Short Report

High prevalence of the IVS 1 + 1 G to A/GJB2 mutation among Czech hearing impaired patients with monoallelic mutation in the coding region of GJB2


Biallelic pathogenic GJB2 gene mutations cause pre-lingual genetic hearing loss in up to 50% of individuals with bilateral sensorineural hearing loss worldwide. Sequencing of the entire GJB2 gene-coding region in Czech patients with pre-lingual bilateral hearing loss revealed that 10.3% of Czech patients carry only one monoallelic pathogenic mutation in the coding region of the GJB2 gene, which is significantly more than the population frequency of 3.4%. The 309-kb GJB6 deletion, frequent in Spain and France, is very rare in the Czech population. In order to evaluate the impact of the IVS1 + 1 G to A splice site mutation in the non-coding part of the GJB2 gene among Czech patients, we tested all available patients with pre-lingual hearing loss with only one monoallelic mutation in the coding part of GJB2. By sequencing of the exon 1 region of the GJB2 gene and HphI restriction analysis in 20 Czech patients we identified nine patients carrying IVS1 + 1 G to A. Testing for this mutation explained deafness in 45% of Czech GJB2 monoallelic patients. This mutation represents now 4% of GJB2 pathogenic mutations in Czech patients and is the third most common GJB2 mutation found in our cohort of 242 unrelated Czech patients with prelingual hearing loss. A similar frequency may also be expected in other Central European or Slavic populations.

Mutations in the GJB2 gene account for about 15–50% of pre-lingual bilateral non-syndromic genetic hearing loss (1–6). In different published studies a substantial fraction of patients (10–15%) carried only one pathogenic mutation in the GJB2 gene despite using direct sequencing of the entire coding region of the gene (7, 8). A 309-kb deletion involving the GJB6 gene, now called del(GJB6-D13S1830) was shown to be the second, previously overlooked, causal mutation in these monoallelic heterozygous patients in Spain and France (9, 10). We had previously tested Czech patients with only one monoallelic mutation in the coding part of GJB2 for the presence of this mutation, but it revealed to be a very rare cause in the Czech population and is not the second major unknown factor in our monoallelic patients (11). Similar results also from Austria have been published (12).

The splice site mutation IVS1 + 1 G to A, also called the – 3170 G to A in the GJB2 gene, was originally reported by Denoyelle et al. (13) and was generally considered to be rare. Not all laboratories are therefore testing for this particular mutation outside the coding region of GJB2 gene. Some published studies have included this mutation while others have not.

This splice site mutation has been found in several populations (2, 14–16) and is predicted to disrupt splicing, yielding no detectable mRNA (14).

To clarify the impact of the IVS1 + 1 G to A mutation among Czech patients with pre-lingual
hearing loss, we tested all available patients with pathogenic mutation on only one allele of the coding region of \textit{GJB2} gene.

\textbf{Patients and methods}

Twenty patients of Czech origin were previously tested by direct sequencing of the entire coding region of the \textit{GJB2} gene. In all 20 of them the 35delG mutation was detected on only one allele. All 20 of these patients also tested negative for the presence of the del(\textit{GJB6-D13S1830}) mutation.

The exon 1 and flanking donor splicing site were amplified with primers Cx26Ex1 F (5'-TCC GTA ACT TTC CCA GTC TCC GAG GGA AGA GG-3') and Cx26 Ex1 R (5'-CCC AAG GAC GTG TGT TGG TCC AGC CCC-3') described previously by Denoyelle et al. (13). Polymerase chain reaction (PCR) products were sequenced using the same primers and the ABI Big Dye Terminator Ver.3 kit (ABI, Foster City, CA) and subsequently analyzed on an ABI 3100 Avant Genetic Analyzer (ABI).

New PCR products from patients carrying the IVS1 + 1 G to A mutation were also digested by \textit{HphI} restriction enzyme to confirm the presence of the mutation.

To clarify the heterozygotes frequency in Czech hearing population we tested 600 anonymous unrelated DNA samples (1200 chromosomes) from individuals without hearing impairment history by \textit{HphI} restriction enzyme digestion of PCR products amplified by primers Cx26 Ex1 F and Cx26 Ex1 R mentioned above. The same control cohort was also tested for the 35delG mutation using fluorescence PCR and fragment analysis.

Furthermore, 180 unrelated Czech patients with pre-lingual hearing loss without any pathogenic mutation in the \textit{GJB2} coding region were tested for the presence of the IVS1 + 1 G to A mutation using a primer extension reaction SNaPShot (ABI) following the manufacturer's instructions.

\textbf{Results}

We detected nine patients out of 20 tested as carrying the IVS1 + 1 G to A mutation in heterozygous state in addition to their already known 35delG mutation. Testing for the IVS1 + 1 G to A mutation or exon 1 and flanking sequences explained previously unclear hearing loss in 45% of Czech patients with one pathogenic mutation in only one allele of the coding region of the \textit{GJB2} gene. In three of the compound heterozygotes the separate segregation of each allele could be confirmed either in the parents or the offspring. The IVS1 + 1 G to A mutation represents 4% of all pathogenic mutations in \textit{GJB2} in our total cohort of 242 unrelated Czech patients with pre-lingual hearing loss and is now the third most common pathogenic mutation detected among Czech patients.

After inclusion of the IVS1 + 1 G to A mutation in our detection procedure, the percentage of individuals with bilateral sensorineural hearing loss with only one monoallelic mutation in the \textit{GJB2} decreased from 10% to only 4%.

We did not detect the IVS1 + 1 G to A mutation in any of the 180 patients without pathogenic mutation in \textit{GJB2} nor in any of the 600 samples from normal hearing controls without hearing loss history. In the same control cohort we detected six 35delG heterozygotes, representing surprisingly only 1%, which is in contrast to our previous results on a different control cohort (6).

\textbf{Discussion}

By testing for the previously known splice site mutation IVS1 + 1 G to A in the non-coding part of the \textit{GJB2} gene, we realized the importance and prevalence of this mutation in the Czech population with pre-lingual hearing loss. We have shown, that the IVS1 + 1 G to A mutation is more common in the Czech population than in many other countries. This mutation should be tested in all patients carrying only one monoallelic mutation in the \textit{GJB2} coding region.

In the initial study on 156 Czech patients with pre-lingual non-syndromic hearing loss (NSHL) (6), concentrating only on the coding exon 2 of the \textit{GJB2} gene showed we clearly underestimated the importance of the IVS1 + 1 G to A mutation at the splice donor site of the non-coding exon 1. This procedure without testing for this splice site mutation was chosen by several other authors in published studies from Germany, UK and Austria (4, 7, 17–19) but several other studies included testing for the \textit{GJB2} exon 1 region (2, 12, 15, 16, 20). Interestingly, the study from Austria, a country with a common history with the Czech Republic, failed to detect this mutation among 393 NSHL being negative or heterozygous for the \textit{GJB2} coding region mutations (12). This could be caused for example by the mixed origin of the selected patients, where patients from several different countries were included. Another study from eastern Austria (19, 21) showed, that 11.1% of hard hearing familiar patients are heterozygous carriers of the \textit{GJB2} 35delG mutation compared with the carrier frequency 1.7% in the normal Austrian population.
population. But in this study, the IVS1 + 1 G to A mutation was not examined, leaving the probability that the hearing loss in a substantial part of these patients could be explained and is caused by this mutation in the non-coding part of GJB2. A similar situation is shown by the results in a recent study in the UK (7). On the other hand a recent study from the Netherlands, which also included the IVS1 + 1 G to A mutation test, showed that this mutation is the third most common mutation in Dutch NSHL patients, after the 35delG and del(GJB6-D13S1830) (15).

Compared to the most common GJB2 mutation in the Czech Republic, namely the 35delG, which represents 83% of all pathogenic alleles (6), the IVS1 + 1 G to A mutation seems to be roughly 20 times less frequent. The observed heterozygotes frequency for 35delG in the Czech hearing population is 1: 29.6 (17 heterozygotes among 504 samples tested) (6). Therefore we expected to detect at least one sample heterozygous for the IVS1 + 1 G to A mutation among 600 control samples tested, but this failed to be true. However in the same cohort the heterozygous frequency for 35delG was only 1%, contrasting to our previous results on another control cohort. By testing for the IVS1 + 1 G to A mutation also among all our NSHL patients negative for any pathogenic mutation on both alleles of the coding region of GJB2, we excluded the possibility that we could have missed some patients homozygous for the IVS1 + 1 G to A. To our best knowledge such a genotype has never been reported. This result shows that it is probably not necessary to test for this mutation in all patients, but to perform it in those who are heterozygous for only one pathogenic mutation.

Further studies in neighboring countries will be needed to clarify the origin and prevalence of this mutation if it is specific to Slavic populations or to Central European or others. Data from Poland, Slovakia and Russia about the occurrence and frequency of this mutation however, are still lacking.

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References


IVS1 + 1 G to A GJB2 mutation in Czech