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USH2A mutational spectrum causing syndromic and non-syndromic retinal dystrophies in a large cohort of Mexican patients

Vianey Ordoñez-Labastida,[1,2,3] Oscar F. Chacon-Camacho,[2,4] Victor R. Lopez-Rodriguez,[2] Juan C. Zenteno[1,2,5]

[1]Rare Disease Diagnostic Unit, Faculty of Medicine, National Autonomous University of Mexico (UNAM), Mexico City, Mexico; [2]Department of Genetics, Institute of Ophthalmology "Conde de Valenciana," Mexico City, Mexico; [3]Faculty of Medicine, Autonomous University of the State of Morelos (UAEM), Morelos, Mexico; [4]Carrera Médico Cirujano, Facultad de Estudios Superiores Iztacala, UNAM, Mexico City, Mexico; [5]Department of Biochemistry, Faculty of Medicine, UNAM, Mexico City, Mexico

Background: Mutations in the *USH2A* gene are the leading cause of both non-syndromic autosomal recessive retinitis pigmentosa (RP) and Usher syndrome, a syndromic form of RP characterized by retinal dystrophy and sensorineural hearing loss. To contribute to the expansion of the *USH2A*-related molecular spectrum, the results of genetic screening in a large cohort of Mexican patients are presented.

Methods: The study population comprised 61 patients with a clinical diagnosis of either non-syndromic RP (n = 30) or Usher syndrome type 2 (USH2; n = 31) who were demonstrated to carry biallelic pathogenic variants in *USH2A* in a three-year period. Genetic screening was performed either by gene panel sequencing or by exome sequencing. A total of 72 available first- or second-degree relatives were also genotyped for familial segregation of the identified variants.

Results: The *USH2A* mutational spectrum in RP patients included 39 distinct pathogenic variants, most of them of the missense type. The most common RP-causing variants were p.Cys759Phe (c.2276G>T), p.Glu767Serfs*21 (c.2299delG), and p.Cys319Tyr (c.956G>A), which together accounted for 25% of all RP variants. Novel *USH2A* mutations included three nonsense, two missense, two frameshift, and one intragenic deletion. The *USH2A* mutational spectrum in USH2 patients included 26 distinct pathogenic variants, most of them of the nonsense and frameshift types. The most common Usher syndrome-causing variants were p.Glu767Serfs*21 (c.2299delG), p.Arg334Trp (c.1000C>T), and c.12067–2A>G), which together accounted for 42% of all USH2-related variants. Novel Usher syndrome *USH2A* mutations included six nonsense, four frameshift, and two missense mutations. The c.2299delG mutation was associated with a common haplotype for SNPs located in exons 2–21 of *USH2A*, indicating a founder mutation effect.

Conclusions: Our work expands the *USH2A* mutational profile by identifying 20 novel pathogenic variants causing syndromic and non-syndromic retinal dystrophy. The prevalent c.2299delG allele is shown to arise from a founder effect. Our results emphasize the usefulness of molecular screening in underrepresented populations for a better characterization of the molecular spectrum of common monogenic diseases.

Retinitis pigmentosa (RP) is a neurodegenerative genetic disease of the retina that can lead to blindness due to loss of the light-sensing rod and cone photoreceptors [1,2]. RP has an estimated prevalence of 1 in 4,000 people, is the most common type of retinal dystrophy, and is one of the most common causes of blindness among working-age adults. At the clinical level, the disease is highly variable regarding age of onset, severity of clinical symptoms, and progression rate. RP is one of the most genetically heterogeneous disorders in humans; causal mutations in at least 69 different genes have been described so far (RetNet accessed on September 2nd, 2022).

Correspondence to: Juan C. Zenteno, Department of Genetics, Institute of Ophthalmology "Conde de Valenciana," Chimalpopoca 14, Col. Obrera, Cuauhtemoc, 06800, Mexico City, Mexico; email: jczenteno@institutodeoftalmologia.org

Homozygous or compound heterozygous mutations in the *USH2A* gene, located in 1q41 and encoding for usherin, a protein expressed in retina and cochlea [3], are the cause of 12%–25% of autosomal recessive RP cases [4,5]. Typically, *USH2A*-related RP develops in the first or second decade of life, initiating with nyctalopia (night blindness), followed by constriction of peripheral visual fields, which leads to tunnel vision and eventually loss of central vision. Biallelic mutations in *USH2A* also cause Usher syndrome, a syndromic form of retinal dystrophy characterized by RP and sensorineural hearing loss [6,7]. Usher syndrome is the main cause of blindness in combination with deafness and has an estimated prevalence of 3.2 to 6.2 cases per 100,000 people [8], although it can be as frequent as 1 in 6,000 in the general American population [9]. So far, causative mutations in at least 16 different genes have been identified in relation to the three different types of Usher syndrome, which are classified

according to their hearing and vestibular phenotypes [10]. Usher syndrome type 2 (USH2), the most common form of the disease, is characterized by the development of RP in the first or second decade of life, moderate-to-severe non-progressive congenital sensorineural hearing loss, and normal vestibular reflexes [11]. To date, three USH2 causative genes have been identified: *USH2A, ADGRV1*, and *WHRN* [12]. *USH2A* is the most prevalent USH2 causal gene, accounting for around 57%–79% of cases, as well as roughly 50% of the three types of Usher syndrome [13].

USH2A is a large gene composed of 72 exons encoding a 5202-aa matrix protein predominantly expressed in the retina and the cochlea [14]. The *USH2A* mutational spectrum is very heterogenous and includes approximately 1,700 variants classified as pathogenic or likely pathogenic by the ACMG (LOVD and HGMD databases, accessed in October 2022). Disease-causing mutations have been reported along the whole *USH2A* gene and include missense, nonsense, deletions, duplications, and splicing variants [15].

While many efforts have been made to characterize the spectrum of *USH2A* genetic variants and their associated clinical features, most of the available data were obtained from RP or USH2 patients from North America, Western Europe, and Eastern Asia [15–23]. There are limited or no data from other ethnic groups, such as Hispanics, South Asians, and Africans. Understudied populations may display a distinct allelic architecture and thus represent a possible source for the identification of both novel disease-causing variants and specific founder mutation effects in monogenic disorders.

In this work, we describe the molecular findings from a large group of Latino patients from Mexico with syndromic (USH2A) and non-syndromic (RP) retinal dystrophies caused by biallelic mutations in the *USH2A* gene. Our results expand the *USH2A* mutational profile by identifying 20 novel pathogenic variants.

METHODS

The study population comprised 61 patients with a clinical diagnosis of either non-syndromic RP or Usher syndrome who were demonstrated to carry biallelic causative variants in *USH2A* from the period of January 2020 to September 2022. The study was approved by the Institutional Review Board of the Conde de Valenciana Institute of Ophthalmology (Mexico City, Mexico) and adhered to the tenets of the Declaration of Helsinki. Medical records were reviewed to collect information about symptoms and other clinical data, such as age, gender, age of onset, family history, and age of detection of night blindness and hearing loss. All patients had a clinical diagnosis of RP based on the following criteria: a history of night blindness and diminished visual acuity associated with typical clinical signs (optic disc pallor, bone spicule pigmentation, retinal vessel attenuation). Information about self-reported hearing loss and the use of hearing aids was also obtained.

Thirty RP probands (11 males and 19 females) pertaining to 30 unrelated pedigrees, including seven families with two or more affected individuals, were ascertained. Age at genetic testing ranged from 31 to 80 years, with a mean age of 51 years. In addition, 31 USH2 probands (16 males and 15 females) pertaining to 31 unrelated families, including four pedigrees with two or more affected individuals, were studied. Age at genetic screening ranged from 21 to 62 years, with a mean age of 39 years.

Genetic analyses: USH2A disease-causing mutations were identified by either gene panel or exome sequencing. Briefly, genomic DNA (gDNA) was extracted from peripheral blood leukocytes from all analyzed individuals using the QIAaMP DNA Blood kit (Qiagen), according to the manufacturer's recommendations. Targeted genomic sequencing was performed using a hybridization-based protocol with the Illumina technology (Illumina, San Diego CA). Sequence analysis and deletion/duplication testing was performed in 298 genes included in the Invitae Inherited Retinal Disorders Panel (Invitae, San Francisco, CA). Targeted regions were sequenced with ≥50x depth, and reads were aligned to GRCh37 (Hg19) human genome sequence. Exonic deletions and duplications were called using an in-house algorithm (Invitae) that determines copy number at each target by comparing the read depth for each target in the proband sequence with both mean read depth and read depth distribution, obtained from a set of clinical samples. For exome sequencing, library preparation was performed by means of the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA); DNA was fragmented and purified using an Agencourt AMPure XP kit (Beckman Coulter Genomics, Chaska, MN). DNA fragment ends were repaired, and adaptor sequences were added to the 5′ and 3′ ends of all fragments. Subsequently, each library was purified, amplified, and hybridized to the SureSelect Human All Exon V6 probes. Index adaptors were ligated to the 5′ and 3′ ends of each sample. DNA fragments were amplified, and fragments from 250 to 350 bp were isolated. The quality of the libraries was assessed through a Bioanalyzer 2100 (Agilent Technologies). Lastly, 100 bp paired-end sequencing was performed using a HiSeq NGS platform (Illumina). The average target region coverage for the samples was >98% at ≥50x depth. Exome sequencing data were filtered using the Franklin

platform (Genoox, Palo Alto, CA). Designation of pathogenic or likely pathogenic variants was performed according to the American College of Genetics and Genomics guidelines. Variants of clinical significance were confirmed using Sanger sequencing. Copy number variant (CNV) analyses were also conducted using the Franklin platform algorithm *Rainbow*. Additionally, a total of 48 first- and second-degree healthy relatives of the probands were screened for carrier status and for confirmation of *trans* configuration of variants in cases of compound heterozygosity.

Determinations of USH2A c.2299delG-linked haplotypes: To determine the haplotype for the recurrent c.2299delG allele in *USH2A*, five single-nucleotide polymorphisms (SNPs) within the *USH2A* gene, exons 2–21, were characterized. Three SNPs were coding (c.373G>A; c.504A>G; c.4457G>A) and two were non-coding (c.3157+35G>A and c.4082–66A>C). A total of five samples carrying the c.2299delG allele were genotyped.

RESULTS

A total of 61 probands, including 30 non-syndromic RP and 31 Usher syndrome cases, carrying biallelic pathogenic or likely pathogenic *USH2A* variants were identified (Appendix 1). All patients included in the study were of Mexican–Mestizo descent and originated mainly from central and southern Mexico. According to the pedigree structure, 22 RP probands occurred sporadically and 8 were familial cases. For Usher syndrome, 27 patients were sporadic cases and 4 were familial cases.

Molecular spectrum in USH2A-related non-syndromic RP: Of 30 RP subjects, 21 were molecularly diagnosed through gene panel sequencing, while the remaining 9 were characterized by exome sequencing. From the total of 60 disease-causing alleles (Appendix 1), the most common types of variants were missense (n = 41), frameshift (n = 7), and nonsense (n = 5). A total of 39 distinct pathogenic variants were identified, including 8 previously unpublished *USH2A* variants (Table 1). Mutations located in exons 13, 63, and 6 together accounted for 52% of all 60 RP-related alleles.

The three most common RP-causing variants were p.Cys759Phe (c.2276G>T), p.Glu767Serfs*21 (c.2299delG), and p.Cys319Tyr (c.956G>A), which together accounted for 25% of all RP variants (Table 2).

Interestingly, there were 28 compound heterozygous and only 2 homozygous genotypes. Previously unpublished *USH2A* mutations included three nonsense, two missense, two frameshift, and one intragenic deletion, with each variant occurring once in the RP cohort (Table 1).

Molecular spectrum in USH2A-related Usher syndrome: Of 31 Usher syndrome subjects, 19 were diagnosed through gene panel sequencing, and the remaining 12 were characterized by exome sequencing. From the total of 62 disease-causing alleles (Appendix 1), the most common types of variants were missense (n = 19), frameshift (n = 19), acceptor splice site (n = 12), and nonsense (n = 10). A total of 26 distinct pathogenic variants were identified, including 12 previously unpublished *USH2A* variants (Table 1). Mutations located in exons 13 and 6 and introns 10 and 61 together accounted for 63% of all 62 Usher syndrome-related alleles.

The three most common Usher syndrome-causing variants were p.Glu767Serfs*21 (c.2299delG), p.Arg334Trp (c.1000C>T), and c.12067–2A>G, which together accounted for 42% of all USH2-related variants (Table 2).

There were 18 compound heterozygous and 13 homozygous genotypes. Previously unpublished Usher syndrome *USH2A* mutations included six nonsense, four frameshift, and two missense mutations (Table 1).

USH2A genetic screening in relatives: A total of 72 first- or second-degree relatives of RP or USH2 probands were genotyped. Of them, biallelic *USH2A* causal variants were confirmed in six RP-affected individuals and in four USH2 cases. Fifty-three subjects were demonstrated to be heterozygous carriers for an *USH2A* pathogenic or likely pathogenic mutation, with 20 of them carrying an RP-associated variant and 33 carrying an USH2-associated variant. In addition, nine first-degree relatives were demonstrated to carry two wild-type *USH2A* alleles.

USH2A c.2299delG-linked haplotypes: In all five samples available for analysis, the c.2299delG mutation was associated with one core haplotype, A-G-A-C-A, for SNPs located in exons 2–21 of the *USH2A* gene (Table 3). This indicates a founder mutation underlying the highly prevalent c.2299delG allele in *USH2A*-related diseases in our population.

DISCUSSION

Hispanic/Latino populations, such as Mexicans, possess a complex genetic structure that reflects recent admixture among and a potentially ancient substructure within Native American, European, and West African source populations. These admixed populations offer an opportunity for the characterization of distinct mutational profiles causing monogenic disease when compared with more well-characterized ethnic groups, such as Caucasians or Asians.

In this work, we described the disease-causing mutational spectrum of *USH2A*, a gene that is clinically relevant because its mutations are the most frequent cause

Table 1. Novel RP- and USH2-causing variants in the *USH2A* gene identified in this study.

Exon #	cDNA change	Protein change	ACMG classification	Allele frecuency
Retinitis pigmentosa				
12	c.2146A>T	p.Lys716Ter	pathogenic	1
13	c.2779C>T	p.Gln927*	pathogenic	1
15	c.2996G>T	p.Cys999Phe	likely pathogenic	1
18	c.4016T>G	p.Val1339Gly	likely pathogenic	1
38	c.7168G>T	p.Gly2390*	pathogenic	1
41	c.7940del	p.Pro2647Leufs*27	pathogenic	1
39–47	deletion		pathogenic	1
50	c.9914_9915del	p.Glu3305Valfs*40	likely pathogenic	1
Usher syndrome				
intron 10	c.1645–2A>G		likely pathogenic	2 (1 homoz.)
11	c.1860C>A	p.Cys620*	likely pathogenic	2 (1 homoz.)
11	c.1850G>A	p.Cys617Tyr	likely pathogenic	1
34	c.6638_6641del	p.Lys2213Ilefs*15	likely pathogenic	1
41	c.7809C>A	p.Cys2603*	likely pathogenic	1
46	c.9187A>T	p.Lys3063*	likely pathogenic	1
49	c.9602_9611del	p.Lys3201Ilefs*13	pathogenic	1
59	c.11516del	p.Gln3839Argfs*4	pathogenic	1
63	c.1139A>G	p.Tyr380Cys	likely pathogenic	1
63	c.13000C>T	p.Gln4334*	pathogenic	1
63	c.12313_12319del	p.Asp4105Serfs*7	likely pathogenic	1
63	c.13272C>A	p.Cys4424*	pathogenic	1

Table 2. Most common RP- and USH2-causing variants in the *USH2A* gene identified in this study.

Retinitis pigmentosa				**Usher syndrome**			
Exon #	**cDNA change**	**Protein change**	**Number of alleles (%)**	**Exon #**	**cDNA change**	**Protein change**	**Number of alleles (%)**
13	c.2276G>T	p.Cys759Phe	7 (12)	13	c.2299del	p.Glu767Serfs*21	14 (23)
13	c.2299del	p.Glu767Serfs*21	4 (7)	6	c.1000C>T	p.Arg334Trp	6 (10)
6	c.956G>A	p.Cys319Tyr	4 (7)	Intron 61	c.12067–2A>G		6 (10)
55	c.10820A>C	p.His3607Pro	3 (5)	6	c.956G>A	p.Cys319Tyr	4 (6)
63	c.12574C>T	p.Arg4192Cys	3 (5)	50	c.9799T>C	p.Cys3267Arg	3 (5)
63	c.12575G>A	p.Arg4192His	3 (5)	10	c.1841–2A>G		3 (5)

Table 3. Core haplotype associated with the *USH2A* c.2299delG allele in unrelated Mexican patients.

Sample#	Exon 2	Exon 3	Exon 13	Intron 15	Intron 18	Exon 21
	c.373G>A, p.Ala125Thr	c.504A>G, p.Thr168Thr	c.2299delG, p.Glu767fs	c.3157+35G>A	c.4082–66A>C	c.4457G>A, p.Arg1486Lys
1	A	G	+	A	C	A
2	A	G	+	A	C	A
3	A	G	+	A	C	A
4	A	G	+	A	C	A
5	A	G	+	A	C	A

of both autosomal recessive non-syndromic RP and USH2. Several *USH2A* mutations are recurrent and can be recognized frequently in distinct populations. For example, the c.2299delG (p.Glu767Serfs*21) allele is the most common deleterious mutation, accounting for up to 25% of observed pathogenic alleles [13]. Another common pathogenic variant is c.2276G>T (p.Cys759Phe), identified in approximately 5% of alleles. In our study, the c.2299delG and p.Cys759Phe alleles were observed in 12% and 7% of RP alleles, respectively, and in 22.5% and 3% of USH2 alleles, respectively. This indicates that, irrespective of the ethnic group studied, the *USH2A* c.2299delG and p.Cys759Phe alleles are major disease-causing alleles.

Our study also revealed that other *USH2A* pathogenic alleles are common in our population, such as the p.Cys319Tyr and p.His3607Pro variants, which together accounted for 12% of RP alleles, and the p.Arg334Trp and c.12067–2A>G, which together accounted for 19% of USH2 alleles in our series. The p.Cys319Tyr mutation was identified homozygously in a Hispanic American family with Usher syndrome diagnosis [24]. Further, the p.His3607Pro mutation was identified in a compound heterozygote state with other *USH2A* pathogenic variants in a Mexican individual with RP [25]. The p.Arg334Trp variant was homozygously present in six Usher syndrome patients from a consanguineous Jewish family from Morocco [26] and in two Jewish families of North African descent, suggesting a common origin for this mutation [20]. Interestingly, the p.Arg334Trp mutation was the second-most frequent mutation identified after c.2299delG in a cohort of 52 Colombian Usher syndrome patients [27]; the c.12067–2A>G mutation was identified in three Bukharian Jewish families with Usher syndrome, one in homozygosity and two in heterozygosity [20], and was also heterozygously identified in three Spanish individuals with pathogenic variants in the other *USH2A* allele [28–30].

The c.2299delG mutation has been reported in patients from Northern and Southern Europe, North and South America, North and South Africa, and China [15], with a particularly high allelic frequency of 30.6% in Scandinavia [19]. Previous studies have indicated a single haplotype shared by alleles bearing the c.2299delG mutation [31,32]. Dreyer et al. showed that the widespread geographic distribution of the c.2299delG mutation in patients from 14 countries was the result of an ancestral mutation that spread throughout Europe and into the New World because of migration [31]. In our work, haplotype analysis of samples carrying the c.2299delG allele indicated a common A-G-A-C-A core haplotype for SNPs located in exons 2–21 of the *USH2A* gene. This haplotype is in accordance with the core haplotype characterized previously in c.2299delG alleles from other ethnic groups, supporting the notion that it has spread by migration.

Previous studies have suggested that genotypes with at least one copy of the p.Cys759Phe allele are associated with isolated RP [33] and that the truncating c.2299delG variant leads to a more severe phenotype with hearing loss [7,33]. In our cohort, the p.Cys759Phe allele accounted for 7% of RP alleles and for 3% of USH2 alleles; in turn, the c.2299delG allele occurred in 22.5% of USH2 alleles and in 12% of RP alleles, thus confirming previous genotype–phenotype observations on these common variants.

The presence of two *USH2A* null or truncating alleles has been associated with a more severe visual/auditory phenotype in diverse series compared to the presence of one or two non-truncating mutations [34–36]. In our cohort, null alleles occurred in approximately 70% of USH2 alleles compared to 32% of RP alleles. In contrast, missense variants occurred in 68% of RP alleles and in 31% of USH2 alleles. Nevertheless, as previously noted, an identical *USH2A* genotype may also lead to very different phenotypes between patients.

In our study group, more than 60% of disease-causing *USH2A* mutations (either RP or USH2) occurred in only five exons: 6, 10, 13, 62, and 63. This is a relevant finding that will allow a more directed and cost-saving strategy for screening syndromic and non-syndromic RP patients in our population.

Genetic analyses in rare disease patients from underrepresented populations are important for the characterization of novel disease-causing variants and for the identification of population-specific founder effects. In the present study, 33% of individuals carried a novel *USH2A* allele and 20 novel *USH2A* pathogenic mutations were identified, thus expanding the current knowledge of *USH2A* genetic defects leading to non-syndromic and syndromic RP. Interestingly, each of these unpublished mutations occurred once in our study group, probably indicating a recent origin in this ethnic group. A larger cohort of patients would indicate whether any of these variants are frequent in Mexico, especially in regions of the country that are underrepresented in this work.

Another interesting finding in our cohort was the distinct genotypic profiles between RP and USH2 patients. Notably, only 7% of our RP subjects had a homozygous genotype, in sharp contrast with the 42% of homozygosity observed among USH2 cases. Although this could suggest that carriers of different RP-causing *USH2A* variants are widely distributed in our population, additional studies on the geographic origin and family structure of our patients are required.

In an RP patient, a novel CNV, consisting of deletion of exons 39–47 of the *USH2A* gene, was demonstrated.

While CNVs are rarely described in *USH2A*, they should be suspected particularly in subjects with a single monoallelic variant after standard NGS bioinformatic analysis. Recent data indicate that CNVs could account for up to 10% of *USH2A* pathogenic alleles, with deletion of exons 22–24 being the most common [37].

Finally, carrier detection for recessive genetic conditions is extremely useful, especially in countries like Mexico, in which endogamy and consanguineous marriage are still common in some geographic areas. In our study, a total of 62 first- or second-degree healthy relatives were tested for the respective pathogenic variant, allowing the identification of 53 heterozygous carriers of the particular *USH2A* variant and 9 wild-type homozygotes. This information is of great importance for genetic counselling and reproductive decisions in these families.

In conclusion, this paper reports the results of the largest study on *USH2A*-causing mutations in a Hispanic population. Twenty novel *USH2A* mutations causing syndromic or non-syndromic RP were demonstrated, indicating the value of conducting genetic screening in understudied populations for a better knowledge of the mutational profile leading to monogenic diseases.

APPENDIX 1. *USH2A* PATHOGENIC AND LIKELY PATHOGENIC VARIANTS IDENTIFIED IN THE PRESENT STUDY.

To access the data, click or select the words "Appendix 1."

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