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Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan

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Autosomal recessive childhood-onset non-syndromic deafness is one of the most frequent forms of inherited hearing impairment. Recently five different chromosomal regions, 7q31, 11q13.5, 13q12, 14q and the pericentromeric region of chromosome 17, have been shown to harbour disease loci for this type of neurosensory deafness. We have studied a large family from Pakistan, containing several consanguineous marriages and segregating for a recessive non-syndromic childhood-onset deafness. Linkage analysis mapped the disease locus (DFNB8) on the distal long arm of chromosome 21, most likely between D21S212 and D21S1225 with the highest lod score of 7.31 at θ = 0.00 for D21S1575 on 21q22.3.

INTRODUCTION

Deafness is one of the most common human sensory defects affecting ~1 in 1000 live births and is inherited in more than half of the cases (for a recent review see ref. 1). There are many different forms of genetic deafness, some of which may themselves be heterogeneous, largely variable in expression and/or present with incomplete penetrance. In ~70% of the cases, genetic deafness is non-syndromic, that is it is not associated with other clinically recognizable features. The most common pattern of inheritance is autosomal recessive, accounting for >75% of the cases. Recently linkage analysis has led to the identification of five different chromosomal regions which harbour loci for recessive non-syndromic forms of deafness, 7q31, 11q13.5 (DFNB2), 13q12 (DFNB1), 14q and the pericentromeric region of chromosome 17 (DFNB3) (2–6).

Here we report linkage data suggesting that another recessive locus maps on the distal long arm of chromosome 21.

RESULTS

We have ascertained a large family (1DF) in Pakistan consisting of 41 members, of which eight are affected by a profound childhood-onset non-syndromic deafness. The age of onset of disease was 10–12 years and hearing was completely lost within 4–5 years. As shown in Figure 1, the family contains several consanguineous marriages.

After excluding the disease locus from all known 'deafness regions', a genome screening was undertaken using the Mappairs set (Research Genetics, Version 5A) of microsatellite markers. Positive two-point maximum lod scores ranging from 2.14–7.31 at θ = 0.00–0.10 have been obtained between the disease locus (DFNB8) and several loci assigned to chromosome 21q22.2-qter (Table 1 and Fig. 2) strongly suggesting that the disease gene is located on the distal long arm of chromosome 21. The highest lod score of 7.31 at θ = 0.00 was calculated between DFNB8 and D21S1575, which mapped <120 kb from the telomere-specific repeat-sequence on 21q (7,8). We have constructed haplotypes of all family members using the alleles at loci D21S212, D21S1575 and D21S1225 (Fig. 1). For D21S212, only the patient in branch 1 is homozygous (for allele 5) whereas patients in branches 2, 3 and 4 are heterozygous with alleles 1–4, 1–5 and 4–5 respectively, suggesting that (in each case at least one) recombination occurred between the disease locus and D21S212. Indeed, the mother in nuclear family 3 carries allele 5, that seems to be in phase with the mutated allele of the deafness gene in 1DF, whereas the patient has a recombinant 'mutant' paternal chromosome 21 with allele 1 of D21S212. As the patient is non-recombinant for the other loci tested distal to D21S212 (Fig. 1), the crossing over should have occurred between D21S212 and D21S1259 placing the disease locus most likely distal to D21S212 on 21q.

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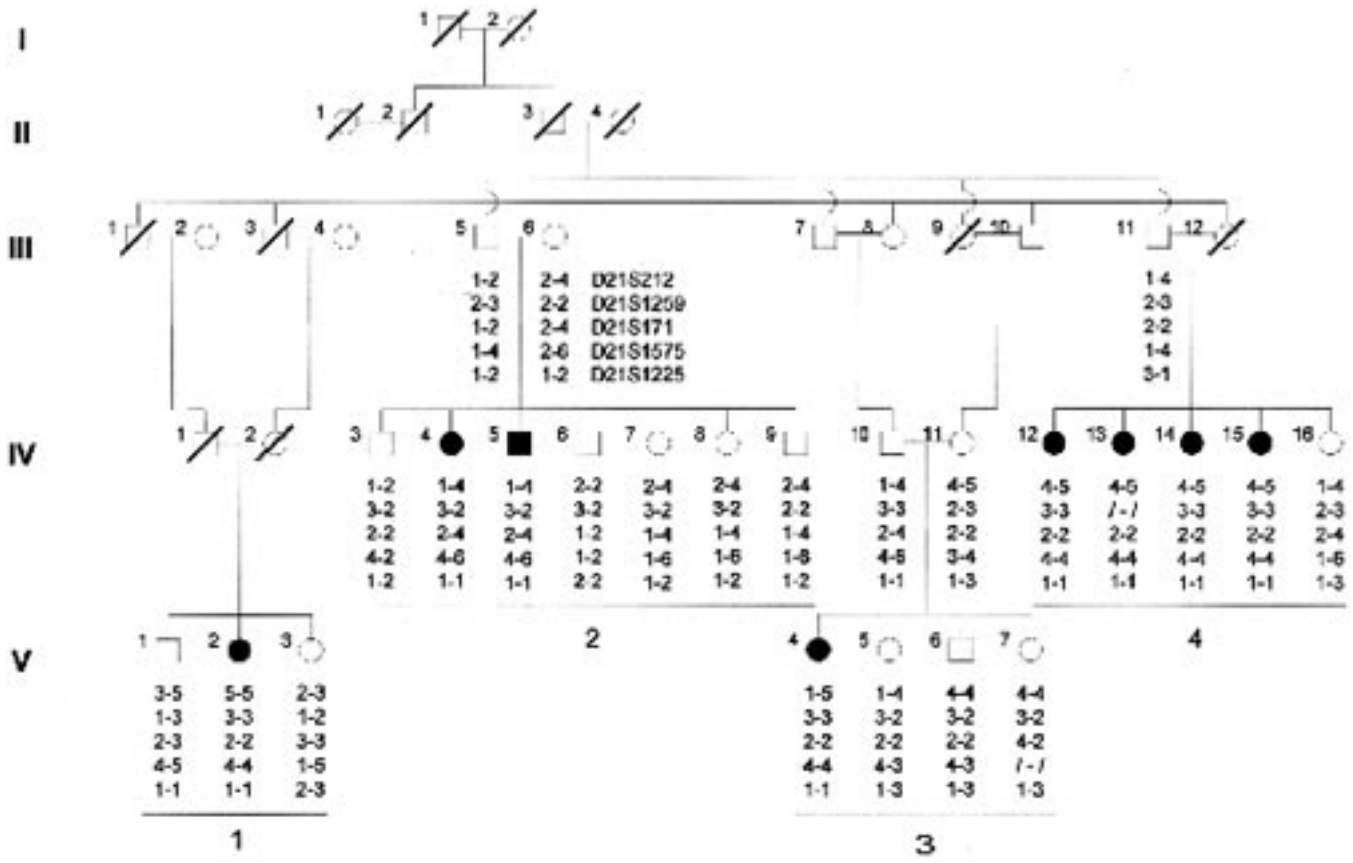

Figure 1. Pedigree of the family studied with alleles at D21S212, D21S1259, D21S171, D21S1575 and D21S1225 arranged as most likely haplotypes. Bottom lines and numbers mark the four different nuclear families (branches) referred to in text.

Table 1. Results of pairwise linkage analyses between DFNB8 and eight loci from 21q in family 1DF

Locus	θ	Z_{max}	0.00	0.01	0.05	0.1	0.2	0.3	0.4
D21S1255	0.06	2.48	1.11	2.03	2.47	2.39	1.77	0.95	0.21
D21S156	0.10	2.14	–∞	0.70	1.91	2.14	1.78	1.06	0.30
D21S266	0.05	2.24	0.87	1.89	2.24	2.11	1.52	0.87	0.30
D21S212	0.05	3.03	–∞	2.62	3.03	2.80	1.89	0.94	0.25
D21S1259	0.00	3.21	3.21	3.14	2.87	2.52	1.79	1.08	0.45
D21S171	0.00	4.51	4.51	4.41	4.01	3.49	2.4	1.47	0.61
D21S1575	0.00	7.31	7.31	7.15	6.52	5.71	4.07	2.48	1.04
D12S1225	0.04	3.54	–∞	3.26	3.52	3.25	2.40	1.45	0.59

All patients in branches 1, 3 and 4 are homozygous for allele 4 of the polymorphism at D21S1575 and all parents carry allele 4 on (at least) one chromosome 21. However, the two patients in branch 2 are heterozygotes with genotypes 4–6. The same is true for the genotypes at D21S1259 and D21S171, two further loci showing no recombination with the disease locus in 1DF (Fig. 1 and Table 1). The two affected individuals in branch 2 carry alleles 2-3 and 2-4 for D21S1259 and D21S171 respectively, while all other patients in the family are homozygous 3-3 and 2-2 respectively. Nevertheless, as the mother of these two patients in branch 2 is not related to her husband, heterozygosity of patients for marker alleles does not mean obligatory recombinations between disease and marker loci (see Discussion). As the oldest sibling in branch 1, a healthy male carries the same alleles for D21S1225 as his affected sister, this recombinant places DFNB8 most likely proximal to D21S1225.

While both D21S1225 and D21S1575 have been placed on the very distal end of 21q on the consensus linkage map of chromosome 21, the relative position of the two loci has not been established yet (Fig. 2 and ref. 8). The analysis of multiple informative meioses in 1DF suggests that D21S1225 maps distal to D21S1575 as this order would require the least number of recombinants to explain the genotype of all family members.

DISCUSSION

The linkage data presented in this communication suggest that a gene for autosomal recessive non-syndromic deafness is on the distal long arm of chromosome 21, most likely in 21q22.3. In case of recessive traits, the resolution power of genetic mapping by pairwise linkage data is largely limited both by the moderate total number of fully informative meioses as the genotype of healthy siblings at the disease locus can not be determined and by the possibility of non-allelic genetic heterogeneity. In case of

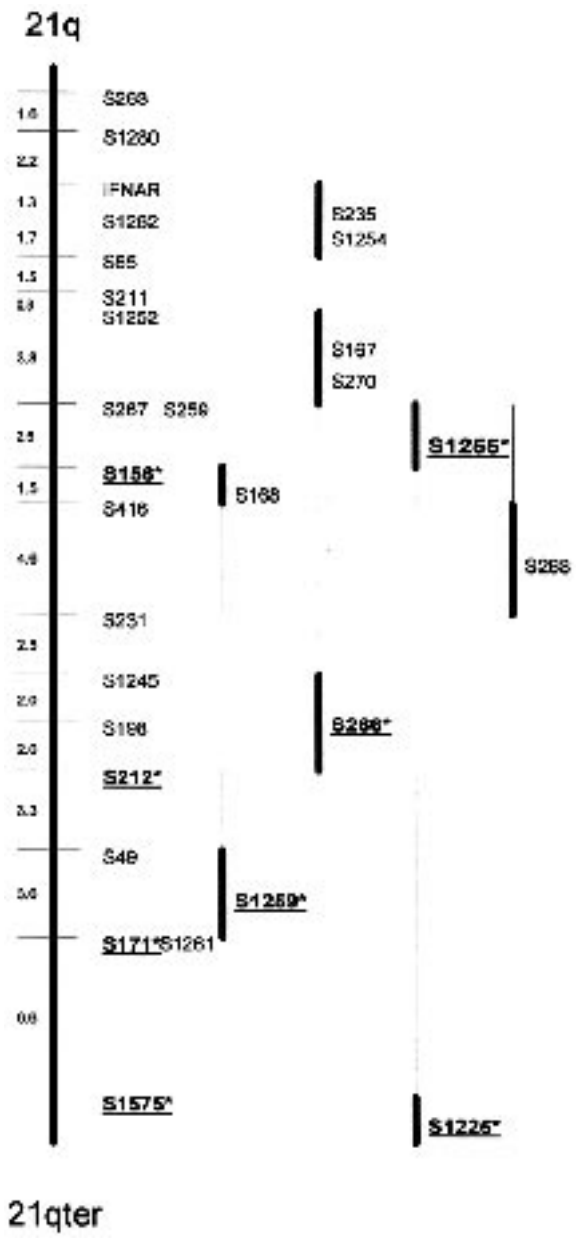

Figure 2. Simplified human chromosome 21 sex-averaged linkage map (based on the data presented in ref. 8). Distances between adjacent markers are in cM. Vertical lines show the probable location of marker loci which could not be positioned with (at least) 1000:1 probability, with a broad line for the most likely interval. Linkage data for loci underlined are given in Table 1.

consanguinity of the parents, all patients should be homozygous for a certain chromosomal region around the disease gene and genetic mapping can be further refined by the analysis of linkage disequilibrium and homozygosity-by-descent for alleles at closely linked polymorphic loci. In 1DF, several different possibilities should be considered to explain the absence of homozygosity for D21S1259, D21S171 and D21S1575 in the affected individuals in branch 2 of the family. As the two patients are related through their father with the rest of the kindred and because their alleles 3, 2 and 4 for D21S1259, D21S171 and D21S1575 respectively, are of paternal origin (Fig. 1), our assumption on close linkage between disease locus and the three loci will still hold. Alleles 2 (D21S1259), 4 (D21S171) and 6 (D21S1575) were transmitted to both patients in branch 2 from their mother who, according to the information given by the family, is not related to her husband. Yet, since they come from the same larger inbred 'clan', the most likely possibility is that the mother is distantly related to the entire family and carries a recombinant chromosome 21 with alleles 2 (D21S1259), 4 (D21S171) and 6 (D21S1575) in phase with the mutant allele of the deafness gene. Alternatively, the mother may carry the same mutation having occurred independently or a different mutation of the same gene, in both cases on a different genetic background. Although it is not very likely, one can not exclude the possibility that the deafness of the two patients in branch 2 is due to a defect in another recessive gene. Examples of non-allelic genetic heterogeneity in extended consanguineous pedigrees segregating for autosomal recessive deafness (2) or for another extremely heterogeneous genetic disorder, retinitis pigmentosa, have been observed recently (9,10).

The location of a recessive deafness gene in 21q22.3 is further substantiated by an independent clinical observation. The locus (EPM1) for progressive myoclonic epilepsy of the Unverricht–Lundberg type, an autosomal recessive disorder of the central nervous system, has been mapped to 21q22.3 by linkage studies (11). Latham and Munro reported a complex syndrome in a family in which the parents were second cousins and five out of eight children had progressive myoclonic epilepsy and early-onset deafness (12). Assuming that this syndrome is due to a larger structural rearrangement (e.g. a deletion or inversion) of chromosome 21, these data also suggest that a gene for childhood-onset deafness is in 21q22.3, probably in the physical proximity of the gene for progressive myoclonic epilepsy.

MATERIALS AND METHODS

Ascertainment of the family and deaf patients

All patients and their living relatives were examined by a specialist for audiometry. For patients, pure-tone audiometry was done in a 'sound chamber' using a Glazer audiometer. Frequencies tested by bone and air conduction were 125, 250, 500, 1000, 2000, 4000 and 8000 Hz with intensities up to 120 dB (maximum audio threshold in both ears of patients in this family was 105 dB at 1000 Hz). The age of onset of the disease was 10–12 years. By age of 14–16 years thresholds were at severe levels across the entire frequency range leading to profound hearing loss. Audiometric configurations for both ears were similar with all deaf persons affected bilaterally.

Linkage analysis

Linkage analysis was done using MLINK (V 5.1) in its FASTLINK implementation (V 2.2). For the analysis, a disease allele frequency of 0.01 was assumed. The mode of inheritance was taken to be autosomal recessive. Due to computational complexity of the multiple inbred pedigree and the resulting excessive amounts of computing time needed, multipoint analysis was not feasible.

Genotyping

Genomic DNA was available from 24 family members indicated in Figure 1. All polymorphic markers were typed using standard PCR amplification conditions. Upon a first indication of linkage

to chromosome 21, the family was genotyped for a total of 25 chromosome 21-specific markers to map the disease locus as precisely as possible (Table 1, Fig. 2 and data not shown).

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