Novel *USH1G* homozygous variant and atypical phenotype. Still Usher 1 Syndrome?

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INTRODUCTION

Usher syndrome (USH, types 1 to 3: MIM276900, MIM276905, MIM605472) is a clinically and genetically heterogeneous autosomal recessive disorder (OMIM, Bonnet & El Amraoui 2012) characterised by different degrees of sensorineural hearing impairment coupled with retinitis pigmentosa. It accounts for more than a half of the cases of inherited deafblindness, with a prevalence estimated as being between 1/6000 and 1/25000 (Boughman et al. 1983, Hope et al. 1997, Spandau & Rohrschneider 2002, Kimberling et al. 2010). Although transmission is traditionally considered as autosomal recessive, digenic forms have been described (Bonnet et al. 2011). Type 1 (USH1) is the most severe form, with profound congenital hearing loss, rapidly progressing retinitis pigmentosa (usually manifesting in the first decade) and vestibular dysfunction as an added feature. USH1 represents 30%-40% of USH cases in the European population (Hope et al. 1997, Spandau et al. 2002).

Through the last three decades, several USH1 loci have been identified and at present seven are the known genes related to this type of Usher syndrome, including *MYO7A* (USH1B), *USH1C* (USH1C), *CDH23* (USH1D), *PCDH15* (USH1F), *USH1G* (USH1G), *CIB2* (USH1J), *ESPN* (USH1M) (Omim, Retnet, Ahmed et al. 2018).

Mutations in various Usher genes have also been shown to cause non-syndromic deafness, non-syndromic RP and atypical USH forms (Ahmed et al. 2003).

Although percentages can be very variable due to populations genetic specificity, approximately 35–39% of the observed causing mutations are found at the USH1B and USH1D loci, followed by 11% in USH1F and 7% USH1C in non-Acadian alleles, while USH1G, depending on different studies, accounts for 7% or is considered rare (Ouyang et al. 2005, Roux et al. 2006, Aller et al. 2007), with 29 pathogenic variants described since its characterization (Weil et al. 2003, HGMD). Oonk et al. (2015) describe a family affected by isolated non-syndromic hearing loss (NSHL) caused by compound heterozygous mutations in *USH1G*. A single family so far is described as affected by the *ESPN*-related form of Usher Syndrome (Ahmed et al. 2018)

Proteins encoded by Usher genes have different natures and through their protein-protein interacting domains they are integrated in a network defined as the "Usher-interactome". Usher proteins mainly colocalize at the level of stereocilia or hair bundle of the inner ear hair cells and of the synaptic and periciliary areas of photoreceptors (Millàn et al. 2011, Kremer et al. 2006, Zou et al. 2017).

USH1G gene encompasses 7.2 kb and comprises three exons, two of which are coding. The open reading frame (ORF) encodes a scaffolding protein that contains three ankyrin-like domains and a SAM (sterile alpha motif) domain and its C-terminal end presents a class I PDZ-binding motif. The SANS protein interacts with

harmonin, myosin VIIa and whirlin. These interactions integrate SANS in the Usher protein functional network responsible for the correct cohesion of the hair bundles in the inner ear (Kremer et al. 2006, Aller et al. 2007, Sorousch et al. 2017). SANS plays a role in regulating endocytosis-dependent ciliogenesis. In USH1G patients, mutations in SANS eliminate Magi2 binding and thereby deregulate endocytosis, lead to defective ciliary transport modules and ultimately disrupt photoreceptor cell function inducing retinal degeneration (Bauss et al. 2014).

Although the presence of allelic disorders is well described for genes *MYO7A*, *USH2A*, *USH1C*, *CDH23*, *PCDH15* and *CIB2*, there are only few reports of *USHIG* phenotypic variability, in which the lack of typical manifestations of the type 1 form of Usher syndrome is considered as a less severe form. Kalay et al. 2005 described affected patients bearing the D458V mutation in SANS as displaying hearing loss, mild retinitis pigmentosa symptoms and normal vestibular function. Bashir et al. (2010) described an *USH1G* deletion in a family affected by a relatively mild form of Usher syndrome, resembling more to a type 2 form. Comparing to the previously reported missense variant (Kalay et al. 2005), they conclude that both missense and deletion mutations in SANS can result in atypical Usher syndrome and that the location or type of mutation does not predict the severity of the disorder explaining the phenotypic variability as an effect of modifications by non-defined genetic or epigenetic factors.

METHODS

The subject of our study is a female patient, aged 27 at the time of our examination, born to consanguineous parents (fig. 2), presenting with profound congenital hearing loss, visual impairment (nyctalopia, concentric visual field reduction) with a reported onset at the age of 22 years, already with a clinical diagnosis of Usher syndrome. No delayed onset of independent walking (> 18months) and/or other signs of vestibular dysfunction were reported. Clinical history was taken particularly regarding the time of onset and reported symptoms of visual impairment. Parents had normal hearing, didn't report any delayed dark adaptation and had no signs of retinal degeneration on fundus examination. We performed a full ophthalmological, audiological and genetic characterization following accurate pre-test genetic counselling and the signing of a specific informed consent to the use of her anonymised clinical and genetic results in scientific reports. All the reported data were collected as part of routine diagnosis and did not require specific ethical committee approval. The research adhered to the tenets of the Declaration of Helsinki.

Audiological phenotyping. The patient underwent a complete auditory and vestibular examination. Pure tone audiometry (PTA) was performed in order to define pure-tone air and bone-conduction thresholds for frequencies ranging from 125 to 8 kHz expressed in decibel hearing level and assessment of perception by verbal speech audiometry.

The evaluation then continued with objective methods, including impedance measurement with tympanometry and stapedius reflex measurement, otoacoustic emissions (OAE), auditory evoked potentials (AEP) and vestibular examination.

ABR (Auditory Brainstem Responses) was performed, to identify the electrophysiological responses from the acoustic nerve to the sub-thalamus region. The following parameters were adopted:

• Headphones stimulation with 0.1 ms click broad-spectrum and 73 ms ISI;

• Decreasing stimulation intensity starting from 90 dB nHL, with a step of 10dB;

• Derivation: homolateral vertex-mastoid (Cz-M), bandwidth 10-4000 Hz).

The result was a total absence of all the waves of the track (from the I to V), thus confirming a neural lesion in addition to the cochlear lesion.

Since tympanogram was normal, it was possible to perform Distortion Products Otoacustic Emission (DPOAE) in order to study the function of outer hair cells. DPOAE resulted bilaterally absent, consistent with the degree of hearing loss. DPOAE was measured with a Madsen Cappella instrument, which generates two primary frequency tones, 2f1 and 2f2, with a stimulus frequency separation of f1/f2. Intensity of the custom stimulus was 40 dB sound pressure level (SPL) at both frequencies. The DPOAE was recorded by automatic scanning of the 250–8000 Hz frequency interval focused on the pure tone audiometric test frequencies.

Vestibular function was evaluated through statokinetic tests (Romberg, sensitized Romberg), detection of spontaneous nystagmus by video nystagmography, detection of evoked nystagmus by the head shaking test (HST) and the bithermal caloric test.

The latter was performed using the Fitzgerald Hallpike method (Rundle & McCabe 1961). The ears were stimulated by irrigation with hot water (44 °C) and cold (30 °C) water.

Ophthalmological phenotyping. The ophthalmologic evaluation included: best corrected visual acuity measurement, biomicroscopy, dilated fundus ophthalmoscopy, visual field evaluation, OCT, fundus autofluorescence (FA), electroretinogram (ERG). Full-field electrophysiology testing (including visual evoked potentials and both photopic and scotopic electroretinogram recordings) were performed according to ISCEV standards (Retimax, CSO, Florence, Italy).

Multicolor imaging, fundus autofluorescence imaging of 50 field of view and Spectral-domain OCT scan through the fovea were performed simultaneously with a confocal scanning laser ophthalmoscope (OCT-SLO Spectralis, Heidelberg Engineering, Germany). Electrodiagnostic testing included

Genetic testing. The family pedigree has been defined (fig. 2). DNA was extracted from whole blood using a commercial kit (E.Z.N.A. Blood DNA kit Omega Bio-tek; Norcross, GA, USA). The following 13 genes known to be associated with Usher syndrome (types 1, 2 and 3) were analysed by massive parallel sequencing (Illumina MiSeq, PE 2x150 bp protocol) using a custom designed panel: *GPR98* (OMIM *602851), *PDZD7* (OMIM *612971), *CIB2* (OMIM *605564), *USH1G* (OMIM *607696), *USH1C* (OMIM *605242), *DFNB31* (OMIM *607928), *MYO7A* (OMIM

*276903), *HARS* (OMIM *142810), *CDH23* (OMIM *605516), *ABHD12* (OMIM *613599), *USH2A* (OMIM *608400), *CLRN1* (OMIM *606397), *PCDH15* (OMIM *605514).

The human reference sequence GRCh38 was used to map the obtained sequences. Sequence variant calling was then performed using Varscan (version v2.3), Bcftools of SAMTools (version 0.1.19-44428cd) and GATK Unified Genotyper, followed by filter-based annotation using Annovar software and public databases such as 1000 Genomes (http://www.1000genomes.org/), Exome Variant Server (evs.gs.washington.edu/EVS) and dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) databases. Specific databases such as Human Gene Mutation Database (HGMD), LOVD (Leiden Open Variation Database) and HumsVar (http://omictools.com/humsavar-tool) were searched for pathogenic variations. For each variant, *in silico* pathogenicity prediction was performed using SIFT (Sorting Intolerant From Tolerant) and PolyPhen-2 (Polymorphism Phenotyping v2) algorithms through the Ensembl Variant Effect Predictor tool (McLaren et al., 2016) and Mutation Taster (http://www.mutationtaster. org/). Identified variants were confirmed through Sanger sequencing (CEQ8800 Sequencer, Beckman Coulter).

RESULTS

Audiological phenotyping confirmed the presence of a profound bilateral symmetrical cochlear sensorineural hearing loss (SNHL) resulting in a severe speech

impairment. Notedly our patient (and her parents when she was minor) always refused a cochlear implant. Previous reports of cochlear implant in USH patients highlight that the best speech results are directly associated with cochlear implantation before the age of 5 years and with emphasis on pre- and post-implantation oral communication therapy (Liu et al. 2008, Jatana et al. 2013). Tympanometry (performed at a frequency of 226Hz) underlined a normal function of the middle ear, with an intact ossicular tympanic system, in absence of effusion or inflammatory disease affecting the eardrum (Type A tympanogram). Acoustic reflex was absent in both ears, ipsilaterally and contralaterally, as a confirmation of the profound sensorineural hearing impairment. Our patient had no otoacoustic emissions and ABR (Auditory Brainstem Responses) resulted in a total absence of all the waves of the track (from the I to V).

Vestibular function examinations detected the absence of spontaneous and positional nystagmus.

Best corrected visual acuity (BCVA) expressed in LogMAR was: Right Eye (RE) 0.1 (cyl. + 2 α 90°); Left Eye (LE) 0.1 (cyl. + 1.50 α 105°). A subcapsular posterior lens opacity was present in both eyes, having an effect on fundus images quality. No cystoid macular oedema was present.

Electrodiagnostic tests showed a marked impairment of the scotopic component and near to normal photopic ERG. Flash visual evoked potentials showed normal responses (data not shown).

Multicolor imaging showed normal colour reflectivity in both eyes in macular area (Fig.1, A-D). Morphological assessment of retinal structure in the macular area (Mitamura et al. 2012) was carried out through Fundus Autofluorescence imaging (FAF) (Fig.1, B-E) and optical coherence tomography (OCT) (Fig.1, C-F).

FAF shows in both eyes typical hyperautofluorescent rings located inside the vascular arcades (better visualization in the left eye) and hypoautofluorescent spots in the mid-periphery.

In both eyes, SD-OCT revealed preserved external limiting membrane (ELM) and inner segment ellipsoid (ISe) across the fovea.

We observed spatial correlations between inner/outer ring borders and ISe/ELM disruption. In fact, inner ring border was located at approximately the same level as ISe disruption and outer ring border was located at approximately the same location as ELM disruption (where ELM disappeared or fused with RPE). SD- OCT images corresponding to the ring, suggest the absence of outer segments this area. The ring can be considered as an early stage of disease with relatively preserved central visual function (Fakin et al. 2012). In accordance with previous studies we believe that photoreceptor inner segments or cell bodies could be the location of abnormal bisretinoid accumulation, possibly contributing to photoreceptor apoptosis (Cottet & Schorderet 2009, Maeda et al. 2008).

Persistence of ISe was seen in both eyes in the area within the rings showing good spatial correlation with the area of normal autofluorescence.

Patient's parents reported to be second cousins (Fig.2) and had no clinical and/or instrumental signs of audiologic or visual impairment.

The mean NGS coverage of targeted bases was 297.77 X with 98.82% being covered to at least 25 X. Genetic testing of the proband revealed the following homozygous variant: *USH1G*: NM_173477: c.1187T>A: p.Leu396Gln. The variation was subsequently confirmed by Sanger sequencing (Fig. 3).

DISCUSSION

Variations in the *USH1G* gene have commonly been associated with clinical phenotypes consistent with complete Usher syndrome type 1, therefore characterised by congenital sensorineural hearing loss coupled with vestibular dysfunction and prepubertal onset of retinitis pigmentosa. The same phenotype can be caused by six other Usher syndrome type 1 genes (*MYO7A*, *CDH23*, *USH1C*, *PCDH15* and *CIB2*, plus the recently added gene *EPTN*) also known to be involved in non-syndromic hearing impairment, indicating a genetic and phenotypic variability in the spectrum of USH1 genes.

USH1G mutations are a rare cause of Usher syndrome and a limited number of cases have been described in the literature. Despite this, a certain phenotypic variability has already been reported (Kalay et al. 2005, Bashir et al. 2010). The phenotypic heterogeneity seen in patients carrying *USH1G* mutations does not appear to depend on the type of variation, suggesting that clinical manifestations may be modified by other genetic or epigenetic factors (Bashir et al., 2010).

The *USH1G* variant here reported is novel and has never been associated with Usher syndrome or other isolated inner ear impairments or retinopathies. We did not find it in any of the databases searched, which included ExAC, 1000 Genomes and EVS. The minor allele frequency is therefore unknown. The aliphatic and hydrophobic amino acid leucine in position 396 is highly conserved across species (Fig. 3), and its replacement by polar glutamine is predicted to be disease causing, deleterious and probably damaging by Mutation Taster, Sift and Polyphen *in silico* prediction software, respectively.

Significantly, the variant lies in the sterile alpha motif (SAM) domain (aa positions 384-446) (Weil et al., 2003) of the SANS protein that interacts with the post synaptic density, disc large, zonula occludens (PDZ1) domain of the harmonin protein, encoded by *USH1C*, forming the harmonin/Sans complex (Yan et al., 2010). Mutations that destabilize the harmonin/Sans complex have been shown to impair the development and function of sensory cells in the inner ear and retina (Yan et al., 2010).

Our findings expand the spectrum of pathogenic variants in *USH1G*, a rare cause of USH1 syndrome, highlighting at the same time the high variability of phenotypic expression in genes belonging to the USH complex.

Our proband showed severe hearing loss and mild second decade onset RP with normal vestibular function. When compared with other non-typical cases described in the literature (Kalay et al. 2005, Bashir et al. 2010), the phenotype appears similar to

that of a consanguineous Pakistani family described by Bashir et al. (2010). In both cases, the clinical picture largely suggested a diagnosis of USH type 2.

In our and in other previously described cases, the absence of vestibular dysfunction, instrumentally verified, coupled with the relative mildness of retinitis pigmentosa, leads to the conclusion that *USH1G* should be considered as a gene that can cause both Type 1 and Type 2-like Usher syndrome.