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Prevalence of 35delG mutation in GJB2 gene in the Moldovan population

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Abstract

Background: Guanine deletion 35delG in GJB2 exon 2 is the pathogenic mutation responsible for up to 70% of cases of congenital non-syndromic sensorineural hearing loss (NSHL) among Europeans. The early molecular diagnostic of hearing loss nature has become important while considering the cochlear implants. The purpose of this study was to establish the frequency of 35delG deletion in GJB2 gene among patients with severe NSHL and its prevalence among Moldovan residents with normal hearing.
Material and methods: 40 patients with congenital bilateral profound NSHL and 300 individuals with normal hearing were examined for deletion 35delG, by using Custom TaqMan SNP genotyping Assay.
Results: 12 (30%) patients with homozygous genotype for 35delG mutation were identified, whereas 8 patients (20%) were heterozygous. The study reported 4 (1.33%) carriers of 35delG mutation among 300 Moldovan individuals with normal hearing.
Conclusions: The present study results suggest a need for including the 35delG molecular testing into the national program of neonatal screening of hearing loss. Considerations on the genetic carrier testing should be made in genetic counseling and family planning.
Key words: GJB2, 35delG mutation, non-syndromic deafness.

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Introduction

Hearing loss (HL) or deafness is the most frequent congenital sensory impairment in humans, which is a very heterogeneous trait. Based on the World Health Organization's (WHO) data, in 2020 around 466 million people worldwide had disabling hearing loss, and 34 million of these people were children [1]. The incidence of severe to profound deafness in the first two decades of life is about 1 in 650 newborns.

Over 150 associated loci and about 80 different protein-coding genes are involved in the perception of sound, up to 1% of these causative human genes have been mapped. Mutations in these genes can lead to similar clinical manifestations of hearing impairment. Non-syndromic HL accounts for up to 70% of genetic deafness, which is almost exclusively monogenic [2].

More than half of cases of congenital autosomal recessive non-syndromic HL resulted from mutations in the DFNB1 locus. Prelingual non-progressive sensorineural forms of deafness are expressed as moderate to profound [3].

The DFNB1 (OMIM 220290) locus on chromosome 13 (13q11-q12) contains two genes, GJB2 (OMIM 121011) and GJB6 (OMIM 604418) [4]. Encoding connexin 26 (Cx26) and connexin 30 (Cx30), respectively are members of intracellular gap junction β proteins family. In the inner ear, the six monomers of Cx26 or Cx30 oligomerize to form as homo- or heteromeric connexons, which are involved in the recycling of ions K+ between hair cells and endolymph [5, 6]. This is believed to play a crucial role in efficient generation of action potentials in mechanosensory transduction of sound.

The literature review demonstrated that more than 100 pathogenic mutations in the GJB2 gene have been identified with a significant contribution of the frameshift and nonsense mutations [7, 8]. The frequency of the individual mutations associated with HL varies within ethnic groups [9]. In Caucasians, the most frequent mutation is 35delG (rs80338939) a point deletion of one of six guanines at the codon position 30-35 of the second exon. This deletion leads to shifted reading frame, creation of stop codon, and premature termination of the protein Cx26 synthesis [10, 11].

This study was aimed to establish the carrier frequency of 35delG deletion in GJB2 gene among patients with severe NSHL and among Moldovan population with normal hearing.

Material and methods

40 children with prelingual non-syndromic HL from the Republic of Moldova and Romania and 300 unrelated participants with normal hearing were included in the study. Written informed consent was signed by all the minors' parents and by healthy participants.

A total 40 hearing impaired children (aged 1-14 years) underwent audiological analysis. The degree of their hearing loss was evaluated as severe (71-90 dB) to profound (>90dB), according to the HL Classification from WHO.

All examined volunteers with normal hearing were aged between 18-29 years. Venous blood samples were taken from all participants.

Genomic DNA isolation from 100 µL of peripheral blood was performed with proteinase K and spin column purification protocol (#K0722, ThermoFisher Scientific). The purity and concentration of DNA samples were detected using a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific). The average DNA concentration of each sample was adjusted to 2 ng/µL.

The targeted search for the 35delG mutation was detected using a Custom TaqMan SNP Genotyping Assay Kit (#4351379, ThermoFisher Scientific) [12]. The primers and MBG probes for allelic discrimination assay were designed using software Primer3 web version [13].

The molecular-genetic analysis was performed in a total reaction volume of 5 µL on 384-well plates. All samples analysis was performed on the QuantStudio 6 flex device (Applied Biosystems, ThermoFisher Scientific), according to manufacturer's protocol. The allelic discrimination data were analyzed using a TaqMan Genotyper Software application (v.1.3.1., Applied Biosystems, ThermoFisher Scientific). The successful genotyping call rate was <96%, the undetermined samples were automatically eliminated from allelic discrimination analysis.

Statistical analysis was performed using the Statistica v.6.0 software.

Results

The molecular screening for the pathological allele in GJB2 gene among the group of children revealed 12 (30%) patients with homozygous genotype for 35delG mutation and 8 patients (20%) with heterozygous form. Genotyping among 300 individuals with normal hearing identified 4 subjects with carrier rate of 1.33%. The distribution of allele frequencies of the rs80338939 samples analyzed is shown in fig. 1.

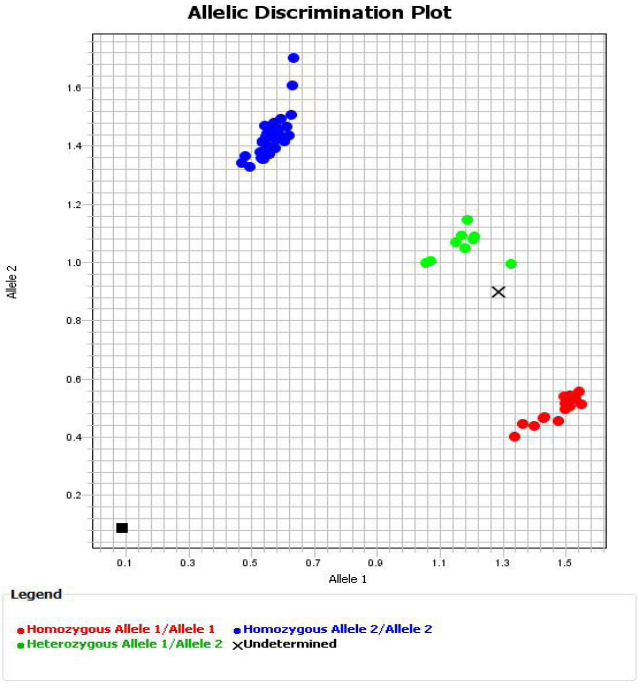

Fig. 1. Allelic Discrimination Plot showing 35delG/rs80338939 Assay

Notes: Representative plot showing performance of allelic Discrimination of 35delG/rs80338939 SNP.
Separation between the signals derived from allele 1 (VIC) and allele 2 (FAM). The red cluster represents homozygous mutants containing copies of the 35delG mutant allele. The green cluster includes individuals having one normal and mutant allele (heterozygous). The blue cluster represents samples homozygous with normal sequence for both alleles.

Discussion

The major pathogenic mutation is point deletion 35delG (rs80338939), which accounts for about 70% of recessive mutations of GJB2 associated with NSHL in populations of European origin, with a carrier frequency of 2-4% [9-11]. Other ethnic groups may have additional or different specific mutations, such as 235delC (rs80338943) in Japanese and Koreans, 167delT (rs80338942) among Ashkenazi Jews, R143W (rs80338948) in Africans [14-16]. W24X mutation has a high frequency in Indian and Roma populations [17]. Almost half of the recessive mutations are frameshift of nonsense type. They have no specific localization and can affect all domains of Cx26 protein [3, 7].

These study findings showed that the prevalence of 35delG mutation in GJB2 gene among volunteers with normal hearing was around 1.33% (4/300). This is in concordance with similar data obtained for other European populations. According to our data, the 35delG deletion was identified in the homo- or heterozygous state in 50% of the patients.

On the other hand, 20% of samples tested were found to have only one mutant allele, 50% of patients had non-carrier rate of 35delG mutation. These results could be explained by the fact that other recessive mutations were present in GJB2 gene in homozygous or compound heterozygous form. It is known that, the NSHL may also develop in compound heterozygous form of the GJB2 mutations with the second mutation in GJB6 (Cx30) gene in the DFNB1 locus [9]. In the case of compound heterozygote of two genes, the expression of these genes is probably affected which is not due to digenic inheritance, as previously assumed [2, 8].

Therefore, it is clinically important to explore other recessive disease-causing mutations in GJB2 gene and to establish a relationship between genotype and phenotype correlation [18].

Due to high frequency of worldwide hearing impairment and its public health impact, early identification and stratification of patients with HL becomes an important

issue. In addition to main directions, research should also focus on the optimization of methods for molecular diagnostics, gene expressions in choosing gene therapy of hereditary pathologies, as well as on prediction of the correct treatment strategy and the estimation of risk of disease recurrence in families.

Conclusions

Our study results support the position that 35delG mutations are a cause in the etiology of non-syndromic hearing loss in other European populations.

In addition, genetic defects that underline deafness could become a good foundation for gene therapy development, treatment strategy, and treatment success. It should be used as a tool in molecular testing to detect genetic origin of deafness in population of Moldova. Our findings suggest including the 35delG testing into the national program of neonatal hearing loss screening. Taking clinical and social impact of NSHL into consideration it is advisable to introduce carrier detection testing in genetic counseling and family planning.

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Authors' contribution: AB analyzed the data and drafted the first manuscript; AB analyzed primers and probes designed for SNP Custom genotyping assay; SP designed the study, participants recruiting and audiological testing; AB, CB, DGA analyzed organized laboratory tests realization; DGA genotyping optimization; AB, CB, GDA, GC performed results interpretation and conclusion elaboration; GC provided logical support and critical discussion of the manuscript. LR rendered substantial help in this bilateral scientific study. All the authors revised and approved the final version of the manuscript.

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