Published in final edited form as:
Am J Med Genet A. 2005 February 15; 133A(1): 18–22. doi:10.1002/ajmg.a.30508.

A Novel Autosomal Recessive Nonsyndromic Hearing Impairment Locus (DFNB42) Maps to Chromosome 3q13.31-q22.3

Muhammad Aslam[1], **Muhammad Wajid**[1], **Maria H. Chahrour**[2], **Muhammad Ansar**[1], **Sayedul Haque**[1], **Thanh L. Pham**[2], **Regie P. Santos**[2], **Kai Yan**[2], **Wasim Ahmad**[1], and **Suzanne M. Leal**[2,*]

[1] Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan

[2] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas

Abstract

A consanguineous family with autosomal recessive nonsyndromic hearing impairment (NSHI) was ascertained in Pakistan and displayed significant evidence of linkage to 3q13.31-q22.3. The novel locus (DFNB42) segregating in this kindred, maps to a 21.6 cM region according to a genetic map constructed using data from both the deCode and Marshfield genetic maps. This region of homozygosity is flanked by markers D3S1278 and D3S2453. A maximum multipoint LOD score of 3.72 was obtained at marker D3S4523. DFNB42 represents the third autosomal recessive NSHI locus to map to chromosome 3.

Keywords

3q13.31-q22.3; DFNB42; nonsyndromic hearing impairment; Pakistan

INTRODUCTION

Genetic hearing impairment can be classified as either syndromic or nonsyndromic. Several hundred syndromes for which hearing impairment is one of their clinical features have been described. They account for 30% of hearing impairment cases with a genetic etiology. Among hereditary nonsyndromic hearing impairment (NSHI), autosomal recessive inheritance predominates and accounts for approximately 75%–80% of the cases, while autosomal dominant inheritance is observed in 15% of cases. Forms of NSHI with mitochondrial and X-linked inheritance are more rarely encountered [Morton, 1991]. Autosomal dominant NSHI is usually post-lingual and progressive, whereas autosomal recessive nonsyndromic forms are generally severe to profound with prelingual onset [Petit, 1996]. NSHI is the most heterogeneous trait known. Thus far over 90 loci have been mapped and 35 genes have been identified [Van Camp and Smith, 2003]. Here is presented the mapping of a novel locus in a Pakistani kindred segregating autosomal recessive NSHI.

[*]Correspondence to: Dr. Suzanne M. Leal, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building N1619.01, Houston, TX 77030. sleal@bcm.tmc.edu.

MATERIALS AND METHODS

Family History

Before the onset of the study, approval was obtained from both the Quaid-I-Azam University and the Baylor College of Medicine Institutional Review Boards. Informed consent was obtained from all family members who participated in the study. The pedigree structure is based upon interviews with multiple family members and personal interviews with key figures in the kindred clarified consanguineous relationships. Pedigree 4012, from the Punjab province in Pakistan (Fig. 1), provided convincing evidence for autosomal recessive mode of inheritance in the form of pseudo-dominant inheritance. Although autosomal dominant hearing impairment can occur in consanguineous families, we believe this is not the case for family 4012; since the parents, grandparents, and great-grandparents of individual 11 are all unaffected. The parents (individuals 7 and 8) of individual 11 and his wife (individual 12) who is also his first cousin must all be heterozygous carriers for the hearing impairment mutation. In addition the phenotype of the hearing impaired family members is consistent with autosomal recessive NSHI. Medical history and physical examination of the affected individuals were performed by trained otolaryngologists affiliated with governmental hospitals. All affected individuals have a history of prelingual hearing impairment that involves all frequencies. The ages upon ascertainment of hearing impaired individuals range from 12 to 50 years. Regardless of age, all affected family members grossly display the same level of hearing impairment, which may indicate that the hearing impairment is not progressive. All family members who participated in the study were asked a series of medical history questions to help elucidate if hearing impairment was due to an environmental factor or was part of a syndrome. All affected individuals underwent ophthalmologic examinations. Defects in ear morphology, dysmorphic facial features, skin and hair conditions, eye disorders including night blindness and tunnel vision, limb deformities, mental retardation, and other clinical features that could indicate that hearing impairment was syndromic were not identified. There were no observations of disturbance in gait, thus suggesting there is no gross vestibular involvement. Maternal conditions such as rubella and drug use during pregnancy, perinatal trauma and meningitis were ruled out by history. In addition, all available medical records of the affected individuals were scrutinized for evidence of perinatal or childhood illness. Figure 2 displays an audiogram taken from hearing impaired individual 17, who was 12-years-old at the time of audiometric testing. Pure tone audiometry was performed in a soundproof booth with a calibrated machine, and fulfilled International Organization for Standardization (ISO) requirements. Individual 17 has bilateral sensorineural hearing impairment, with moderate hearing impairment at the low frequencies that is steeply sloping from severe at mid-frequency range to profound at 2–4 kHz. The hearing impairment was reported by the parents to be prelingual in onset and is probably congenital.

Extraction of Genomic DNA and Genotyping

Venous blood samples were obtained from nine family members five of which were hearing impaired. Genomic DNA was extracted from whole blood following a standard protocol [Grimberg et al., 1989], quantified by spectrophotometric readings at optical density 260 and diluted to 40 ng/μl for PCR amplification. A genome scan was carried out on seven DNA samples at the Center for Inherited Disease Research (CIDR). A total of 388 fluorescently labeled short tandem repeat (STR) markers were genotyped. These markers are spaced ~10 cM apart and are located on the 22 autosomes and the X and Y chromosomes. Two additional unaffected family members, individuals 18 and 19 were ascertained after the completion of the genome scan and were genotyped for markers within the region which was suggestive for linkage.

Linkage Analysis

Two-point linkage analysis was carried out using the MLINK program of the FASTLINK computer package [Cottingham et al., 1993] and multipoint linkage analysis was performed using ALLEGRO [Gudbjartsson et al., 2002]. Haplotypes were constructed using SIMWALK2 [Weeks et al., 1995; Sobel and Lange, 1996]. An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were used for the analysis. Marker allele frequencies were estimated from the founders and reconstructed genotypes of founders from this family and twelve additional families from Pakistan which underwent a genome scan at the same time at CIDR. For the fine mapping markers it was not possible to estimate allele frequencies from the founders, because these markers were only genotyped in this family. Moreover, since false positive results can be obtained when analyzing the data using a too low of an allele frequency for the allele segregating with the disease locus [Freimer et al., 1993], a sensitivity analysis was carried out for the multipoint linkage analysis by varying the allele frequency of the allele that is segregating with the disease locus from 0.2 to 0.8 for the fine mapping markers.

In addition, there was concern that some consanguineous relationships within the pedigree were not reported, which would affect the LOD score results. Individuals 6 and 10 who married into the family are not from the same village as their spouses, and therefore it is unlikely that they are related to their spouses or other family members. Although it was reported that individuals 3 and 4 are not related to each other but since they are from the same village we cannot rule out that this mating is not consanguineous. Therefore, besides carrying out the linkage analysis with the reported pedigree structure, we also analyzed the pedigree assuming individuals 3 and 4 are first cousins, individuals 1 and 2 (the pedigree founders) are first cousins, and assuming both scenarios had occurred. Only two-point LOD scores were calculated to make the comparisons, since in order to calculate multipoint LOD scores with these additional relationships, the pedigree structure would have to be broken into two parts in order to analyze it using ALLEGRO. This could also bias the results. It should be noted that for the original analysis the pedigree structure was analyzed in its entirety.

A genetic map was constructed for the multipoint analysis using both the Centre d'Etude du Polymorphisme Humain (CEPH) [Dausset et al., 1990] and deCode [Kong et al., 2002] genotype data using MAP-O-MAT [Lander and Green, 1987; Matise and Gitlin, 1999], because not all markers that were used for fine mapping appeared on either the Marshfield [Broman et al., 1998] or deCode genetic maps.

RESULTS

Analysis of the genome scan data identified an area of interest on chromosome 3q. Two-point analysis generated a maximum LOD score of 2.6 for marker D3S4523 at recombination fraction 0, and multipoint linkage analysis also produced a maximum LOD score of 2.6 at this same marker. In addition, maximum multipoint LOD scores of 1.4, 2.5, and 1.6 were obtained at chromosomes 7, 8, and 21 respectively. None of the other chromosomes gave LOD scores above 0.8. An additional number of markers were chosen in the regions of interest on chromosomes 7 (14 markers), 8 (26 markers), and 21 (11 markers), were genotyped in all nine samples and linkage analyses were performed. The two-point and multipoint linkage analysis of the additional and the genome scan markers for chromosomes 7, 8, and 21 did not give significant results, with maximum multipoint LOD scores of 1.0, 1.7, and 0.09, respectively.

In order to establish linkage and fine map the DFNB42 locus on chromosome 3, 27 additional markers were selected from the Marshfield and deCode genetic maps; 14 markers

are proximal to D3S4523 (D3S4551, D3S2496, D3S2422, D3S4018, D3S1518, D3S1278, D3S3529, D3S1558, D3S2460, D3S3649, D3S1303, D3S3703, D3S2302, and D3S3513) and 13 of the markers are distal (D3S3720, D3S4011, D3S2316, D3S1765, D3S3636, D3S3584, D3S3607, D3S1541, D3S2322, D3S3657, D3S1238, D3S1590, and D3S2453). Only six of the fine mapping markers were informative for linkage, although the markers had an average heterozygosity of 0.71. Table I summarizes the two-point LOD scores obtained for six fine mapping markers and three genome scan markers. The maximum two-point LOD score remained at genome scan marker D3S4523, however, it increased to 3.21 ($\theta = 0$) with the genotyping of the two additional individuals, 18 and 19. Multipoint linkage analysis for the family gave a maximum LOD score of 3.72 also at marker D3S4523. When the marker allele frequencies were varied for the fine mapping markers from 0.2 to 0.8, the maximum multipoint LOD score remained at marker D3S4523 and varied from 3.90 to 3.23, respectively.

When two-point linkage analysis was carried out assuming additional consanguineous relationships, the LOD score at marker D3S4523 changed only slightly from the LOD score obtained using the original pedigree structure. Assuming individuals 3 and 4 are first cousins the maximum two-point LOD score is 3.17, when the founders individuals 1 and 2 are considered to be first cousins the LOD score is 3.18, and for both scenarios combined the LOD score is 3.14.

The 3-unit support interval contained a 21.6 cM region which spanned form marker D3S1278 to marker D3S2453. Haplo-types were then constructed to determine the critical recombination events. The disease haplotype (region of homozygosity) was the same region contained within the 3-unit support interval. This region corresponds to a physical map distance of 21.2 Mb [International Human Genome Sequence Consortium, 2001]. The critical recombinations defining the co-segregating interval occurred in affected individuals. The centromeric boundary of this interval was defined by a recombination between markers D3S1278 and D3S4523 observed in individual 16. The telomeric boundary of this interval corresponds to a historic recombination event between markers D3S1765 and D3S2453, which was observed in individual 11.

DISCUSSION

The majority of families from Pakistan with deafness that this research group is studying are consanguineous. Of the 196 families studied thus far, 94% of the pedigrees display one or more consanguineous matings. Family 4012 (Fig. 1) presented with two consanguinity loops. The parents of affected individual 11 (individuals 7 and 8) are first cousins and the inbreeding coefficient for individual 11 is 0.0625. Individuals 11 and 12, parents of individuals 13 through 19, are first cousins, and the inbreeding coefficient for individuals 13 through 19 is 0.0781.

Phenotypic information and the pedigree structure gave initial evidence of autosomal recessive inheritance for family 4012. Autosomal recessive inheritance was further supported by the results of this study. The probability of observing by chance a 21.6 cM region of homozygosity in all five hearing impaired family members and in none of the four hearing family members is extremely low ($P < 0.0001$).

The linkage data presented suggest that a gene for autosomal recessive nonsyndromic prelingual deafness is located on chromosome 3q13.31-q22.3. Four other loci for inherited deafness DFNB6 (3p14-p21) [Fukushima et al., 1995], DFNB15 (3q21-q25) [Chen et al., 1997], DFNA18 (3q22) [Bonsch et al., 2001], and DFNA44 (3q28-q29) [Modamio-Hoybjor et al., 2003] have been previously mapped on chromosome 3. For these four loci only one

gene, *TMIE*, has been identified for DFNB6. A loss of function mutation in *TMIE* gene is responsible for deafness in humans and mice indicating its critical role in the auditory system [Naz et al., 2002].

The DFNB42 (3q13.31-q22.3) locus overlaps with DFNA18 (3q22). Although two genes in close proximity might cause DFNB42 and DFNA18, it is also possible that hearing loss in DFNB42/DFNA18 families is caused by different mutations in the same gene. It has been observed for several forms of NSHI that different mutations in the same gene cause an autosomal dominant and recessive form of NSHI (e.g., *GJB2*, *MYO7A*, *TECTA*, and *TMC1*) [Van Camp and Smith, 2003]. It should also be noted that the region of homozygosity for DFNB42 does not overlap with that of the DFNB15 locus. The DFNB42 region starts at marker D3S4523 (at 122, 212, 964 Mb) to marker D3S1765 (at 125, 689, 392 Mb); while the first marker indicated in the DFNB15 region is D3S1290 (at 134, 311, 827 Mb) [Chen et al., 1997].

The DFNB42 interval contains a number of known genes. Among the candidate genes for nonsyndromic deafness, are solute carrier family members *SLC21A2* (MIM 601460) and *SLC15A2* (MIM 602339). These genes encode transport proteins and show homology with *SLC26A4* (MIM 605646). Mutations in the *SLC26A4* gene are responsible for both NSHI (DFNB4) and Pendred syndrome and account for approximately 5% of recessive deafness in south Asians and other populations [Park et al., 2003]. Additional candidates within the region include *LSAMP* (MIM 603241) which codes for a neural cell adhesion molecule [Pimenta et al., 1996]. Proximal Myotonic Myopathy (PROMM) (MIM 602668) has also been mapped in the region 3q13.3-q24 and *ZNF9* has been implicated as the causative gene. Hearing loss has been described as one of the features of PROMM phenotype, however it should be noted that *ZNF9* was recently excluded as the cause of NSHI in a family segregating DFNA18 [Bonsch et al., 2003]. The promoter and exonic regions of *SLC15A2* and *ZNF9* were sequenced in two hearing impaired individuals and one unaffected individual from family 4012 and no causal variants were found. Localizing DFNB42 is the first step in identifying the causative gene underlying the HI phenotype.

Acknowledgments

Grant sponsor: Higher Education Commission (HEC) Pakistan; Grant sponsor: American Hearing Research Foundation; Grant sponsor: NIH-National Institute of Deafness and other Communication Disorders; Grant number: DC03594; Grant sponsor: National Institutes of Health (to The Johns Hopkins University); Grant number: N01-HG-65403.

We wish to thank the family members for their invaluable participation and cooperation. Genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, Contract Number N01-HG-65403.

References

Bonsch D, Neumann C, Lang-Roth R, Witte O, Lamprecht-Dinnesen A, Deufel T. PROMM and deafness: Exclusion of ZNF9 as the disease gene in DFNA18 suggests a polygenic origin of the PROMM/DM2 phenotype. Clin Genet 2003;63:73–75. [PubMed: 12519376]

Bonsch D, Scheer P, Neumann C, Lang-Roth R, Seifert E, Storch P, Weiller C, Lamprecht-Dinnesen A, Deufel T. A novel locus for autosomal dominant, non-syndromic hearing impairment (DFNA18) maps to chromosome 3q22 immediately adjacent to the DM2 locus. Eur J Hum Genet 2001;9:165–170. [PubMed: 11313754]

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL. Comprehensive human genetic maps: Individual and sex-specific variation in recombination. Am J Hum Genet 1998;63:861–869. [PubMed: 9718341]

Chen A, Wayne S, Bell A, Ramesh A, Srisailapathy CR, Scott DA, Sheffield VC, Van Hauwe P, Zbar RI, Ashley J, Lovett M, Van Camp G, Smith RJ. New gene for autosomal recessive non-syndromic hearing loss maps to either chromosome 3q or 19p. Am J Med Genet 1997;71:467–471. [PubMed: 9286457]

Cottingham RW Jr, Indury RM, Schaffer AA. Faster sequential genetic linkage computations. Am J Hum Genet 1993;53:252–263. [PubMed: 8317490]

Dausset J, Cann H, Cohen D, Lathrop M, Lalouel JM, White R. Centre d'etude du polymorphisme humain (CEPH): Collaborative genetic mapping of the human genome. Genomics 1990;6:575–577. [PubMed: 2184120]

Freimer NB, Sandkuijl LA, Blower SM. Incorrect specification of marker allele frequencies: Effects on linkage analysis. Am J Hum Genet 1993;52:1102–1110. [PubMed: 8503444]

Fukushima K, Ramesh A, Srisailapathy CR, Ni L, Wayne S, O'Neill ME, Van Camp G, Coucke P, Jain P, Wilcox ER. An autosomal recessive non-syndromic form of sensorineural hearing loss maps to 3p-DFNB6. Genome Res 1995;5:305–308. [PubMed: 8593615]

Grimberg J, Nawoschik S, Bellusico L, McKee R, Turck A, Eisenberg A. A simple and efficient non-organic procedure for the isolation of genomic DNA from Blood. Nucleic Acid Res 1989;17:83–90.

Gudbjartsson DF, Jonasson K, Frigge ML, Kong A. Allegro, a new computer program for multipoint linkage analysis. Nat Genet 2002;25:12–13. [PubMed: 10802644]

International Human Genome Sequence Consortium. Initial sequence and analysis of the human genome. Nature 2001;409:860–921. [PubMed: 11237011]

Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. A high-resolution recombination map of the human genome. Nat Genet 2002;31:241–247. [PubMed: 12053178]

Lander ES, Green P. Construction of multi-locus genetic linkage maps in humans. Proc Natl Acad Sci 1987;84:2363–2367. [PubMed: 3470801]

Matise TC, Gitlin JA. MAP-O-MAT: Marker-based linkage mapping on the World Wide Web. Am J Hum Genet 1999;65:A2464.

ModamSio-Hoybjor S, Moreno-Pelayo MA, Mencia A, del Castillo I, Chardenoux S, Morais D, Lathrop M, Petit C, Moreno F. A novel locus for autosomal dominant nonsyndromic hearing loss (DFNA44) maps to chromosome 3q28-29. Hum Genet 2003;112:24–28. [PubMed: 12483295]

Morton NE. Genetic epidemiology of hearing impairment. Ann NY Acad Sci 1991;630:16–31. [PubMed: 1952587]

Naz S, Giguere CM, Kohrman DC, Mitchem KL, Riazuddin S, Morell RJ, Ramesh A, Srisailpathy S, Deshmukh D, Riazuddin S, Griffith AJ, Friedman TB, Smith RJ, Wilcox ER. Mutations in a novel gene, *TMIE*, are associated with hearing loss linked to the DFNB6 locus. Am J Hum Genet 2002;71:632–636. [PubMed: 12145746]

Park HJ, Shaukat S, Liu XZ, Hahn SH, Naz S, Ghosh M. Origins and frequencies of SLC26A4 (PDS) mutations in east and south Asians: Global implications for the epidemiology of deafness. J Med Genet 2003;40:242–248. [PubMed: 12676893]

Petit C. Genes responsible for human hereditary deafness: Symphony of a thousand. Nat Genet 1996;14:385–391. [PubMed: 8944017]

Pimenta AF, Fischer I, Levitt P. cDNA cloning and structural analysis of the human limbic-system-associated membrane protein (LAMP). Gene 1996;170:89–195.

Sobel E, Lange K. Descent graphs in pedigree analysis: Applications to haplotyping, location scores, and marker-sharing statistics. Am J Hum Genet 1996;58:1323–1337. [PubMed: 8651310]

Van Camp, G.; Smith, RJH. Hereditary Hearing Loss Homepage. 2003. URL: http://www.uia.ac.be/dnalab/hhh

Weeks DE, Sobel E, O'Connell JR, Lange K. Computer programs for multilocus haplotyping of general pedigrees. Am J Hum Genet 1995;56:1506–1507. [PubMed: 7762577]

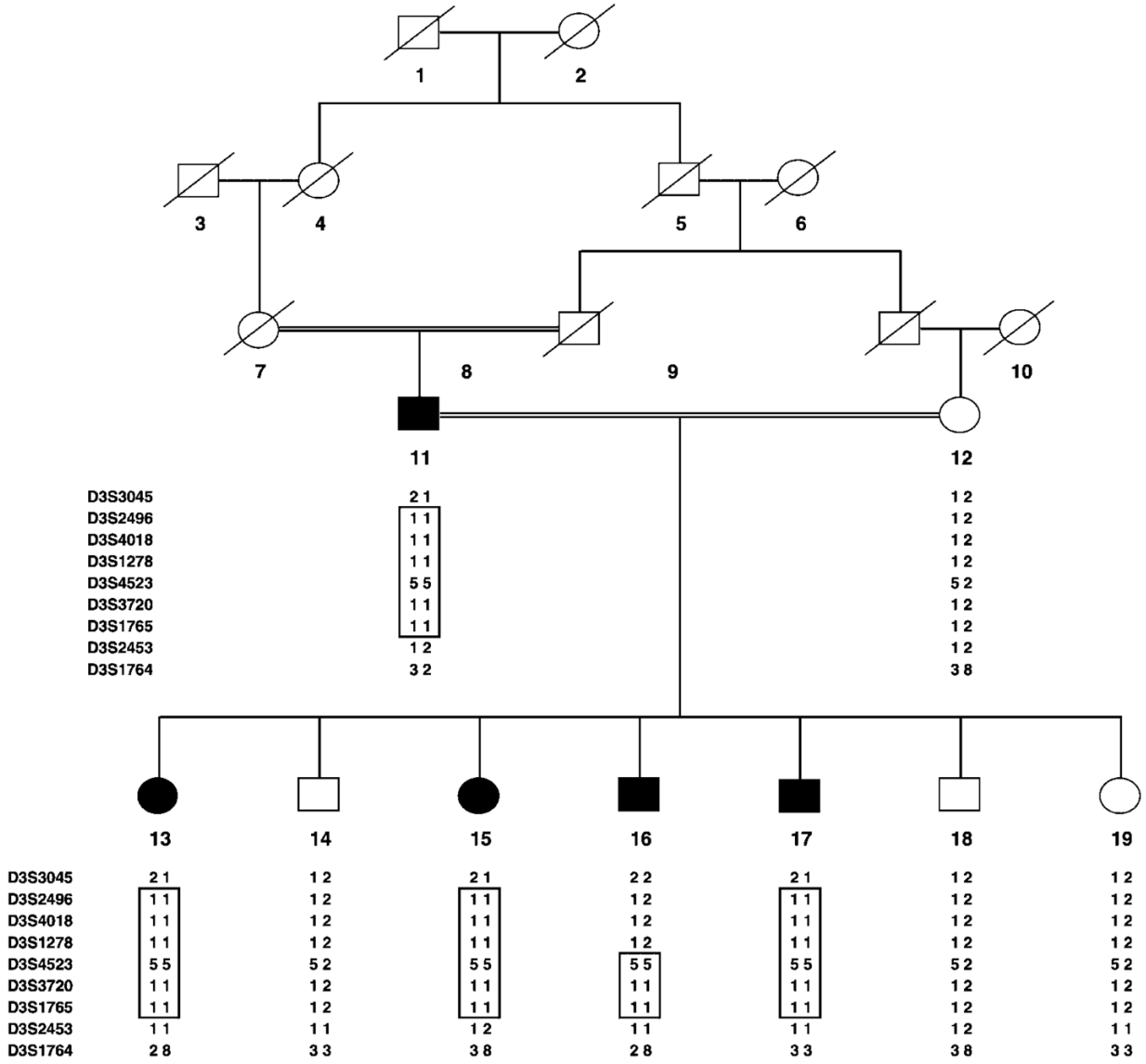

Fig. 1.

Pedigree 4012, which segregates DFNB42. Black symbols represent individuals with hearing impairment due to DFNB42. Clear symbols represent unaffected individuals. Haplotypes for the most closely linked STRs are shown below each symbol. DFNB42 haplotype is displayed in a box.

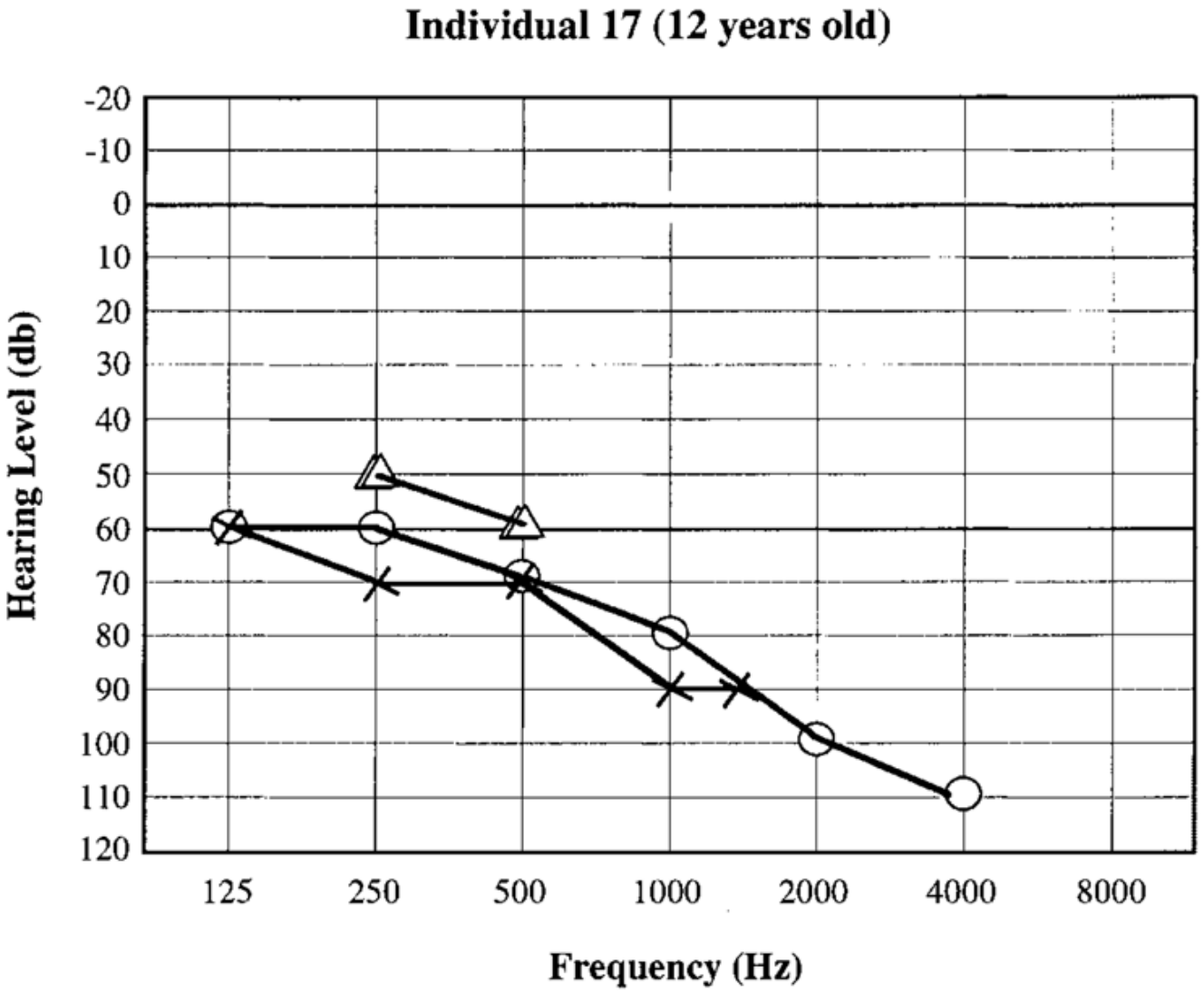

Fig. 2.

Audiogram from affected individual 17 of pedigree 4012. The age denoted represents the age at which the audiogram was taken. The following symbols represent: o, air conduction for right ear; ×, air conduction for left ear; Δ, bone conduction for both ears.

TABLE I

Two-Point LOD Score Results Between the DFNB42 Locus and Chromosome 3 Markers

Marker	Marshfield map position[a]	deCode map position[b]	Combined map (MAP-O-MAT)[c]	Physical map position[d]	LOD score at θ = 0.0	0.02	0.04	0.05	0.1	0.2	0.3
D3S3045	**124.16**	**117.29**	**0.0**	**108,310,758**	**−1.65**	**−0.60**	**−0.36**	−0.28	**−0.09**	**0.01**	**0.02**
D3S2496	126.83	119.99	2.4	112,383,739	−∞	0.59	0.81	0.86	0.94	0.81	0.55
D3S4018	127.89	120.11	2.8	112,975,115	−∞	0.59	0.81	0.86	0.94	0.81	0.55
D3S1278	129.73	—	4.2	116,445,055	−∞	0.59	0.81	0.86	0.94	0.81	0.55
D3S4523	—	**126.98**	**11.2**	**122,212,964**	**3.21**	**3.09**	**2.96**	**2.90**	**2.58**	**1.92**	**1.24**
D3S3720	139.12	127.80	11.5	123,048,811	2.38	2.28	2.19	2.14	1.90	1.41	0.91
D3S1765	139.12	—	11.5	125,689,392	2.38	2.28	2.19	2.14	1.90	1.41	0.91
D3S2453	—	144.11	25.8	137,598,675	−∞	−3.09	−2.25	−1.98	−1.19	−0.51	−0.20
D3S1764	**152.62**	**145.53**	**27.5**	**140,509,197**	−∞	**−3.67**	**−2.58**	**−2.24**	**−1.25**	**−0.46**	**−0.18**

[a] Markers displayed in italic flank the haplotype. Genome scan markers are shown in bold.

[b] Sex-averaged kosambi cM map distance from the Marshfield genetic map.

[c] Sex-averaged kosambi cM map distances estimated from the CEPH and deCode data using MAP-O-MAT.

[d] Sequence-based physical map distance in bases according to Human Genome Project, Santa Cruz.

Am J Med Genet A. Author manuscript; available in PMC 2010 July 23.