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OTOLOGY

TMPRSS3 mutations in autosomal recessive nonsyndromic hearing loss

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Abstract Nonsyndromic genetic deafness is highly heterogeneous in its clinical presentation, pattern of inheritance and underlying genetic causes. Mutations in *TMPRSS3* gene encoding transmembrane serine protease account for <1 % of autosomal recessive nonsyndromic hearing loss (ARNSHL) in Caucasians. Targeted next generation sequencing in the index family with profound deaf parents and a son, and Sanger sequencing of selected *TMPRSS3* gene regions in a cohort of thirty-five patients with suspected ARNSHL was adopted. A son and his mother in the index family were homozygous for *TMPRSS3* c.208delC (p.His70Thrfs*19) variant. Father was digenic compound heterozygote for the same variant and common *GJB2* c.35delG variant. Three additional patients from the ARNSHL cohort were homozygous for *TMPRSS3* c.208delC. *TMPRSS3* defects seem to be an important cause of ARNSHL in Slovenia resulting in uniform phenotype with profound congenital hearing loss, and satisfactory hearing and speech recognition outcome after cochlear implantation. Consequently, *TMPRSS3* gene analysis should be included in the first tier of genetic investigations of ARNSHL along with *GJB2* and *GJB6* genes.

Keywords Autosomal recessive nonsyndromic hearing loss · Next generation sequencing · TMPRSS3

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Introduction

Nonsyndromic genetic deafness is highly heterogeneous in its clinical presentation (age at onset, progression, audiological characteristics), pattern of inheritance and underlying genetic causes [1]. Mutations in several genes result in similar clinical presentation of nonsyndromic hearing loss. On the other hand, mutations in the same gene can result in various clinical presentations [2, 3]. Autosomal recessive nonsyndromic hearing loss (ARNSHL) is usually prelingual, nonprogressive and severe to profound [4]. Up to date, it was accounted to mutations in 55 genes (Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org), where the number is rapidly rising. Most frequently mutated gene in ARNSHL is *GJB2*, followed by *SLC26A4*, *MYO7A*, *OTOF*, *CDH23* and *TMC1* genes. In Slovenians, 26.6 % of congenitally deaf patients [5] and 11 % of progressive hearing loss patients [6] had biallelic *GJB2* mutations, where other genetic causes of ARNSHL were not exploited so far. Mutations in *TMPRSS3* gene encoding transmembrane serine protease are associated with two ARNSHL phenotypes, DFNB8 with childhood onset and DFNB10 where hearing loss is congenital and severe [7]. *TMPRSS3* mutations are predicted to account for <1 % of ARNSHL in Caucasians [8] but are more frequent among Pakistani (1.8 %) [9], Tunisian (5 %) [10], Korean (5.9 %) [11] and especially Turkish patients (12 %) [12].

Materials and Methods

Patients

The protocol for the evaluation of hearing-impaired patients comprised a detailed family history, a medical

history focusing on potential causes of acquired hearing loss (acoustic trauma, intrauterine infections, perinatal complications, meningitis, mumps, prenatal and postnatal ototoxic drug exposure), and a complete audiological history including the age of the onset of hearing loss, the rate of progression and other audiological symptoms [13]. Written informed consent/assent was obtained from the participants or parents/minors prior to the study that was conducted according to the principles in the Declaration of Helsinki. The index family with congenitally deaf parents and their son was studied initially, followed by 35 patients (23 females, aged 1.5–29 years, mean age 9.5) with ARNSHL and no mutation identified in *GJB2* or *GJB6* genes.

DNA isolation and quantification

Whole blood samples were used for isolation of genomic DNA with established laboratory protocols based on FlexiGene DNA isolation kit (Qiagen, Hilden, Germany). Isolated DNA samples were tested for purity and concentration using established spectroscopic protocols. The A260/A280 absorption ratio above 1.7 was the indication of successful DNA sample purification. One hundred ng/μL stock solution was prepared from each sample and 20 ng/μL working solution was used for next generation sequencing (NGS) analysis.

Library preparation and NGS analysis

The regions of interest were enriched using TruSight One library enrichment kit (Illumina, San Diego, USA) following manufacturer's instructions. DNA samples of patients were fragmented, tagged, amplified and pooled to yield 12 pM loading library sample. NGS sequencing was performed using MiSeq desktop sequencer coupled with MiSeq Reagent kit v3 (both Illumina, San Diego, USA). 300 cycles paired-end run using Phred quality threshold >30 was executed followed by onboard primary analysis. Detected genetic variants exceeding >20× coverage threshold setting were analyzed with Variant Studio 2.2 software (Illumina). Further evaluation of variants was restricted to those located in 100 genes related to hearing loss, both syndromic and nonsyndromic, as reported in Hereditary Hearing Loss Homepage, among them, there were 52 genes related to ARNSHL. Among known variants, we eliminated those with minor allele frequency above 1 % from further evaluations.

Sanger sequencing

The presence of candidate causative variant was confirmed with Sanger sequencing. Exon 3 of the *TMPSSS3* gene was PCR amplified with in-house designed primers TMPRSS3e3F (gacagggacgcaatttcaata) and TMPRSS3e3R (tacagatgggaagggtcagg), exon 6 with primers TMPRSS3e6F (ttcaaagagggggaaatagaa) and TMPRSS3e6R (ctgagggcaaggagatagga). Amplicons were sequenced using a BigDye Terminator v3.1 sequencing kit and an ABI Genetic Analyzer 3500 (both Applied Biosystems, Foster City, USA).

Results

Clinical characteristics of the patients

Family with profound hearing loss, parents and their son

The index family comprised congenitally deaf parents and their son. All others possible causes of hearing loss were excluded [13]. The father has never received hearing aids, but mother has had hearing aids for <2 years in her early childhood without any hearing improvement and still refuses to use them. When Slovenian cochlear implant program started, both parents were adults with only sign language developed. They never complained regarding vestibular disorders. The son is their only offspring and was conceived at their age of 37 years. He was referred to an audiologist after he failed at the National newborn hearing screening program where evoked otoacoustic emissions were not present in first 2 days after birth. He underwent a clinical otorhinolaryngological examination, including ear microscopy, with a systematic search for signs of a syndromic form of hearing loss. All others possible causes of hearing loss were excluded [13]. Before each hearing test, a tympanogram was performed and middle ear causes of hearing loss were excluded. The brain stem evoked acoustic potentials (BERA) were not detected even at stimulation with 110 dB click. He underwent ophthalmological and neurological examinations. They revealed no other pathology. He was immediately fitted with bilateral hearing aids at his age of 5 months but without any benefit. As he was a candidate for cochlear implantation, computed aid tomography (CT scan) of the temporal bone was performed and showed normal medial and inner ear. The cochleography measurements were negative and the electrically evoked auditory brain responds (EABR) were not detectable. For the stimulation, the stimulating golf electrode was placed on round window and reversed electrical stimuli with intensity from 200 μA to 1 mA and duration of 200 μs were used. He received cochlear implant (CI) on his right side at age of 11 months. Now, he has pure tone audiometry hearing thresholds in speech frequencies at 25 dB and he is integrated in regular school. In all of them, *GJB2*, *GJB6* variants and

Table 1 Clinical characteristics of patients with homozygous c.207delC *TMPRSS3* mutation (BERA, brain evoked response audiology; residual hearing in isolated frequencies was assessed with behavioral audiometry in open field)

Patient	Year of birth	BERA	Profound hearing loss onset	Residual hearing (dB)	Age at cochlear implantation (months)	Hearing with cochlear implant (dB)
1-mother	1977	Negative	Congenital	80–110	/	/
1-son	2007	Negative	Congenital	80–110	11	25
2	2008	Negative	Congenital	95–110	30	45
3	2001	Negative	Congenital	80–100	13	25
4	2004	Negative	Congenital	70–85	11	25

mitochondrial A1555G variant were previously excluded. Only in the father, common c.35delG variant in *GJB2* gene in a heterozygous state was identified.

Patients with suspected ARNHL

After identifying *TMPRSS3* variant in the index family, the same variant was also screened in other Slovenian congenitally deaf patients with suspected ARNHL. Thirty-five patients were included, all with normally hearing parents and no signs of syndromic forms of hearing loss. In all of them, *GJB2*, *GJB6* mutations and mitochondrial A1555G mutation were previously excluded. All were congenitally deaf, confirmed with BERA measurements. They all received CI as children at average age of 3 years. All except one had good hearing with cochlear implant at the average level 35 DB; in one, the hearing with CI was immeasurable due to severe mental disability not accounted to syndromic forms of hearing loss. Clinical characteristics of patients with identified *TMPRSS3* mutations were summarized in the Table 1.

Identification of the disease associated variant

In all three family members, NGS sequencing revealed *TMPRSS3* variants that were subsequently confirmed with Sanger sequencing. The son and his mother carried c.208delC (p.His70Thrfs*19) variant on both alleles. It was detected with read depth 62/65 in the son and 31/32 in the mother. Father was compound heterozygous for the same c.208delC variant (read depth 31/66) and additional c.579dupA (p.Cys194Metfs*17) variant (read depth 27/66). c.208delC variant is a known frame-shift variant associated with ARNSHL. Sanger sequencing of the group of patients with suspected ARNHL identified three additional patients with c.208delC variant in homozygous state. c.579dupA variant (rs397517376) is also a frame-shift variant but is located in noncoding exon and has no known association with ARNHL. Therefore, in the father digenic compound heterozygousity of *TMPRSS3,* c.208delC variant and previously detected *GJB2*c.35delG variant was proposed.

Discussion

The family history is usually not as evident in ARNSHL as it is in dominant nonsyndromic hearing loss. In the investigated family, both parents and their son were congenitally deaf, and we could not reliably predict the inheritance and suggest more limited targeted genetic testing for autosomal recessively or dominantly inherited deafness. Additionally, father was known to carry *GJB2* variant, which was implying possible digenic inheritance in the family. Therefore, NGS sequencing targeting all genes, related to both syndromic and nonsyndromic hearing loss was used successfully. Targeted NGS sequencing seems suitable for identification of causative mutations in hearing-loss patients due to many genes involved in its etiology and various mode of inheritance. It was previously reported as efficient in identifying mutations in 33 % [14] or 12.7 % [15] of sensorineural hearing-loss cases.

Estimated frequency of *TMPRSS3* mutations in general childhood Caucasian deaf population is 0.38 % [8]. c.207delC variant was so far reported in homozygous state in one Spanish [8], one Dutch [16], 6 Pakistani and 8 Newfoundland patients, each from one family [17]. In compound heterozygous state, the mutation was reported in one Greek [8], one Dutch [16] and 2 Newfoundland patients [17]. This one nucleotide deletion is leading to frame-shift at the amino acid position 160 and premature termination after 18 unrelated amino acids. The mutation is located just after the transmembrane domain and the truncated protein is lacking LDLRA, SRCR and protease domain [8] suggesting that variant is seriously affecting the function of the truncated protein. Minor allele frequency according to Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) in apparently healthy European-American population is 0.06 where it is not present in homozygous state. This is further confirming its pathogenicity.

The phenotype resulting from the *DMPRSS3* variants is dependent on residual proteolitic activity of the mutated protein [18]. Combination of two severe variants on separated alleles leads to profound deafness with prelingual onset, while the combination of severe and mild variant leads to milder phenotype with postlingual onset [16]. In

concordance, patients presented here with homozygous c.207delC variant had the same level of profound hearing loss.

Variant c.208delC was detected in homozygous state in two patients from the index family and in three additional ARNHL patients, altogether 5 out of 38 patients (13.1 %). Therefore, *TMPRSS3* defects seem to be an important cause of ARNHL in Slovenians, even though it was previously reported to be very rare in Caucasians [8]. Consequently, *TMPRSS3* gene analysis should be included in the first tier of genetic investigations of ARNSHL along with *GJB2* and *GJB6* genes. Identification of causative variants in hearing-loss patients is important to at least partly predict the severity of hearing loss and assist in the decision regarding cochlear implant. Patients with *TMPRSS3* mutations have satisfactory speech recognition after the cochlear implantation [16]. The son from the index family received cochlear implant at the age of 13 months with an excellent outcome. During the 6 years of using the implant, he developed good verbal communication and attends regular school. The three additional patients from ARNSHL group with the same mutation were also congenitally deaf and received cochlear implants at the age of 11–30 months with comparably favorable outcome.

In conclusion, *TMPRSS3* mutations seem to be an important cause of ARNSHL in Slovenia resulting in rather uniform phenotype with profound congenital hearing loss, and satisfactory hearing and speech recognition outcome after cochlear implantation.

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Conflict of interest None declared.

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