


Original Investigation

Clinical Aspects of Usher Syndrome and the *USH2A* Gene in a Cohort of 433 Patients

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IMPORTANCE A new statistical approach is needed to describe the clinical differences between type I and type II Usher syndrome and between the 2 most frequent mutations in the *USH2A* gene.

OBJECTIVES To describe the primary phenotypic characteristics and differences between type I and type II Usher syndrome and to establish a phenotype-genotype correlation for the 2 most frequent mutations in the *USH2A* gene.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study at a genetics department, in which clinical evaluations were performed for 433 patients (297 unrelated families) who were classified as having type I, II, III, atypical, or unclassified Usher syndrome according to their clinical history, pedigree data, results from ophthalmological studies, and audiological, neurophysiological, and vestibular test results. Molecular studies were performed for 304 patients (256 unrelated families). The Mann-Whitney *U* test or the χ^2 test was used for calculating the differences between mean values for the analyzed parameters.

MAIN OUTCOMES AND MEASURES Age at diagnosis; age at onset of night blindness, visual field loss, visual acuity loss, and cataracts; and severity and age at diagnosis of hearing loss.

RESULTS The comparison between patients with type I Usher syndrome and those with type II Usher syndrome revealed $P < .001$ for most items analyzed. The most frequent mutations in the *USH2A* gene were the p.Glu767Serfs*21 and p.Cys759Phe mutations, with an allelic frequency of 23.2% (63 of 272 alleles) and 8.1% (22 of 272 alleles), respectively. The phenotypic analysis for patients carrying p.Cys759Phe showed $P < .001$ for most items analyzed when compared with patients carrying p.Glu767Serfs*21 and when compared with patients carrying other mutations in the *USH2A* gene. None of the p.Cys759Phe patients exhibited a severe hearing loss phenotype, and more than 60% had only mild hearing loss. Most patients carrying the p.Glu767Serfs*21 mutation (72.1%) were moderately deaf.

CONCLUSIONS AND RELEVANCE Our study presents the clinical differences between type I and type II Usher syndrome and between the 2 most frequent mutations in the *USH2A* gene. Detailed genotype-phenotype correlations, as presented in our study, allow for a better correlation of clinical signs with a known genotype and can improve the clinical management, genetic counseling, and risk assessment of patients with Usher syndrome because an estimated prognosis of their disease can be made.

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Usher syndrome is an autosomal recessive disorder with a prevalence of 3.2 to 6.2 cases per 100 000 people.¹⁻³ It is characterized by sensorineural hearing loss, retinitis pigmentosa (RP), and variable vestibular dysfunction. Usher syndrome is clinically and genetically heterogeneous and is the most common form of genetic deafness and blindness, representing 50% of all cases.⁴

The majority of patients with Usher syndrome usually fall into 1 of 3 clinical categories.⁵ Type I Usher syndrome is the most severe form and is characterized by severe to profound congenital deafness, vestibular areflexia, and prepubertal onset of RP; type II Usher syndrome manifests as moderate to severe hearing loss, absence of vestibular dysfunction, and subsequent onset of RP; and type III Usher syndrome is characterized by progressive postlingual hearing loss, variable onset of RP, and variable vestibular response. However, some cases are not easily classifiable under these categories and could be categorized as atypical Usher syndrome.^{6,7}

To date, 10 genes responsible for Usher syndrome have been identified: 6 genes responsible for type I Usher syndrome (*MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, and *CIB2*); 3 genes responsible for type II Usher syndrome (*USH2A*, *GPR98* [also known as *VLGR1*], and *DFNB31* [or *WHRN*]); and 1 gene responsible for type III Usher syndrome (*USH3A* or *CLRN1*),^{8,9} although the *HARS* gene has been recently proposed as a novel gene responsible for type III Usher syndrome.¹⁰ Another gene, *PDZD7*, was shown to contribute to type II Usher syndrome by modifying the retinal phenotype on a *USH2A* background or in digenic inheritance with *GPR98*.¹¹

With the exception of some inbred populations, in which 1 or 2 mutations account for most of the syndromic cases (eg, p.Tyr173* in the *CLRN1* gene in Finns; c.733C>T, p.Arg245* in the *PCDH15* gene in Ashkenazi Jews; and c.216G>A in the *USH1C* gene in Acadians),¹²⁻¹⁴ most of the mutations responsible for Usher syndrome are private and are found in 1 or a few families. Only the mutation c.2299delG (p.Glu767Serfs*21) on the *USH2A* gene accounts for a high proportion of cases of type II Usher syndrome of European origin with allele frequencies of 15% to 50%,¹⁵ as well as mutation c.2276C>G (p.Phe759Cys) on the *USH2A* gene, which is implicated in a 4% of mutated alleles in autosomal recessive RP in Spanish patients.¹⁶

Although the auditory and vestibular functions are the distinguishing features of the different types of Usher syndrome, RP is the main ophthalmic manifestation shared by all the types of Usher syndrome. Some authors^{1,17-20} have reported an earlier onset and more severe form of RP in patients with type I Usher syndrome. However, it is uncertain whether these differences are highly prevalent or clearly distinguishable among the different types of Usher syndrome, and some authors²¹⁻²⁵ have argued that the prognosis for visual function is not significantly different in the 2 most prevalent types (type I and type II Usher syndrome).

To date, many genetic advances have been made in the molecular diagnosis of Usher syndrome. However, the indistinguishable symptoms within the same clinical type of Usher syndrome and the similar symptoms among the different clinical types of Usher syndrome hamper this molecular diagnostic.

In the present study, we evaluated 433 patients (belonging to 297 unrelated families) who received a diagnosis of Usher syndrome. Of these 433 patients, 304 patients (256 families) underwent molecular analysis. Our aim was to quantitatively describe the primary phenotypic characteristics and differences between type I and type II Usher syndrome in this cohort and to establish a phenotype-genotype correlation for the most frequent mutations in the *USH2A* gene. This information would be useful in determining the prognosis for affected patients and would assist in genetic counseling and in assigning phenotypes for molecular study.

Methods

Audiological, Vestibular, and Ophthalmological Studies

A total of 297 unrelated families (433 patients) that were suspected of having Usher syndrome presented to our genetics department during the period from 1992 to 2012. Written informed consent was obtained from all patients and family members. All procedures were reviewed and approved by the ethics committee of the hospital and adhered to the tenets of the Declaration of Helsinki (59th World Medical Association General Assembly; Seoul, Korea; October 2008).

Patients were classified according to their clinical history, pedigree data, results from ophthalmological studies,^{18-20,25} audiological test results^{17,24,25} (or self-reported hearing loss), and neurophysiological and vestibular test results.^{17,24,25} Based on all this information, all patients were assigned to 1 of the following 4 groups: type I, type II, type III, and atypical Usher syndrome; when the available data were not sufficient for clinical classification, patients were assigned to the unclassified Usher syndrome group. Ophthalmological data were available for 193 patients with type II Usher syndrome, 57 patients with type I Usher syndrome, and 39 patients with atypical Usher syndrome (for a total of 289 patients).

To facilitate the comprehension of the *USH2A* screening results, we divided those patients with mutations in *USH2A* into 3 categories: category 1 consists of all patients presenting with 1 or 2 mutated alleles in the *USH2A* gene (including patients with type II, atypical, or unclassified Usher syndrome); category 2 consists of patients with type II Usher syndrome presenting with at least 1 mutated allele in the *USH2A* gene; and category 3 consists of all patients with type II Usher syndrome, regardless of whether or not a mutated allele was detected.

Ophthalmological status was established according to data obtained following an established protocol. These data consisted of the patient's own ophthalmological history and the ophthalmological history of his or her family (eg, onset of symptoms, age at diagnosis, and diagnosis) obtained from ophthalmological studies,^{18-20,25} including (at least) assessment of night blindness, progressive loss of peripheral vision (computerized central and peripheral visual-field testing), best-corrected visual acuity, fundus compatible with RP^{26,27} (ophthalmoscopic examination after pupillary dilation), and electrophysiological examination with pathologic electroret-

Table 1. Clinical Data (Ocular and Audiological Symptoms) for Patients With Type I or Type II Usher Syndrome

Data	Type I Usher Syndrome		Type II Usher Syndrome		P Value ^a
	Patients Analyzed, No.	Age, Mean (SD), y	Patients Analyzed, No.	Age, Mean (SD), y	
Diagnosis of disease	53	15.6 (10.4)	180	26.8 (11.4)	<.001
Onset of NB	55	9.5 (8)	187	18.1 (9.7)	<.001
Onset of VF loss	59	11.2 (8.1)	193	21.8 (11.2)	<.001
Onset of VA loss	37	19.1 (15.9)	140	25.7 (13.5)	.001
Diagnosis of cataracts	27	33.4 (13)	110	37 (13.2)	.10
Diagnosis of hearing loss	72	1.1 (0.5)	194	9.2 (11)	<.001

Abbreviations: NB, night blindness; VA, visual acuity; VF, visual field.

^a Determined by use of the Mann-Whitney *U* test.

inogram showing a marked reduction in rod or rod and cone signal²⁸ (full-field electroretinogram according to the standards of the International Society for Clinical Electrophysiology of Vision [http://www.iscev.org]).

Data on hearing loss were available for 213 patients with type II Usher syndrome, 75 patients with type I Usher syndrome, and 24 patients with atypical Usher syndrome (for a total of 312 patients). Hearing loss severity was established according to audiological tests,^{17,24,25} and patients were classified as having mild (between >25 and ≤40 dB), moderate (between >40 and ≤70 dB), or severe/profound (>70 dB) hearing loss.

Genetic Analysis

A total of 433 patients (belonging to 297 unrelated families) who received a diagnosis of Usher syndrome were evaluated for genetic study. At least 1 affected family member from each of 256 recruited families underwent molecular testing (eTable in the Supplement). Only 129 patients (from 41 unrelated families) were not analyzed owing to different causes (eg, a molecular diagnosis had already been given to an affected sibling or data on DNA were not available) (eTable in the Supplement).

Patients were analyzed using a specific Usher syndrome genotyping microarray for mutation screening (http://www.asperbio.com/asper-ophthalmics/usher-syndrome-genetic-testing)²⁸ (versions from October 2007 to December 2012). The Usher syndrome microarray detects at least 1 mutation in about 34% of the patients because it only detects those previously identified mutations that have been incorporated into the microarray,^{29,30} followed by Sanger direct sequencing of Usher syndrome genes for confirmation of mutations found on the microarray (*MYO7A*³¹ [NM_000260.3], *CDH23*^{32,33} [NM_022124.5], *PCDH15*^{34,35} [NM_033056.3], *USH1C*³⁶ [NM_005709.3], *USH1G*³⁷ [NM_173477.2], *USH2A*^{7,38} [NM_206933.2], *GPR98*³⁹ [NM_032119.3], *DFNB31*³⁹ [NM_015404.3], and *CLRN1*⁴⁰ [NM_046380.1]). Segregation (in all cases in which it was possible) and complete sequencing of the gene, if only 1 mutation was found in that gene, were performed.

Other molecular analyses included currently available commercial multiplex ligation-dependent probe amplification (MLPA) assays for Usher syndrome (the SALSA MLPA [MRC-Holland] P361-A1/P362-A2 and P292-A2 probe mixes) and the direct sequencing of genes in selected cases according to a prioritization algorithm.⁴¹ Genetic variants were classified as clearly pathological, UV4 (unclassified variant [UV] likely pathogenic), UV3 (UV of uncertain pathogenicity), UV2 (UV

likely nonpathogenic), UV1 (UV likely neutral), and clearly neutral according to the Leiden Open Variation Database (https://grenada.lumc.nl/LOVD2/Usher_montpellier/index.php).⁴²

Statistical Analysis

The statistical analysis of the differences between mean values was calculated for the different parameters analyzed. The Mann-Whitney *U* test included in SPSS version 15.0 (SPSS Inc) was used to calculate the differences in the onset of night blindness, visual field loss, and visual acuity loss and in the age at diagnosis of cataracts and of hearing loss because these variables did not present a normal distribution. The differences in severity of hearing loss were analyzed using a χ^2 test included in Epidat 4.0.

Results

As shown in the eTable in the Supplement, 61.2% (95% CI, 55.7%-66.7%) of the analyzed patients (186 of 304) carried at least 1 mutated allele in 1 Usher syndrome gene: 45 patients with type I Usher syndrome, 114 patients with type II Usher syndrome, 25 patients with atypical Usher syndrome, 1 patient with type III Usher syndrome, and 1 patient with unclassified Usher syndrome (changes classified as neutral, UV1, or UV2 have not been taken into account). The number and percentage of patients classified according to the clinical subtype and genotype, and their molecular characterization, are shown in the eTable in the Supplement.

The comparisons between the type I and type II Usher syndrome phenotypes revealed $P < .001$ for most of the items analyzed and $P = .02$ for the age at onset of visual acuity loss (Table 1). The only analyzed item that did not show $P < .05$ was age at diagnosis of cataracts ($P = .20$). The mean (SD) age at diagnosis, age at onset of night blindness, age at onset of visual field loss, age at onset of visual acuity loss, age at diagnosis of cataracts, and age at diagnosis of hearing loss for patients with type I or type II Usher syndrome are shown in Table 1.

Data on severity of hearing loss among patients with type I or type II Usher syndrome are shown in eFigure 1 in the Supplement; all patients with type I Usher syndrome (except 1 patient who reported moderate hearing loss) had severe to profound hearing loss (98.7% [95% CI, 97.4%-100.0%]), whereas patients with type II Usher syndrome had moderate hearing loss (80.3% [95% CI, 75.0%-85.6%]). The statistical analysis showed $P < .001$ for the comparison of the severity of

Table 2. Clinical Diagnosis for Patients With Mutations in the *USH2A* Gene

Type of Usher Syndrome, Mutation	Patients, ^a No.
Type II	
p.Glu767Serfs*21	47
p.Cys759Phe	9
Other	56
Total	112 ^b
Atypical	
p.Glu767Serfs*21	4 ^c
p.Cys759Phe	10
Other	9
Total	23 ^d
Unclassified	
p.Glu767Serfs*21	1 ^e
Total	136 ^f

^a These patients underwent molecular testing and presented with at least 1 mutated allele.

^b One allele was detected in 53 patients, and 2 alleles were detected in 59 patients.

^c Double heterozygous patients (p.Glu767Serfs*21 and p.Cys759Phe) have been included in the p.Glu767Serfs*21 group.

^d One allele was detected in 12 patients, and 2 alleles were detected in 11 patients.

^e Two alleles were detected.

^f One allele was detected in 65 patients, and 2 alleles were detected in 70 patients.

hearing loss between patients with type I Usher syndrome and patients with type II Usher syndrome.

Regarding the *USH2A* phenotype, 112 of 114 molecularly characterized patients with type II Usher syndrome (98.2% [95% CI, 95.8%-100.0%]) carried at least 1 mutated allele in the *USH2A* gene; a similar percentage was found for 23 of 25 molecularly characterized patients with atypical Usher syndrome (92.0% [95% CI, 81.4%-100.0%]). The clinical diagnosis received by patients who carried at least 1 *USH2A* mutated allele was type I or atypical Usher syndrome, except for 1 of the patients with unclassified Usher syndrome who was homozygous for *USH2A* (c.2299delG; p.Glu767Serfs*21) (Table 2).

Among the 136 *USH2A* mutated cases (including cases of type II, atypical, and unclassified Usher syndrome), we detected 76.1% (95% CI, 71.0%-81.2%) of the expected mutations (category 1; 207 of 272 alleles) (Table 3). Also, we found that *USH2A* accounted for 76.3% (95% CI, 70.8%-81.9%) of the mutated alleles in those cases of type II Usher syndrome with at least 1 detected mutation (category 2: 171 of 224 alleles) and for 45.5% (95% CI, 40.4%-50.5%) of the total type II Usher syndrome alleles included in our study (category 3: 171 of 376 alleles).

The most frequent mutation was p.Glu767Serfs*21, which accounted for 23.2% (95% CI, 18.1%-28.2%), 25.5% (95% CI, 19.7%-31.2%), and 15.2% (95% CI, 11.5%-18.8%) for category 1, category 2, and category 3, respectively (Table 3). The p.Cys759Phe mutation accounted for 8.1% (95% CI, 4.8%-11.3%) for category 1, 4% (95% CI, 1.4%-6.6%) for category 2, and 2.4% (95% CI, 0.8%-3.9%) for category 3 (Table 3). The rest of the mutations in *USH2A* accounted for less than 5% in all categories

(Table 3). The phenotypes associated with p.Glu767Serfs*21 and p.Cys759Phe mutations are shown in Table 4.

Patients carrying mutations in the *USH2A* gene (patients with type II or atypical Usher syndrome; clinical data were not available for the patient with unclassified Usher syndrome) were divided into 3 groups for phenotype analysis: the p.Glu767Serfs*21 group (those with at least 1 allele with the p.Glu767Serfs*21 mutation), the p.Cys759Phe group (those with at least 1 allele with the p.Cys759Phe mutation), and “other” group (those who did not have either the p.Glu767Serfs*21 mutation or the p.Cys759Phe mutation).

Patients carrying at least 1 mutated allele with p.Cys759Phe had either the type II Usher syndrome phenotype (40.9% [95% CI, 20.4%-61.4%]) or the atypical Usher syndrome phenotype (59.1% [95% CI, 38.6%-79.6%]). The p.Glu767Serfs*21 mutation in the *USH2A* gene showed a positive predictive value of 90.4% for the type II Usher syndrome phenotype, in molecularly characterized patients presenting with at least 1 mutated allele in the *USH2A* gene.

The mean (SD) age at diagnosis, age at onset of night blindness, age at onset of visual field loss, age at onset of visual acuity loss, age at diagnosis of cataracts, and age at onset of hearing loss for p.Glu767Serfs*21, p.Cys759Phe, and “other” patients are shown in Table 5. The comparison between groups p.Glu767Serfs*21 and “other” showed $P > .05$ for all analyzed parameters (data not shown). The mean values for the p.Glu767Serfs*21 group were very similar to those found in the general type II Usher syndrome group (Tables 1 and 5). The analysis of p.Cys759Phe vs p.Glu767Serfs*21 revealed $P < .01$ for most of the ophthalmological items analyzed (Table 5), with p.Cys759Phe showing a milder ophthalmological phenotype.

Although most of the patients carrying the p.Cys759Phe mutation had only mild hearing loss, and none of them had the severe hearing loss phenotype, most patients carrying the p.Glu767Serfs*21 mutation were moderately deaf (72.1% [95% CI, 58.7%-85.5%]). Data on the severity of hearing loss among patients with *USH2A* mutations are shown in eFigure 2 in the Supplement. The comparison of the severity of hearing loss between p.Glu767Serfs*21 and p.Cys759Phe patients resulted in $P < .001$, and the comparison of the severity of hearing loss between “other” and p.Cys759Phe patients resulted in $P < .001$. No difference was found in the severity of hearing loss between p.Glu767Serfs*21 and “other” patients (data not shown).

Discussion

Clinical differences between the Usher syndrome subtypes were established long ago.^{1,5} Nevertheless, it is not always easy to classify patients under any single subtype.⁶ In our analysis, it has been observed that all visual impairment in patients with type I Usher syndrome (except cataracts) appears at around adolescence, whereas for patients with type II Usher syndrome, symptoms are diagnosed in the third decade of life (Table 1). Hearing loss in patients with type I Usher syndrome is diagnosed in the first year of life, whereas for patients with type II Usher syndrome, the mean age at diagnosis of hearing loss is 9 years. These phenotypic differences can help not only with regard to the ini-

Table 3. Results Among the 136 *USH2A* Gene Mutated Cases Regarding Allelic Frequency for the *USH2A* Gene's Most Frequent Mutations^a

Mutation(s)	<i>USH2A</i> Gene Mutated Alleles, No.	Allelic Frequency of <i>USH2A</i>					
		Global Allelic Frequency of <i>USH2A</i> Among Patients With Usher Syndrome (Category 1)		Among Patients With Type II Usher Syndrome and Mutation (Category 2)		Among 188 Patients With Type II Usher Syndrome Who Underwent Molecular Testing (Category 3)	
		% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.
c.2299delG, p.Glu767Serfs*21	63	23.2 (18.1-28.2)	63/272	25.5 (19.7-31.2)	57/224	15.2 (11.5-18.8)	57/376
c.2276C>G, p.Cys759Phe	22	8.1 (4.8-11.3)	22/272	4 (1.4-6.6)	9/224	2.4 (0.8-3.9)	9/376
c.9799 t > C, p.Cys3267Arg	13	4.8 (2.2-7.3)	13/272	5.4 (2.4-8.3)	12/224	3.2 (1.4-5.0)	12/376
c.5776 + 1G>A	8	2.9 (0.9-4.9)	8/272	3.4 (1.1-6.0)	8/224	2.1 (0.7-3.6)	8/376
c.10273_10274dupTT, p.Cys3425Phefs*4	8	2.9 (0.9-4.9)	8/272	3.1 (0.8-5.4)	7/224	1.9 (0.5-3.2)	7/376
c.2431_2432delAA, p.Lys811Aspfs*11	7	2.6 (0.7-4.5)	7/272	1.8 (0.1-3.5)	4/224	1.1 (0.0-2.1)	4/376
c.10712C>T, p.Thr3571Met	6	2.2 (0.5-4.0)	6/272	2.7 (0.6-4.8)	6/224	1.6 (0.3-2.9)	6/376
c.1841-2A>G, p.Gly614Aspfs*6	5	1.8 (0.2-3.4)	5/272	2.2 (0.3-4.2)	5/224	1.3 (0.2-2.5)	5/376
Other ^b	75	27.6 (22.3-32.9)	75/272	28.1 (22.2-34.0)	63/224	16.6 (13.0-20.5)	63/376
Total	207	76.16 (71.0-81.2)	207/272	76.3 (70.8-81.9)	171/224	45.5 (40.4-50.5)	171/376

^a To facilitate the comprehension of the *USH2A* screening results, we divided those patients with mutations in *USH2A* into 3 categories: category 1 consists of all patients presenting with 1 or 2 mutated alleles in the *USH2A* gene (including patients with type II, atypical, or unclassified Usher syndrome); category 2 consists of patients with type II Usher syndrome presenting with at

least 1 mutated allele in the *USH2A* gene; and category 3 consists of all patients with type II Usher syndrome, regardless of whether or not a mutated allele was detected.

^b Less than 5 mutations.

Table 4. *USH2A* Gene p.Glu767Serfs*21 and p.Cys759Phe Mutations: Associated Phenotypes^{a,b}

Frequent Mutations in <i>USH2A</i>	Patients With Type II Usher Syndrome		Patients With Atypical Usher Syndrome	
	Molecular Study	Phenotype Analysis	Molecular Study	Phenotype Analysis
p.Glu767Serfs*21/p.Glu767Serfs*21	10	10	0	0
p.Glu767Serfs*21/other ^c	15	14	0	0
p.Glu767Serfs*21/unknown	22	20	1	1
p.Glu767Serfs*21/p.Cys759Phe			3	3
c.2299delG, p.Glu767Serfs*21, No./Total No. (% [95% CI]) of patients	47/51 (92.2 [84.8-99.5])	46	4 (7.8 [0.5-15.2])	4
p.Cys759Phe/other ^c	5	5	5	5
p.Cys759Phe/unknown	4	3	5	5
p.Cys759Phe, No./Total No. (% [95% CI]) of patients	9/22 (40.9 [20.4-61.4])	8	13 (59.1 [79.6-38.6])	13

^a Data are No. of patients, unless otherwise indicated.

^b The patient with unclassified Usher syndrome who was homozygous for the c.2299delG (p.Glu767Serfs*21) mutation has not been included.

^c Patients with a different mutation at p.Glu767Serfs*21 or p.Cys759Phe for the second mutated allele are referred to as "other" patients.

tial clinical classification but with regard to the molecular study approach, because vestibular data are not always available for all patients and because clinical data can be incomplete for all the items needed for classification.

We must keep in mind that all items analyzed, except for type I Usher syndrome hearing loss, have a variable age at onset in both type I and type II Usher syndrome groups. However, given that all the phenotypic variables analyzed showed statistical differences between type I and type II Usher syndrome (with the exception of cataracts), we can say that the onset of clinical symptoms (visual and audiological impairment) occurs later in patients with type II Usher syndrome than in patients with type I Usher syndrome. Moreover, hearing loss

in patients with type II Usher syndrome is said to be prelingual,^{17,43} although diagnosis (or awareness by patients) is usually postlingual.⁴⁴ This could be due to various reasons, such as conversational frequencies that can be only mildly affected in these patients⁴⁴ (patients may not be aware of hearing loss), very slow progression of hearing impairment (reported by some patients), and/or damaging environmental agents that can worsen hearing loss (reaching a level of impairment that the patient can notice).

When analyzing the phenotype associated with the most frequent mutations in the *USH2A* gene, we found that an evident difference appears between p.Glu767Serfs*21 and p.Cys759Phe. The p.Glu767Serfs*21 group mean values for all

Table 5. Clinical Data (Ocular and Audiological Symptoms) for Patients Carrying p.Glu767Serfs*21, p.Cys759Phe, and Other Mutations on *USH2A*^a

Data	p.Glu767Serfs*21		p.Cys759Phe		Other		P Value ^b	
	Mean (SD), y	Patients, No.	Mean (SD), y	Patients, No.	Mean (SD), y	Patients, No.	p.Glu767Serfs*21 vs p.Cys759Phe	p.Cys759Phe vs Other
Diagnosis of disease	25.2 (10.4)	34	35.1 (12.1)	19	24.8 (11.8)	51	.006	.001
Onset of NB	18.4 (9.2)	36	26.3 (11.1)	18	17.9 (10.4)	52	.009	.001
Onset of VF loss	20.4 (9.9)	40	28.2 (11.8)	19	22 (12.8)	55	.02	.01
Onset of VA loss	23.8 (15.2)	23	28.4 (12.8)	14	25.9 (12.4)	39	.20	.60
Diagnosis of cataracts	34.9 (12.0)	21	48.7 (16.6)	9	34.6 (14.2)	28	.02	.01
Onset of hearing loss	9.1 (11.0)	42	41.2 (20.0)	1	11.6 (17.6)	54	<.001	<.001

Abbreviations: NB, night blindness; VA, visual acuity; VF, visual field.

^a The mutation p.Glu767Serfs*21 is c.2299delG (p.Glu767Serfs*21), and the mutation p.Cys759Phe is c.2276C>G (p.Cys759Phe), in the *USH2A* gene. "Other" refers to patients carrying a mutation in the *USH2A* gene, but these

patients did not have either the p.Glu767Serfs*21 mutation or the p.Cys759Phe mutation.

^b Determined by use of the Mann-Whitney *U* test.

analyzed signs are very similar to the mean values for the total number of patients with type II Usher syndrome and for patients carrying other mutations of *USH2A* other than p.Cys759Phe (eFigure 2 in the Supplement; Tables 1 and 4). This observation is expected because p.Glu767Serfs*21 is the most frequent mutation found in our patients with type II Usher syndrome.

With regard to the p.Glu767Serfs*21 allele, we have found a global frequency for patients with type II Usher syndrome (15.2% [95% CI, 11.6%-18.8%]) that is similar to those previously reported in our population.^{15,45} To our knowledge, this is the first work comparing the phenotypic differences, both audiological and ophthalmological, between carriers of p.Glu767Serfs*21 and carriers of p.Cys759Phe.

The p.Glu767Serfs*21 mutation was present in 92.2% (95% CI, 84.8%-99.5%) of patients who received a clinical diagnosis of type II Usher syndrome but only in 7.8% (95% CI, 0.5%-15.2%) of patients who received a clinical diagnosis of atypical Usher syndrome. On the other hand, the p.Cys759Phe mutation was present in 40.9% (95% CI 20.4-61.4) of patients with type II Usher syndrome and 59.1% (95% CI 38.6-79.6) of patients with atypical Usher syndrome. The p.Cys759Phe mutation was initially reported to cause autosomal recessive RP without hearing impairment.⁴⁶ Our group and others^{7,16,42,46-48} have shown that compound heterozygotes of p.Cys759Phe with other *USH2A* mutations can cause autosomal recessive RP or type II Usher syndrome.

We did not find the p.Cys759Phe mutation in the homozygous state in our cohort of patients with type II Usher syndrome. However, according to our data, those compound heterozygotes carrying p.Cys759Phe appear to display an atypical type II Usher syndrome phenotype.

The phenotype of patients with type II Usher syndrome who carry the p.Cys759Phe mutation is characterized by a later onset of RP, compared with the general population of patients with type II Usher syndrome, and mild to moderate (but not severe) hearing loss. These facts can explain the late diagnosis of some patients with Usher syndrome who received an unclear diagnosis because hearing loss can be overlooked or can be diagnosed later (being interpreted as presbycusis).

In addition, variability in sensorineural hearing loss for p.Cys759Phe has already been described.^{7,38}

According to our data, patients with RP who carrying p.Cys759Phe should undergo an audiological examination (and follow-up) by an expert, to discard or correctly diagnose Usher syndrome, because a correct diagnosis has substantial implications for hearing prognosis and clinical management, and also for genetic counseling and family risk. Usher syndrome is a clinically and genetically heterogeneous disease. Our study presents, in an objective way, the clinical differences between type I and type II Usher syndrome and between the 2 most frequent *USH2A* gene mutations.

Conclusions

Many efforts have been made to improve the clinical and genetic diagnosis of Usher syndrome because the correct classification of patients as having type I, type II, or type III Usher syndrome is a very important issue not only in molecular genetic studies, defining the genetic testing strategy, but from a clinical point of view; it is crucial for the prognosis, management, and counseling of patients. In the field of molecular diagnosis, the Usher syndrome microarray was implemented to detect mutations underlying Usher syndrome cases in a cost-efficient way, independent of the clinical type. Moreover, in spite of its limitations, as only detecting previously identified mutations that have been incorporated to the microarray, its low mutation detection rate, and its need for regular updating, the microarray has proved to be a good first choice for diagnostic testing for the past few years.

Regarding the correct classification of Usher syndrome, molecular genetic diagnostic has allowed for a better understanding of this complex disease. However, diagnosis and classification remain difficult for some patients, and a great effort still needs to be made in the field of genotype-phenotype correlations. As presented in our study, detailed genotype-phenotype correlations allow for a better correlation of clinical signs with a known genotype and can improve the clinical management of Usher syndrome because then an estimated prognosis of disease can be made.

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REFERENCES

- Hope CI, Bunday S, Proops D, Fielder AR. Usher syndrome in the city of Birmingham—prevalence and clinical classification. *Br J Ophthalmol*. 1997;81(1):46-53.
- Cohen M, Bitner-Glindzicz M, Luxon L. The changing face of Usher syndrome: clinical implications. *Int J Audiol*. 2007;46(2):82-93.
- Espinós C, Millán JM, Beneyto M, Nájera C. Epidemiology of Usher syndrome in Valencia and Spain. *Community Genet*. 1998;1(4):223-228.
- Vernon M. Sociological and psychological factors associated with hearing loss. *J Speech Hear Res*. 1969;12(3):541-563.
- Littlefield JW, de Grouchy J, Ebling FJG. *Birth Defects: Proceedings of the Fifth International Conference, Montreal, Canada, 21-27 August, 1977*. Amsterdam, Netherlands: Excerpta Medica; 1978.
- Otterstedde CR, Spandau U, Blankenagel A, Kimberling WJ, Reisser C. A new clinical classification for Usher's syndrome based on a new subtype of Usher's syndrome type I. *Laryngoscope*. 2001;111(1):84-86.
- Aller E, Jaijo T, Beneyto M, et al. Identification of 14 novel mutations in the long isoform of *USH2A* in Spanish patients with Usher syndrome type II. *J Med Genet*. 2006;43(11):e55.
- Millán JM, Aller E, Jaijo T, Blanco-Kelly F, Gimenez-Pardo A, Ayuso C. An update on the genetics of Usher syndrome. *J Ophthalmol*. 2011;2011:417217.
- Riazuddin S, Belyantseva IA, Giese AP, et al. Alterations of the CIB2 calcium- and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. *Nat Genet*. 2012;44(11):1265-1271.
- Puffenberger EG, Jinks RN, Sougnez C, et al. Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One*. 2012;7(1):e28936.
- Ebermann I, Phillips JB, Liebau MC, et al. *PDZD7* is a modifier of retinal disease and a contributor to digenic Usher syndrome. *J Clin Invest*. 2010;120(6):1812-1823.
- Ben-Yosef T, Ness SL, Madeo AC, et al. A mutation of *PCDH15* among Ashkenazi Jews with the type 1 Usher syndrome. *N Engl J Med*. 2003;348(17):1664-1670.
- Joensuu T, Hämäläinen R, Yuan B, et al. Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. *Am J Hum Genet*. 2001;69(4):673-684.
- Ouyang XM, Hejtmancik JF, Jacobson SG, et al. *USH1C*: a rare cause of USH1 in a non-Acadian population and a founder effect of the Acadian allele. *Clin Genet*. 2003;63(2):150-153.
- Aller E, Larrieu L, Jaijo T, et al. The *USH2A* c.2299delG mutation: dating its common origin in a Southern European population. *Eur J Hum Genet*. 2010;18(7):788-793.
- Bernal S, Ayuso C, Antiñolo G, et al. Mutations in *USH2A* in Spanish patients with autosomal recessive retinitis pigmentosa: high prevalence and phenotypic variation. *J Med Genet*. 2003;40(1):e8.
- Kumar A, Fishman G, Torok N. Vestibular and auditory function in Usher's syndrome. *Ann Otol Rhinol Laryngol*. 1984;93(6, pt 1):600-608.
- Piazza L, Fishman GA, Farber M, Derlacki D, Anderson RJ. Visual acuity loss in patients with Usher's syndrome. *Arch Ophthalmol*. 1986;104(9):1336-1339.
- Fishman GA, Anderson RJ, Lam BL, Derlacki DJ. Prevalence of foveal lesions in type 1 and type 2 Usher's syndrome. *Arch Ophthalmol*. 1995;113(6):770-773.
- Edwards A, Fishman GA, Anderson RJ, Grover S, Derlacki DJ. Visual acuity and visual field impairment in Usher syndrome. *Arch Ophthalmol*. 1998;116(2):165-168.
- Grøndahl J, Mjøs S. Usher syndrome in four Norwegian counties. *Clin Genet*. 1986;30(1):14-28.
- Matthews TW, Poliquin J, Mount J, MacFie D. Is there genetic heterogeneity in Usher's syndrome? *J Otolaryngol*. 1987;16(2):61-66.
- Kimberling WJ, Möller CG, Davenport SL, et al. Usher syndrome: clinical findings and gene localization studies. *Laryngoscope*. 1989;99(1):66-72.
- Möller CG, Kimberling WJ, Davenport SL, et al. Usher syndrome: an otoneurologic study. *Laryngoscope*. 1989;99(1):73-79.
- Seeliger M, Pfister M, Gendo K, et al. Comparative study of visual, auditory, and olfactory function in Usher syndrome. *Graefes Arch Clin Exp Ophthalmol*. 1999;237(4):301-307.
- Marmor MF. Visual loss in retinitis pigmentosa. *Am J Ophthalmol*. 1980;89(5):692-698.
- Marmor MF. Visual acuity and field loss in retinitis pigmentosa. *Arch Ophthalmol*. 1991;109(1):13-14.
- Marmor MF, Zrenner E; International Society for Clinical Electrophysiology of Vision. Standard for clinical electroretinography (1999 update). *Doc Ophthalmol*. 1998-1999;97(2):143-156.
- Cremers FP, Kimberling WJ, Külm M, et al. Development of a genotyping microarray for Usher syndrome. *J Med Genet*. 2007;44(2):153-160.
- Jaijo T, Aller E, García-García G, et al. Microarray-based mutation analysis of 183 Spanish families with Usher syndrome. *Invest Ophthalmol Vis Sci*. 2010;51(3):1311-1317.
- Weston MD, Kelley PM, Overbeck LD, et al. Myosin VIIA mutation screening in 189 Usher syndrome type 1 patients. *Am J Hum Genet*. 1996;59(5):1074-1083.
- Bolz H, von Brederlow B, Ramírez A, et al. Mutation of *CDH23*, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat Genet*. 2001;27(1):108-112.
- Bork JM, Peters LM, Riazuddin S, et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene *CDH23*. *Am J Hum Genet*. 2001;68(1):26-37.
- Ahmed ZM, Riazuddin S, Bernstein SL, et al. Mutations of the protocadherin gene *PCDH15* cause Usher syndrome type 1F. *Am J Hum Genet*. 2001;69(1):25-34.
- Ahmed ZM, Riazuddin S, Aye S, et al. Gene structure and mutant alleles of *PCDH15*.

nonsyndromic deafness DFNB23 and type 1 Usher syndrome. *Hum Genet.* 2008;124(3):215-223.

36. Verpy E, Leibovici M, Zwaenepoel I, et al. A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nat Genet.* 2000;26(1):51-55.

37. Weil D, El-Amraoui A, Masmoudi S, et al. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet.* 2003;12(5):463-471.

38. van Wijk E, Pennings RJ, te Brinke H, et al. Identification of 51 novel exons of the Usher syndrome type 2A (*USH2A*) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. *Am J Hum Genet.* 2004;74(4):738-744.

39. Weston MD, Lujendijk MW, Humphrey KD, Möller C, Kimberling WJ. Mutations in the *VLGR1* gene implicate G-protein signaling in the

pathogenesis of Usher syndrome type II. *Am J Hum Genet.* 2004;74(2):357-366.

40. Adato A, Vreugde S, Joensuu T, et al. *USH3A* transcripts encode clarin-1, a four-transmembrane-domain protein with a possible role in sensory synapses. *Eur J Hum Genet.* 2002;10(6):339-350.

41. Garcia-Garcia G. *Usher Syndrome: Molecular Analysis of the USH2 Genes and Development of Next-Generation Sequencing Platform.* [doctoral thesis] Valencia, Spain: Universidad de Valencia; 2013.

42. Garcia-Garcia G, Aparisi MJ, Jaijo T, et al. Mutational screening of the *USH2A* gene in Spanish USH patients reveals 23 novel pathogenic mutations. *Orphanet J Rare Dis.* 2011;6:65.

43. Keats BJB, Lentz J. Usher syndrome type II. In: Pagon RA, Adam MP, Ardinger HH, et al, eds. GeneReviews [Internet]. Seattle, Washington: University of Washington; 1993-2014. <http://www.ncbi.nlm.nih.gov/books/NBK1341/>. Initial Posting December 10, 1999. Last Update August 29, 2013. Accessed October 1, 2014.

44. Abadie C, Blanchet C, Baux D, et al. Audiological findings in 100 USH2 patients. *Clin Genet.* 2012;82(5):433-438.

45. Beneyto MM, Cuevas JM, Millán JM, et al. Prevalence of 2314delG mutation in Spanish patients with Usher syndrome type II (*USH2*). *Ophthalmic Genet.* 2000;21(2):123-128.

46. Rivolta C, Sweklo EA, Berson EL, Dryja TP. Missense mutation in the *USH2A* gene: association with recessive retinitis pigmentosa without hearing loss. *Am J Hum Genet.* 2000;66(6):1975-1978.

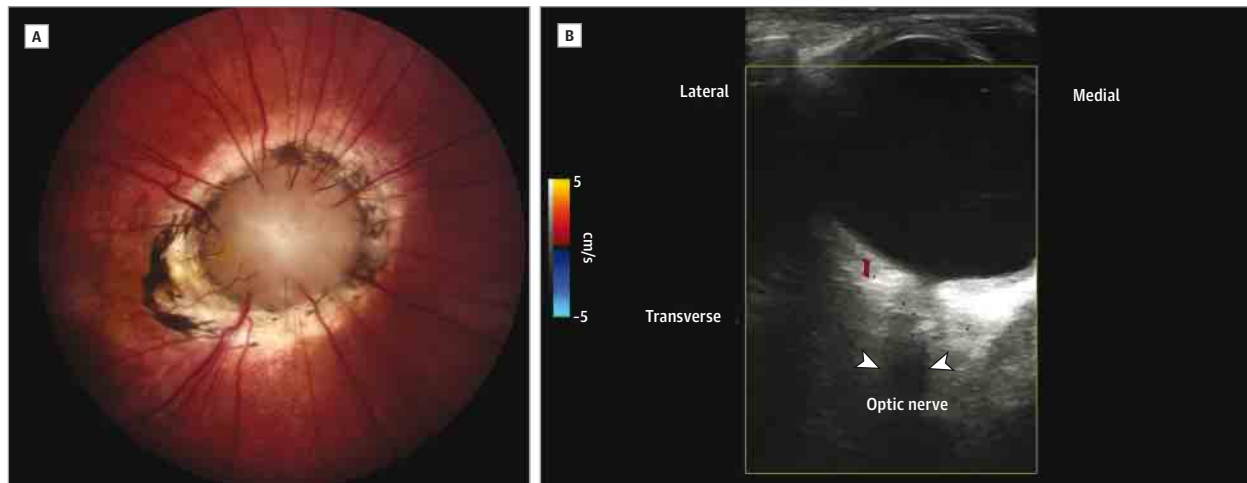
47. Herrera W, Aleman TS, Cideciyan AV, et al. Retinal disease in Usher syndrome III caused by mutations in the clarin-1 gene. *Invest Ophthalmol Vis Sci.* 2008;49(6):2651-2660.

48. Baux D, Larrieu L, Blanchet C, et al. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. *Hum Mutat.* 2007;28(8):781-789.

OPHTHALMIC IMAGES

The Moyamoya Optic Disc

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A, Morning glory disc anomaly may be associated with ipsilateral carotid dysgenesis and moyamoya disease (Japanese for the "something hazy like a puff of cigarette smoke" appearance created by dilated collateral vessels on angiography). Absence of central retinal vasculature gives rise to compensatory collateralization of chorioretinal anastomoses (cilioretinal vessels) to also impart a "moyamoya" bypass system that is ophthalmoscopically visible within the distal optic nerve. B, Color Doppler ultrasonography demonstrates the absence of vasculature within the central optic nerve (Video 1 and Video 2). Arterial flow within 1 cilioretinal artery is imaged in red.