Non-syndromic X-linked deafness is a rare form of genetic deafness accounting for a small proportion of all hereditary hearing loss. It is both clinically and genetically heterogeneous and five loci have been described to date but only two of these have been mapped. DFN2 represents a locus for congenital profound sensorineural hearing loss that has yet to be mapped. We describe a four generation family with this phenotype in which female carriers have a mild/moderate hearing loss affecting the high frequencies. The mutant gene has been mapped to Xq22 using polymorphic microsatellite markers. A maximum two point lod score of 2.91 at θ = 0 was observed with a fully informative dinucleotide repeat at COL4A5, and flanking recombinations were observed at DXS990 and DXS1001.

INTRODUCTION

Deafness is one of the most common human sensory defects affecting ~1 in 1000 live births and is thought to be inherited in more than half of cases (1). Of these, ~70% are considered to be non-syndromic, that is, not associated with other clinically recognisable features outside the auditory system. X-linked deafness accounts for only a few percent of all non-syndromic deafness but nevertheless is genetically heterogeneous. McKusick has categorised X-linked deafness into four types according to age of onset and audiogram (2) but recent genetic work has shown this classification to be oversimplified. Genetically, the locus DFN1 has been assigned to the phenotype of progressive sensorineural hearing loss, MIM 304700 (3), but recent restudy of the same family has revealed the additional clinical features of blindness, dystonia, spasticity and fractures, which define this clinical entity as a new syndrome, Mohr–Tranebjærg Syndrome (4). This syndrome has been mapped to Xq22 (4). The symbol DFN2 has been assigned to profound congenital sensorineural deafness (MIM 304500) and is unmapped. The locus DFN3, to which the majority of families with non-syndromic X-linked deafness map, was assigned to a gene causing progressive mixed deafness with perilymphatic gusher at stapes surgery (MIM 304400) and mapped to Xq13–q21 (5,6,7). It is now known that both mixed and pure sensorineural deafness may be caused by mutations in the same gene, POU3F4, at this locus (8,9) and that they share the same radiological phenotype (10). However, not all cases of mixed or sensorineural deafness which map to Xq13–q21 are accounted for by mutations in POU3F4 and there may be another gene in this region, mutation in which results in deafness (11,12). DFN4 has been mapped to Xp21.2 in a region containing the Duchenne muscular dystrophy locus and mutation at this locus results in congenital, profound sensorineural deafness in males and mild to moderate high frequency sensorineural hearing loss of adult onset in females (15).

We describe the re-evaluation of a four generation British–American family with congenital profound sensorineural hearing loss in males, similar to that ascribed to the unmapped locus DFN2. We present evidence of linkage of the mutant gene in this family to markers in Xq22 and describe a smaller family which does not show linkage to any of the loci described previously.

RESULTS

Family 1

This family was typed using markers from Xq13–q21, to exclude the DFN3 locus. DXS995 and DXS1002 are known to flank the POU3F4 gene at DFN3 (13). DXS995 lies within 5 cM of DFN3 (14) and physical data has shown that it actually lies within 20 kb of the POU3F4 gene (8). Recombinations between individuals IV_7 and V_4, and individuals III_8 and IV_11, with DXS995 and recombinant between III_8 and IV_11 with DXS1002 effectively excludes DFN3 as the causative locus. Lod scores are detailed in Table 1.

Both families were typed for microsatellite markers which span the DMD gene in Xp21.1 in order to exclude linkage to DFN4. Typing revealed different haplotypes in affected males across the entire DMD locus, excluding DFN4 as the causative locus in family 1.

Re-examination of previous linkage data prompted a search for linkage on the long arm of the X chromosome. Typing with commercially available primer pairs detecting microsatellite polymorphisms revealed a region of linkage at Xq22.

The two-point lod scores between the disease gene and marker loci, generated by LIPED, are shown in Table 1. A maximum two-point LOD score of 2.91 at zero recombination was observed with dinucleotide repeats at COL4A5 and at DXS1106. Likely recombinations occurred between individuals III_8 and IV_11 between the markers DXS990 and DXS1106, and between individual IV_11 and her sons V_8 and V_9 between DXS1220 and DXS1001.
Figure 1. Family 2. Probable haplotypes across the DFN4 locus at Xp21.1 assuming the least number of recombinations. DFN4 is encompassed by the 5′ end of the DMD gene and a region between intron 50 (STR50) and the 3′ untranslated region (15).

Table 1. Pairwise LOD scores between the disease locus and Xq chromosome markers

<table>
<thead>
<tr>
<th>Locus</th>
<th>LOD score at θ = 0</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Z_max</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS995</td>
<td>-99.99</td>
<td>-1.002</td>
<td>-0.509</td>
<td>-0.127</td>
<td>0.007</td>
<td>0.037</td>
<td>0.037</td>
</tr>
<tr>
<td>DXS1002</td>
<td>-99.99</td>
<td>0.057</td>
<td>0.293</td>
<td>0.429</td>
<td>0.390</td>
<td>0.243</td>
<td>0.429</td>
</tr>
<tr>
<td>DXS990</td>
<td>-99.99</td>
<td>0.057</td>
<td>0.293</td>
<td>0.429</td>
<td>0.390</td>
<td>0.243</td>
<td>0.429</td>
</tr>
<tr>
<td>DXS1106</td>
<td>2.913</td>
<td>2.671</td>
<td>2.42</td>
<td>1.89</td>
<td>1.32</td>
<td>0.70</td>
<td>2.913</td>
</tr>
<tr>
<td>PTP</td>
<td>1.408</td>
<td>1.297</td>
<td>1.182</td>
<td>0.93</td>
<td>0.66</td>
<td>0.35</td>
<td>1.408</td>
</tr>
<tr>
<td>DXS17</td>
<td>1.12</td>
<td>1.002</td>
<td>0.869</td>
<td>0.562</td>
<td>0.230</td>
<td>-0.016</td>
<td>1.12</td>
</tr>
<tr>
<td>DXS1230</td>
<td>0.505</td>
<td>0.461</td>
<td>0.416</td>
<td>0.322</td>
<td>0.22</td>
<td>0.116</td>
<td>0.505</td>
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<tr>
<td>COL4A5</td>
<td>2.913</td>
<td>2.671</td>
<td>2.42</td>
<td>1.89</td>
<td>1.32</td>
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<tr>
<td>DXS1220</td>
<td>1.138</td>
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<td>0.749</td>
<td>0.527</td>
<td>0.280</td>
<td>1.138</td>
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<tr>
<td>DXS1001</td>
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<td>-0.148</td>
<td>0.284</td>
<td>0.504</td>
<td>0.412</td>
<td>0.166</td>
<td>0.504</td>
</tr>
</tbody>
</table>

Family 2

Analysis of this family with markers from Xq13–q21 showed multiple recombinations and excluded POU3F4 (DFN3) as the disease gene, as indicated in Table 2. DXS986 lies within 6 cM of DFN3 (14) and so DFN3 can be excluded as the disease gene if a lod score of −2 is taken as evidence against linkage (Haldane’s mapping function).

The DFN4 locus has previously been mapped between DXS992, a microsatellite marker which lies between intron 50 of the DMD gene and the 3′ untranslated region, and DXS1068 which lies within 0.01 cM of the 5′ end of the gene (15). Family 2 was typed for the following microsatellite markers which span the DMD gene from the 5′ brain promoter to the 3′ untranslated region in Xp21.1 in order to exclude linkage to DFN4: 5′ DYS1, intron 44, intron 45, intron 49, intron 50, introns 62/63 and 3′ STR-HI. Typing revealed different haplotypes in affected males across the entire DMD locus, excluding DFN4 as the causative locus in Family 2. The haplotypes are shown in Figure 1 and show that the affected males, II 2 , II 5 , II 7 and III 4 do not share a common haplotype in this region, effectively excluding linkage.

Subsequent to the observation of linkage of Family 1 to Xq22, Family 2 was typed for the marker at COL4A5. As shown in Figure 2, three of the affected males, II 5 , II 7 and III 4 have different alleles, indicating that the COL4A5 gene is an unlikely candidate for this disorder in Family 2.
Figure 2. Microsatellite analysis of Family 2 with the dinucleotide repeat at COL4A5. The three affected males II_5, II_7, and III_4 have different alleles effectively excluding this as the disease gene. Individuals III_2, III_3, III_5, IV_1, and IV_2 were not typed for this marker since DNA was not available.

Table 2. Two point LOD scores between the disease gene and marker loci for Family 2

<table>
<thead>
<tr>
<th>Locus</th>
<th>Lod score at θ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>COL4A5</td>
<td>–99.99</td>
</tr>
<tr>
<td>DXS986</td>
<td>–99.99</td>
</tr>
<tr>
<td>DXS1002</td>
<td>–99.99</td>
</tr>
</tbody>
</table>

DISCUSSION

Linkage of Family 1 to Xq22 provides a map location for DFN2. Several syndromic forms of deafness map to Xq22, including Alport syndrome, Mohr–Tranebjærg syndrome (MTS) and Pelizaeus–Merzbacher disease (PMD). At present it is not known whether our family represents an allelic form of one of these conditions although phenotypically this is very unlikely.

Normal ophthalmologic examination, normal urinalysis and lack of family history of renal disease, as well as the age of onset and pattern of hearing loss makes Alport syndrome unlikely. Nevertheless, screening of the COL4A5 gene is being undertaken in Family 1 to exclude an unusual allelic variant; mutation in the COL4A5 gene has been described in a family with characteristic renal disease but without hearing loss (16). The gene COL4A6 which lies in a head to head configuration with COL4A5, remains a possible candidate, although deletions of the 5' end of this gene and part of the neighbouring COL4A5 gene are reported to cause diffuse leiomyomatosis (DL) and Alport’s syndrome (17). However, no mutations in COL4A6 alone have yet been described, so the phenotype associated with mutations in the gene is unknown. By itself, Family 2 is too small to establish or exclude linkage to a chromosomal region but recombinations with the intragenic marker in COL4A5 exclude this as a candidate gene in this family.

The Mohr–Tranebjærg syndrome (MTS), which has been recently mapped to Xq22 (4), was originally described by Mohr in 1960 in a family with a non-syndromic form of progressive sensorineural deafness. However, recent re-evaluation has revealed additional clinical features which include progressive dystonia, spasticity, mental deterioration and visual symptoms leading to blindness. None of these features have been observed in Family 1, none having been present in the affected males of generation II or in individuals IV_1 and IV_12 who are now in their thirties and forties. Furthermore, there is no evidence of progression of the deafness in this family. Whether or not MTS represents an allelic form of Pelizaeus–Merzbacher disease (PMD) is not yet known. This is a possibility since mutations in the proteolipid protein gene responsible for PMD have also been reported in families with complex X-linked spastic paraplegia (18).

It is therefore possible that the deafness in our Family 1 is caused by mutation in an unknown gene in Xq22. Expressed sequences in this region have been identified during work undertaken in the identification of the btk gene, mutations in which cause X-linked agammaglobulinaemia (19,20). Such ESTs will be considered as potential positional candidate genes during the course of future work.

Figure 3. (a) Audiogram of affected male, Family 1, individual IV_1. (b) A typical audiogram of an obligate female carrier (IV_7) from Family 1.
Figure 4. Family tree for Family 1, showing X-linked inheritance and probable haplotypes across Xq21–q24, assuming the least number of recombinations. Likely recombination has occurred between individuals III 8 and IV 11 between the markers DXS990 and DXS1106, and between individual IV 11 and her sons V 8 and V 9 between DXS1220 and DXS1001. An x symbol in the haplotype indicates that the individual was not typed for that marker.

Audiological testing of obligate female carriers in this family has shown that III 2, III 6, III 8, III 10, IV 7 and IV 11 have a mild to moderate hearing loss (an example of which is shown in Fig. 3b). During the course of this work, linkage analysis has identified several females who carry the affected haplotype (IV 2, IV 5, IV 9, IV 13, V 4, V 6 and V 7). Of these, individuals IV 13 and V 6 are reported to have a mild hearing loss on pure tone audiometry and the hearing status of IV 2 and V 7 is unknown. Individual IV 5 who is aged 37 years, gives a history of subjective hearing loss and has a mild/moderate hearing loss on pure tone and Bekesy audiometry; IV 9 (aged 41 years), however, has a normal audiogram but very small otoacoustic emissions as does her daughter V 5 (data not shown). Previous clinical studies of obligate carriers of X-linked deafness have shown that such females do not always have audiometrically detectable hearing loss (13,21). Thus, normal pure tone audiometry in a female does not exclude carrier status; the value of other forms of audiometric testing, such as otoacoustic emission data, in detecting carriers needs to be evaluated.

If new X-linked deafness genes are identified, Family 2 can be analysed for exclusion of a candidate gene. At the present time the small family size precludes the possibility of independently establishing or refuting linkage. However, clinical investigation may reveal the carrier status of females and their children in this family, which would enable more meioses to be scored. Individuals IV 1 and IV 2 are too young to show a hearing loss detectable by pure tone audiometry, based upon the natural history of hearing loss seen in other hearing impaired males in the family.

The nomenclature of non-syndromic X-linked deafness is confusing as it preceded molecular analysis of families. DFN1 was originally assigned to the phenotype of progressive non-syndromic deafness (3) which has subsequently been shown to be a form of syndromic deafness (4). Similarly, DFN3 represented mixed deafness with perilymphatic gusher but has since been shown to encompass the phenotype of pure sensorineural deafness (8,9). The locus DFN2 has traditionally been allocated to a gene causing congenital sensorineural deafness (22–27), which could encompass Family 1. Hopefully, the molecular dissection of non-syndromic X-linked deafness will allow classification based upon genetic loci alone and elucidate a genotype-phenotype correlation.
MATERIALS AND METHODS

Family 1 (Fig. 4) has been described previously as family 7 (7). Re-evaluation of this family revealed that individual IV12 has congenital profound sensorineural hearing loss and also suffers from Down’s Syndrome. In the past this individual was typed as unaffected because little was known about him. Individual II4, previously shown to be affected with hearing loss but with no other family history, is shown as unaffected here since the sudden onset of her hearing loss was revealed to have dated from a severe head injury sustained in her teens. Affected males in this family suffer from a severe/profound pure sensorineural hearing loss which is prelingual in onset. The audiogram of an affected male (individual IV1) is shown in Figure 3a. A CT scan of the petrous temporal bone in affected males showed no abnormality. All of the obligate female carriers have shown a mild/moderate hearing loss, more pronounced in the higher frequencies but none has complained of a subjective hearing loss. An example of this is shown in Figure 3b. There was no family history of renal disease and urinalysis was normal. Ophthalmic evaluation was also normal.

Family 2 has not been reported previously (Fig. 1). Affected males have a moderate to severe sensorineural hearing loss of childhood onset (age 3–9 years at diagnosis) and all have developed speech. Obligate female carriers have a mild hearing loss. CT scan of the petrous temporal bone of an affected male showed no abnormality.

PCR analysis

DNA was extracted from peripheral blood lymphocytes by standard techniques and was amplified in the polymerase chain reaction (PCR) by primers flanking dinucleotide repeat polymorphisms. Reaction mix consisted of 250 ng genomic DNA, 50 pmol of each primer, buffer consisting of 1.5 mM Tris pH 8.3, 1.5 mM KCl, 0.2 mM dGTP, dATP, dTTP and 0.02 mM dCTP, 1 µl [32P]dCTP (3000 Ci/mmol) per 1 ml of reaction mix, and 1 U Taq polymerase (Bioline) in a reaction mix of 50 µl. Products were separated on a 6% acrylamide–7 M urea denaturing gel at 60 W for 2–3 h. Dried gels were exposed to X-ray film (X-OMAT, Kodak) for 24 h.

All primer sequences are available from Généthon (28) except for PLP (29), COL4AS5 (30), DXS17 (31), 5′ DYS 1 (32) STR 44/45/49/50 (33), STR H1 and STR 62/63. (J. Taylor and A. Brinke, pers. comm.).

Conditions for thermal cycling consisted of denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 1 min at the annealing temperature of the individual primer pair, 1 min at 72°C, followed by a final extension step of 72°C for 10 min.

The annealing temperatures of primer pairs were as follows: 5′ DYS I 44°C; DXS1106, 50°C; 5′ STR HI, 51°C; DXS1230 and DXS1001, 53°C; STR 44,45,49 and 50, 54°C; DXS995, 55°C; COL4AS and DXS990, 56°C; DXS986, DXS1220 and PLP, 58°C; DXS1210, 59°C and STR 62/63, 60°C.

Linkage analysis

Pairwise lod scores were calculated between the disease gene and the marker loci using LIPED. Penetrance was taken as 100% in males. Frequency of the deafness allele was estimated at 0.0001.

Locus order and interloci distances were determined from the most recently available data (13). Figure 5. Postulated order of X chromosome markers used in this study (13).

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