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# Comprehensive sequence analysis of nine Usher syndrome genes in the UK National Collaborative Usher Study

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## ABSTRACT

**Background** Usher syndrome (USH) is an autosomal recessive disorder comprising retinitis pigmentosa, hearing loss and, in some cases, vestibular dysfunction. It is clinically and genetically heterogeneous with three distinctive clinical types (I–III) and nine Usher genes identified. This study is a comprehensive clinical and genetic analysis of 172 Usher patients and evaluates the contribution of digenic inheritance.

**Methods** The genes *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, *USH2A*, *GPR98*, *WHRN*, *CLRN1* and the candidate gene *SLC4A7* were sequenced in 172 UK Usher patients, regardless of clinical type.

**Results** No subject had definite mutations (nonsense, frameshift or consensus splice site mutations) in two different USH genes. Novel missense variants were classified UV1–4 (unclassified variant): UV4 is ‘probably pathogenic’, based on control frequency <0.23%, identification *in trans* to a pathogenic/probably pathogenic mutation and segregation with USH in only one family; and UV3 (‘likely pathogenic’) as above, but no information on phase. Overall 79% of identified pathogenic/UV4/UV3 variants were truncating and 21% were missense changes. *MYO7A* accounted for 53.2%, and *USH1C* for 14.9% of USH1 families (USH1C: c.496+1G>A being the most common USH1 mutation in the cohort). *USH2A* was responsible for 79.3% of USH2 families and *GPR98* for only 6.6%. No mutations were found in *USH1G*, *WHRN* or *SLC4A7*.

**Conclusions** One or two pathogenic/likely pathogenic variants were identified in 86% of cases. No convincing cases of digenic inheritance were found. It is concluded that digenic inheritance does not make a significant contribution to Usher syndrome; the observation of multiple variants in different genes is likely to reflect polymorphic variation, rather than digenic effects.

## INTRODUCTION

Usher syndrome (USH) is an autosomal recessive disease characterised by the association of sensorineural hearing loss, retinitis pigmentosa (RP) and in some cases by vestibular dysfunction. The disorder is divided into three clinical types: type I (USH1) characterised by profound congenital hearing loss, absent vestibular function and onset of RP usually within the first decade of life; type II (USH2),

characterised by congenital, moderate to severe hearing loss, with normal vestibular function and onset of RP around or after puberty; and type III (USH3), defined by postlingual progressive hearing loss and variable vestibular response together with RP.<sup>1–2</sup> In addition there remain patients whose disease does not fit into any of these three subtypes, because of atypical audiovestibular or retinal findings, who are said to have ‘atypical Usher syndrome’.

Eleven loci and nine genes are associated with USH and cases of digenic inheritance have been described.<sup>3–16</sup> For USH1, five associated genes have been cloned—*MYO7A* (USH1B), *USH1C*, *CDH23* (USH1D), *PCDH15* (USH1F), and *USH1G* (USH1G). Three associated genes—*USH2A* (USH2A), *GPR98* (USH2C), and *WHRN* (USH2D)—have been found to be responsible for USH2 and mutations in the *CLARIN/CLRN1* gene were found in cases of USH3. Proteins encoded by Usher genes belong to different classes and are organised in the common synaptic and periciliary areas of the photoreceptors, and in the stereocilia or hair bundle of the inner ear hair cells.<sup>1–17</sup> Digenic cases of Usher have been reported with description of possibly pathogenic variants in two different USH genes, but the pathogenicity of these variants is often questionable, with at least one of the genes often containing a missense variant.<sup>18–20</sup> Only one study describes an Usher type II case, with one truncating mutation in *GPR98* and one truncating mutation in a novel USH modifier gene, *PDZD7*.<sup>21</sup>

The National Collaborative Usher Study (NCUS) was initiated in order to examine the molecular epidemiology of USH in the UK in anticipation of treatments for the condition, and to look specifically for the contribution of digenic inheritance in Usher syndrome. This report describes sequence analysis of nine USH genes *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, *USH2A*, *GPR98*, *WHRN*, *CLRN1* and a candidate gene *SLC4A7* in the ethnically heterogeneous UK population. The *SLC4A7* gene encodes the stilbene-insensitive electroneutral sodium bicarbonate co-transporter (NBC3) and loss of NBC3 in mice causes degeneration of sensory cells in the inner ear and eye.<sup>22</sup> At the beginning of this study *SLC4A7* was considered as a potential positional candidate gene for type 2

Usher syndrome in humans. All 384 exons of these 10 genes were sequenced by bidirectional capillary sequencing in one affected individual from each family, regardless of their clinical type, as well as in 48 CEPH (Centre d'Etude du Polymorphisme Humain) controls. The study contributes 774 sequence variants to the locus specific database (LSDB) for USH genes.<sup>23</sup> Molecular diagnosis with one or two pathogenic or likely pathogenic variants was established in 86% of USH families; 295 variants residing in the exons or exon/intron boundaries were novel. We found no convincing cases of digenic inheritance, although the polymorphic nature of some of the genes means that many individuals had variants in more than one gene.

## PATIENTS AND METHODS

### Patient and control DNAs

A total of 188 probands and 456 family members (parents and sibs) were collected and studied as part of the UK NCUS. The protocol of the study adhered to the provisions of the Declaration of Helsinki and had multicentre research ethics approval granted for recruitment through Moorfields Eye Hospital, Great Ormond Street Hospital (who both also approved the study), the support organisation Sense, or as self-referrals. Informed consent to the study was obtained from all participants.

Patients were classified as Usher type I (USH1), II (USH2), III (USH3) or atypical based on ophthalmologic, audiometric and vestibular tests. Control DNA cohorts consisted of 381 unrelated UK blood donors (European Collection of Cell Cultures, ECCAC), 48 CEPH control DNAs (Caucasian, Utah, USA), and 57 individuals of Pakistani origin (courtesy of Professor Eamonn R Maher, Birmingham, UK).

Ophthalmic examination was performed in all affected individuals to confirm the presence of RP and included best corrected visual acuities, slit lamp biomicroscopy, colour vision testing with Hardy-Rand-Rittler colour plates, and Goldmann perimetry using the V4e, I4e and I4e targets. Retinal imaging with digital colour fundus photography, optical coherence tomography (6mm scans centred on the fovea; Stratus OCT3; Carl Zeiss Meditec, Dublin, California, USA) and fundus autofluorescence (FAF) imaging (HRA, Heidelberg, Germany) was also performed. Pattern and full field electroretinograms (ERGs) were performed in some cases using international standards.<sup>24 25</sup>

Audiologic evaluation included pure tone audiometry, tympanometry, stapedia reflex measurement, transient evoked otoacoustic emission recordings, and auditory brain stem evoked response recording using standard protocol.<sup>26–31</sup> Subjective pure tone air and bone conduction thresholds were determined at 0.25, 0.5, 1, 2, 4, and 8 kHz using a GSI 61 audiometer (Guymark, Cradley Heath, UK), TDH39 supra aural earphones (Sennheiser UK, Ltd, High Wycombe, UK), and the British Society of Audiology recommended procedure. Audiometric descriptors of mild, moderate, severe, and profound hearing loss were calculated according to the British Society of Audiology descriptors. Vestibular function was evaluated with infrared video nystagmography, a rotary chair system (Neurokinetics, Pittsburgh, Pennsylvania, USA), and vestibulo-ocular reflex responses.<sup>30</sup> Binaural bithermal caloric testing with water was undertaken using the British Society of Audiology recommended protocol (<http://www.thebsa.org.uk/docs/RecPro/CTP.pdf>),<sup>29</sup> and the departmental normative data for peak slow component velocity were used to determine normality. Canal paresis (>17%) and directional preponderance (>16%) were calculated according to Jongkees formulae,<sup>32</sup> and vestibular hypofunction was defined by total eye velocity <78°/s. All parameters were defined by departmental normative data. Bilateral horizontal

semicircular canal function was assessed using sinusoidal (60° peak velocity and 0.05 Hz) and step rotation testing (acceleration, 0°–60°/s constant velocity in <1 s). A gain of either <0.23 in test or time constant of <8 s on impulsive rotation was considered vestibular hypofunction.

### DNA sequencing

Genomic DNA of patients and family members was extracted from peripheral blood by standard methods. The 188 NCUS probands and 48 CEPH controls underwent bidirectional DNA sequencing of nine Usher genes (*MYO7A*, *CDH23*, *PCDH15*, *USH1C*, *USH1G*, *USH2A*, *GPR98*, *WHRN*, *CLRN1*) and the candidate gene *SLC4A7*. Exons and flanking sequences were amplified by PCR using 578 primer sets and sequenced at the Wellcome Trust Sanger Institute as part of the ExoSeq project. Primers were designed for all the exons of the transcripts whose NM numbers are given below under Accession Numbers. Primers covered the entire region of each of these transcripts and primer sequences are available on request.

Direct sequencing was performed using the BigDye Terminator Cycle Sequencing on an ABI 3100 (Applied Biosystems, Foster City, California, USA). Sequences were analysed using GAP4,<sup>33</sup> and SeqMan softwares (DNASTAR Inc, Madison, Wisconsin, USA). Further details of the ExoSeq protocols and instructions on data access are available from: <http://www.sanger.ac.uk/resources/downloads/human/exoseq.html>

All sequence variants except *SLC4A7* will be submitted to the LSDB for Usher Syndrome (USHbases).<sup>23</sup>

### Analysis of USH variants in parents, sibs and control DNAs

A total of 365 variants were genotyped by Sequenom using Sequenom iPLEX Gold assay on the MassARRAY Platform (Sequenom, San Diego, California, USA) in probands and available family members to ascertain parental origin, and in controls to assess minimum allele frequency (MAF); 320 pathogenic and putatively pathogenic variants were identified in probands during Sanger sequencing and 45 were highly polymorphic single nucleotide polymorphisms (SNPs) selected from the SNP database (supplementary table 1).

Genotyped variants and family pedigrees were imported into Progeny Lab software (Progeny, LLC) where haplotypes were constructed from a combination of intragenic variants and polymorphic SNPs in individual families.

### Assessment of pathogenicity

To facilitate clear description of variants with respect to their pathogenicity, we have used a grading system along the guidelines provided by the Clinical and Molecular Genetics Society, a constituent member of the British Society of Human Genetics.

Variants were graded using two definite pathogenicity grades, that is, pathogenic and neutral. Variants which could not be confidently classified as either pathogenic or neutral were called 'unclassified variants' (UV1–UV4), with UV4 being probably pathogenic and UV1 being probably neutral.

All frame shift mutations, nonsense mutations, and mutations of the first two nucleotides of canonical intron splice acceptor or donor sites have been classified as pathogenic. A missense or intronic change was described as pathogenic if it fulfilled *all* of the following criteria: it occurred in controls with a frequency <0.236%, was identified *in trans* to a pathogenic/probably pathogenic mutation and it was either novel and segregated with USH in more than two families, or was previously published as pathogenic/likely pathogenic. The benchmark frequency of 0.236% was determined based on the MAF of the

most common USH mutation USH2A:p.Glu767SerfsX21 in 846 control chromosomes assayed in this study.

If a novel variant fulfilled the above criteria, but segregated with USH in only one family, it was deemed to be probably pathogenic and was classified as 'UV4'. Missense variants were classed as 'UV3' (likely pathogenic) if the frequency in control chromosomes was <0.236%, but phase of the variant could not be ascertained due to missing family data. Missense and silent changes of the last nucleotide of the exon that are likely to affect splicing were also described as 'UV3' if they were found in the same gene as another 'pathogenic' or 'UV4' variant. Our determination of a variant as pathogenic, is therefore stringent.

Variants with uncertain pathogenicity were described as 'UV2'. UV2 variants fulfilled the criteria described for UV3, but were only genotyped in 96 CEPH control chromosomes. A missense variant was also classified as UV2 if it was the only possibly pathogenic variant in the gene. Furthermore, novel intronic variants residing three nucleotides from the start/end of the exon and not found in 846 control chromosomes were also classified as UV2.

'UV1' (probably neutral variants) variants were found in patients who already had two other pathogenic/probably pathogenic mutations or did not segregate with disease. The MAF of UV1 in control chromosomes was either <0.236% or was not assessed. We cannot exclude the possibility that such variants may modify disease phenotype.

'Neutral' variants did not segregate with disease, were either previously published as neutral, or were found in controls with a frequency >0.236%. A box diagram detailing the grading system is in supplemental data 1.

Novel missense variants, classified as UV2–UV4 and pathogenic, were also assessed using Usher Syndrome Missense Analysis. Usher Syndrome Missense Analysis is a web-based tool dedicated to analysis of missense variants in Usher genes available through USHbases. (<https://194.167.35.160/cgi-bin/USMA/USMA.fcgi>). The software compiles orthologue analysis, alignment of protein domains, secondary structure and 3D predictions.<sup>23 34</sup> Output is given by number and percentage of orthologues conserved to those surveyed.

### Accession numbers

Sequence variants were described following Human Genome Variation Society's recommendations according to the following accession numbers: NM\_000260.3 (*MYO7A*), NM\_153676.2 (*USH1C*), NM\_022124.5 (*CDH23*), NM\_033056.3 (*PCDH15*), NM\_173477.2 (*USH1G*), NM\_206933.2 (*USH2A*), NM\_032119.3 (*GPR98*), NM\_015404.3 (*WHRN*), NM\_174878.2 (*CLRN1*), and NM\_003615.3 (*SLC4A7*).

### USH1C:c.496+1G>A assay

PCR primers were designed to target the splice mutation, USH1C:c.496+1G>A, using the amplification created restriction site method (see supplemental data 2). These primers were designed to avoid the VNTR in intron 5.<sup>5</sup>

### Multiplex ligation dependent probe amplification

Multiplex ligation dependent probe amplification (MLPA) designed by MRC Holland was used to confirm suspected large genomic deletion in *PCDH15*. The SALSA MLPA 292-A1 kit was used according to manufacturer's instructions. MLPA is a multiplex PCR based method of DNA copy number quantification. (<http://www.mlpa.com/>). Two oligonucleotides complementary to a target sequence hybridise next to each other on the target, separated by a single base. When both hybridise

they may be ligated to each other to form a single complete probe. PCR primers complementary to each arm of the probe amplify the hybridised oligonucleotide target. Each probe is of a unique length and can be resolved by capillary sequencing and quantified relative to standards of known copy number by ratio.

### In silico splice site prediction

Novel synonymous changes in direct proximity to splice sites and novel synonymous changes which were absent in controls were evaluated *in silico* (Human Splicing Finder, <http://www.umd.be/HSF/> and Splice Site Prediction by Neural Network, [http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)), supplementary tables 2 and 3).

## RESULTS

### Clinical evaluation

A total of 188 families were recruited for the NCUS study: 47 were diagnosed with USH1 on the basis of having profound congenital hearing loss on audiometry, absent vestibular function on formal testing and typical RP; 121 were diagnosed with USH2 on the basis of sloping moderate to severe congenital sensorineural hearing loss on audiometry and normal vestibular function on formal testing with typical RP; four were diagnosed with USH3 on the basis of progressive sensorineural hearing loss and typical RP. Four NCUS probands had typical RP, but their hearing loss was not typical for any type of Usher syndrome. A further seven had both RP and hearing loss which were considered atypical for Usher syndrome. Another five NCUS probands were thought not have Usher syndrome, but were diagnosed with autosomal-recessive RP (one proband), Alström syndrome (one proband), sector RP and hearing loss (one proband<sup>35</sup>), and an unknown syndromic disorder (two probands). All underwent sequencing.

### Analysis of variants

We recorded a total of 774 sequence variants in exons and exon/intron boundaries of the 188 NCUS probands; 319 intronic variants resided more than 20 nucleotides from a splice donor or acceptor site and were not analysed in detail. The remaining 455 variants (295 novel and 160 previously published) were classified as 'pathogenic' (115 variants), 'UV4' (15 variants), 'UV3' (11 variants), 'UV2' (18 variants), 'UV1' (201 variants) and 'neutral' (95 variants). Novel variants were classified as UV4, based on a frequency <0.236% in controls, identification *in trans* to a pathogenic/probably pathogenic mutation and segregation with USH in only one family (ie, probably pathogenic), and UV3 (likely pathogenic) as above but phase of the variant could not be ascertained due to missing family data.

Pathogenic and UV2–UV4 variants per patient are described in tables 1–3. Details of 295 novel variants are described in supplementary table 2 (UV2–UV4, pathogenic) and supplementary table 3 (UV1, neutral).

### Molecular diagnosis in Usher type 1 families (N=47)

Diagnosis with two pathogenic/UV4/UV3 variants was established in 37/47 (78.7%) USH1 families, and a single pathogenic/UV4 variant was found in four families (8.5%). In another three families (6.4%) we suspect involvement of USH1 genes based on haplotype analysis (family 168 shown in supplemental figure 1, and families 104, 206). Molecular diagnosis was unclear in a further three families (6.4%) with either UV2 (uncertain pathogenicity) variants (family 705) or no mutations identified (families 129, 340). Genotypes are detailed in table 1.



**Table 1** Genotypes of Usher syndrome type 1 probands (novel variants are in bold)

| Family     | Gene           | Allele 1*                 | Allele 2*                       | Allele 1 score | Allele 2 score | Ethnicity†            |
|------------|----------------|---------------------------|---------------------------------|----------------|----------------|-----------------------|
| 107        | <i>CDH23</i>   | <b>c.6712+1G&gt;A</b>     | Unknown                         | Pathogenic     |                | Caucasian             |
| 140‡       | <i>CDH23</i>   | p.Arg2107X                | p.Arg2107X                      | Pathogenic     | Pathogenic     | Pakistani (Sindi)     |
| 163        | <i>CDH23</i>   | p.Arg2107X                | <b>p.Leu2436ThrfsX3</b>         | Pathogenic     | Pathogenic     | Caucasian             |
| 407        | <i>CDH23</i>   | <b>c.2177-2A&gt;G</b>     | <b>P.Leu3041Pro</b>             | Pathogenic     | UV4            | Caucasian             |
| 555        | <i>CDH23</i>   | <b>c.7362G&gt;A§</b>      | <b>c.6254_6254-3delCAGGinsT</b> | UV3            | Pathogenic     | Caucasian             |
| 168‡       | <i>CDH23¶</i>  | Unknown¶                  | Unknown¶                        |                |                | Turkish Cypriot       |
| 30         | <i>MYO7A</i>   | p.Lys1255ArgfsX8          | p.Ala26Glu**                    | Pathogenic     | Pathogenic     | Caucasian             |
| 146        | <i>MYO7A</i>   | p.Lys1255ArgfsX8          | p.Asp521GlnfsX8                 | Pathogenic     | Pathogenic     | Caucasian             |
| 444        | <i>MYO7A</i>   | p.Lys1255ArgfsX8          | p.Lys542GlnfsX5                 | Pathogenic     | Pathogenic     | Caucasian             |
| 68         | <i>MYO7A</i>   | p.Gly214Arg               | p.Arg212His                     | Pathogenic     | Pathogenic     | Caucasian             |
| 100        | <i>MYO7A</i>   | <b>c.3108+1G&gt;A</b>     | <b>c.3108+1G&gt;A</b>           | Pathogenic     | Pathogenic     | Indian                |
| 111        | <i>MYO7A</i>   | p.Arg669X                 | c.5944G>A††                     | Pathogenic     | Pathogenic     | Caucasian             |
| 132        | <i>MYO7A</i>   | p.Arg972X                 | p.Arg972X                       | Pathogenic     | Pathogenic     | Iranian               |
| 93         | <i>MYO7A</i>   | c.3504-1G>C**             | p.Leu1858Pro                    | Pathogenic     | Pathogenic     | Caucasian             |
| 262        | <i>MYO7A</i>   | p.Asp1613ValfsX32         | <b>p.Lys420X</b>                | Pathogenic     | Pathogenic     | Caucasian             |
| 287        | <i>MYO7A</i>   | <b>p.Gly1942X</b>         | p.Gly25Arg                      | Pathogenic     | Pathogenic     | Caucasian             |
| 435        | <i>MYO7A</i>   | p.Arg1240Gln              | c.133-2A>G                      | Pathogenic     | Pathogenic     | Caucasian             |
| 578        | <i>MYO7A</i>   | p.Arg669X                 | p.Lys542GlnfsX5                 | Pathogenic     | Pathogenic     | Caucasian             |
| 731        | <i>MYO7A</i>   | p.Ala2009ProfsX32         | <b>c.-48A&gt;G‡‡</b>            | Pathogenic     | Pathogenic     | Caucasian             |
| 732        | <i>MYO7A</i>   | <b>p.Glu117SerfsX33</b>   | c.592+1G>T                      | Pathogenic     | Pathogenic     | Caucasian             |
| 516        | <i>MYO7A</i>   | <b>p.Gly1378TrpfsX6</b>   | <b>p.Glu968Asp</b>              | Pathogenic     | UV4            | Caucasian             |
| 69         | <i>MYO7A</i>   | <b>p.Arg241Pro</b>        | c.5944G>A††                     | UV4            | Pathogenic     | Caucasian             |
| 35         | <i>MYO7A</i>   | p.Arg2024X                | <b>p.Asp75His</b>               | Pathogenic     | UV4            | Caucasian             |
| 79         | <i>MYO7A</i>   | p.Arg1240Gln              | <b>p.Leu2193Phe</b>             | Pathogenic     | UV4            | Caucasian             |
| 257        | <i>MYO7A</i>   | p.Cys31X                  | p.Arg1883Gln                    | Pathogenic     | UV4            | Asian                 |
| 706‡       | <i>MYO7A</i>   | p.Phe1963del              | p.Phe1963del                    | UV4            | UV4            | Black African (Ghana) |
| 182        | <i>MYO7A</i>   | <b>p.Pro2126LeufsX5</b>   | p.Arg1240Trp                    | Pathogenic     | UV3            | Caucasian             |
| 500        | <i>MYO7A</i>   | <b>p.Trp1431X</b>         | p.Ala826Thr§§                   | Pathogenic     | UV4            | Caucasian             |
| 692        | <i>MYO7A</i>   | p.Arg212His               | <b>c.1798-3C&gt;G</b>           | Pathogenic     | UV2            | Caucasian             |
| 676        | <i>MYO7A</i>   | <b>p.Arg241Pro</b>        | <b>p.Glu380Lys</b>              | UV4            | UV2            | Caucasian             |
| 42         | <i>MYO7A</i>   | p.Arg1701X                | Unknown                         | Pathogenic     |                | Turkish Cypriot       |
| 104‡       | <i>MYO7A¶¶</i> | Unknown¶¶                 | Unknown¶¶                       |                |                | Caucasian             |
| 206‡       | <i>MYO7A¶¶</i> | p.Tyr2015His              | p.Tyr2015His                    | UV2            | UV2            | Caucasian             |
| 705        | <i>MYO7A</i>   | <b>c.1793-3C&gt;G</b>     | Unknown                         | UV2            |                | Greek Cypriot         |
| 291‡       | <i>PCDH15</i>  | <b>p.Gly942ValfsX22</b>   | <b>p.Gly942ValfsX22</b>         | Pathogenic     | Pathogenic     | South Pacific         |
| 313        | <i>PCDH15</i>  | c.3717+1G>A               | <b>Exon 9-18 deleted***</b>     | Pathogenic     | Pathogenic     | Caucasian             |
| 399‡       | <i>PCDH15</i>  | <b>c.3501+2T&gt;C</b>     | <b>c.3501+2T&gt;C</b>           | Pathogenic     | Pathogenic     | Caucasian             |
| 119        | <i>PCDH15</i>  | <b>Exon 10 deleted†††</b> | <b>Exon 10 deleted†††</b>       | Pathogenic     | Pathogenic     | Caucasian             |
| 87         | <i>USH1C</i>   | p.Arg80ProfsX69           | c.496+1G>A                      | Pathogenic     | Pathogenic     | Caucasian             |
| 461        | <i>USH1C</i>   | p.Arg80ProfsX69           | p.Arg80ProfsX69                 | Pathogenic     | Pathogenic     | Jewish                |
| 4 families | <i>USH1C</i>   | c.496+1G>A                | c.496+1G>A                      | Pathogenic     | Pathogenic     | Caucasian             |
| 530‡       | <i>USH1C</i>   | <b>p.Glu149del</b>        | <b>p.Glu149del</b>              | Pathogenic     | Pathogenic     | Indian                |
| 129, 340   | Unknown        |                           |                                 |                |                | Caucasian             |

\*Unless stated otherwise, the alleles were not observed in control chromosomes.

†Caucasian: UK and European.

‡Consanguineous family.

§Last nucleotide of exon (possibly affects splicing).

¶See supplemental figure 1.

\*\*Found in 1/872 (0.11%) control chromosomes.

††Last nucleotide of exon; causes MYO7A:p.Val1953GlnfsX12.<sup>23</sup>

‡‡IVS1-2A>G.

§§Found in 2/826 (0.23%) control chromosomes (both heterozygotes are Pakistani controls).

¶¶Consanguineous family demonstrating linkage to Usher type1 genes. The causative mutations were either not found or were of uncertain pathogenicity (UV2).

\*\*\*See supplemental figure 2. Deletion of *PCDH15* exons 9–18 was confirmed by MLPA.

†††A homozygous deletion of *PCDH15* exon 10 was confirmed by MLPA. The family is not knowingly consanguineous.

MLPA, multiplex ligation dependent probe amplification; UV unclassified variant.

Mutations in *MYO7A* were the most frequent cause of USH1 in our cohort, representing the molecular cause of USH in 25 (53.2%) out of 47 USH1 families (supplemental data 3). In 22 out of 47 families (46.8%) we identified two *MYO7A* pathogenic/UV4/UV3 variants and in three families (6.4%) only one such variant. Twenty-eight out of 47 (59.6%) mutated alleles were predicted to code for prematurely truncated proteins, 17 (36.2%) were missense mutation, and two were in-frame deletions.

Overall, two pathogenic variants in *USH1C* were identified in seven out of 47 (14.9%) USH1 families, making this the second most common USH1 gene. Remarkably, *USH1C*:c.496+1G>A<sup>36</sup> was the most frequent USH1 mutation in our cohort, accounting for 9.6% (9/94) of all expected USH1 mutations. Analysis of three polymorphic *USH1C* SNPs showed that the c.496+1G>A is associated with a common haplotype—that is, ‘A-C-G’ (rs2072227-rs2240488-rs2883581). It was found in

**Table 2** Genotypes of Usher type 2 and of Usher type 3 probands (novel variants in bold)

| Family   | USH type | Gene  | Allele 1*                | Allele 2*                   | Allele 1 score | Allele 2 score | Ethnicity†        |
|----------|----------|-------|--------------------------|-----------------------------|----------------|----------------|-------------------|
| 3        | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Arg4971X</b>           | P              | P              | Caucasian         |
| 21       | 2        | USH2A | p.Glu767SerfsX21         | p.Arg626X                   | P              | P              | Caucasian         |
| 26, 46   | 2        | USH2A | p.Glu2288X               | Unknown                     | P              |                | Caucasian         |
| 29       | 2        | USH2A | p.Arg4192His‡            | p.Arg4192His‡               | UV2            | UV2            | Caucasian (Italy) |
| 32       | 2        | USH2A | p.Glu767SerfsX21         | p.His308SerfsX16            | P              | P              | Caucasian         |
| 38       | 2        | USH2A | p.Glu767SerfsX21         | p.Arg34X                    | P              | P              | Caucasian         |
| 45       | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Ser1173X</b>           | P              | P              | Caucasian         |
| 53§      | 2        | USH2A | p.Cys1452LeufsX25        | p.Cys1452LeufsX25           | P              | P              | Indian            |
| 57       | 2        | USH2A | p.Glu767SerfsX21         | p.Cys536Arg                 | P              | P              | Caucasian         |
| 61       | 2        | USH2A | p.Pro560LeufsX31         | p.Glu2265_Tyr2266del insAsp | P              | P              | Caucasian         |
| 64       | 2        | USH2A | <b>p.Trp1607X¶</b>       | <b>p.Cys620Phe</b>          | P              | P              | Caucasian         |
| 82       | 2        | USH2A | <b>c.11047 + 1G&gt;A</b> | <b>p.Cys3575Tyr</b>         | P              | UV4            | Caucasian         |
| 113      | 2        | USH2A | <b>p.Cys982LeufsX2</b>   | p.Trp3955X**                | P              | P              | Arab              |
| 147      | 2        | USH2A | p.Gln3959AsnfsX53        | <b>p.Asn4762Ser</b>         | P              | UV3            | Caucasian         |
| 151      | 2        | USH2A | p.Gln1063SerfsX15        | Unknown                     | P              |                | Caucasian         |
| 155      | 2        | USH2A | p.Glu767SerfsX21         | p.Arg1946X                  | P              | P              | Caucasian         |
| 165      | 2        | USH2A | p.Glu767SerfsX21         | p.Trp2945X                  | P              | P              | Caucasian         |
| 171      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Lys4816X</b>           | P              | P              | Caucasian         |
| 179      | 2        | USH2A | p.Arg1504LysfsX26        | <b>p.Trp2744X</b>           | P              | P              | Caucasian         |
| 187      | 2        | USH2A | p.Glu767SerfsX21         | p.Gln1063SerfsX15           | P              | P              | Caucasian         |
| 192      | 2        | USH2A | <b>p.Pro746Ala</b>       | c.7595-3C>G††               | UV4            | P              | Caucasian         |
| 193      | 2        | USH2A | p.His308SerfsX16         | <b>c.9371 + 1G&gt;C¶</b>    | P              | P              | Caucasian         |
| 194      | 2        | USH2A | p.Thr4439Ile             | p.Cys3267Arg                | P              | UV4            | Caucasian         |
| 200      | 2        | USH2A | p.Glu767SerfsX21         | <b>c.11390-1G&gt;C</b>      | P              | P              | Caucasian         |
| 203§     | 2        | USH2A | <b>p.Cys870X</b>         | <b>p.Cys870X</b>            | P              | P              | Turkish Cypriot   |
| 205§     | 2        | USH2A | Unknown                  | Unknown                     |                |                | Indian            |
| 212, 702 | 2        | USH2A | p.Glu767SerfsX21         | p.Cys419Phe                 | P              | P              | Caucasian         |
| 215      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Ala4153Thr¶</b>        | P              | P              | Caucasian         |
| 219, 672 | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Ala1872LeufsX58</b>    | P              | P              | Caucasian         |
| 220      | 2        | USH2A | <b>p.Arg3689X</b>        | p.Trp3521Arg                | P              | P              | Caucasian         |
| 221      | 2        | USH2A | <b>Exon 47 deleted‡‡</b> | <b>Exon 47 deleted‡‡</b>    | P              | P              | Greek             |
| 225      | 2        | USH2A | p.Asn346His              | Unknown                     | P              |                | Caucasian         |
| 239      | 2        | USH2A | p.Thr4809Ile             | Unknown                     | P              |                | Caucasian         |
| 247      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Gln3959AsnfsX53</b>    | P              | P              | Caucasian         |
| 296      | 2        | USH2A | p.Glu767SerfsX21         | p.Gln675X                   | P              | P              | Caucasian         |
| 314§     | 2        | USH2A | c.1841-2A>G              | c.1841-2A>G                 | P              | P              | Caucasian         |
| 321      | 2        | USH2A | p.Thr4439Ile             | p.Asn346His                 | P              | P              | Caucasian         |
| 332      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Cys620Phe</b>          | P              | P              | Caucasian         |
| 334, 386 | 2        | USH2A | p.Glu4458AspfsX3         | c.7595-3C>G††               | P              | P              | Caucasian         |
| 345      | 2        | USH2A | p.Glu1492X               | <b>c.11047 + 1G&gt;A</b>    | P              | P              | Caucasian         |
| 347      | 2        | USH2A | p.Glu2288X               | <b>p.Gly268Arg</b>          | P              | UV3            | Unknown           |
| 355      | 2        | USH2A | p.Glu767SerfsX21         | p.Glu4458AspfsX3            | P              | P              | Caucasian         |
| 359      | 2        | USH2A | p.Cys5153X               | p.Trp3521Arg                | P              | P              | Caucasian         |
| 367, 17  | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Gln4541X</b>           | P              | P              | Caucasian         |
| 369      | 2        | USH2A | p.Arg1504LysfsX26        | p.Glu767SerfsX21            | P              | P              | Caucasian         |
| 374      | 2        | USH2A | p.Pro560LeufsX31         | <b>p.His340Leu</b>          | P              | UV3            | Caucasian         |
| 377      | 2        | USH2A | <b>p.Arg1777Trp</b>      | <b>p.Asn2285Ser</b>         | UV3            | UV2            | Indian            |
| 385      | 2        | USH2A | <b>p.Glu3305ArgfsX41</b> | p.Asn346His                 | P              | P              | Caucasian         |
| 387      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Ile2754AsnfsX15</b>    | P              | P              | Caucasian         |
| 389      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Tyr4801X</b>           | P              | P              | Caucasian         |
| 394      | 2        | USH2A | p.Glu767SerfsX21         | p.Glu284AspfsX38            | P              | P              | Caucasian         |
| 398      | 2        | USH2A | p.Gln675X                | <b>p.Gln4541X</b>           | P              | P              | Caucasian         |
| 401      | 2        | USH2A | p.Glu767SerfsX21         | p.Glu2288X                  | P              | P              | Caucasian         |
| 408§     | 2        | USH2A | p.Cys419Phe              | p.Cys419Phe                 | P              | P              | Caucasian         |
| 417      | 2        | USH2A | p.Glu767SerfsX21         | p.Thr4439Ile                | P              | P              | Caucasian         |
| 418      | 2        | USH2A | p.Arg63X                 | p.Arg1549X                  | P              | P              | Caucasian         |
| 427      | 2        | USH2A | p.Cys1452LeufsX25        | Unknown                     | P              |                | Afro-Caribbean    |
| 440      | 2        | USH2A | <b>p.Cys620Phe</b>       | Unknown                     | P              |                | Caucasian         |
| 455      | 2        | USH2A | <b>p.Ser4377X</b>        | p.Cys419Phe                 | P              | P              | Caucasian         |
| 490      | 2        | USH2A | p.Arg1281X               | p.Met1280Ile                | P              | UV4            | Caucasian         |
| 509      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Trp4713X</b>           | P              | P              | Caucasian         |
| 545      | 2        | USH2A | p.Glu767SerfsX21         | <b>p.Leu1378Pro</b>         | P              | UV4            | Caucasian         |

Continued

Table 2 Continued

| Family | USH type | Gene             | Allele 1*                    | Allele 2*                    | Allele 1 score | Allele 2 score | Ethnicity†           |
|--------|----------|------------------|------------------------------|------------------------------|----------------|----------------|----------------------|
| 546    | 2        | <i>USH2A</i>     | <b>p.Cys3281Phe</b>          | Unknown                      | UV2            |                | Caucasian            |
| 549    | 2        | <i>USH2A</i>     | p.Gly4403ProfsX15            | <b>p.Ser1588HisfsX5</b>      | P              | P              | Caucasian/Philippino |
| 558§   | 2        | <i>USH2A</i> ‡   | <b>p.Thr281Lys</b>           | <b>p.Thr281Lys</b>           | UV2            | UV2            | Turkish Cypriot      |
| 568    | 2        | <i>USH2A</i>     | p.Gly4403ProfsX15            | Unknown                      | P              |                | Caucasian            |
| 591    | 2        | <i>USH2A</i>     | p.Asn346His                  | p.Trp3521Arg                 | P              | P              | Caucasian            |
| 595    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | p.Trp3521Arg                 | P              | P              | Caucasian            |
| 601    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | <b>p.Ser1136Asn</b>          | P              | UV4            | Caucasian            |
| 611    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | <b>c.651 + 1G&gt;A</b>       | P              | P              | Caucasian            |
| 620    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | <b>p.Cys999LeufsX9</b>       | P              | P              | Caucasian            |
| 644    | 2        | <i>USH2A</i>     | <b>p.Asn1967TrpfsX5</b>      | <b>p.Arg1578Cys</b>          | P              | UV4            | Caucasian            |
| 648    | 2        | <i>USH2A</i>     | p.Arg1504LysfsX26            | p.Cys419Phe                  | P              | P              | Caucasian            |
| 651    | 2        | <i>USH2A</i>     | <b>p.Arg1946LeufsX22</b>     | Unknown                      | P              |                | Caucasian            |
| 657    | 2        | <i>USH2A</i>     | p.Arg63X                     | Unknown                      | P              |                | Caucasian            |
| 670    | 2        | <i>USH2A</i>     | <b>p.Gly1751Val</b>          | <b>p.Gly2017Cys§§</b>        | UV2            | UV2            | Indian               |
| 680    | 2        | <i>USH2A</i>     | p.Asn346His                  | p.Cys419Phe                  | P              | P              | Caucasian            |
| 683§   | 2        | <i>USH2A</i>     | <b>Exons 50–55 deleted‡‡</b> | <b>Exons 50–55 deleted‡‡</b> | P              | P              | Kashmiri             |
| 5 fams | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | p.Glu767SerfsX21             | P              | P              | Caucasian            |
| 465    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | c.10585G>A§§                 | P              | UV3            | Caucasian            |
| 432    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | <b>c.12295-3T&gt;A</b>       | P              | UV2            | Indian               |
| 531    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | <b>p.Gly257Arg</b>           | P              | UV2            | Unknown              |
| 669    | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | <b>p.Phe1868Cys</b>          | P              | UV2            | Unknown              |
| 9 fams | 2        | <i>USH2A</i>     | p.Glu767SerfsX21             | Unknown                      | P              |                | Caucasian            |
| 136§   | 2        | <i>GPR98</i>     | <b>Exon 83 deleted¶¶</b>     | <b>Exon 83 deleted¶¶</b>     | P              | P              | Arab Palestinian     |
| 170    | 2        | <i>GPR98</i>     | <b>p.Arg2286X</b>            | <b>p.Ser3339Asn</b>          | P              | UV3            | Caucasian            |
| 271    | 2        | <i>GPR98</i>     | <b>p.Arg4802X</b>            | <b>p.Ile3325Thr</b>          | P              | UV2            | Caucasian            |
| 275    | 2        | <i>GPR98</i>     | <b>p.Asp1375His</b>          | Unknown                      | UV2            |                | Caucasian            |
| 300§   | 2        | <i>GPR98</i>     | <b>p.Glu2103X</b>            | p.Gln2301X                   | P              | P              | Caucasian            |
| 357    | 2        | <i>GPR98</i>     | <b>p.Arg800X</b>             | <b>c.13433G&gt;T§§</b>       | P              | UV3            | Caucasian            |
| 481    | 2        | <i>GPR98</i>     | <b>p.Ser5048ArgfsX29</b>     | <b>p.Val2321AlafsX4</b>      | P              | P              | Caucasian            |
| 665    | 2        | <i>GPR98</i>     | <b>p.Ala3579ValfsX6</b>      | p.Val3363AspfsX11            | P              | P              | Caucasian            |
| 697    | 2        | <i>GPR98</i>     | <b>c.9623 + 1G&gt;A</b>      | Unknown                      | P              |                | Caucasian            |
| 222    | 2        | <i>GPR98</i> *** | Unknown                      | Unknown                      |                |                | Indian               |
| 110    | 2        | <i>MYO7A</i>     | p.Gly1942X                   | Unknown                      | P              |                | Caucasian            |
| 49     | 3        | <i>CLRN1</i>     | p.Ser50LeufsX12              | p.Ser50LeufsX12              | P              | P              | Caucasian            |
| 82     | 3        | <i>CLRN1</i>     | p.Asn48Lys                   | p.Asn48Lys                   | P              | P              | Ashkenazi Jewish     |

For family 29, *USH2A* haplotypes are not homozygous. It is possible they are p.Arg4192His hemizygous and have a deletion on the other allele.

\*Unless stated otherwise, the alleles were not observed in control chromosomes.

†Caucasian: UK and European.

‡Parental origin could not be determined. Patient and affected sib are homozygous for the mutation.

§Consanguineous family.

¶Found in 1/872 (0.11%) control chromosomes.

\*\*Found in 2/860 (0.23%) control chromosomes.

‡‡Splice mutation; causes *USH2A*:p.Pro2533Asnfs\*5.<sup>23</sup>

‡‡Large deletion speculated based on patient's homozygosity of *USH2A* haplotypes and failure to amplify exon.

§§Last nucleotide of the exon.

¶¶Deletion strongly suspected based on homozygosity for *GPR98* markers (*USH2A* excluded based on haplotype analysis), and apparent non-inheritance of *GPR98* SNPs in the family and PCR non-amplification of patient's as well as affected sib's DNAs (supplemental figure 3).

\*\*\*Not reported as a consanguineous family. Usher is compatible with mutation in *GPR98* (affected sibs are homozygous for a *GPR98* haplotype); *USH2A* is excluded by haplotype analysis.

P, Pathogenic; UV unclassified variant.

a homozygous state in four families and in a compound heterozygous state with p.Arg80ProfsX69 in one family. It did not occur in 234 ethnically matching control chromosomes.

Mutations in *CDH23* were determined as the cause of USH1 in five (10.6%) families. In family 407 we identified a likely pathogenic (UV4) novel missense variant *CDH23*:p.Leu3041Pro *in trans* with a pathogenic splice mutation *CDH23*:c.2177-2A>G together with a previously published pathogenic mutation, *MYO7A*:p.Arg302His.<sup>37 38</sup>

Mutations of *PCDH15* occurred in only four (8.5%) families; two pathogenic variants were detected in all four cases and involved deletions causing frame shifts and splice mutations, all predicted to result in a truncated protein. In two out of these four families (119 and 313) haplotype analysis indicated the presence of large genomic deletions which were confirmed by MLPA (table 1 and supplemental figure 2).

We did not find any pathogenic or potentially pathogenic variants in *USH1G* in the entire cohort.

### Molecular diagnosis in Usher type 2 families (N = 121)

Mutations in *USH2A* were the molecular cause of USH2 in 96 out of 121 (79.3%) of USH2 families; two pathogenic/UV4/UV3 variants were found in 73/121 (60.3%) of all USH2 families and only one pathogenic/UV4/UV3 variant was identified in 23/121 (19%) of USH2 families, in spite of full sequence analysis. In a further four families, mutations in *USH2A* were suspected as the cause of USH as we identified variants of uncertain pathogenicity (UV2) and one consanguineous family (family 205) was linked to *USH2A*, but we could not find any likely pathogenic variants (table 2).

The *USH2A*:p.Glu767SerfsX21 mutation was the most common mutation in the entire USH cohort, accounting for

**Table 3** Genotypes of atypical and non-Usher patients (novel variants are in bold)

| Family     | Diagnosis                  | Gene         | Allele 1*          | Allele 2*    | Pathogenicity Allele_1 | Pathogenicity Allele_2 | Ethnicity† |
|------------|----------------------------|--------------|--------------------|--------------|------------------------|------------------------|------------|
| 9 families | Atypical Usher             | Unknown      |                    |              |                        |                        |            |
| 520        | Atypical Usher             | <i>MYO7A</i> | p.Leu326Gln        | Unknown      | UV2                    |                        | Indian     |
| 633        | Atypical Usher             | <i>USH1C</i> | <b>p.Arg339Gln</b> | Unknown      | <b>UV2</b>             |                        | Caucasian  |
| 542        | ARRP                       | <i>USH2A</i> | p.Cys759Phe        | p.Cys3358Tyr | Pathogenic             | UV3                    | Caucasian  |
| 505‡       | Sector RP and hearing loss | <i>USH1C</i> | p.Arg103His        | c.2227-1G>T  | Pathogenic             | Pathogenic             | Caucasian  |
| 448        | Alström syndrome           | Unknown      |                    |              |                        |                        |            |
| 569, 502   | Unknown, not Usher         | Unknown      |                    |              |                        |                        |            |

\*Unless stated otherwise, the alleles were not observed in control chromosomes.

†Caucasian: UK and European.

‡Saihan *et al.*<sup>35</sup>

ARRP, autosomal recessive retinitis pigmentosa; RP, retinitis pigmentosa; UV, unclassified variant.

31% of all *USH2* alleles and 33.7% of identified pathogenic/UV4/UV3 *USH2A* alleles. Five families were homozygous for p.Glu767SerfsX21, 35 families were compound heterozygotes, and in 12 families p.Glu767SerfsX21 was the only pathogenic variant identified. We also observed other mutations occurring multiple times; segregating in four families were previously published p.Glu2288X and p.Trp3521Arg. The latter was not found in control chromosomes (0/836) and always appeared *in trans* with a pathogenic variant. Novel changes segregating in three families were p.Cys620Phe and p.Gln4541X. The remaining mutations were private, appearing in one or two families only.

*GPR98* mutations accounted for eight (6.6%) of *USH2* families, with two pathogenic/UV3 variants identified in six families and one variant in two families. In an additional ninth family (consanguineous family 222), we suspect mutations in the *GPR98* as the cause of USH as the two affected sibs share a homozygous haplotype consisting of three informative *GPR98* SNPs (rs1700510, rs10942605, rs2438351); *USH2A* was excluded based on haplotype analysis. Twelve out of 14 *GPR98* mutations were novel: three deletions causing frame shift mutations, four nonsense mutations, two splice mutations, one missense, and a homozygous large deletion (table 2). In consanguineous family 136 we suspect a large deletion of exon 83 (supplemental figure 3) as the two affected sibs in this family shared a homozygous *GPR98* haplotype; parents were heterozygous for the same haplotype. Despite affected sibs showing homozygosity for the *GPR98* haplotype throughout the gene, the mother was 'genotype C' for rs3098356 residing in intron 83, while the father was 'genotype A'; the assay failed in both affected sibs. PCR primers designed to amplify *GPR98* exon 83 in the affected sibs also failed, supporting a homozygous deletion of exon 83, for which parents are likely to be hemizygous. Intron 83 is 101 kb in size and mapping of breakpoints is in progress.

Interestingly, one patient (110) with an *USH2* phenotype and no mutations in *USH2* genes had a single *MYO7A*:p.Gly1942X nonsense mutation. In this family, we could not confirm association of the disease with any USH gene by SNP analysis as the proband has no sibs. The proband has good speech and bilateral severe hearing loss with an audiometric configuration more consistent with an *USH2* phenotype. There was also no history of delayed motor milestones and vestibular testing demonstrated normal vestibular function.<sup>39</sup>

In nine out of 121 (7.4%) *USH2* families, no possibly pathogenic variants were identified.

#### Molecular diagnosis in Usher type 3 families (N=4)

Very few subjects in our cohort were found to have mutations in *CLRN1*. Molecular diagnosis was clear in two families (table 2). Family 83 segregated a previously published *CLRN1* homozy-

gous mutation p.Asn48Lys,<sup>15</sup> the prevalent mutation in Ashkenazi Jews, and family 49 segregated *CLRN1*:p.Ser50LeufsX12 in the homozygous state.<sup>40</sup> Neither family was known to be consanguineous. While the proband from family 49 had moderate hearing loss and normal vestibular function, the proband from family 83 had vestibular hypofunction with moderate to severe hearing loss.

In family 482, clinically classified as Usher type 3 because of progressive hearing loss, we found four missense variants of uncertain pathogenicity (two in *WHRN* and two in *CDH23*), none of which were found in 96 control chromosomes. The variants in *WHRN* were *in trans* (p.Glu137Gln and p.Gln252Arg), and so were the two variants in *CDH23* (p.Glu1113Gln and p.Gly2908Arg). The predicted p.Gly2908Arg change (c.8722G>A) occurs at the last nucleotide of *CDH23* exon 60 and might act by altering splicing (supplementary table 3). Based on haplotype segregation analysis, neither *CDH23* nor *WHRN* could be excluded as the causative gene. Phenotypically, patient 482 had mild RP, profound hearing loss and vestibular function within normal limits.

One proband (429) classified clinically as having Usher syndrome type 3 had no mutations in any USH genes or the candidate gene *SLC4A7*.

#### Non-Usher cases and NCUS probands with atypical RP and hearing loss

Four NCUS probands had typical RP, but their hearing loss was not typical for any type of Usher syndrome. None of these had any mutations in the 10 sequenced genes. A further seven had both RP and hearing loss which were considered atypical for Usher syndrome; one had a single *MYO7A*:p.Leu326Gln missense variant which was previously published as a likely pathogenic variant (UV3) in a Pakistani family<sup>30</sup>; a second had a novel *USH1C*:p.Arg339Gln UV2 variant (supplementary table 2) and five had no mutations.

Of the remaining five NCUS probands who were not thought to have Usher syndrome, the patient with autosomal-recessive RP had two known *USH2A* mutations—that is, p.Cys759Phe and p.Cys3358Tyr; and the one with sector RP and hearing loss had two *USH1C* mutations. In this family the proband had moderate hearing loss and borderline bilateral vestibular hypofunction, whereas a younger affected sibling had severe hearing loss and normal vestibular function.<sup>35</sup> We found no mutations in the patient diagnosed with Alström syndrome or the two patients with an unknown syndromic disorder.

#### DISCUSSION

Molecular diagnosis in Usher syndrome is hindered by significant genetic heterogeneity, the large size of some of the Usher



genes, and the number of missense changes in genes such as *MYO7A* and *USH2A*. To this is added the further potential complexity of digenic inheritance which has been proposed in some cases of Usher syndrome and described in other retinal diseases.<sup>18 20 21</sup> Although a major undertaking in terms of time and expense, we decided at the beginning of the study to sequence all the known Usher genes in all subjects, regardless of clinical subtype, in order to assess evidence for, and contribution of, digenic inheritance and the extent of polymorphic sequence variation within the genes.

With digenic inheritance in mind, demonstrating that novel missense changes are truly pathogenic, rather than neutral variants is often difficult in the absence of functional studies; this is particularly so in the case of polymorphic genes in an ethnically diverse population. We applied a stringent assignment of pathogenicity to novel missense changes. A novel missense change was described as pathogenic only if it occurred in controls with a frequency <0.236%, was identified *in trans* to a pathogenic/probably pathogenic mutation, and it segregated with USH in more than two families. If the variant did not fulfil all of the mentioned criteria, it was classified as UV4/UV3 (supplemental data 1).

Although a number of molecular studies of Usher cohorts have been published to date, only one smaller study has been designed in a way that would systematically detect digenic inheritance and whether or not this is a significant or recurring phenomenon.<sup>18</sup> Bonnet *et al* described 10 (out of 54) USH patients with presumably pathogenic mutations in two different USH genes. Seven of them had biallelic mutations in one gene, and carried an additional mutation in a second and, for one of them, also a third USH gene. However, none of these had definite pathogenic mutations (ie, nonsense, frame shifting or splice) in two different genes. In all cases, one of the *heterozygous* mutations was a missense change which could have been a rare benign variant or possibly a disease modifier. For example, CDH23:p.R1060W, reported as presumably pathogenic in a digenic USH case,<sup>18</sup> has previously been published as likely non-pathogenic.<sup>20 41 42</sup> A possible case of digenic inheritance is reported in one (out of 75) USH patients who segregated CDH23:p.T1209A and PCDH15:p.T1867del variants<sup>20</sup>; however, the p.T1209A variant was also found in 48/904 (MAF=5.3%) alleles in the 1000 Genomes Project which suggests that it is unlikely to be pathogenic (<http://browser.1000genomes.org/index.html>).

The polymorphic variation present in Usher genes means that multiple variants are likely to be found if multiple genes are sequenced. In our study, many patients had a number of variants across multiple genes, and there are several interesting examples of two pathogenic variants in one gene and a missense variant, previously reported as a missense mutation, in a different gene. For example, CDH23:p.Arg3175His, previously published as disease-causing,<sup>43</sup> was identified in an USH2 family (219) together with two *USH2A* truncating mutations. Another variant, more recently published as a pathogenic missense change, CDH23:p.Ala366Thr,<sup>42 44–46</sup> occurred in 1/96 CEPH chromosomes and was found in an Usher syndrome type 1 patient 146 who has two pathogenic *MYO7A* mutations (p.Asp521GlufsX8 and p.Lys1255ArgfsX8). Also CDH23:p.His755Tyr was regarded as pathogenic,<sup>18 44</sup> but we identified it in a consanguineous USH2 family (203) segregating a homozygous *USH2A* nonsense mutation. So although the findings of others are similar to ours, their interpretation is different. We found no convincing evidence for digenic inheritance in this study; no subject had two definitely pathogenic alleles (nonsense, frameshifts or splice mutations) in different genes,

which given the overall spectrum of mutations in Usher syndrome (79% of identified pathogenic/UV4/UV3 variants were truncating mutations and 21% were missense changes) one might expect to find in genuine digenic inheritance. If digenic inheritance exists, it must be an occurrence too rare to be taken into account in genetic counselling. The only example of an USH2 patient described by Ebermann *et al*, who carried a single truncating mutation in *GPR98* and a truncating mutation in *PDZD7*<sup>21</sup> explained as 'digenic inheritance', could also be accounted for by an unidentified second mutation in *GPR98* in combination with a modifier allele in *PDZD7*. Since our study was completed before mutations in *PDZD7* were published as a cause of USH, this gene was not sequenced in our cohort.

We detected at least one pathogenic/likely pathogenic mutant allele in 86% of all Usher probands studied, indicating that there is no other Usher gene of major impact in the population. However, in the USH1 cohort, only a single pathogenic/UV4/UV3 variant was identified in 4/47 (8.5%) of families and in the USH2 cohort we observed a comparatively much higher number of missing alleles with only one pathogenic/UV4/UV3 variant identified in 26/121 (21.5%) of USH2 families. Undetected large genomic rearrangements, undetected pathogenic variants in the promoter and intronic regions, misdiagnosed USH syndrome, and human as well as computer software errors during sequence analysis are likely to underlie these 'missing alleles'. Certainly gross deletions and duplications have been well documented in genes such as *PCDH15* where they account for 37% of *PCDH15* mutations,<sup>34</sup> and 13% of USH1 cases.<sup>36</sup> Large genomic deletions and duplications have also been reported in *MYO7A*, *CDH23*, *GPR98*, and *USH2A*.<sup>34 47 48</sup> To analyse such rearrangements reliably, other methods such as MLPA and oligonucleotide array based comparative genomic hybridisation could be used in future.<sup>49</sup>

Our future research will focus on detection of large genomic rearrangements and mutations causing splicing aberrations at the mRNA level and will aim to clarify further the molecular diagnosis in the NCUS cohort.

Although probands with a clinical classification of Usher syndrome type 1 were screened for all USH genes, the causative mutations were only found in USH1 genes. In probands clinically classified as USH2, only 1/121 patients had a nonsense mutation in *MYO7A*, an USH1 gene. In another family who entered the study with a diagnosis thought unlikely to be Usher syndrome, we identified two *USH1C* mutations and affected sibs were subsequently diagnosed as having sector RP and hearing loss.<sup>35</sup> Therefore, regarding cases with atypical presentation, the mutation detection rate is low, but even these cases can harbour mutations in the known genes and produce unexpected phenotypes. Thus clinical classification, particularly that of type 1 Usher, is generally very robust, so screening all genes is unnecessary for molecular diagnosis in most cases and segregation analysis using haplotypes will be valuable for selecting candidate genes.<sup>34 50</sup>

Because 52.5% of pathogenic and likely pathogenic variants were novel, the use of microarray chips for molecular diagnosis in a disorder with a large number of private mutations such as USH is limited. It can, however, serve as a useful initial screen, although hybridisation techniques are being superseded by massively parallel sequencing, with the ability to generate large datasets. The existence of LSDBs for nine Usher genes (USHbases) combining international datasets is a valuable tool for molecular genetic studies of USH. The database enables integration of published and unpublished data, is regularly updated, and currently encompasses >4500 entries with 900



## Electronic database information

- ▶ USHbases—LSDB for Usher syndrome genes: [https://grenada.lumc.nl/LOVD2/Usher\\_montpellier/USHbases.html](https://grenada.lumc.nl/LOVD2/Usher_montpellier/USHbases.html)
- ▶ Clinical and Molecular Genetics Society: <http://www.cmgs.org/BPGs/pdfs%20current%20bpgs/UV%20GUIDELINES%20ratified.pdf>
- ▶ Usher Syndrome Missense Analysis: <https://194.167.35.160/cgi-bin/USMA/USMA.fcgi>
- ▶ 1000 Genomes Browser: <http://browser.1000genomes.org/index.html>
- ▶ British Society of Audiology Recommended Procedure for the Caloric Test: <http://www.thebsa.org.uk/docs/RecPro/CTP.pdf>
- ▶ MRC Holland for MLPA: (<http://www.mlpa.com/>)
- ▶ Splice Site Prediction: Human Splicing Finder, <http://www.umd.be/HSF/> and Splice Site Prediction by Neural Network, [http://www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)

unique pathogenic, neutral and unclassified variants.<sup>23</sup> We have 295 novel variants to submit to USHbases (137 are missense changes). Integration of large datasets such as this with data from all groups studying Usher syndrome, combined with haplotype and segregation analysis in families, and functional analysis of variants, will enable more reliable detection of truly pathogenic USH variants as well as the discovery of likely modifier genes.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** Ethics approval was provided by NRES Committee London South East (MREC).

**Contributors** All authors contributed to the conception and design of the study, acquisition of data, or analysis and interpretation of data, and all were involved in critical review of the manuscript for intellectual content and reviewed the final version.

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**Data sharing statement** All data will be provided to Locus Specific Databases (LSDBs) Usher genes (USHbases) which is publicly accessible.

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## Supplemental Data 1. Pathogenicity grades

|  | Pathogenic     | UV4 | UV3     | UV2     | UV1            | Neutral        |
|--|----------------|-----|---------|---------|----------------|----------------|
| Frequency in ethnically matching controls (N=876 chromosomes) $\leq 0.236\%$                 | yes            | yes | yes     | yes*    | unknown        | no             |
| <i>In trans</i> with a 'Pathogenic' or 'UV4' mutation  | yes            | yes | unknown | unknown | unknown        | no             |
| Segregates with disease in >2 NCUS families or previously published as 'Pathogenic' or 'UV4' | yes            | no  | no      | no      | no             | no             |
| Novel, segregates with disease in only one NCUS family                                       | Not applicable | yes | yes     | yes     | no             | no             |
| Identified in proband with one or two other mutations with a higher pathogenicity grade      | no             | no  | no      | no      | yes or unknown | yes or unknown |

\*Genotyped in 96 CEPH control chromosomes

## Supplemental Data 2. USH1C:c.496+1G>A assay

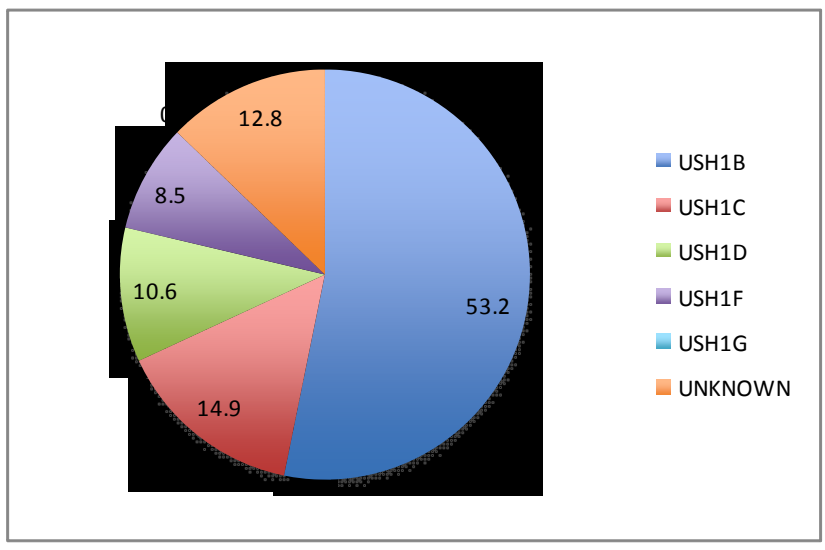
The following primers were used: forward 5'-TAGGGGACGAGATCGTCCGGATCA-3' and reverse (introduced mutation is underlined): 5'-

TGGAGTACTGCCCTGCTCTGGCCTCACACA-3'. The reverse primer was designed in order to create an artificial restriction site in c.496+1G>A mutant sequence, but not the wild type, using the web-based program dCAPS Finder v2.[21] For each PCR reaction 1ng/μl genomic DNA was amplified with 0.5pmol/μl of each primer, 1M betaine (Sigma-Aldrich), 0.25mM dNTPs (Microzone Ltd), 1x buffer, 1.5mM MgCl<sub>2</sub> and 0.5% BIOTAQ<sup>TM</sup> DNA polymerase (all Bioline). The PCR program consisted of a denaturation step of 10 min at 95°C, followed by 37 cycles of 30 sec at 95°C, 30 sec at 61°C, 40 sec at 72°C and a final extension at 72°C for 10 min. Restriction digests were prepared using *PciI* (New England Biolabs) according to manufacturer's instructions. The digested product was run on 4% agarose gel.

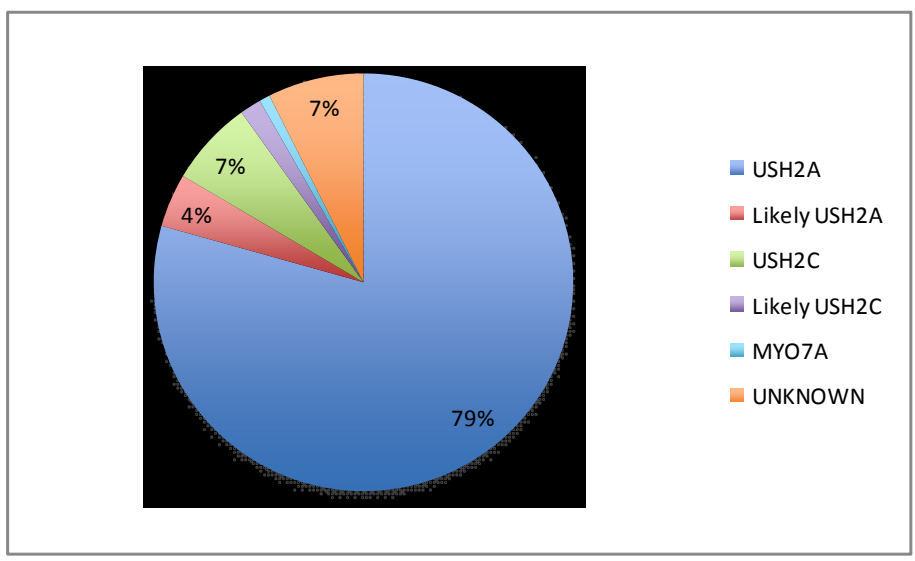


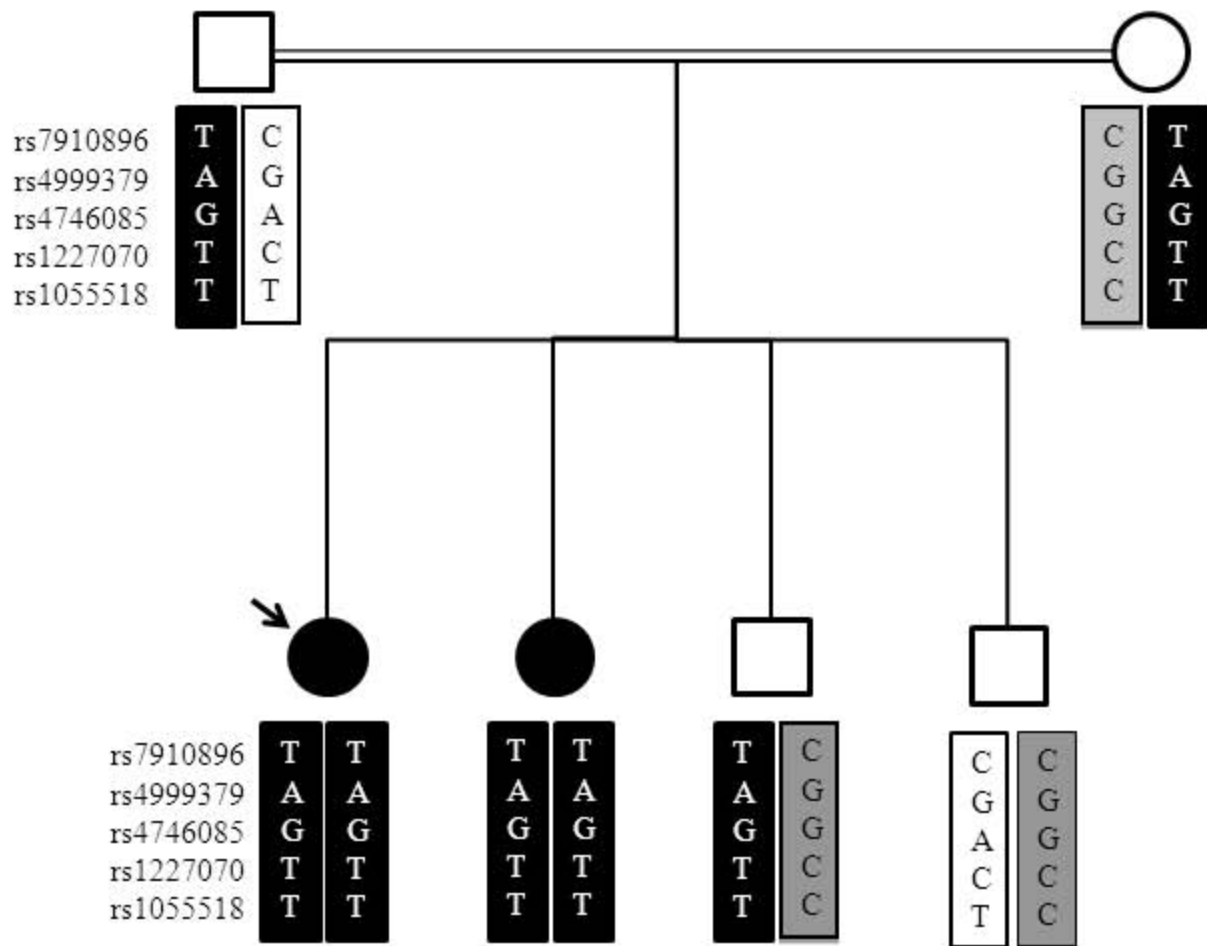
Supplemental Data 3.

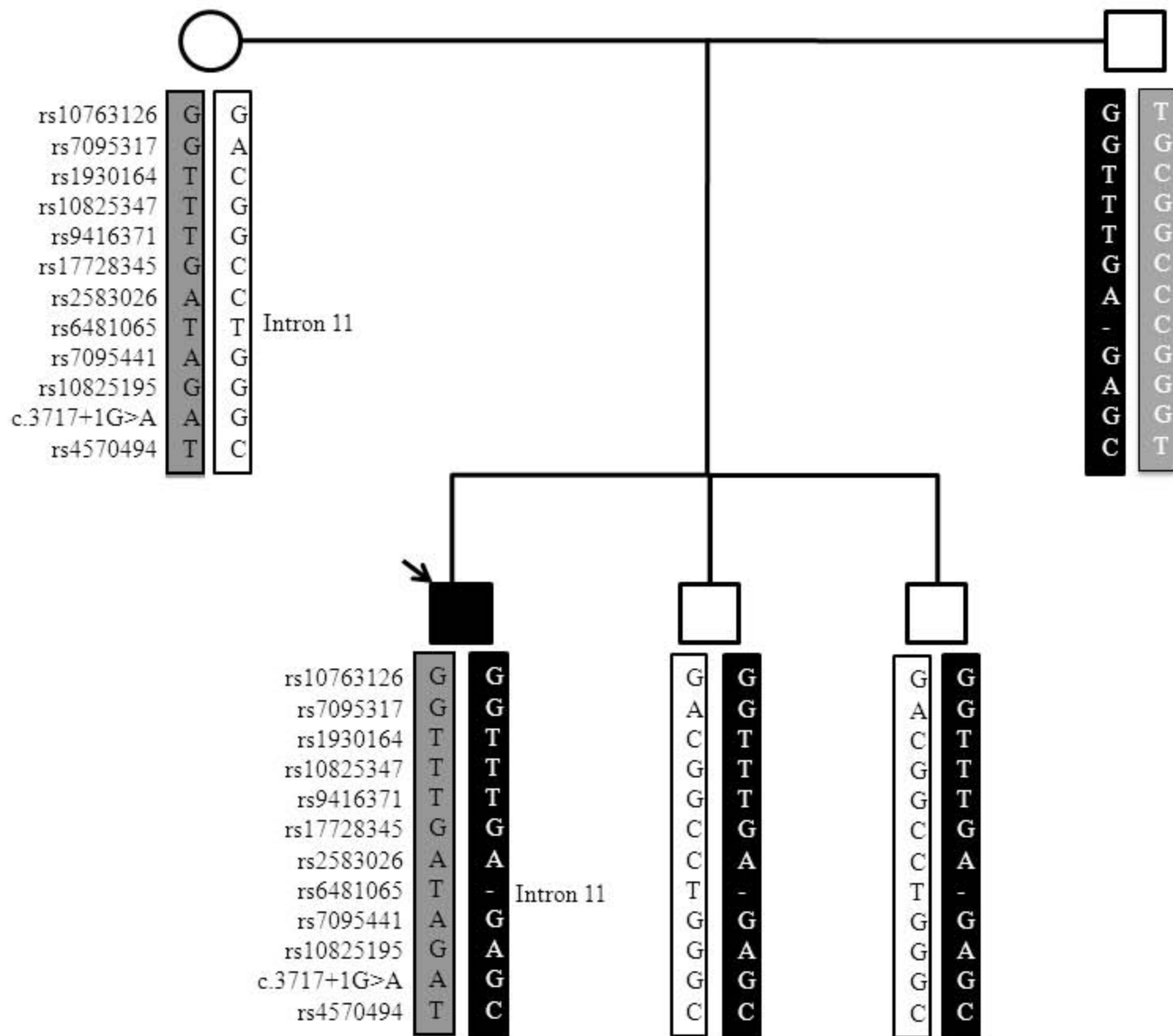
a) Relative proportions of molecular subtypes in Usher Type 1

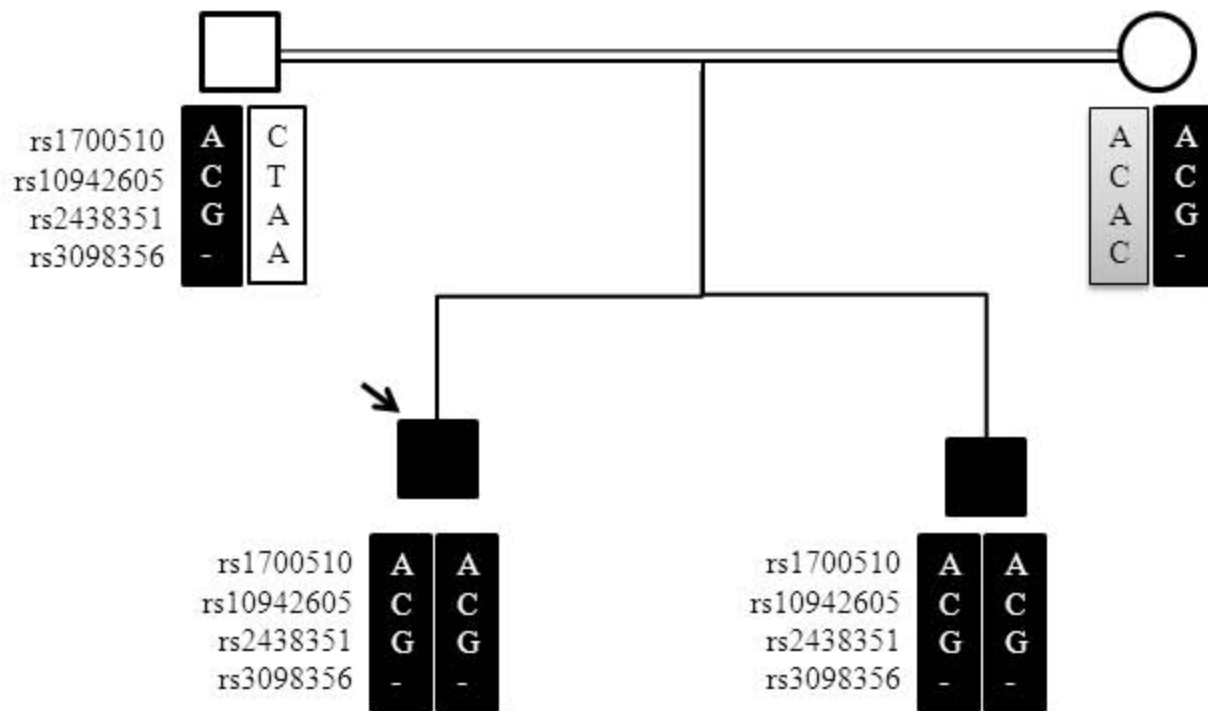


b) Relative proportions of molecular subtypes in Usher Type 2











**Supplementary Table 1. Polymorphic SNPs genotyped at Sequenom.**

| Assay_Name | Gene          |
|------------|---------------|
| rs1227070  | <i>CDH23</i>  |
| rs1867998  | <i>CDH23</i>  |
| rs4999379  | <i>CDH23</i>  |
| rs723274   | <i>CDH23</i>  |
| rs1055518  | <i>CDH23</i>  |
| rs4746085  | <i>CDH23</i>  |
| rs7910896  | <i>CDH23</i>  |
| rs10942605 | <i>GPR98</i>  |
| rs1700510  | <i>GPR98</i>  |
| rs2010355  | <i>GPR98</i>  |
| rs2438351  | <i>GPR98</i>  |
| rs3098356  | <i>GPR98</i>  |
| rs4944143  | <i>MYO7A</i>  |
| rs6592711  | <i>MYO7A</i>  |
| rs12793189 | <i>MYO7A</i>  |
| rs2156568  | <i>MYO7A</i>  |
| rs10825347 | <i>PCDH15</i> |
| rs1930164  | <i>PCDH15</i> |
| rs2583026  | <i>PCDH15</i> |
| rs4570494  | <i>PCDH15</i> |
| rs6481065  | <i>PCDH15</i> |
| rs7095441  | <i>PCDH15</i> |
| rs10763126 | <i>PCDH15</i> |
| rs10825195 | <i>PCDH15</i> |
| rs17728345 | <i>PCDH15</i> |
| rs1937392  | <i>PCDH15</i> |
| rs7095317  | <i>PCDH15</i> |
| rs9416371  | <i>PCDH15</i> |
| rs9828914  | <i>SLC4A7</i> |
| rs1494420  | <i>SLC4A7</i> |
| rs2072227  | <i>USH1C</i>  |
| rs2240488  | <i>USH1C</i>  |
| rs2883581  | <i>USH1C</i>  |
| rs12033676 | <i>USH2A</i>  |
| rs2168924  | <i>USH2A</i>  |
| rs2168924  | <i>USH2A</i>  |
| rs12635944 | <i>USH3A</i>  |
| rs6801898  | <i>USH3A</i>  |
| rs718841   | <i>WHRN</i>   |
| rs942520   | <i>WHRN</i>   |

|            |             |
|------------|-------------|
| rs10759697 | <i>WHRN</i> |
| rs10982231 | <i>WHRN</i> |
| rs4979379  | <i>WHRN</i> |
| rs7034891  | <i>WHRN</i> |
| rs7036125  | <i>WHRN</i> |

**Supplementary Table 2. Novel UV2-UV4 and pathogenic variants.**

| Gene name     | DNA change                | Protein change           | MAF (%) in controls <sup>a</sup> | Ortholog Conservation <sup>d</sup> | 1000 Genomes <sup>f</sup> | Pathogenicity |
|---------------|---------------------------|--------------------------|----------------------------------|------------------------------------|---------------------------|---------------|
| <i>CDH23</i>  | c.2177-2A>G               | p.?                      | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>CDH23</i>  | c.6254_6254-3delCAGGinsT  | p.?                      | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>CDH23</i>  | c.6712+1G>A               | p.?                      | 0                                | NA                                 | No                        | Pathogenic    |
| <i>CDH23</i>  | c.7305dup                 | p.Leu2436ThrfsX3         | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>CDH23</i>  | c.7362G>A*                | p.Thr2454Thr             | 0                                | NA                                 | No                        | UV3           |
| <i>CDH23</i>  | c.9122T>C                 | p.Leu3041Pro             | 0                                | 16 / 17 (94.12%)                   | No                        | UV4           |
| <i>MYO7A</i>  | c.1138G>A                 | p.Glu380Lys              | 0 [0/93 CEPH]                    | 21 / 23 (91.30%)                   | No                        | UV2           |
| <i>MYO7A</i>  | c.1258A>T                 | p.Lys420X                | 0 [0/96 CEPHs]                   | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.1798-3C>G               | p.?                      | 0 [0/96 CEPH]                    | NA                                 | No                        | UV2           |
| <i>MYO7A</i>  | c.223G>C                  | p.Asp75His               | 0                                | 24 / 24 (100%)                     | No                        | UV4           |
| <i>MYO7A</i>  | c.3108+1G>A               | p.?                      | 0                                | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.338_348dup              | p.Glu117SerfsX33         | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.4131dup                 | p.Gly1378TrpfsX6         | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.4293G>A                 | p.Trp1431X               | 0                                | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.4838delA                | p.Asp1613ValfsX32        | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.-48A>G (IVS1-2A>G)      | p.?                      | 0                                | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.5824G>T                 | p.Gly1942X               | 0                                | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.6377delC                | p.Pro2126Leuf sX5        | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>MYO7A</i>  | c.6577C>T                 | p.Leu2193Phe             | 0                                | 23 / 23 (100.00%)                  | No                        | UV4           |
| <i>MYO7A</i>  | c.722G>C                  | p.Arg241Pro              | 0                                | 23 / 23 (100.00%)                  | No                        | UV4           |
| <i>PCDH15</i> | c.2823delG                | p.Gly942ValfsX22         | 0 [0/96 CEPH]                    | NA                                 | No                        | Pathogenic    |
| <i>PCDH15</i> | c.3501+2T>C               | p.?                      | 0                                | NA                                 | No                        | Pathogenic    |
| <i>PCDH15</i> | Large deletion: Exon 9-18 | p.?                      | NA                               | NA                                 | No                        | Pathogenic    |
| <i>PCDH15</i> | Large deletion: Exon 10   | p.?                      | NA                               | NA                                 | No                        | Pathogenic    |
| <i>USH1C</i>  | c.1016G>A                 | p.Arg339Gln <sup>b</sup> | 0                                | 17 / 21 (80.95%)                   | No                        | UV2           |
| <i>USH1C</i>  | c.2227-1G>T <sup>c</sup>  | p.?                      | 0                                | NA                                 | No                        | Pathogenic    |

|              |                        |                       |                  |                                  |    |            |
|--------------|------------------------|-----------------------|------------------|----------------------------------|----|------------|
| <i>USH1C</i> | c.446_448delA<br>GG    | p.Glu149del           | NA               | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.1019A>T              | p.His340Leu           | 0                | 17 / 17<br>(100.00%)             | No | UV3        |
| <i>USH2A</i> | c.6049G>T*             | p.Gly2017Cys          | 0                | 15/17 (88.24%)                   | No | UV2        |
| <i>USH2A</i> | c.10724G>A             | p.Cys3575Tyr          | 0                | 17 / 17<br>(100.00%)             | No | UV4        |
| <i>USH2A</i> | c.11047+1G>A           | p.?                   | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.11065C>T             | p.Arg3689X            | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.11390-1G>C           | p.?                   | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.11872_11873<br>delAC | p.Gln3959Asn<br>fsX53 | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.12295-3T>A           | p.?                   | 0 [0/96<br>CEPH] | NA                               | No | UV2        |
| <i>USH2A</i> | c.12457G>A             | p.Ala4153Thr          | 0.12             | 13 / 17<br>(76.47%) <sup>c</sup> | No | UV4        |
| <i>USH2A</i> | c.13130C>A             | p.Ser4377X            | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.13621C>T             | p.Gln4541X            | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.14139G>A             | p.Trp4713X            | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.14285A>G             | p.Asn4762Ser          | 0                | 17 / 17<br>(100.00%)             | No | UV3        |
| <i>USH2A</i> | c.14403C>G             | p.Tyr4801X            | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.14446A>T             | p.Lys4816X            | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.14911C>T             | p.Arg4971X            | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.1859G>T              | p.Cys620Phe           | 0                | 17 / 17<br>(100.00%)             | No | Pathogenic |
| <i>USH2A</i> | c.2236C>G              | p.Pro746Ala           | 0                | 17 / 17<br>(100.00%)             | No | UV4        |
| <i>USH2A</i> | c.2610C>A              | p.Cys870X             | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.2942_2943ins<br>T    | p.Cys982Leufs<br>X2   | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.2994_3007del         | p.Cys999Leufs<br>X9   | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.3407G>A              | p.Ser1136Asn          | 0                | 17 / 17<br>(100.00%)             | No | UV4        |
| <i>USH2A</i> | c.3518C>A              | p.Ser1173X            | 0                | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.4133T>C              | p.Leu1378Pro          | 0                | 15 / 17<br>(88.24%)              | No | UV4        |
| <i>USH2A</i> | c.4354dup              | p.Cys1452Leu<br>fsX25 | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.4732C>T              | p.Arg1578Cys          | 0                | 15 / 17<br>(88.24%)              | No | UV4        |
| <i>USH2A</i> | c.4761delG             | p.Ser1588Hisf<br>sX5  | 0 [0/96<br>CEPH] | NA                               | No | Pathogenic |
| <i>USH2A</i> | c.4821G>A              | p.Trp1607X            | 0.12             | NA                               | No | Pathogenic |



|              |                               |                       |                   |                      |                 |            |
|--------------|-------------------------------|-----------------------|-------------------|----------------------|-----------------|------------|
| <i>USH2A</i> | c.5252 G>T                    | p.Gly1751Val          | 0                 | 14 / 17<br>(82.35%)  | No              | UV2        |
| <i>USH2A</i> | c.5329 C>T                    | p.Arg1777Trp          | 0                 | 6 / 17 (35.29%)      | No              | UV3        |
| <i>USH2A</i> | c.5603T>G                     | p.Phe1868Cys          | 0 [0/96<br>CEPH]  | 11 / 17<br>(64.71%)  | No              | UV2        |
| <i>USH2A</i> | c.5614_5620del<br>GCTGTCG     | p.Ala1872Leuf<br>sX58 | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.5834_5835ins<br>TC          | p.Arg1946Leu<br>fsX22 | 0                 | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.5898_5899del<br>AA          | p.Asn1967Trp<br>fsX5  | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.651+1G>A                    | p.?                   | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.6854A>G                     | p.Asn2285Ser          | 0 [0/96<br>CEPH]  | 16 / 17<br>(94.12%)  | No              | UV2        |
| <i>USH2A</i> | c.6928A>C                     | p.Thr2310Pro          | 0 [0/96<br>CEPH]  | 17 / 17<br>(100.00%) | Yes<br>(1/1258) | UV2        |
| <i>USH2A</i> | c.769G>A                      | p.Gly257Arg           | 0 [0/96<br>CEPH]  | 16 / 17<br>(94.12%)  | No              | UV2        |
| <i>USH2A</i> | c.8231G>A                     | p.Trp2744X            | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.8261delT                    | p.Ile2754Asnf<br>sX15 | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.842C>A                      | p.Thr281Lys           | 0 [0/93<br>CEPH]  | 15 / 17<br>(88.24%)  | No              | UV2        |
| <i>USH2A</i> | c.9371+1G>C                   | p.?                   | 0.12              | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.9459C>A                     | p.Cys3153X            | NA                | NA                   | No              | Pathogenic |
| <i>USH2A</i> | c.9842G>T                     | p.Cys3281Phe          | 0 [0/96<br>CEPHs] | 17 / 17<br>(100.00%) | No              | UV2        |
| <i>USH2A</i> | c.9912dup                     | p.Glu3305Arg<br>fsX41 | 0                 | NA                   | No              | Pathogenic |
| <i>USH2A</i> | Large deletion:<br>Exon 47    | p.?                   | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>USH2A</i> | Large deletion:<br>Exon 50-55 | p.?                   | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>GPR98</i> | c.10016G>A                    | p.Ser3339Asn          | 0                 | 17 / 19<br>(89.47%)  | No              | UV3        |
| <i>GPR98</i> | c.10736_37delC<br>C           | p.Ala3579Valf<br>sX6  | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>GPR98</i> | c.13433G>T*                   | p.Ser4478Ile          | 0                 | 19 / 20<br>(95.00%)  | No              | UV3        |
| <i>GPR98</i> | c.14404C>T                    | p.Arg4802X            | 0                 | NA                   | No              | Pathogenic |
| <i>GPR98</i> | c.15144delC                   | p.Ser5048Argf<br>sX29 | 0 [0/96<br>CEPH]  | NA                   | No              | Pathogenic |
| <i>GPR98</i> | c.2398C>T                     | p.Arg800X             | 0.11              | NA                   | No              | Pathogenic |
| <i>GPR98</i> | c.4123G>C                     | p.Asp1375His          | 0                 | 14 / 20<br>(70.00%)  | No              | UV2        |
| <i>GPR98</i> | c.6307G>T                     | p.Glu2103X            | 0                 | NA                   | No              | Pathogenic |
| <i>GPR98</i> | c.6856C>T                     | p.Arg2286X            | NA                | NA                   | No              | Pathogenic |

|              |                            |                  |                   |                     |    |            |
|--------------|----------------------------|------------------|-------------------|---------------------|----|------------|
| <i>GPR98</i> | c.6962_63delT<br>G         | p.Val2321AlafsX4 | NA                | NA                  | No | Pathogenic |
| <i>GPR98</i> | c.9623+1G>A                | p.?              | 0                 | NA                  | No | Pathogenic |
| <i>GPR98</i> | c.9974T>C                  | p.Ile3325Thr     | 0 [0/96<br>CEPHs] | 17 / 19<br>(89.47%) | No | UV2        |
| <i>GPR98</i> | Large deletion:<br>Exon 83 | p.?              | 0 [0/96<br>CEPHs] | NA                  | No | Pathogenic |

### Legend to Supplementary Table 2.

\*Last nucleotide of an exon. Variant is predicted to significantly lower efficiency of adjacent splice site as predicted by *in silico* analysis (Human Splicing Finder, <http://www.umd.be/HSF/> and Splice Site Prediction by Neural Network, [www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)).

NA-Not Applicable

<sup>a</sup> Minimum Allele Frequency in 876 control chromosomes unless stated otherwise

<sup>b</sup> Identified in NCUS proband with RP atypical for Usher syndrome

<sup>c</sup> Identified in NCUS proband who was diagnosed with sector RP and hearing loss.[22]

<sup>d</sup> Alignments were made using Usher Syndrome Missense Analysis tool (USMA). Full alignments are available via a link from LSDB for Usher syndrome. Unless stated otherwise, the mutated residue was not found in ortholog species

<sup>e</sup> Mutated residue was found in *Ciona intestinalis* and *Ciona savignyi* (family of sea squirts)

<sup>f</sup> Accessed on July 13th 2011 (<http://browser.1000genomes.org/index.htm>)

**Supplementary Table 3. Novel UV1 and Neutral variants.**

| <b>Gene name</b> | <b>DNA change</b>               | <b>Protein change</b>            | <b>MAF (%) in controls<sup>a</sup></b> | <b>Pathogenicity</b> |
|------------------|---------------------------------|----------------------------------|--|----------------------|
| <i>CDH23</i>     | c.10036G>C                      | p.Glu3346Gln                     | 0.11                                   | UV1                  |
| <i>CDH23</i>     | c.129C>T <sup>+</sup>           | p.Ser43Ser                       | NA                                     | UV1                  |
| <i>CDH23</i>     | c.1307G>A                       | p.Ser436Asn                      | 0.34                                   | Neutral              |
| <i>CDH23</i>     | c.1369C>T                       | p.Arg457Trp                      | 0.23                                   | UV1                  |
| <i>CDH23</i>     | c.1595C>T                       | p.Thr532Met                      | 0                                      | UV1                  |
| <i>CDH23</i>     | c.173A>G                        | p.Gln58Arg                       | NA                                     | UV1                  |
| <i>CDH23</i>     | c.1752+6G>A                     | p.?                              | NA                                     | UV1                  |
| <i>CDH23</i>     | c.1919C>T                       | p.Thr640Met                      | 0                                      | UV1                  |
| <i>CDH23</i>     | c.198G>A                        | p.Val66Val                       | NA                                     | UV1                  |
| <i>CDH23</i>     | c.204C>T                        | p.Gly68Gly                       | NA                                     | UV1                  |
| <i>CDH23</i>     | c.2235C>T <sup>+</sup>          | p.Ile745Ile                      | 0                                      | UV1                  |
| <i>CDH23</i>     | c.2289+20G>C                    | p.?                              | NA                                     | UV1                  |
| <i>CDH23</i>     | c.2970C>T                       | p.Asp990Asp                      | 0.69                                   | Neutral              |
| <i>CDH23</i>     | c.3337G>C                       | p.Glu1113Gln                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.3664G>A                       | p.Ala1222Thr                     | NA                                     | UV1                  |
| <i>CDH23</i>     | c.3801C>T                       | p.Thr1267Thr                     | 0.11                                   | UV1                  |
| <i>CDH23</i>     | c.3845A>G                       | p.Asn1282Ser                     | 0.93                                   | Neutral              |
| <i>CDH23</i>     | c.3895G>A                       | p.Val1299Ile                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.4231G>A                       | p.Glu1411Lys                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.4287C>T                       | p.Pro1429Pro                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.442G>A<br>[ENST00000224721]   | p.Gly148Arg<br>[ENST00000224721] | NA                                     | UV1                  |
| <i>CDH23</i>     | c.444+3T>C<br>[ENST00000224721] | p.?                              | NA                                     | UV1                  |
| <i>CDH23</i>     | c.4786C>T                       | p.Arg1596Cys                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.4846-19G>A                    | p.?                              | NA                                     | UV1                  |
| <i>CDH23</i>     | c.4890C>T <sup>+</sup>          | p.Asn1630Asn                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.4947G>A                       | p.Thr1649Thr                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.5067+15G>A                    | p.?                              | NA                                     | UV1                  |
| <i>CDH23</i>     | c.551G>A                        | p.Arg184His                      | 0                                      | UV1                  |
| <i>CDH23</i>     | c.5541C>T                       | p.Asn1847Asn                     | 0.12                                   | UV1                  |
| <i>CDH23</i>     | c.5660C>T                       | p.Thr1887Ile                     | 0.34                                   | Neutral              |
| <i>CDH23</i>     | c.6197G>A                       | p.Arg2066Gln                     | 0.36                                   | Neutral              |
| <i>CDH23</i>     | c.6648C>T <sup>+</sup>          | p.Ala2216Ala                     | 0                                      | UV1                  |
| <i>CDH23</i>     | c.67+19G>A                      | p.?                              | NA                                     | UV1                  |
| <i>CDH23</i>     | c.7722C>T                       | p.Tyr2574Tyr                     | 0.34                                   | Neutral              |

|              |              |              |                  |         |
|--------------|--------------|--------------|------------------|---------|
| <i>CDH23</i> | c.8722G>A*   | p.Gly2908Arg | 0                | UV1     |
| <i>CDH23</i> | c.9014C>T    | Ala3005Val   | 0                | UV1     |
| <i>CDH23</i> | c.9015G>A    | Ala3005Ala   | 0.11             | UV1     |
| <i>CDH23</i> | c.9238G>A    | Ala3080Thr   | 0.23             | UV1     |
| <i>CDH23</i> | c.9319+11G>A | p.?          | NA               | UV1     |
| <i>CDH23</i> | c.9973C>G    | Arg3325Gly   | 0                | UV1     |
| <i>MYO7A</i> | c.1081-10G>C | p.?          | NA               | UV1     |
| <i>MYO7A</i> | c.1407G>A    | Val469Val    | NA               | UV1     |
| <i>MYO7A</i> | c.133-7C>T   | p.?          | NA               | UV1     |
| <i>MYO7A</i> | c.1543A>C    | Lys515Gln    | 0.12             | UV1     |
| <i>MYO7A</i> | c.1554+8G>A  | p.?          | NA               | UV1     |
| <i>MYO7A</i> | c.1817G>A    | p.Arg606His  | 0                | UV1     |
| <i>MYO7A</i> | c.1969C>T    | p.Arg657Trp  | 0                | UV1     |
| <i>MYO7A</i> | c.2057G>A    | p.Arg686His  | 0.11             | UV1     |
| <i>MYO7A</i> | c.2120G>A    | p.Arg707His  | 0                | UV1     |
| <i>MYO7A</i> | c.2617C>T    | p.Arg873Trp  | 0.11             | UV1     |
| <i>MYO7A</i> | c.2886G>C    | p.Gln962His  | 0                | UV1     |
| <i>MYO7A</i> | c.3453G>A    | p.Leu1151Leu | NA               | UV1     |
| <i>MYO7A</i> | c.359 G>A    | p.Arg120His  | 0                | UV1     |
| <i>MYO7A</i> | c.4023C>T    | p.Pro1341Pro | 0                | UV1     |
| <i>MYO7A</i> | c.4074C>T    | p.Ser1358Ser | 0.47             | Neutral |
| <i>MYO7A</i> | c.4161C>A    | p.Asp1387Glu | 0                | UV1     |
| <i>MYO7A</i> | c.4461C>T    | p.Asn1487Asn | 0.23             | UV1     |
| <i>MYO7A</i> | c.4505 A>G   | p.Asp1502Gly | 0                | UV1     |
| <i>MYO7A</i> | c.4619 C>T   | p.Ala1540Val | 0                | UV1     |
| <i>MYO7A</i> | c.4845C>A    | p.Pro1615Pro | NA               | UV1     |
| <i>MYO7A</i> | c.4950C>T    | p.Asn1650Asn | 0                | UV1     |
| <i>MYO7A</i> | c.495G>A     | p.Thr165Thr  | 0.12             | UV1     |
| <i>MYO7A</i> | c.5122C>A    | p.Arg1708Ser | 0                | UV1     |
| <i>MYO7A</i> | c.5216G>A    | p.Arg1739Gln | NA               | UV1     |
| <i>MYO7A</i> | c.5245C>T    | p.Arg1749Trp | 0.12             | UV1     |
| <i>MYO7A</i> | c.54G>C      | p.Gln18His   | 0.12             | UV1     |
| <i>MYO7A</i> | c.5619G>A    | p.Arg1873Arg | NA               | UV1     |
| <i>MYO7A</i> | c.569T>G     | p.Leu190Trp  | 0 [0/96<br>CEPH] | UV1     |
| <i>MYO7A</i> | c.5866G>A    | p.Val1956Ile | 0.24             | UV1     |
| <i>MYO7A</i> | c.6051+16C>G | p.?          | NA               | UV1     |
| <i>MYO7A</i> | c.6509C>T    | p.Thr2170Ile | NA               | UV1     |
| <i>MYO7A</i> | c.6626G>A    | p.Arg2209Gln | 0                | UV1     |
| <i>MYO7A</i> | c.6640G>A    | p.Gly2214Ser | 0.11             | UV1     |
| <i>MYO7A</i> | c.973A>T     | p.Ile325Phe  | 0                | UV1     |

|               |                          |                                |      |         |
|---------------|--------------------------|--------------------------------|------|---------|
| <i>MYO7A</i>  | c.974T>G                 | p.Ile325Ser                    | 0    | UV1     |
| <i>MYO7A</i>  | c.5598C>A                | p.Leu1866Leu                   | 0.23 | UV1     |
| <i>PCDH15</i> | c.2625G>C                | p.Ser875Ser                    | NA   | UV1     |
| <i>PCDH15</i> | c.319-20A>T              | p.?                            | NA   | UV1     |
| <i>PCDH15</i> | c.3502-8C>T              | p.?                            | NA   | UV1     |
| <i>PCDH15</i> | c.3795A>G                | p.Glu1265Asp                   | 0    | UV1     |
| <i>PCDH15</i> | c.3983+12T>C             | p.?                            | NA   | UV1     |
| <i>PCDH15</i> | c.4974A>C                | p.Ser1658Ser                   | 0.11 | UV1     |
| <i>PCDH15</i> | c.5247_5249de<br>ITCC    | p.Pro1752del                   | 0.57 | Neutral |
| <i>PCDH15</i> | c.5263C>T                | p.Pro1755Ser                   | NA   | UV1     |
| <i>PCDH15</i> | c.5358_5359in<br>sCCTCTT | p.Ile1786_Pro17<br>87insProLeu | NA   | UV1     |
| <i>PCDH15</i> | c.5359C>T                | p.Pro1787Ser                   | NA   | UV1     |
| <i>PCDH15</i> | c.5398G>A                | p.Val1800Ile                   | NA   | UV1     |
| <i>PCDH15</i> | c.5550C>A                | p.Thr1850Thr                   | NA   | UV1     |
| <i>PCDH15</i> | c.5707A>G                | p.Ile1903Val                   | NA   | UV1     |
| <i>USH1C</i>  | c.1086-13G>T             | p.?                            | NA   | UV1     |
| <i>USH1C</i>  | c.1430G>A                | p.Arg477Gln                    | 0    | UV1     |
| <i>USH1C</i>  | c.2340C>T                | p.Val780Val                    | NA   | UV1     |
| <i>USH1C</i>  | c.324T>C                 | p.Phe108Phe                    | 0    | UV1     |
| <i>USH1G</i>  | c.1152C>T                | p.Asp384Asp                    | 0    | UV1     |
| <i>USH1G</i>  | c.388A>G                 | p.Lys130Glu                    | 0    | UV1     |
| <i>USH1G</i>  | c.501C>G                 | p.Arg167Arg                    | 0.12 | UV1     |
| <i>USH1G</i>  | c.566G>A                 | p.Arg189Gln                    | 0.12 | UV1     |
| <i>USH1G</i>  | c.678C>A                 | p.Gly226Gly                    | 0    | UV1     |
| <i>USH1G</i>  | c.705G>A                 | p.Glu235Glu                    | 0.12 | UV1     |
| <i>USH1G</i>  | c.83C>T                  | p.Pro28Leu                     | NA   | UV1     |
| <i>GPR98</i>  | c.10260C>T <sup>+</sup>  | p.Phe3420Phe                   | 0    | UV1     |
| <i>GPR98</i>  | c.10796+9A><br>G         | p.?                            | NA   | UV1     |
| <i>GPR98</i>  | c.10873C>G               | p.Leu3625Val                   | NA   | UV1     |
| <i>GPR98</i>  | c.10927A>G               | p.Thr3643Ala                   | 0.11 | UV1     |
| <i>GPR98</i>  | c.11599G>A               | p.Glu3867Lys                   | NA   | UV1     |
| <i>GPR98</i>  | c.12121-<br>16A>G        | p.?                            | NA   | UV1     |
| <i>GPR98</i>  | c.13232-7A>G             | p.?                            | NA   | UV1     |
| <i>GPR98</i>  | c.13358A>G               | p.His4453Arg                   | 0    | UV1     |
| <i>GPR98</i>  | c.13919G>A               | p.Gly4640Glu                   | 0    | UV1     |
| <i>GPR98</i>  | c.14309G>A               | p.Arg4770His                   | 0.92 | Neutral |
| <i>GPR98</i>  | c.14856C>T               | p.His4952His                   | 0    | UV1     |
| <i>GPR98</i>  | c.15105C>T <sup>+</sup>  | p.Ser5035Ser                   | NA   | UV1     |
| <i>GPR98</i>  | c.15301G>A <sup>+</sup>  | p.Gly5101Arg                   | 0.36 | Neutral |
| <i>GPR98</i>  | c.15343C>T               | p.Leu5115Leu                   | 0    | UV1     |

|              |                        |              |                         |         |
|--------------|------------------------|--------------|-------------------------|---------|
| <i>GPR98</i> | c.16164A>G             | p.Arg5388Arg | 1.54                    | Neutral |
| <i>GPR98</i> | c.16312A>G             | p.Thr5438Ala | 0.48                    | Neutral |
| <i>GPR98</i> | c.18026G>A             | p.Arg6009Gln | 0                       | UV1     |
| <i>GPR98</i> | c.18040T>C             | p.Phe6014Leu | 0                       | UV1     |
| <i>GPR98</i> | c.18273A>G             | p.Ala6091Ala | 0.23                    | UV1     |
| <i>GPR98</i> | c.18311-8_12delTTTT    | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.1849G>A              | p.Val617Met  | NA                      | UV1     |
| <i>GPR98</i> | c.18746T>G             | p.Leu6249Arg | 0                       | UV1     |
| <i>GPR98</i> | c.18753C>T             | p.Ala6251Ala | 0                       | UV1     |
| <i>GPR98</i> | c.18782T>C             | p.Leu6261Ser | 0                       | UV1     |
| <i>GPR98</i> | c.18802+9G>A           | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.18803-13A>G          | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.2185A>G              | p.Ile729Val  | 0                       | UV1     |
| <i>GPR98</i> | c.2284C>T              | p.Arg762Cys  | 0                       | UV1     |
| <i>GPR98</i> | c.2516T>C              | p.Val839Ala  | 0.12                    | UV1     |
| <i>GPR98</i> | c.2553+11T>A           | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.2735-10C>A           | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.2834G>A              | p.Gly945Glu  | NA                      | UV1     |
| <i>GPR98</i> | c.3022+8T>C            | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.3255T>C              | p.Asp1085Asp | 0.12                    | UV1     |
| <i>GPR98</i> | c.327C>T               | p.Asp109Asp  | 0.24                    | UV1     |
| <i>GPR98</i> | c.3289G>A*             | p.Gly1097Ser | 0.11                    | UV1     |
| <i>GPR98</i> | c.3635-10C>A           | p.?          | NA                      | UV1     |
| <i>GPR98</i> | c.3805T>C              | p.Phe1269Leu | 0                       | UV1     |
| <i>GPR98</i> | c.380T>G               | p.Leu127Arg  | 2.22%<br>[2/90<br>CEPH] | UV1     |
| <i>GPR98</i> | c.3975G>A              | p.Thr1325Thr | 0                       | UV1     |
| <i>GPR98</i> | c.4214C>T <sup>+</sup> | p.Ser1405Ser | NA                      | UV1     |
| <i>GPR98</i> | c.4260A>G              | p.Glu1420Glu | 0.84                    | Neutral |
| <i>GPR98</i> | c.5104C>T              | p.Pro1702Ser | 0                       | UV1     |
| <i>GPR98</i> | c.5221T>C <sup>+</sup> | p.Leu1741Leu | 0                       | UV1     |
| <i>GPR98</i> | c.5525-7C>T            | p.?          |                         | UV1     |
| <i>GPR98</i> | c.5785G>T              | p.Ala1929Ser | 0.24                    | UV1     |
| <i>GPR98</i> | c.6012G>T              | p.Leu2004Phe | NA                      | UV1     |
| <i>GPR98</i> | c.6289C>T              | p.Arg2097Cys | 1.19                    | Neutral |
| <i>GPR98</i> | c.6317C>T              | p.Ala2106Val | 0.69                    | Neutral |
| <i>GPR98</i> | c.6318G>A              | p.Ala2106Ala | 0.68                    | Neutral |
| <i>GPR98</i> | c.6608 T>C             | p.Val2203Ala | 0.34                    | Neutral |
| <i>GPR98</i> | c.6695A>G              | p.Tyr2232Cys | NA                      | UV1     |

|              |                       |              |                     |         |
|--------------|-----------------------|--------------|---------------------|---------|
| <i>GPR98</i> | c.6938C>T             | p.Pro2313Leu | 0                   | UV1     |
| <i>GPR98</i> | c.6994A>T             | p.Ile2332Phe | 0                   | UV1     |
| <i>GPR98</i> | c.7176C>T             | p.Ser2392Ser | 0.24                | UV1     |
| <i>GPR98</i> | c.7179C>T             | p.Asp2393Asp | 1.48                | Neutral |
| <i>GPR98</i> | c.7229A>G             | p.Tyr2410Cys | 0.11                | UV1     |
| <i>GPR98</i> | c.7293C>T             | p.Ala2431Ala | 0                   | UV1     |
| <i>GPR98</i> | c.7576 A>G            | p.Ile2526Val | 0                   | UV1     |
| <i>GPR98</i> | c.7821G>A             | p.Glu2607Glu | 0.36                | Neutral |
| <i>GPR98</i> | c.7874G>A             | p.Arg2625His | NA                  | UV1     |
| <i>GPR98</i> | c.9280G>A             | p.Val3094Ile | NA                  | UV1     |
| <i>GPR98</i> | c.9650C>T             | p.Ala3217Val | 0.8                 | Neutral |
| <i>GPR98</i> | c.9743G>A             | p.Gly3248Asp | NA                  | UV1     |
| <i>USH2A</i> | c.10062G>C            | p.Val3354Val | 0.11                | UV1     |
| <i>USH2A</i> | c.10510 C>G           | p.Pro3504Ala | 0.12                | UV1     |
| <i>USH2A</i> | c.11597C>T            | p.Ala3866Val | 0                   | UV1     |
| <i>USH2A</i> | c.1174C>T             | p.Pro392Ser  | 0                   | UV1     |
| <i>USH2A</i> | c.11928G>A            | p.Thr3976Thr | NA                  | UV1     |
| <i>USH2A</i> | c.12505A>G            | p.Thr4169Ala | 0                   | UV1     |
| <i>USH2A</i> | c.12823T>A            | p.Ser4275Thr | 0.11                | UV1     |
| <i>USH2A</i> | c.12910G>A            | p.Glu4304Lys | 0                   | UV1     |
| <i>USH2A</i> | c.13631G>T            | p.Gly4544Val | 0                   | UV1     |
| <i>USH2A</i> | c.1453A>G             | p.Ile485Val  | 0                   | UV1     |
| <i>USH2A</i> | c.1731C>T             | p.Cys577Cys  | 0.24                | UV1     |
| <i>USH2A</i> | c.2844 C>G            | p.Cys948Trp  | 0                   | UV1     |
| <i>USH2A</i> | c.2844C>G             | p.Cys948Trp  | 0                   | UV1     |
| <i>USH2A</i> | c.3261 C>T            | p.Ile1207Ile | 0                   | UV1     |
| <i>USH2A</i> | c.3945T>C             | p.Asn1315Asn | 1.12                | Neutral |
| <i>USH2A</i> | c.4115C>A             | p.Pro1372His | 0.12                | UV1     |
| <i>USH2A</i> | c.4560C>T             | p.Ile1520Ile | 0.46                | Neutral |
| <i>USH2A</i> | c.5142 T>C            | p.Asn1714Asn | 0                   | UV1     |
| <i>USH2A</i> | c.550A>C              | p.Thr184Pro  | 0 [0/96<br>CEPH]    | UV1     |
| <i>USH2A</i> | c.7130A>G             | p.Asn2377Ser | 0.81                | Neutral |
| <i>USH2A</i> | c.7584C>T             | p.Thr2528Thr | 0.11                | UV1     |
| <i>USH2A</i> | c.785-17_-<br>14delAT | p.?          | 0                   | UV1     |
| <i>USH2A</i> | c.8681+18A><br>G      | p.?          | 4.35 [4/92<br>CEPH] | UV1     |
| <i>USH2A</i> | c.9228C>A             | p.Asp3076Glu | 0                   | UV1     |
| <i>USH3A</i> | c.126G>A              | p.Thr42Thr   | 0                   | UV1     |
| <i>USH3A</i> | c.6A>C                | p.Pro2Pro    | 0.46                | Neutral |
| <i>WHRN</i>  | c.1166+18<br>G>A      | p.?          | NA                  | UV1     |
| <i>WHRN</i>  | c.1365T>C             | p.Ser455Ser  | NA                  | UV1     |



|               |                         |              |   |         |
|---------------|-------------------------|--------------|---|---------|
| <i>WHRN</i>   | c.1653 C>T <sup>†</sup> | p.Gly551Gly  | NA                                      | UV1     |
| <i>WHRN</i>   | c.2118A>G               | p.Pro706Pro  | NA                                      | UV1     |
| <i>WHRN</i>   | c.2485 G>A              | p.Ala829Thr  | NA                                      | UV1     |
| <i>WHRN</i>   | c.409 G>C               | p.Glu137Gln  | 0 [0/134<br>ECCAC]                      | UV1     |
| <i>WHRN</i>   | c.755A>G                | p.Gln252Arg  | 0 [0/98<br>ECCAC;<br>0/84<br>Pakistani] | UV1     |
| <i>SLC4A7</i> | c.1740-3T>C             | p.?          | NA                                      | UV1     |
| <i>SLC4A7</i> | c.2314C>T               | p.Pro772Ser  | 1.72                                    | Neutral |
| <i>SLC4A7</i> | c.3624G>A               | p.Val1208Val | 0                                       | UV1     |
| <i>SLC4A7</i> | c.402-7C>T              | p.?          | NA                                      | UV1     |
| <i>SLC4A7</i> | c.459T>C                | p.Tyr53Tyr   | NA                                      | UV1     |
| <i>SLC4A7</i> | c.756A>T                | p.Glu252Asp  | 0.12                                    | UV1     |

### Legend to Supplementary Table 3.

\*Last nucleotide of an exon. CDH23:c.8722G>A, (p.Gly2908Arg), and GPR98:c.3289G>A (p.Gly1097Ser) had no significant effect on predicted use of splice sites.

<sup>†</sup>Novel synonymous changes in which there was no other contributing evidence to their pathogenicity ie. the patient had a single or no other pathogenic/UV3/UV4 mutations accounting for their disease in the same or another gene *and* they do not appear or have not been assessed in controls, were also analysed *in silico* (Human Splicing Finder, <http://www.umd.be/HSF/> and Splice Site Prediction by Neural Network, [www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)). There was no significant effect on splicing except for the variant *WHRN*:c.1653C>T, (p.Gly551Gly); this increased splice score by >20% for creation of a novel splice site (Human Splicing Finder, <http://www.umd.be/HSF/>) - initial score of 65 for wild-type C increasing to 92 for variant T. However this variant showed no effect on splicing using Splice Site Prediction by Neural Network, [www.fruitfly.org/seq\\_tools/splice.html](http://www.fruitfly.org/seq_tools/splice.html)).

<sup>a</sup> Minimum Allele Frequency in 878 control chromosomes unless stated otherwise; NA-not assessed