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Clue to a New Deafness Gene: A Large Chinese Nonsyndromic Hearing Loss Family Linked to DFNA4

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Hereditary hearing loss is one of the most common neurosensory defects in humans. Approximately 70% of cases are nonsyndromic and could be inherited in autosomal dominant, autosomal recessive, mitochondrial, X-linked, and Y-linked manners (Wang et al., 2004; Alford, 2011). The autosomal dominant type, comprising 15%–20% of nonsyndromic hearing loss, is monogenic and genetically heterogeneous. Since the first dominant deafness locus (DFNA1) was identified in 1992, a total of 64 DFNA loci have been mapped (DFNA1–DFNA64), and 27 corresponding genes have been identified (<http://hereditaryhearingloss.org>). Previous studies have revealed that one deafness locus can be linked to more than one gene (Bayazit and Yilmaz, 2006), and the question “one locus, how many genes?” was first raised about a decade ago (Van-Hauwe et al., 1999). So far, several loci, including DFNA2 and DFNA3, have been shown to be related to one or more genes, showing high genetic heterogeneity in hereditary hearing loss (Grifa et al., 1999; Goldstein and Lalwani, 2002; Yan et al., 2011).

The DFNA4 locus was mapped to chromosome 19q13 with progressive sensorineural hearing loss (Chen et al., 1995; Mirghomizadeh et al., 2002; Pusch et al., 2004). However, it took almost 10 years to uncover the corresponding gene; finally, the nonmuscle myosin heavy-chain gene *MYH14* was identified as a causal gene of DFNA4 deafness (Bearer et al., 2000; Donaudy et al., 2004; Mhatre et al., 2004). However, causal mutations of the *MYH14* gene have been revealed only in families from Western European countries (Fig. S1). Genetic analysis in the American family in which this locus was originally defined excluded *MYH14* as the possible causal gene. The DFNA4 locus has been proposed to be associated with other responsible genes including the *CEACAM16* gene (Zheng et al., 2011). In this study, we ascertained and located a large Chinese family with autosomal dominant nonsyndromic hearing loss. Linkage analysis and mutation screening identified the linkage to the DFNA4 locus rather than the known DFNA4 genes, *MYH14* and *CEACAM16*. Our

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SUPPLEMENTARY DATA

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results support the hypothesis that there is another DFNA gene upstream of the *MYH14* gene and it may be linked to the DFNA4 locus.

We first collected and evaluated a six-generation Chinese family (family Z002) with autosomal dominant sensorineural hearing loss living in a county of South China. Phenotype of this family was similar but different to that of the reported DFNA4 families. Affected family members showed postlingual, symmetrical, and bilateral nonsyndromic sensorineural hearing loss without vestibular symptoms (Fig. 1A). Hearing loss occurred in the second to third decades of life. Ages of the affected members in family Z002 ranged from 22 to 61 years at the time of our investigation. Hearing loss varied between the fourth and fifth generations. This audiological variation between generations indicated progressive hearing loss with increasing age, which began in the high frequencies and gradually developed to all frequencies (Table S1). The level of hearing loss became severe at later ages. Speech discrimination scores were low in all patients. Vestibular function was normal in all affected family members, and high-resolution computed tomography (HRCT) of the temporal bone in the proband (IV:1) showed normal inner and middle ear structures, including the vestibular aqueduct and internal auditory canal. None of the affected members had a history of exposure to aminoglycosides, noise, or other causes that may account for the hearing impairment. Electrocardiography studies and color Doppler cardiogram indicated normal cardiovascular functions in affected members.

To identify the causal gene, we performed the genome-wide linkage scan, fine mapping, and haplotype analysis in family Z002. The linkage study located the deafness gene on the long arm of chromosome 19 (19q13.33-q13.4) and excluded the remainder of the genome. Positive two-point LOD scores of 3.54 and 3.03 ($\theta = 0$) were observed at markers D19S902 and D19S420, respectively. Fine mapping of the region using additional microsatellite markers confirmed the linkage. A maximum two-point LOD score of 5.30 ($\theta = 0$) was obtained for marker D19S879 (Table S2). Haplotype analysis disclosed a key recombination event between markers D19S902 and D19S879 in subject V:9, defining the centromeric limit of the critical interval (Fig. S2). The same recombination was observed in subject V:15. However, she was 10 years old, below the age of onset of the deafness in the family, and therefore her diagnosis was not clear. Subjects IV:4, IV:6, IV:8, IV:9, IV:13, and IV:16 provided the telomeric boundary of the linked interval at marker D19S571. These recombinations refined the disease-linked region to 11.36 cM (4.97 Mb), flanked by markers D19S902 (centromeric) and D19S571 (telomeric). This candidate region showed partial overlap with the critical intervals identified in the previously studied American and German DFNA4 large families, and included the reported deafness-causal gene *MYH14* (Fig. 1B).

To test whether *MYH14* is responsible for the hearing loss phenotype in family Z002, we did direct sequencing of the gene and revealed no disease-causing mutations. However, 20 nucleotide variants were detected in both affected and unaffected family members (Fig. S1). Among these variants, two synonymous substitutions, 657G > A (rs4801822) in exon 5 and 2127A > G (rs1651553) in exon 17, were detected. The other 18 intronic nucleotide variants identified in the family were all known SNPs. These SNPs did not co-segregate with the deafness phenotype of family Z002.

To further define the candidate region of this family, we performed SNP haplotype analysis. The SNP analysis revealed an important recombination between D19S879 and rs55934167 (Fig. 1C, horizontal arrow). In the SNP haplotype of subject V:9, an important recombination was present between marker D19S879 and the *MYH14* gene, mapping the disease gene telomeric to *MYH14*. The patient was clearly diagnosed with hereditary deafness, but had a different SNP haplotype (112211) compared with the other affected family members. This haplotype had been transmitted from his unaffected mother (IV:9).

This recombination excluded the *MYH14* gene from the candidate region of family Z002 and placed the region between the proximal boundary marker D19S902 and the distal boundary *MYH14* gene, defining a critical interval of 2.5 Mb.

The refined critical region of family Z002 included 121 known genes according to the MapViewer database. However, *CEACAM16* was excluded from the area, and coding sequence screening of this gene also found no causative mutations. No other nonsyndromic deafness genes have been mapped to this critical region, with the exception of *MYH14*. Among these, we selected three potassium channel genes, *KCNA7*, *KCNJ14*, and *KCNC3*, as candidates for mutation screening in family Z002. Genes located around the refined region, such as *KPTN*, *ERCC1*, and *KCNN4*, were also screened. No deafness-related mutations were detected in the coding regions or splice sites of these six genes.

Autosomal dominant nonsyndromic hearing loss has very complicated genotype–phenotype correlations because of its extreme heterogeneity (Lenz and Avraham, 2011). One locus harboring more than one gene is not uncommon. The DFNA2 locus, which contains the two identified causative genes *GJB3* and *KCNQ4*, is a good example (Xia et al., 1998; Coucke et al., 1999). Previous studies indicate that DFNA4 might be one such complex locus containing multiple deafness genes (Fig. 1B). Our genetic analysis showed that Z002 is the sixth reported family that maps to the DFNA4 locus. This locus has a distinct audiologic phenotype including later onset, slower progression, and wider affected frequency range (from high to all frequencies) of sensorineural hearing loss with reduced speech discrimination compared with the other DFNA4 families. These audiologic characteristics, when correlated with subsequent genetic findings, could provide further evidence for heterogeneity at the DFNA4 locus. Four of the previously reported families from Western European countries presented with causal mutations in the known DFNA4 gene, *MYH14* (Donaudy et al., 2004; Yang et al., 2005). Further study excluded *MYH14* and identified *CEACAM16* as a causal gene of the American DFNA4 family (Yang et al., 2005; Zheng et al., 2011). We screened the coding and noncoding exons and 5′-upstream regulatory region of *MYH14*, as well as the *CEACAM16* gene, in family Z002. However, no causal mutations were detected. Haplotype transmission analysis of intragenic SNPs has refined the critical interval to 2.5 Mb and excluded the two known DFNA4 genes as candidates for family Z002, implying the existence of a new deafness gene at the DFNA4 locus.

According to these results, we deduced that the causal gene in family Z002 is proximal to *MYH14*. This refined candidate region contains more than 100 functional genes. We have screened several genes as candidates according to their functions or specific expression in the inner ear of mice within and even around the refined region. However, no pathologic mutations were detected, with the exception of reported SNPs (Table S3), in these genes, suggesting that they are not involved in DFNA4 deafness. In addition, there are six genes (*RPL13A*, *RPS11*, *HRC*, *NUP62*, *FTL*, and *IL4I1*) within the interval included in the expression database for the human cochlea (http://www.brighamandwomens.org/bwh_hearing/default.aspx) that are thought to be good candidates. *SLC17A7*, which encodes the vesicular glutamate transporter VGLUT1, is a paralog for *SLC17A8*, which encodes the vesicular glutamate transporter-3 (VGLUT3) and is responsible for human deafness DFNA25 (Ruel et al., 2008). *SLC17A7* is specifically expressed in the neuron-rich regions of the brain and mediates the uptake of glutamate into synaptic vesicles at presynaptic nerve terminals of excitatory neural cells. Thus, it may be involved in glutamate metabolism in the inner ear (Schenck et al., 2009). Another possibility is *GRIN2D*, which belongs to the ionotropic glutamate receptor, *N*-methyl-D-aspartate (NMDA) receptor-channel gene family. Studies have shown that the glutamate receptor plays an essential role in high-frequency hearing and ionic homeostasis in the basal cochlea, and glutamate receptor antagonists can have a protective effect on the inner ear (Gao et al., 2007). Screening of

these genes is currently being performed. With the development of next generation sequencing technology in recent years (Zhang et al., 2011), target next-generation sequencing of the critical region would be an appropriate choice for us to identify the causal gene of family Z002.

In conclusion, our results provide further clues to the identification of the new DFNA4 gene. Because of the diversity of functional genes and the proteins encoded that are involved in the hearing system, identification of this novel DFNA4 gene may provide new insight into the molecular mechanism of hearing loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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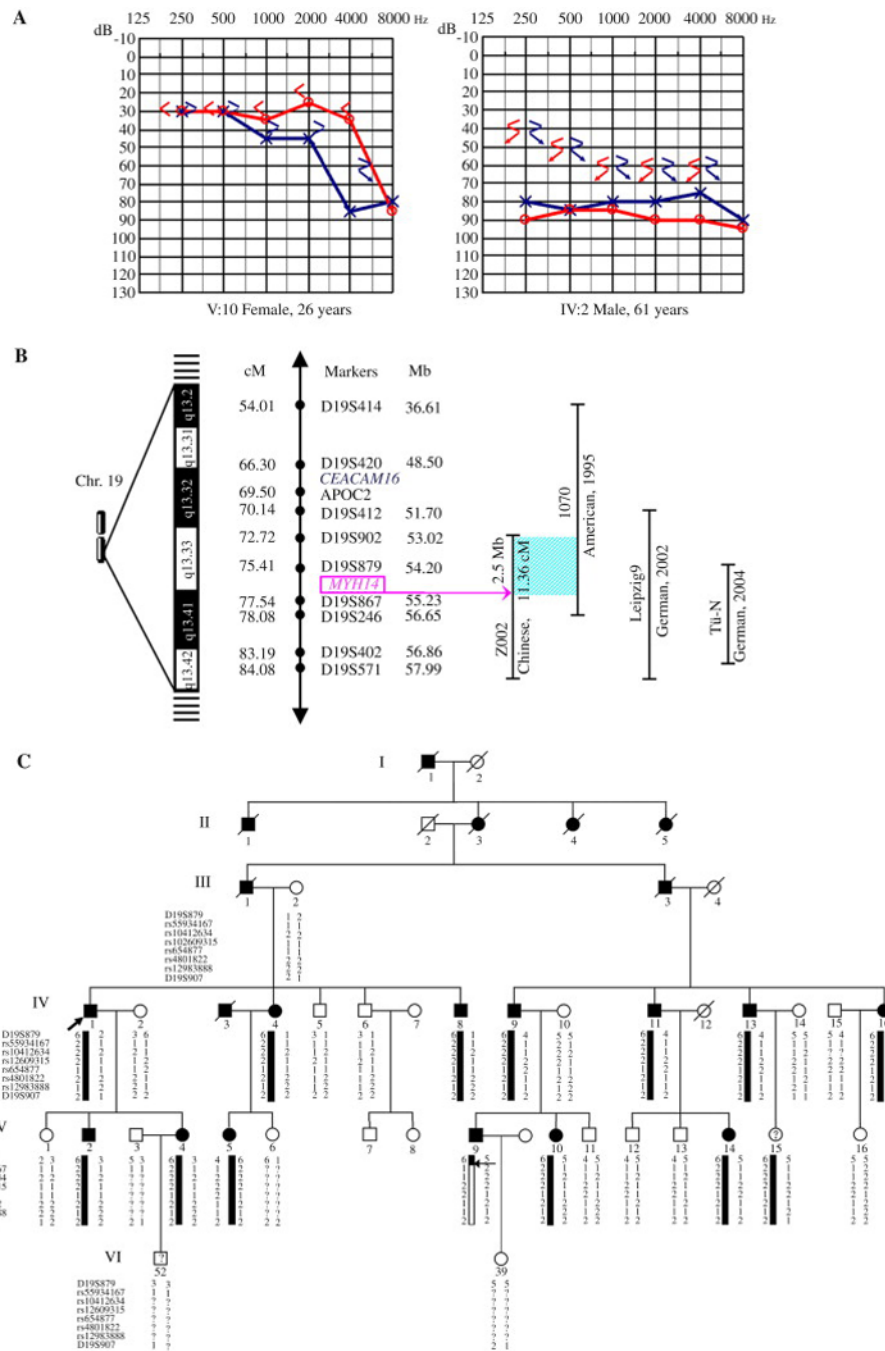


Fig. 1. Gene fine mapping and screening in family Z002 and the other DFNA4 families
A: typical pure-tone audiograms of both ears from two affected subjects (V:10 and IV:2) in family Z002. '○' connected by lines, right-ear air conduction; '×' connected by lines, left-ear air conduction; '<' , right-ear bone conduction; '>' , left-ear bone conduction; '↙' and '↘' , no response at maximum bone conduction output. **B:** schematic genetic and physical map of the DFNA4 locus on chromosome 19q13.2-q13.4 in families Z002, 1070, Leipzig9, and Tü-N. The shadowed area represents a 2.5-Mb region from marker D19S902 to the MYH14 gene shared by families Z002 and 1070. The genetic distances of microsatellite markers were obtained from the Marshfield genetic maps, and the physical distances of the

markers were obtained from the NCBI database. cM, centimorgan; Mb, megabase. C: SNP haplotype analysis in family Z002. The analysis indicates a recombination (denoted by the horizontal arrow) between D19S879 and rs55934167 in affected subject V:9. The recombination allows exclusion of MYH14 from the candidate region of family Z002 and refines the region to ~2.5 Mb.