Connexin26 deafness in several interconnected families


Abstract

Mutations in the connexin26 gene are the basis of much autosomal recessive sensorineural deafness. There is a high frequency of mutant alleles, largely accounted for by one common mutation, 35delG. We have studied a group of families, who had been brought together through marriages between Deaf persons, in which there are more than 30 Deaf people in four generations. We show that many of the several cases of deafness are the result of 35delG homozygosity or 35delG/Q57X compound heterozygosity at the connexin26 locus. A considerable range of audiographic phenotypes was observed. The combined effects of a high population frequency of mutant alleles, and of positive assortative marriage among the Deaf, led to an infrequently observed recessive pedigree pattern.

Keywords: genetic deafness; connexin26; DFNB1

Mutation in the connexin26 (CX26) gene is, in some populations at least, the basis of around half of all recessively inherited prelingual sensorineural deafness. One common allele, designated 35delG, accounts for three quarters or more of all CX26 mutations: the deletion of one guanine (G) in a sequence of six Gs at nucleotides 30-35. The heterozygosity rate for a mutant CX26 allele in a general population can be estimated from generally accepted figures for the frequency of congenital deafness (1/1000), the proportion of all congenital deafness which is supposed to be genetic (two thirds), the fraction of this which is autosomal recessive (about 80%), and assuming approximately half of this recessive deafness to be the result of CX26. From these figures, CX26 recessive deafness occurs in 1 in 3750 children and a heterozygote frequency of 3.3% (1 in 30) is calculated. For the 35delG mutation, the calculation comes to 1 in 40. In a direct observation of 280 hearing subjects from general populations in Italy and Spain, 1 in 31 were 35delG heterozygotes (Italian 1/25, Spanish, 1/43). The important contribution to deafness of CX26 is illustrated in a study we report here of a group of several interconnected families, in which, owing to the marriage of Deaf persons, it is apparent that...
several different entry points of CX26 mutation have come together.

Subjects and methods

KINDRED REPORT

The kindred, of New Zealand residence, had originally come to the attention of one of us (RJMG) when a member presented for genetic counselling. The pedigree is illustrated in fig 1. Most family members with a severe-profound loss communicate by sign language. Altogether, there have been nine marriages between Deaf persons. Information on hearing status was obtained from family members and from audiology clinic records. In the majority (III.3-8 and descendants), the deafness was regarded as having been congenital and non-progressive. An audiogram in II.5 is reported to have had a “flat” pattern with a 50-60 decibel loss. In III.2 and descendants, a progressive hearing loss was reported. We have audiological documentation to confirm this for III.2, V.7, and V.8, but only anecdotal evidence (that is, information from other family members) that V.1-5 and V.10 also have a progressive loss. II.1, IV.4, and IV.12 are considered to have an acquired deafness. IV.2 has a sib with a severe loss, as does she herself, and a nephew of hers has a moderate hearing loss; these people have not been studied.

MOLECULAR ANALYSIS

Blood samples were obtained from 21 family members and the DNA was prepared as previously described. The conditions used for amplification were as follows: 95°C for 45 seconds, 62°C for 45 seconds, and 72°C for 45 seconds, for 35 cycles using TaqI enzyme (Boehringer Mannheim) on a Corbett 960 thermocycler (Sydney). The 35delG mutation was screened by sequencing the 5' region of the 681 bp product using primer CX26P1 with the Ther-mosequenase kit (Amersham) and separated on an 8% acrylamide gel. The observation of a 35delG mutation was confirmed by sequencing the reverse strand using primer CX26P15 on the original amplified product (CX26P15: 5'-AGGACGGTGAGCCAGATCT-3').

Mutations other than 35delG were searched for in III.2, IV.5, 6, 12, 14, 15, V.7, 8, 18, 19, and 20 by sequencing the whole CX26 sequence using primers CX26P1, CX26P5, and CX26P6 (CX26P6: 5'-GCAGACATCTTCTCCGGGTCA-3'). In some subjects, this mutation screen detected a C→T change at nt169, which is predicted to cause a premature termination (Q57X). The presence of the Q57X mutation was confirmed by amplifying the product with CX26P1 and CX26P3 (CX26P3: 5'-GACACGAGATCAGCTGAG-3'), producing a 314 bp product, which was then digested with TaqI (Boehringer Mannheim). A normal allele produces three TaqI fragments of sizes 48 bp, 140 bp, and 126 bp, whereas the Q57X allele produces two TaqI fragments of 48 bp and 266 bp.

Results

Fourteen Deaf and seven hearing subjects from generations III-V were tested for CX26 gene mutations. Among the Deaf persons, there were five 35delG homozygotes (IV.10, 11, 13, V.13, 21) and four 35delG/Q57X compound heterozygotes (IV.14, V.18, 19, 20). Their hearing losses were mostly, but by no means universally, of severe to profound degree, and some showed considerable asymmetry between ears (fig 2). Of four subjects with a progressive

![Figure 2](http://jmg.bmj.com/) Audiograms of eight family members, CX26 homozygotes or compound heterozygotes, for whom this documentation was available. Hearing threshold in decibels on x axis, frequency in Hertz on y axis. Audiograms grouped according to CX26 genotype: 35delG/35delG homozygotes (above) and 35delG/Q57X compound heterozygotes (below).
hearing loss, two tested as 35delG heterozygotes (III.2, IV.5) and in two no CX26 mutation was found (V.7, 8). One hearing parent of Deaf children (III.6) and four hearing children of Deaf parents (V.14, 15, 16, 17), tested 35delG/normal. One hearing brother of Deaf sibs (IV.15) tested Q57X/normal. These subjects were assumed to be unaffected carriers. One hearing parent of Deaf children (IV.6) and one “marrying in” Deaf spouse with acquired deafness (IV.12) tested homozygous normal.

**Discussion**

There is considerable genetic heterogeneity in inherited deafness, and to date over 30 loci and eight genes have been identified. Much the predominant contributor, at least in European/Mediterranean populations, is recessive deafness resulting from mutation in the connexin26 gene (the locus also known as DFNB1), and so the observation of a number of unrelated families with CX26 related deafness is, in any such population, to be expected. This, along with the marked preference of Deaf persons for a Deaf spouse, endows considerable potential for marriages between Deaf persons in both of whom the basis of the deafness would reside in a CX26 genotype. Deafness could come to be transmitted vertically in these families, and the pedigree may not at all resemble the horizontal pattern that typically characterises non-consanguineous recessive inheritance.

We presume this to be the explanation, in part at least, for the pedigree pattern recorded here. For the right hand part of the pedigree (III.3-8 and descendants) there are five Deaf-Deaf unions. One marrying in partner was diagnosed deaf because of childhood meningitis. Thus, the marriages in this part of the pedigree include one CX26-nonCX26 union and four CX26-CX26 unions. Consistent with this interpretation, the children born of these unions have all been deaf or have all been hearing (that is, segregation ratios of 1.0). While the hearing loss in most is of a severe to profound degree, examples of moderate loss are also noted, as are examples of considerable asymmetry between the ears (fig 2).

We lack a definitive genetic interpretation for the left hand branches of the pedigree (II.1-5 and descendants). Coincidental CX26 heterozygosity is to be expected in a pedigree structure such as we present, and this may be the explanation in III.2 and IV.5. A mutation in a dominant gene (such as, for example, POU4F3) might be responsible. Nevertheless, a case for CX26 remains open. It is perplexing that, in other studies, quite a number of Deaf persons have had only one mutant CX26 allele identifiable. If this is not coincidental heterozygosity, then some other scenario must apply, such as digenic inheritance, with perhaps the involvement of another connexin locus, or there may exist other types of CX26 mutation (possibly in the promoter sequence of the gene) yet to be discovered.

With this new molecular information concerning CX26, it is now possible to provide family members in families such as this with clearer advice concerning their own and a partner’s genetic status. Not all family members may wish to exercise this choice and the Deaf community is assertive in declaring that deafness is not to be “medicalised” (a reason, we believe, for the less than complete response we have had to requests for DNA sampling in the present study). The challenge to those who provide genetic counselling in this context is to maintain sympathetic responses to sensitivities of members of the Deaf community and to the concerns of the hearing members of Deaf families.

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