Application of next-generation sequencing to identify mitochondrial mutations: Study on m.7511T>C in patients with hearing loss

URSZULA LECHOWICZ1, AGNIESZKA POLLAK1, AGNIESZKA FRĄCZAK1, MAŁGORZATA RYDZANICZ2, PIOTR STAWIŃSKI1, ARTUR LORENS3, PIOTR H. SKARŻYŃSKI4-6, HENRYK SKARŻYŃSKI7, RAFAŁ PŁOSKI2* and MONIKA OŁDAK1*

1Department of Genetics, World Hearing Center, Institute of Physiology and Pathology of Hearing, 02-042 Warsaw; 2Department of Medical Genetics, Center for Biostructure, Medical University of Warsaw, 02-106 Warsaw; 3Department of Implants and Auditory Perception; 4World Hearing Center, Institute of Physiology and Pathology of Hearing, 02-042 Warsaw; 5Department of Heart Failure and Cardiac Rehabilitation, Second Faculty, Medical University of Warsaw, 03-242 Warsaw; 6Institute of Sensory Organs, 05-830 Nadarzyn; 7Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, 02-042 Warsaw, Poland

Received June 29, 2017; Accepted October 24, 2017

DOI: 10.3892/mmr.2017.8064

Abstract. Interruptions in the activity of mitochondria induced by mutations in the mitochondrial genome (mtDNA) can be the source of numerous diseases including hearing loss (HL). One of the mitochondrial variants responsible for HL is the m.7511T>C mutation located in the mitochondrially encoded tRNA serine 1 (UCN) gene. Next-generation sequencing was used to search for the HL mutations in the whole mtDNA of 2 patients with maternal inheritance and real time-polymerase chain reaction was applied for population screening of the m.7511T>C mutation in a group of 1,644 patients with HL. Sequencing of the whole mtDNA in 2 probands revealed a homoplasmic m.7511T>C mutation. Inheritance of the m.7511T>C mutation has been confirmed in examined matrilineal relatives in both families. The mean age of HL onset was 14.1 years old with the mean degree of HL equaling 74.8 dB. A large-scale search for the m.7511T>C mutation among the patients with HL established the frequency of the m.7511T>C mutation at 0.12% among Polish patients with HL. In conclusion, this first report on central European patients harboring the m.7511T>C mutation reveals that the m.7511T>C may be important when diagnosing patients with maternally inherited HL.

Introduction

Hundreds to thousands of mitochondria are present on average in a human cell. Each of the mitochondrion contains several copies of inherited maternally 16 569 -base pair, circular, double-stranded DNA molecule (mtDNA). The main role of mitochondria is to provide energy to the cell in a process of oxidative phosphorylation, running through specialized complexes located in the mitochondrial electron transport chain. Approximately 10% of proteins necessary for mitochondrial activity (13 respiratory chain peptides, 22 transfer RNAs and 2 mitochondrial ribosomal RNAs) are encoded by its own genome. The majority of proteins found in mitochondria are encoded by the nuclear genes (1,2). Disturbances in effective functioning of mitochondria resulting from mutations in the mitochondrial genome can lead to various disorders including hearing loss (HL). It can occur as an isolated feature or a part of a genetic syndrome, e.g., mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERFF) or Kearns-Sayre syndrome (KSS), which is a mitochondrial myopathy, characterized by ptosis and ophthalmoplegia and other symptoms such as proximal muscle weakness, cerebellar ataxia, diabetes mellitus and/or endocrinopathies (3).

Identifying the genetic background of isolated, sensorineural hearing loss (SNHL) is challenging because deafness may result from mutations in many different genes (4). In patients with isolated, prelingual and recessive SNHL mutations in the deafness, autosomal recessive 1 (DFNB1)
locus, containing the gap junction protein beta 2 (GJB2) and the gap junction protein beta 6 (GJB6), are responsible for about 50% of cases (4,5). Conversely, isolated, congenital maternally inherited SNHL is as rare as 1% (6) but the prevalence of postlingual, maternally inherited HL has been estimated even up to 30% (7-9). The most common mtDNA mutations in the Polish HL patients are m.1555A>G in the MT-RNR1 gene and m.3243A>G in the MT-TL1 gene with the prevalence of approximately 1% for each of them (10,11). The vast majority of mtDNA mutations cause postlingual, bilateral and symmetrical HL, which may progress over time. Along with HL, tinnitus and/or vertigo are also observed in many individuals (12-15). Mutations in mtDNA related to HL are homoplasmic (pure population of mutated mtDNA) but various levels of heteroplasmy (mixture of wild-type and mutant mtDNA) are also found.

MT-TS1 gene (MIM 590080) encoding tRNASer\(^{\text{UCN}}\), a small 69 nucleotide RNA represents another HL-related hot spot in the mitochondrial genome. Two mutations of this gene, i.e., m.7510T>C and m.7511T>C are located in the acceptor arm of the tRNA molecule. They disrupt the highly conserved structure of the acceptor stem of the tRNASer\(^{\text{UCN}}\). The m.7511T>C mutation reduces by approximately 80% the level of tRNA synthesis, strongly affecting the mitochondrial protein translation (16) leading to an impaired oxidative phosphorylation. Heretofore, m.7511T>C mutation has been identified only in 7 HL families worldwide (1,17-24).

Since introduction of next-generation sequencing (NGS), analysis of the whole mitochondrial DNA is no longer labor intensive. Having a complete sequence of the mitochondrial genome, the involvement of other mtDNA mutations in the development of HL can be excluded and patients can obtain an accurate result of mitochondrial genetic testing.

In this study we have identified the m.7511T>C mutation in two unrelated HL families using NGS of the whole mtDNA. This is the first report on Polish patients harboring m.7511T>C, which includes their detailed audiological characteristics as well as the frequency of this mutation among more than 1,600 Polish HL individuals.

Materials and methods

Patients. Two DNA samples derived from unrelated probands (family A and B; both of Caucasian origin) with a maternal type of HL inheritance were selected for NGS sequencing of the whole mtDNA (Fig. 1). Mutations at the DFNB1 locus for which HL patients at the Department of Genetics (Institute of Physiology and Pathology of Hearing, Warsaw, Poland) are tentively tested according to the EMQN recommendations (25) and mitochondrial DNA mutations (m.1555A>G, m.3243A>G) as well as major pre- and perinatal HL risk factors (i.e., severe prematurity, congenital rubella, mumps or cytomegalovirus infection, severe neonatal hyperbilirubinemia) were not identified among the surveyed members from both families.

For screening of the m.7511T>C mutation a group of 1,644 unrelated HL patients of Caucasian origin, with a various degree of HL and without common GJB2 mutations (c.35delG, c.334_c.335delAA, c.358_c.360delGAG, c.167delT, c.313_326del) was selected. Based on the age of onset (AO), the group was divided into subgroups of 600 patients with prelingual (<6 years) and 1,044 patients with postlingual (>6 years) HL.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local Ethics Committee (IFPS/KB/04/2012). Informed consent was obtained from all individual participants included in the study.

Audiological assessment in patients with the m.7511T>C mutation. Hearing levels were determined by pure-tone audiometry at frequencies of 0.5, 1, 2, 4 and 8 kHz and classified according to the pure tone average (PTA) as mild (21-40 dB HL), moderate (41-70 dB HL), severe (71-90 dB HL) and profound (>90 dB HL) (26). Temporal bone computer tomography (CT) or magnetic resonance imaging (MRI) scans were obtained preoperatively from patients with cochlear implants (CI). Preoperatively, and at intervals of 1, 2 and 3 years postimplantation, patients were evaluated using pure-tone audiometry on both ears in the unaided condition and were tested for Polish monosyllabic word recognition (prerecorded on compact disc) and presented at 70 dB Sound Pressure Level (SPL) in quiet and in 10 dB signal-to-noise ratio (SNR) speech spectrum noise (27). Forty words (2 lists of 20 words) were presented from a loudspeaker placed 1.5 m from the subject in an Industrial Acoustics Company Inc. (Winchester, UK) double-walled booth. Lists were chosen at random, with no list being used twice with the same subject. Preoperatively, subjects were tested with two hearing aids (HAs). Postoperatively, subjects were tested using the CI with the contralateral ear HA.

DNA isolation. DNA was isolated from peripheral blood by a standard salting out method (28).

Next-generation and Sanger sequencing of mtDNA. In the first step two overlapping amplicons of the whole mtDNA (~9 kb each) were produced by a long range PCR using Takara LA Taq Hot Start polymerase (Takara Bio Inc., Kusatsu, Japan) with the specific primers: Amt123F, 5’CTT TGATTCCTGCCCTACCC; Amt8663R, 5’GGTGTGTTGTTA TTAGTCCGTTGTG; Bmt8467F, 5’CTACAATTCCCTCCACT AAAGC; and Bmt824R, 5’ATCAGGGTGTGTTCCTCCCTTGG designed with Primer3 software (http://bioinfo.ut.ee/prime3-0.4.0/prime3/) (29,30) based on the mtDNA revised Cambridge Sequence (rCRS; GenBank accession no. NC_012920). PCR was performed under the following conditions: An initial 2 min incubation at 94°C was followed by 40 cycles of PCR with 10 sec of denaturation at 98°C and 18 min of annealing and extension at 68°C. The reaction was completed by 1 cycle of final extension at 72°C for 10 min: 1.5 µl of PCR product was analyzed on 1.5% agarose gel with 1 kb plus DNA ladder (Invitrogen; Thermofisher Scientific, Inc., Waltham, MA, USA). Next, DNA libraries were prepared with the NexteraXT Sample preparation kit according to the manufacturer’s instructions (Illumina Inc., San Diego, CA, USA) and a paired-end index sequencing (2x150 bp) was performed on the MiSeq sequencer (Illumina Inc.). The obtained data were
analyzed by the CASAVA software (Illumina Inc.) for demultiplexing and FASTQ file generation. Reads were aligned to rRS using the Burrows-Wheeler Alignment Tool and processed further by the Picard and Genome Analysis Toolkit (31-33). Population frequency of the identified variants and the corresponding phenotypic information were derived from the online mitochondrial genome database (http://www.mitomap.org) and converted to the MS Excel format for final manual analyses (34). NGS results were inspected with Integrative Genomics Viewer (IGV) (35). The average coverage for both samples was above 400x. Variants described in the MitoMap database (http://www.mitomap.org/MITOMAP) as pathogenic were selected for primary analysis. Next, variants with low population frequency in MitoMap and Human Mitochondrial Genome Database (http://www.mtdb.igp.uu.se/), non-synonymous or located in highly conserved and/or functionally important domains of tRNA or rRNA were considered as potentially pathogenic (36).

Presence of the identified mutations was confirmed by Sanger sequencing. PCR primers Mt7271F, 5’-GGCTCA TTCATTCTCTAACAGC-3’ and Mt7649R, 5’-GGCGGT GATCATGAGGTG-3’ for the region encompassing m.7511T>C were designed with the Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) based on rRS. PCR was performed under the following conditions: an initial 5-min incubation at 95˚C was followed by 32 cycles of PCR with 30 sec of denaturation at 95˚C, 55 sec of annealing at 56˚C and 1 min of extension at 72˚C. The reaction was completed by 1 cycle of final extension at 72˚C for 10 min.

Heteroplasmy level, penetration score and determination of haplogroups. Heteroplasmy level of m.7511T>C was estimated based on the electropherograms from Sanger sequencing or IGV data. Penetration score was calculated as a number of HL individuals among all matrilineal relatives in a family. Haplogroups were determined with HaploGrep 2 (v2.1.0) and Mitomaster tools (37,38).

Real time PCR. Real time PCR screening for the m.7511T>C mutation was performed on the Viia7 system (Life Technologies, Carlsbad, CA, USA) using an assay on demand containing a respective primer pair encompassing m.7511T and fluorescently-labelled TaqMan probes for the discrimination of m.7511T and C (Life Technologies). As a positive control DNA sample with a confirmed m.7511T>C mutation was used.

Statistical analysis. Mutation frequency among different populations was compared by Chi-square statistics. Correlation between the heteroplasmy level and AO was determined with the Spearman Rank Correlation (Spearman's rho) test (39). The data was considered statistically significant at P<0.05.

Results

Sequencing of the whole mtDNA in two probands from unrelated HL families with pedigrees suggesting maternal inheritance, revealed a homoplasmic m.7511T>C mutation in the mitochondrial MT-TS1 gene (Fig. 2). Presence of the mutation was confirmed by Sanger sequencing (data not shown).

To investigate the segregation of m.7511T>C with HL, available DNA samples from the family members have been Sanger sequenced. Inheritance of m.7511T>C has been confirmed over generations in matrilineal relatives in both families. From nine DNA samples collected from family A seven had the m.7511T>C mutation in a homoplasmic state, in one 50% heteroplasmy was detected and the other one (A.II.10) who did not carry the mutation also did not have HL

Figure 1. Pedigrees of Family A and B. Circles represent females, squares represent males and diamonds depict individuals of undetermined gender. The filled symbols indicate affected individuals and asterisks indicate subjects tested for m.7511T>C mutation. Probands are marked with arrows and symbols with a diagonal line through it indicate deceased family members.
Table I. Detailed clinical characterization of patients harboring the m.7511T>C mutation and heteroplasmy level among patients.

| Patient number | Sex | Present AO age (years) | Mean HL RE | Mean HL LE | Ototoxic drugs | Vertigo | Tinnitus | Heteroplasmy level (%) | Additional information |
|----------------|-----|------------------------|------------|------------|---------------|---------|----------|------------------------|------------------------|
| A.I.2          | F   | 87                     | 3.5        | 86         | 113           | -       | -        | -                      | 50                     |
| A.II.2         | M   | 57                     | -          | -          | -             | -       | -        | -                      | 100                    |
| A.II.5         | F   | 55                     | 37         | 84         | 85            | +       | +        | +                      | 100                    |
|                |     |                        |            |            |               |         |          |                        | CI (RE) and HA (LE); preoperative MRI of temporal bones-no abnormalities |
| A.II.6         | M   | 54                     | -          | -          | -             | -       | -        | -                      | 100                    |
| A.II.8         | M   | 51                     | 40         | 57         | 59            | -       | -        | +                      | 100                    |
| A.II.12        | F   | 47                     | 2.5        | 30         | 48            | -       | +        | -                      | 100                    |
| A.III.8        | F   | 28                     | 3          | 69         | 65            | -       | -        | 100 HA                 |
| A.III.9        | F   | 34                     | 0          | 85         | 79            | +       | -        | -                      | 100                    |
| B.II.3         | M   | 76                     | 27         | 92         | 97            | -       | -        | -                      | 100                    |
| B.III.1        | M   | 66                     | 7          | 68         | 79            | -       | +        | +                      | 100                    |
| B.III.7        | M   | 64                     | -          | -          | -             | -       | -        | -                      | 100                    |
| B.III.10       | F   | 60                     | -          | -          | -             | -       | +        | -                      | 100                    |
| B.IV.9         | M   | 36                     | 7          | nd         | nd            | -       | -        | -                      | 100                    |

M, male; F, female; HL, hearing loss; nd, no data available; AO, age of onset; CI, cochlear implant; HA, hearing aids; RE, right ear; LE, left ear; CT, computer tomography; MRI, magnetic resonance imaging.

(Figs. 1 and 2). Among patients with m.7511T>C mutation in family A, HL was observed in six out of eight individuals and there was an interfamilial variability in its AO (0–40 years) and severity (from moderate to profound) (Table I). All tested matrilineal relatives from family B had a homoplasmic m.7511T>C mutation. In three individuals postlingual HL has been diagnosed, while the remaining two carriers of the mutation did not suffer from HL.

Among the tested individuals in both families only patients with m.7511T>C had HL. The penetration score was calculated as 64% (7/11) for family A and 60% (6/10) for family B, which makes an average value of 62% (13/21). The mean age of HL onset in the individuals was 14.1 years with the mean degree of HL being 74.8±20.3 dB, which corresponds to a moderate HL. There was a weak, positive correlation between the level of heteroplasmy and AO, but it was not statistically significant.
Some patients in both families suffered from vertigo or/and tinnitus (Table I).

Some of the affected individuals are using HA or CI and HA as a treatment (Table I). In two patients with partial deafness (PD) (40,41) (Fig. 3) cochlear implantation was successfully applied (42). No structural abnormalities were observed on the temporal bone CT scans. At the age of 51, patient A.II.5 received CI (Med-El Concerto Flex 24, Med-El, Austria) in the right ear (RE). Before surgery the monosyllabic speech discrimination in quiet and noise in the bilaterally best fitted HAs was 60 and 15% correct responses, respectively. According to the hearing preservation classification, acoustic hearing was preserved completely after implantation (43). Over a period of 3 years, monosyllabic word recognition increased from 60 to 95% under quiet conditions and from 15 to 40% under noisy conditions. Patient A.III.9 with bilateral mild-to-profound HL obtained CI (CI24RE (SRA); Cochlear, Melbourne, Australia) in the RE at the age of 18 years (44). Before surgery the monosyllabic speech discrimination in quiet and noise in the bilaterally best fitted HAs was 30 and 0%, respectively. After implantation hearing was partially preserved. Over a period of 3 years, monosyllabic word recognition increased from 30 to 100% under quiet conditions and from 0 to 75% under noisy conditions. Both patients substantially benefited from CI.

Prevalence of m.7511T>C in the Polish HL population and in other populations. Searching for the m.7511T>C mutation among both subgroups of HL patients (n=1644) did not identify any additional cases of m.7511T>C mutation. Taking into account that our probands also fulfilled criteria for real time PCR screening, we have estimated the frequency of m.7511T>C mutation at 0.12% (2/1646) among HL patients with excluded common GJB2 mutations.

We have compared our data to the Chinese HL and Japanese HL families to 84% in the African-American pediatric (17,20), French (18), Japanese (17,20) and Chinese (19,21) origin, but heretofore it has not been reported in HL patients from central Europe.

In this study we assessed for the first time the frequency of m.7511T>C in the group of Polish HL patients. Its frequency of 0.12% is not significantly different from that of m.7445A>G (0.4%, 1/250 Polish nonsyndromic HL patients) (45), which is considered a major HL causative mutation in the MT-TSI gene. Two other common mtDNA mutations in Polish HL patients are m.1555A>G in MT-RNR1 detected at a frequency of 1.3% (20/1499) and m.3243A>G in MT-TL1 with a frequency of 1% (16/1499) (10,11). Based on the results, the total percentage of mitochondrial HL-related mutations in Polish patients can be estimated at almost 3%.

Various m.7511T>C penetration levels have been reported in respect to HL. It ranges from 30% in some French or Japanese families to 84% in the African-American pedigree (17,18,20,24). In the Polish families the penetration rate was established at an average of 62%. It is a rather high score.
### Table II. Summary of the clinical data of families with the m.7511T>C mutation.

| Author, year | Family origin | AO (years) | HL type | Penetration (%) | Heteroplasmy level (%) | Other symptoms | (Refs.) |
|--------------|---------------|------------|---------|-----------------|------------------------|----------------|---------|
| Friedman *et al*, 1999; Li *et al*, 2004; Sue *et al*, 1999 | African-American | 4-44 | Mild to severe; symmetric; gradually progressive | 84 (36/43) | 94-100 | Three individuals with insulin-dependent diabetes mellitus | (1,16,24) |
| Ishikawa *et al*, 2002; Ishikawa *et al*, 2006; Li *et al*, 2005 | Japanese 1 | 3-30 | Mild to severe; mostly down-sloping audiograms; progressive; in few cases asymmetry | 67 (14/21) | 84-92 | Tinnitus; in one subject post mortem temporal bone findings revealed severe loss of spiral ganglion cells and loss of neuronal filaments in Rosenthal's canal; diabetes mellitus; intracerebral hemorrhage | (17,22,23) |
| Yamasoba *et al*, 2007 | Japanese 2 | 2-45 | Normal-moderate-severe; mild to high frequencies affected | 30 (7/23) | 100 | No | (20) |
| Chapiro *et al*, 2002 | French 1 | 3-33 | Mild to severe; stable; U-shaped curves audiograms | 43 (9/21) | 100 | One tinnitus patient (with no HL) | (18) |
| Chapiro *et al*, 2002 | French 2 | <2 | Mild to severe; stable; uni- and bilateral | 30 (6/20) | 51-85 (in blood, urine, hair root cells, mouthwash samples) | Bilateral and permanent tinnitus since 20 years of age | (18) |
| Chen *et al*, 2015 | Chinese 1 | 14-25 | Mild to severe; flat audiometric configuration; progressive | 82 (9/11) | Various levels | No | (21) |
| Tang *et al*, 2015 | Chinese 2 | 6 | Moderate; flat; progressive | 60 (3/5) | 100 | No | (19) |
| Lechowicz *et al* | Polish 1 (family A) | 0-40 | Mild to profound | 64 (7/11) | 50-100 | Vertigo, tinnitus | Present study |
| Lechowicz *et al* | Polish 2 (family B) | 7 | Mild to severe; progressive | 60 (6/10) | 100 | Tinnitus | Present study |

AO, age of onset; HL, hearing loss.
particularly when compared with other Caucasian families (36.5%). It is not easy to explain such differences in penetration score, mainly because there is still a gap in our knowledge about the precise pathomechanisms of m.7511T>C mutation and/or coexistence of possible modifying factors.

**MT-TS1** deafness mutations often occur in homoplasmy or high levels of heteroplasmy, suggesting a high heteroplasmic threshold for pathogenicity. Interestingly, in both Polish families we have observed patients with homoplasmic m.7511T>C mutation, among whom no HL has been so far diagnosed (individuals: A.II.2, A.II.6, B.III.7, B.III.10; Table I). On the other hand, the subject A.I.2 who had an early onset and profound HL was found to have just 50% heteroplasmic level of the m.7511T>C mutation. Sequencing of the entire mtDNA and determining that both our families belong to different mitochondrial haplogroups suggest that factors modifying the AO, degree and progression of HL are rather not located in mtDNA. Only in three previous reports of the m.7511T>C mutation, the authors have sequenced the whole mtDNA, thereby excluding a direct role of other mtDNA variants or their co-involvement in the pathogenesis of HL. The results suggest that other polymorphisms and/or mutations in the nuclear genome as well as environmental factors may modulate the phenotypic variation (18,24) but the triggering or modifying mechanisms still await elucidation.

It is known that some mutations in the mtDNA (e.g., m.1555A>G) create an increased susceptibility to aminoglycosides, which results in ototoxicity and HL (10,46). Except for one patient (Table I), none of the other studied individuals were treated with aminoglycosides. Thus, their role as a factor triggering HL in patients with m.7511T>C mutation can be excluded based on our and other studies (1,18). There is a great variation in the HL AO in our patients with m.7511T>C and in the majority of m.7511T>C HL patients that were reported worldwide (Table II). The AO varied from prelingual to a late postlingual usually in the third or fourth decade of life (20).

Medical records of previously reported families with the m.7511T>C mutation showed no other clinical features usually associated with mitochondrial diseases, i.e., muscular diseases, visual loss or neurological disorders, but few patients were diagnosed with diabetes mellitus (17,24). Similarly to the French and Japanese families, some patients in our study suffered from vertigo and tinnitus (Table I). Temporal bone CT or head MRI of our patients did not reveal any significant abnormalities of the cochlear structures. At the time of writing the manuscript there was only one report on temporal bone histopathologic findings in a patient with m.7511T>C mutation. In the Japanese male severe loss of spiral ganglion cells in the cochlea accompanied by severe loss of neuronal filaments in Rosenthal's canal and partial atrophy of stria vascularis in all turns of cochlea was described (22).

From the theoretical point of view, severe loss of spiral ganglion cells, as the main cause of sensorineural HL associated with the m.7511T>C mutation, could hinder the benefit from cochlear implantation as a sufficient number of intact ganglion cells is necessary for a CI to enable good speech discrimination (47).

CI is an electronic hearing prosthesis designed for individuals with profound deafness. In the newest application, electric stimulation from a CI is used in case of PD, defined as a mild to moderate low frequency SNHL sloping to a profound HL in the higher frequencies (40). In each case of cochlear implantation an individual surgical approach is required. The key issue is to avoid the inner ear trauma and consequently to prevent apoptosis (48). Therefore the correct choice of electrode and its insertion depth is essential. According to published data insertion of 16-20 mm is recommend (49). An individual, yet multi-featured approach is required especially in case of progressive HL, where maintaining the structure of the patient's inner ear is crucial. Typically, in case of severe HL, the preferred electrode insertion depth is 28 mm (47), but in case of PD the recommended insertion depth is 20-25 mm (50). Low frequencies hearing preservation after CI facilitates combining the electric and acoustic stimulation of the auditory system (40). Contrary to the theoretical assumption, results of the current study show that patient with the m.7511T>C mutation and PD can have substantial benefit from CI. This findings is in line with the studies where other mtDNA (e.g., m.1555A>G) mutation were identified among successful CI users with PD (51,52). Good outcomes of CI in these patients suggest that those with mutations in the mtDNA are good candidates for CI. Due to the fact that there is a huge variability in CI outcomes and many factors are thought to be involved in post-implantation performance, genetic testing would be helpful in predicting a CI benefit and facilitating the decision about treatment choices.

The main goal of this project was to search for mtDNA HL mutations, other than m.1555A>G and m.3243A>G, among Polish HL patients. As there were no reports describing the prevalence of m.7511T>C in the Polish population so far, presence of the m.7511T>C was not checked by Sanger sequencing in the analyzed patients prior to their inclusion into the NGS study, which might be considered as a limitation of this study.

In conclusion, the m.7511T>C mutation is an important cause of maternally inherited isolated SNHL, which may manifest from early childhood to middle age. The mutation occurs usually as homoplasmic and rarely as heteroplasmic. There is no statistically significant correlation between m.7511T>C heteroplasmey level and the HL AO. The present study provides two additional HL families with the m.7511T>C, which further supports the pathogenic character of the mutation and indicates how to treat such patients.

**Acknowledgements**

The present study was supported by National Science Centre (grant nos. 2012/05/N/ZS5/02629 and 2013/09/D/NZ5/00251).

**References**

1. Friedman RA, Bykhovskaya Y, Sue CM, DiMauro S, Bradley R, Fallis-Cunningham R, Paradies N, Pensak ML, Smith RJ, Groden J, et al.: Maternally inherited nonsyndromic hearing loss. Am J Med Genet 84: 369-372, 1999.
2. Lightowlers RN, Taylor RW and Turnbull DM: Mutations causing mitochondrial disease: What is new and what challenges remain? Science 349: 1494-1499, 2015.
3. Van Camp G and Smith RJ: Maternally inherited hearing impairment. Clin Genet 57: 409-414, 2000.
4. Nance WE: The genetics of deafness. Ment Retard Dev Disabil Res Rev 9: 109-119, 2003.
5. Snoeckx RL, Huygen PL, Feldmann D, Marlin S, Denoyelle F, Waligora J, Mueller-Malesinska M, Pollak A, Ploski R, Murgia A, et al: GJB2 mutations and degree of hearing loss: A structural and functional approach. Am J Hum Genet 78: 945-957, 2006.

6. Smith RJ, Bale JF Jr and White KR: Sensorineural hearing loss in children. Lancet 365: 879-890, 2005.

7. Hutchin TP, Thompson KR, Parker M, Newton V, Bittner-Glindzicz M and Mueller RF: Prevalence of mitochondrial DNA mutations in childhood/congenital onset non-syndromal sensorineural hearing loss. Int J Hum Genet 9: 229-231, 2001.

8. Jacobs HT, Hutchin TP, Käppi T, Gillies G, Minkkinen K, Walker J, Thompson K, Rovio AT, Carella M, Melchionda S, et al: Mitochondrial DNA mutations in patients with postlingual, nonsyndromic hearing impairment. Eur J Hum Genet 13: 26-33, 2005.

9. Yelverton JC, Arnos K, Xia XJ, Nanve WE, Pandya A and Dodson KM: The clinical and audiological features of hearing loss due to mitochondrial mutations. Otologyngol Head Neck Surg 148: 1017-1022, 2013.

10. Iwanicka-Pronicka K, Pollak A, Skórka A, Lechowicz U, Korniszewski L, Westfal P, Skarżyński H and Ploski R: Audio profiles in mitochondrial deafness m.1555A>G and m.3243A>G show distinct differences. Med Sci Monit 21: 694-700, 2015.

11. Iwanicka-Pronicka K, Pollak A, Skórka A, Lechowicz U, Pajdowska M, Furmanek M, Rzeski M, Korniszewski L, Skarżyński H and Ploski R: Postlingual hearing loss as a mitochondrial 3243A>G mutation phenotype. PLoS One 7: e44054, 2012.

12. Guan MX: Molecular pathogenetic mechanism of maternally inherited deafness. Ann NY Acad Sci 1011: 259-271, 2004.

13. Kokotas H, Petersen MB and Willems PJ: Mitochondrial deafness. Clin Genet 71: 379-391, 2007.

14. Kokotas H, Petersen MB and Willems PJ: Maternally inherited hearing loss in a large kindred with a novel tRNA, UAC C3243A>G mutation. Otolaryngol Head Neck Surg 138: 851-856, 2008.

15. Cao L, Shihtara H, Horii T, Nagao Y, Imai H, Abe K, Hara T, Hayashi J and Yonekawa H: The mitochondrial bottleneck occurs without reduction of mtDNA content in female mouse germ cells. Nat Genet 39: 386-390, 2007.

16. Taylor RW and Turnbull DM: Mitochondrial DNA mutations in human disease. Nature Rev Genet 6: 389-402, 2005.

17. Li X, Fischel-Ghodsi N, Schwartz F, Yan Q, Friedman RA and Guan MX: Biochemical characterization of the mitochondrial tRNA(Ser) (UCN) T7511C mutation associated with nonsyndromic deafness. Nucleic Acids Res 32: 867-877, 2004.

18. Skarżyński H and Płoski R, Pollak A, Gawęcki W, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

19. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

20. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

21. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

22. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

23. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

24. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

25. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.

26. Skarżyński H, Skarzyński PH, et al: Maternal and de novo mutations external association of POU3F4 with distinct clinical and radiological phenotype of hearing loss. PLoS One 11: e0166618, 2016.
46. Prezant TR, Agapian JV, Bohlman MC, Bu X, Oztas S, Qiu WQ, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, et al: Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nat Genet 4: 289-294, 1993.

47. Skarzynski H, Lorens A, Zgoda M, Piotrowska A, Skarzynski PH and Szkielkowska A: Atraumatic round window deep insertion of cochlear electrodes. Acta Otolaryngol 131: 740-749, 2011.

48. De Seta D, Torres R, Russo FY, Ferrary E, Kazmitcheff G, Heymann D, Amiaud J, Sterkers O, Bernardeschi D and Nguyen Y: Damage to inner ear structure during cochlear implantation: Correlation between insertion force and radio-histological findings in temporal bone specimens. Hear Res 344: 90-97, 2017.

49. Gantz BJ, Dunn C, Walker E, Van Voorst T, Gogel S and Hansen M: Outcomes of adolescents with a short electrode cochlear implant with preserved residual hearing. Otol Neurotol 37: e118-e125, 2016.

50. Nordfalk KF, Rasmussen K, Bunne M and Jablonski GE: Deep round window insertion versus standard approach in cochlear implant surgery. Eur Arch Otorhinolaryngol 273: 43-50, 2016.

51. Usami S, Miyagawa M, Nishio SY, Moteki H, Takumi Y, Suzuki M, Kitano Y and Iwasaki S: Patients with CDH23 mutations and the 1555A>G mitochondrial mutation are good candidates for electric acoustic stimulation (EAS). Acta Otolaryngol 132: 377-384, 2012.

52. Miyagawa M, Nishio SY and Usami S: A comprehensive study on the etiology of patients receiving cochlear implantation with special emphasis on genetic epidemiology. Otol Neurotol 37: e126-e134, 2016.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.