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Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene

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Prelingual non-syndromic (isolated) deafness is the most frequent hereditary sensory defect. In >80% of the cases, the mode of transmission is autosomal recessive. To date, 14 loci have been identified for the recessive forms (DFNB loci). For two of them, DFNB1 and DFNB2, the genes responsible have been characterized; they encode connexin 26 and myosin VIIA, respectively. In order to evaluate the extent to which the *connexin 26* gene (*Cx26*) contributes to prelingual deafness, we searched for mutations in this gene in 65 affected Caucasian families originating from various countries, mainly Tunisia, France, New Zealand and the UK. Six of these families are consanguineous, and deafness was shown to be linked to the DFNB1 locus, 10 are small non consanguineous families in which the segregation of the trait has been found to be compatible with the involvement of DFNB1, and in the remaining 49 families no linkage analysis has been performed. A total of 62 mutant alleles in 39 families were identified. Therefore, mutations in *Cx26* represent a major cause of recessively inherited prelingual deafness since according to the present results they would underlie approximately half of the cases. In addition, one specific mutation, 30delG, accounts for the majority (~70%) of the *Cx26* mutant alleles. It is therefore one of the most frequent disease mutations so far identified. Several lines of evidence indicate that the high prevalence of the 30delG mutation arises from a mutation hot spot rather than from a founder effect. Genetic counselling for prelingual deafness has been so far considerably impaired by the difficulty in distinguishing genetic and non genetic deafness in families presenting with a single deaf child. Based on the results presented here, the development of a simple molecular test could be designed which should be of considerable help.

INTRODUCTION

Profound or severe prelingual deafness affects one child in a thousand in developed countries (1). It is a major impairment as

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it impedes oral language acquisition. According to studies performed in a U.S. population of children with non-syndromic (isolated) prelingual deafness and in whom an obvious environmental cause has been excluded, up to two-thirds of cases have a genetic basis (2). These forms are mainly sensorineural and are almost exclusively monogenic. The major mode of inheritance is autosomal recessive (DFNB), involving 72–85% of cases, this fraction increasing to 90% when only profound deafness is taken into account. Autosomal recessive prelingual deafness is known to be genetically highly heterogeneous. Estimates of the number of DFNB loci vary from 30 to 100 (3, for a review), of which 14 have so far been mapped to the human chromosomes (4 for review; 5,6; M. Mustapha, personal communication).

A majority of the families attending genetic counselling clinics consist of normal hearing parents with a single deaf child who wish to know the probability of recurrence of the condition. In most cases, given the important role of environmental causes of prelingual deafness, it is not possible even to recognize whether the hearing loss is of genetic origin. Genetic counselling in such families would be greatly improved by an ability to detect DFNB mutations. In this respect, the high degree of genetic heterogeneity of the condition represents a major obstacle.

After the initial identification of the DFNB1 locus on 13q11 in a large consanguineous Tunisian family (7), two studies performed on collections of New Zealand/Australian families (8) and on Italian/Spanish families (9) suggested that this locus might be a major contributor to prelingual deafness in these populations. Recently, the *Cx26* gene, which encodes a gap junction protein, connexin 26, has been shown to underlie DFNB1 deafness. Two different G→A substitutions resulting in premature stop codons in three DFNB1-linked consanguineous Pakistani families have been reported (10). This result has offered the opportunity to directly assess the contribution of DFNB1 in autosomal recessive prelingual deafness. We therefore undertook a characterisation of the spectrum and prevalence of mutations present in the *Cx26* gene in 65 Caucasian families, from several parts of the world, affected with autosomal recessive prelingual deafness.

RESULTS

Mutations in consanguineous Tunisian and Lebanese DFNB1 families

In these families the involvement of the DFNB1 locus could be demonstrated by linkage analysis. In four of the five families from Tunisia (S15, S19, 20 and 60), and in the Lebanese family (LH), the same mutation was detected in all affected children on both *Cx26* alleles, namely, a deletion of a guanosine (G) in a sequence of six G extending from position 30 to 35 (position 1 being the first base of the initiator codon) (Table 1). This mutation is hereafter referred to as 30delG mutation according to the nomenclature proposed by Beaudet and Tsui (11). It creates a frameshift which results in a premature stop codon at nucleotide position 38. The mutation segregating in the fifth family from Tunisia (ST) was identified as a G→T transversion at nucleotide position 139 creating a premature stop codon (GAG→TAG) at codon 47, and was designated E47X. In each family, normal hearing parents were found to be heterozygous for the corresponding mutation.

Mutations in small non-consanguineous New Zealand and Australian families consistent with DFNB1 linkage

In these families, segregation analysis has previously been reported as compatible with the involvement of the DFNB1 locus (8). Deaf individuals from six of the ten families [51, 1160, 1608 (III.20), 1873 (II.3), 1877 and 1879] were homozygous for the 30delG mutation. The deaf siblings from family 1773 were heterozygous for 30delG. Deaf individual II.2 from family 1873 (see 'Materials and Methods', and Table 1) was heterozygous for a deletion of 38 bp beginning at nucleotide position 31, designated 31del38. No other mutation was detected in the deaf children of family 1773 and the deaf individual (II.2) in family 1873. Nevertheless, in this individual, a deletion of the polymorphic marker immediately proximal to the *Cx26* gene (locus D13S175) had previously been observed (8) which may indicate that a DNA rearrangement has impaired the functioning of the other *Cx26* allele. In family 9670, compound heterozygosity for a missense mutation (R184P) and an in frame single-codon deletion (delE138) was observed in affected siblings (Table 1). In only one family (1548) was no *Cx26* mutation detected (not shown).

Mutations in small families uncharacterised for DFNB1 linkage, living in France, New Zealand and the UK

Nineteen families living in France (some of them having foreign ancestors, see Materials and Methods), two in New Zealand and 28 in the UK were studied. In these families, cosegregation of the deafness with polymorphic markers had not been analysed. Deaf children from six families living in France were found to be homozygous for the 30delG mutation as well as affected children from two families from the UK (Table 1). In addition, children from four families from France were found to be heterozygous for this mutation; no other mutation was detected in these families. Deaf children from one family from New Zealand and 11 families from the UK were also heterozygous for this mutation. In family 19053 from the UK, the other allele carries a 14 bp deletion (310del14). In all the other families, heterozygotes for the 30delG mutation, the presence of the second allele was detected by sequencing analysis, preceded or not by DNA subcloning of the two alleles (see Materials and Methods). In the 25 remaining families from the different populations, no mutation in the *Cx26* gene was found.

Analysis of control individuals

To exclude the possibility that the 30delG mutation was simply a common polymorphism in the population, we screened a set of control DNA samples for mutations in the coding part of *Cx26* gene. No mutation was detected from a panel of DNA from 51 unrelated parents or grand-parents of CEPH families (102 chromosomes) from various Caucasian groups and from 68 unrelated individuals living in France (136 chromosomes).

Table 1. Mutations in the *Cx26* coding exon in individuals affected with familial forms of prelingual deafness

Family (geographical origin)	30delG mutation	Other mutation	Deafness
DFNB1-linked families			
S15 (sTu)	homozygous	–	profound
S19 (sTu)	homozygous	–	profound
ST (sTu)	–	homozygous E47X	profound
20 (nTu)	homozygous	–	profound
60 (nTu)	homozygous	–	profound
LH (Leb)	homozygous	–	severe-profound
Families consistent with DFNB1 linkage			
51 (NZ)	homozygous	–	severe-profound
1160 (NZ)	homozygous	–	moderate-severe[a]
1608 individual III.20 (NZ)	homozygous	–	profound[b]
1773 (NZ)	heterozygous	–	profound
1873 individual II.3 (NZ)	homozygous	–	moderate
1873 individual II.2 (NZ)	–	heterozygous 31del38	profound
1877 (NZ)	homozygous	–	profound
1879 (NZ)	homozygous	–	severe-profound
9670 (Aus)	–	delE118/R148P	moderate-severe
Families uncharacterised for DFNB1 linkage			
P1 (Fr)	homozygous	–	severe-profound
P3 (Leb)	homozygous	–	severe-profound
P5 (Fr)	homozygous	–	profound
P6 (Fr)	heterozygous	–	severe-profound
P9 (Por)	homozygous	–	severe-profound
P10 (Fr)	homozygous	–	severe-profound
P11 (Fr)	heterozygous	–	moderate-severe
P14 (Alg)	heterozygous	–	moderate-severe
P16 (mother/Fr, father/Pol)	homozygous	–	severe[b]
P17 (Fr)	heterozygous	–	severe[c]
1885 (NZ)	heterozygous	–	profound
19002 (UK)	homozygous	–	moderate-severe[d]
19010 (UK)	heterozygous	–	profound
19011 (UK)	homozygous	–	profound
19012 (UK)	heterozygous	–	profound
19020 (UK)	heterozygous	–	moderate[e]
19025 (UK)	heterozygous	–	profound
19029 (UK)	heterozygous	–	moderate-severe[d]
19038 (UK)	heterozygous	–	profound
19039 (UK)	heterozygous	–	moderate[f]
19048 (UK)	heterozygous	–	moderate[e]
19053 (UK)	heterozygous	heterozygous 310del14	profound
19054 (UK)	heterozygous	–	profound
19073 (UK)	heterozygous	–	moderate-severe

The analysis reported here concerns deaf children of the various families except for family 1873 (see Materials and Methods).
[a]Moderate in one ear, severe in the other ear.
[b]Moderate hearing loss in mother (bordering on severe at high frequencies).
[c]Mild hearing loss in father, these parents being heterozygous carriers for the 30delG mutation.
[d]Moderate-severe in one ear, severe in the other ear.
[e]Moderate in one ear, profound in the other ear.
[f]Moderate in one ear, severe in the other ear.
Geographical origins: (Alg) Algeria, (Aus) Australia, (Fr) France, (Leb) Lebanon, (NZ) New Zealand, (Pol) Poland, (Por) Portugal, (nTu) North Tunisia, (sTu) South Tunisia.

DISCUSSION

The present data allow an estimate of the frequency of DFNB1-associated deafness among prelingual autosomal recessive deafness in three geographic populations, New Zealand/Australian, French and British. Previous indications that the DFNB1 locus might be an important contributor to recessive prelingual deafness in a New Zealand/Australian population (8) are confirmed here; we found *Cx26* mutations in 10 Anglo-Celtic families from a study group of 22 families from New Zealand/Australia (see Materials and Methods). This 10/22 fraction shown to carry a *Cx26* mutation is comparable with the native UK population fraction (13/28), as is the fraction (10/19) in the families living in France, of varied ethnic make-up (see Materials and Methods). Therefore, these results suggest that *Cx26* mutations underlie half of the cases of prelingual autosomal recessive forms of deafness.

Moreover, the present study establishes that the contribution of the DFNB1 locus predominantly results from a newly identified 30delG mutation. The prevalence of the 30delG mutation relative to all *Cx26* mutations can be tentatively estimated by pooling the results obtained from the 39 mutation-positive families and by allowing one independent chromosome for each of the six consanguineous homozygous families and two independent chromosomes for each of the nonconsanguineous families. Of the 72 mutated chromosomes thus defined, 50 (69%) carry the 30delG mutation. Every other identified mutation occurs only once. According to the estimated frequency of recessively inherited prelingual deafness (1/2000) the 30delG mutation is one of the most frequent disease mutations so far described.

Is the 30delG mutant allele due to founder effect, or can the same mutation recur due to particular qualities of the gene DNA sequence ('hot spot of mutation')? We favour the latter hypothesis for the following reasons. Firstly, another *Cx26* mutation, a 38 bp deletion was detected, in which the last nucleotide before the deleted sequence was the same 30G. Secondly, there are characteristics of the *Cx26* DNA sequence at the 30G site or in the immediate vicinity that indicate a 'mutation hot spot' propensity. A Chi consensus motif (GCTGGTGG) extends from 19 bp downstream of nucleotide 30G and this sequence, which is known to promote recombination in the *Escherichia coli* genome (12) and mammalian immunoglobulin genes (13) has already been found in mutation hot spots responsible for β-thalassemia (14). There is also, at position 29, a TGGGG sequence, which is involved in the immunoglobulin class switch and this sequence has been found associated with a deletion hot spot in the β-globin gene (see 15, for review). In addition, it is possible that the G-deletion might arise from polymerase slippage due to the stretch of six Gs, as already suggested for *PAX2* mutations in renal-coloboma syndrome (16). Thirdly, the mutation has been observed in families of several European-Mediterranean 'ethnicities' (French, Tunisian, Lebanese, Portuguese, Polish, Anglo-Celtic). Haplotyping of the region in the immediate proximity or within the *Cx26* gene itself should clarify the issue.

The fact that our analyses would not have picked up mutations in the 5′ and 3′ non-coding sequences or in the promoter region, may account for the present failure to identify a presumed partnering *Cx26* mutation in 16 deaf children heterozygous for the 30delG mutation. Alternatively, an interaction with a mutation in a different connexin gene may be the explanation: most connexins are able to form intercellular channels with other connexins at gap junctions ('heterotypic channels') (17).

We have stressed the difficulties encountered in genetic counselling for prelingual non-syndromic deafness due to the inability to distinguish genetic and non-genetic deafness, in those families presenting with a single deaf child. Our findings indicate a useful role for a 30delG mutation search in such families. Since this would be a focused search for just the one mutation, this should be a pratical proposition for most molecular laboratories. It will be important to confirm a high incidence of the 30delG mutation in other populations of various origins. Finally, given the observation that a moderate hearing loss can affect some individuals homozygous for the 30delG mutation (see Table 1), further studies should not necessarily be limited to families in which the deafness is of severe or profound degree.

MATERIALS AND METHODS

Patients

Sixty-five affected Caucasian families from various geographical regions, mainly France, New Zealand and Australia, Tunisia and Lebanon, and the UK, were studied. They could be classified into three categories: (i) consanguineous families each having a significant linkage to the DFNB1 locus; (ii) small non-consanguineous families in which linkage analysis was compatible with the involvement of DFNB1; and (iii) small families in which no linkage analysis had been undertaken.

The first category consists of six large families living in geographically isolated regions. Five are from Tunisia, two from the north and three from the south. Linkage to the DFNB1 locus in the two families from northern Tunisia (families 20 and 60) has previously been reported (7); the three families from southern Tunisia (S15, S19 and ST) and the family from Lebanon (LH) comprise a total of three, five, two and five deaf children, respectively, the deafness being of severe or profound degree. The marriages were between first cousins (20, 60, S15, ST and LH) and between first and second cousins (S19). Linkage analysis of these six families resulted in individual lod scores ranging from 2.5 to 10 with polymorphic markers from the DFNB1 region (D13S175, D13S141, D13S143 and D13S115).

The second category of patients comprises eight New Zealand families with at least two deaf siblings (families 51, 1160, 1548, 1608, 1773, 1873, 1877, 1879) and one Australian family (9670). Family 1608 is atypical in that four siblings, sharing the same DFNB1 marker haplotypes, have a mild to moderate deafness (bordering on severe at high frequencies), with the child of one of them (III:20) being profoundly deaf. In family 1873, the unrelated parents (individuals II.2 and II.3) are deaf as are their two children, and we therefore considered this as two families, bringing to 10 the total of independent families. Apart from families 1608 and 1873, no parent acknowledged any hearing impairment. These nine families showed cosegregation between deafness and polymorphic markers of the DFNB1 region, with maximum individual lod scores ranging from 0.6 to 1.2. Ten other families in the original study of Maw *et al*. (8) had shown no cosegregation with the DFNB1 locus. The New Zealand families were all of Caucasian origin, with no known Polynesian admixture. According to the antecedent family names, the ancestral proportions among the families reflected that of the general Caucasian New Zealand population, with the great

predominance being of Anglo-Celtic patrimony and a small fraction due to migration from continental Europe. Neither parental consanguinity nor links between any of the families were recognized. In the Australian case, the father was from Northern Ireland and the mother from Yorkshire, UK.

The third category is composed of 19 families living in France, two in New Zealand and 28 in UK, each with at least two affected children. No parent acknowledged any hearing impairment, except for the mother in family P16 and the father in family P17, each of whom had a moderate and progressive high-frequency hearing loss. Five of the families living in France had foreign ancestors from Lebanon (family P3), Turkey (family P4, not shown), Portugal (family P9), Algeria (family P14) and Poland (father in family P16). In two of the families (P7, not shown and P14), the parents were distantly related.

Amplification of the coding exon of *Cx26*

PCR amplifications were carried out on genomic DNA using a set of primers that allowed the amplification of the entire coding sequence of the *Cx26* gene, which consists of a single coding exon (10). Primer sequences were as follows: 5′-TCTTTTCCAGAG-CAAACCGCC-3′ and 5′-TGAGCACGGGTTGCCTCATC-3′. PCR conditions were: 35 cycles of 95°C 1 min, 58°C 1 min, 72°C 2 min. The expected size of the PCR product was 777 bp. In addition, amplification products of the DNA from deaf children from families P6, P11, P14 and P17 were subcloned.

DNA sequencing

Sequencing of the PCR products and some subcloned PCR products was performed as previously described (18) using the dideoxy chain terminator method on an Applied Biosystems DNA sequencer ABI373 with fluorescent dideoxynucleotides. The primers used were the same as those for the PCR amplification plus two internal primers (5′-GACACGAAGATCAGCTGCAG-3′ and 5′-CCAGG-CTGCAAGAACGTGTG-3′). All PCR products were sequenced on both strands, thus allowing to detect the normal and the mutated allele in the normal hearing parents and deaf children heterozygous for the 30delG mutation.

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