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Frequencies of two common mutations (c.35delG and c.167delT) of the connexin 26 gene in different populations of Hungary

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Abstract. The most common form of non-syndromic autosomal recessive deafness (NSRD) is caused by mutations in the gene GJB2, encoding the protein connexin 26 (Cx26). The mutation c.35delG is found in 30-70% of Caucasian NSRD cases, and is abundant (allele frequency of 0.5-2%) in several European populations, while c.167delT is found in the Ashkenazi Jewish population with about 2% frequency. In the current study, using simple PCR-based tests we established an allele frequency of 0.6% in the Hungarian average, and 0.4% in the Romani (Gypsy) populations for the c.35delG mutation, and an allele frequency of 2.4% in the Ashkenazi population for the c.167delT mutation. Our results do not differ significantly from the published data for Caucasian and non-European Ashkenazi populations and they present figures for the Romani population for the first time. Both mutations may be significant causative factors among the NSRD cases of the respective populations in Central Europe.

Introduction

Congenital deafness occurs in about 1 in 1000 live births, of which about 50% has a hereditary cause. Approximately 80% of hereditary deafness cases are non-syndromic with autosomal recessive inheritance. Previous studies identified at least 30 human, non-syndromic recessive deafness (NSRD, OMIM#220290) loci (DFNB1-30) (1,2). Despite the extreme genetic heterogeneity, a large proportion of NSRD families was linked to DFNB1 on chromosome 13q11, whereas other loci were only found in isolated families. Mutations in gene GJB2, encoding the gap-junction protein connexin 26 (Cx26, OMIM ref. number:121011), were shown to be responsible for DFNB1-related deafness. A large number of studies have carried out mutation detection of the Cx26 gene among NSRD-affected populations with different geographical origins. The Cx26 mutation database (3) listed a total of 121 different mutations (102 with recessive, 8 with dominant and 11 with unknown pattern of inheritance) in May 2004. However, outstandingly prevalent point mutations have been identified in geographically different patient populations. The frequencies of these mutations have also been tested in the respective general populations. Such data are listed in Table I. The most extensively studied genetic alteration, a deletion of a guanine within a stretch of six guanine residues between nucleotide positions 30-35 of the GJB2 cDNA (c.35delG), was found in 30-70% of NSRD cases, and shown to have a high carrier frequency (1-4%) in several Caucasian populations (4-6) (Table I). Among Ashkenazi Jews of American and Israeli origin, another deletion (c.167delT) was shown to be the most common GJB2 mutation with about 50% allele frequency among NRSD cases and 4% carrier frequency in random populations (7-10). However, no data are currently available on the frequencies of the above mutations in European Ashkenazi and Romani (Gypsy) populations.

The aim of our study was to determine the frequencies of GJB2 c.35delG and c.167delT mutations in representative randomly selected, Romani and Ashkenazi populations in Hungary.

Materials and methods

The control group consisted of 163 consecutive, volunteer first time blood donors from Central (Budapest) and Southern (Szeged) parts of Hungary (87 males and 76 females, mean age: 24±8 years) (11). The Romani study group consisted of 346 unrelated, randomly selected healthy Romani individuals (172 males and 175 females, mean age: 29.2±10 years). This group contained three well defined sub-groups based on geographic location of residence in Hungary: 166 participants were from the North-East, 149 from the South and 32 from the Central region (Budapest) of Hungary. The Ashkenazi group consisted of 186 unrelated, randomly selected

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Table I. Comprehensive summary of population studies of the most frequent disease causing mutations of the Cx26 gene in geographically distinct general populations.

Mutation	Population	Number	Number of heterozygotes	Allele frequency (%)	Reference
c.235delC					
	Japanese	399	5	0.63	(22-24)
	Chinese	150	2	0.67	(18)
	Korean	100	1	0.50	(25)
Trp24Stop					
	Indian	205	5	1.22	(17)
c.167delT					
	Palestinian	400	1	0.13	(26)
	Ashkenazi (USA)	546	22	2.01	(7)
	Ashkenazi (Israeli)	467	14	1.50	(8)
	Ashkenazi (USA)	1012	40	1.98	(10)
	Ashkenazi (Israeli)	268	20	3.73	(9)
c.35delG					
	Caucasian (Austria)	1212	11	0.45	(27)
	Caucasian (Turkey)	674	12	0.89	(28)
	Caucasian (Australia)	1000	10	0.50	(29)
	Caucasian (Poland)	551	4	0.36	(6)
	Caucasian (Czech Rep.)	195	4	1.03	(6)
	Caucasian (Hungary)	52	5	4.81	(19)

Ashkenazi individuals (40 males and 146 females, mean age: 84±10 years) from central Hungary (Budapest). All of our study populations were representative of the general population of the respective ethnic group. No significant actual disease e.g. hearing impairment was known about the participants. However, no audiological tests were carried out so slight hearing alterations may have been unnoticed. Enrolment to both latter ethnic groups was based on self-identification. Blood samples were collected with written informed consent of the participants and, DNA samples were used without personal identifiers.

Genomic DNA was extracted from anticoagulated peripheral blood by standard 'salting-out' procedure. For the detection of the c.35delG mutation, PCR amplification was performed with the primer pair Cx26-EcoRII and Cx26-P1 as described using 35 cycles of 95°C for 1 min, 56°C for 1 min, and 72°C for 1 min (12). PCR products were digested with *Eco*RII under conditions recommended by the manufacturer. For the detection of the c.167delT, newly designed primer pairs Cx26f1 (5'-TTG GAA AGA TCT GGC TCA CC) and Cx26r1 (5'-CTC CCC CTT GAT GAA CTT CC) were used. PCR amplifications consisted of 35 cycles (95°C for 45 sec, 60°C for 45 sec and 72°C for 45 sec), and the PCR products were digested with *Pst*I restriction endonuclease. Digested PCR products were size-separated by electrophoresis on 3.5% agarose gels and visualised by ethidium bromide staining. The primer pair for the detection of c.167delT was not reported previously in the literature, this PCR-RLFP method was validated by direct sequencing.

Allele frequencies are presented as % ±95% confidence interval (CI). The Fisher's Exact Test was used to compare the prevalence of the tested connexin 26 mutations in the different ethnic groups.

Results

Figs. 1A and B show representative results of PCR-RFLP determinations of the c.35delG and c.167delT mutations. The genotypes of the identified c.167delT hetero- and homozygous, and some of the wild-type individuals were confirmed by direct sequencing giving identical results in all cases (not shown).

We screened 163 Hungarian first time, randomly selected, healthy blood donors as an average Hungarian population, 346 healthy Romani and 186 Ashkenazi unrelated individuals to determine the carrier frequency of the mutation c.35delG in Hungary. As shown in Table II, we found 2 heterozygous individuals among the blood donors, giving an allele frequency of 0.6% (CI 0-1.5%) for the control Hungarian population. We identified 3 heterozygous individuals in the Romani population, giving an allele frequency of 0.4% (CI 0-0.9%) in this group. No carrier was found for the c.35delG mutation in the Ashkenazi population.

To determine the c.167delT mutation carrier frequency, we screened all three above mentioned groups. In the Ashkenazi group, 7 heterozygous and 1 homozygous individuals were identified, which gives an allele frequency of 2.4% (CI 0.8-4.0%), whereas no c.167delT carrier was

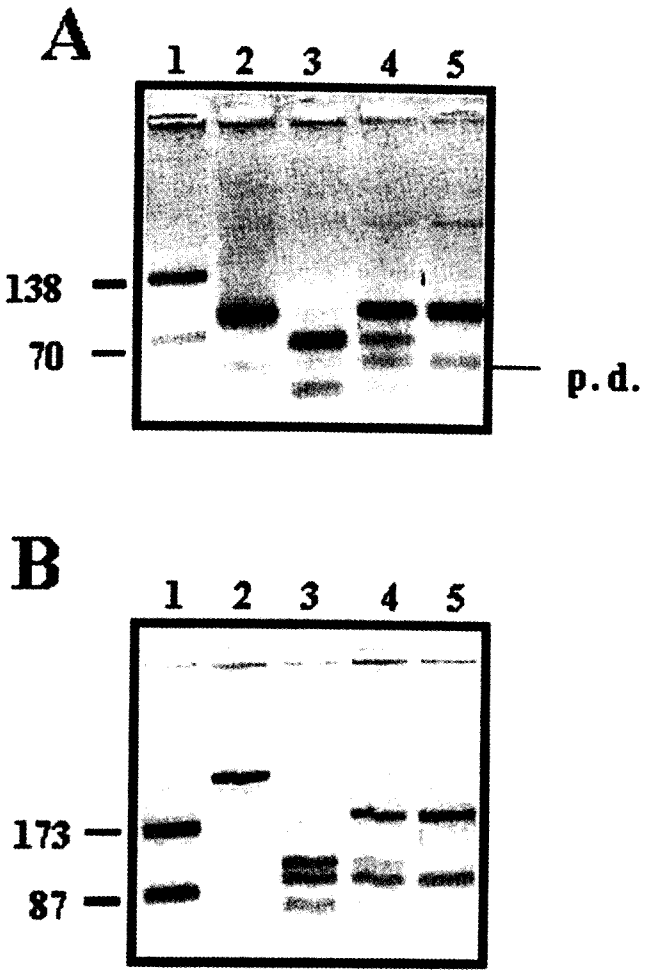

Figure 1. Detection of mutations c.35delG and c.167delT of the connexin 26 gene by simple PCR-RFLP techniques. To test for c.35delG (A) and for c.167delT (B) point mutations, simple PCR-RFLP determinations were carried out as described in the Materials and methods section. Samples with identical genotypes for the respective mutation were analysed in the same order in both determinations. Lanes 1, Size markers, exact sizes are given on the left. The exact fragment sizes [in basepairs (bp)] of the samples are as follows: Lanes 2, undigested PCR products 89 (panel A) and 272 (panel B); lanes 3, wild genotypes 60/29 (panel A) and 112/91/69 (panel B); lanes 4, heterozygous genotypes 89/60/29 (panel A) and 181/112/91/69 (panel B); lanes 5, homozygous genotypes 89 (panel A) and 181/91 (panel B). p.d., primer dimer.

detected in the blood donor group and the Romani population (Table II.). The difference between the Ashkenazi and the control group was statistically significant ($p<0.05$) as was the difference between the c.35delG and c.167delT frequencies in the Ashkenazi group.

Discussion

By May 2004, 121 different mutations of GJB2 had already been identified (3), but so far only four genetic alterations have high prevalence in different general ethnic groups (c.35delG in the Caucasian, c.167delT in the Ashkenazi c235delC and Trp24Stop (W24X) in the Oriental populations see Table I). The stretch of six consecutive guanines between position 30-35 in GJB2 was suggested to represent a mutational 'hot spot' causing the high prevalence of c.35delG (13,14). The fact that no conservative haplotype was observed in the flanking regions of c.35delG chromosomes further supported this hypothesis (7). However later haplotype studies using flanking markers rather support several independent founder effects affecting the same gene (15,16). A large scale screening for the c.35delG mutation (among 3270 random controls from 17 European countries) detected a declining carrier frequency of c.35delG from South to North in Europe and a carrier frequency of 1 in 35 was reported for southern and of 1 in 79 for Central and Northern Europe (5,6). This observation and the absence of c.35delG in non-European populations also suggested a single origin for c.35delG somewhere in Europe or in the Middle East. In the case of the c.167delT, the conservation of haplotypes flanking the mutation clearly suggests a single origin of this mutation (founder effect) (7) and similar conclusions were made in population studies of the two frequent oriental mutations (17,18).

The c.35delG allele frequency of 0.6% (approximately 1/80 heterozygous ratio), found in the average Hungarian population of the current study is similar to that reported for other Central European countries. However, Toth *et al* (19) reported an extremely high carrier frequency of the c.30delG (indistinguishable from c.35delG) mutation in a relatively

Table II. Genotyping results, allele frequencies and 95% confidence intervals for the c.35delG and c.167 delT mutation screening in three different Hungarian populations.

		c.35delG			c.167delT		
Population	Number of individuals tested	Number of heterozygous individuals	Number of homozygous individuals	Allele frequency (95% CI)	Number of heterozygous individuals	Number of homozygous individuals	Allele frequency (95% CI)
Hungarian	163	2	0	0.6 (0-1.5)	0	0	0
Romani (Gypsy)	346	3	0	0.4 (0-0.9)	0	0	0
Ashkenazi	186	0	0	0	7	1	2.4 (0.8-4.0)

CI, 95% confidence interval.

small North-Eastern Hungarian control population. In our Ashkenazi study group no c.35delG carrier was found.

The prevalence of the c.35delG mutation in the Romani (Roma) population was previously not reported. Population genetic studies have generally concluded that Roma are genetically distinct from other European populations, and believed to have a common Asian origin. Roma migrated out of India and arrived in the Byzantine Empire in the 10-11th century. Their early migration from the Balkans into Central and Western Europe was completed by the 15th century (20). We detected an allele frequency of 0.6% (or 1/80 heterozygous ratio) for the c.35delG mutation in the healthy Romani population in Hungary, which is similar to data of our control population. The frequency of c.35delG in Asian populations is extremely low (15,18,21). Based on examples of other populations (e.g. low but detectable presence of c35delG in Ashkenazi and Oriental populations) it is likely that, the similar c.35delG prevalence in the Romani and the Hungarian populations is caused by population admixture (genetic influx during the migration or after the settlement)

We detected the c.167delT mutation exclusively in the Ashkenazi population with similar allele frequency as reported in the literature, while this mutation was absent in the Hungarian control and Romani populations.

Although non-syndromic deafness usually occurs sporadically, a genetic cause is responsible in up to 60% of cases. Clinical and audiological features do not usually allow reliable discrimination between genetic and acquired causes. Introducing the testing of the two most common European GJB2 mutations in Hungary could facilitate diagnosis of congenital deafness, genetic counselling of the affected families and allow early treatment for individuals with hearing impairment. Our novel data for these three distinct Central European populations substantially extend the earlier population genetic observations and further support the importance of genetic testing in affected individuals with hearing impairment.

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References

1. Keats BJB and Berlin CI: Genomics and hearing impairment. Genome Res 9: 7-16, 1999.
2. Usami S, Koda E, Tsukamoto K, *et al*: Molecular diagnosis of deafness: impact of gene identification. Audiol Neurootol 7: 185-190, 2002.
3. Calvo J, Rabionet R, Gasparini P, *et al*: Connexins and deafness Homepage. World wide web URL: http://www.crg.es/deafness.
4. Green GE, Scott DA, McDonald JM, *et al*: Carrier rates in the Midwestern United States for GJB2 mutations causing inherited deafness. J Am Med Association 281: 2211-2216, 1999.
5. Gasparini P, Rabionet R, Barbujani G, *et al*: High carrier frequency of the c.35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 c.35delG. Eur J Hum Genet 8: 19-23, 2000.
6. Lucotte G and Mercier G: Meta-analysis of GJB2 mutation c.35delG frequencies in Europe. Genet Test 5: 149-152, 2001.
7. Morell JR, Kim HJ, Hood LJ, *et al*: Mutations in the connexin 26 gene (GJB2) among Ashkenazi Jews with non syndromic recessive deafness. N Eng J Med 339: 1500-1505, 1998.
8. Sobe T, Erlich P, Berry A, *et al*: High frequency of the deafness-associated 167delT mutation in the connexin 26 (GJB2) gene in Israeli Ashkenazim. Am J Med Genet 86: 499-500, 1999.
9. Lerer I, Sagi M, Malamud E, *et al*: Contribution of connexin 26 mutations to non syndromic deafness in Ashkenazi patients and the variable phenotypic effect of the mutation c.167delT. Am J Med Genet 95: 53-56, 2000.
10. Dong J, Katz DR, Eng CM, *et al*: Nonradioactive detection of the common Connexin 26 167delT and 35delG mutations and frequencies among Ashkenazi Jews. Mol Genet Metab 73: 160-163, 2001.
11. Andrikovics H, Kalmár L, Bors A, *et al*: Genotype screening for hereditary hemochromatosis among voluntary blood donors in Hungary. Blood Cells Mol Dis 27: 334-341, 2001.
12. Wilcox SA, Osborn AH and Dahl HM: A simple PCR test to detect the common c.35delG mutation in the connexin 26 gene. Mol Diagn 5: 75-78, 2000.
13. Denoyelle F, Weil D, Maw MA, *et al*: Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. Hum Mol Genet 12: 2173-2177, 1997.
14. Kelley PM, Harris DJ, Comer BC, *et al*: Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet 62: 792-799, 1998.
15. Ohtsuka A, Yuge I, Kimura S, *et al*: GJB2 deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet 112: 329-333, 2003.
16. Van Laer L, Coucke P, Mueller RF, *et al*: A common founder for the 35delG GJB2 gene mutation in connexin 26 hearing impairment. J Med Genet 38: 515-518, 2001.
17. RamShankar M, Girirajan S, Dagan O, *et al*: Contribution of connexin26 (GJB2) mutations and founder effect to non-syndromic hearing loss in India. J Med Genet 40: e68, 2003.
18. Liu XZ, Xia XJ, Ke XM, *et al*: The prevalence of connexin 26 (GJB2) mutations in the Chinese population. Hum Genet 111: 394-397, 2002.
19. Toth T, Kupka S, Esmer H, *et al*: Frequency of the recessive 30delG mutation in the GJB2 gene in Northeast-Hungarian individuals and patients with hearing impairment. Int J Mol Med 8: 189-192, 2001.
20. Gresham D, Morar B, Underhill PA, *et al*: Origins and divergence of the Roma (Gypsies). Am J Hum Genet 69: 1314-1331, 2001.
21. Wang YC, Kung CY, Su MC, *et al*: Mutations of Cx26 gene (GJB2) for prelingual deafness in Taiwan. Eur J Hum Genet 10: 495-498, 2002.
22. Fuse Y, Doi K, Hasegawa T, *et al*: Three novel connexin26 gene mutations in autosomal recessive non-syndromic deafness. Neuro Report 23: 1853-1857, 1999.
23. Abe S, Usami S, Shinkawa H, *et al*: Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet Genet 37: 41-43, 2000.
24. Kudo T, Ikeda K, Kure S, *et al*: Novel mutations in the Connexin 26 gene (GJB2) responsible for childhood deafness in the Japanese population. Am J Med Genet 90: 141-145, 2000.
25. Park HJ, Hahn SH, Chun YM, *et al*: Connexin26 mutations associated with nonsyndromic hearing loss. Laryngoscope 110: 1535-1538, 2000.
26. Shahin H, Walsh T, Sobe T, *et al*: Genetics of congenital deafness in the Palestinian population: multiple connexin 26 alleles with shared origins in the Middle East. Hum Genet 110: 284-289, 2002.
27. Janecke AR, Nekahm D, Loffler J, *et al*: *De novo* mutation of the connexin 26 gene associated with dominant non-syndromic sensorineural hearing loss. Hum Genet 108: 269-270, 2001.
28. Tekin M, Akar N, Cin S, *et al*: Connexin 26 (GJB2) mutations in the Turkish population: implications for the origin and high frequency of the 35delG mutation in Caucasians. Hum Genet 108: 385-389, 2001.
29. Dahl HH, Saunders K, Kelly TM, *et al*: Prevalence and nature of connexin 26 mutations in children with non-syndromic deafness. Med J Aust 175: 191-194, 2001.