The Genetics of Usher Syndrome in the Israeli and Palestinian Populations

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Submitted: August 16, 2017
Accepted: January 17, 2018


PURPOSE. Usher syndrome (USH) is the most common cause for deaf-blindness. It is genetically and clinically heterogeneous and prevalent in populations with high consanguinity rate. We aim to characterize the set of genes and mutations that cause USH in the Israeli and Palestinian populations.

METHODS. Seventy-four families with USH were recruited (23 with USH type 1 [USH1], 33 with USH2, seven with USH3, four with atypical USH, and seven families with an undetermined USH type). All affected subjects underwent a full ocular evaluation. A comprehensive genetic analysis, including Sanger sequencing for the detection of founder mutations, homozygosity mapping, and whole exome sequencing in large families was performed.

RESULTS. In 79% of the families (59 out of 74), an autosomal recessive inheritance pattern could be determined. Mutation detection analysis led to the identification of biallelic causative mutations in 51 (69%) of the families, including 21 families with mutations in USH2A, 17 in MYO7A, and seven in CLRN1. Our analysis revealed 28 mutations, 11 of which are novel (including c.802G>A, c.8558+1G>T, c.10211del, and c.14023A>T in USH2A; c.285+2T>G, c.2187+1G>T, c.3802G>A, c.5069-5070insC, c.5101C>T, and c.6196C>T in MYO7A; and c.1549del in GPR98).

CONCLUSIONS. We report here novel homozygous mutations in various genes causing USH, extending the spectrum of causative mutations. We also prove combined sequencing techniques as useful tools to identify novel disease-causing mutations. To the best of our knowledge, this is the largest report of a genetic analysis of Israeli and Palestinian families (n = 74) with different USH subtypes.

Keywords: Usher syndrome, mutation, whole exome sequencing

Usher syndrome (USH) is an autosomal recessive disease characterized by the association of sensorineural hearing loss (SNHL), retinitis pigmentosa (RP), and in some cases, vestibular dysfunction. USH is the leading genetic cause of deaf-blindness, with a prevalence in the range of 1 to 4 people per 25,000.1 USH is classified into three types based on the diverse clinical symptoms observed in patients, which is mainly via the severity and progression of the hearing loss and the age of onset of RP.2 USH type 1 (USH1) patients are defined as having congenital severe-to-profound SNHL, vestibular areflexia, and an onset of RP within the first decade of life. USH2 patients show congenital moderate-to-severe SNHL, normal vestibular function, and onset of RP within the second decade of life. In USH3 patients, the hearing loss, vestibular dysfunction, and onset of RP are progressive, sporadic, and variable, respectively. Lately, cumulative USH cases with an atypical presentation not fitting any of the classical subtypes have been described,3,4 as well as a combination of cone-rod degeneration and SNHL.5–8 Early symptoms of RP are night blindness and loss of peripheral vision caused by the degeneration of rod photoreceptors. As RP progresses, the cone photoreceptors also degenerate, contributing to blindness.2 The loss of central (cone-mediated) vision results in USH patients becoming legally or completely blind, and there is currently no known cure for the disease.1

To date, 12 genes are known to cause USH (http://hereditaryhearingloss.org, in the public domain). There are six genes linked to USH1: MYO7A,11 USH1C,12 CDH23,13 PCDH15,14 USH1G,15 and CIB2.16 MYO7A is the main gene responsible for USH1, since mutations in this gene alone account for 70% of USH1.17–18 Mutations in three genes (USH2A,19 GPR98,20 and WHRN21) are known to cause USH2. Mutations in USH2A are the most common in USH2 patients, accounting for 85% of USH2 cases, while mutations in GPR98 account for 6% of USH2 in the French and other Caucasian populations.22,23 CLRN1 is the most frequent mutated gene in USH3 patients.24 A mutation in CEP250, encoding a ciliary protein, was found to cause
atypical USH with early-onset SNHL and a relatively mild RP in an Iranian Jewish family. In addition, c.133G>T mutation in the ARSG gene was found recently to cause atypical USH in three Yemenite Jewish families. The Israeli population consists of different ethnicities, including Jews, Christians, Muslims, Bedouins, and Druze; however, founder mutations causing different USH types have been reported in the Jewish population. The most common are a missense mutation (c.144T>G, p.N48K) in the CLRN1 gene and a nonsense mutation (c.733C>T, p.R245*) in the PCDH15 gene in Ashkenazi Jews, as well as four USH2A mutations: a splicing mutation (c.12067-2A>G), a frameshift (p.T806fs*28), a nonsense mutation (c.2209C>T, p.R737*), and a missense mutation (c.1000C>T, p.R334W) in non-Ashkenazi Jews of various origins. A large exonic deletion in MYO7A (c.6355–16_6648*162, p.Q2119_K2215del) was reported to cause USH1 in North African Jews. It was only recently that the genetic basis of USH in the Arab-Muslim population was studied, and a few sporadic cases were reported.

Ocular evaluation included a full ophthalmological examination, Goldmann perimetry, ERG, color vision testing using the Ishihara 38-panel and Farnsworth-Munsell D-15 tests, color and infrared fundus photos, optical coherence tomography (OCT), and fundus autofluorescence (FAF) imaging performed as previously described.

**METHODS**

**Patient Recruitment and Clinical Evaluation**

All subjects were treated in accordance with the tenets of the Declaration of Helsinki. Prior to donation of a blood sample, a written informed consent was obtained from all individuals who participated in this study after explanation of the nature and possible consequences of the study. The research was approved by the institutional review board at the Hadassah Medical Center.

DNA was extracted from the index patients, as well as from other affected and unaffected family members, using the FlexiGene DNA kit (QIAGEN, Hilden, Germany). Sanger sequencing of PCR products was performed using specific primers designed using the Primer3 software and the UCSC website (Supplementary Table S1). Whole exome sequencing (WES) analysis of 11 families (including at least two affected and one unaffected individuals per family) was performed at the Otogenetics Corporation using a paired-end sample preparation kit (NimbleGen V2, 44.1 Mbp; Roche, Basel, Switzerland) and HiSeq2000 (Illumina, San Diego, CA, USA) at a 50× and 100× coverage (ranging from 0 to 524 reads per nucleotide). Sequence reads were aligned to the human genome reference sequence (build hg19) and variants were called and annotated using the DNAnexus software package (http://drorsharon1.wixsite.com/the-sharon-lab/data, in the public domain). Dataset files, including the annotated information, were analyzed using ANNOVAR software according to the dbsNP database (build 135) with the following filtering steps (see Supplementary Table S2): (1) variant type, only the following variant types were included: missense, nonsense, insertions and deletions within the coding region, and splice-
# Table: The Mutations Identified in Patients With Usher Syndrome in the Current Study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation Name</th>
<th>Reference</th>
<th>Mutation Detection Method</th>
<th>Family No.</th>
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Spectrum of Mutations That Cause Usher Syndrome

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site; (2) variants found within segmental duplications were excluded; (3) variants with a minor allele frequency (MAF) greater than 0.05 in the ExAC Project were excluded (http://exac.broadinstitute.org/, in the public domain); (4) variants with a SIFT (http://sift.jcvi.org/, in the public domain) score greater than 0.05 in the ExAC Project were excluded (http://exac.broadinstitute.org/, in the public domain); (5) variants with a PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/, in the public domain) score less than 0.05 were excluded; (5) variants with a SIFT (http://sift.jcvi.org/, in the public domain) score less than 0.85 were excluded; and (6) single heterozygous variants that do not fit an autosomal recessive inheritance pattern were excluded. Suspected pathogenic mutations were verified using Sanger sequencing. All WES samples were analyzed for copy number variants, but no homozygous deletion was identified. In addition, they perfectly cosegregated in the corresponding family and obtained the highest pathogenicity scores. MJ, Moroccan Jew; NAJ, North African Jew; AM, Arab Muslim; SJ, Samarian Jew; OJ, Oriental Jew; YJ, Yemenite Jew; TJ, Tunisian Jew; AC, Arab Christian; BJ, Buchanan Jew; AD, Arab Druze; AJ, Ashkenazi Jew.

† Genomic position according to GRCh37 reference.

The two novel missense putative pathogenic mutations (MYO7A-p.G1298R and USH2A-p.G268R) were tested in a set of 408 individuals with WES data and found to be negative. In addition, they perfectly cosegregated in the corresponding family and obtained the highest pathogenicity scores. MJ, Moroccan Jew; NAJ, North African Jew; AM, Arab Muslim; SJ, Samarian Jew; OJ, Oriental Jew; YJ, Yemenite Jew; TJ, Tunisian Jew; AC, Arab Christian; BJ, Buchanan Jew; AD, Arab Druze; AJ, Ashkenazi Jew.

† Genomic position according to GRCh37 reference.

## RESULTS

Our cohort contained 1542 families with various inherited retinal diseases, and 74 of the index cases (4.8%) were diagnosed with USH. A breakdown of our USH cohort based on the clinical type (external circle in Fig. 1) revealed 31% with USH1 and 45% with USH2, followed by USH3 (9%). In 59 of the USH families (80%), the inheritance pattern was determined as autosomal recessive (AR), and the remaining index cases were isolate cases. We reported previously the identification of disease-causing mutations in USH2A (in five of the families),28,34 CEP250 (in a single family),3 and ARSG (in two families),25 and we describe here the genetic analysis of the remaining 66 families. The ocular and retinal function in 26 USH patients and hearing in four patients with identified disease-causing mutations were evaluated (Supplementary Table S3) and, as reported previously, found to be within the phenotypic range of each USH type.

Aiming to identify the genetic cause of disease in these families, we performed a set of analyses, including screening for founder mutations, homozygosity mapping (HM) using whole genome SNP arrays (Supplementary Table S4), and WES analysis (Supplementary Fig. S1; Supplementary Table S4). We were able to identify 28 mutations in 41 of the 66 families (Table; Fig. 2, Supplementary Fig. S2), 11 of which are novel. We also employed functional prediction software, MutationTaster (http://www.mutationtaster.org/, in the public domain) and PolyPhen2, that indicated that these variants are damaging (Supplementary Table S5). The methods used in each studied family is listed in Supplementary Table S6.

As a first step in the genetic analysis, we screened the patients for 13 mutations (Supplementary Table S1) that were reported previously to cause USH in the corresponding ethnic groups (Table). The analysis revealed 11 of the mutations in 25 of the families. Interestingly, since the screen was performed by Sanger sequencing of the exons, including the previously published mutations, other mutations in the same exons could be identified. Further analyses confirmed these findings.
be detected by chance. Indeed, in three additional families (MOL0881, MOL0841, and MOL0365), we identified novel disease-causing mutations (Table; Fig. 3) (p.R1701* and c.285+2T>G in MYO7A and p.R4675* in USH2A).

We subsequently performed SNP array analysis in members of eight of the remaining families with reported consanguinity; in seven of the families (Supplementary Table S4) the disease-causing mutation was identified by further analyses. This was followed either by mutation analysis of candidate genes (families MOL594, MOL0620, and MOL0973) or WES analysis (families MOL0104, MOL0512, MOL0514, and MOL0962). The combination of SNP arrays and mutation analysis revealed two founder mutations in USH2A (p.Q81Yfs*28 and IVS61-2A>G; Table) that were known in other ethnic groups, and therefore the analysis expanded the set of origins to be screened for these mutations. The SNP array–WES combined analysis revealed the identification of four novel mutations, a frameshift deletion (c.15494delA) in GPR98 (in which mutations were not reported previously in the Israeli population) and three MYO7A mutations: a frameshift insertion (c.5069_5070insC), a missense mutation (c.3892G>A), and a splice-site mutation (c.2187+1G>T), as well as one known mutation, c.2307del (p.N769Kfs*5) in MYO7A (Fig. 3).

In addition, WES was performed on the index case of family members from 11 unrelated families (Supplementary Table S2). Analysis of the exome sequencing data by filtering out nonpathogenic variants (based on the criteria detailed in the Methods section; see Supplementary Table S4) revealed the identification of six different novel mutations. In three USH2 families, we identified novel USH2A mutations (splice-site mutations c.5776+1G>A and c.7595-2144A>G, a missense mutation c.802G>A, and a frameshift deletion c.10211delC; see Table and Supplementary Table S5), as well as a known mutation (p.R737* and p.C4808*), and in the USH1 families, we identified two novel MYO7A mutations (c.3892G>A and c.6196C>T). Aiming to examine whether these mutations are founder mutations in the corresponding population, we screened these mutations in additional USH patients of the same origin and identified additional families with the same mutations, as shown in the Table.

MOL0853 is a consanguineous Druze family including three siblings affected with USH2 (Fig. 2). Clinical examination at ages 21 to 30 of the three siblings showed relatively preserved visual acuity, mild myopia, and a nondetectable scotopic and photopic ERG (one patient had severely reduced, but not extinguished, cone ERG responses). Fundus imaging of two of the patients showed a typical presentation of RP, including peripheral retinal atrophy with bone spicule-like pigmentation, narrowing of blood vessels, and pale optic discs (Figs. 4A–B). FAF imaging demonstrated atrophic changes that can be seen as hypofluorescent areas in the mid and near periphery, encroaching the macula, which is more evident with age (Figs. 4C–D). The atrophic changes are seen in more detail using the Heidelberg FAF images (Figs. 4E–F), with a hyperfluorescent ring around the fovea and with hypofluorescence in the macular area. OCT horizontal cross-sections revealed atrophy
of the outer nuclear layer with sparing of the foveal region (Figs. 4G–H). Since no mutations have been reported as a cause of USH in the Druze population, we performed WES analysis of the index case with an average coverage of 50X. The analysis revealed over 23,000 sequence variants that were filtered (as detailed in the Methods section). Seventy-four variants passed the filtering steps, one of which was a homozygous transversion in USH2A, c.14424C>A (Fig. 3A), leading to a premature stop codon (p.C4808*) that fully cosegregated in the family.

MOL1087 is a consanguineous Arab-Muslim family with a unique structure (Fig. 2): four of five siblings were affected with USH2, as well as a daughter of MOL1087-1. All five patients had congenital hearing loss with normal vestibular function and typical RP. ERG testing performed on two of the patients at the age of 34 showed severe photopic reduction and no rod response (Supplementary Table S3). Aiming to identify the cause of disease, we performed WES analysis on three family members and identified two novel USH2A mutations (Table; Fig. 3). The four affected siblings were found to be homozygous for c.802G>A (p.G268R), whereas MOL1087-2 was found to be a compound heterozygous for p.G268R and c.10211delC (p.P3404Qfs*23). The missense variant has a very low MAF (1*10^{-5} at ExAC), and Gly268 is highly conserved along evolution (Fig. 3B), making this variant a very likely pathogenic mutation. In addition, a subsequent screen for this variant revealed another family (MOL0594), including six subjects who participated in the study, of whom four were affected: MOL0594-1, MOL0594-2, and MOL0594-3 diagnosed with the USH2 phenotype were found to be homozygous for p.G268R, whereas MOL0594-5, who is 10 years old and presented with RP without hearing loss, was found to be heterozygous for p.G268R. These genetic findings indicate that the causative mutation/gene in MOL0594-5 is different from USH2A, an already known phenomenon in a highly consanguineous family.

**DISCUSSION**

We report here the largest analysis of Israeli and Palestinian families (n = 74) with USH, to our knowledge, reported thus far. The clinical distribution of our USH cohort based on types of the syndrome (45% USH2, 31% USH1, 9% USH3, 8% USH2/3, 7% atypical) is different from other cohorts that have been reported to show a large variability in different populations: USH2 has been reported to be relatively common in Germany, China, Italy, and England (82%, 77%, 65% and 64% of all USH cases, respectively), 10,22,35,36 compared to only 45% in our cohort. On the other hand, USH3 is relatively common in our cohort (9%), as also has been reported in a few populations due to a founder effect, including Finland, Norway, and the Ashkenazi Jewish population (46%, 14%, and 40%, respectively).37–39 In addition to the known types, we have reported previously a family MOL0028 harboring a mutation in a novel USH-causing gene, CEP250,5 and recently mutation in ARSG gene in three Yemenite Jewish families causing atypical USH phenotype.25

The genetics of USH in different populations revealed that mutations in MYO7A are the most common cause of USH1 (ranging from 29% to 59%): 29%–39% in the United States and United Kingdom,17,40 41% in Germany and Denmark, 41 and 54% in North Africa and Eastern Europe.42 In the current study, MYO7A was causative in 17 of the 23 USH1 families (74%). Similarly, USH2A mutations were reported to be the most frequent cause of USH2 (44%–75% in different populations),43,44 and in 21 of 35 of our USH2 families (64%).
FIGURE 4. Fundus photos of patients from MOL0853. Color ultrawide field Optos photos (A, B), FAF Optos photos (C, D), FAF of the posterior pole (E, F), and horizontal cross-sections by OCT (G, H) of the right eye of patients MOL0853-3 (left column, age 26 years) and MOL0853-1 (right column, age 33 years).
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Direct Sanger sequencing of PCR products is still considered a classical and useful technique for screening of founder mutations that are prevalent in the studied population. However, the relatively large size of most of the genes that are associated with USH (e.g., USH2A with 71 coding exons, MYO7A with 48 exons, USH1G with 28 exons, etc.) makes screening of entire genes a complicated task. We therefore designed a screening approach in which known founder mutations are initially screened, and if negative, HM or WES are used, followed by Sanger sequencing of the candidate genes or suspected mutations. This analysis revealed 28 different causative mutations in 41 families (63% of the USH families included in this report), 11 of which are novel, including the first report, to our knowledge, of a GPR98 mutation in the studied population as the cause of USH2. Mutations in this gene have been reported to cause up to 5.7% of USH2 patients. Our findings extend the spectrum of USH-causing mutations, improving the genetic workup of these patients. In addition, this may have implications regarding patient inclusion in future clinical interventional trials.

High-throughput sequencing techniques, including gene panels and WES, were reported to be useful in the identification of novel mutations in previously known genes. In addition, cumulative data show that WES can be useful in the discovery of novel causative genes in different medical disciplines, including ophthalmology. Next generation sequencing was also reported to be efficient in identifying mutations in known as well as novel USH-related genes. Moreover, the combination of HM and WES has been proven to be highly efficient in identifying disease-causative mutations. Here, we identified three novel mutations in MYO7A using the combination of HM and WES and additional novel mutations in both MYO7A and USH2A using WES alone. These techniques, as well as new developments to detect intronic mutations in heterozygous copy number variants, will allow a better yield in the near future.

USH results in a frustrating phenotype with the end result of losing the two major senses, hearing and vision. The auditory defect can be corrected in most cases by cochlear implants, however, no cure is currently available for the visual loss. It should be noted that the majority of mutations identified in this study are expected to yield no functional protein, that is, splice-site, nonsense, and frameshift mutations. Most USH genes, however, are challenging in the respect of future potential gene therapy, mainly due to gene size and complex alternative splicing mechanism. Identifying the vast majority of disease-causing mutations in specific USH genes might shed a light on the most important domains and better target regions for applying therapy.

Unfortunately, we could not establish a phenotype-genotype correlation in our patients by overviewing the audiologic and the ocular signs and symptoms together with the genetic findings. Previous works were controversial in their ability to identify such a correlation due to various presentations of subjects harboring the same causative mutation. Such phenotypic variation of Mendelian diseases is thought to be caused by other gene modifiers and regulators. We assume that recently developed ocular imaging technologies developed utilizing adaptive optics, high resolution OCT, OCT-A, and microperimetry, might be helpful in accurate phenotyping of the USH patients at tissue and cell levels, allowing for the establishment of phenotype-genotype correlation. In conclusion, we report here an analysis of a large cohort of USH patients using a combined genetic approach for the identification of founder and novel disease-causing mutations. Our analysis revealed the identification of known as well as novel mutations in various USH genes, extending the spectrum of USH-causing mutations.

Acknowledgments

The authors thank all patients and family members for their participation in this study and thank Michelle Grunin for copy editing the manuscript.

Disclosure: A. Khalaileh, None; A. Abu-Diab, None; T. Ben-Yosef, None; A. Raas-Rothschild, None; I. Lerer, None; Y. Alsawalit, None; I. Chowers, None; E. Banin, None; D. Sharon, None; S. Khateb, None

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