Short reports

A novel C202F mutation in the connexin26 gene (*GJB2*) associated with autosomal dominant isolated hearing loss

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Abstract

Mutations in the *GJB2* gene encoding connexin26 (CX26) account for up to 50% of cases of autosomal recessive hearing loss. In contrast, only one *GJB2* mutation has been reported to date in an autosomal dominant form of isolated prelingual hearing loss. We report here a novel heterozygous 605G→T mutation in *GJB2* in all affected members of a large family with late childhood onset of autosomal dominant isolated hearing loss. The resulting C202F substitution, which lies in the fourth (M4) transmembrane domain of CX26, may impair connexin oligomerisation. Finally, our study suggests that *GJB2* should be screened for heterozygous mutations in patients with autosomal dominant isolated hearing impairment, whatever the severity of the disease.

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Keywords: C202F mutation; connexin26 gene (*GJB2*); autosomal dominant hearing loss

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Serious hearing impairment is detected in about 1/1000 children before 1 year of age. Deafness is of genetic origin in approximately half of these patients.[1 2] To date, 31 dominant loci (DFNA), 28 recessive loci (DFNB), and four X linked loci (DFN) have been shown to be associated with non-syndromic deafness.[2 3] Mutations in *GJB2* are detected in up to 50% of people with autosomal recessive non-syndromic hearing loss.[4–7] In contrast, only one *GJB2* mutation (W44C) has previously been identified in two unrelated families with an autosomal dominant form of isolated prelingual hearing impairment.[8] The M34T *GJB2* mutation, initially thought to be responsible for dominant isolated hearing loss, in fact appears to be a polymorphism.[8–10] Thus, the exact role of *GJB2* mutations in autosomal dominant hearing loss remains largely unknown.

We report here a French family with 15 affected members presenting isolated, mild to moderate, postlingual hearing impairment (fig 1). In this family, hearing loss was detected between 10 and 20 years of age in most of the affected persons. We found intrafamilial variability for the severity of hearing loss, which was restricted to high frequencies during the first decade and progressed to middle frequencies between 10 and 50 years of age.

We performed genetic linkage analysis in this family using a set of microsatellite markers linked to dominant DFNA1-15 loci and recessive DFNB1-17 loci.[11] We obtained significant positive lod scores of 3.88 at $\theta=0$ and 3.27 at $\theta=0$ with D13S175 and D13S141 microsatellite markers, respectively. Since these two markers are closely linked to *GJB2*, we screened the whole *GJB2* coding sequence for mutations in our patients. Single strand conformation polymorphism (SSCP) analysis of the *GJB2* coding sequence showed an abnormal pattern in affected subjects. Direct sequencing showed a heterozygous G→T mutation at nucleotide 605, resulting in a cysteine to phenylalanine substitution at codon 202 (C202F, fig 2A). The remaining coding sequence showed no SSCP anomaly. The 605G→T mutation resulted in the loss of an *Sfa*NI site. Restriction site analysis using *Sfa*NI showed heterozygosity for the 605G→T mutation in all affected family members (fig 2B). In contrast, this mutation was absent in healthy family members and in 95 controls.

CX26 belongs to a large family of proteins which form intercellular channels and allow rapid exchange of small molecules between adjacent cells.[12 13] CX26 is also thought to play an important part in auditory transduction, by recycling endolymphatic potassium ions. In the rat and mouse cochlea, CX26 is highly expressed in the supporting cells of the sensory epithelium and in the fibrocytes lining the cochlear duct.[2 14 15] Several lines of evidence suggest that the C202F mutation reported here may play an important role in CX26 function. Firstly, the C202F mutation affects an amino acid which is invariable among 17 vertebrate connexins.[16] This mutation lies in the fourth (M4) transmembrane domain of CX26, which seems to be important for protein folding.[17] In addition, a heterozygous mutation (C201R) affecting the CX32 analogous cysteine underlies severe X linked Charcot-Marie-Tooth disease.[18] Moreover, a truncation mutation, thought to result in the deletion of the M4

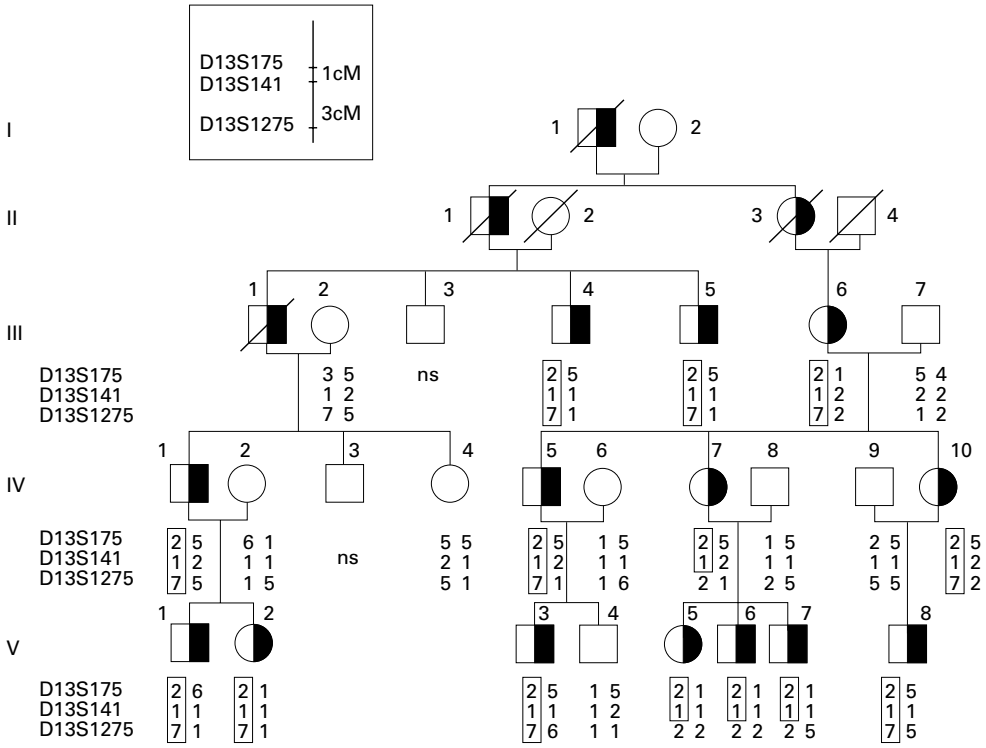

Figure 1 Pedigree of the family and haplotypes for three microsatellite markers on chromosome 13. Filled symbols represent affected subjects. ns: not studied. The haplotype segregating with the disease is boxed. Insert: genetic distances between microsatellite markers.

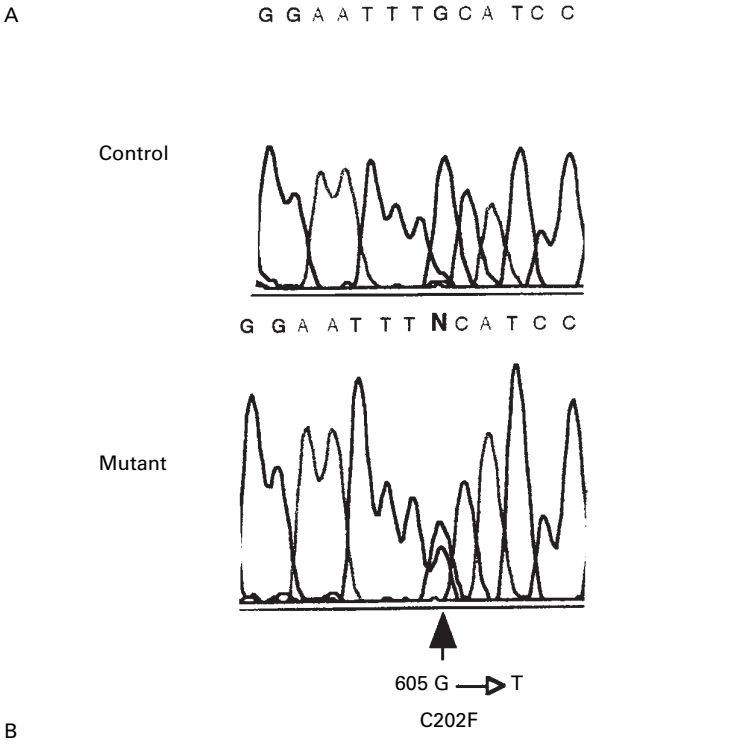

Figure 2 (A) DNA sequence showing the 605G→T GJB2 mutation in an affected subject. (B) SfaNI restriction site analysis of PCR amplified DNA from family members. The 260 bp fragment corresponds to the mutant allele. Affected members are indicated by an asterisk.

domain of CX31, is associated with dominant, late onset hearing loss.[16] As six CX26 molecules oligomerise to form a hemichannel or connexon,[17] the C202F mutation might disturb the interaction between the M4 domain of one mutant CX26 and the M2 domain of the neighbouring connexin, thus resulting in the formation of a non-functional channel.

Heterozygous *GJB2* mutations have previously been found in families with palmoplantar keratoderma and sensorineural hearing loss,[19 20] and in dominant non-syndromic deafness in only one instance.[8] This latter heterozygous *GJB2* mutation (W44C), which is associated with profound prelingual and progressive non-syndromic deafness, lies in the E1 extracellular loop of the protein involved in interactions between connexons of adjacent cells.[8] In contrast, the heterozygous C202F *GJB2* mutation reported here in late onset hearing impairment lies in the M4 transmembrane domain of the protein, thought to be important for connexin oligomerisation.

Finally, our observation suggests that screening for mutations in *GJB2*, which account for up to 50% of autosomal recessive hearing loss cases, should also be performed in autosomal dominant hearing loss cases, even in late onset forms of the disease.

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