International Journal of Pediatric Otorhinolaryngology 76 (2012) 1681–1684

Contents lists available at SciVerse ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

DFNB35 due to a novel mutation in the *ESRRB* gene in a Czech consanguineous family

Dana Šafka Brožková [a,*], Jana Laštůvková [b], Eliška Machalová [a], Jana Lisoňová [a], Marie Trková [c], Pavel Seeman [a]

[a] DNA laboratory, Department of Child Neurology, Charles University, 2nd Medical School and University Hospital Motol, Prague, Czech Republic
[b] Department of Medical Genetics, Masaryk Hospital, Regional Health Corporation, Ústí nad Labem, Czech Republic
[c] Centre for Medical Genetics and Reproductive Medicine GENNET, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form 2 August 2012
Accepted 3 August 2012
Available online 27 August 2012

Keywords:
DFNB35
ESRRB
Homozygosity mapping
Non-syndromic hearing loss

ABSTRACT

Objectives: Non-syndromic hearing loss (NSHL) is a genetically heterogeneous disorder with mostly autosomal recessive inheritance. So far 40 genes and the same amount of loci with as yet unknown genes were described with autosomal recessive NSHL.
Patients and methods: A consanguineous Czech family with a child with NSHL was genotyped using SNP array and homozygous regions were compared with previously reported DFNB loci.
Results: *GRXCR1* and *ESRRB* genes associated with autosomal recessive NSHL were located in two of the eight homozygous regions detected by SNP array genotyping.

Mutation p.R291L in a homozygous state was found in the deaf child, the parents were heterozygous. The entire coding region of the *ESRRB* gene was sequenced in additional 39 patients of Czech origin with early NSHL and only two variants, p.V413I and p.P386S, were found in homozygous state, but are considered to be polymorphisms.
Conclusion: Homozygosity mapping is a powerful method for identification of genes in heterogeneous recessive diseases. This is the first report of DFNB35 mutations in the Czech Republic and it seems to be a rare cause of NSHL. Additional mutations in *ESRRB* gene were reported in Pakistan, Tunisia and Turkey.

1. Introduction

Nonsyndromic hearing loss (NSHL) is a genetically very heterogeneous disease with 40 genes associated with autosomal recessive (AR) types already reported and many more loci with the genes yet unknown [1]. The hearing loss in patients with AR NSHL is usually severe to profound and early. By far the most frequent cause of AR NSHL are mutations in the *GJB2* gene [2,3]. Frequently, a prevalent mutation is found in particular populations for the *GJB2* gene [4].

Homozygosity mapping is a powerful tool for finding the causal mutations in AR disorders especially amongst families with consanguinity [5]. This approach was used recently to find the new genes: GPSM2 and GRXCR1 associated with NSHL [6,7]. Our group proved the method as successful by finding the causal mutation in the *MARVELD2* gene responsible for DFNB49 [8]. In all these cases homozygosity mapping using SNP whole genome arrays was successful in finding the regions with disease causing mutations.

DFNB35 locus was reported in 2003 by Ansar et al. [9]. Five years later Collin et al. [10] found the causal gene and reported five mutations in the *ESRRB* gene as the cause of DFNB35. The sixth mutation in the *ESRRB* was reported last year by Ben Said et al. [11] and novel deletion in the *ESRRB* gene was reported last year [12].

There were no additional reports on DFNB35 and *ESRRB* mutations published since that time. Here we report a consanguineous Czech family with a novel variation in the *ESRRB* gene showing evidence to be causal for DFNB35. So far the mutations in the *ESRRB* gene were reported only in Turkey, Tunisia and Pakistan. This is the first report of DFNB35 mutation in the Czech Republic and also in Europe.

2. Patients and methods

All patients involved in this study signed informed consent for DNA examination in connection with non-syndromic hearing loss. All patients were previously tested negative by sequencing for the

* Corresponding author at: DNA laboratory, Department of Child Neurology, Charles University 2nd Medical School and University Hospital Motol, V Úvalu 84, 15006 Prague, Czech Republic. Tel.: +420 224436789.
E-mail address: dana.brozkova@seznam.cz (D. Šafka Brožková).

0165-5876/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijporl.2012.08.006

most frequent cause of NSHL, mutations in the coding part of the *GJB2* gene.

A double consanguineous family from the common Czech population was referred to genetic counselling because of profound hearing loss in their child.

The genomic DNA from parents (V.1, V.2) and affected child VI.1 from family 1 (Fig. 1) was genotyped using Illumina HumanCytoSNP-12v2.1 and the resulting data were analysed by GenomeStudio V2011.1. Homozygous regions larger than 2MB were screened for known regions associated with the NSHL. We focused on regions which were homozygous in the patient and heterozygous in his parents.

All coding exons and intron boundaries of *ESRRB* gene were sequenced with a set of eight pairs of primers. All coding exons of the *GRXCR1* gene were sequenced with four pairs of primers. The resulting PCR products were sequenced with the Big Dye Terminator v3.1 and analysed on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Two reference sequences NM_004452.3 and NM_001080476.2 were used for *ESRRB* and *GRXCR1* gene, respectively.

The entire coding region (all eight coding exons) of gene *ESRRB* was sequenced in the additional 39 hearing loss patients of Czech origin with previously excluded mutations in exon 2 of the *GJB2* gene by sequencing.

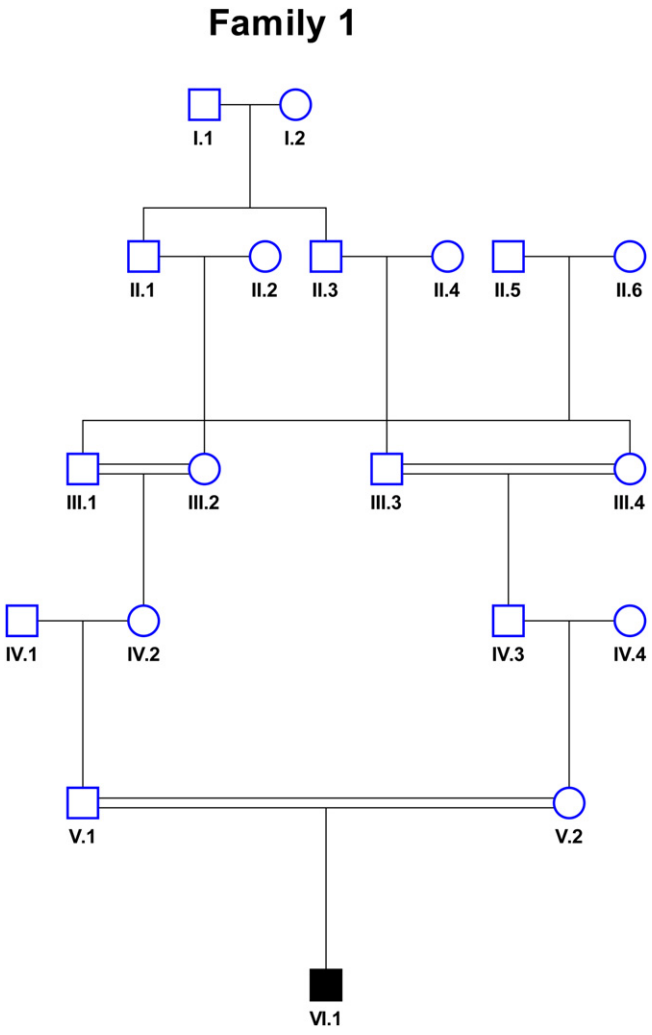

Fig. 1. Pedigree of Czech consanguineous family with non-syndromic hearing loss DFNB35. Filled symbols – affected patients, clear symbols – healthy persons.

The prediction programmes SIFT and PolyPhen-2 were used for the pathogenicity classification of the mutations. A score below 0.05 (SIFT) and closer to 1 (PolyPhen-2) means the mutation has pathogenic effect. Exome Variant Server (EVS) [13], 1000 Genomes [14,15] and SNP database [16,17] were used to find the information about identified variants.

3. Results

3.1. Molecular genetic findings

Overall, eight homozygous regions larger than 2MB were detected across the patient's genome. These areas were homozygous in the patient and heterozygous in the parents. Comparison of the homozygous regions with the loci of genes already associated with the AR NSHL revealed overlap for two regions: DFNB25 containing the *GRXCR1* gene and DFNB35 with the *ESRRB* gene. Sanger sequencing of the *GRXCR1* gene did not reveal any variation. Subsequent sequencing of the *ESRRB* gene detected the variation c.872 G>T (p.R291L) in exon 5. The patient was homozygous for this variation, whereas the parents were heterozygous carriers of the p.R291L variation.

The computer prediction of the pathogenicity scored the p.R291L deleterious by SIFT (0.00) and probably damaging by PolyPhen2 (1.00). This variant is not listed in the 1000 Genomes Project nor in the EVS (5379 exomes data). This position p.R291 is highly conserved amongst species (Fig. 2).

Two other nonsynonymous variants were found during sequencing of *ESRRB* in the additional 39 NSHL patients. Mutation c.1237G>A (p.V413I) was found in a homozygous state in one patient, but it has its number in the db SNP (rs146351534) and reported frequency for heterozygotes, which is 0.016. The second variation c.1156C>T (p.P386S) was found in two patients in homozygous and heterozygous state and has its number in the db SNP (rs61742642) and reported frequency for heterozygotes 0.091. Moreover both variants were scored as tolerated and benign by SIFT (1.00 and 0.06) and Polyphen2 (0.016 and 0.481).

One synonymous variation was found: c.81C>A ((*p.*(=)), rs35544003) in two patients.

3.2. Clinical findings

Clinical examination revealed profound and very early bilateral hearing loss in the proband (VI.1) at the age of six months. According to the parents, the hearing loss was congenital, but no objective hearing examination was undertaken before this age. The transient otoacoustic emission spectra (TEOAEs) were bilaterally absent and Steady State Evoked potentials (SSEP) were bilaterally without response at the age of one year. No other dysmorphic or abnormal findings indicating syndromic hearing loss were present.

Both parents have normal hearing.

4. Discussion

In this study we report a novel mutation in the *ESRRB* gene as the cause of DFNB35 and as the first case of DFNB35 reported not only in the Czech Republic, but also in Europe. Overall eight pathogenic mutations are so far known in the *ESRRB* gene as the cause of DFNB35 worldwide (Table 1). DFNB35 seems to be a rare cause of AR NSHL; examination of 39 additional Czech hearing loss patients revealed only two other variations in homozygous state considered to be polymorphisms due to the in silico prediction and mainly due to the high frequency in the population. A similar observation was reported in Turkey where homozygosity mapping of the DFNB35 region in 83 consanguineous NSHL patients revealed the homozygosity only in seven of them and sequencing