.4:c.9469C>T
NG_009497.2:g.611351C>T		NP_996816.3:p.(Gln3157Ter)
NC_000001.10:g.215990440G>A	NC_000001.11:g.215817098G>A	1	215990440
.	G	A	1	215817098	.	G	A	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11516A>G	MANE	NM_206933.4:c.11516A>G
NG_009497.2:g.685240A>G		NP_996816.3:p.(Gln3839Arg)
NC_000001.10:g.215916551T>C	NC_000001.11:g.215743209T>C	1	215916551
.	T	C	1	215743209	.	T	C	USH2A
HGNC:12601	Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11875_11876del		MANE	NM_206933.4:c.11875_11876del
	NG_009497.2:g.700228_700229del
NP_996816.3:p.(Gln3959AsnfsTer53)	NC_000001.10:g.215901565_215901566del
NC_000001.11:g.215728223_215728224del	1	215901561	.	TTG	T
1	215728219	.	TTG	T	USH2A	HGNC:12601	Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.14131C>T	MANE	NM_206933.4:c.14131C>T
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NG_009497.2:g.757475C>T NP_996816.3:p.(Gln4711Ter)
NC_000001.10:g.215844316G>A NC_000001.11:g.215670974G>A 1 215844316
. G A 1 215670974 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.1618C>T MANE NM_206933.4:c.1618C>T
NG_009497.2:g.106540C>T NP_996816.3:p.(Gln540Ter)
NC_000001.10:g.216495251G>A NC_000001.11:g.216321909G>A 1 216495251
. G A 1 216321909 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.6159del MANE NM_206933.4:c.6159del
NG_009497.2:g.379911del NP_996816.3:p.(Glu2054LysfsTer10)
NC_000001.10:g.216221881del NC_000001.11:g.216048539del 1 216221879
. CT C 1 216048537 . CT C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.10342G>A MANE NM_206933.4:c.10342G>A
NG_009497.2:g.641734G>A NP_996816.3:p.(Glu3448Lys)
NC_000001.10:g.215960057C>T NC_000001.11:g.215786715C>T 1 215960057
. C T 1 215786715 . C T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11403_11404delinsTTT MANE
NM_206933.4:c.11403_11404delinsTTT
NG_009497.2:g.685127_685128delinsTTT
NP_996816.3:p.(Glu3802LeufsTer12) NC_000001.10:g.215916663_215916664delinsAAA
NC_000001.11:g.215743321_215743322delinsAAA 1 215916663 . CG
AAA 1 215743321 . CG AAA USH2A HGNC:12601 Homo
sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11815G>A MANE NM_206933.4:c.11815G>A
NG_009497.2:g.700168G>A NP_996816.3:p.(Glu3939Lys)
NC_000001.10:g.215901623C>T NC_000001.11:g.215728281C>T 1 215901623
. C T 1 215728281 . C T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12152_12153insTT MANE NM_206933.4:c.12152_12153insTT
NG_009497.2:g.748158_748159insTT
NP_996816.3:p.(Glu4051AspfsTer2) NC_000001.10:g.215853632_215853633insAA
NC_000001.11:g.215680290_215680291insAA 1 215853632 . T TAA
1 215680290 . T TAA USH2A HGNC:12601 Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12232G>T MANE NM_206933.4:c.12232G>T
NG_009497.2:g.748238G>T NP_996816.3:p.(Glu4078Ter)
NC_000001.10:g.215853553C>A NC_000001.11:g.215680211C>A 1 215853553
. C A 1 215680211 . C A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.13335_13347delinsCTTG MANE
NM_206933.4:c.13335_13347delinsCTTG
NG_009497.2:g.753873_753885delinsCTTG
NP_996816.3:p.(Glu4445_Ser4449delinsAspLeu)
NC_000001.10:g.215847906_215847918delinsCAAG
NC_000001.11:g.215674564_215674576delinsCAAG 1 215847906 .
AGAGTCCATGTTC CAAG 1 215674564 . AGAGTCCATGTTC CAAG
USH2A HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA

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NM_206933.4:c.13466dup          MANE    NM_206933.4:c.13466dup
NG_009497.2:g.754004dup                 NP_996816.3:p.(Glu4491GlyfsTer6)
NC_000001.10:g.215847788dup     NC_000001.11:g.215674446dup     1       215847786
.       G       GC      1       215674444       .       G       GC      USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.14885dup          MANE    NM_206933.4:c.14885dup
NG_009497.2:g.787808dup                 NP_996816.3:p.(Glu4963GlyfsTer38)
NC_000001.10:g.215813984dup     NC_000001.11:g.215640642dup     1       215813982
.       C       CT      1       215640640       .       C       CT      USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.2299del           MANE    NM_206933.4:c.2299del
NG_009497.2:g.181354del                 NP_996816.3:p.(Glu767SerfsTer21)
NC_000001.10:g.216420437del     NC_000001.11:g.216247095del     1       216420436
.       TC      T       1       216247094       .       TC      T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.6670G>T           MANE    NM_206933.4:c.6670G>T
NG_009497.2:g.435294G>T                 NP_996816.3:p.(Gly2224Cys)
NC_000001.10:g.216166497C>A     NC_000001.11:g.215993155C>A     1       216166497
.       C       A       1       215993155       .       C       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.802G>A            MANE    NM_206933.4:c.802G>A
NG_009497.2:g.100812G>A                 NP_996816.3:p.(Gly268Arg)
NC_000001.10:g.216500979C>T     NC_000001.11:g.216327637C>T     1       216500979
.       C       T       1       216327637       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.9424G>T           MANE    NM_206933.4:c.9424G>T
NG_009497.2:g.611306G>T                 NP_996816.3:p.(Gly3142Ter)
NC_000001.10:g.215990485C>A     NC_000001.11:g.215817143C>A     1       215990485
.       C       A       1       215817143       .       C       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.10636G>T          MANE    NM_206933.4:c.10636G>T
NG_009497.2:g.646303G>T                 NP_996816.3:p.(Gly3546Ter)
NC_000001.10:g.215955488C>A     NC_000001.11:g.215782146C>A     1       215955488
.       C       A       1       215782146       .       C       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11266G>A          MANE    NM_206933.4:c.11266G>A
NG_009497.2:g.669731G>A                 NP_996816.3:p.(Gly3756Ser)
NC_000001.10:g.215932060C>T     NC_000001.11:g.215758718C>T     1       215932060
.       C       T       1       215758718       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12284G>A          MANE    NM_206933.4:c.12284G>A
NG_009497.2:g.748290G>A                 NP_996816.3:p.(Gly4095Asp)
NC_000001.10:g.215853501C>T     NC_000001.11:g.215680159C>T     1       215853501
.       C       T       1       215680159       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12283G>A          MANE    NM_206933.4:c.12283G>A
NG_009497.2:g.748289G>A                 NP_996816.3:p.(Gly4095Ser)
NC_000001.10:g.215853502C>T     NC_000001.11:g.215680160C>T     1       215853502
.       C       T       1       215680160       .       C       T       USH2A
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HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.13018G>C          MANE    NM_206933.4:c.13018G>C
NG_009497.2:g.753556G>C                 NP_996816.3:p.(Gly4340Arg)
NC_000001.10:g.215848235C>G     NC_000001.11:g.215674893C>G     1       215848235
.       C       G       1       215674893       .       C       G       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.13207_13208del            MANE    NM_206933.4:c.13207_13208del
        NG_009497.2:g.753745_753746del
NP_996816.3:p.(Gly4403ProfsTer15)       NC_000001.10:g.215848046_215848047del
NC_000001.11:g.215674704_215674705del   1       215848044       .       GCC     G
1       215674702       .       GCC     G       USH2A   HGNC:12601      Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3547_3548del              MANE    NM_206933.4:c.3547_3548del
        NG_009497.2:g.228558_228559del
NP_996816.3:p.(Ile1183PhefsTer19)       NC_000001.10:g.216373236_216373237del
NC_000001.11:g.216199894_216199895del   1       216373231       .       AAT     A
1       216199889       .       AAT     A       USH2A   HGNC:12601      Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.6847_6848insATCA          MANE    NM_206933.4:c.6847_6848insATCA
        NG_009497.2:g.457714_457715insATCA
NP_996816.3:p.(Ile2283AsnfsTer49)       NC_000001.10:g.216144077_216144078insGATT
NC_000001.11:g.215970735_215970736insGATT       1       216144076       .       A
ATGAT   1       215970734       .       A       ATGAT   USH2A   HGNC:12601      Homo
sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.15496A>G          MANE    NM_206933.4:c.15496A>G
NG_009497.2:g.799612A>G                 NP_996816.3:p.(Ile5166Val)
NC_000001.10:g.215802179T>C     NC_000001.11:g.215628837T>C     1       215802179
.       T       C       1       215628837       .       T       C       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4714C>T           MANE    NM_206933.4:c.4714C>T
NG_009497.2:g.331322C>T                 NP_996816.3:p.(Leu1572Phe)
NC_000001.10:g.216270469G>A     NC_000001.11:g.216097127G>A     1       216270469
.       G       A       1       216097127       .       G       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4714del           MANE    NM_206933.4:c.4714del
NG_009497.2:g.331322del                 NP_996816.3:p.(Leu1572PhefsTer3)
NC_000001.10:g.216270469del     NC_000001.11:g.216097127del     1       216270468
.       AG      A       1       216097126       .       AG      A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.5018T>C           MANE    NM_206933.4:c.5018T>C
NG_009497.2:g.343602T>C                 NP_996816.3:p.(Leu1673Pro)
NC_000001.10:g.216258189A>G     NC_000001.11:g.216084847A>G     1       216258189
.       A       G       1       216084847       .       A       G       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.9433C>T           MANE    NM_206933.4:c.9433C>T
NG_009497.2:g.611315C>T                 NP_996816.3:p.(Leu3145Phe)
NC_000001.10:g.215990476G>A     NC_000001.11:g.215817134G>A     1       215990476
.       G       A       1       215817134       .       G       A       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.13355del          MANE    NM_206933.4:c.13355del
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NG_009497.2:g.753893del NP_996816.3:p.(Leu4452CysfsTer9)
NC_000001.10:g.215847899del NC_000001.11:g.215674557del 1 215847897
. CA C 1 215674555 . CA C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.2310_2311delinsC MANE NM_206933.4:c.2310_2311delinsC
NG_009497.2:g.181365_181366delinsC
NP_996816.3:p.(Lys770AsnfsTer18) NC_000001.10:g.216420425_216420426delinsG
NC_000001.11:g.216247083_216247084delinsG 1 216420425 . CT
G 1 216247083 . CT G USH2A HGNC:12601 Homo
sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.2431A>T MANE NM_206933.4:c.2431A>T
NG_009497.2:g.181486A>T NP_996816.3:p.(Lys811Ter)
NC_000001.10:g.216420305T>A NC_000001.11:g.216246963T>A 1 216420305
. T A 1 216246963 . T A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.5603T>G MANE NM_206933.4:c.5603T>G
NG_009497.2:g.355179T>G NP_996816.3:p.(Phe1868Cys)
NC_000001.10:g.216246612A>C NC_000001.11:g.216073270A>C 1 216246612
. A C 1 216073270 . A C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3532C>G MANE NM_206933.4:c.3532C>G
NG_009497.2:g.228543C>G NP_996816.3:p.(Pro1178Ala)
NC_000001.10:g.216373248G>C NC_000001.11:g.216199906G>C 1 216373248
. G C 1 216199906 . G C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.8431C>A MANE NM_206933.4:c.8431C>A
NG_009497.2:g.549558C>A NP_996816.3:p.(Pro2811Thr)
NC_000001.10:g.216052233G>T NC_000001.11:g.215878891G>T 1 216052233
. G T 1 215878891 . G T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.9815C>T MANE NM_206933.4:c.9815C>T
NG_009497.2:g.629399C>T NP_996816.3:p.(Pro3272Leu)
NC_000001.10:g.215972392G>A NC_000001.11:g.215799050G>A 1 215972392
. G A 1 215799050 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11411del MANE NM_206933.4:c.11411del
NG_009497.2:g.685135del NP_996816.3:p.(Pro3804LeufsTer13)
NC_000001.10:g.215916657del NC_000001.11:g.215743315del 1 215916655
. AG A 1 215743313 . AG A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.14272C>T MANE NM_206933.4:c.14272C>T
NG_009497.2:g.777786C>T NP_996816.3:p.(Pro4758Ser)
NC_000001.10:g.215824005G>A NC_000001.11:g.215650663G>A 1 215824005
. G A 1 215650663 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.1679del MANE NM_206933.4:c.1679del
NG_009497.2:g.136113del NP_996816.3:p.(Pro560LeufsTer31)
NC_000001.10:g.216465679del NC_000001.11:g.216292337del 1 216465677
. AG A 1 216292335 . AG A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA

NM_206933.4:c.4106C>T MANE NM_206933.4:c.4106C>T
NG_009497.2:g.231751C>T NP_996816.3:p.(Ser1369Leu)
NC_000001.10:g.216370040G>A NC_000001.11:g.216196698G>A 1 216370040
. G A 1 216196698 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4438_4439del MANE NM_206933.4:c.4438_4439del
NG_009497.2:g.253008_253009del
NP_996816.3:p.(Ser1480HisfsTer6) NC_000001.10:g.216348782_216348783del
NC_000001.11:g.216175440_216175441del 1 216348781 . GCT G
1 216175439 . GCT G USH2A HGNC:12601 Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.7244C>G MANE NM_206933.4:c.7244C>G
NG_009497.2:g.493777C>G NP_996816.3:p.(Ser2415Ter)
NC_000001.10:g.216108014G>C NC_000001.11:g.215934672G>C 1 216108014
. G C 1 215934672 . G C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.7475C>T MANE NM_206933.4:c.7475C>T
NG_009497.2:g.528255C>T NP_996816.3:p.(Ser2492Leu)
NC_000001.10:g.216073536G>A NC_000001.11:g.215900194G>A 1 216073536
. G A 1 215900194 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.775_776del MANE NM_206933.4:c.775_776del
NG_009497.2:g.63487_63488del
NP_996816.3:p.(Ser259PhefsTer63) NC_000001.10:g.216538305_216538306del
NC_000001.11:g.216364963_216364964del 1 216538302 . ACT A
1 216364960 . ACT A USH2A HGNC:12601 Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.7883dup MANE NM_206933.4:c.7883dup
NG_009497.2:g.539683dup NP_996816.3:p.(Ser2629LysfsTer7)
NC_000001.10:g.216062110dup NC_000001.11:g.215888768dup 1 216062107
. T TG 1 215888765 . T TG USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.920_921insGCCA MANE NM_206933.4:c.920_921insGCCA
NG_009497.2:g.102921_102922insGCCA
NP_996816.3:p.(Ser307ArgfsTer17) NC_000001.10:g.216498869_216498870insTGGC
NC_000001.11:g.216325527_216325528insTGGC 1 216498869 . G
GTGGC 1 216325527 . G GTGGC USH2A HGNC:12601 Homo
sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.917_918insGCTG MANE NM_206933.4:c.917_918insGCTG
NG_009497.2:g.102918_102919insGCTG
NP_996816.3:p.(Ser307LeufsTer17) NC_000001.10:g.216498873_216498874insAGCC
NC_000001.11:g.216325531_216325532insAGCC 1 216498872 . G
GCAGC 1 216325530 . G GCAGC USH2A HGNC:12601 Homo
sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12752G>T MANE NM_206933.4:c.12752G>T
NG_009497.2:g.753290G>T NP_996816.3:p.(Ser4251Ile)
NC_000001.10:g.215848501C>A NC_000001.11:g.215675159C>A 1 215848501
. C A 1 215675159 . C A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3381del MANE NM_206933.4:c.3381del

NG_009497.2:g.228392del NP_996816.3:p.(Thr1128ProfsTer10)
NC_000001.10:g.216373400del NC_000001.11:g.216200058del 1 216373398
. TG T 1 216200056 . TG T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.1055C>T MANE NM_206933.4:c.1055C>T
NG_009497.2:g.103056C>T NP_996816.3:p.(Thr352Ile)
NC_000001.10:g.216498735G>A NC_000001.11:g.216325393G>A 1 216498735
. G A 1 216325393 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.10974_10975insTA MANE NM_206933.4:c.10974_10975insTA
NG_009497.2:g.661695_661696insTA
NP_996816.3:p.(Thr3659Ter) NC_000001.10:g.215940095_215940096insTA
NC_000001.11:g.215766753_215766754insTA 1 215940095 . T TTA
1 215766753 . T TTA USH2A HGNC:12601 Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11299A>T MANE NM_206933.4:c.11299A>T
NG_009497.2:g.669764A>T NP_996816.3:p.(Thr3767Ser)
NC_000001.10:g.215932027T>A NC_000001.11:g.215758685T>A 1 215932027
. T A 1 215758685 . T A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.13010C>T MANE NM_206933.4:c.13010C>T
NG_009497.2:g.753548C>T NP_996816.3:p.(Thr4337Met)
NC_000001.10:g.215848243G>A NC_000001.11:g.215674901G>A 1 215848243
. G A 1 215674901 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.13316C>T MANE NM_206933.4:c.13316C>T
NG_009497.2:g.753854C>T NP_996816.3:p.(Thr4439Ile)
NC_000001.10:g.215847937G>A NC_000001.11:g.215674595G>A 1 215847937
. G A 1 215674595 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.15017C>T MANE NM_206933.4:c.15017C>T
NG_009497.2:g.789259C>T NP_996816.3:p.(Thr5006Met)
NC_000001.10:g.215812532G>A NC_000001.11:g.215639190G>A 1 215812532
. G A 1 215639190 . G A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.15063_15081delinsGC MANE
NM_206933.4:c.15063_15081delinsGC
NG_009497.2:g.793756_793774delinsGC
NP_996816.3:p.(Thr5022GlnfsTer150) NC_000001.10:g.215808017_215808035delinsGC
NC_000001.11:g.215634675_215634693delinsGC 1 215808017 .
CTTTTTCCCAGGAGTTGTT GC 1 215634675 . CTTTTTCCCAGGAGTTGTT
GC USH2A HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2,
mRNA
NM_206933.4:c.5118G>A MANE NM_206933.4:c.5118G>A
NG_009497.2:g.343702G>A NP_996816.3:p.(Trp1706Ter)
NC_000001.10:g.216258089C>T NC_000001.11:g.216084747C>T 1 216258089
. C T 1 216084747 . C T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.7931G>A MANE NM_206933.4:c.7931G>A
NG_009497.2:g.539731G>A NP_996816.3:p.(Trp2644Ter)

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NC_000001.10:g.216062060C>T     NC_000001.11:g.215888718C>T     1       216062060
.       C       T       1       215888718       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.8522G>A           MANE    NM_206933.4:c.8522G>A
NG_009497.2:g.549649G>A                 NP_996816.3:p.(Trp2841Ter)
NC_000001.10:g.216052142C>T     NC_000001.11:g.215878800C>T     1       216052142
.       C       T       1       215878800       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.8981G>A           MANE    NM_206933.4:c.8981G>A
NG_009497.2:g.582551G>A                 NP_996816.3:p.(Trp2994Ter)
NC_000001.10:g.216019240C>T     NC_000001.11:g.215845898C>T     1       216019240
.       C       T       1       215845898       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.10561T>C          MANE    NM_206933.4:c.10561T>C
NG_009497.2:g.645687T>C                 NP_996816.3:p.(Trp3521Arg)
NC_000001.10:g.215956104A>G     NC_000001.11:g.215782762A>G     1       215956104
.       A       G       1       215782762       .       A       G       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11105G>A          MANE    NM_206933.4:c.11105G>A
NG_009497.2:g.668663G>A                 NP_996816.3:p.(Trp3702Ter)
NC_000001.10:g.215933128C>T     NC_000001.11:g.215759786C>T     1       215933128
.       C       T       1       215759786       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11864G>A          MANE    NM_206933.4:c.11864G>A
NG_009497.2:g.700217G>A                 NP_996816.3:p.(Trp3955Ter)
NC_000001.10:g.215901574C>T     NC_000001.11:g.215728232C>T     1       215901574
.       C       T       1       215728232       .       C       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3309C>A           MANE    NM_206933.4:c.3309C>A
NG_009497.2:g.221169C>A                 NP_996816.3:p.(Tyr1103Ter)
NC_000001.10:g.216380622G>T     NC_000001.11:g.216207280G>T     1       216380622
.       G       T       1       216207280       .       G       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.3368A>G           MANE    NM_206933.4:c.3368A>G
NG_009497.2:g.228379A>G                 NP_996816.3:p.(Tyr1123Cys)
NC_000001.10:g.216373412T>C     NC_000001.11:g.216200070T>C     1       216373412
.       T       C       1       216200070       .       T       C       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.5385T>A           MANE    NM_206933.4:c.5385T>A
NG_009497.2:g.350173T>A                 NP_996816.3:p.(Tyr1795Ter)
NC_000001.10:g.216251618A>T     NC_000001.11:g.216078276A>T     1       216251618
.       A       T       1       216078276       .       A       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.6084T>A           MANE    NM_206933.4:c.6084T>A
NG_009497.2:g.379836T>A                 NP_996816.3:p.(Tyr2028Ter)
NC_000001.10:g.216221955A>T     NC_000001.11:g.216048613A>T     1       216221955
.       A       T       1       216048613       .       A       T       USH2A
HGNC:12601      Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.7132_7133del              MANE    NM_206933.4:c.7132_7133del
        NG_009497.2:g.493665_493666del
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NP_996816.3:p.(Tyr2378HisfsTer39) NC_000001.10:g.216108125_216108126del
NC_000001.11:g.215934783_215934784del 1 216108124 . GTA G
1 215934782 . GTA G USH2A HGNC:12601 Homo sapiens
usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.10407C>A MANE NM_206933.4:c.10407C>A
NG_009497.2:g.645533C>A NP_996816.3:p.(Tyr3469Ter)
NC_000001.10:g.215956258G>T NC_000001.11:g.215782916G>T 1 215956258
. G T 1 215782916 . G T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.1139A>G MANE NM_206933.4:c.1139A>G
NG_009497.2:g.103140A>G NP_996816.3:p.(Tyr380Cys)
NC_000001.10:g.216498651T>C NC_000001.11:g.216325309T>C 1 216498651
. T C 1 216325309 . T C USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.11819A>C MANE NM_206933.4:c.11819A>C
NG_009497.2:g.700172A>C NP_996816.3:p.(Tyr3940Ser)
NC_000001.10:g.215901619T>G NC_000001.11:g.215728277T>G 1 215901619
. T G 1 215728277 . T G USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.4108G>C MANE NM_206933.4:c.4108G>C
NG_009497.2:g.231753G>C NP_996816.3:p.(Val1370Leu)
NC_000001.10:g.216370038C>G NC_000001.11:g.216196696C>G 1 216370038
. C G 1 216196696 . C G USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.653T>A MANE NM_206933.4:c.653T>A
NG_009497.2:g.63365T>A NP_996816.3:p.(Val218Glu)
NC_000001.10:g.216538426A>T NC_000001.11:g.216365084A>T 1 216538426
. A T 1 216365084 . A T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.8143del MANE NM_206933.4:c.8143del
NG_009497.2:g.539943del NP_996816.3:p.(Val2715Ter)
NC_000001.10:g.216061850del NC_000001.11:g.215888508del 1 216061847
. AC A 1 215888505 . AC A USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.12569T>C MANE NM_206933.4:c.12569T>C
NG_009497.2:g.753107T>C NP_996816.3:p.(Val4190Ala)
NC_000001.10:g.215848684A>G NC_000001.11:g.215675342A>G 1 215848684
. A G 1 215675342 . A G USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA
NM_206933.4:c.15433G>A MANE NM_206933.4:c.15433G>A
NG_009497.2:g.799549G>A NP_996816.3:p.(Val5145Ile)
NC_000001.10:g.215802242C>T NC_000001.11:g.215628900C>T 1 215802242
. C T 1 215628900 . C T USH2A
HGNC:12601 Homo sapiens usherin (USH2A), transcript variant 2, mRNA