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Short Report

DFNB49 is an important cause of non-syndromic deafness in Czech Roma patients but not in the general Czech population

Šafka Brožková D, Laštůvková J, Štěpánková H, Krůtová M, Trková M, Myška P, Seeman P. DFNB49 is an important cause of non-syndromic deafness in Czech Roma patients but not in the general Czech population. Clin Genet 2011. © John Wiley & Sons A/S, 2011

Due to endogamy, the Roma have a higher risk for autosomal recessive (AR) disorders. We used homozygosity mapping on single-nucleotide polymorphism chips in one Czech Roma consanguineous family with non-syndromic hearing loss (NSHL). The second largest homozygous region in a deaf patient was mapped to the previously reported DFNB49 region. The *MARVELD2* gene was recently reported as a causal gene for NSHL DFNB49. Sequencing of the *MARVELD2* gene revealed a previously reported homozygous mutation c.1331+2 T>C (IVS4 + 2 T>C) in the deaf child. Subsequently, the same mutation was found in two more Roma families from an additional 19 unrelated Czech Roma patients with deafness tested for the *MARVELD2* gene. To explore the importance of *MARVELD2* mutations and DFNB49 for the general Czech and Central European population with early hearing loss we also tested 40 unrelated Czech patients with AR NSHL. No pathogenic mutation in the *MARVELD2* gene was found in a group of 40 Czech non-Roma patients. Mutations in the *MARVELD2* gene seem to be a significant cause of early NSHL in Czech Roma and this gene should be tested in this group of patients after *GJB2*.

Conflict of interest

The authors declare no conflict of interest.

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Key words: deafness – DFNB49 – homozygosity mapping – *MARVELD2* – non-syndromic hearing loss – Roma

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Non-syndromic hearing loss (NSHL) is genetically extremely heterogeneous, but in the vast majority of cases follows autosomal recessive (AR) inheritance. Mutations in the *GJB2* gene are the most common cause of AR NSHL named as DFNB (1).

The Roma or Gypsies are a population of Indian origin, with their exodus from India dated to approximately the 5th to 10th century AD. The vast majority of Roma live in Europe, especially in the Balkans and southeast Europe (2). The Roma population is

known to have a higher risk for AR diseases. In most recessive disorders, Roma patients are homozygous for causal mutations. Most Roma patients with NSHL are homozygous for the founder mutation p.Trp24X (W24X) in the *GJB2* gene (3–5). Recently, several AR diseases were reported in the Roma due to a founder mutation – hereditary motor and sensory neuropathy-type Lom (6), congenital glaucoma (7), congenital cataracts, facial dysmorphism and neuropathy syndrome (8), and congenital myasthenic syndromes (9).

The DFNB49 region was reported in 2004 by Ramzan et al (10). Two years later, the gene *MARVELD2* was discovered to harbour the causal mutations in the DFNB49 region in eight families from Pakistan by Riazuddin et al (11). Chishti et al (12) described in 2008 an additional three Pakistani families with one novel and two already reported mutations in the *MARVELD2* gene. *MARVELD2* is the name in the official nomenclature, but originally the name was *TRIC* and this name is also used as an unofficial synonym. The frequency of *MARVELD2* mutations in the European population with early NSHL is completely unknown.

We used homozygosity mapping in a Czech Roma family with a deaf child and a known consanguinity of parents where mutations in the *GJB2* gene were previously excluded.

Materials and methods

Genomic DNA was extracted from peripheral blood according to a standard protocol. All patients signed informed consent for DNA testing for clarification of the cause of early deafness. The patients were previously tested negative for mutations in the *GJB2* gene by sequencing of the entire coding region. The genomic DNA samples from family 1, the affected patient (VI.1), both his parents (V.1 and V.2) and an unaffected sister (VI.2), were hybridized on the Affymetrix GeneChip Human Mapping 250K NspI arrays (Affymentrix, Santa Clara, CA). Data were analysed by the Affymetrix software – Genotyping Console v4.0. We searched for homozygous segments in the affected patient which were heterozygous in both parents and not the same homozygous in the unaffected sister. Homozygous segments of 2 Mb size or larger were selected for further analysis.

The six coding exons and flanking intron boundaries of *MARVELD2* were amplified with a set of seven pairs of primers designed with ExonPrimer (http://ihg.helmholtz-muenchen.de/ihg/ExonPrimer.html). The products were sequenced with Big Dye Terminator v3.1 chemistry and analysed on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). The reference sequence NM_001038603.1 was used for assigning the mutation. The exon 4 was sequenced from DNA anonymized controls from Roma individuals from Czech Republic and Slovakia (Czechoslovakia in the past) for the c.1331+2 T>C mutation.

Audiometric examination of hearing loss was performed by the steady-state evoked potentials (SSEP) method due to the patient's young age. SSEP (also known as ASSR) is a method for objective audiometry, similar to ABR. The result from the examination is an estimated audiogram with quantification of hearing loss 10–130 dB on different frequencies (500, 1000, 2000 and 4000 Hz).

Results

Molecular genetic findings

Homozygosity mapping in the Czech Roma family with known consanguinity revealed 20 homozygous regions of 2 Mb size or larger in the affected patient VI.1 which were heterozygous in the parents and healthy sister (Fig. 1, the homozygous regions). The 20 homozygous regions were compared to the chromosomal regions and genes already associated with AR NSHL (DFNB loci). In one of the largest homozygous regions on chromosome 5 (9 Mb: 64.218.736–73.223.416 according to a build 36), only one gene previously associated with hearing loss was found, namely *MARVELD2*. Sequencing of all six coding exons and flanking intron parts of *MARVELD2* gene in our Roma patient revealed the splice-site mutation c.1331+2 T>C (IVS 4+2 T>C).

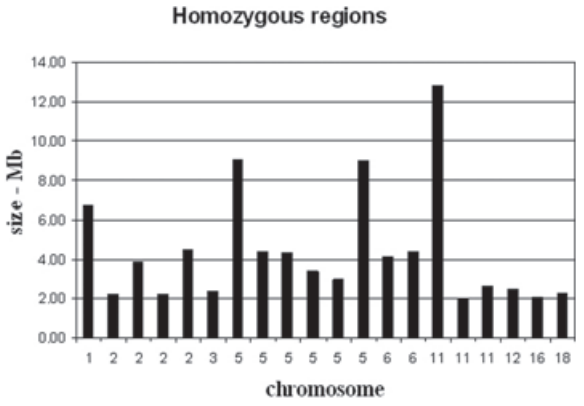

Fig. 1. Twenty homozygous regions found in the family 1. On the *x*-axis is the chromosome number and on the *y*-axis are the sizes of regions in mega base pairs.

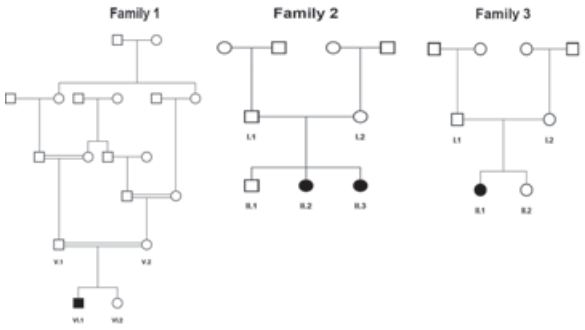

Fig. 2. Pedigrees of three Czech Roma families with recessive non-syndromic hearing loss DFNB49. The consanguinity is displayed in family 1. The additional affected patients in families 1 and 2 are not shown in the pedigrees. They were not available for testing, since they live in other countries. Filled symbols – affected patients, clear symbols – healthy persons.

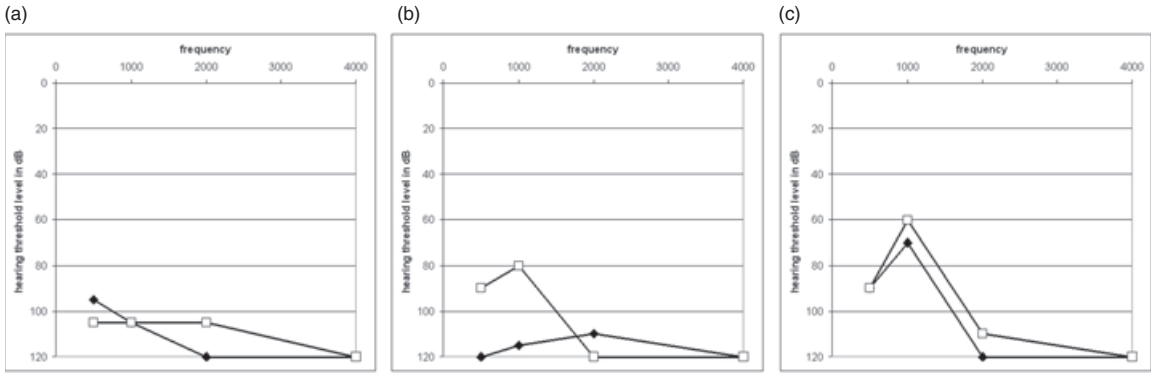

Fig. 3. Estimated audiograms obtained by SSEP audiometry examination. Left ear, ♦; right ear, □; (**a**) patient VI.1 from family 1, (**b**) patient II.2 and (**c**) patient II.3 from family 2. The tested frequencies were 500, 1000, 2000 and 4000 Hz; the hearing threshold levels are in dB. Profound hearing loss higher than 80, respectively 60, was found in all patients in left and right ears.

Table 1. Summary of all the pathogenic mutations in *MARVELD2*[a]

Mutation	Alternative mutation name	Origin of family	References
c. 1183-1G>A	IVS3-1G>A	–	(11)
c. 1331+1G>A	IVS4+1G>A	Pakistan	(12)
c.1331+2delTGAG	IVS4+2delTGAG	–	(11)
c.1331+2delTGAG	IVS4+2delTGAG	–	(11)
c. 1331+2T>C	IVS4+2T>C	Pakistan	(11)
c. 1331+2T>C	IVS4+2T>C	–	(11)
c. 1331+2T>C	IVS4+2T>C	–	(11)
c. 1331+2T>C	IVS4+2T>C	–	(11)
c. 1331+2T>C	IVS4+2T>C	Pakistan	(12)
c. 1331+2T>C	IVS4+2T>C	Pakistan	(12)
c. 1331+2T>C	IVS4+2T>C	Roma, Czech Republic	This report
c. 1331+2T>C	IVS4+2T>C	Roma, Czech Republic	This report
c. 1331+2T>C	IVS4+2T>C	Roma, Czech Republic	This report
c. 1498C>T	p.R500X	–	(11)

[a]To date only five mutations have been reported in the *MARVELD2* gene. Some of them were found in several families as shown in the table. Some of the patients with mutations are very probably also from Pakistan, but in the article by Riazuddin et al., the ethnicity is not stated precisely, so the place of origin is undetermined. In all cases the mutations cause non-syndromic, early and severe to profound hearing loss.

This mutation was found in the homozygous state only in the patient (VI.1). The parents (V.1 and V.2) and sister (VI.2) were heterozygotes for this mutation (Fig. 2, pedigrees). Testing of further 19 Czech Roma deaf patients without *GJB2* mutations revealed the same mutation in a further two, unrelated Roma patients (families 2 and 3), showing that DFNB49 is probably an important cause of deafness in the Roma population. Further testing of 40 non-Roma patients with non-syndromic prelingual hearing loss, without *GJB2* mutation, did not reveal any other *MARVELD2* mutation, showing that DFNB49 is a very rare cause of deafness in the general Czech–Slavic population.

Additional testing of 48 anonymized DNA samples from unrelated, healthy Roma individuals revealed one heterozygous sample for the c.1331+2 T>C mutation.

Clinical findings

Available clinical examination of the three affected patients revealed profound and very early or even congenital bilateral hearing loss. The examination of patient VI.1 in family 1 (Fig. 2, pedigrees) at age 4 revealed profound hearing loss between 95–120 dB. The patients II.2 and II.3 from family 2 were examined at age 3 and 14 months with hearing loss 80–110 dB (Fig. 3, estimated audiograms).

Discussion

These are probably the first cases of DFNB49 in the Roma population (Table 1, the summary of all *MARVELD2* mutations). The same mutation c.1331+2 T>C (IVS4 + 2 T>C) in the *MARVELD2* gene was described by Riazuddin (11) and Chishti (12) in six Pakistani families. In addition, the functional study as exon-trapping and RNA analysis from lymphoblastoid cells showed aberrant splicing with frameshift and truncated protein (11). The findings of the same splice-site mutations c.1331+2 T>C in Pakistani families and Roma in the Czech Republic may represent a very old mutation deriving from a common ancestor as

was reported in other genes. Although independent mutational events on the same nucleotide can not be excluded, we think it is much less probable (5, 13). Haplotype analysis of Pakistani and Czech families may clarify this situation in the future.

Sensorineural hearing loss in homozygous carriers of the mutation is congenital and profound (90–110 dB). It is the same corresponding result as was reported for this mutation earlier. The *MARVELD2* gene encodes the tight-junction protein present in many epithelial tissues and seems to be necessary for the formation of the epithelial barrier. Surprisingly, the phenotype of patients with mutations found in the *MARVELD2* present only with deafness (11–12).

The same splice-site mutation c.1331+2 T>C was found in three unrelated Czech Roma families. The additional 17 Roma families examined did not have any mutation in the *MARVELD2*. No mutation was found in the group of 40 Czech patients of non-Roma origin with prelingual non-syndromic deafness without *GJB2* mutations. In a group of DNA controls the frequency of heterozygotes for mutation c.1331+2 is 1:48 (2%). The frequency for mutation W24X in the *GJB2* gene in Czech Roma controls is 2:41 (4.9%) and the total for European Roma is 4.6% (14). DFNB1 is evidently more frequent in Roma than DFNB49. There might be a small deviation because of the small statistic sample of the control group. This supports our results and points to a frequent mutation in Czechoslovak Roma.

Homozygosity mapping using single-nucleotide polymorphism chips followed by sequencing of candidate genes within the largest homozygous intervals proved to be a powerful tool for finding causal mutations in Roma families with AR disorders. Our results show that the mutations in the *MARVELD2* gene are an important cause of NSHL in Czech Roma and similarly may also apply for Roma in other countries.

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