Short Report

High frequency of autosomal-recessive DFNB59 hearing loss in an isolated Arab population in Israel


High frequency of autosomal-recessive DFNB59 hearing loss in an isolated Arab population in Israel.

Autosomal-recessive non-syndromic hearing impairment (DFNB) is usually of prelingual onset with a moderate to profound degree of hearing loss. More than 70 DFNB loci have been mapped and ∼40 causative genes have been identified. Non-syndromic hearing impairment caused by mutations of DFNB59 (encoding pejvakin) has been described in a couple of families in which affected individuals presented with either auditory neuropathy or hearing loss of cochlear origin. We have identified and clinically evaluated three consanguineous families of Israeli Arab origin with prelingual non-syndromic hearing impairment and absent otoacoustic emissions in a total of eight affected individuals. All the families originate from the same village and bear the same family name. We have identified a c.406C>T (p.R136X) nonsense mutation in the DFNB59 gene in affected individuals from these families. Among the inhabitants of the village, we found an exceptionally high carrier frequency of ∼1 in 12 individuals (7/85; 8.2%). The high prevalence of hearing impairment can be explained by a founder effect and the high consanguinity rate among the inhabitants of this village.

Conflict of interest

The authors have no conflicts of interest to disclose.

With an estimated prevalence of 1 in 1000 newborns, bilateral sensorineural hearing impairment is a frequent neurosensory disorder. Hearing loss may be conductive, sensorineural, or mixed; syndromic or non-syndromic (isolated); and of pre- or post-lingual onset. More than 50% of prelingual hearing impairment is non-syndromic and of genetic origin, mostly with autosomal-recessive inheritance. For non-syndromic autosomal-recessive hearing loss (termed DFNB), the hearing impairment is usually bilateral, has prelingual onset, involves all frequencies and is of moderate to profound severity (1). Mutations in ∼40 genes are known to cause autosomal-recessive hearing impairment (2).

Autosomal-recessive diseases are common in the Arab population of Israel, mostly as a consequence of the high rate of consanguinity. Among Israeli Arabs (who represented 16.8% of the Israeli population in 2008 (3)), up to 44% of the marriages used to be between relatives, approximately half of which were between first cousins,
Borck et al.

although these numbers are somewhat lower today (4–6). In these communities some otherwise rare autosomal-recessive diseases are relatively frequent and can be limited to one extended kindred, one village, one region or one religious community (3, 7). Identifying the molecular basis of severe genetic conditions in genetically isolated populations may allow for improved genetic counseling and for genetic screening (8–10). In the case of hearing impairment, establishing a molecular genetic diagnosis of a non-syndromic form can help to avoid expensive and sometimes invasive clinical investigations that might be necessary during clinical work-up in suspected or confirmed syndromic forms (11). Early molecular diagnosis in at-risk newborns can also help to start hearing rehabilitation within the first months of life.

Non-syndromic hearing impairment caused by \textit{DFNB59} mutations (OMIM 610220; http://www.ncbi.nlm.nih.gov/omim) has been described in a couple of consanguineous families with auditory neuropathy or hearing impairment of cochlear origin (12–17). In this study, we report on an unusually high frequency of the p.R136X nonsense mutation in the \textit{DFNB59} gene among the inhabitants of an Israeli Arab village.

Materials and methods

Homozygosity mapping and haplotype analysis

The study was performed as a part of clinical genetic testing for families with hearing impairment originating from a Muslim Israeli Arab village of more than 10,000 inhabitants, who belong to 20 clans of various ethnic and geographical origins. Written informed consent was obtained from all subjects and the study was performed following an ethics committee approval for studies on the molecular genetics of hearing impairment.

Four hundred microsatellite markers, spaced at \(\sim 10\) cM intervals, from the ABI PRISM LINKAGE-MAPPING SET version 2.5 (Applied Biosystems, Foster City, CA) were amplified by multiplex polymerase chain reaction (PCR). Amplified markers were electrophoresed on an ABI 3700 DNA capillary sequencer and analyzed with GENESCAN and GENOTYPER software (Applied Biosystems). Primer sequences and physical positions of additional microsatellite markers used for fine-mapping and haplotype analysis were obtained from the UCSC Genome Bioinformatics database.

Mutation analysis

For sequence analysis of the \textit{DFNB59} gene, we designed intronic primers to PCR amplify the six coding exons (GenBank transcript NM_001042 702.3) and respective exon–intron boundaries. Primer sequences are available on request. PCR products were sequenced on an ABI 3730 DNA Analyzer using BigDye chemistry v3.1 (Applied Biosystems).

Evaluation of the mutation frequency

We determined the carrier frequency of the \textit{DFNB59} c.406C>T mutation among 85 randomly selected inhabitants of the village using a customized Taqman genotyping assay (Applied Biosystems) using the PCR primers F: 5'-ACCAA ACATGAAGTGAAGTATCAACA-3' and R: 5'-TGTAATGGAAGTTATACCTAAAGTATTTAA TGAAAAACTGA-3' followed by detection of the reference allele with the reporter sequence 5'-ACTGACCGTGTAAT-3' and the mutant allele with the reporter sequence 5'-ATTATACTGACCACTGTAAT-3' (where the underlined nucleotides correspond to the wildtype and mutant allele of the c.406C>T mutation, respectively). This control group consisted of anonymous individuals who had undergone genetic carrier screening for other diseases in the village as part of a routine preventive genetic screening program (8, 9).

Results

Clinical evaluation

We clinically evaluated three families segregating autosomal-recessive hearing impairment comprising eight affected individuals (families A–C, Fig. 1). Although the parents in each family were not aware of a common ancestry, all three families originate from the same Israeli Arab village, belong to the same clan and bear the same family name, making a distant relationship possible. Affected individuals had bilateral sensorineural hearing impairment of prelingual onset. The degree of hearing impairment was moderate to severe with no evidence for progression. No subjective vestibular symptoms were reported, and on clinical examination there were no additional clinical signs or symptoms. In two individuals tested at the ages of 4 and 9 months, otoacoustic emissions (OAEs) were absent.

Homozygosity mapping and identification of a \textit{DFNB59} nonsense mutation

A genome-wide linkage analysis with homozygosity mapping followed by fine-mapping located the candidate region to the chromosomal region 272.
High frequency of autosomal-recessive DFNB59 hearing loss

Fig. 1. Haplotypes of polymorphic markers on chromosome 2 studied in three Israeli Arab sibships with autosomal-recessive hearing impairment. The boxed regions indicate the homozygous regions in each affected individual. The DFNB59 gene is located at 179.3 Mb between D2S2173 and D2S324. DFNB, autosomal-recessive non-syndromic hearing loss.

2q31.1-q31.3 between the flanking markers D2S2314 and D2S2978 (Fig. 1), defining a critical region of 4.25 Mb. This region includes the DFNB59 gene. Because all affected individuals from the three families were homozygous for the same allele at four consecutive microsatellite markers (D2S138 to D2S324; Fig. 1), we expected them to be homozygous for the same mutation inherited from a common ancestor. Sequencing of the DFNB59 coding exons and splice sites indeed revealed a homozygous c.406C>T (p.R136X) nonsense mutation in all affected individuals, whereas all tested parents were heterozygous mutation carriers. This mutation has previously been identified in a distinct Palestinian family segregating autosomal-recessive non-syndromic hearing impairment (17).

Mutation frequency and haplotype analysis

Among the 85 anonymous individuals born in the village whom we screened, we detected 7 carriers of the c.406C>T (p.R136X) mutation (8.2%; ∼1 in 12). We next asked whether the occurrence of this mutation in the village and in the previously reported family originating from the West Bank (17) was due to a founder effect. Genotyping of six microsatellite markers covering 1.6 Mb at the DFNB59 locus showed that the mutation-bearing haplotype is different on the proximal side of the mutation between the Israeli Arab families and the Palestinian family living in the West Bank, whereas the genotypes at three markers located distally to the mutation are identical (Table 1).

Discussion

We have identified a causative DFNB59 nonsense mutation in hearing-impaired individuals from an Israeli Arab village. DFNB59 encodes pejvakin, a member of the gasdermin family expressed in inner and outer hair cells and a subset of supporting cells as well as in the spiral ganglion and in structures of the afferent auditory pathway (12, 16).

Auditory neuropathy is a sensorineural disorder characterized by normal OAEs but absent or abnormal auditory brainstem responses and/or hearing impairment as assessed by a pure-tone audiogram (18). Normal OAEs in auditory neuropathy reflect a preserved function of outer hair cells. Hearing loss and/or poor speech-perception
Table 1. Microsatellite haplotypes at the DFNB59 locus of affected members of the Israeli Arab and West Bank families with autosomal-recessive hearing impairment due to a p.R136X mutation

<table>
<thead>
<tr>
<th>Microsatellite marker/gene</th>
<th>Physical position in Mb (UCSC hg19)</th>
<th>Allele 1 affected individuals</th>
<th>Allele 2 affected individuals</th>
<th>Allele 1 affected individuals</th>
<th>Allele 2 affected individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S148</td>
<td>178.2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MS1-22CA</td>
<td>178.8</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MS2-20CA</td>
<td>178.9</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MS3-19GT</td>
<td>179.3</td>
<td>1a</td>
<td>1a</td>
<td>1a</td>
<td>1a</td>
</tr>
<tr>
<td>D2S324</td>
<td>179.6</td>
<td>2a</td>
<td>2a</td>
<td>2a</td>
<td>2a</td>
</tr>
<tr>
<td>MS4-21CA</td>
<td>179.8</td>
<td>2a</td>
<td>2a</td>
<td>2a</td>
<td>2a</td>
</tr>
</tbody>
</table>

DFNB, autosomal-recessive non-syndromic hearing loss.

*Genotypes that are homozygous for the same allele in affected individuals from both families.

Table 2. Reported DFNB59 mutations and associated phenotypes

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Ethnic origin of hearing-impaired individuals</th>
<th>Hearing impairment phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous c.547C&gt;T; p.R183W</td>
<td>Iranian</td>
<td>Non-syndromic, prelingual sensorineural hearing impairment</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Auditory neuropathy</td>
<td></td>
</tr>
<tr>
<td>Homozygous c.161C&gt;T; p.T54I</td>
<td>Turkish</td>
<td>Congenital severe to profound hearing impairment with absent ABR</td>
<td>(13)</td>
</tr>
<tr>
<td>Homozygous c.113dupT; p.Lys41GlufsX8</td>
<td>Moroccan</td>
<td>Early childhood onset slowly progressive hearing loss; OAE and ABR tests both abnormal, impaired central vestibular function</td>
<td>(14)</td>
</tr>
<tr>
<td>Homozygous c.499C&gt;T; p.R167X</td>
<td>Turkish</td>
<td>Severe to profound sensorineural hearing impairment, OAE and ABR absent, no vestibular dysfunction</td>
<td>(13)</td>
</tr>
<tr>
<td>Homozygous c.726delT; p.F242LfsX7</td>
<td>Iranian</td>
<td>Non-syndromic sensorineural hearing impairment</td>
<td>(15)</td>
</tr>
<tr>
<td>Homozygous c.988delG; p.V330LfsX7</td>
<td>Iranian</td>
<td>Absent ABR and OAE, suggesting hearing loss of cochlear origin</td>
<td>(15)</td>
</tr>
<tr>
<td>Homozygous c.122delA; p.K41SfsX18</td>
<td>Iranian</td>
<td>Non-syndromic moderate to profound progressive hearing impairment</td>
<td>(16)</td>
</tr>
<tr>
<td>Homozygous c.406C&gt;T; p.R136X</td>
<td>Palestinian</td>
<td>Sensorineural hearing impairment</td>
<td>(17)</td>
</tr>
<tr>
<td>Homozygous c.406C&gt;T; p.R136X</td>
<td>Israeli Arab</td>
<td>Absent ABR and OAE, suggesting hearing loss of cochlear origin</td>
<td>This study</td>
</tr>
</tbody>
</table>

ABR, auditory brainstem responses; DFNB, autosomal-recessive non-syndromic hearing loss; OAE, otoacoustic emissions.
Hearing impairment is among the few genetic disorders listed in the Israeli National Genetic Database (http://goldenhelix.org/server/israeli/) that have a relatively high prevalence in all three major communities of the non-Jewish population, i.e. Muslim Arabs, Christian Arabs and Druze (3). The relatively high frequency of hearing impairment in the village (this report) has been known for many years, although the exact prevalence has not been established. Screening of DNA from randomly selected inhabitants revealed a p.R136X carrier frequency of 8.2% among individuals born in the village, consistent with the high frequency of hearing impairment. This high carrier frequency is similar to the carrier frequencies for mutations in SMN1 (causing spinal muscular atrophy), IGHMBP2 (causing spinal muscular atrophy with respiratory distress 1) and CC2D1A (causing non-syndromic mental retardation), which are 13.1%, 9.9% and 8.9%, respectively, in the same village (8, 9, 19). In this village, 28.6% of all marriages are consanguineous and 16.5% of the total are between first cousins (9). Among the village founders were families whose ancestors immigrated from Sudan and descendants of families who came to Palestine from Egypt along with Muhammad Ali’s troops in 1834. The DFNB59 mutation carriers belong almost exclusively to two extended families whose ancestors migrated from Sudan to Israel in the 19th century. This means that the mutation must have occurred in a common ancestor of these two families before they settled in the village, which was over 150 years ago. Interestingly, recent studies using exome sequencing have estimated that any given individual is heterozygous for >20 nonsense, canonical splice site and frameshift mutations as well as for many more presumably deleterious missense variants (20). This is expected to also apply to the village founders. While some of these mutations have probably been lost in subsequent generations or do not cause an obvious phenotype even in the case of homozygosity, other alleles will remain in the population. It may be inferred that the basis of the high prevalence of hearing impairment are mainly a founder effect and the high rate of intermarriage among the descendents of these two families. An additional reason for the high prevalence of hearing loss is that these same two extended families have a large number of intellectually disabled individuals (9), and therefore members of other families are unwilling to marry into these two families. As a result, the members of these families frequently marry among themselves. Of note, the observed carrier frequency for the p.R136X mutation is higher than carrier frequencies for the GJB2 mutations c.35delG (7.8%), p.V37I (4.8%) and p.W77R (2.4%) that cause autosomal-recessive hearing impairment in a Muslim Arab village in the Galilee (21).

The p.R136X mutation was previously identified in an unrelated family segregating autosomal-recessive hearing impairment that originated from the West Bank (17). Our haplotype analyses failed to clearly show whether the occurrence of the mutation at two distinct geographical places was the result of an old founder effect or of recurrence of the mutation affecting a hypermutable CpG dinucleotide. The high carrier frequency would be in agreement with an old founder effect, consistent with data obtained from the study of 12 mutations in five genes present in a distinct Muslim Arab village in Israel; in such villages, frequent mutations are old because they have been introduced soon after the foundation of the village (22). The DFNB59 p.R136X has not been identified outside Israel and the West Bank. This is consistent with observations regarding the majority of autosomal-recessive diseases present in non-Jewish populations of Israel in which a single homozygous mutation accounts for all individuals affected by a given disorder. In the case of Muslim Arabs in Israel, of the 195 autosomal-recessive disorders listed in the Israeli National Genetic Database in 2010, 121 (62%) were due to a single homozygous mutation (3). Of note, however, even in this rather isolated population hearing loss shows a considerable genetic and allelic heterogeneity (17).

In summary, our study shows that a specific DFNB59 nonsense mutation causes hearing impairment in an Israeli Arab village. This finding has implications for genetic counseling in this village—in which there is a high prevalence of at least four autosomal-recessive neurological and neurosensory conditions—for early diagnosis of hearing impairment aiming at early intervention for hearing rehabilitation, and for the study of autosomal-recessive conditions in isolated populations in general.

Acknowledgements

We are grateful to the families who participated in this study. G. B. was supported by the Deutsche Forschungsgemeinschaft (DFG; BO2985/3-1).

References

Borck et al.