Non-syndromic Hearing Impairment in a Hungarian Family with the m.7510T>C Mutation of Mitochondrial tRNA^{Ser(UCN)} and Review of Published Cases

Katalin Komlósi • Anita Maász • Péter Kisfali •
Kinga Hadzsiev • Judit Bene • Béla I. Melegh •
Mária Ablonczy • Krisztina Németh • György Fekete •
Béla Melegh

Received: 03 May 2012 / Revised: 19 September 2012 / Accepted: 24 September 2012 / Published online: 02 November 2012
© SSIEM and Springer-Verlag Berlin Heidelberg 2012

Abstract The m.7510T>C mitochondrial DNA (mtDNA) mutation is a tRNA^{Ser(UCN)} alteration leading to matrilineal isolated hearing impairment. The current paper reviews the available reports on the m.7510T>C mtDNA mutation, with special attention to phenotypic variations and haplogroup background. A Hungarian family, the fourth family reported in the literature, is presented, in which analysis of three generations with bilateral isolated hearing loss revealed the m.7510T>C tRNA^{Ser(UCN)} mutation in homoplasmic form in the affected members. Haplogroup analysis verified an unnamed subgroup of mitochondrial haplogroup H. Previously reported Spanish and North American Caucasian families belong to different subgroups of haplogroup H. Analyzing our biobank of Hungarian patients with sensorineural hearing loss, we did not detect this mutation in any other patient, nor was it found in Caucasian haplogroup H control samples. Comparing the cases reported so far, there is interfamilial variability in the age of onset, accompanying symptoms, and haplogroup background. Our case adds further genetic evidence for the

pathogenicity of the m.7510T>C mutation and underlines the need to include full mtDNA sequencing in the screening for unexplained hearing loss.

Introduction

Inherited hearing impairment is among a highly heterogeneous group of disorders (Willems 2000). In the majority of cases it is non-syndromic, but it can sometimes be associated with abnormalities of other organ systems as part of a syndrome. Inherited as a matrilineal trait, sensorineural hearing loss (SNHL) can be attributed to mtDNA mutations. Isolated, non-syndromic hearing loss (OMIM #500008) has been associated classically with mitochondrial 12S rRNA (Prezant et al. 1993) and tRNA^{Ser(UCN)} genes (Friedman et al. 1999), but recent genetic and functional studies suggest the role of further mitochondrial genes (Zheng et al. 2012), such as the ND1 of complex I of the respiratory chain (m.3388C>A), the tRNA^{Ile} (m.4295A>G), subunit COII of complex IV (m.8078G>A), the tRNA^{Ser(AGY)} (m.12236G>A), the tRNA^{His} (m.12201T>C) (Yan et al. 2011), and Cytochrome B, subunit of complex III (m.15077G>A) (Gutiérrez Cortés et al. 2012). Certain mutations can cause syndromic or isolated hearing impairment, as in the m.7445A>G mutation, leading to hearing loss with or without palmoplantar keratoderma (Reid et al. 1994; Maász et al. 2008), or in the m.7472insC mutation, responsible for hearing loss sometimes associated with a neurological disorder (Verhoeven et al. 1999).

Accumulating data from affected families of different ethnic backgrounds, and analysis of control cohorts may gradually confirm the pathogenic role of other rare variants.

Communicated by: Eva Morava

Competing interests: None declared

K. Komlósi (✉) • A. Maász • P. Kisfali • K. Hadzsiev • J. Bene •
B.I. Melegh • B. Melegh
Department of Medical Genetics, University of Pécs, József A. út 7,
Pécs H-7623, Hungary
e-mail: komlosi.katalin@pte.hu

M. Ablonczy • K. Németh • G. Fekete
2nd Department of Pediatrics, Semmelweis University, Budapest,
Hungary

The m.7510T>C alteration in the tRNA^{Ser(UCN)} was previously regarded as a rare variant, originally identified in three large families with matrilineal non-syndromic SNHL (Hutchin et al. 2000; Castillo et al. 2002; Labay et al. 2008). In the originally reported Caucasian family from the United Kingdom, the mutation was present in very high levels of heteroplasmy in blood with no available data on the haplogroup background (Hutchin et al. 2000). The second extended Spanish family carried the homoplasmic mutation on sub-haplogroup H1 (Castillo et al. 2002), while the third reported family, of North American origin, carried the mutation on an unnamed subgroup of mitochondrial haplogroup H (Labay et al. 2008). The m.7510T>C mtDNA mutation was not detected in normal Caucasian controls (Hutchin et al. 2000), nor in haplogroup- or sub-haplogroup-matched controls (Labay et al. 2008). Although it was not found in some large screening populations with non-syndromic SNHL (Jacobs et al. 2005; Lvque et al. 2007), several families have already been described.

The current report reviews the available data on the m.7510T>C mutation with special attention to phenotypic variations and haplogroup background. Further, a Hungarian family is presented, the fourth family reported in the literature, in which the analysis of three generations with bilateral isolated SNHL revealed the m.7510T>C tRNA^{Ser(UCN)} mutation.

Materials and Methods

This study was approved by our Institutional Review Board. Written informed consent was obtained from all adults, and parents or legal guardians of all children. Patients examined at our Department since 2001 with SNHL served as controls.

Genomic DNA was prepared from venous blood samples obtained from three members of the family, the index patient, his mother, and his maternal grandmother (Fig. 1). Sequencing for mtDNA mutations was done as described previously (Masz et al. 2008). The sequences were compared with the revised Cambridge reference sequence (<http://www.ncbi.nlm.nih.gov/entrez>; GenBank: #J01415) and haplogroup phylogeny from MITOMAP (<http://www.mitomap.org>). Heteroplasmy for m.7510T>C was assessed by *Hinf*I digestion (Fig. 3) (Hutchin et al. 2000; Castillo et al. 2002).

Patients

Phenotype of the Hungarian family

We examined a four generation Hungarian Caucasian non-consanguineous family with five affected family members.

Fig. 1 Phenotypic findings of the Hungarian family. Filled symbols denote the members of the family with non-syndromic hearing impairment. II/2., III/2., and IV/1. were available for genetic analysis which showed the m.7510T>C mutation in the tRNA^{Ser(UCN)} gene of the mitochondrial DNA. The index patient is indicated by an arrow

The segregation pattern of the hearing loss raised the possibility of matrilineal inheritance (Fig. 1). Our index patient (Fig. 1, IV/1.) is a 7-year-old boy with normal physical appearance, who was born by vaginal delivery at 40 weeks of gestation, weighing 3,200 g. The perinatal period was uneventful. His early psychomotor development was age-appropriate: he stood upright at 11 months of age, walked unaided by 12 months, started to utter words at 16 months and speak fluently at 2 years of age. At the age of 4.5 years he was investigated for hearing loss. At that age it was noticed that he had coordination problems and a delay in fine motor skills: he was very clumsy in drawing, he was unable to stand on one foot and ride the bike and was uneasy climbing the stairs. Detailed physical and neurological examination did not show any alteration apart from his clumsiness, laboratory investigations were all within the normal range (repeated lactate and pyruvate levels and kidney and liver functions were normal), ophthalmologic and cardiologic investigation showed no pathologic finding, brain MRI and MR angiography as well as EEG were normal. Conductive hearing loss was ruled out by otoscopic examination, tympanometry with acoustic reflex testing, and the use of tuning fork tests. Pure tone audiometry confirmed bilateral, sensorineural, symmetrical hearing loss with an average loss of 40 dB HL in both ears (Fig. 4) at the age of 5 years. His hearing loss showed a slowly progressive and asymmetrical course, showing an average of 70 dB HL in the right ear, 80 dB HL in the left ear at the age of 6 years and 75 dB HL in the right ear and 90 dB HL in the left ear at the age of 7 years (Fig. 4). The patient received a hearing aid at the age of 5.5 years. The index patient has a healthy sister (Fig. 1, IV/2.) 3 years of age, no signs of hearing loss have been noted in her so far.

Fig. 2 Electropherograms of the index patient and an unaffected control. Sequence of the index patient and a control. The arrow shows the mutant 7510C and wild-type 7510T nucleotides of the mitochondrial tRNA^{Ser(UCN)} gene in the affected proband and a control

Fig. 3 *HinfI* PCR-RFLP assay for m.7510T>C heteroplasmy in the members of the Hungarian SNHL family. Mitochondrial DNA was PCR amplified and digested with *HinfI* as described in previous publications (Hutchin et al. 2000, Castillo et al. 2002). Digestion product sizes (bp) are shown on the right. **Lane 1:** Lambda DNA/*EcoRI*+*HindIII* Marker. **Lane 2:** Wild-type amplification product containing a single *HinfI* cleavage site that results in 181 bp and 82 bp fragments after digestion. **Lane 3, 4, and 5:** the index patient, his mother, and his grandmother: the m.7510T>C mutation creates a *HinfI* site within the 181 bp fragment that results in 132 bp and 49 bp products. Since no 181 bp fragment can be detected, all three family members harbor the mutation in homoplasmic form

The mother, the maternal grandmother, the maternal great-aunt and the maternal great-grandmother (Fig. 1) all developed severe symmetrical isolated sensorineural hearing loss by the age of 30 years, no audiograms were available from them. The mother (Fig. 1, III./2.) noticed a slowly progressive hearing loss in her early 20s, pure tone audiometry at the age of 30 years confirmed bilateral,

sensorineural, symmetrical hearing loss with an average loss of 70 dB HL in both ears, she received a hearing-aid in her late twenties. Both of her pregnancies and especially the deliveries worsened her hearing deficit. Detailed physical and neurological examination did not show any other alteration, laboratory investigations were all within the normal range (repeated lactate and pyruvate levels and kidney and liver functions were normal), and ophthalmologic and cardiologic investigation showed no pathologic finding. The maternal grandmother (Fig. 1, II./2.) and her sister (Fig. 1, II./3.) also claim to have first noticed their hearing impairment in their early 20s, no precise information was available about the maternal great-grandmother. Hearing loss was slowly progressive in the affected family members, no episodes of sudden hearing loss, of worsening of the symptoms after noise exposure, or episodes of vertigo were noted, the mother and the maternal grandmother reported to have intermittent tinnitus.

Genotype of the Hungarian family

The sequence analysis of the patient, his mother, and grandmother was performed on DNA extracted from blood leukocytes. No other tissues were available for investigation. During sequence analysis of the mtDNA in the index patient, we detected the m.7510T>C mutation in the tRNA^{Ser(UCN)} (Fig. 2 all mtDNA sequence variants are listed in Table 2). We then screened the mother and maternal grandmother and confirmed the presence of the mutation in their blood samples. Unfortunately, the great-aunt and the sister of our index patient were not available for testing. A specified RFLP assay of all three investigated

Fig. 4 Air conduction audiograms of the index patient carrying the m.7510T>C mutation at age 5, 6, and 7 years (y). Squares, right ear; triangles, left ear. Audiograms of the other affected family members were not available

family members confirmed the mutation in homoplasmic form (Fig. 3).

Haplotype analysis (Herrnstadt et al. 2002) of the index patient and his family members suggested that the family carries the mutation on an unnamed subgroup of the mitochondrial haplogroup H. We assigned the m.7510T>C mutation containing mitochondrial sequence in our Hungarian family to haplogroup H, based on the SNP 7028C, which is specific for all branches of haplogroup H (Herrnstadt et al. 2002). Our sequence contains 4793G, which defines a rare unnamed sub-haplogroup that is not among the major subgroups of H (Herrnstadt et al. 2002). In addition, we excluded the SNPs defining subgroups H1, H2, H3 and H4, and several other SNPs defining other rare unnamed subgroups of haplogroup H. We genotyped 60 Caucasian samples from our SNHL biobank but failed to detect the m.7510T>C mutation. Only the m.7445A>G mutation and the m.3243A>G mutations were detected previously

among these samples (Maász et al. 2008). The m.7510T>C alteration was also absent in 30 Caucasian haplogroup H healthy controls from our biobank.

In addition, the common deafness associated nuclear alteration (35delG in the *GJB2* gene) was investigated, and gave negative results in the affected family members.

Phenotype and genotype details of the previously reported families with the m.7510T>C mutation are listed in Table 1.

Discussion

In this study, we describe a fourth family with matrilineal transmission of non-syndromic SNHL attributable to the m.7510T>C transition of the mitochondrial tRNA^{Ser(UCN)} gene. Results of previous studies give functional evidence of an unfavorable effect of m.7510T>C on pre-tRNA^{Ser(UCN)} processing (Yan et al. 2006), which may lead to decreased levels of the mature tRNA^{Ser(UCN)} overall, thereby limiting mitochondrial protein synthesis and consequently respiration as demonstrated for other tRNA mutations (Yarham et al. 2010). One of these studies demonstrated that cells bearing the tRNA^{Ser(UCN)} m.7445A>G mutation also have a decreased synthesis rate of some mitochondrial proteins, especially the sixth subunit of the NADH dehydrogenase (ND6), and a significant reduction in the amount of ND6 mRNA (Li et al. 2005). This observation suggests that the mRNA of the ND6 is derived from the same precursor as the tRNA^{Ser(UCN)}. The same mechanism may apply for the m.7510T>C variant, since it also disrupts a highly conserved base pair in the aminoacyl stem of the tRNA^{Ser(UCN)} (Hutchin et al. 2000).

The m.7510T>C mutation has been described in three families so far (Table 1). In all published families, symptoms appeared mainly as isolated hearing loss. There were no reports of cardiac, ophthalmologic, or renal involvement or movement disorder in the affected family members (Hutchin et al. 2000; Castillo et al. 2002; Labay et al. 2008). Our index patient had a delay in fine motor skills and coordination at the age of 4.5 years when his hearing loss became evident, but no other organ involvement (renal, cardiac, ophthalmologic, endocrine) could be detected. As accompanying neurological symptoms, episodes of sudden, severe, and self-limiting vertigo; chronic or intermittent spontaneous bilateral tinnitus; and worsening of the symptoms after noise exposure were reported in the third North American family (Labay et al. 2008). Penetrance and age of onset of the disease differed within the pedigrees reported so far (Hutchin et al. 2000; Castillo et al. 2002; Labay et al. 2008). In the second, Spanish, family, the largest reported so far, the mutation was confirmed in 26 members of the family and auditory impairment was shown in 21 out of 26 carriers. According

Table 1 Phenotype and genotype data of published families bearing the m.7510T>C mitochondrial mutation. The table gives a comparison of the so far published families bearing the m.7510T>C mutation, showing the proportion of the mutation, the haplogroup background, the age at onset of symptoms, the penetrance, the number of affected family members, and their accompanying symptoms. The numbers refer to the number of affected family members. The asterisk indicates the percentage of the mutation within the limits of detection

Families	Status of the mutation and haplogroup	Onset of the disease	Number of affected family members, penetrance	Clinical symptoms				References
				Hearing loss	Trigger factor	Tinnitus	Vertigo	
UK	Heteroplasmic* (>95% and 90%) haplogroup: n.a.	15 months to 70 years	11 members affected , complete penetrance	2: bilateral asymmetrical mild 2: bilateral asymmetrical profound SNHL	No data available	No data available	No data available.	Hutchin et al. 2000
Spanish	Homoplasmic haplogroup: H1	First two decades of life	26 members carriers ; 22 affected , 4 asymptomatic carriers , incomplete penetrance	Progressive moderate to profound symmetrical SNHL 3: asymmetrical	1: measles 4 females: hearing loss worsened with pregnancy 2: febrile illness 1: measles 5: blunt head trauma	Most affected family members	Not reported	Castillo et al. 2002
North American	Homoplasmic haplogroup: rare H variant 3333T	2 to 40 years of age	16 affected , complete penetrance	16 : bilateral progressive SNHL		7: chronic or intermittent	2: sudden, severe, self-limiting	Labay et al., 2008
Hungarian	Homoplasmic rare H variant 4793G	5 to 20 years of age	5 members affected , no complete penetrance	5: bilateral mild to profound SNHL	Pregnancy, delivery	2: intermittent	No	Current report

n.a. SNHL sensorineural hearing loss

Table 2 Alterations in the mtDNA sequence of the index patient

mtDNA variation	Category	Interpretation
m.709G>A	Polymorphism	MT-RNR1 (12S rRNA)
m.4769A>G	Polymorphism	p.Met100Met in MT-ND2
m.4793A>G	Polymorphism	p.Met92Met in MT-ND2 rare haplogroup H variant
m.7510T>C	Known mutation	MT-TS1 (tRNA Ser 1)
m.8251G>A	Polymorphism	p.Gly222Gly in MT-CO2
m.11938C>T	Polymorphism	p.Leu393Leu in MT-ND4
m.15954A>G	Polymorphism	MT-NC10 noncoding

Apart from the m.7510T>C mutation, no other pathological alteration was detected in the index patient

to the symptoms and audiograms, four carriers were reported to be asymptomatic: at the age of 11, 15, 16, and 31 air conduction audiograms did not reveal an impairment (Castillo et al. 2002). Although no further data is available on the asymptomatic carriers, given the intrafamilial variable onset of the symptoms, it can be speculated that the carriers did not yet reach the age at which symptoms would appear. No complete penetrance can be demonstrated in our Hungarian family either. Although the so far unaffected, 3-year-old sister of the index patient was not available for testing, she might be just too young to show symptoms of hearing loss.

The wide range of age at onset, and the variability among individuals in the affected families, also suggests that other mitochondrial or nuclear factors as well as environmental agents may contribute to the symptoms in these carriers. The mutation was present in homoplasmic form in the Spanish and North American family (Castillo et al. 2002; Labay et al. 2008), and also in our Hungarian patients, while it was reported to be present in a high level of heteroplasmy (>95% and 90% respectively) in the original UK family (Hutchin et al. 2000). Only blood leukocytes were available in this work, and no other tissues were examined in previous reports. However, the proportion of the normal and the mutant alleles may vary in the auditory cells, explaining incomplete penetrance and the difference in the age of onset.

The m.7510T>C mutation has been reported to be a rare finding and was not found in some large screening populations with non-syndromic SNHL (Jacobs et al. 2005; Lévêque et al. 2007). It was originally interpreted as a benign polymorphism defining a deep branch in the mitochondrial DNA phylogeny (Labay et al. 2008). However, it was not detected in normal Caucasian controls (Hutchin et al. 2000), or in haplogroup- and sub-haplogroup-matched controls (Labay et al. 2008). Our

own investigations demonstrate that it was absent in 60 other Caucasian patients with SNHL and in 30 Caucasian healthy controls of haplogroup H. Since several families with isolated SNHL have been described to harbor the m.7510T>C mutation, it can be regarded as a disease-causing mutation. Haplotype analysis in our Hungarian family suggested an unnamed subgroup of haplogroup H, delineated by the SNP m.4793A>G, which is different from the haplogroups of the previously reported Spanish and North American families, and the UK family with a sequence variant (m.4336A>G) not present in the other three families. The fact that the m.7510T>C mutation arose on different mitochondrial subgroups provides further evidence for the pathogenicity of this mutation. Evolutionary studies showed that deleterious mitochondrial mutations are influenced very strongly by selection and are eliminated rapidly, so their appearance should be the result of very recent mutational events and transmitted through very few generations; thus, founder events are not likely to be found (Torroni et al. 2003). The finding that all four so far described families carry the m.7510T>C mutation on different European mtDNA haplogroup backgrounds indicates that European haplogroups do not increase or reduce the risk of expressing the disease phenotype. Since there is no marked phenotypic variability among the families, the haplogroup data may support that phenotypic expression is not necessarily influenced by haplogroup background.

To our knowledge, this is the fourth family in the literature with the m.7510T>C tRNA^{Ser(UCN)} mutation. Comparing the cases reported so far (Table 1), there is some variability in the age of onset, the accompanying symptoms such as tinnitus or increased sensitivity to noise, ototoxicity, and the haplogroup background. Given the fact that hearing loss resulting from mitochondrial mutations can exhibit incomplete penetrance and a broad phenotypic spectrum, screening for unexplained hearing loss should include full mtDNA sequencing (encompassing the tRNA^{Ser(UCN)} mutations) in all cases in which maternal inheritance cannot be clearly ruled out.

Acknowledgments We are grateful to Anna Erdélyi and Edit Papp for their skilled technical assistance in the mitochondrial DNA analysis. This work was supported by the Grants OTKA T 73430 and ETT 2009 243–07. We are grateful to the family for their cooperation.

All authors declare no conflict of interest relating to the work in the manuscript.

Synopsis of the Article

We present the fourth family reported in the literature with bilateral isolated hearing loss due to the m.7510T>C tRNA^{Ser(UCN)} mutation in an unnamed subgroup of

mitochondrial haplogroup H providing further evidence for the pathogenic role of the m.7510T>C mitochondrial mutation.

References

- Castillo FJ, Villamar M, Moreno-Pelayo MA et al (2002) Maternally inherited non-syndromic hearing impairment in a Spanish family with the 7510T>C mutation in the mitochondrial tRNA^{Ser(UCN)} gene. *J Med Genet* 39(12):e82
- Friedman RA, Bykhovskaya Y, Sue CM et al (1999) Maternally inherited nonsyndromic hearing loss. *Am J Med Genet* 84(4):369–372
- Gutiérrez Cortés N, Pertuiset C, Dumon E et al (2012) Novel mitochondrial DNA mutations responsible for maternally inherited non-syndromic hearing loss. *Hum Mutat* 33(4):681–689
- Hernstadt C, Elson JL, Fahy E et al (2002) Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian, and European haplogroups. *Am J Hum Genet* 70(5):1152–1171
- Hutchin TP, Parker MJ, Young ID et al (2000) A novel mutation in the mitochondrial tRNA^{Ser(UCN)} gene in a family with non-syndromic sensorineural hearing impairment. *J Med Genet* 37(9):692–694
- Jacobs HT, Hutchin TP, Kappi T et al (2005) Mitochondrial DNA mutations in patients with postlingual, nonsyndromic hearing impairment. *Eur J Hum Genet* 13(1):26–33
- Labay V, Garrido G, Madeo AC et al (2008) Haplogroup analysis supports a pathogenic role for the 7510T>C mutation of mitochondrial tRNA^{Ser(UCN)} in sensorineural hearing loss. *Clin Genet* 73(1):50–54
- Lévêque M, Marlin S, Jonard L et al (2007) Whole mitochondrial genome screening in maternally inherited non-syndromic hearing impairment using a microarray resequencing mitochondrial DNA chip. *Eur J Hum Genet* 15(11):1145–1155
- Li X, Zhang LS, Fischel-Ghodsian N, Guan MX (2005) Biochemical characterization of the deafness-associated mitochondrial tRNA^{Ser(UCN)} A7445G mutation in osteosarcoma cell hybrids. *Biochem Biophys Res Commun* 328(2):491–498
- Maász A, Komlósi K, Hadzsiev K et al (2008) Phenotypic variants of the deafness-associated mitochondrial DNA A7445G mutation. *Curr Med Chem* 15(13):1257–1262
- Prezant TR, Agopian JV, Bohlman MC et al (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nat Genet* 4(3):289–294
- Reid FM, Vernham GA, Jacobs HT (1994) A novel mitochondrial point mutation in a maternal pedigree with sensorineural deafness. *Hum Mutat* 3(3):243–247
- Torroni A, Campos Y, Rengo C et al (2003) Mitochondrial DNA Haplogroups Do Not Play a Role in the Variable Phenotypic Presentation of the A3243G Mutation. *Am J Hum Genet* 72(4):1005–1012
- Verhoeven K, Ensink RJ, Tiranti V et al (1999) Hearing impairment and neurological dysfunction associated with a mutation in the mitochondrial tRNA^{Ser(UCN)} gene. *Eur J Hum Genet* 7(1):45–51
- Willems PJ (2000) Genetic causes of hearing loss. *N Engl J Med* 342(15):1101–1109
- Yan H, Zareen N, Levinger L (2006) Naturally occurring mutations in human mitochondrial pre-tRNA^{Ser(UCN)} can affect the transfer ribonuclease Z cleavage site, processing kinetics, and substrate secondary structure. *J Biol Chem* 281(7):3926–3935
- Yan X, Wang X, Wang Z et al (2011) Maternally transmitted late-onset non-syndromic deafness is associated with the novel heteroplasmic T12201C mutation in the mitochondrial tRNA^{His} gene. *J Med Genet* 48(10):682–690
- Yarham JW, Elson JL, Blakely EL, McFarland R, Taylor RW (2010) Mitochondrial tRNA mutations and disease. *Wiley Interdiscip Rev RNA* 1(2):304–324
- Zheng J, Ji Y, Guan MX (2012) Mitochondrial tRNA mutations associated with deafness. *Mitochondrion* 12:406–413