ORIGINAL RESEARCH

Cochlear Implants in Deaf Patients with Novel TMPRSS3 Gene Mutation

Rong Yu, MD; Kai Wang, MD; Liujun You, MD; Junxin Kang, MD; Honghui Ai, MD; Hongqun Jiang, MD

ABSTRACT

Objective • To investigate the effect of cochlear implants in deaf patients with *TMPRSS3* gene pathogenic variations.

Methods • Variations of deafness genes were detected in 2 patients with profound hearing loss. Both received unilateral cochlear implantation. Hearing and speech abilities were evaluated and analyzed before and 3 and 6 months after surgery. The analysis included post-surgery evaluation of auditory behavior (Categories of Performance [CAP]) and Speech Intelligibility Rating (SIR).

Results • In the 2 patients, 3 pathogenic single nucleotide variations (SNVs) of *TMPRSS3* gene and a large deletion in 21q22.3 were detected. The CAP and SIR grades increased with the recovery time.

Conclusion • Cochlear implants have a good effect in patients with *TMPRSS3* gene mutation deafness. Preoperative gene testing has a certain reference significance for the prognosis in patients with the deafness gene mutation. (*Altern Ther Health Med.* 2023;29(5):102-106).

Rong Yu, MD; Liujun You, MD; Junxin Kang, MD; Honghui Ai, MD; Hongqun Jiang, MD; Department of Otorhinolaryngology, First Affiliated Hospital of Nanchang University, Nanchang, China. **Kai Wang, MD;** Department of Otorhinolaryngology, The 908th Hospital of Chinese People's Liberation Army, Joint Logistic Support Force, Nanchang, China.

Corresponding author: Hongqun Jiang, MD
E-mail: jianghongqun@sohu.com
Corresponding author: Honghui Ai, MD
E-mail: aihh@ncu.edu.cn

INTRODUCTION

Deafness is a major public health problem worldwide, and hearing protection is a major issue for China's social and economic development. There are many etiologies for deafness, but genetics is the major cause. In congenital deafness with genetic heterogeneity, the key step to reduce the rate of birth defects is to discover the molecular etiology of deafness and explain its mechanism of action. Among patients with hereditary hearing loss, 70% are non-syndromic, and autosomal recessive non-syndromic deafness (DFNB) is usually characterized by severe to profound pre-lingual sensorineural hearing loss.

The *TMPRSS3* gene is expressed in the Corti organ, stria vascularis and spiral ganglia or cochlea,[1] and encodes a transmembrane serine protease. Pathogenic variations of the *TMPRSS3* gene caused severe to profound sensorineural deafness with onset at pre- or post-lingual (DFNB8/DFNB10) age. At present, 76 pathogenic variations have been reported in *TMPRSS3*. Cochlear implantation (CI) is an important and effective treatment for deaf patients with *TMPRSS3* gene variations.[2] Individual differences exist in the postoperative effects, which are related to many factors, such as the duration of deafness, patient age at implantation, residual hearing, rehabilitation training, inner ear malformation and posterior cochlear lesions.[3,4] The results of CI in patients with *TMPRSS3* deafness are inconsistent.

In this study, we reported 2 patients with *TMPRSS3* gene mutations during CI presenting with pre- and post-lingual deafness and identified 3 SNVs of the *TMPRSS3* gene and a large deletion in 21q22.3, providing reference for the effect of CI in patients with *TMPRSS3*-induced deafness, providing diagnosis and genetic counseling for these families and enriching the spectrum of pathogenic mutations of the *TMPRSS3* gene.

MATERIALS AND METHODS

Clinical Data

In our study, the proband of family 1 was a 4-year-old girl of Han nationality who had poor response to sound after age 3 years; proband of family 2 was an 8-month-old boy of Han nationality who had poor response to sound after birth. They both received CI from the department of otolaryngology in First Affiliated Hospital of Nanchang University in China.

This study was approved by the ethics committee of our hospital, and the patients' guardians provided written informed consent.

The index case 's family came from Jiangxi Province, China. A detailed medical history of the proband was obtained along with a detailed physical examination, including an examination by an ear specialist, physical examination and clinical classification. Peripheral venous blood was collected from the proband and family members, and venous blood samples from 50 normal controls were obtained from our hospital molecular genetic database. All the index cases received an MRI and auditory brainstem response testing.

Genetic Analysis

The probands and their parents underwent genetic analysis. The purified DNA was sent to Boao Laboratory, Markham, Ontario, Canada for high-throughput DNA sequencing to detect hearing loss genes. The average sequencing coverage was above 99% using the illumina Novaseq (San Diego, California USA) sequencing platform. The peripheral blood of the proband was extracted for deafness gene detection by high-throughput sequencing. A total of 227 deafness genes were included in high-throughput sequencing. Genome Analysis Tool Kit (GATK) Best Practice workflow was used to develop variants. Annovar was used for annotation and then the annotation results were integrated, and preliminary filtering and normalized modification were carried out according to the annotation results. The filtering process included removing loci with a low mutation ratio and low sequencing depth. The variations were screened and compared with NCBI dbSNP database, gnomAD exon database, 1000 Genome database and ExAC database to exclude the known polymorphic loci with a normal frequency higher than 5%. Then we used the Genome Variation Database (DGV) (http://dgv.tcag.ca/dgv/app/home), Decipher(https://www.deciphergeno mics.org /browser) and Clinvar (https://www.nCBI.Gov/clINVar clinvar) to search the reported status and pathogenicity of mutation loci, exclude the reported benign mutation loci, and bioinformatics protein function prediction software PROVEAN, SIFT, Mutationtaster etc were used to predict biological function. Pathogenicity analysis and diagnosis were performed according to the guidelines recommended by the American College of Medical Genetics and Genomics and Association for Molecular Pathology.

Cochlear Implantation

All patients underwent a standard transmastoid posterior tympanotomy with electrode array (CI512) inserted through a round window. The CI was placed into the skull fixation slot. The electrode was inserted into the round window with electrode tweezers and slowly inserted along the drum. Corticosteroids are injected into the round window to reduce the cochlear inflammatory response. The gap between the round window and the electrode was filled with muscle fragments, and no outflow of perilymph was observed after several minutes. The skin was sutured and bandaged. Postoperative neural response telemetry (NRT) and impedance detection were performed; 2 points were detected by NRT and impedance was notably good.

Efficacy Indicator

The efficacy indicator included the Categories of Auditory Performance (CAP)[5] and Speech Intelligibility Rating (SIR)[6] scales. The efficacy indicators in the 2 patients were measured before surgery, and 3 and 6 months after surgery. The CAP is divided into 9 grades and is used to assess the hearing ability of hearing-impaired children at different times on a regular basis. SIR is divided into 5 grades to assess the speech intelligibility of hearing-impaired children at different periods.

RESULTS

Family and Clinical Evaluations

The proband of family 1 had pre-lingual hearing loss. An auditory brainstem response (ABR) hearing test performed at 3 years of age showed severe hearing loss. Her older brother had normal hearing. The family 2 proband has pre-lingual profound hearing loss; an ABR failed to elicit a response at 95dB nHL.

Sequencing Results of *TMPRSS3*

A heterozygous variation c.371C>T (p.Ser124Leu) of the *TMPRSS3* gene was identified in the proband of family 1, which was inherited from his father, and a large deletion about 4.67Mb in chromosome 21q22.3(43. 41Mb-48.08Mb) including *TMPRSS3* gene was also detected in this patient.

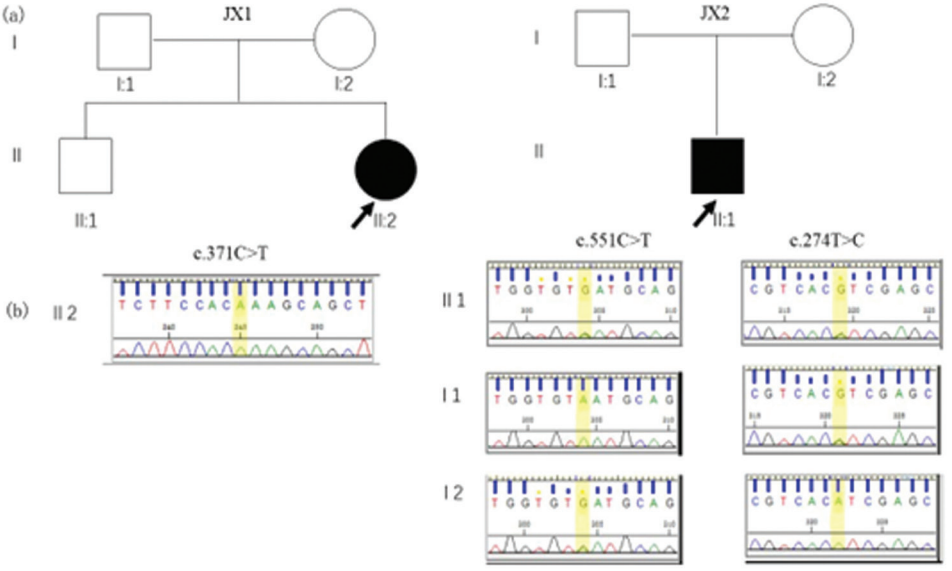
Compound heterozygous variations c. 551T>C(p. Leu184Ser) and c. 274T>C(p,Cys94Arg) were identified in the proband of family 2. Variation c.551T>C, which was previously reported as pathogenic came from his mother and the novel variation c.274T>C was inherited from his father.

These data, together with the clinical presentation of the affected siblings and consistent autosomal recessive inheritance and chromosome 21 of the mutations in the affected and unaffected members, indicated that the *TMPRSS3* mutations, *TMPRSS3*:c.371C>T, deletion in chromosome 21q22.3 (43.41Mb-48.08Mb), *TMPRSS3*:c.551T>C and *TMPRSS3*: c.274T>C were the cause of hearing impairment in these families (Figure 1). *TMPRSS3*:c.371C>T and *TMPRSS3*: c.551T>C were already known, and deletion in chromosome 21q22.3(43.41Mb-48.08Mb) and *TMPRSS3*:c.274T>C were reported as novel and classified as likely pathogenic.

Effects After Cochlear Implantation

CI was successful in both patients and there were no complications. At different postoperative time points, the postoperative cochlear hearing threshold test score was improved compared with before surgery, and the CAP and SIR scale scores were also improved. The scores were both 0 points before surgery and 3 points 3 months after surgery;

Figure 1. Pedigree of Chinese family with mutation analysis of *TMPRSS3*. (**1A**)The proband is indicated by an arrow. Subjects II:2, II:1 were tested by NGS. Other family members were tested by Sanger verification. (**1B**) DNA sequencing profile showing the c.371C>T, c.551C>T, and c.274T>C mutations in *TMPRSS3*.



Abbreviations: NGS, next generation sequencing.

proband 1 was 6 points and proband 2 was 4 points at 6 months after surgery.

DISCUSSION

Congenital hearing loss is the most common sensory impairment in humans, with an incidence of approximately 1 in 1000 live births. Genetic factors account for approximately 50%,[7] while non-syndromic deafness accounts for approximately 80% of inherited deafness, and is inherited by autosomal recessive (DFNB), autosomal dominant (DFNA) and X-linked inheritance (DFN).[8]

A *TMPRSS3* gene defect can lead to DFNB[8] (post-lingual deafness) and DFNB10 (pre-lingual deafness),[9-10] which is autosomal recessive. It is also the first deafness gene discovered so far, but the exact biological function of this protease is not clear at present. This gene is located in the q22.3 region of chromosome 21, is 24KB in length, contains 13 exons, coding from exon 2 and has 4 alternative splicing transcripts in humans. Encoding approximately 454, 327, 327 and 344 amino acids,[11] respectively, *TMPRSS3* encodes a transmembrane serine protease (TTSP) type II, which contains an n-terminal transmembrane domain. The low-density lipoprotein receptor A (LDLRA) domain, cysteine-rich scavenger receptor domain and carboxy-terminal protease domain[12] are expressed in a variety of tissues, including inner ear hair cells, stria vascularis and spiral ganglion. It can activate sodium channel EnaC, promote protein lysis and participate in the maintenance of low sodium ion (Na+) concentration levels in endolymphatic tissues.[13-15] It has also been suggested that *TMPRSS3* protein dysfunction reduces the expression of potassium channels, which play a critical role in maintaining sound editing and resting potential stabilization of inner hair cells.[16] According to a literature review, in the GJB2 negative autosomal recessive deaf population, the percentage of the occurrence of the*TMPRSS3* gene in the Turkish, Slovenian, European and Korean population is 12%, 13.1%, 0.45% and 5.9%, respectively,[17-20] while in the chromosomal recessive deaf population, the occurrence of the *TMPRSS3* gene in the population of Pakistan, Tunisia, South Korea and China was 1.8%, 5%, 2.5% and 4.6%,[21-24] respectively.

The data shows that the *TMPRSS3* gene is still an important pathogenic factor in hereditary deafness that cannot be ignored. Especially when the common GJB2, SLC26A4, mtDNA gene mutations are excluded, this gene should be considered. More than half of congenital deafness is related to genetic factors, and most hereditary deafness is severe or extremely severe sensorineural deafness. CI is the most effective treatment and rehabilitation method for deafness; however, the efficacy of CI varies with different pathogenic genes. Before CI surgery, the molecular pathogenesis of the deafness can be identified through genetic testing, and the efficacy of CI can be preliminarily predicted by selecting targeted treatment programs.

Up until April 2021, the Human Gene Mutation Database (HGMD®)database (http://www.hgmd.cf.ac.uk/ac/index.php) included a total of 84 kinds of *TMPRSS3* gene mutations, including 65 species of missense mutation, 5 kinds of splice mutations, 4 small deletion mutations, small fragments into 4 and 6 other types of mutations. It has been suggested that *TMPRSS3* gene mutations can be classified as mild or severe, and hearing phenotype depends on the binding type of mutation site. A combination of mild and severe heterozygous mutations can lead to post-lingual hearing loss (DFNB8),

while 2 severe mutations can lead to pre-lingual hearing loss (DFNB10).[25]

In family 1, the proband had post-lingual hearing loss, which was a compound heterozygous mutation and c.371C>T in *TMPRSS3* was a missense mutation, which resulted in the transformation of serine 124 into leucine [p.Ser124Leu]. This variation has been reported previously.[26] Further copy number variation (CNV) analysis revealed that heterozygous deletion of 4.67 Mb fragment size was suspected at q22.3(43.41 Mb-48.08Mb) on chromosome 21 of the autosomal chromosome. Regional deletions in 21Q22.3 have not been reported in the general population genomic polymorphism database (Database of Genomic Variations [DGV]). The DECIPHER and ClinVar databases included several cases of pathogenicity or potential pathogenicity variants with deletion of relevant regions. According to CNV evaluation criteria, the loss of copy number in the q22.3 region of chromosome 21 was identified as possibly pathogenic.

In pedigree 2, the proband had pre-lingual hearing loss, which was a heterozygous mutation. There were 2 heterozygous mutations in the TMPRSS3 gene (c.551T>C, c.274T> C). TMPRSS3: c.551T>C mutation was a missense mutation, which resulted in the transformation of leucine 184 into serine [p.Leu184Ser], which was reported in patients with Hodgkin's lymphoma as a pathogenic variation.[27,28] TMPRSS3: c.274T>C lead to the encoding peptide 92 cysteine to arginine [p.Cys92Arg], which has not yet been reported. This variation was not included in gnomAD, Thousand People database or the Exome Aggregation Consortium (ExAC) database. Computer-aided analysis predicted that the mutation was likely to affect protein structure/function. Based on the above evidence, we recommend that the mutation be classified as likely pathogenic in accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines [PM2, PM3, PP1, PP3].

A CI is the most effective biomedical engineering device for severe sensorineural hearing loss, but there are still some patients with unsatisfactory results after CI. In addition to patient age at implantation being the main variable, different damage sites ranging from the cochlea to the auditory nerve caused by different gene mutations may be another key factor. With the continuous progress of genetic detection technology for deafness, genetic diagnosis has become an important tool for preoperative evaluation and postoperative effect prediction in patients receiving a CI. Based on the genetic information of patients with hereditary deafness, accurate classification and diagnosis of deafness can be designed for patients with individualized prevention and treatment programs, and accurate medical treatment of hereditary deafness can be realized.

An important factor in the effectiveness of CI is the presence of retrocochlear lesions. CI electrodes can help patients regain hearing by stimulating the neurons of the spiral ganglion in the inner ear, and the lesions of the spiral ganglion, auditory nerve and its posterior conduction pathway have a great influence on the effect of a CI. The *TMPRSS3* gene is expressed in inner hair cells, outer hair cells, Sertoli cells, spiral ganglion and stria vascularis in the cochlea. The effect of an *TMPRSS3* gene mutation on spiral ganglion may be one of the pathogenic mechanisms, and a CI in patients with the *TMPRSS3* gene mutation may be affected by it. The results of CI implantation in patients with *TMPRSS3* deafness are mixed. Weegerink, et al. evaluated the effects of CI in 7 patients with different phenotypes (DFNB8 and DFNB10) and genotypes, and the scores of syllables and words were higher than those of the 2 controls, resulting in good speech recognition ability after CI.[29] Xue, et al. reported a case of CI in a patient age 6 years whose hearing and language abilities were significantly improved after surgery, and her residual hearing was well maintained.[30]

Battelino, et al. reported 5 cases of extremely severe deafness with an average patient age of 3 years who received CI.[31] Except for 1 patient with a mental disability, the other 4 patients had an average postoperative hearing threshold of 35dB, and one of them enrolled in regular school. Miyagawa, et al. reported that 2 patients who received sonoelectric stimulation had good speech recognition and perception after CI.[32,33] Elbracht, et al. reported that 4 siblings with post-lingual deafness in a family had good results following CI.[34] However, among the 10 cases reported by Eppsteiner, et al. and Eliot Shearer, et al, 9 cases had poor effect, and 10 cases all had post-speech deafness. The average age at CI was 50.4 years.[35-37]

The probands in both families showed no structural abnormalities in the cochlea. The CAP and SIR scores of the 2 probands improved well in half a year after surgery, with CAP score reaching 6 points and SIR score reaching 4 points.

In this study, we reported the specific type of mutation in 2 deaf patients with a novel *TMPRSS3* mutation, and both patients experienced significant hearing improvement after CI, which provided clinical experience for the treatment of those deaf patients with *TMPRSS3* genetic defects.

Study Limitations

The most notable limitation of this study is the small number of persons with an identifiable genetic cause of hearing loss.

CONCLUSION

Our study data support the fact that patients with the *TMPRSS3* gene mutation had good effects after CI. Preoperative gene testing may be helpful in the prognosis of patients who have a deafness gene mutation.

FUNDING

This study was supported by National Natural Science Foundation of China (No. 82101237).

REFERENCES

1. Guipponi M, Vuagniaux G, Wattenhofer M, et al. The transmembrane serine protease (TMPRSS3) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. *Hum Mol Genet*. 2002;11(23):2829-2836. doi:10.1093/hmg/11.23.2829
2. Gates GA, Miyamoto RT. Cochlear implants. *N Engl J Med*. 2003;349(5):421-423. doi:10.1056/NEJMp038107
3. Gong S, Wang M. Factors related to the effect of cochlear implantation. *Chinese Sci J Hearing Speech Rehab*. 2015;(4):241-244.

4. Morton CC, Nance WE. Newborn hearing screening--a silent revolution. *N Engl J Med*. 2006;354(20):2151-2164. doi:10.1056/NEJMra050700
5. Wang D, Zhou H, Zhang J. Evaluation of auditory behavior in children with prespeech deafness after cochlear implantation. *Clin Otolaryngol Head Neck Surg*. 2015;29(5):441-444.
6. Yu W, Tao P, et al. Establishment and reliability test of Chinese language intelligibility classification criteria. *J Audiol Speech Disord*. 2013;21(05):465-468.
7. Petersen MB, Willems PJ. Non-syndromic, autosomal-recessive deafness. *Clin Genet*. 2006;69(5):371-392. doi:10.1111/j.1399-0004.2006.00613.x
8. Petit C. Genes responsible for human hereditary deafness: symphony of a thousand. *Nat Genet*. 1996;14(4):385-391. doi:10.1038/ng1296-385
9. Veske A, Oehlmann R, Younus F, et al. Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan. *Hum Mol Genet*. 1996;5(1):165-168. doi:10.1093/hmg/5.1.165
10. Bonné-Tamir B, DeStefano AL, Briggs CE, et al. Linkage of congenital recessive deafness (gene DFNB10) to chromosome 21q22.3. [J]. *Am J Hum Genet*. 1996;58(6):1254-1259.
11. Scott HS, Kudoh J, Wattenhofer M, et al. Insertion of β-satellite repeats identifies a transmembrane protease causing both congenital and childhood onset autosomal recessive deafness. *Nat Genet*. 2001;27(1):59-63. doi:10.1038/83768
12. Rawlings ND. Peptidase specificity from the substrate cleavage collection in the MEROPS database and a tool to measure cleavage site conservation. *Biochimie*. 2016;122:5-30. doi:10.1016/j.biochi.2015.10.003
13. Couloigner V, Fay M, Djelidi S, et al. Location and function of the epithelial Na channel in the cochlea. *Am J Physiol Renal Physiol*. 2001;280(2):F214-F222. doi:10.1152/ajprenal.2001.280.2.F214
14. Guipponi M, Vuagniaux G, Wattenhofer M, et al. The transmembrane serine protease (*TMPRSS3*) mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. *Hum Mol Genet*. 2002;11(23):2829-2836. doi:10.1093/hmg/11.23.2829
15. Wattenhofer M, Sahin-Calapoglu N, Andreasen D, et al. A novel *TMPRSS3* missense mutation in a DFNB8/10 family prevents proteolytic activation of the protein. *Hum Genet*. 2005;117(6):528-535. doi:10.1007/s00439-005-1332-x
16. Molina L, Fasquelle L, Nouvian R, et al. Tmprss3 loss of function impairs cochlear inner hair cell Kcnma1 channel membrane expression. *Hum Mol Genet*. 2013;22(7):1289-1299. doi:10.1093/hmg/dds532
17. Wattenhofer M, Sahin-Calapoglu N, Andreasen D, et al. A novel *TMPRSS3* missense mutation in a DFNB8/10 family prevents proteolytic activation of the protein. *Hum Genet*. 2005;117(6):528-535. doi:10.1007/s00439-005-1332-x
18. Battelino S, Klancar G, Kovac J, Battelino T, Trebusak Podkrajsek K. *TMPRSS3* mutations in autosomal recessive nonsyndromic hearing loss. *Eur Arch Otorhinolaryngol*. 2016;273(5):1151-1154. doi:10.1007/s00405-015-3671-0
19. Wattenhofer M, Di Iorio MV, Rabionet R, et al. Mutations in the *TMPRSS3* gene are a rare cause of childhood nonsyndromic deafness in Caucasian patients. *J Mol Med (Berl)*. 2002;80(2):124-131. doi:10.1007/s00109-001-0310-6
20. Chung J, Park SM, Chang SO, et al. A novel mutation of *TMPRSS3* related to milder auditory phenotype in Korean postlingual deafness: a possible future implication for a personalized auditory rehabilitation. *J Mol Med (Berl)*. 2014;92(6):651-663. doi:10.1007/s00109-014-1128-3
21. Ahmed ZM, Li XC, Powell SD, et al. Characterization of a new full length *TMPRSS3* isoform and identification of mutant alleles responsible for nonsyndromic recessive deafness in Newfoundland and Pakistan. *BMC Med Genet*. 2004;5(1):24. doi:10.1186/1471-2350-5-24
22. Masmoudi S, Antonarakis SE, Schwede T, et al. Novel missense mutations of *TMPRSS3* in two consanguineous Tunisian families with non-syndromic autosomal recessive deafness. *Hum Mutat*. 2001;18(2):101-108. doi:10.1002/humu.1159
23. Lee J, Baek JI, Choi JY, Kim UK, Lee SH, Lee KY. Genetic analysis of *TMPRSS3* gene in the Korean population with autosomal recessive nonsyndromic hearing loss. *Gene*. 2013;532(2):276-280. doi:10.1016/j.gene.2013.07.108
24. Gao X, Huang SS, Yuan YY, et al. Identification of *TMPRSS3* as a significant contributor to autosomal recessive hearing loss in the Chinese population. *Neural Plast*. 2017;2017:3192090. doi:10.1155/2017/3192090
25. Weegerink NJ, Schraders M, Oostrik J, et al. Genotype-phenotype correlation in DFNB8/10 families with *TMPRSS3* mutations. *J Assoc Res Otolaryngol*. 2011;12(6):753-766. doi:10.1007/s10162-011-0282-3
26. Lechowicz U, Gambin T, Pollak A, et al. Iterative Sequencing and Variant Screening (ISVS) as a novel pathogenic mutations search strategy - application for TMPRSS3 mutations screen. *Sci Rep*. 2017;7(1):2543. doi:10.1038/s41598-017-02315-w
27. Li X, Tan B, Wang X, et al. Identification of a complex genomic rearrangement in *TMPRSS3* by massively parallel sequencing in Chinese cases with prelingual hearing loss. *Mol Genet Genomic Med*. 2019;7(6):e685. doi:10.1002/mgg3.685
28. Sang S, Ling J, Liu X, et al. Proband whole-exome sequencing identified genes responsible for autosomal recessive non-syndromic hearing loss in 33 Chinese nuclear families. *Front Genet*. 2019;10:639. doi:10.3389/fgene.2019.00639
29. Weegerink NJ, Schraders M, Oostrik J, et al. Genotype-phenotype correlation in DFNB8/10 families with *TMPRSS3* mutations. *J Assoc Res Otolaryngol*. 2011;12(6):753-766. doi:10.1007/s10162-011-0282-3
30. Gao X, Yuan YY, Wang GJ, et al. Novel mutations and mutation combinations of *TMPRSS3* cause various phenotypes in one Chinese family with autosomal recessive hearing impairment. *BioMed Res Int*. 2017;2017:4707315. doi:10.1155/2017/4707315
31. Battelino S, Klancar G, Kovac J, Battelino T, Trebusak Podkrajsek K. *TMPRSS3* mutations in autosomal recessive nonsyndromic hearing loss. *Eur Arch Otorhinolaryngol*. 2016;273(5):1151-1154. doi:10.1007/s00405-015-3671-0
32. Miyagawa M, Nishio SY, Sakurai Y, et al. The patients associated with *TMPRSS3* mutations are good candidates for electric acoustic stimulation. *Ann Otol Rhinol Laryngol*. 2015;124(1_suppl) (suppl 1):193S-204S. doi:10.1177/0003489415575056
33. Zhang X, Xu Y, Liu D, et al. A modified multiplex ligation-dependent probe amplification method for the detection of 22q11.2 copy number variations in patients with congenital heart disease. *BMC Genomics*. 2015;16(1):364. doi:10.1186/s12864-015-1590-5
34. Elbracht M, Senderek J, Eggermann T, et al. Autosomal recessive postlingual hearing loss (DFNB8): compound heterozygosity for two novel *TMPRSS3* mutations in German siblings. *J Med Genet*. 2007;44(6):e81. doi:10.1136/jmg.2007.049122
35. Eppsteiner RW, Shearer AE, Hildebrand MS, et al. Prediction of cochlear implant performance by genetic mutation: the spiral ganglion hypothesis. *Hear Res*. 2012;292(1-2):51-58. doi:10.1016/j.heares.2012.08.007
36. Shearer AE, Tejani VD, Brown CJ, et al. In vivo electrocochleography in hybrid cochlear implant users implicates *TMPRSS3* in spiral ganglion function. *Sci Rep*. 2018;8(1):14165. doi:10.1038/s41598-018-32630-9
37. Shearer AE, Eppsteiner RW, Frees K, et al. Genetic variants in the peripheral auditory system significantly affect adult cochlear implant performance. *Hear Res*. 2017;348:138-142. doi:10.1016/j.heares.2017.02.008