Characterisation and genetic mapping of a new X linked deafness syndrome

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Abstract

Background—Hereditary forms of hearing loss are classified as syndromic, when deafness is associated with other clinical features, or non-syndromic, when deafness occurs without other clinical features. Many types of syndromic deafness have been described, some of which have been mapped to specific chromosomal regions.

Methods—Here we describe a family with progressive sensorineural hearing loss, cognitive impairment, facial dysmorphism, and variable other features, transmitted by apparent X linked recessive inheritance. Haplotype analysis of PCR products spanning the X chromosome and direct sequencing of candidate genes were used to begin characterising the molecular basis of features transmitted in this family. Comparison to known syndromes involving deafness, mental retardation, facial dysmorphism, and other clinical features was performed by review of published reports and personal discussions.

Results—Genetic mapping places the candidate locus for this syndrome within a 48 cM region on Xq1-21. Candidate genes including *COL4A5*, *DIAPH*, and *POU3F4* were excluded by clinical and molecular analyses.

Conclusions—The constellation of clinical findings in this family (deafness, cognitive impairment, facial dysmorphism, variable renal and genitourinary abnormalities, and late onset pancytopenia), along with a shared haplotype on Xq1-21, suggests that this represents a new form of syndromic deafness. We discuss our findings in comparison to several other syndromic and non-syndromic deafness loci that have been mapped to the X chromosome.

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are currently over 70 known loci for non-syndromic deafness, the responsible gene has been identified for fewer than half.²

X linked deafness accounts for about 5% of all congenital deafness,³ is genetically heterogeneous, and is categorised into seven types (*DFN1*, *DFN2*, *DFN3*, *DFN4*, *DFN6*, *DFN7*, and *DFN8*), according to age of onset and type of hearing loss (progressive versus non-progressive, conductive versus sensorineural; see OMIM: <http://www.ncbi.nlm.nih.gov/Omim> and <http://hgins.uia.ac.be/dnalab/hhh>). Of the seven non-syndromic deafness loci (*DFNs*) originally mapped to the X chromosome, one (*DFN1/TIMM8A* or Mohr-Tranebjærg syndrome) has been associated with other clinical features and is now considered syndromic. The other five known deafness loci are classically non-syndromic, some of which map to Xp (*DFN4*, *DFN6*), while others (*DFN2* and *DFN3/POU3F4*) map to Xq. The precise location of *DFN8* has not yet been disclosed.

We describe here a family with a previously unrecognised, apparently X linked form of syndromic congenital bilateral sensorineural deafness associated with cognitive impairment, facial dysmorphism, and variable involvement of other organ systems. There are some X linked deafness syndromes associated with multiple other clinical problems including cognitive impairment.⁴ Similarly, among the multiple X linked loci for mental retardation, several have deafness as an associated clinical feature. However, none of the previously described deafness-mental retardation syndromes exhibit the specific constellation of clinical findings present in this family. Genetic mapping places the region involved for this syndrome on the long arm of the X chromosome. Review of publications showed no other reports of families with clinical features similar to those present in our family. We present the clinical features in this family, suggest a potential map location, and discuss a number of possible candidate genes for this novel syndrome.

Methods

CLINICAL EVALUATION

Three males ranging from 12 to 54 years of age in one kindred were evaluated for a syndrome associated with hearing loss at the request of a female relative desiring preconception counselling. Review of their pedigree was most consistent with X linked recessive inheritance, as the affected males, with strikingly similar features, spanned three generations and were related through unaffected mothers (fig 1). No consanguinity was noted, and no other family members, including a son of a presumed obligate carrier mother, had known hearing loss or

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Approximately one in every 1000 infants is born with severe to profound hearing loss, and about half of these cases are believed to have a genetic basis.¹ Roughly 70% of all cases of genetic deafness occur in the absence of other non-auditory anomalies (non-syndromic deafness), while the remaining 30% have additional congenital abnormalities (syndromic deafness). The presence of additional symptoms and signs often allows physicians to recognise the particular gene(s) involved even without extensive genetic linkage analysis. While there

Figure 1 Pedigree of family. Age at initial evaluation and carrier status is shown. Horizontal line above symbols indicates subjects who were clinically evaluated.

learning disabilities. The mother of the 31 year old man had isolated congenital unilateral cleft lip. Features in all of the affected males are listed in table 1. Hearing loss in each affected male was identified in early childhood and categorised as bilateral, sensorineural, severe to profound. The two older males exhibited non-progressive hearing loss, whereas the 12 year old boy experienced progressive hearing loss, increasing from severe to profound over a 10 year period, with subsequent cochlear implantation at the age of 15. Computerised tomography of his temporal bone, done before cochlear implantation, showed normal anatomy except for underdeveloped, non-pneumatised mastoid cells. He was reported at 8 months post-implantation to be able to detect multiple environmental sounds, with less improvement so far in speech reception. All three males had normal tympanograms, indicating adequate middle ear functioning. Obligate carrier females exhibited no hearing loss.

Mild facial dysmorphism, unique to affected males, was also noted, including telecanthus, hypertelorism, epicanthic folds, broad mouth, and low set ears (fig 2). Additional features

included mild to severe cognitive impairment, telangiectasias, widely spaced, hypoplastic nipples, umbilical hernias, and dermatoglyphics characterised by a high number of arches. In addition, the two older men (aged 31 and 54) had microcephaly, short stature, and pancytopenia, which were more severe in the 54 year old man. The clinical course of the 54 year old man was also complicated by hypothyroidism, diagnosed at the age of 16, and splenomegaly, with normal megakaryocytes, slight hypocellularity, and erythroid hyperplasia on bone marrow examination. The 12 year old boy also had a cleft soft palate.

Abnormalities of the genitourinary tract were present in the 12 year old boy, who exhibited congenital bifid scrotum, small undescended testicles and phallus, chordee, and absence of the vas deferens and epididymis. Testicular biopsy performed during surgical chordee repair and orchidopexy showed normal testicular tissue. Abdominal ultrasound at birth was notable for dysplasia of the left kidney. Proteinuria was identified at the age of 11, and he was treated with angiotensin converting enzyme inhibitors to prevent hyperfiltration through the right kidney. The 54 year old man had a history of renal insufficiency and intravenous pyelogram at the age of 46 showed small kidneys (right 7 cm, left 8 cm) with smooth renal margins and faint opacification consistent with chronic glomerulonephritis. The 31 year old man showed no haematuria or proteinuria on repeated urine analyses and had no genitourinary or renal abnormalities, as shown by normal abdominal ultrasound at the age of 25.

The degree of cognitive impairment varied among affected subjects. The 54 year old man was sociable, mild mannered, and exhibited moderate to severe mental retardation with limited speech, but responded to gesture commands, was cooperative, and social. He attended a school for the deaf from 5 to 10 years of age and completed third grade. The 31 year old was educated in mainstream school starting at third grade, graduated from high school, and subsequently attended a technical college. The

Table 1 Clinical features of affected subjects in the pedigree

	Patient 1 (54 y)	Patient 2 (29 y)	Patient 3 (12 y)
Bilateral sensorineural hearing loss	+	+	+
Microcephaly	+	+	–
Telecanthus	+	+	+
Epicanthic folds	–	+	+
Narrow palpebral fissures	+	+	–
Broad nasal root	+	+	+
Malar hypoplasia	+	+	+
Dental malocclusion	+	+	+
Abnormal teeth	+	+	+
Full lower lip	–	+	+
Micrognathia	–	+	+
Low set ears	+	+	+
Myopia	+	+	–
Short stature	+	+	–
Umbilical hernia	+	+	+
Widely spaced, hypoplastic nipples	+	+	+
Dermatoglyphics, >5 low arches	+	+	+
Abnormal distal interphalangeal creases	+	+	+
Telangiectasias	+	+	–
Decreased cognitive functioning	+	+	+
Pancytopenia	Severe	Mild	–
Karyotype	46,XY	46,XY	46,XY

Figure 2 Affected family members, aged 12 (upper row), 31 (middle row), and 54 (bottom row) years. Note telecanthus, epicanthic folds, hypertelorism, wide nasal root, malar hypoplasia, low set ears, and broad mouth.

12 year old boy exhibited some learning disabilities but attended mainstream education classes.

Ophthalmological evaluations were notable for mild to severe myopia in the two older affected males. The 54 year old man also underwent left cataract removal and lens implantation. None of the affected males exhibited structural ocular malformations.

Chromosome analysis of a buccal smear from the 12 year old boy performed at birth for ambiguous genitalia suggested a mosaic karyotype of 46,XY/45,X. Repeat karyotype of peripheral blood at 1 week of age showed normal 46,XY chromosomes (100 cells examined). Prophase analysis (750-850 bands) of peripheral blood chromosomes from the 31 year old man showed no visible microdeletions. Chromosomal analyses of peripheral blood and bone marrow from the 54 year old man were also normal.

HAPLOTYPE MAPPING

DNA from each subject was prepared from peripheral blood leucocytes with Clontech Nucleospin columns (Palo Alto, California). Thirty eight primer sequences defining 19 loci for well mapped, highly polymorphic markers spanning the X chromosome were used to genotype affected males in a stepwise fashion in this kindred.⁵ Allele sizes were scored visually against one another and a control.

PCR for the first and last exons of *COL4A5* was performed as previously described.⁶ Primers were designed to amplify two exons of the *DIAPH2* gene based on the partial genomic sequence of this gene⁷ to look for gene deletions (Genbank direct submission by P Wray, Accession No Z86061, Sanger Centre, Hinxton, Cambridgeshire, UK).

PCR reactions were performed with the markers DXS169, DXS26, and 71:21, as previously described,⁸ to rule out a deletion

Figure 3 Chromatogram of the X chromosome with key markers shown on the left. Distances of markers in cM from the telomeric end of Xp are shown in parentheses. The solid vertical bar shows the common haplotype among affected subjects in this family. Chromosomal locations for all genetic markers and candidate genes are available at <http://www.ncbi.nlm.nih.gov/genome/guide/HsChrX.shtml>.

proximal to the deafness gene, *POU3F4*.^{8,9} PCR primers were designed to amplify the entire coding sequence of *POU3F4* (Genbank direct submission by R Deadman, Accession No Z82170, Sanger Centre, Hinxton, Cambridgeshire, UK). Products were purified and sequenced at the University of Michigan DNA Sequencing Core.

Results

Genetic mapping of the initial 19 markers indicated that the three affected subjects share a common haplotype spanning a 48 cM region of Xq1-21 (fig 3). Several deafness loci have previously been mapped to this region, including *DFN1/TIMM8A*, *DFN2*, *COL4A5*, *DIAPH2*, and *DFN3/POU3F4*. A second round of PCR screening was performed to rule out deletions and point mutations in *DFN3/POU3F4* and other candidate regions of the X chromosome among affected subjects. This analysis showed no deletions or point mutations in or around *POU3F4*. No altered PCR products in the *COL4A5* or *DIAPH2* genes were identified. These reactions showed that both the *COL4A5* and *DIAPH2* genes are grossly intact in affected members of this pedigree.

Discussion

All three maternally related affected males in this pedigree have severe bilateral congenital sensorineural hearing loss, facial dysmorphism, mild to moderate cognitive impairment, umbilical hernias, and abnormal dermatoglyphics. The older two patients also developed pancytopenia that became progressively worse with age. Extensive review of published reports has shown no similar reports to suggest a specific diagnosis, implying that these people probably have a previously unreported deafness syndrome. Environmental influences in the intrauterine, perinatal, or childhood period must be considered as possible causes for the features seen in this family. However, there was no history of medication use or exposure to radiation or teratogens during the pregnancies of the two younger males. Moreover, the three affected subjects span a range of over 40 years, and environmental influences common to all three would be expected to have influenced their respective sibs too. Indeed, some features in these subjects are most probably the result of additional unrelated medical problems, such as the hypothyroidism in the 54 year old man.

The features common to all three subjects (sensorineural hearing loss, short stature, facial dysmorphism, cognitive impairment, and variable other features including late onset pancytopenia, abnormal dermatoglyphics, and microcephaly) are consistent with a previously undescribed syndrome. These features are present in all three affected males in this family and absent in females. There is no male to male transmission to suggest autosomal dominant inheritance, although the pedigree size is limited. Mitochondrial inheritance cannot be definitively excluded, especially given the multisystem involvement of clinical findings and variability among affected subjects. However, there is no strong evidence of lactic acidosis, failure to thrive, encephalopathy, neuropathy, myopathy, seizures, stroke-like episodes, retinitis, or renal tubule disease as is commonly seen in mitochondrial disease, and there is no evidence of matrilineal transmission.

Transmission of the clinical features in this family is most consistent with X linked inheritance. We therefore focused our initial mapping efforts on the X chromosome. PCR analysis of 19 polymorphic markers on the X chromosome⁵ showed that all three affected subjects share a common haplotype that spans a 48 cM region of the long arm of the X chromosome. The likelihood that all males would share this common haplotype by chance alone is 1 in 64, strongly suggesting that alteration of a gene or genes within Xq1-21 is responsible for the features seen in this syndrome.

A number of known syndromic and non-syndromic deafness loci have been previously mapped to this region. *DFN3* maps to Xq21 and is a non-syndromic form of mixed deafness caused by mutations in or around the *POU3F4* gene.¹⁰ Microdeletions and a duplication involving a region 5' of the *POU3F4* gene have been identified in subjects with *DFN3*. These subjects also exhibit a characteristic deficiency of bone between the basal turn of the cochlea

and the internal auditory meatus that is evident on high resolution CT scanning.¹¹ This radiologically detectable cochlear anomaly was absent in the 12 year old boy. Moreover, PCR analysis of a number of markers in this region and complete sequencing of the *POU3F4* coding region indicated that the *POU3F4* gene and surrounding regions are grossly intact in affected subjects in this pedigree.¹⁰

Alport syndrome, caused by mutations in the *COL4A5* gene on chromosome Xq21-22, was recently identified in a family with haematuria, sensorineural hearing loss, and additional features including mental impairment and facial dysmorphism.¹² Molecular analysis in this family showed a contiguous gene deletion that leads to a complete absence of *COL4A5* and presumably affects one or more adjacent loci.¹² The features seen in patients with Alport syndrome in their report were also different from those present in our patients. Moreover, the two youngest men in our pedigree have no haematuria, making a variant of Alport syndrome less likely. PCR analysis of the *COL4A5* gene performed on an affected family member of our pedigree showed that the 5' and 3' ends of *COL4A5* are intact in this family. Therefore, large deletions which include the 5' and 3' ends of *COL4A5* do not explain the symptoms seen in our patients. Nevertheless, submicroscopic deletions within the coding region or other sequence alterations in *COL4A5* might have gone undetected by our analysis.

DIAPH1, a human homologue of the *Drosophila* gene *diaphanous*, is mutated in non-syndromic deafness *DFNA1* (OMIM 124900). A second *diaphanous* homologue, *DIAPH2*, maps to Xq22 and has been proposed as a candidate for *DFN2*.⁷ Using primers based on the published genomic sequence of *DIAPH2*, we assayed for large deletions in an affected family member in this pedigree and found no altered PCR products compared to control DNA.

DFN1/Mohr-Tranebjærg (*DFN1/MTS*) is a syndrome of progressive, sensorineural hearing loss that also presents with neurodegenerative symptoms, dystonia, spasticity, and visual impairment. *DFN1/MTS* is associated with mutations in the deafness/dystonia peptide (*TIMM8A*) gene.¹³ The type of neurodegenerative symptoms and the progressive nature of hearing loss in *DFN1/MTS* were not seen in our family. Similarly, all three affected subjects in the pedigree we describe have symptoms and signs not previously characterised in subjects with *DFN1/MTS*.¹³ Nevertheless, we cannot rule out possible genetic heterogeneity within *DFN1* families, and future analysis of this family is necessary to exclude mutations in the *TIMM8A* gene.

DFN2, an additional deafness locus that maps to this region, is associated with congenital deafness or non-syndromic progressive postlingual sensorineural hearing loss. Mapping originally placed this locus at Xq21-22¹⁴ and the addition of another affected family refined the interval to a 9.2 Mb region of Xq21.¹⁵ No other associated features have been reported in *DFN2* families, although there may be clinical heterogeneity within this locus.

Several other syndromes with deafness/cognitive impairment as associated features map to chromosomal regions within Xq1-21 and were considered during clinical evaluation of this family. Juberg-Marsidi syndrome, caused by mutations in *ATRX*, includes mental retardation, deafness, and microgenitalia.¹⁶ While these features were present in some affected members of our family, this diagnosis would not adequately explain the dysmorphism observed in affected males in this family.

So far, we have been unable to find any large deletions in several known candidate genes for familial deafness that map to Xq1-21 (*POU3F4*, *COL4A5*, and *DIAPH2*). Therefore, we conclude that this syndrome is either the result of an unusual mutation not previously reported in a known gene (thus producing a novel phenotype) or, more likely, a mutation in an as yet unidentified gene or genes that are critical for normal hearing and cognition. On prophase karyotype analysis, one band corresponds to about 5-10 million base pairs or five to 50 average sized genes. Thus, any region smaller than this would not be visible by standard Giemsa staining techniques. A number of contiguous gene deletions have been identified in this area^{17,18} raising the possibility that the disorder we describe here represents a new contiguous gene deletion syndrome. This hypothesis is particularly attractive considering there are a number of mental retardation loci that have been mapped to the X chromosome but have yet to be identified. The syndrome we describe here may be the result of microdeletions not detectable by prophase karyotype analysis. Such a microdeletion may involve the *DFN2* or *DFN3* loci, positioned within the 48 cM region where our syndrome maps, or one or more other genes on Xq1-21. More refined genetic analysis of this family or identification of other similarly affected subjects and families will help elucidate the underlying molecular basis of this syndrome and may provide critical knowledge about the molecular basis of hearing loss and cognitive impairment.

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