A New Pathogenic Variant in the TRIOBP Associated with Profound Deafness Is Remediable with Cochlear Implantation

Ahmet M. Tekin, Geert de Ceulaer, Paul Govaerts, Yıldırım Bayazit, Wim Wuyts, Paul Van de Heyning, Vedat Topsakal

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Sensorineural hearing loss · Cochlear implantation · Hereditary hearing loss · Genetic deafness · Hereditary

Introduction

Severe sensorineural hearing loss (SNHL) is a common sensory deficit that affects at least 1 in 1,000 newborns, and up to 60% of all cases are considered to be of genetic origin [Liu et al., 2001; Morton and Nance, 2006]. Genetic hearing loss can be divided into syndromic and nonsyndromic SNHL [Hochman et al., 2010]. Hereditary hearing impairment without any other associated clinical features is referred to as “nonsyndromic” and is a genetically heterogeneous condition [Friedman and Griffith, 2003]. More than 80 genes have been shown to cause nonsyn-
dromic hereditary hearing loss [Miyagawa et al., 2016]. Nevertheless, in only one-third of SNHL patients and in one-fourth of patients with cochlear implants, pathogenic mutations in common hearing loss genes can be identified [Wu et al., 2008a; Wu et al., 2011; Miyagawa et al., 2013].

A nonfrequent type of deafness is TRIOBP-associated nonsyndromic autosomal recessive hereditary hearing loss. In 2006, Riazuddin et al. [2006] and Shahin et al. [2006] mapped DFNB28 to chromosome 22q13.1 and found that pathogenic mutations in TRIOBP were diverging with hearing loss in 15 families. The TRIOBP gene encodes TRIO- and filamentous-actin-binding proteins, which take a significant role in the durability and stiffness of hair cell stereocilia in the cochlea [Kitajiri et al., 2010]. Stereocilia are mechanosensorial structures which are embedded in the apical surface and roots of inner ear hair cells and the cuticular plate [Katsuno et al., 2019]. Sound-induced deflections of the stereocilia bundle change the open probability of the mechanotransduction channel and thereby initiate electrochemical signals that are transmitted via the eighth nerve to the auditory cortex [Zhao and Müller, 2015]. Rigidity and flexibility of the stereocilia bundle during stimuli are of great importance for functioning, and its absence or dysfunction leads to hearing loss due to degeneration of hair cells with mechanic sensor. Although the length of stereocilia differs according to their place in the cochlea, their rootlet dimensions are identical. There is evidence for a physical connection between the rootlets and the lateral wall. This relation and other characteristics of the cytoskeleton in the apex account for somatic motility in the cochlear amplifier. Some fitting strategies and implant design take this into account in application of cochlear implants [Landsberger et al., 2016]. Its stiffness and durability are secured by its rootlets, pliable structures that harbor the base of the stereocilia into the cuticular plate. Rootlets are formed by densely packed, tapered actin filaments at the base of each stereocilium [Furness et al., 2008].

It has been revealed that the Triobp mouse mutant TriobpΔex8/Δex8 with an engineered deletion of exon 8 (orthologous to human exon 6) fails to form normal rootlets, even though parts of the stereocilia develop normally. Upon stimulation of stereocilia of the TriobpΔex8/Δex8 mouse, hyperflexibility of the stereocilia and decreased pivot rigidity were noticed, followed by progressive stereocilia degeneration. Consequently, TriobpΔex8/Δex8 mice are profoundly deaf from an early age [Kitajiri et al., 2010]. This mimics DFNB28 in humans and explains the severity and early prelingual onset of hearing loss. Various isoforms of the protein, diverging in total length and expression pattern, have been explored [Riazuddin et al., 2006; Shahin et al., 2006]. Both human and mouse isoforms are classified into long (TRIOBP-3, TRIOBP-5, and TRIOBP-6) and short (TRIOBP-1, TRIOBP-2, and TRIOBP-4) isoforms. Interestingly, no part of the protein is shared between TRIOBP-1 and TRIOBP-4 [Seipel et al., 2001]. Such a variety of isoforms encoded by a single gene may be explained by the presence of 6 accepted alternative promoters [Thierry-Mieg and Thierry-Mieg, 2006]. TRIOBP-1 is ubiquitously expressed in different tissues and was found in the whole brain, liver, spleen, kidney, retina, and inner ear [Riazuddin et al., 2006]. TRIOBP-1 plays an important role in regulation of adherent junctions as well as reorganization of the actin cytoskeleton, particularly in stress fibers and cortical F-actin [Shahin et al., 2006]. TRIOBP-4 and TRIOBP-5 were particularly found in the adult cochlea and retina of both humans and mice. In the inner ear, TRIOBP-4 and TRIOBP-5 are expressed in stereocilia rootlets. Furthermore, TRIOBP-4 is also localized along the whole length of stereocilia. Proper structure of the rootlets is important for stereocilia rigidity and stiffness, thereby allowing normal process of sound transmission [Kitajiri et al., 2010].

Cochlear implantation (CI) is currently regarded as the regular treatment modality for severe to profound SNHL. CI has well-documented benefits for spoken language, reading skills, and cognitive development [Niparko et al., 2010], but the hearing results after CI can vary among individuals. Outcomes of CI are highly variable depending on numerous factors such as age at onset of the auditory problem, CI age, and amount of residual hearing [Francis et al., 2004; Vlahović and Šindija, 2004]. Another probable factor that can affect the outcomes of the cochlear implants is the etiology of hearing loss. Etiologies including neural and/or central damage to the auditory system have poor outcomes after CI than those primarily affecting the hair cells like hereditary nonsyndromic deafness [Pyman et al., 2000; Francis et al., 2004; Taitelbaum-Sweed et al., 2006]. In this study, we report a pathogenic variant in the TRIOBP gene and hearing outcomes after CI and a rather novel fitting strategy in all 3 affected siblings.

Materials and Methods

Subjects
Of all the affected siblings, 2 brothers and 1 sister, part of an Afghan refugee consanguineous family that arrived in Belgium early 2017, were referred to the outpatient clinic of our tertiary re-
ferral center for otology and neurotology for diagnostic workup and candidacy selection for CI. All study cases were under the age of 18 years; therefore, both parents have signed a written informed consent for surgery, genetic testing, and anonymized use of data for scientific purposes for all children and their own data. The study was conducted ethically in accordance with good clinical practice according the World Medical Association Declaration of Helsinki. Since this was a retrospective chart study not requiring any extra visits, interventions, or examinations of the participants, the study has been granted an exemption from requiring ethics approval. At the time of their registration at the clinic, the 3 affected children were 5, 9, and 12 years old, and hereafter the subjects are referred to as S5, S9, and S12, respectively. These children were referred to a special education system in their home country and have not been involved in the genetic study.

Audiological Evaluation

Clinical audiometry was performed to obtain nonaided pure-tone air and bone conduction thresholds according to ISO 8253-1 [2010] standards. The hearing thresholds were determined using pulsed pure-tones in the frequency range from 125 Hz to 8 kHz. Aided sound field audiometry was performed according to ISO 8253-2 [2009] standards. The thresholds were determined using warble tones in the frequency range from 250 Hz to 6 kHz.

Speech audiometry was performed following ISO 8253-3 [2012] standards. The Flemish version of the Göttingen speech lists was used to assess the children’s speech perception [Wouters et al., 1994]. This test consists of 12 lists each containing 10 consonant-vowel-consonant (CVC) words. The percentage of correctly repeated phonemes in the open-set condition was scored. Monosyllable words were presented at discrete intensities of 40, 55, 70, and 85 dB SPL, and a weighted averaged phoneme speech index (EaSI, Eargroup Speech Index) was calculated over the intensities (phoneme score at 70 dB SPL receives double weight).

Spectral discrimination capacity of the aided ears (fitted with hearing aids or with a cochlear implant) was assessed using the A§E-Phoneme discrimination test. This test was first described by Govaerts et al., 2006 and was part of the psychoacoustic test suite that is incorporated in the Audiqueen audiological database software (Otoconsult NV, Antwerp, Belgium).

Auditory brainstem response testing was done using the BioLogic® Navigator Pro system (Natus, Pleasanton, CA, USA). Otoacoustic emission testing was done using the Otoport registration device (Otodynamics Ltd., Hatfield, UK).

Molecular Analysis

Molecular analysis was performed on DNA extracted from fresh blood using standard techniques. Variant analysis was performed by next generation sequencing (NGS) on the NextSeq500 sequencer (Illumina, San Diego, CA, USA) after Haloplex enrichment of a gene panel consisting of 99 genes known to be impli-
cated in nonsyndromal hearing loss. Sequence data were analyzed with SeqNext Analysis Software (JSI medical systems, Ettenheim, Germany). For all individual genes, a 30× coverage was obtained for >95% of the coding sequence, and for the total gene panel, a 30× coverage was obtained for >98% of the total coding sequences of all genes. Minimal minor allele frequency threshold for variant detection is not based on frequency in a database but on frequency in the generated sequence reads. Potentially pathogenic variants were confirmed by Sanger sequencing. Classification of variants was performed according to ACMG guidelines [Richards et al., 2015]. Parental origin of detected variants was done by conventional Sanger sequencing on the ABI3130XL genetic analyzer (Applied Biosystems).

Results

Molecular Analysis

Molecular analysis showed a homozygous c.1342C > T p. (Arg448*) pathogenic variant in exon 7 of the TRIOBP gene (reference sequence NM_001039141.2) in all 3 affected siblings. No other shared variants providing another possible explanation for the hearing loss were observed. Both parents were shown to be heterozygous carrier of this variant.

Preoperative Audiological Findings

All 3 affected siblings presented with bilateral profound SNHL with pure-tone averages between 83 and 90 dB HL. The hearing loss was first detected in their home country around the age of 2, strongly suggesting a congenital onset. S12 received his first hearing aid as late as the age of 8 years, S9 at the age of 5 years, and S5 at the age of 4 years. The pedigree and corresponding audiograms of the siblings are shown in Figure 1.

Preoperatively, the A§E-Phoneme discrimination test was performed to assess the spectral discrimination capacity of the children’s ears fitted with state-of-the-art power hearing aids. S5 was able to discriminate only 55% of the 20 presented phoneme contrasts using her hearing aids, S9 could discriminate 95% of the contrasts, and S12 was able to discriminate 75% of the contrasts (Fig. 2).

Speech audiometry tests could not be performed preoperatively in any of the children because none had developed oral speech by the moment of testing. In the process for candidacy for CI, all 3 siblings also underwent ABR testing. No peaks could be identified on the traces up to the maximum output of the ABR testing apparatus (90 dB nHL). No distortion product oto-acoustic emissions (DPOAEs) could be recorded from the 6 ears.

Audiological Outcome after CI

All 3 children were implanted unilaterally at the University Hospital of Antwerp. All received a Nucleus CI532 implant on the right ear. The first activation of the implant speech processor took place 2 weeks after surgery. A Nucleus CP1000 speech processor was fitted according to the FOX target-driven, computer-assisted approach as described [Govaerts et al., 2010; Battmer et al., 2015; Buechner et al., 2015].

Through this procedure, the sound field thresholds with the implant system in place quickly improved and reached near-normal values. One month after activation, the pure-tone averages with the CI processor were between 30 and 23 dB HL, and the pure-tone averages 1 year after activation are shown in Figure 3.

The spectral discrimination using the A§E-Phoneme discrimination test was repeated after CI. One month after activation, S5 could discriminate 85% of the contrasts, and S9 and S12 could discriminate 90% and 100%, respectively. S5 and S9 further improved their discrimination capacity, and after 3 months, they could discriminate 95 and 100% of the presented phoneme contrasts, respectively. Finally, at 5 months after CI activation, S5 was also able to discriminate all 20 contrasts (100%) (Fig. 4).
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Fig. 3. Postoperative audiometric thresholds with the Nucleus CP1000 processor. a One month after activation (yellow) and 1 year after activation (red) results for S5. b One month after activation (yellow) and 1 year after activation (red) results for S9. c One month after activation (yellow) and 1 year after activation (red) results for S12.

Fig. 4. Postoperative A§E-Phoneme discrimination scores with the Nucleus CP1000 processor. a One month, 3 months, and 5 months after activation results for S5 (in the direction of the arrow increased discrimination scores). b One month and 3 months after activation results for S9 (in the direction of the arrow increased discrimination score). c One month and 3 months after activation results for S12 (in the direction of the arrow not changed discrimination score). In gray the phoneme pairs that could be discriminated, and in black the contrasts that could not be discriminated.
By the start of 2019, 10 months after the first activation of the implant, the open-set speech audiometry test could be performed for the first time by the 2 younger CI recipients (S5 and S9). Both children could identify up to a maximum of 77% phonemes correctly. The complete speech audiometric curves indicated an average speech score (EaSI) of 63% correct for S5 and 71% correct for S9, as shown in Figure 5. The older sibling (S12) could not yet perform open-set speech audiometry at that moment.

Discussion

This study presents a homozygous p. (Arg448*) variant detected in the TRIOBP gene in an Afghan family where 3 affected siblings suffer from a profound hearing loss. The 3 siblings (2 males, 1 female) were implanted successfully and showed clear benefit after CI.

The TRIOBP gene encodes for a structure protein that has various isoforms. Most mutation-related hearing loss usually originates from the dysfunction of sensory hairy cells in the cochlea. The relationship between TRIOBP-1 and diseases has not been elucidated as much as TRIOBP-4 and TRIOBP-5. Although TRIOBP-1 and TRIOBP-4 have been reported to have completely different functions, both have been shown to be associated with cancer [Park et al., 2018]. It has been shown that TRIOBP-4 and/or TRIOBP-5 is required for hearing, whereas TRIOBP-1 is necessary for the viability and development of the embryo [Kitajiri et al., 2010]. Pathogenic variants in the TRIOBP gene are not among the most common causes of hearing loss. In the literature, 22 families [Diaz-Horta et al., 2012; Fardaei et al., 2015; Gu et al., 2015; Yan et al., 2016; Naz et al., 2017] and 2 isolated cases [Wesdorp et al., 2017] were reported to show induced hearing loss due to the mutation in the TRIOBP gene, and this is found in subjects from USA, China, India, Iran, Pakistan, Palestine, South Africa, Turkey, and the Netherlands. Our patients originate from Afghanistan.

TRIOBP-5 which is an isoform TRIOBP is reported to be expressed itself at roots of stereocilia, and TRIOBP-4 is reported to be expressed along the root and the whole stereocilia [Kitajiri et al., 2010]. TRIOBP-4 and TRIOBP-5 should function correctly for morphologic and functional durability and continuity, and the mutation of these isoforms in DFNB28 leads to stereociliary fusion which arises from the impairment of actin netstat apical sites of inner ear hairy cells [Park et al., 2018]. In a study conducted with rats, inactivation of TRIOBP-5 and TRIOBP-4 was shown to cause impairment in the structure of stereocilia bundles in hair cells and also in the supportive cells that have important functions for normal sound transduction in the organ of Corti, facilitating its necessary mechanic flexibility [Katsuno et al., 2019]. Genetic, physiologic, and morphologic studies show that TRIOBP-5 and TRIOBP-4 play an active role in these sensory and nonsensory cells in the inner ear.

The region of exon 7 is defined as hot point and shown to be more susceptible to mutations due to the accumulation of repeated sequences [Pollak et al., 2017]. The TRIOBP c.1342C > T p. (Arg448*) variant detected in our patient’s mutation is located in exon 7. This variant is reported (1/249546 alleles) in the gnomAD population in a European (non-Finnish) subject. As TRIOBP loss of function variants have been described before as the cause of hearing loss and the homozygous variant segregated in

Fig. 5. Postoperative open-set speech audiometry results. a Ten months after the first activation of the implant, the average speech score (EaSI) was 63% correct for S5. b Ten months after the first activation of the implant, the average speech score (EaSI) was 71% correct for S9.
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by factors such as the duration and the time course of deafening, residual hearing, early implanted children (1st year of life), cognitive and biographic parameters, and acute cochlear trauma during CI [Dalbert et al., 2016; Lenarz, 2017]. Postoperative speech discrimination score is one of the most important indicators of success of the operation [Farhood et al., 2017]. In our patients, the two young siblings (S5 and S9) were quicker in rehabilitation at postoperative 5th month discrimination when compared to the older sibling (S12). This result shows that in patients with prelingual deafness due to genetic causes, younger patients may expect a better CI result than older patients [Miyagawa et al., 2016]. One of the benefits of early cochlear implant application is to minimize the gap between the age of language development and chronological age and to learn hearing information during sensitive hearing and language development periods [Ciscare et al., 2017]. Postoperative follow-up period is as important as preoperative period. A good follow-up of CI operation may be yielded with psychosocial support by evaluating the results together with the family and the audiology team at least every 6 months [Riahi et al., 2013].

Finally, this study describes a new potential variant affecting the TRIOBP gene in an Afghani family where 3 affected siblings suffer from a profound hearing loss. We also describe the preoperative audiological findings and the audiological outcome after CI. Based on the improvement of the all 3 affected siblings, we conclude that patients with hereditary hearing loss due to the TRIOBP mutation appear to be good candidates for CI.

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Statement of Ethics

All study cases were under the age of 18 years; therefore, both parents have signed a written informed consent for surgery, genetic testing, and anonymized use of data for scientific purposes for all children and their own data. The study was conducted ethically in accordance with good clinical practice according the World Medical Association Declaration of Helsinki. Since this was a retrospective chart study not requiring any extra visits, interventions, or examinations of the participants, the study has been granted an exemption from requiring ethics approval.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.
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Author Contributions

Vedat Topsakal, Paul Van de Heyning, Geert de Ceulaer, and Paul Govaerts performed interventions, data validation, and data analysis and approved the final version of the manuscript. Ahmet M. Tekin, Yıldırım Bayazıt, and Vedat Topsakal were involved in study design, data analysis, and writing. Wim Wuyts and Vedat Topsakal were involved in genetic evaluation and interpretation.

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