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A novel locus for autosomal dominant nonsyndromic hearing loss identified at 5q31.1-32 in a Chinese pedigree

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Abstract Hearing impairment is an extremely heterogeneous disorder. A total of 35 loci and 17 related genes for autosomal dominant nonsyndromic hearing loss have been identified. In a Chinese pedigree characterized by autosomal dominant inheritance with bilateral, postlingual, progressive, and sensorineural nonsyndromic hearing impairment, the putative disease gene locus was localized to chromosome 5q31.1-32 by a genome-wide scan. Fine mapping indicated that the disease gene was located within an 8.8-cM region between markers *D5S2056* and *D5S638*, with a maximum two-point logarithm of differences (LOD) score of 6.89 ($\theta = 0$) at *D5S2017*. By the candidate gene approach, mutation screening of the *DIAPH1* and *POU4F3* genes at 5q31 was performed. No mutation was found, suggesting that this is a novel deafness locus, which has been named DFNA42.

Key words Autosomal dominant nonsyndromic hearing loss · Linkage analysis · Logarithm of differences (LOD) score · DFNA42 · Heterogeneous disorder

Introduction

Hearing impairment is an extremely heterogeneous disorder that affects about 1 in 2000 newborns. Hereditary hearing loss can be classified as syndromic (SHL) or nonsyndromic (NSHL). Nonsyndromic hearing loss is a common sensory deafness and can be divided into two types: prelingual and postlingual deafness. Within the prelingual NSHL category, 75%–80% are autosomal recessive, 20%–25% are dominant, and 1%–1.5% are X-linked. In nearly all hereditary postlingual NSHL families, inheritance patterns are autosomal dominant.

To date, 71 NSHL loci have been reported, including 35 autosomal dominant, 29 recessive, 5 X-linked, and 2 mitochondrial loci. Among them, 28 related genes for NSHL have been identified. A total of 17 genes for autosomal dominant NSHL have been isolated, including *GJB2* (Kelsell et al. 1997), *GJB3* (Xia et al. 1998), *GJB6* (Grifa et al. 1999), *COCH* (Robertson et al. 1998), *COL11A2* (McGuirt et al. 1999), *DFNA5* (Van Laer et al. 1998), *DIAPH1* (Lynch et al. 1997), *KCNQ4* (Kubisch et al. 1999), *MYO7A* (Liu et al. 1997), *POU4F3* (Vahava et al. 1998), *TECTA* (Verhoeven et al. 1998), *MYH9* (Lalwani et al. 2000), *EYA4* (Wayne et al. 2001), *MYO6* (Melchionda et al. 1998), *DSPP* (Xiao et al. 2001), *WFS1* (Bespalova et al. 2001; Young et al. 2001), and *TMC1* (Kurima et al. 2002). These genes perform a wide range of functions, such as intercellular communication via gap-junction formation, regulation of actin polymerization by *DIAPH1*, transcription regulation by *POU4F3*, tectorial membrane integrity, and anchoring of the actin cytoskeleton by mysin, thus suggesting that many different processes are involved in auditory functioning and development. Identification of different NSHL disease-causing genes will help to elucidate the molecular basis of hearing and hearing loss.

The Chinese kindred presented here displays autosomal dominant, bilateral, postlingual, progressive and sensorineural hearing loss. We report here the results of a linkage analysis and mutation detection of this family that revealed a novel locus for DFNA, which we have named DFNA42.

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Subjects and methods

Family

A Chinese family with hearing impairment was identified by the Department of Otorhinolaryngology of the Xiangya Hospital of Central South University. Written informed

consent was obtained from all study participants. All subjects, recruited from Hunan Province, China, underwent pure-tone audiometry (PTA) and distortion product otoacoustic emission (DPOAE) testing, and a complete physical examination. Pure-tone thresholds were determined with air conduction at 125–8000 Hz and bone conduction at 250–8000 Hz. The criterion used to determine whether an individual was affected was bilateral sensorineural hearing loss of more than 30 dB at at least one frequency. Some patients underwent computerized tomography (CT) and vestibular testing. Previous audiological tests were collected if available.

Allelotyping

Blood samples were collected from 37 pedigree members. Genomic DNA was extracted from peripheral blood by standard phenol extraction protocols. Samples were quantified by spectrophotometry and diluted to 30 ng/μL for polymerase chain reaction (PCR) amplificaton. Genome-wide screening was performed with 382 markers spaced an average of 10 cM apart (ABI PRISM Linkage Mapping Set, Version 2.0, Foster City, CA, USA). Fine mapping was accomplished using fluorescently labeled primers from the Généthon linkage map (Dib et al. 1996). Multiplex PCR was carried out in a 5 μl reaction mixture containing 30 ng of genomic DNA, 1 × PCR buffer, 200 μM of each dNTP, 3.0 mM $MgCl_2$, 80 pmol each of forward and reverse primers, and 0.2 U of Ampli *Taq* Gold DNA polymerase. Reaction products were loaded onto a 6% denaturing polyacrylamide gel (7 M urea) and visualized on an ABI 377XL sequencer. Alleles were analyzed by Genescan analysis version 3.0 software and Genotyper version 2.1 software.

Linkage analysis

A two-point linkage analysis was conducted using the MLINK program of the Linkage 5.1 package (Lathrop and Lalouel 1984). The disease allele frequency was set at 0.0001, with the recombination fraction (θ) in men and women being considered equal. The disease was assumed to be fully penetrant and autosomal dominant on the basis of the pedigree information. The most likely haplotype was constructed by the Cyrillic program.

Mutation analysis

Primers were designed to amplify all exons and flanking intronic splicing sites of the *DIAPH1* and *POU4F3* genes. PCR was carried out in standard reaction mixtures, and amplified fragments were bidirectionally sequenced using an ABI 377XL sequencer.

Results

Clinical data

Thirty-seven members of the family were included in the study, of which 14 were affected; the earliest age of onset was 24 years old (V-9). Additionally, the PTA showed high-frequency hearing loss of 25 dB at 6000–8000 Hz in two other family members (IV-10, age 22; V-6, age 23), leading us to assume that they were possibly affected. No patients showed malformations of the inner ear by CT examination or abnormal function by vestibular testing. The characteristics of hearing loss in this pedigree was consistent with bilateral, postlingual, progressive, and sensorineural

Table 1. Two-point logarithm of differences (LOD) scores between the disease and chromosome 5q markers

	LOD score at θ = [a]							
Marker	.0	.01	.05	.10	.20	.30	.40	Z_{max} [b]
D5S1984	—	−.54	1.20	1.64	1.56	1.04	.41	1.70
D5S2002	—	1.64	2.64	2.74	2.25	1.42	.44	2.75
D5S2117	—	.92	2.00	2.19	1.90	1.28	.51	2.19
D5S2056	1.42	3.48	3.77	3.54	2.70	1.65	.57	3.77
D5S2115	3.08	3.09	3.01	2.80	2.21	1.48	.68	3.09
D5S1983	4.56	4.48	4.14	3.71	2.80	1.84	.88	4.56
D5S2116	6.05	5.95	5.51	4.95	3.73	2.40	1.00	6.05
D5S658	4.08	4.01	3.72	3.30	2.34	1.32	.48	4.08
D5S2017	6.89	6.77	6.27	5.61	4.19	2.64	1.04	6.89
D5S643	1.54	1.54	1.51	1.40	1.07	.66	.24	1.54
D5S638	5.45	5.35	4.95	4.44	3.33	2.13	.87	5.45
D5S436	3.62	3.55	3.27	2.91	2.15	1.37	.61	3.62
D5S2033	—	1.80	2.30	2.30	1.86	1.19	.46	2.33
D5S2090	4.62	4.59	4.37	4.00	3.10	2.03	.89	4.62
D5S636	—	.85	2.52	2.85	2.53	1.74	.77	2.86
D5S640	—	1.46	2.67	2.89	2.50	1.70	.73	2.89
D5S410	—	−.82	.35	.67	.68	.44	.17	.68

[a] LOD scores were calculated under an autosomal dominant mode of inheritance, with a penetrance of 100%. IV-10 and V-6 were calculated as possibly affected and III-6 as normal
[b] Z_{max} was calculated using the ILINK program of the Linkage 5.1 package

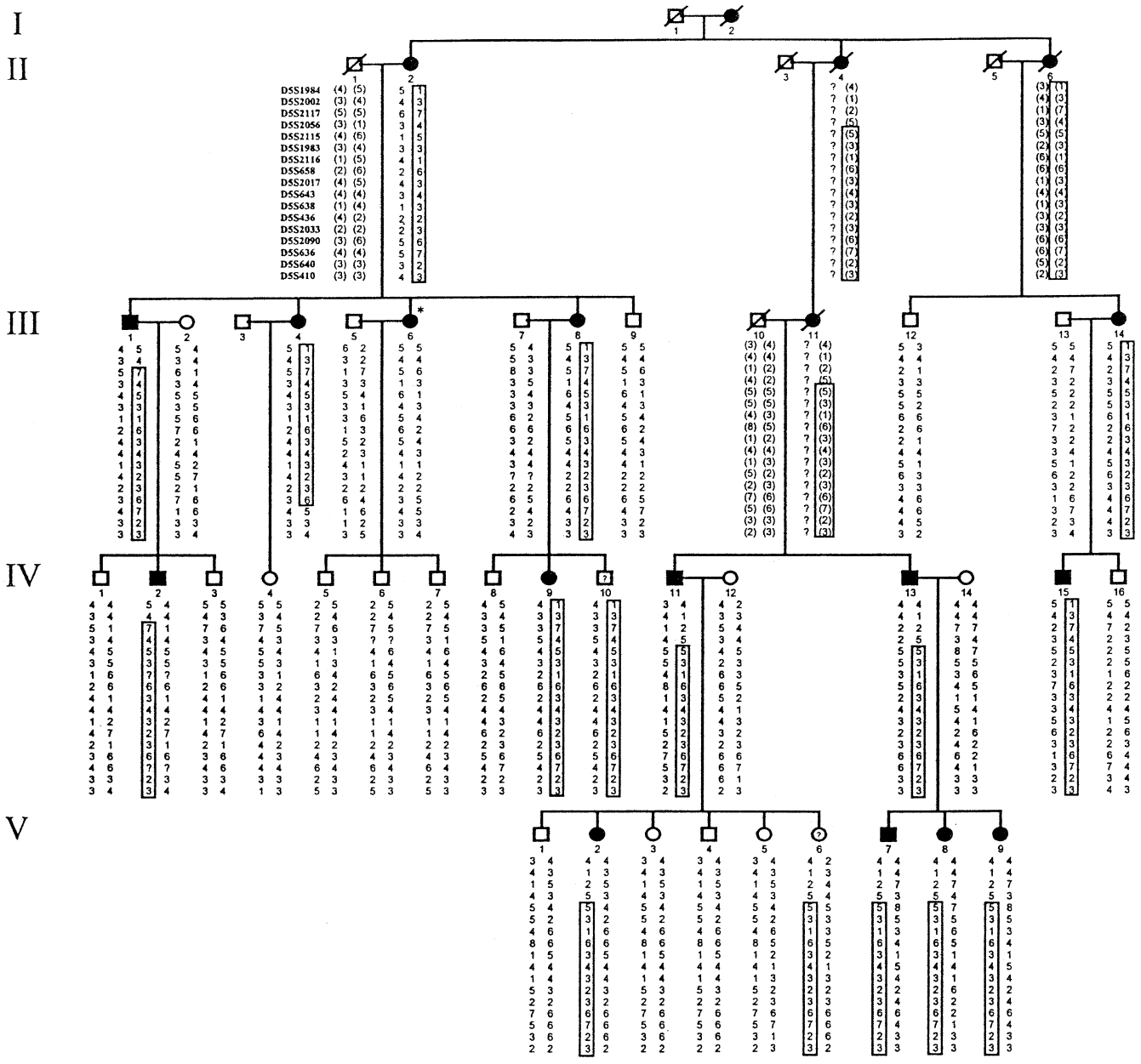

Fig. 1. Haplotype analysis of Chinese Hunan nonsyndromic hearing loss (NSHL) pedigree illustrating recombination events between the disease locus and chromosome 5q markers. Seventeen markers from chromosome 5 are shown at the upper left. The haplotype linked to deafness is *boxed*. Inferred alleles are in *parentheses*. A *question mark* (*?*) in *circles* or *squares* indicates possibly affected (IV-10, V-6). An *asterisk* (*) indicates a member with a phenocopy (III-6)

nonsyndromic hearing impairment. Examination of the history and clinical records of affected members suggested that high-frequency loss in this pedigree begins at the second or third decade and progresses to profound deafness involving all frequencies.

Two-point logarithm of differences scores

A maximum two-point logarithm of differences (LOD) score of 3.62 with marker *D5S436* ($\theta = 0$) was obtained. Two markers flanking *D5S436*, *D5S410* and *D5S2115* produced maximum LOD scores of 0.68 ($\theta = 0.2$) and 3.08 ($\theta = 0$). Fourteen additional markers around *D5S2115* were selected from the Généthon linkage map for fine mapping. A maximum two-point LOD score of 6.89 ($\theta = 0$) was obtained at *D5S2017*. Combined pairwise LOD scores between relevant markers and the disease locus are given in Table 1.

Haplotype analysis

The most likely haplotype in our pedigree was constructed using the Cyrillic program (Fig. 1). In most affected family members, a linked haplotype was found. In the inferred

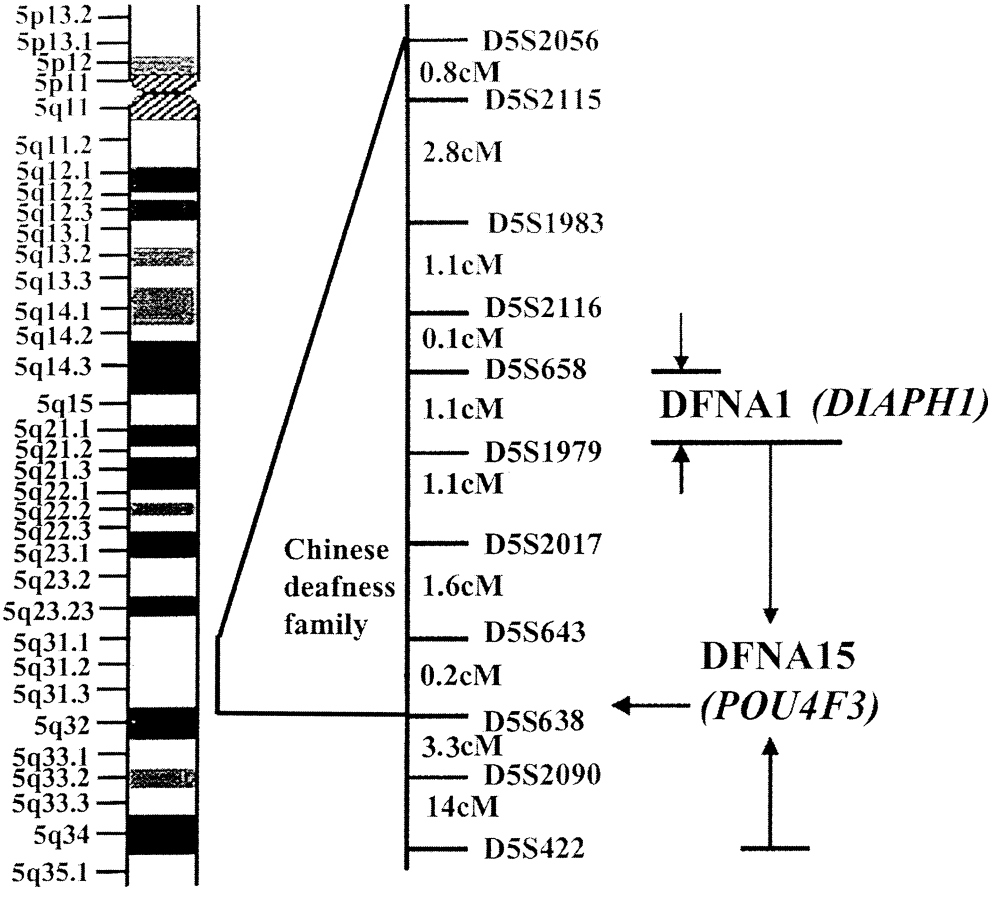

Fig. 2. Map of chromosome 5q containing DFNA1, DFNA15, and candidate regions for the Chinese Hunan deafness pedigree. Genetic distances between the markers are from the Généthon linkage map. The links between the genetic and cytogenetic maps are inferred from the genome database report

haplotype of individual II-4, a recombination was present between *D5S2056* and *D5S2115*, mapping the disease gene centromeric to marker *D5S2056*. Her affected descendents III-11, IV-11, and IV-13 have inherited this recombinant chromosome. Another important recombination event was present between *D5S643* and *D5S638* in a single unaffected individual (IV-8). He is 42 years old with a normal PTA. Because his mother is homozygous at *D5S643*, it is not known whether the recombination event occurred between *D5S2017* and *D5S643* or between *D5S643* and *D5S638*. Thus, we estimate that the interval is 8.8 cM, flanked by *D5S2056* and *D5S638* at 5q31.1-q32.

Mutation analysis

Two genes, *DIAPH1* and *POU4F3* for DFNA1 and DFNA15, respectively, have been localized to 5q31 (Fig. 2). We analyzed these two genes by direct sequencing. The amplified DNA fragments containing the exons and flanking intronic splicing sites of *DIAPH1* and *POU4F3* were gel-purified and sequenced. No mutation was found in this family.

Discussion

In this study, we identified DNFA42 at chromosome 5q31.1-q32 in a Chinese NSHL family. Thirteen affected members have inherited the disease haplotype; only one affected woman (Fig. 1, III-6) does not carry the haplotype linked to deafness. As the woman is 60 years old and has three children who are normal in hearing, her hearing impairment may be a phenocopy caused by nongenetic factors such as aging. Two individuals, IV-10 and V-6, were confirmed to carry the deafness-linked haplotype, having a hearing loss of 25 dB only at 6000–8000 Hz. We considered them as possibly affected individuals because both of them were less than 24 years old.

Two other loci, DFNA1 (Leon et al. 1992) and DFNA15 (Vahava et al. 1998), have been localized to 5q31. DFNA1 causes a type of nonsyndromic, dominant, postlingual progressive hearing loss, and is caused by mutations in the human homolog of the *Drosophila* gene *diapharous* (*DIAPH1*) (Lynch et al. 1997). *DIAPH1* is one member of the formin gene family, involved in cytokinesis and the establishment of cell polarity. *DIAPH1* is located in the candidate region that we identified (Fig. 2), but no mutation of the *DIAPH1* gene was found in this family. The affected individuals in this family show a high-frequency hearing impairment onset at 24 years old, whereas DFNA1 is characterized by the onset of low-frequency hearing loss at 10

years of age, progressing to profound bilateral deafness involving all frequencies by the age of 30. Therefore, *DIAPH1* can be excluded as a candidate for deafness in this family.

DFNA15 is another locus mapped to 5q31, in which hearing loss begins between ages 13 and 30 years, with moderate to severe defects in hearing occurring by age 50 years. Mutations in *POU4F3* have been reported to be associated with DFNA15 (Vahava et al. 1998). The locus for DFNA15 overlaps with the candidate region of this family (Fig. 2), and *POU4F3* is localized closely to *D5S436*; however, it is not within the critical region of this NSHL locus. *POU4F3* was also analyzed for mutations, but no mutation was found. Thus, the locus for this deafness pedigree is probably a novel locus, and we named it DFNA42.

To determine the gene responsible for hearing impairment in this family, a database search was performed (www.genome.ucsc.edu). In the region 5q31.1-q32, there are a number of genes that are expressed in the developing human inner ear: the early growth response 1 gene (*EGR1*), the human homolog of the *Drosophila* gene *diaphanous* (*DIAPH1*), the pou-domain transcription factor brn3c gene (*POU4F3*), antiquitin (*ATQ1*), the protein phosphatase 2A catalytic subunit gene (*PPP2CA*), and the osteonectin gene (*SPARC*). There are also a number of genes mapped to this region that are expressed in the developing inner ear of nonhuman vertebrates: genes for glucocorticoid receptor (*GCR*), bumetanide-sensitive Na-K-CL cotransporter (*SLC12A2*), neurogenin 1 (*NEUROD3*), integrin alpha-2 (*ITGA2*), catenin alpha-1 (*CTNNA1*), and fibroblast growth factor 1 (*FGF1*). Of the above genes, *CTNNA1* is a good candidate for DFNA42, because E-cadherin-mediated cell–cell adhesion is affected by three cytoplasmic proteins known as alpha, beta, and gamma catenins. These catenins are thought to function as connectors that anchor E-cadherin to the cytoskeletal actin bundle through the cadherin cytoplasmic domain. The cadherin 23 gene (*CDH23*) has been confirmed to be the cause of type 1D Usher syndrome and DFNB12 (Bolz et al. 2001; Bork et al. 2001).

It has been reported that mouse *slc12a2* is related to deafness and inner-ear defects (Delpire et al. 1999), but human *SLC12A2* was excluded as it is located centromerely to D5S2078, which is 3.4 cM away from the DNFA42 locus. Mutation analysis of the gene responsible for DNFA42 is underway.

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