Establishing Genotype–phenotype Correlation in USH2A-related Disorders to Personalize Audiological Surveillance and Rehabilitation

*†Leslie P. Molina-Ramírez, *††Eva Lenassi, *††Jamie M. Ellingford, *††Panagiotis I. Sergouniotis, †Simon C. Ramsden, §§Iain A. Bruce, and *††Graeme C. M. Black

Objective: USH2A-related disorders are characterised by genetic and phenotypic heterogeneity, and are associated with a spectrum of sensory deficits, ranging from deafblindness to blindness with normal hearing. It has been previously proposed that the presence of specific USH2A alleles can be predictive of unaffected hearing. This study reports the clinical and genetic findings in a group of patients with USH2A-related disease and evaluates the validity of the allelic hierarchy model.

Patients and Intervention: USH2A variants from 27 adults with syndromic and nonsyndromic USH2A-related disease were analyzed according to a previously reported model of allelic hierarchy. The analysis was replicated on genotype–phenotype correlation information from 197 individuals previously reported in 2 external datasets.

Main Outcome Measure: Genotype–phenotype correlations in USH2A-related disease.

Results: A valid allelic hierarchy model was observed in 93% of individuals with nonsyndromic USH2A-retinopathy (n = 14/15) and in 100% of patients with classic Usher syndrome type IIa (n = 8/8). Furthermore, when two large external cohorts of cases were combined, the allelic hierarchy model was valid across 85.7% (n = 78/91) of individuals with nonsyndromic USH2A-retinopathy and 95% (n = 123/129) of individuals with classic Usher syndrome type II (p = 0.012, χ² test). Notably, analysis of all three patient datasets revealed that USH2A protein truncating variants were reported most frequently in individuals with hearing loss.

Conclusion: Genetic testing results in individuals suspected to have an USH2A-related disorder have the potential to facilitate personalized audiological surveillance and rehabilitation pathways. Key Words: Personalized medicine—Hearing loss—Retinitis pigmentosa—USH2A-related disease—Usher syndrome.

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Defects in the Usher syndrome type IIa (USH2A) gene are an important cause of visual and auditory sensory impairment (1). To date, more than 1,050 disease-causing DNA variants (Human Gene Mutation Database, accessed July 16, 2019) have been reported. These are associated with significant clinical heterogeneity: biallelic USH2A mutations have been linked to combined congenital sensorineural hearing loss (SNHL) and
retinitis pigmentosa (USH2), nonsyndromic retinitis pigmentosa (nsRP), (2) and nonsyndromic SNHL (3).

Current understanding of the molecular mechanisms that underpin this variability remains incomplete despite the fact that recent observations on large cohorts of patients with USH2A-related disease have provided important insights (4–6). Notably, a model aiming to predict the audiological phenotype from the USH2A genotype has been previously proposed (4). This allelic hierarchy model suggests that genotypes including at least one “nsRP-enriched” allele are significantly more prevalent in individuals with nsRP than in individuals with USH2. Preservation of hearing function has also been attributable to the predicted variant consequence at the protein level: USH2A missense variants have shown a tendency to occur in association with development of retinopathy with normal hearing or less severe SNHL (5,6). Conversely, USH2A protein truncating variants have been consistently linked to the development of more severe SNHL (6). Overall, this emerging evidence may suggest that genotypic information can be used to personalise audiological surveillance of individuals with USH2A-disease.

In this study, we assessed the impact of USH2A genotypes on hearing function by investigating genotype–phenotype correlations and assessing the validity of the USH2A allelic hierarchy model. These results were then compared and combined with previous findings from two large external cohorts of patients with USH2A-related disease (5,7).

SUBJECTS AND METHODS

Study Subjects

Unrelated patients with a diagnosis of nsRP or USH2 were ascertained retrospectively through the database of the Manchester Regional Genetic Laboratory Service, Manchester, United Kingdom. Individuals with presumed biallelic disease-causing variants in USH2A were selected. A total of 27 unrelated patients with USH2A-related disease were included in the study: 15 had nsRP, 8 had classic USH2, and 4 had atypical USH2 (please see below for relevant definitions). DNA analysis was performed using gene panel testing in all 27 cases. Fourteen of these patients were tested on a 105-gene panel and 13 samples were tested on a 176-gene panel using previously described methods (8,9). A single proband also underwent genome sequencing as previously described (10).

Variant interpretation (11). Collection and retrospective analysis of available clinical data such as visual acuity, fundoscopic findings, and, in selected cases, optical coherence tomography findings was performed. Age of onset for visual and hearing symptoms, pure-tone audiograms, and/or notes with information on previous audiological evaluations were extracted from the medical records. For the purposes of this study, patients were classified as follows: 1) nsRP, patients with retinitis pigmentosa and no complaint of hearing loss, 2) classic USH2, patients with congenital/prelingual SNHL and retinitis pigmentosa, and 3) atypical USH2, patients with retinitis pigmentosa manifesting SNHL later in life.

Characterization and Classification of USH2A Variants

From This Cohort and the Biomedical Literature

The variants identified with this study were combined with previously reported USH2A variants present in the LOVD-USHBases (12) and the Human Gene Mutation Database (13). To reduce the risk of including misclassified variants and to avoid the significant effects of sampling variance at very low allele counts, we classified the following variants as “unknown/novel”: 1) all changes for which their phenotypes were not obtained and/or 2) all changes present in less than two probands. All other variants were classified as “nsRP-enriched” or “nonspecific.” The distinction between these two categories was based on the following ratio: [probands with USH2 carrying a specific USH2A variant]/[probands with nsRP carrying this USH2A variant]. To determine a cut-off value we evaluated the c.2276G > T variant, one of the most common mutations identified in patients with nsRP and a change repeatedly reported to be enriched in individuals with nonsyndromic disease (2). Following extensive curation of the literature, the c.2276G > T variant was found in 96 individuals with nsRP and in 20 individual with Usher syndrome (4). Thus, all alleles with a ratio of 1/5 or less were considered “nsRP-enriched” while alleles exceeding this ratio were considered “nonspecific.” Importantly, the above definitions suggest that nsRP-enriched alleles are expected to have a significantly smaller effect on hearing but they might still be encountered in patients with USH2.

Assessment of the USH2A Allelic Hierarchy Model

Following classification of each allele, the USH2A allelic hierarchy model was evaluated. We considered the model to be valid in two instances: 1) an individual with nsRP carrying at least one nsRP-enriched variant, 2) an individual with Usher syndrome that has no nsRP-enriched alleles (Table 1).

In addition to testing the USH2A allelic hierarchy model in our cohort, we tested it in external datasets obtained from two studies reporting findings in large cohorts of individuals with USH2A-related disease: Pierrache et al., 2016 (148 patients; 33 nsRP, 73 USH2) and Carss et al., 2017 (49 patients, 34 nsRP, 15 USH) (5,7). These studies were selected as they contain significant numbers of patients with USH2A-related disease (i.e., both Usher syndrome and USH2A-associated nsRP) and they report their clinical and genetic findings in detail in the corresponding supplementary material sections.

Statistical analyses were performed using R version 3.5.0. Fisher’s or χ² tests were used for categorical variables where applicable. A p value < 0.05 was considered statistically significant.

TABLE 1. USH2A allelic hierarchy model hypothesis

| USH2A-Phenotype | USH2A Allele Combination |
|-----------------|-------------------------|
| nsRP (patients with nsRP and no complaint of SNHL) | nsRP-enriched Any* |
| Classic Usher syndrome type IIa (RP and congenital SNHL) | Nonspecific Nonspecific |

*Any with either an nsRP-enriched, unspecific, unknown/novel allele.

nsRP indicates nonsyndromic retinitis pigmentosa; RP, retinitis pigmentosa; SNHL, sensorineural hearing loss.
| Patient-ID | Year of Birth | Sex | Clinical Diagnosis | Onset of SNHL (Decade) | Onset of Visual Symptoms (Yrs) | VA OD | VA OS | Main Ophthalmology Examination Findings | Main Ophthalmology Examination Findings | USH2A Allele 1 | USH2A Allele 2 |
|------------|---------------|-----|-------------------|------------------------|-----------------------------|-------|-------|----------------------------------------|----------------------------------------|----------------|----------------|
| 11012656   | 1984          | M   | USH2              | 1st                    | 13                          | 0.6   | 0.5   | Blunted foveal reflexes, attenuated vessels, peripheral pigmentum | p.(Cys20Alafs*71) | c.1558delT | p.(Cy520Alafs*71) |
| 12000462   | 1967          | M   | USH2              | 1st                    | 42                          | 0.04  | 0.02  | Bone spicule pigmentary changes and attenuated retinal arterioles | p.(Cys620Phe) | c.1859G>T | p.(Gly173Gly) |
| 0087001    | 1983          | F   | USH2              | 1st                    | 20                          | 1.1   | 1     | Profoundly reduced peripheral vision, peripheral pigment, attenuated vessels | NA | c.2299delG | p.(Glu767Serfs*21) |
| 11013807   | 1971          | F   | USH2              | 1st                    | 20                          | 1     | 0.48  | NA | NA | c.4474G>T | p.(Glu1492*) |
| 13008753   | 1986          | F   | USH2              | 1st                    | 19                          | 0.3   | 0     | ERG widespread photoreceptor dystrophy, bone spicule pigmentation in mid retinal periphery | p.(Glu1492*) | c.4645C>T | p.(Arg1549*) |
| 15014727   | 1990          | F   | USH2              | 1st                    | 23                          | 0.1   | 0.1   | Mid-peripheral pigmentum, ERG extinguished pattern | p.(Glu2288*) | c.6862G>T | p.(Arg1549*) |
| 10003406   | 1994          | F   | USH2              | 1st                    | 24                          | 0.02  | 0     | ERGs extinguished | NA | c.5776+1G>A | p.(Arg1549*) |
| 16017684   | 1967          | M   | USH2              | 1st                    | 22                          | 0.32  | 0.32  | Peripheral pigment, cystoid changes | p.(Glu2288*) | c.2299delG | p.Glu767Serfs*21 |
| 14014093   | 1979          | F   | Atypical USH2     | 4th                    | 33                          | 0.2   | 0.2   | Bone spicule pigmentination in nasal retina | p.(Glu2288*) | c.2299delG | p.Glu767Serfs*21 |
| 10004715   | 1970          | M   | Atypical USH2     | 5th                    | 19                          | 0.2   | 0.1   | Macule hole dry, severe peripheral pigmentum | p.(Glu2288*) | c.2276G>T | p.(Cys759Phe) |
| 13007042   | 1944          | M   | Atypical USH2     | 7th                    | 65                          | 0.3   | 0     | Retinal pigmentary changes, peripheral vision severely affected; ERGs significant receptor dystrophy | p.(Glu2288*) | c.2276G>T | p.(Cys759Phe) |
| 12008422   | 1964          | M   | Atypical USH2     | 5th                    | 20                          | 0.3   | 0.5   | Lens opacities | p.(Glu2288*) | c.6446G>A | p.(Glu2288*) |
| 14020775   | 1972          | F   | mRP               | No complaint            | 18                          | 0.4   | 0.4   | Bilateral RPE changes, macular cystic changes, dry fovea | p.(Glu2288*) | c.10073G>A | p.(Glu2288*) |
| 0070378    | 1973          | F   | mRP               | No complaint            | 31                          | 1     | 1     | ERG widespread retinopathy, peripheral pigmentum | p.(Glu2288*) | c.10073G>A | p.(Glu2288*) |
| 13015666   | 1956          | M   | mRP               | No complaint            | 17                          | NA    | NA    | NA | NA | c.13441A>G | p.(Arg448Gly) |
| 12011272   | 1965          | F   | mRP               | No complaint            | 46                          | 0.1   | 0.1   | Focal areas of bone spicules, nasal pigmentum | p.(Glu2288*) | c.1323G>A | p.(Glu2288*) |
| 15002225   | 1960          | M   | mRP               | No complaint            | 40                          | 0.3   | 0.3   | Peripheral pigmentum, preservation of central area | p.(Glu2288*) | c.14791G>T | p.(Glu2288*) |
| 13004912   | 1974          | F   | mRP               | No complaint            | 26                          | 1.4   | 1.5   | Bone spicules, attenuated vessels, cystoid macular edema | p.(Glu2288*) | c.1223G>T | p.(Glu2288*) |
| 12008423   | 1987          | M   | mRP               | No complaint            | 18                          | 0     | 0.2   | NA | NA | c.4222C>G | p.(Glu1408*) |
| 11006504   | 1962          | M   | mRP               | No complaint            | 47                          | 0.9   | 0.8   | Peripheral pigment, epiretinal membranes with retinal thickening in the left eye and left cystoid macular edema | p.(Glu1408*) | c.9413G>A | p.(Glu3138Asp) |
RESULTS

Clinical Findings

Ophthalmic findings were in keeping with USH2A-related disease in all study subjects (Table 2). The documented mean age of onset for eye disease in the 15 individuals with nsRP and 8 with classic USH2 was 31.69 and 22.88 years, respectively. The mean age of onset for the atypical USH2 subjects was 34.25 years.

All the eight individuals with classic USH2 presented with congenital-onset SNHL. Atypical USH2 patients complain of postlingual SNHL (Table 2). Pure-tone audiometry data were available in seven of eight patients with classic USH2 and in three of four patients with atypical USH2. These audiometric findings revealed bilateral, downward-sloping, moderate-to-severe SNHL patterns across frequencies 0.5, 1, 2, 4, and 8 kHz. (Fig. 1A). The mean pure-tone average hearing threshold among individuals with classic USH2 was 59.7 dBHL (SD 23.8) for low frequencies (0.25 kHz–2 kHz), 70.1 dBHL (SD 24.7) for extended mid-frequencies (0.5 – 4 kHz) and 80.9 dBHL (SD 20.7) for high frequencies (2–8 kHz). Patients with atypical USH2 showed better hearing thresholds in comparison to the individuals with classic USH2 (Fig. 1B). For this group, the mean pure-tone average was 22.5 dBHL (SD 12.4) for low frequencies (0.25–2 kHz), 31.9 dBHL (SD 16.1) for extended mid-frequencies (0.5–4 kHz), and 50.5 dBHL (SD 17.5) for high frequencies (2–8 kHz). Six of the eight classic USH2 patients received hearing amplification with hearing aids and in one case, rehabilitation with unilateral cochlear implantation was required.

Molecular Results in USH2A-related Disease

Genetic analyses of the 27 patients in our cohort identified a total of 35 likely pathogenic and pathogenic variants (Fig. 2). Of these 35 variants, 19 (54.28%) were previously identified in the literature and 15 (42.85%) were novel at the time of analysis. The most prevalent mutation was the c.2276G>T p.(Cys759Phe) missense change; this was identified in 10 study subjects with nsRP. The second most prevalent change was the c.2299delG p.(Glu767Serfs/C321) variant, identified in three individuals with Usher syndrome type II.

TABLE 2 (Continued)

| Patient-ID | Year of Birth | Sex | Clinical Diagnosis | Onset of SNHL (Yrs) | Onset of Visual Symptoms (Yrs) | VA OD | VA OS | Main Ophthalmology Examination Findings | USH2A Allele 1 | USH2A Allele 2 |
|------------|--------------|-----|-------------------|---------------------|-------------------------------|------|------|-----------------------------------------|----------------|----------------|
| 15008755   | 1951         | F   | nsRP              | 48                  | NA                            | 0.1  | 0.2  | RP changes                              | c.4645C>T       | c.4106C>T     |
| 15008804   | 1966         | M   | nsRP              | 25                  | NA                            | 0.2  | 0.2  | Peripheral pigmentum and atrophy         | c.10342G>A      | c.10342G>A    |
| 15011185   | 1973         | M   | nsRP              | 20                  | 0.8                           | 0.1  | 0.1  | Retinal pigmentary epithelium           | c.2276G>T       | c.2276G>T     |
| 15017064   | 1945         | M   | nsRP              | 56                  | NA                            | 0.2  | 0.2  | Limited RP                              | c.12574C>T      | c.12574C>T    |
| 15021183   | 1946         | F   | nsRP              | 20                  | 0.1                           | 0.1  | NA   | Bilateral dry macula                     | c.13331delC     | c.13331delC   |
| 15005941   | 1985         | F   | nsRP              | 20                  | NA                            | 0.1  | 0.1  | Bilateral dry macula                     | Exon10 to14 del | c.9974G>A     |
| 16003144   | 1946         | F   | nsRP              | 20                  | 0.1                           | 0.1  | NA   | Severe RP                               | c.3485C>A       | c.3485C>A     |

Atypical USH2 indicates retinitis pigmentosa and hearing loss complaint in adulthood; ERG, electroretinogram; HM, hands movement; NA, not available information; nsRP, nonsyndromic retinitis pigmentosa; OD, right eye; OS, left eye; RP, retinitis pigmentosa; RPE, retinal pigmentary epithelium; SNHL, sensorineural hearing loss; USH2, Classic Usher syndrome type IIa; VA, visual acuity.

Molecular Analyses

To analyze 35 USH2A-related variants, we categorized 4 of 35 as nsRP-enriched (11%), 11 of 35 variants as nonspecific (31%), and 20 of 35 variants as typical USH2A variants.
unknown/novel (57%). The localization of these variants in the respective protein domains is shown in Figure 2. We found that the USH2A-allelic hierarchy model was valid in 14 of 15 cases (93%) with nsRP. Also, all the eight cases with atypical USH2 (100%) had nonspecific alleles in keeping with the model. Of note, the four cases with atypical USH2 carried either an nsRP-enriched allele or an unknown/novel allele.

We then combined our cohort with two previously reported, large external datasets (Table S2, http://links.lww.com/MAO/A933) (5,7). These 3 cohorts altogether included 220 individuals with USH2A-related disease: 91 with nsRP (41.3%) and 129 individuals with classic USH2 (54.5%); the 4 cases with atypical USH2 from the present cohort were excluded from this analysis. A total of 172 USH2A alleles were reported accounting for disease in all these individuals. Twelve USH2A variants (14%) were categorized as nsRP-enriched alleles, 61 (32%) as nonspecific alleles, and 99 (57%) remained in the unknown/novel category (Table S1, http://links.lww.com/MAO/A932). The allelic hierarchy model was valid in 14 of 15 cases (93%) with nsRP. Also, all the eight cases with classic USH2: 

**USH2A Variants and Predicted Consequence at Protein Level**

After examining the proportion of USH2A variants based on their predicted consequence at protein level, we observed a tendency for two missense USH2A variants to be found in probands with nsRP in comparison with those with classic USH2: 

These were observed in 42% (n = 38/91) of the nsRP cases and in 11% (14/129) of the USH2 cases. In contrast, presumed two protein truncating variants, in homozygous or compound heterozygous state, were reported in 6% (6/91) of nsRP cases and in 48% (62/129) of cases with classic USH2.

**DISCUSSION**

Personalized medicine proposes to optimize patient care based on individual conditions and molecular diagnosis. Confirmation of a molecular diagnosis in individuals with inherited SNHL is swiftly gaining a role in clinical care as it has the potential to enable prompt, accurate, and personalized diagnosis and prognosis (14) as well as a personalized decision-support for rehabilitation strategy planning (15). Furthermore, presymptomatic, newborn, and preimplantation genomic testing are...
gaining momentum (16) and, as a result, predicting the natural history of a disorder from a specific genotype is becoming increasingly more relevant.

The present study illustrates the importance of establishing a molecular diagnosis in USH2A-related disease, a clinically and genetically heterogeneous condition frequently associated with SNHL. The presence or absence of congenital hearing loss is a key clinical feature that impacts management and quality of life of patients affected by this disorder. Aiming to evaluate the extent to which the audiological phenotype can be predicted by the USH2A genotype, we performed a detailed genotype–phenotype correlation study and assessed the validity of a previously proposed USH2A-allelic hierarchy model (4). This model classifies USH2A disease-causing alleles into three categories (nsRP-enriched, nonspecific, and unknown/novel) according to each allele’s prevalence in each phenotype. The model is an extension of a concept first described by Rivolta et al. (2) who identified that the c.2276G > T variant is not significantly associated with hearing loss. Overall, we observed that patients harboring at least one nsRP-enriched or unknown/novel allele (presumed to be nsRP-enriched) were consistently reported not to have prelingual-onset SNHL. The allelic hierarchy model was valid in 86% for individuals with nsRP in a combined dataset including the present cohort and two external cohorts (5,7). The common c.2276G > T allele was identified in 42 (38/91) of cases with nsRP and only 4.6% (6/129) of cases with classic USH2. We speculate that nsRP-enriched alleles allow complete or partial preservation of USH2A protein function in outer hair cell stereocilia leading to normal hearing or mild SNHL. As a result, affected individuals may develop normal speech and require fewer audiological assessments. Notably, individuals presumed to have USH2A-related nsRP may manifest SNHL later in life. While it is important to consider that the presence of SNHL in nonsyndromic retinopathy may be linked to additional extrinsic (e.g., infection, trauma, etc.) or intrinsic mechanisms (e.g., patient 13007042 manifesting likely age-related hearing loss), one cannot exclude the possibility of this being associated with underlying defects in USH2A. Future functional investigations of mutant USH2A variants (and their interaction with the rest of the Usher syndrome complex) are expected to provide important insights into the role of USH2A in photoreceptors and stereocilia. The identification of biallelic nonspecific alleles in an individual should alert the clinician to conduct a closer and detailed audiological evaluation. Notably, progression of hearing loss has been documented in USH2A-related disorders (17–19). A recent study reported individuals carrying nonspecific alleles, such as c.1256G > T or c.2299delG, to have more rapid progression and more severe hearing thresholds (6,19). It can be speculated that faster progression is associated with the presence of nonspecific alleles or protein truncating variants. As a result, there might be a
link between the need and timing of cochlear implantation and the USH2A genotype. Further studies should determine whether early intervention in patients with specific genotypes would be beneficial.

The USH2A allelic hierarchy model has a number of limitations. First, many individuals affected by USH2A-related disorders carry at least one unknown/novel variant. This reflects the frequency of previously unreported USH2A alleles and, to a lesser extent, the scarce phenotypic information available in some scientific reports. The value of the model is limited in such cases as an accurate prediction can only be made when a previously unreported change is combined with nsRP-enriched alleles. We believe that this issue may be partly addressed with the increasing availability of well-phenotyped cohorts of patients with USH2A-related disease. Second, the allelic hierarchy model is probabilistic; even if someone carries an nsRP-enriched allele, they might still present with childhood-onset SNHL—it is just that the likelihood of developing more severe hearing deterioration is significantly reduced. Outcome prediction based on genotype is a clearly complex and multidimensional task, even for a monogenic disorder such as USH2A-related diseases. The clinical presentation is likely to be due to a complex interplay of the inherited USH2A variants, changes in other genes related with SNHL, and/or environmental factors. Wider adoption of genomic testing in clinic will enable the identification of more patients with USH2A-related disorders, enabling more refined/accurate models to be developed. Lastly, due to the retrospective design of the study, data from the three datasets were combined without accounting for the methodological heterogeneity of the ascertained groups.

In summary, our findings replicate the USH2A-allelic hierarchy model and propose that careful analysis and classification of variants in USH2A-related disorders can guide targeted audiological surveillance. Detailed audiological phenotyping in large genotyped cohorts, functional work on the effect of variants in cochlear hair cell function, and study of the interaction between Usher syndrome associated proteins are expected to provide important insights. Finally, further research should be undertaken to determine whether USH2A genotype can predict the need to perform cochlear implantation in individuals with USH2A-related disorders.

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