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Report

A Novel Locus (DFNA23) for Prelingual Autosomal Dominant Nonsyndromic Hearing Loss Maps to 14q21-q22 in a Swiss German Kindred

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DFNA23, a novel locus for autosomal dominant nonsyndromic hearing loss, was identified in a Swiss German kindred. DNA samples were obtained from 22 family members in three generations: 10 with hearing impairment caused by the DFNA23 locus, 8 unaffected offspring, and 4 spouses of hearing-impaired pedigree members. In this kindred, the hearing-impaired family members have prelingual bilateral symmetrical hearing loss. All audiograms from hearing-impaired individuals displayed sloping curves, with hearing ability ranging from normal hearing to mild hearing loss in low frequencies, normal hearing to profound hearing loss in mid frequencies, and moderate to profound hearing loss in high frequencies. A conductive component existed for 50% of the hearing-impaired family members. The majority of the hearing-impaired family members did not display progression of hearing loss. The DFNA23 locus maps to 14q21-q22. Linkage analysis was carried out under a fully penetrant autosomal dominant mode of inheritance with no phenocopies. A maximum multipoint LOD score of 5.1 occurred at Marker D14S290. The 3.0–LOD unit support interval is 9.4 cM and ranged from marker D14S980 to marker D14S1046.

Nonsyndromic hearing loss (NSHL) is the most genetically heterogeneous trait known (Van Camp et al. 1997). Over 60 loci have been mapped and a total of 15 genes have been identified (Hereditary Hearing Loss Homepage). This extreme genetic heterogeneity suggests that there are many different processes that can malfunction within the inner ear to cause hearing loss (Heller and Hudspeth 1998). Because of this extreme genetic heterogeneity, an important strategy in mapping novel NSHL loci has been to analyze individual kindreds that can independently establish linkage.

Thus far, for autosomal dominant nonsyndromic hearing loss (ADNSHL), a total of 30 loci have been mapped, and 11 genes (COCH [MIM 601369], COL11A2 [MIM 120290], DFNA5 [MIM 600994], DIAPH1 [MIM 602121], GJB2 [MIM 121011], GJB3 [MIM 603324], GJB6 [MIM 604418], KCNQ4 [MIM 603537], MYO7A [MIM 276903], POU4F3 [MIM 602460], and TECTA [MIM 602574]) have been isolated. For ADNSHL only a handful of families have been identified that segregate a particular locus with the exception of those loci for which only one family has been identified (i.e., DFNA1 [MIM 124900]).

The Swiss-German kindred presented here (101) displays autosomal dominant bilateral early-onset hearing loss. Kindred 101 (figure 1) was ascertained through the Department of Otorhinolaryngology at the University Hospital of Zurich. Informed consent was obtained from all study participants and from the parents of underaged study participants. A family history was obtained through an oral interview. Audiometric and DNA samples were obtained from pedigree members in three generations.

Blood samples were collected from 19 pedigree members, and buccal swabs were collected from 3 family members. Of the study subjects from whom a DNA sample was obtained, 10 individuals were hearing impaired because of the DFNA23 locus, 8 individuals were unaffected offspring, and 4 individuals were spouses of

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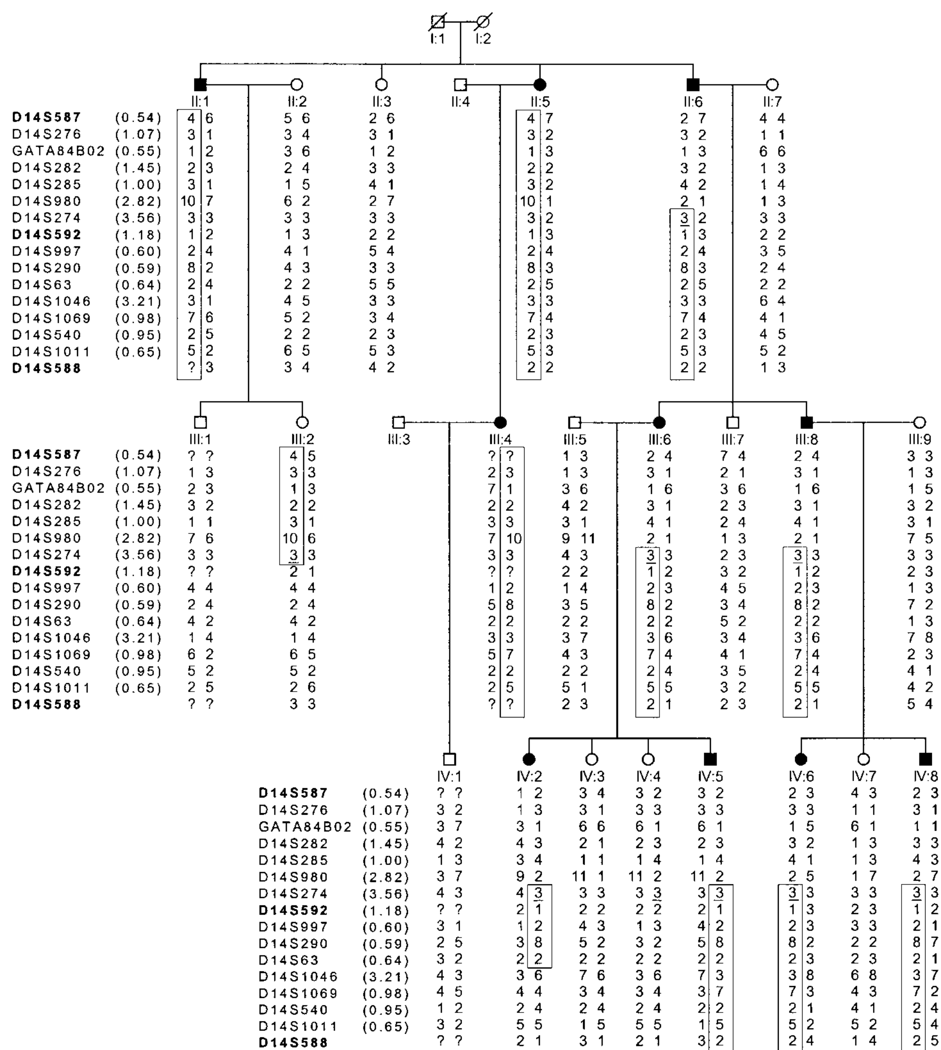

Figure 1 Pedigree drawing of Swiss-German kindred 101, which segregates DFNA23. The sexes of some of the family members have been changed to protect the anonymity of the family. The genetic-map distance in centimorgans (cM) to the next marker are given in parenthesis next to the marker name. Markers shown in bold were genotyped for the genome scan. Filled symbols represent individuals with hearing impairment caused by DFNA23. Gray symbols represent individuals whose hearing status is unknown. Unfilled symbols represent unaffected individuals. Haplotypes for the most–closely linked STRPs are shown below each symbol. Question marks indicate an unknown allele. The haplotype segregating with the disease allele appears in rectangles. The 3 allele from marker D14S274 is underlined for those individuals where it is not possible to elucidate whether marker D14S274 is part of the disease haplotype.

hearing-impaired pedigree members. Auditory thresholds were determined by standard pure-tone audiometry with air conduction at 125–8000 Hz and bone conduction at 250–4000 Hz. In addition, where possible, previous audiological tests were collected. The history and records of the hearing-impaired family member suggested a prelingual onset at birth or in the first years of life. The majority of the affected family members did not display progression of hearing loss during a followup period which varied between 6 and 32 years. The hearing loss showed progression in only three cases. Audiometry displayed a bilateral and symmetrical hearing loss. In five cases there existed a conductive component (which produced an air-bone gap between 10 dB and 40 dB). All audiograms showed sloping curves, with normal hearing to mild hearing loss in low frequencies, normal hearing to profound hearing loss in middle frequencies, and moderate to profound hearing loss in high frequencies.

DNA was extracted from 10 ml of blood by use of standard techniques (Miller et al. 1988). A genome scan was carried out on 19 DNA samples at the National Heart, Lung, and Blood Institute (NHLBI) mammalian genotyping service, using Marshfield screening set 9

(Yuan et al. 1997). A total of 386 fluorescently labeled simple tandem repeat (STRP) markers were genotyped, with an average heterozygosity of 0.76. These markers are spaced ~10 cM apart and are located on the 22 autosomes and the X and Y chromosomes.

Linkage analysis was carried out under a fully penetrant autosomal dominant mode of inheritance with no phenocopies. A disease frequency of .001 was used in the analysis. The marker-allele frequencies were estimated from the data by means of both observed and reconstructed genotypes of founders within this pedigree and other Swiss-German pedigrees. It should be noted that, since almost all family members are genotyped, pedigree 101 is very robust to changes in marker allele frequencies, varying marker allele frequencies having little or no effect on the LOD score results. Two-point linkage analysis was carried out on all autosomal markers from the genome scan by means of the MLINK program of the FASTLINK computer package (Cottingham et al. 1993). A maximum two-point LOD score of 3.9 with marker D14S592 was obtained. No other marker gave a LOD score $\geqslant 3$. Flanking markers to D14S592, D14S587, and D14S588 produced maximum LOD scores of 0.71 (at recombination fraction [θ] = .22) and 1.3 (at θ = .09), respectively. Multipoint linkage analysis, performed with the LINKMAP program of the FASTLINK computer package, ruled out the possibility that the DFNA23 locus lies between D14S587 and D14S306 or between D14S588 and D14S53 (data not shown).

For fine mapping, 13 additional markers were selected from the Marshfield map (Broman et al. 1998); 6 of these markers—D14S276, GATA84B02, D14S282, D14S285, D14S980, and D14S274—lie between D14S587 and D14S592, and an additional 7 markers—D14S997, D14S290, D14S63, D14S1046, D14S1069, D14S540, and D14S1011—lie between D14S592 and D14S588 (fig. 1). No other markers were available from this region that would allow for finer mapping of the DFNA23 locus. For these markers, 22 DNA samples were genotyped: the 19 DNA samples included in the genome scan plus three additional DNA samples—III.1, III.4, and IV.1—for which only a limited amount of DNA was available, since a buccal swab had been collected. The data were reanalyzed using two-point and multipoint linkage analysis. The results of the two-point linkage analysis are displayed in table 1. A maximum two-point LOD score of 5.1 was obtained at θ = 0 for marker D14S290. A graph of the results of the multipoint analysis is displayed in figure 2. Since marker D14S290 is fully informative an identical maximum multipoint lod score of 5.1 was obtained at this marker. Marker D14S290 maps to 68.6 cM from the telomere. The 3.0-LOD unit support interval for DFNA23 ranges from 60.4 cM to 69.8 cM (9.4 cM).

SIMWALK2 (Weeks et al. 1995; Sobel and Lange 1996) was used to generate the haplotypes with the highest likelihood (fig. 1). The interval that DFNA23 maps to is flanked by recombination events at marker D14S980 (60.4 cM) and D14S1046 (69.8 cM). The interval between these two markers is 9.4 cM. If the haplotypes (fig. 1) are examined, marker D14S274 cannot be placed within the disease haplotype unequivocally. For those individuals for whom it is not possible to elucidate whether D14S274 is part of the disease haplotype, the 3 allele is underlined. On the basis of haplotype reconstruction of the parents of II.1, II.2, II.3, II.5, and II.6 (data not shown), the parent carrying the disease allele was homozygous at marker D14S274. Therefore, for individual II.6, it is not possible to tell whether a recombination event occurred between markers D14S980 and D14S274 or between D14S274 and D14S592. Furthermore, for individuals III.2 and IV.4, the affected parents are also homozygous at marker D14S274, so it is impossible to elucidate whether a recombination event occurred between markers D14S980 and D14S274 or between D14S274 and D14S592. If marker D14S274 is not part of the disease haplotype, then the haplotype for DFNA23 is 6.6 cM. However, since it is impossible to rule out that marker D14S274 is part of the disease haplotype, a fairer estimate of the haplotype length is 9.4 cM.

In considering candidate genes for DFNA23, the most interest lies in those genes which map to 14q21-q22 and are expressed in the cochlea. Although genes expressed in other portions of the auditory pathway could also be involved in causing NSHL, genes expressed in the cochlea are good candidates for DFNA23, since all known mutations leading to deafness in humans occur in genes

Table 1

Two-Point LOD Score Results between the DFNA23 Locus and Chromosome 14 Markers

	LOD Score at θ =						
Marker	.0	.01	.05	.10	.20	.30	.40
D14S587	$-\infty$	−2.14	−.25	.37	.71	.63	.38
D14S276	$-\infty$	−1.46	−.16	.28	.49	.41	.21
GATA84B02	$-\infty$	−1.09	.20	.63	.81	.68	.39
D14S282	$-\infty$	−2.13	−.23	.41	.77	.70	.41
D14S285	$-\infty$	−1.55	.30	.88	1.09	.87	.45
D14S980	$-\infty$	−.95	.86	1.39	1.50	1.16	.61
D14S274	.76	.76	.71	.64	.47	.27	.08
D14S592	3.91	3.85	3.58	3.23	2.46	1.61	.73
D14S997	4.21	4.14	3.86	3.48	2.67	1.78	.85
D14S290	5.12	5.03	4.69	4.25	3.28	2.19	1.01
D14S63	2.41	2.36	2.19	1.95	1.44	.87	.3
D14S1046	$-\infty$	−5.49	−2.21	−.94	.05	.35	.31
D14S1069	$-\infty$	.75	1.86	2.08	1.87	1.31	.59
D14S540	$-\infty$	1.37	1.9	1.96	1.7	1.2	.57
D14S1011	1.53	1.53	1.48	1.37	1.07	.69	.27
D14S588	$-\infty$	.67	1.19	1.25	1.07	.75	.4

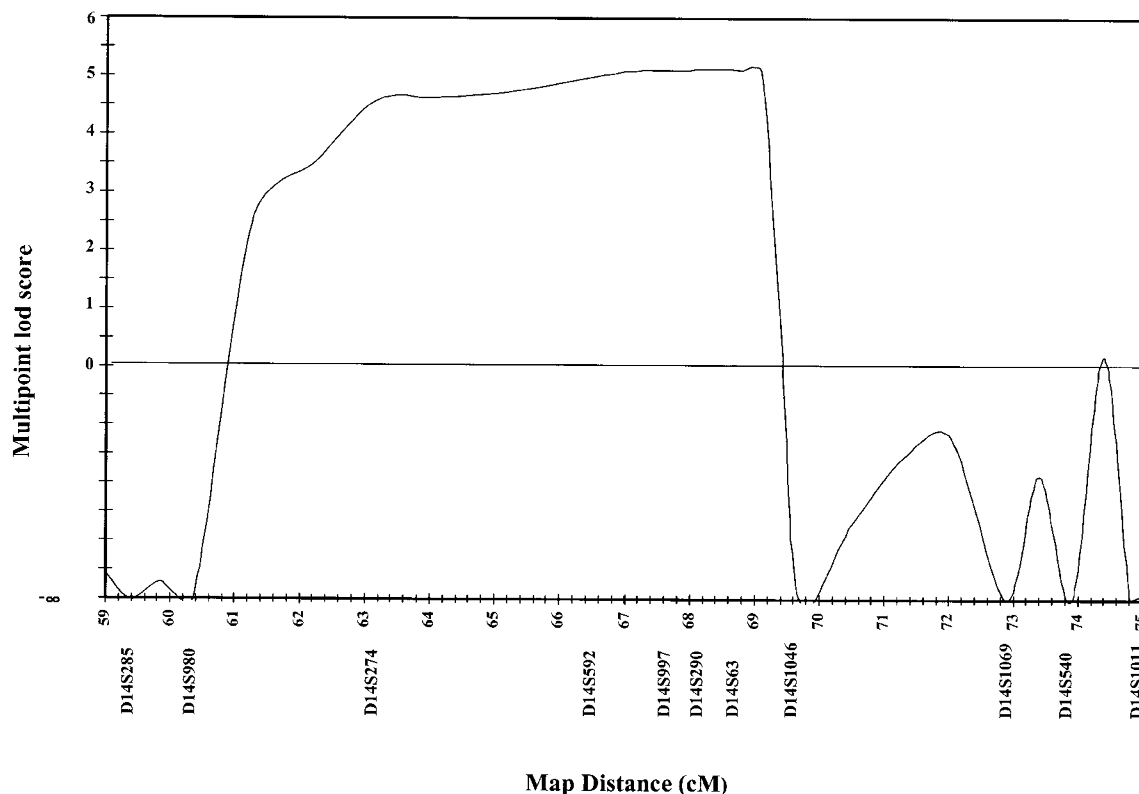

Figure 2 Results from the multipoint linkage analysis. A maximum multipoint LOD score of 5.1 occurred at marker D14S290 at 68.6 cM. The interval that DFNA23 maps to is flanked by recombination events at marker D14S980 and D14S1046.

that are expressed in the specialized cell types of the cochlea.

In the region 14q21-q22, there are a number of genes that are expressed in the human fetal cochlea: human protein kinase C-η gene (Quan and Fisher 1999); reticulon (RTN1 [MIM 600865]); kinectin (KTN1 [MIM 600381]); heat shock 10-KD protein (HSPE1 [MIM 600141]), hypoxia-inducible factor 1, alpha subunit (HIF1A [MIM 603348]); and nidogen 2 (NID2 [Kohfeldt et al. 1998]) (Human Cochlear cDNA Library and EST Database). There are also genes that map to this region that are expressed in the cochlea of nonhuman vertebrates: alpha-actinin (alpha 1) (ACTN1 [MIM 102575]), bone morphogenetic protein 4 (BMP4 [MIM 112262]), and homolog of *Drosophila* orthodenticle 2 (OTX2 [MIM 600037]) (see Bussoli and Steel, Table of Gene Expression in the Developing Ear). Among these many genes, the strongest candidate genes for DFNA23 are ACTN1, NID2, and KTN1.

ACTN1 was found to be expressed in adult rat cuticular plate, the organ of Corti, the stereocilia of the outer and inner hair cells, pillar cells, and supporting cells (Zine and Romand 1993). Not only does the expression information on ACTN1 make it an attractive candidate for DNA23, but also the fact that another widely expressed actin-associated protein, the human homologue of *Drosophila melanogaster* diaphanous (DIAPH1 [MIM 602121]), is responsible for NSHL due to DFNA1 (MIM 124900) further strengthens the evidence that ACTN1 is a candidate for DFNA23.

NID2 is also a good candidate for DFNA23, since it has been shown that NID2 can bind the networks of collagen IV and laminin in situ and thereby stabilize the basement-membrane architecture (Kohfeldt et al. 1998). Mutations within COL4A3 (MIM 120070), COL4A4 (MIM 120131), COL4A5 (MIM 303630), and COL4A6 (MIM 303631) are responsible for Alport syndrome (MIM 203780), for which one of the features is progressive sensorineural hearing loss. NID2 has also been shown to bind to collagen I (Kohfeldt et al. 1998). Mutations in COL1A1 (MIM 120150) have been shown to cause osteogenesis imperfecta congenita (OIC [MIM 166210]) with sensorineural and/or conductive hearing loss.

KTN1 is another strong candidate for DFNA23. This gene encodes a human protein with extensive coiled-coil structure, which is similar to the coiled-coil tail of the Myosin-II family (Print et al. 1994). Mutations in two myosin genes have been found to be responsible for NSHL, myosin VIIA gene (MYO7A [MIM 276903]) is

responsible for DFNB2 (MIM 600060) and DFNA11 (MIM 601317), and the Myosin 15 gene (MYO15 [MIM 602666]) is responsible for DFNB3 (MIM 600316).

Linkage of the DFNA23 locus to 14q21-q22 is an essential first step in gene identification that will provide insight into the pathophysiology of this disorder. Although the gene for DFNA23, like its autosomal dominant counterparts, will not play a major role in the etiology of hearing loss, identification of this gene will aid in understanding the mechanism of hearing and hearing loss.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Bussoli TJ and Steel KP, Table of Gene Expression in the Developing Ear, http://www.ihr.mrc.ac.uk/Hereditary/genetable/index.html (for ACTN1, BMP4, and OTX2)

Hereditary Hearing Loss Homepage, http://hgins.uia.ac.be/dnalab/hhh/ (for current number of hearing-loss loci and genes)

Human Cochlear cDNA Library and EST Database, http://hearing.bwh.harvard.edu/cochlearcdnalibrary.htm (for genes RTN1, KTN1, HSPE1, HIF1A, and NID2)

Marshfield Center for Medical Genetics, http://www.marshmed.org/genetics/ (for the order and genetic distances of STRP loci on 14q)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for ACTN1 [MIM 102575], Alport syndrome [MIM 203780], BMP4 [MIM 112262], COCH [MIM 601369], COL1A1 [MIM 120150], COL4A3 [MIM 120070], COL4A4 [MIM 120131], COL4A5 [MIM 303630], COL4A6 [MIM 303631], COL11A2 [MIM 120290], DFNA1 [MIM 124900], DFNA5 [MIM 600994], DFNA11 [MIM 601317], DFNB2 [MIM 600060], DFNB3 [MIM 600316], DIAPH1 [MIM 602121], GJB2 [MIM 121011], GJB3 [MIM 603324], GJB6 [MIM 604418], HIF1A [MIM 603348], HSPE1 [MIM 600141], KCNQ4 [MIM 603537], KTN1 [MIM 600381], MYO7A [MIM 276903], MYO15 [MIM 602666], OIC [MIM 166210], OTX2 [MIM 600037], POU4F3 [MIM 602460], RTN1 [MIM 600865] and TECTA [MIM 602574])

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