Severe retinal degeneration at an early age in Usher syndrome type 1B associated with homozygous splice site mutations in MYO7A gene

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Abstract

Purpose: Usher syndrome is the most common cause of deafness associated with visual loss of a genetic origin. The purpose of this paper is to report very severe phenotypic features of type 1B Usher syndrome in a Saudi family affected by positive homozygous splice site mutation in MYO7A gene.

Methods: Affected siblings went through detailed history. Complete ophthalmic examination was done. Imaging with colour fundus photography, fundus autofluorescence (AF), and optical coherence tomography (OCT) scans was performed. Full field electroretinogram (ffERG) was recorded. Molecular genetic testing was done using next-generation sequencing.

Results: Visual acuity was more reduced (range 20/300–20/40) in older siblings (age >30 years), than in younger (age <30 years) siblings (range 20/70–20/25). OCT scans showed macular atrophy in all but one case that has cystoid macular edema (CME). AF demonstrated atrophy outside a small foveal area showing high signal. FfERG was flat in all cases. The homozygous splice site mutation c.470+1G>A in intron 5 of the MYO7A gene was detected in all affected siblings.

Conclusions: This mutation manifested with advanced retinal degeneration at a young age. This may have implications regarding future gene therapy in Usher syndrome cases with this genotype.

Keywords: Usher syndrome, Retinitis pigmentosa, Electroretinogram, Fundus autofluorescence, MYO7A mutation

Introduction

Usher syndrome is the most common cause underlying genetically associated deafness and blindness. The syndrome is named after Charles Usher (a British ophthalmologist) and has an estimated incidence of 3–4.4 per 100,000 people. The disorder is transmitted through an autosomal recessive inheritance and is clinically and genetically heterogeneous. Affected persons have congenital sensorineural hearing loss and progressive pigmentary retinopathy. The cilium is a common cellular organelle in the inner hair cells and photoreceptors of the retina. In Usher syndrome, a dysfunction of the cilium results in combined hearing loss and visual dysfunction. Three clinical subtypes of
Usher syndrome have been identified, with mutations in different sets of genes. Usher syndrome type 1 (USH1) is the most severe of the three USH subtypes with profound hearing loss since birth, vestibular areflexia, and retinitis pigmentosa appearing at a prepubescent age. Usher syndrome type 2 (USH2) displays moderate to severe hearing loss, absence of vestibular function, and later onset of retinal degeneration. Usher syndrome type 3 (USH3) shows progressive postlingual hearing loss, variable onset of RP, and variable vestibular response.

All three subtypes of Usher syndrome share the classic ophthalmologic findings seen in retinitis pigmentosa and are described as a triad of attenuated retinal blood vessels, waxy pallor of the optic disc, and intraretinal pigmentation in a bone-spicule pattern. Retinal vascular attenuation was found in 94% of 384 eyes and optic disc pallor in 52% in one series. The macula becomes affected in moderate or advanced disease, when photoreceptor degeneration advances and leads to retinal thinning and loss of visual acuity. Cataracts, especially posterior subcapsular, affect approximately 50% of patients with retinitis pigmentosa. The vitreous may contain a dust-like pigmented substance, compromised of pigment granules. Complete vitreous detachment is more common than in normal subjects.

Usher syndrome involves at least 12 loci among the three different clinical subtypes. Genes identified for the more commonly inherited loci include MYO7A (encoding myosin VIIa), USH2A (encoding usherin), CDH23 (encoding cadherin 23), PCDH15 (encoding protocadherin 15), USH1C (encoding harmonin), USH3A (encoding clarin 1), and USH1G (encoding SANS). Transcripts from all these genes are found in many tissues/cell types other than the inner ear and retina, but all are uniquely critical for retinal and cochlear cell function.

MYO7A encodes for a large (2215 aminoacid actin-based) motor protein expressed in the cochlear hair cells as well as retinal photoreceptors and in the retinal pigment epithelium (RPE). Although not fully understood, myosin VIIa is important for maintaining the structure and function of retinal photoreceptors and retinal pigment epithelium (RPE). In photoreceptors, it is involved in transport of Rhodopsin from the rod inner segments to the outer segments. In the RPE, it is vital for localization of melanosomes in the apical microvilli and normal mobility of phagosomes.

Patients & methods

This is a retrospective study of a large consanguineous Usher syndrome family that consists of 12 children comprising 2 affected daughters, 4 affected sons, 3 unaffected daughters and 3 unaffected sons. The parents are from the same tribe with no history of vision or hearing problems. Informed consent for genetic testing and inclusion in the study was obtained from affected family members. Consent for inclusion in the study was obtained from non-affected family members except for the father who was not available for consultation. Institutional Review Board (IRB)/Ethics Committee approval at King Khaled Eye Specialist Hospital was obtained. The research adhered to the tenets of the Declaration of Helsinki. Table 1 summarizes the demographic and clinical findings in the 6 affected siblings.

The age range of the children was 17–40 years. The age of the affected siblings ranged from 17 to 38 years. A detailed medical and family history was obtained from their mother. This included information regarding age of onset of hearing loss and age of perceived night blindness (Table 1).

All the family members except the father underwent complete ophthalmologic examination including best-corrected visual acuity (VA), intraocular pressure (IOP) measurement, slit lamp examination, and dilated fundus examination. Color fundus photos were obtained for all cases using Topcon fundus camera (Top TRC-50DX). Fundus autofluorescence (AF) imaging was done for all cases using wide field Optos system (Optos 200TX) with 488 nm wavelength.

Retinal structure was analyzed qualitatively with transfoveal horizontal spectral domain optical coherence tomography scans (OCT, Heidelberg Engineering, Inc., Heidelberg, Germany) and wide field imaging (Optos PLC, Dunfermline, UK). Visual field testing was not done because of the severity of deafness and associated speech difficulties that made communication for testing difficult.

Retinal function was evaluated in affected siblings with full-field electroretinography (ffERG, Nicolet Biomedical Instruments, Madison, Wisconsin, USA), in dark adapted and light adapted state according to ISCEV standards, with a few modifications as follows. Full-field electroretinograms were recorded in a Nicolet analysis system (Nicolet Biomedical Instruments, Madison, Wisconsin, USA), after dark adaptation of subjects for 40 min, dilatation of the pupils with topical cyclopentolate 1% and metaxedrine 2.5% and topical anaesthesia, with a Burian Allen bipolar contact lens and a ground electrode applied to the forehead. Responses were

Table 1. Demographics, clinical, and imaging findings in affected siblings from a family with the homozygous donor splice site mutation c.470+1G>A in intron 5 of the MYO7A gene.

| Case | II:2 | II:3 | II:4 | II:8 | II:11 | II:12 |
|------|------|------|------|------|-------|-------|
| Age (age of examination) | 38 | 36 | 34 | 27 | 22 | 17 |
| Gender | Female | Female | Male | Male | Male | Male |
| Age of onset of hearing loss | 6 months | 6 months | 5 months | 4 months | 4 months | 4 months |
| Age of onset of visual symptoms | 6 years | 6 years | 5 years | 5 years | 5 years | 5 years |
| BCVA OD | 20/300 | 20/40 | 20/300 | 20/60 | 20/70 | 20/40 |
| BCVA OS | 20/300 | 20/40 | 20/200 | 20/50 | 20/40 | 20/25 |
| IOP OD | 17 | 17 | 15 | 13 | 13 | 10 |
| IOP OS | 16 | 15 | 14 | 11 | 13 | 11 |
| Foveal thickness OD | 142 | 153 | 210 | 171 | 110 | 393 |
| Foveal thickness OS | 130 | 193 | 199 | 153 | 95 | 340 |
| fERG | Non-recordable | Non-recordable | Non-recordable | Non-recordable | Non-recordable | Non-recordable |

* Central subfield thickness in micro-meters on horizontal transfoveal spectral domain optical coherence tomographyscans. fERG = Full field electroretinography. OD = right eye. OS = left eye. BCVA = best corrected visual acuity.
obtained stimulating with single full-field flash (30 ms) with blue light light (0.81 cd-s/m²: rod response) and with white light (10.02 cd-s/m²: combined rod-cone response). Photopic responses were obtained with a background illumination of 3.4 cd-s/m² in order to saturate the rods.

Audiometry was done for affected siblings and it confirmed profound sensorineural hearing loss in all of them confirming the diagnosis of type 1 Usher syndrome.

For the proband (II:8), molecular genetic testing in the proband included next-generation sequencing (NGS, Illumina HiSeq 1500, performed by Center for Human Genetics Bioscientia, Ingelheim, Germany) of the known genes involved in Usher syndrome, comprising 398 exons: MYO7A/USH1B, USH1C, CDH23/USH10, PCDH15/USH1F, USH1G, USH2A, OFNB31/USH20, GPR98/USH2C, CLRN1/USH3A, PDZ07/digenic and CIB2, as follows: 12-14

Genomic DNA was fragmented, and the coding exons of the analyzed genes as well as the corresponding exon–intron boundaries were enriched using the Roche/NimbleGen sequence capture approach, amplified and sequenced simultaneously by Illumina technology (next-generation sequencing, NGS) using an Illumina HiSeq1500 system. The target regions were sequenced with an average coverage of 472-fold. For 99% of the regions of interest a 20-fold coverage was obtained. NGS data analysis was performed using bioinformatic analysis tools as well as JSI Medical Systems software (version 4.1.2). Identified variants and indels were filtered against external and internal databases and filtered depending on their allele frequency focusing on rare variants with a minor allele frequency (MAF) of 1% or less. Nonsense, frameshift and canonical splice site variants were primarily considered likely pathogenic. Assessment of pathogenicity of identified non-synonymous variants was performed using bioinformatic prediction programs like Mutation Taster, Polyphen-2, Mutation Assessor and FATHMM. Variants that have been annotated as common polymorphisms in databases or in the literature were not considered further.

Putatively pathogenic differences between the wildtype sequence (human reference genome according to UCSC Genome Browser: hg19, GRCh37) and the patient’s

Fig. 1. Pedigree of a family with Usher syndrome and the homozygous donor splice site mutation c.470+1G>A in intron 5 of the MYO7A gene showing the unaffected consanguineous parents, 3 unaffected sons, 3 unaffected daughters, 2 affected daughters, and 4 affected sons.

Fig. 2. Color photos of the affected siblings in a family with the homozygous donor splice site mutation c.470+1G>A in intron 5 of the MYO7A gene. A: right eye of sibling II:2. B: left eye of sibling II:2. C: right eye of sibling II:3. D: left eye of sibling II:3. E: right eye of sibling II:4. F: left eye of sibling II:4. G: right eye of sibling II:8. H: left eye of sibling II:8. I: right eye of sibling II:11. J: left eye of sibling II:11. K: right eye of sibling II:12. L: left eye of sibling II:12. (Note the typical RP features in all cases (pigmentary changes, vascular attenuation, and disc pallor). There are widespread RPE atrophic changes sparing a small central macular area with arrows pointing to the possibly preserved central macular area.)
sequence mentioned and interpreted in this report were validated using polymerase chain reaction (PCR) amplification followed by conventional Sanger sequencing. The resulting sequence data for the $\text{MYO7A}$ gene (OMIM 276903; Locus: Chromosome 11q13.5) were compared to the reference sequence NM_000260.3.

For the siblings (II:2, II:3, II:4, II:11, II:12), the following was done: Genomic DNA was screened for the mutation c.470+1G>A in the $\text{MYO7A}$ gene on chromosome 11q13.5 (OMIM 276903). Therefore, exon 5 of the $\text{MYO7A}$ gene was amplified by polymerase chain reaction (PCR) and analyzed by direct sequencing. The resulting sequence data were compared to the reference sequence NM_000260.3.

Results

NGS of known RP and Usher syndrome genes revealed the homozygous splice site mutation c.470+1G>A in intron 5 of the $\text{MYO7A}$ gene, which was confirmed by conventional Sanger sequencing. This substitution affects the invariant GT donor splice site of intron 5 and has been previously described in homozygous state in a family with Usher syndrome type 1B. Therefore, exon 5 of the $\text{MYO7A}$ gene was amplified by polymerase chain reaction (PCR) and analyzed by direct sequencing. The resulting sequence data were compared to the reference sequence NM_000260.3.

For the siblings (II:2, II:3, II:4, II:11, II:12), the following was done: Genomic DNA was screened for the mutation c.470+1G>A in the $\text{MYO7A}$ gene on chromosome 11q13.5 (OMIM 276903). Therefore, exon 5 of the $\text{MYO7A}$ gene was amplified by polymerase chain reaction (PCR) and analyzed by direct sequencing. The resulting sequence data were compared to the reference sequence NM_000260.3.

In this family (Fig. 1), the above mentioned mutation was associated with a very severe form of Usher syndrome. All the affected siblings experienced profound deafness since their early childhood (before the age of 6 months) resulting in speech difficulties. All the 6 affected siblings complained of reduced night vision starting prior to school age followed by progressive central vision deterioration. All had a typical picture of advanced retinitis pigmentosa with vascular attenuation, optic disc pallor, and intra-retinal pigment migration. There was widespread RPE and photoreceptor atrophy outside the central macular area (Fig. 2).

Fundus autofluorescence imaging (Fig. 3) showed a central macular area of increased AF signal. AF demonstrated reduced signal outside the central island extending to the mid-periphery confirming RPE atrophy.

Spectral domain optical coherence tomography (SD-OCT) imaging demonstrated reduced central foveal thickness in 5/6 cases (Table 1, Fig. 4). This was associated with attenuation of the RPE layer and loss of the photoreceptors layer as well as the ellipsoid zone. Cystoid macular edema was seen in sibling II: 12 and was associated with increased central foveal thickness. It is to be noted that sibling II: 12 is the youngest brother (17 years old). In siblings II:3, II:8, II:11, and II:12, a small central area of preserved photoreceptors layer was seen. In siblings II:2 and II:4, the photoreceptors layer showed tiny remnants, that were not continuous.
FfERG was non-recordable in all affected siblings under both photopic and scotopic conditions (Fig. 5). Imaging was normal in unaffected siblings.

Discussion

The homozygous donor splice site mutation c.470+1G>A in intron 5 of the MYO7A gene detected in this family has been reported before without description of the phenotypic features.\textsuperscript{15,16} Ben-Rebeh and co-workers reported 2 affected cases with the same mutation in Tunisian population but they reported ffERG in 2 cases only (with some preserved cone function) without OCT or AF features.\textsuperscript{17} Jacobson et al. described the clinical, OCT, visual field, and dark adaptometry findings in cohort of 33 cases affected by USH 1B due to MYO7A mutations.\textsuperscript{18} Their cases descended from 25 families of different ethnic origins. Their cases showed a relatively milder form of the disease compared to our cases. An array of mutations affecting MYO7A gene have been reported in their study.\textsuperscript{18}

In this Saudi family, we describe a very severe phenotype of Usher syndrome presenting at an early age. The auditory loss happened in the first 6 months of life, leading in addition to defective development of speech. Their central vision started to suffer at or just after admission to school. Therefore, these cases would be clinically categorized as Usher type 1. This is further supported by the finding of a homozygous splice site MYO7A mutation in all affected siblings.

**Fig. 4.** OCT of the affected siblings of a family with the homozygous donor splice site mutation c.470+1G>A in intron 5 of the MYO7A gene (horizontal scans across the fovea). A: right eye of sibling II:2. B: left eye of sibling II:2. C: right eye of sibling II:3. D: left eye of sibling II:3. E: right eye of sibling II:4. F: left eye of sibling II:4. G: right eye of sibling II:8. H: left eye of sibling II:8. I: right eye of sibling II:11. J: left eye of sibling II:11. K: right eye of sibling II:12. L: left eye of sibling II:12. (Note the reduced central macular thickness in the first 5 affected siblings with loss the ellipsoid zone layer and attenuation of the RPE layer with visible choriocapillaries. In sibling II:12, there is CME with increased central foveal thickness. In siblings II:3, II:8, II:11, and II:12 some preserved central part of photoreceptors layer is appreciated (marked with the yellow arrows).)
Mutations in the MYO7A are the most prevalent cause of Usher syndrome type 1. In the family reported here, six of the siblings were unaffected, which is in keeping with autosomal recessive inheritance.

Central vision was particularly poor in siblings II:2 and II:4 measuring around 20/300. All affected siblings in our presented family (including those with relatively good central VA) could not navigate around without help, most likely because of limited residual functional visual field. Although extensive retinitis pigmentosa has been reported in cases of Usher syndrome, central visual acuity was reported to be preserved until late in life, typically until the 5th to 7th decade with visual acuity of 6/60.19

The retinal findings demonstrated widespread pigmentary degeneration and RPE atrophy in addition to an absent foveal reflex. SD-OCT confirmed the presence of atrophic macular changes in all patients except the youngest patient (sibling II: 12), with CME in his OCT explaining the increased central foveal thickness. Various studies have reported the presence of macular edema and cystoid macular edema in RP and Usher syndrome.20 Another study where 110 patient were enrolled showed that 43% of the patients had atrophic appearing lesions of the retinal pigment epithelium within the fovea of both eyes in patients with RP.21

Autofluorescence findings in affected siblings of this family revealed a small central area of increased AF signal. Such increased signal may indicate ongoing process leading to photoreceptor degeneration resulting in high lipofuscin load in the RPE. The very small size of that central area is a clue for the advanced stage of the disease.

Central vision was relatively better in siblings II:3, II:8, II:11, and II: 12. In these cases, we noticed a larger preserved central macular island compared to the other 2 affected siblings (Fig. 2). We also observed a remaining central area of photoreceptors layer (Fig. 4) in the above cases with better BCVA. There was no correlation between the central foveal thickness and BCVA.

Gene therapy is underway for USH. Some promising results in animal models with MYO7A mutations demonstrated improvement in structure and function of the photoreceptors and RPE with subretinal delivery of AAV vectors.22 Phase1/2 human trials are going on now to assess the safety and efficacy of Lentiviral vectors expressing MYO7A in cases affected by type 1B USH.23 It is crucial to

![Fig. 5. A: Full field electroretinography (ffERG) in an affected patient from a family with the homozygous donor splice site mutation c.470+1G>A in intron 5 of the MYO7A gene., above: Cone response recording from sibling II:3. below: ffERG, Rod response recording from sibling II:3. (Note the flat recording under photopic and scotopic conditions indication severe loss of function of both rods and cones.)](image-url)
select cases for the new gene therapy modalities before end stage retinal degeneration takes place to rescue the surviving photoreceptors and RPE cells. Our findings in this study could have useful therapeutic implications in cases affected by homozygous splice site mutation c.470+1G>A in intron 5 of the MYO7A gene. This is to detect these cases as early as possible to try and save the viable photoreceptors and RPE given the aggressive nature of this genotype of USH. This is perhaps of value for the next generations of this family and similar families. For cases with advanced retinal degeneration (such as the cases presented here), it might be too late to try gene therapy. Other therapeutic options could be suitable for them such as retinal prosthesis.

We acknowledge this this mutation has been reported before. However, the severity of phenotypic features in this family is striking. Limitations of our study include small sample size and lack of genotyping of the unaffected siblings and parents. However the molecular genetic findings all affected patients and negative history in the mother and father, and normal clinical findings in the mother, strongly suggest a recessive inheritance.

Conclusion

Our phenotypic findings in this family confirm a severe form of Usher syndrome at an early age associated with homozygous splice site mutation c.470+1G>A in intron 5 of the MYO7A gene. These findings may have implications for future gene therapy in Usher syndrome caused by this specific mutation, supporting intervention as early as possible, if any.

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Conflict of interest

The authors declare that there is no conflict of interests.

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