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EVIDENCE FOR GENOTYPE-PHENOTYPE CORRELATION FOR *OTOF* MUTATIONS

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Abstract

Objective—The aim of this study is to evaluate the auditory phenotype in subjects with *OTOF* gene mutations to describe genotype-phenotype correlations.

Material-methods—Twenty-two affected members from three families with homozygous *OTOF* mutations were included. Nine subjects were evaluated audiologically with otoscopic examination, pure-tone audiometry, tympanometry with acoustic reflex testing, auditory brain stem responses, and otoacoustic emission tests.

Results—Homozygous c.4718T>C (p.Ile1573Thr) mutation was associated with the auditory neuropathy/auditory dys-synchrony (AN/AD) phenotype and with progressive sensorineural hearing loss in four siblings in one family, while homozygous c.4467dupC (p.I1490HfsX19) was associated with severe to profound sensorineural hearing loss without AN/AD in four relatives in another family. Homozygous c.1958delC (p.Pro653LeufsX13) mutation was associated with moderate sensorineural hearing loss without AN/AD in one affected person in an additional family.

Conclusions—The audiological phenotype associated with different *OTOF* mutations appears to be consistently different suggesting the presence of a genotype-phenotype correlation.

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Keywords

Auditory neuropathy; autosomal recessive; hearing loss; *OTOF*

1. Introduction

Congenital (or prelingual) inherited hearing impairment affects approximately one in 1,000 newborns. Genetic etiologies are responsible for 60-80% of cases with congenital hearing loss. Hereditary hearing loss is classified into two main categories: non-syndromic (90%), of which majority are autosomal recessive (DFNB), and syndromic (10%). The inheritance pattern of non-syndromic hearing loss can be autosomal recessive, autosomal dominant, X-linked and mitochondrial [1]. Mutations in 43 genes, including *OTOF* (MIM 603681), have been implicated in non-syndromic autosomal recessive sensorineural hearing loss (SNHL) [2].

Yasunaga et al. [3] first identified mutations in *OTOF*, which is located on the short arm of human chromosome 2 and codes for otoferlin, as a cause of non-syndromic autosomal recessive SNHL at the DFNB9 locus. To date, 93 mutations in *OTOF* have been reported [4]. Otoferlin was found to be preferentially expressed in sensory hair cells of the cochleathe vestibule, and also expressed in the brain [3,5]. In the murine mature cochlea, otoferlin is present in the inner hair cells and plays an essential role in a late step of synaptic vesicle exocytosis and likely acts as a sensor triggering membrane fusion [6,7]. *OTOF*-related hearing loss is frequently associated with auditory neuropathy/dys-synchrony (AN/AD) [8-11]. The auditory neuropathies are characterized by disturbed pure tone audiometry, auditory brain stem responses (ABR) and preservation of otoacoustic emissions (OAEs). In this disease, auditory nerve and/or inner hair cells are damaged, however, the outer hair cells are intact [12].

Most individuals previously reported with *OTOF* mutations exhibited severe-to-profound SNHL [6, 8, 9, 11, 13-18]. However, available data on genotype-phenotype correlation remain limited. In this study, we analyzed auditory phenotype of three families that we determined as having *OTOF* mutations.

2. Material and methods

This study was approved by the Ankara University Ethics Committee (Turkey) and by the IRB at the University of Miami (USA). All families were Turkish and after careful evaluation, they were negative for the presence of syndromic findings or environmental exposure. Otoscopic examination, pure-tone audiometry, tympanometry measurement with acoustic reflex, and transient otoacoustic emission (TEOAE) test were performed. ABR test was performed on one member in one family. The degree of hearing loss was determined by pure tone audiometry. Degree of hearing impairment was defined by pure tone average (PTA) threshold levels at 0.5, 1, 2 and 4 kHz. Hearing loss was classified as mild (PTA 21-40 dB HL), moderate (PTA 41-70 dB HL), severe (PTA 71-95 dB HL) and profound (PTA > 95 dB HL) (European Concerted Action Project on Genetics of Hearing Impairment

1996). Computed tomography of temporal bone was performed on one affected member in each family.

3. Results

In this study three families with homozygous *OTOF* mutations were studied (Figures 1 and 2). Details of molecular studies have been previously reported [19]. Each mutation co-segregated with hearing loss as a fully penetrant autosomal recessive trait.

The clinical details of the affected children are summarized in Table 1. We determined homozygous p.Pro653LeufsX13 (c.1958delC) mutation in affected members of family 438. The audiological characteristics of this truncating mutation was studied in one affected individual and were prelingual-onset, bilateral symmetric moderate sensorineural hearing loss with flat audiogram configuration (Table 1). Otoacoustic emission tests were evaluated in one person with a history of hearing device usage and were absent.

We found the p.Ile1490HisfsX19 (c.4467dupC), another truncating mutation, in nine individuals from family 766. Audiological findings were studied in four affected subjects. Hearing loss was prelingual-onset, bilateral symmetric profound and sensorineural with flat and fragmentary shape audiogram configuration. Transient evoked otoacoustic emissions were absent. Tympanometry results were normal. Acoustic middle ear muscle reflexes were absent (Table 1). Computed tomography of the temporal bone was normal in one affected person.

We found homozygous p.Ile1573Thr (c.4718T>C), a missense mutation in four individuals from family 796, who were audiologically characterized. Hearing loss was prelingual-onset, bilateral symmetric mild to severe sensorineural with flat-sloping audiogram configuration. ABR performed on one patient who is 9 years old. The hearing levels of all children were consecutively as follows: mild hearing loss in a 9-year-old child, moderate hearing loss in an 11-year and a 13-year-old, and severe hearing loss in a 17-year-old child. Transient evoked otoacoustic emissions were present at least three frequencies in both ear in all children. Also tympanometry results were normal and acoustic middle ear muscle reflexes were absent in all children (Table 1). No ABR waves were obtained in both ears by using click stimuli in the 9 years old child.

Computed tomography of the temporal bone was normal in one affected person from each family.

4. Discussion

In this study we provide details of the auditory phenotype for three *OTOF* mutations. A large number of affected individuals were available with all homozygous mutations, making comparison between different mutations easier. The OAEs were present in all four children of family 796 with the p.Ile1573Thr mutation showing that this missense mutation typically causes AN/AD. Another interesting finding in this family was that the level of hearing worsened as children got older which suggests that the hearing loss due to p.Ile1573Thr is progressive. Progressive hearing loss has been reported associated with another missense

mutation, p.Glu1700Gln, in *OTOF* [13]. In contrast to what we observed in family 796, none of the five affected subjects in the other two families with homozygous p.I1490HfsX19 or p.Pro653LeufsX13 mutations had AN/AD.

OTOF mutations were identified in 56% of patients with AN/AD [18]. However, it is not clear whether the preservation of OHC function is a constant phenotype for hearing loss caused by *OTOF* mutations. It has been reported that *OTOF*-related deafness appears to be an AN/AD in the first years of life and with time OAEs disappear and electrophysiologic testing becomes more consistent with a cochlear defect [8,13, 20]. The reasons behind this finding may be environmental factors, such as usage of hearing aids, and/or genetic factors [8, 21]. All four affected members of family 766 in this study denied using hearing aids but their audiological tests showed OHC loss invariably. Thus, the presence of OAEs in all affected members who were homozygous for a potentially less disruptive mutation (p.Ile1573Thr in family 796) and the absence of OAEs in all affecteds with a severely disrupting mutation (p.Ile1490HisfsX19 in family 766) might suggest that the OHC dysfunction is a direct consequence of the identified *OTOF* mutation. Audiological phenotypes of previously reported homozygous *OTOF* mutations are shown in Table 2. It is interesting to note that the oldest patient with preserved OAEs was 18 years old with a missense mutation [9]. The oldest reported patient with preserved OAEs and a homozygous truncating mutation was 11 years old [8]. Hence, it is possible that truncating mutations cause loss of OAEs at an earlier age, while potentially milder mutations promote the preservation of OAEs at older ages.

5. Conclusion

In this study we summarize the audiological phenotype associated with three *OTOF* mutations. The c.4718T>C (p.Ile1573Thr) mutation is associated with the AN/AD phenotype and with progressive SNHL. The 4467dupC (p.I1490HfsX19) mutation is associated with severe to profound SNHL with no evidence of AN/AD. The c.1958delC (p.Pro653LeufsX13) mutation is associated with moderate SNHL. Further studies are needed to conclude on the genotype-phenotype correlation regarding the mutations in the *OTOF* gene.

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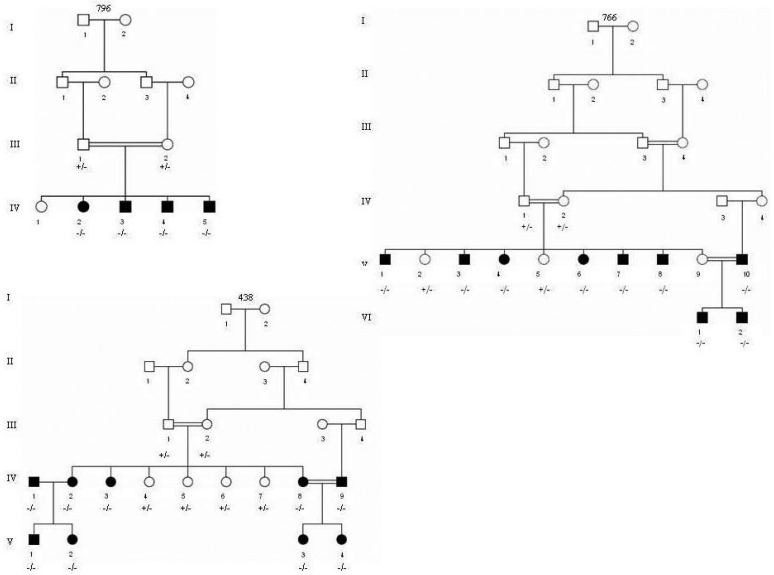

Figure 1.

Pedigrees of families with *OTOF* mutations. Closed symbols: affected individuals. Circles are females squares are males. −/−, +/−, and −/− are homozygous mutant, heterozygous, and homozygous wild type, respectively.

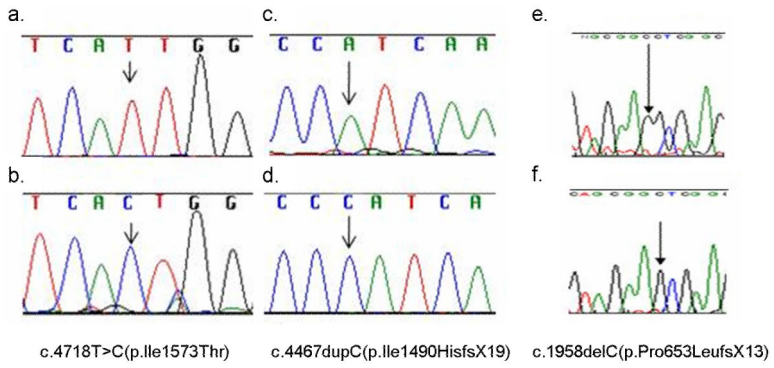

Figure 2.

Electropherograms showing homozygous *OTOF* mutations.

a. wild type.

b. homozygous c.4718T>C(p.Ile1573Thr) in family 796.

c. wild type.

d. homozygous c.4467dupC(p.Ile1490HisfsX19) in family 766.

e. wild type.

f. homozygous c.1958delC(p.Pro653LeufsX13) in family 438.

Table 1

Summary of clinical and audiological data of studied patients

	Subject	Age (Year)	Hearing level	Audiogram shape	OAE	Onset	Mutation
Family 438	IV-9	22	Bilateral-moderate	Flat	Absent	C/P	c.1958delC (p.Pro653LeufsX13)
Family 766	V-1	35	Bilateral-profound	Flat	Absent	C/P	c.4467dupC (p.Ile1490HisfsX19
	V-3	30	Bilateral-severe	Flat	Absent	C/P	
	V-4	16	Right profound, left severe	Right scoop, Left flat	Absent	C/P	
	V-6	14	Bilateral-profound	Fragmentary shape	Absent	C/P	
Family 796	IV-2	17	Bilateral- severe	Flat	Present	C/P	c.4718T>C (p.Ile1573Thr)
	IV-3	13	Bilateral-moderate	Sloping	Present	C/P	
	IV-4	11	Bilateral-moderate	Sloping	Present	C/P	
	IV-5	9	Bilateral-mild	Flat	Present	C/P	

C/P: Congenital or prelingual

Table 2

Audiological phenotypes of previously reported homozygous OTOF mutations

Mutations		Age tested/ Number of Cases	Hearing level	OAE	Reference
Missense	p.Leu1011Pro	6y / 1	Severe-profound	Bil (+)	Tekin et al. [9]
		17y/ 1	Severe-profound	Bil (−)	
		18y/ 1	Severe-profound	Bil (+)	
	p.Phe1795Cys	12-20m/ 1	Profound	Bil (+)	Santeralli et al. [22]
		2y6m/ 1	Profound	Bil (+)	Zadro et al. [23]
	p.Glu1700Gln	1y7m-2y7m/4	Progressive moderate	(+)	Chiu et al. [13]
		2y/ 1	Progressive profound	(−)	
		Birth→2y/1	Progressive profound	(+)→(−)	
	p.G541S	26/1	Temperature sensitive hearing loss	(−)	Varga et al. [6]
	p.R1939Q	1y7m-2y6m/5	Profound	(−)	Matsunaga et al. [18]
		1y9m-2y10m/2	Severe	(−)	
		4m-10m/3	Profound	(+)	Iwasa et al. [25]
Other Mutations	c.1981dupG p.D661GfsX2	na/1	Profound	na	Mahdieh et al. [26]
	p.Gln829Ter	1-11y/3	Profound	Bil (+)	Rodriguez-Ballesteros et al. [8]
		2-9y/3	Profound	Uni (+)	
		2y/ 1	Profound	Bil (+)→ Bil (−)	
		4-85y/7	Profound	Bil (−)	
	c.1886_1887dupA (p.Pro630SerfsX9)	16m-4y/ 2	Profound	Bil (+)	Varga et al. [22]
	IV18+1G>T	3-6y/2	Moderate to severe	Bil (+)	
	IVS28-2A>C	15m-3y/ 2	Profound	Bil (+)	
	IVS9-2T>A	3y/ 1	Profound	Bil (+)	Zadro et al. [23]
	p.Glu1804Del	7-10y/3	Temperature sensitive hearing loss	Bil (+)	Marlin et al. [24]

(+)→(−): Present both ears in the first test became absent later on