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A gene for a dominant form of non-syndromic sensorineural deafness (*DFNA11*) maps within the region containing the *DFNB2* recessive deafness gene

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Hereditary hearing loss is divided into two groups, syndromic and non-syndromic, the latter being more common and highly heterogeneous. Linkage analyses were performed on a Japanese family showing a dominant form of non-syndromic progressive sensorineural hearing loss. This gene (*DFNA11*) was localized within the region of chromosome 11q which contains the second gene for a recessive form of non-syndromic sensorineural hearing loss (*DFNB2*). Since it has been reported that another gene for dominant non-syndromic hearing loss (*DFNA3*) has been mapped to the same region as the first gene for recessive hearing loss (*DFNB1*), it is possible that different mutations in the *DFNB2* gene may result in either dominant or recessive hearing loss.

INTRODUCTION

Hereditary hearing loss is divided into two groups, syndromic and non-syndromic, the latter being more common and highly heterogeneous (1). More than 20 forms of non-syndromic hearing loss have been established based on the mode of inheritance, age of onset, severity of hearing loss, and type of audiogram (2). These clinical distinctions are important for characterizing each form, but it is uncertain as to how many different genes may be responsible for each form of non-syndromic hearing loss.

Based on audiologic findings, non-syndromic hearing loss is subdivided into three categories: sensorineural, conductive, and mixed. Hereditary hearing loss generally presents a difficulty in preserving or restoring hearing ability, except for several forms of conductive or mixed hearing loss. Much remains to be clarified about the mechanisms of non-syndromic sensorineural hearing loss. A mitochondrial ribosomal RNA mutation has recently been identified as one cause of maternally inherited non-syndromic sensorineural hearing loss (3). However, an additional autosomal recessive mutation has been postulated as the other essential factor for phenotypic expression (4), but it has not yet been identified.

Some forms of non-syndromic sensorineural hearing loss occur in childhood or in early adulthood, and gradually become worse. The identification of genes for such forms of hearing loss may offer insights into devising therapies which can arrest or impede the progression of disease. Recent studies have succeeded in mapping genes for non-syndromic sensorineural hearing loss, including six dominant genes (5–10). In these reports, hearing loss was generally progressive. In this study, linkage and haplotype analysis was performed on a Japanese family to localize the gene responsible for a dominant form of non-syndromic progressive sensorineural hearing loss.

RESULTS

Family data and clinical evaluation

Figure 1 shows the pedigree of the affected family from Tochigi prefecture, Japan. The pattern of hearing loss clearly revealed an autosomal dominant inheritance with no evidence of nonpenetrance. Most affected individuals noticed hearing loss in their first decade of life after complete speech acquisition, with subsequent gradual progression. All affected individuals suffer from bilateral sensorineural hearing loss without vertigo or any other associated symptoms. They had symmetric, gently sloping or flat audiograms with hearing loss at all frequencies. Most affected individuals between the ages of 20 and 60 years had moderate hearing loss. Complete audiologic examinations were performed on four affected individuals and demonstrated cochlear involvement. Of the four individuals who underwent caloric testing, two individuals in generations II and III exhibited decreased responses bilaterally, while the other two individuals in generation IV showed normal responses. Affected individuals showed no evidence for other factors predisposing to hearing loss with the exception of individual III-2, who had a history of chronic otitis media in the left ear. She noticed hearing loss long before suffering from chronic otitis media. Myringoplasty of the left ear alleviated the conductive component of hearing loss. Audiologic findings strongly suggested bilateral cochlear involvement for her hearing loss. Therefore, she was included in the linkage analysis as an affected individual.

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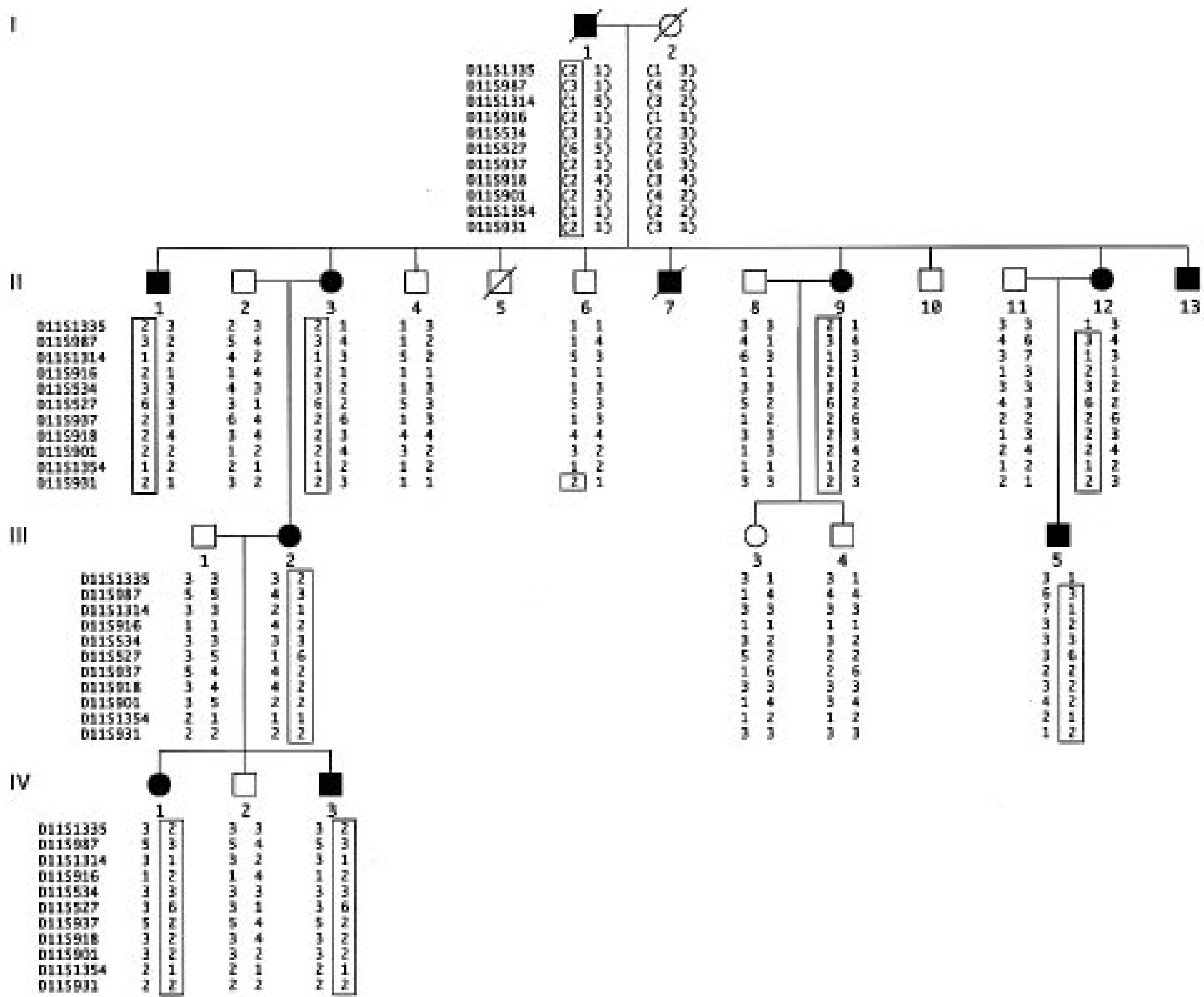

Figure 1. The Japanese family with non-syndromic sensorineural hearing loss. Filled symbols denote affected individuals. The genotypes for the markers on chromosome 11q are given below each individual. Parentheses denote inferred genotypes. Boxes indicate the haplotypes linked to *DFNA11*.

Linkage analysis

Three loci for autosomal dominant non-syndromic sensorineural hearing loss had been reported (5–7) when the linkage analysis was begun. After exclusion of the three loci, we have performed an extensive search of the complete human genome using more than 300 polymorphic microsatellite markers. Linkage was first detected with the marker *D11S2371* on chromosome 11q, which gave a maximal pairwise LOD score of 3.25 at $\theta = 0$. The approximate location of this marker is close to that of *DFNB2* (11), which is the second gene for non-syndromic recessive sensorineural hearing loss. Additional markers covering this region were selected from the Généthon marker collection (12) and from those described in a previous report (11). Since *D11S2371* has not been positioned precisely on the Généthon genetic linkage map, it was not included in the subsequent analyses. Significant linkage also was observed for the other six fully informative markers which gave maximal LOD scores exceeding 3.0 at $\theta = 0$ (Table 1). Various assumptions of the frequencies of the gene for hearing loss and the alleles did not significantly alter the LOD scores (data not shown).

The haplotype analysis in this family is shown in Figure 1 and demonstrates three obligatory recombination events, which localized the gene for hearing loss (*DFNA11*) within the genetic region defined by *D11S1335* and *D11S931*. Multipoint linkage analysis supported this region as the *DFNA11* locus, but could not define the interval any more precisely (Fig. 2).

DISCUSSION

The present study shows evidence for significant genetic linkage between several markers on chromosome 11q and a gene causing dominant, non-syndromic sensorineural hearing loss in a Japanese family. Obligatory recombination events defined the region for the gene for hearing loss, *DFNA11*, between markers *D11S1335* and *D11S931*. These markers have been assigned to chromosome 11q12.3-q13.1 and 11q14.3-q21, respectively (Genome Database). Thus, the *DFNA11* gene maps to chromosome 11q12.3-q21. This region contains another locus for non-syndromic hearing loss associated with recessive inheritance, *DFNB2*, which already has been mapped to an interval between *D11S916* and *D11S937* on chromosome 11q13.5 (11). It is tempting to speculate that this gene may be the common

denominator for both forms of hearing loss, although a number of genes may be located in this region. Another gene responsible for a dominant form of non-syndromic sensorineural hearing loss, *DFNA3*, is noted to have been mapped to the same region as the first gene for recessive hearing loss, *DFNB1* (7). It was suggested that different mutations in the same *DFNB1* gene may cause dominant or recessive hearing loss and that some of the other genes for hearing loss may be responsible for both modes of inheritance (7). In this report, several examples of mutations in the same gene resulting in either dominant or recessive disease were cited (7). A similar situation also has been observed in the von Willebrand factor gene (13), the thyroid hormone receptor β gene (14), and the β-globin gene (15). Considering these facts, we hypothesize that both dominant and recessive mutations within the *DFNB2* gene cause non-syndromic hearing loss.

Table 1. Pairwise LOD scores between *DFNA11* and chromosome 11q marker loci

Locus	Recombination fraction (θ)						
	0.00	0.01	0.05	0.1	0.2	0.3	0.4
D11S1335	–2.75	1.20	1.69	1.71	1.41	0.92	0.37
D11S987	3.25	3.20	2.97	2.67	2.01	1.27	0.49
D11S1314	3.25	3.20	2.97	2.67	2.01	1.27	0.49
D11S916	3.24	3.19	2.96	2.66	2.00	1.26	0.49
D11S534	2.41	2.36	2.19	1.95	1.44	0.87	0.29
D11S527	3.25	3.20	2.97	2.67	2.01	1.28	0.50
D11S937	3.25	3.20	2.97	2.67	2.01	1.28	0.50
D11S918	3.25	3.20	2.97	2.67	2.01	1.28	0.50
D11S901	2.11	2.07	1.91	1.70	1.24	0.73	0.23
D11S1354	0.60	0.58	0.53	0.46	0.31	0.17	0.05
D11S931	–∞	0.37	0.91	1.00	0.84	0.52	0.17

The gene for Usher syndrome type IB (*USH1B*) also maps to the same chromosomal region as *DFNB2* (16,17). The phenotypic difference between them is the absence of visual impairment in DFNB2. Despite this clear difference, it is still possible that USH1B and DFNB2 represent allelic heterogeneity at a single locus (11,18,21). Both the *USH1B* and the *DFNB2* genes were predicted to be the human homologue of the *shaker-1 (sh1)* gene located on mouse chromosome 7 (11,19). *Shaker-1*, USH1B, and DFNB2 present hearing loss and some vestibular abnormalities (11,18). Although no family members presented with vertigo in this study, caloric testing in selected members revealed age-dependent vestibular dysfunction. These findings showed the phenotypic similarity between DFNA11 and DFNB2, and support our hypothesis. The *sh1* gene recently has been found to encode an unconventional myosin molecule of the type VII family (20). Moreover, a human gene encoding myosin VIIa has been reported to be responsible for USH1B (21). Thus, it can be supposed that USH1B, DFNB2, and DFNA11 are caused by different mutations in the same gene which encodes myosin VIIa. Another unconventional myosin, hair-bundle myosin I, is thought to be responsible for the adaptation of mechanoelectrical transduction by hair cells of the inner ear (22,23). Very recently, it has been shown that the mouse *Snell's waltzer* deafness gene encodes an unconventional myosin VI, which appears to be required for maintaining structural integrity of hair cells of the inner ear (24). These reports strongly suggest that unconventional

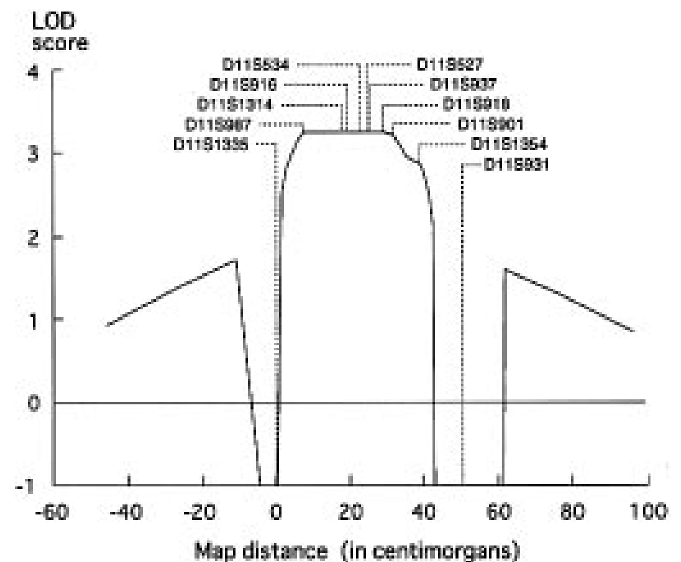

Figure 2. Multipoint linkage analysis between *DFNA11* and the markers on chromosome 11q. The location of *D11S1335* was set at map position zero.

myosins play important roles in various forms of sensorineural hearing loss.

In this study, some of the alleles linked to the *DFNA11* locus were present in very low frequencies in the normal control population living in the same area as the family studied. For instance, the frequencies of allele 6 for *D11S527* and allele 1 for *D11S1314* were 0.01 and 0.02, respectively. Moreover, none of the normal individuals bore the linked alleles for *D11S918* and *D11S987*, as described in Materials and Methods. It is possible that these alleles may be in linkage disequilibrium with *DFNA11*. A large-scale study of families showing linkage to the *DFNA11* locus and normal control individuals in a Japanese population will be required for confirmation of the linkage disequilibrium.

MATERIALS AND METHODS

Family data

All the family members included in this study gave a complete history and underwent otoscopic examination and pure tone audiometry with air and bone conduction. Selected members with hearing loss underwent general medical and audiologic examinations including speech discrimination scores, Békésy audiometry, tympanometry, acoustic reflex thresholds, evoked otoacoustic emissions. In addition, vestibular function was evaluated by bithermal caloric testing.

Genotyping

Peripheral blood samples were taken from each family member after informed consent was obtained. Genomic DNA was extracted and a polymerase chain reaction using polymorphic markers was performed as reported (25) except that the radioactive bands were detected with a Fujix Bioimaging Analyzer BAS2000 (Fuji Film). All primer sequences originated from the Généthon marker collection (12) except for *D11S534* and *D11S527*, which previously have been described (11). In addition, a CHLC human screening set (Weber version 6; Research Genetics) was used in the genomic searches.

Linkage analyses

Pairwise and multipoint LOD scores were calculated using the MLINK and LINKMAP options of the LINKAGE version 5.1 program package (26), assuming a gene for autosomal dominant hearing loss with a frequency of 10^{-4}, a mutation rate of 10^{-6}, and equal recombination rates for females and males. Four liability classes were assigned based on the ages of onset in the family studied; 0, age under 10 years; 0.57, age between 10 and 15 years; 0.86, age between 16 and 25 years; 1.0, age over 25 years. The frequencies of the marker alleles were calculated on the basis of the genotypes of 50 unrelated normal individuals living in Tochigi prefecture, except for those with allele 1 for *D11S918* and allele 3 for *D11S987*, which were not detected in any of the first 50 normal individuals as well as in an additional 24 normal individuals. For these two alleles we assumed a frequency of 0.01. The inferred genotypes of the deceased individuals shown in Figure 1 were not included in the statistical analyses.

Multipoint linkage analysis with the putative disease locus against the fixed map of the eleven markers on chromosome 11 was performed using overlapping five-point analyses (27). Recombination distances between loci are based on the Généthon linkage map (12) and a previous report (11), assuming that *D11S534* is located 3 cM telomeric to *D11S916*. Because of computational constraints, alleles at each locus were recoded with minimal loss of the information.

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