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Identification of a novel homozygous *ARSG* mutation as the second cause of Usher syndrome type 4

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ABSTRACT

Purpose: Usher syndrome is a genetic disease characterized by combined sensorineural hearing loss, retinitis pigmentosa, and vestibular areflexia, with 15 known causative genes. Depending on the severity and onset of the symptoms, 3 different subtypes of the pathology have been classically established, although an increasing number of rare cases are being accumulated as atypical forms. The present work aims to discover the genetic cause in a patient with atypical Usher syndrome, by performing whole exome sequencing in several family members.

Observations: The obtained results identified a novel homozygous missense mutation (p.Asp44Asn) in the *ARSG* gene as the cause of the disease, which was characterized by late-onset progressive symptoms in the patient. A resembling phenotype, recently defined as the novel Usher syndrome type 4, was described in three families sharing another *ARSG* mutation. Both mutations affect two contiguous amino acid residues, which appear to be critical for the correct function of the protein.

Conclusions and Importance: These findings support the identification of the second disease mutation in this gene and a new evidence of the implication of *ARSG* in the genetic basis of Usher syndrome type 4.

1. Introduction

Usher syndrome (USH) is an autosomal recessive disease characterized by combined sensorineural hearing loss (SNHL), retinitis pigmentosa (RP) and, in some cases, vestibular areflexia. It is the leading cause of inherited deaf-blindness (MIM 268000), with an estimated prevalence of 1.6–4.4 per 10,000 individuals worldwide.[1–3] Based on the age of onset, severity, and progression of symptoms, USH has been classified into three categories. Usher syndrome type 1 (USH1) is the most severe form of the disease, characterized by congenital profound SNHL with vestibular areflexia and early-onset RP, within the first decade of life. USH2 is the most prevalent, with moderate non-progressive SNHL, normal vestibular function, and RP starting as early as adolescence. Finally, USH3 presents progressive SNHL, sometimes accompanied by vestibular dysfunction, and variable onset of RP. However, an increasing number of families presenting incompatible phenotypes with these three established categories are being accumulated as atypical forms of USH.[4,5]

Together with this reported phenotypic diversity, USH presents a high genetic and allelic heterogeneity, as observed in the majority of inherited retinal dystrophies (IRD).[6,7] To date, a total of 15 genes and 3 loci have been described as the causes of the different USH forms,[8] although some cases remain genetically undetermined. In this sense, the implementation of next generation sequencing technologies has largely contributed to the improvement of IRD and USH diagnostic rates by identifying new mutations and novel causative genes.[9] Among them, Khateb et al. described for the first time in 2018 a homozygous missense variant in the arylsulfatase G gene (*ARSG*) responsible for an atypical USH, defined as USH4 (OMIM database) and characterized by late-onset RP and usually late-onset progressive SNHL without vestibular involvement.[10] In this work, we describe a novel homozygous missense variant in *ARSG* as the second mutation associated with USH4 phenotype.

2. Case report

The patient, a 44-year-old female of Spanish origin, first reported to the Institut de Microcirurgia Ocular (IMO) (Barcelona, Spain) at the age of 40, complaining of progressive nyctalopia, with a previous history of hearing impairment since infancy. Ophthalmic examination revealed a

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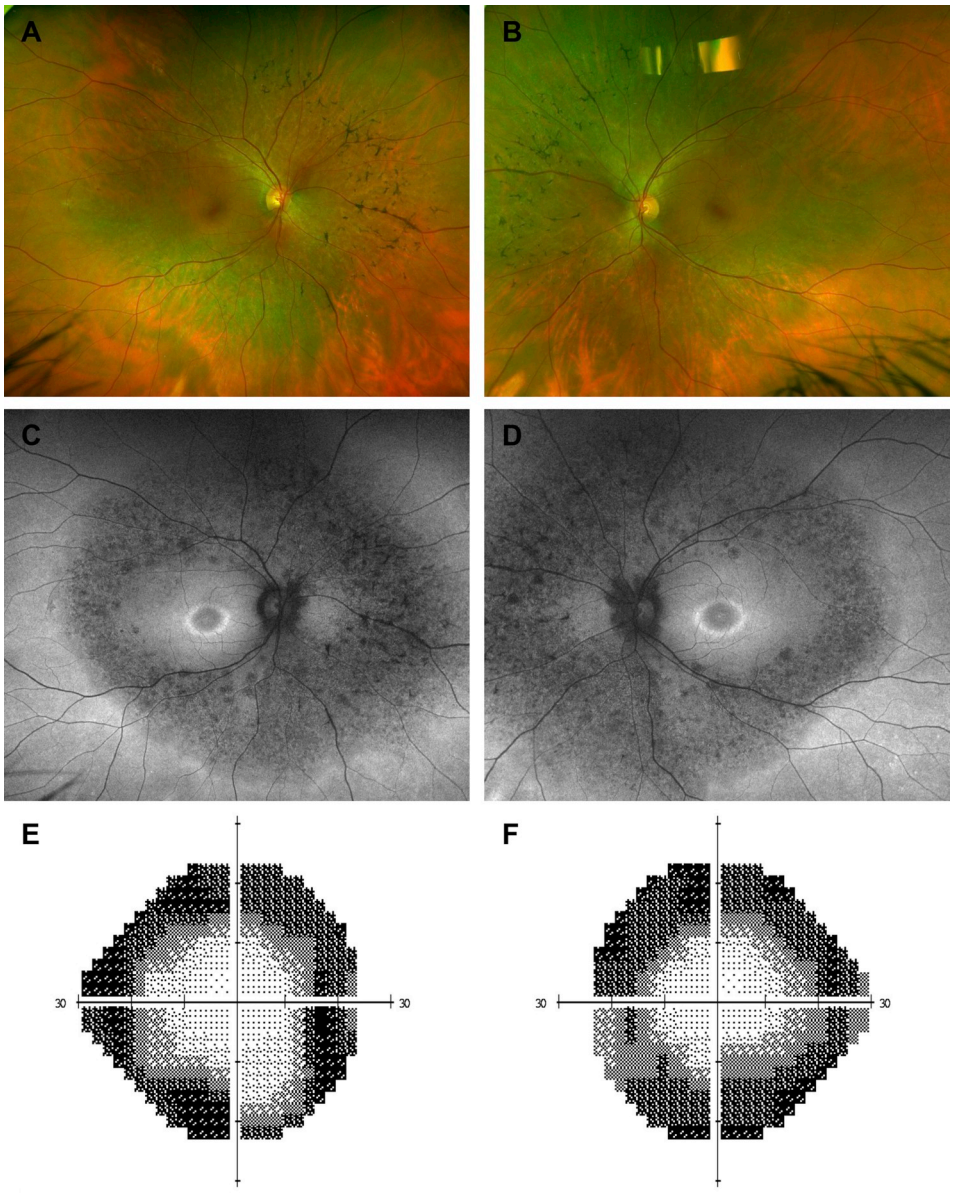

Fig. 1. Clinical evaluation of a patient with Usher syndrome type 4 presenting the homozygous c.130G > A pathogenic variant in *ARSG*. Right eye (left panels) and left eye (right panels) images of a 43-year-old patient: **A, B)** fundus retinographies show pink optic discs with only very mild pallor, normal appearing blood vessels and retinal pigment epithelium with disturbances, and bone spicule-like pigmentations in mid-periphery; **C, D)** fundus autofluorescence images present a hypoautofluorescent area at the level of the vascular arcades and a hyperautofluorescent ring surrounding the macular area; and **E, F)** automated perimetry reveals a bilateral visual field constriction with central preservation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

singular retinal phenotype consisting of a pericentral retinal pigment epithelium alteration that extended beyond the optic nerve, with mid-peripheral bone spicule-like disturbances (Fig. 1A and B). Fundus autofluorescence imaging showed a hypoautofluorescent area at the level of the vascular arcades and a hyperautofluorescent ring surrounding the macular area (Fig. 1C and D). At that time, the best-corrected visual acuity was 20/25 in both eyes, and automated perimetry revealed a bilateral visual field constriction (Fig. 1E and F). During 4 years of follow-up, the area of retinal degeneration did not increase, although fundus autofluorescence images successively obtained throughout that time period revealed that the retinal pigment disturbances became more marked, with an increase of the bone spicule–like pigmentations. Regarding the auditory system involvement, although first acute hearing difficulties appeared during infancy, SNHL was diagnosed following the RP findings. Audiometric screening results, provided by the patient, detected a moderate to severe bilateral SNHL and normal vestibular function. The speech detection and reception thresholds were, respectively, 45 dB and 50 dB in the right ear and 40 dB and 40 dB in the left ear, and the audiometric curves showed a descending pattern. All these results were sustained after three years of follow-up. According to the family history, no other relative was reported to be affected, as the patient was the only member with ocular and audiometric impairment. Moreover, the parents reported to be first cousins.

To identify the molecular cause of the disease in this case, whole-exome sequencing (WES) was performed in DNA samples from the patient, the parents, and the healthy sibling. The obtained individual data were combined and initially analyzed by filtering a panel of 279 genes associated with 13 non-syndromic and 10 syndromic IRD (RetNet and Pubmed databases), as previously described.[11] However, no potential pathogenic variants were detected. Therefore a second screening was carried out including the totality of WES covered genes. In this case, and considering the reported familiar consanguinity, homozygosity by descent was prioritized. The restrictive filtering criteria, based on the deleterious potential and the minor allele frequency (MAF $\leq$ 0.0001), allowed the identification of *ARSG, CDCP1, DTNA, GRID2IP,* and *GUCY1A2* as candidate genes (Table 1), all of which presented a putative pathogenic homozygous missense variant in the patient. Sanger sequencing was performed to further confirm these putative pathogenic variants detected by WES. Additionally, the mRNA expression was assessed for each candidate by RT-PCR, in order to discard any non-retinal gene. The results confirmed the transcription of the five genes in this tissue (data not shown). Concerning the

Table 1
Putative pathogenic variants obtained from WES analyses.

Gene	Position	Nucleotide change	Amino acid change	Existing variation	MAF	Predictors												
						Condel	SIFT	Polyphen	Fathmm	Fathmm-MKL	LRT	M-CAP	MetaLR	MetaSVM	Mut Asse	Mut Tast	PROVEAN	LoFtool
ARSG	chr17:68307623	c.130G > A	p.Asp44Asn	rs199566950	0.00002 (ExAC)	D	D	D	D	D	D	D	D	D	H	D	–	D
CDCP1	chr3:45091224	c.1942G > A	p.Gly648Ser	rs542744518	0.00001 (ExAC)	D	D	D	T	N	D	D	T	T	M	N	D	B
DTNA	chr18:34765979	c.86G > A	p.Arg29His	rs1249921119	0.00001 (GnomAD)	N	T	PD	T	D	D	D	T	T	L	D	N	D
GRID2IP	chr7:6526599	c.755C > T	p.Pro252Leu			N	D	B	T	D	U	D	T	T	L	D	D	–
GUCY1A2	chr11:106939717	c.949A > G	p.Arg317Gly	rs777157547	0.00001 (ExAC)	D	D	B	D	D	U	D	D	D	L	D	D	D

B: benign, D: damaging/deleterious/disease causing/probably damaging, H: high, L: low, M: medium, MAF: minor allele frequency, N: neutral, PD: possibly damaging, T: tolerated, U: unknown, WES: whole-exome sequencing.

pathogenicity of all the changes, the bioinformatic predictors indicated that all variants presented deleterious effects, although the highest deleterious potential was shown by the variant c.130G > A (p.Asp44Asn) in the *ARSG* gene (*NM_014960.4*) (Table 1). According to the available databases, this variant is described as an extremely rare polymorphism, with a very low minor allele frequency (*rs199566950*, MAF = 0.00002). Moreover, our data further supported this frequency, as the variant was not found in an in-house cohort of 261 control individuals. Finally, although by the time of the analyses, none of the candidate genes had been reported to cause any retinal phenotype, the recent work reported by Khateb et al. confirmed our findings and led us to consider the variant c.130G > A in *ARSG* as the most probable genetic cause of the disease in our patient.

3. Discussion

The present work strongly suggests a novel pathogenic variant in *ARSG* as the cause of an atypical USH phenotype. The patient was homozygous for the mutation c.130G > A (p.Asp44Asn) and presented a late-onset RP, consisting of a pericentral localized retinal involvement with bone spicule-like pigmentations, accompanied by a progressive moderate to severe SNHL with normal vestibular function. The auditory abnormalities started during the infancy of the patient and became more severe during the following years, after which it stabilized.

ARSG was found to be associated, for the first time, with USH in 2018, with the identification of a homozygous founder missense variant, c.133G > T (p.Asp45Tyr), in three families of Yemenite Jewish origin diagnosed with a distinctive and atypical form of USH, later defined as USH4.[10] This reported phenotype closely resembles the clinical features of our patient, thus suggesting similar molecular consequences of both pathogenic variants. In this sense, the reported c.133G > T mutation was demonstrated to alter a highly conserved aspartate residue, critical for the correct enzymatic activity of ARSG. Our mutation affects the contiguous aspartate residue, which is also highly conserved in different species and even more preserved in all the 17 human members of the sulfatase family.[10] Hence, the related USH4 phenotypes caused by these two mutations could be due to the similar crucial role of both aspartate residues in the ARSG function, although further functional assays should be performed, such as the analysis of the gene and protein expression levels, the subcellular localization, and the sulfatase activity in the ARSG mutated forms. Even though these shared USH4 phenotype could be *ARSG*-specific, other genotype-phenotype correlations should be considered, as both changes are closely located in the protein, and thus other possible molecular effects could be derived from other *ARSG* alterations. In this sense, future findings are likely to extend the number of pathogenic variants for this gene, some of which could explain other atypical USH forms, and may enforce the reconsideration of the existing subtypes of the disease.

The *ARSG* results previously published, which included an extensive and rigorous functional analysis of the enzyme, were essential for the determination of the novel mutation hereby reported.[10] However, it is worth mentioning that our obtained WES results already indicated that the c.130G > A variant in *ARSG* was the best candidate mutation to explain the disease. Thus, the analysis of combined data from multiple family members and the usage of restrictive criteria of filtering, covering all human genes, appear to be a comprehensive and powerful strategy to identify new genetic basis of pathology in consanguineous families.[12]

In conclusion, these findings represent the second genetic evidence of the *ARSG* implication in Usher syndrome and the second disease mutation causing the recently described USH4 phenotype, enhancing the role of this gene in the inherited deaf-blindness landscape and suggesting an update of the current USH subtypes classification.

4. Patient consent

The patient provided consent for the publication of the case in writing.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Ethics approval

All procedures were in accordance with the Declaration of Helsinki. Ethics approval was received from the Ethics Committee of IMO (ID number 160321_96).

Declaration of competing interest

The following authors have no financial disclosures: VA-M, RN, AB-J, and EP.

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