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The Many Faces of Sensorineural Hearing Loss: One Founder and Two Novel Mutations Affecting One Family of Mixed Jewish Ancestry

Doron M. Behar,[1,2] Bella Davidov,[1] Zippora Brownstein,[3] Tamar Ben-Yosef,[4] Karen B. Avraham,[3] and Mordechai Shohat[1,3]

Dramatic progress has been made in our understanding of the highly heterogeneous molecular bases of sensorineural hearing loss (SNHL), demonstrating the involvement of all known forms of inheritance and a plethora of genes tangled in various molecular pathways. This progress permits the provision of prognostic information and genetic counseling for affected families, which might, nevertheless, be exceedingly challenging. Here, we describe an intricate genetic investigation that included Sanger-type sequencing, BeadArray technology, and next-generation sequencing to resolve a complex case involving one family presenting syndromic and nonsyndromic SNHL phenotypes in two consecutive generations. We demonstrate and conclude that such an effort can be completed during pregnancy.

Introduction

The wealth of genetic heterogeneity known to be causative for sensorineural hearing loss (SNHL) makes the molecular diagnosis of such cases expensive and time-consuming (Smith *et al.*, 2005; Shearer *et al.*, 2010), but is particularly challenging during pregnancy. Divided into syndromic (30%) and nonsyndromic (70%), genetic etiologies make up 70% of all prelingual-onset SNHL (Smith *et al.*, 2005). The single leading cause for hereditary SNHL, in general, and for nonsyndromic prelingual SNHL, in particular, is involvement of the DFNB1 gene *GJB2* (Zelante *et al.*, 1997). The most common cause of syndromic prelingual SNHL, characterized by combined SNHL and vision loss, is the Usher syndrome (USH) (Friedman *et al.*, 2011). Both DFNB1 and USH are recessively inherited and the numerous population-specific causative mutations identified in the respective genes make up the essence of prenatal SNHL prevention programs to date (Smith *et al.*, 2005). The same is true for the various Jewish communities in which different founder mutations have been identified (Brownstein *et al.*, 2009). We report the resolution of a complex case of a family of mixed Ashkenazi and Iraqi Jewish ancestry during pregnancy, illustrating the scope of clinical and molecular work-up of SNHL in the genomic era.

Materials and Methods

Subjects

A 35-year-old woman (II-2) of consanguineous (first cousins) Iraqi Jewish ancestry sought urgent genetic counseling at the beginning of her second pregnancy (Fig. 1). The woman suffers from type 2 Usher syndrome (USH2), characterized by moderate SNHL and retinal degeneration (retinitis pigmentosa), and was known to be homozygous for the founder Iraqi Jewish *USH2A* mutation c.239_240insGTAC (Adato *et al.*, 2000). She reported that her husband (II-3) was of Ashkenazi Jewish origin, that her brother (II-1) suffers from congenital profound SNHL and that her 4-year-old son (III-1) suffers from congenital moderate SNHL with a normal electroretinogram (ERG) at the age of three (Fig. 1). Genetic evaluation of all family members commenced after informed consent. The study was approved by the Helsinki Committee of the Israel Ministry of Health and the Institutional Review Board Committees of the Rabin Medical Center.

DNA analysis

Genomic DNA was isolated from peripheral blood by using the Qiagen kit (Qiagen Ltd.) following the manufacturer's protocol. Known founder mutations were screened as

[1]Raphael Recanati Genetics Institute, Rabin Medical Center, Petah Tikva, Israel.
[2]Molecular Medicine Laboratory, Rambam Medical Center, Haifa, Israel.
[3]Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.
[4]Department of Genetics, The Rappaport Faculty of Medicine and Research Institute, Technion–Israel Institute of Technology, Haifa, Israel.

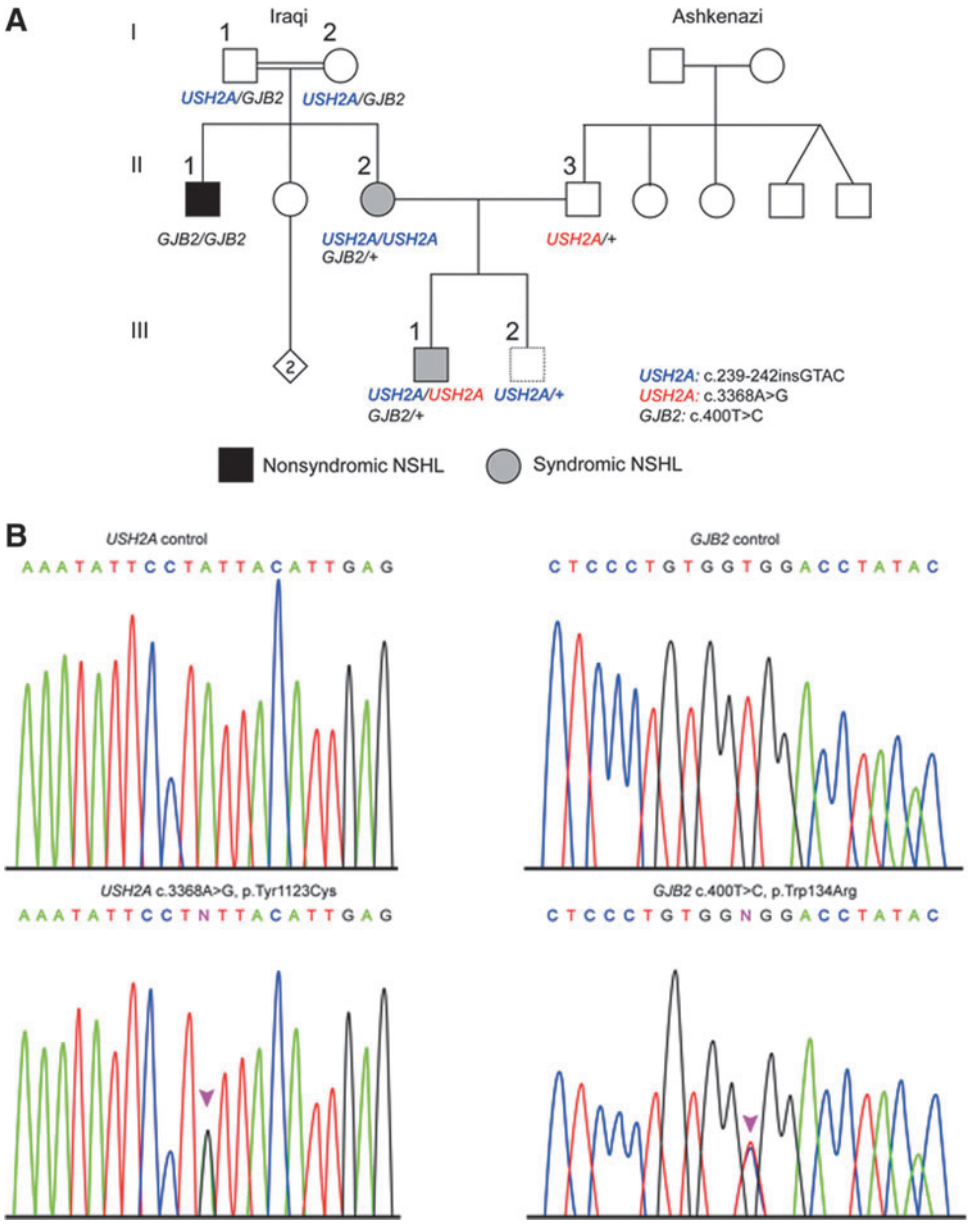

FIG. 1. Presentation of the molecular analysis of the complex family. **(A)** Pedigree of the investigated family. Initial clinical presentation indicated type 2 Usher syndrome (USH2) in the mother II-2, but was suggestive of NSHL in her brother **(II-1)** and son **(III-1)**. The genotypes are indicated for the ascertained individuals. **(B)** Sanger sequencing of the novel *USH2A* and *GJB2* heterozygote mutations c.3368A>G, p.Tyr1123Cys, and c.400T>C, p.Trp134Arg, respectively, in a control individual and III-1. Color images available online at www.liebertpub.com/gtmb

previously summarized (Brownstein *et al.*, 2009). For Sanger sequencing of the *GJB2*, *USH2A*, and *SMPX* genes, DNA was amplified to obtain all coding exons and their flanking regions using conventional PCR techniques. Sequencing was performed on a 3730xl DNA Analyzer (Applied Biosystems). A BeadArray-based screen for 198 common mitochondrial and autosomal NSHL mutations was completed by Asper Biotech Ltd. (www.asperbio.com). A next-generation sequencing (NGS) panel, including a total of 80 genes implicated in NSHL was carried out at Otogenetics Ltd. (www.otogenetics.com).

Results

Molecular investigation of III-1 (Fig. 1) included family screening for all known Iraqi founder *DFNB1* and *USH2A* mutations and for all known Ashkenazi founder *DFNB1*, *USH1F*, and *USH3* mutations (Brownstein *et al.*, 2009), and yielded only the obligatory heterozygous states for the Iraqi *USH2A* mutation (Fig. 1 and Table 1). III-1 underwent a screen for the 198 common mitochondrial and autosomal NSHL mutations included in a diagnostic microarray (www.asperbio.com). No pathogenic mutations were found; the Iraqi *USH2A* c.239_240insGTAC mutation is not included in this array. II-2 requested prenatal diagnosis for SNHL. Considerable maternal stress was witnessed. As there was no evidence for retinal involvement in her son (III-1) and as no Ashkenazi *USH2A* founder mutations were previously reported, sequencing of the *GJB2* gene in her son was performed (Brownstein *et al.*, 2009). However, as the family's pedigree could theoretically be concordant with autosomal recessive, autosomal dominant, or X-linked inheritance modes, and as it was clear that time would not allow for hierarchical Sanger-based screening of additional genes in case of normal *GJB2* sequencing, we opted to use an NGS panel, including a total

Table 1. Clinical Characteristics of the Family Members

Patient	*Phenotype*	USH2A *genotype*	GJB2 *genotype*
I-1	Normal hearing	239–242insGTAC/+	400T>C/+
I-2	Normal hearing	239–242insGTAC/+	400T>C/+
II-1	Profound SNHL		400T>C/400T>C
II-2	Moderate SNHL	239–242insGTAC/239–242insGTAC	400T>C/+
II-3	Normal hearing	3368A>G/+	
III-1	Moderate SNHL; normal ERG at age 3	239–242insGTAC/3368A>G	400T>C/+
III-2	Normal ABR at age 1	239–242insGTAC/+	

SNHL, sensorineural hearing loss; ERG, electroretinogram; ABR, auditory brainstem response.

of 80 genes implicated in NSHL (www.otogenetics.com) on DNA derived from III-1. In addition, Sanger sequencing of the X-linked *SMPX* gene was performed. A novel *GJB2* c.400T>C (NM_004004); p.Trp134Arg (NP_003995) variant was detected in a heterozygous state, with a PolyPhen-2 score of 0.994 (http://genetics.bwh.harvard.edu/pph2/). Whereas it did not explain the SNHL of III-1, the segregation of the mutation clarified the diagnosis of II-1 and explained the degree of HL manifested by his audiogram (not shown), which is compatible with the profound phenotype of many *GJB2* mutations. The NGS panel suggested over 100 additional alterations that were less likely to be deleterious, but the available time made it impossible to validate and segregate all variants. Fourteen days before the date of the scheduled amniocentesis, the clinical signs and symptoms were evaluated and it was concluded that the normal ERG at age three in the child III-1 did not rule out USH2 (Malm *et al.*, 2011). Accordingly, sequencing of the *USH2A* gene, which was not included in the NGS panel, was performed. Partial results obtained a day before the amniocentesis yielded the novel *USH2A* variant c.3368A>G (NM_206933); p.Tyr1123Cys (NP_ 996816), scoring 0.999 by PolyPhen-2, in a heterozygous state in both the husband, II-3, and son, III-1 (Fig. 1). Consequently, we concluded that two different phenotypes and three different genotypes affect the family. The mother II-2 is affected by USH2, due to a homozygous *USH2A* Iraqi founder mutation (Adato *et al.*, 2000), her brother II-1 has a homozygous novel DFNB1-related nonsyndromic SNHL resulting from parental consanguinity, and her son III-1 suffers from USH2A due to a compound heterozygous state of the known Iraqi mutation (Auslender *et al.*, 2008) and a second novel mutation. Amniocentesis was carried out at week 20 and the embryo was found to carry only the Iraqi *USH2A* mutation in a heterozygous state. At 1 year of age (III-2), there were no indications of SNHL, including a normal auditory brainstem response test.

Discussion

The presented case is a compelling example of the complexity and extreme heterogeneity of hereditary hearing loss in the genomic era (Smith *et al.*, 2005; Brownstein *et al.*, 2009; Shearer *et al.*, 2010), illustrating the need to consider various modes of inheritance, syndromic versus nonsyndromic phenotypes, and the use of various genomic technologies to resolve, during pregnancy, the multiple etiologies affecting one family. Clinically, it should be emphasized that normal ERG at the age of 3 years should not rule out the diagnosis of USH (Malm *et al.*, 2011). In this report, the different parental ancestries, the common practice of primarily relying on the identification of founder deleterious mutations among Ashkenazi Jews (Brownstein *et al.*, 2009), the normal ERG, and the hope for a favorable nonsyndromic versus syndromic prognosis for the elder son, were misleading. Hence, while the presented case supports the recommendation to sequence the *GJB2* gene first in each SNHL individual (Brownstein *et al.*, 2009), the sequence of any gene known to affect a family member should be considered even if the odds for its involvement appear to be extremely low. The molecular diagnostics was carried out under a very constricted schedule of ~20 weeks. This required relying solely on prediction algorithms such as PolyPhen-2, rather than functional tests to infer mutagenicity for the detected variants, thus highlighting the need to complete genetic investigations well ahead of the actual pregnancy. It should be reiterated that preconception planning might be particularly important in similar cases because genetic testing for SNHL has raised some unique ethical issues stemming from cultural differences in attitudes toward hearing loss (Dagan *et al.*, 2002) and the fact that treatments such as cochlear implantation are now commonly practiced. Accordingly, genetic counseling should be provided in an appropriate setting after the pathogenicity of the identified mutation is well established and when the parents can fully comprehend and weigh the risks, benefits, and limitations of genetic testing. Finally, it has already been suggested that comprehensive NGS platforms are critical for the molecular analysis of NSHL cases, since the number of currently known genes involved in this phenotype makes it difficult, if not impossible, to offer clinically useful screening based on Sanger-type sequencing (Shearer *et al.*, 2010; Brownstein *et al.*, 2011). In this study, we implemented NGS and identified a novel *GJB2* mutation. The development of comprehensive SNHL gene panels using NGS, including robust analytical tools appropriate for rapid clinical management, are a major challenge. Whereas a number of these platforms consisting of target gene capture and NGS have been developed for deafness and other diseases (Shearer *et al.*, 2010; Brownstein *et al.*, 2011), a timely analysis of the multiple variants is difficult and usually precludes providing a definitive answer within the time frame of a pregnancy. This continues to be a challenge that should be overcome in the coming years.

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Author Disclosure Statement

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Address correspondence to:
Doron M. Behar, MD, PhD
Raphael Recanati Genetics Institute
Rabin Medical Center
Beilinson Campus
Petah Tikva 49100
Israel

E-mail: d_behar@rambam.health.gov.il