Published in final edited form as:

Hum Genet. 2006 August ; 120(1): 85–92. doi:10.1007/s00439-006-0188-z.

***DFNB68*, a novel autosomal recessive non-syndromic hearing impairment locus at chromosomal region 19p13.2**

Regie Lyn P. Santos,

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building N1619.01, Houston, TX 77030, USA. Genetic Epidemiology Unit, Department of Epidemiology and Biostatistics, Erasmus Medical Centre, Rotterdam, The Netherlands

Muhammad Jawad Hassan,

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

Shaheen Sikandar,

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

Kwanghyuk Lee,

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building N1619.01, Houston, TX 77030, USA

Ghazanfar Ali,

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

Protacio E. Martin Jr,

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building N1619.01, Houston, TX 77030, USA

Michael Angelo L. Wambangco,

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building N1619.01, Houston, TX 77030, USA

Wasim Ahmad, and

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

Suzanne M. Leal

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Alkek Building N1619.01, Houston, TX 77030, USA

Suzanne M. Leal: sleal@bcm.tmc.edu

Abstract

From a large collection of families with autosomal recessive non-syndromic hearing impairment (NSHI) from Pakistan, linkage has been established for two unrelated consanguineous families to 19p13.2. This new locus was assigned the name *DFNB68*. A 10 cM genome scan and additional fine mapping were carried out using microsatellite marker loci. Linkage was established for both families to *DFNB68* with maximum multipoint LOD scores of 4.8 and 4.6. The overlap of the homozygous regions between the two families was bounded by D19S586 and D19S584, which limits the locus interval to 1.9 cM and contains 1.4 Mb. The genes *CTL2*, *KEAP1* and *CDKN2D* were screened but were negative for functional sequence variants.

Correspondence to: Suzanne M. Leal, sleal@bcm.tmc.edu.

Regie Lyn P. Santos and Muhammad Jawad Hassan contributed equally to this work.

Introduction

The extreme genetic heterogeneity for hearing impairment (HI) testifies to the inherent complexity of the mammalian inner ear. As more HI genes are identified, the elucidation of the function of the proteins that these genes encode contributes greatly to the understanding of cochlear mechanisms and their role in disease causation. Currently, 37 genes have been identified for non-syndromic hearing impairment (NSHI; Van Camp and Smith, 2005); however, for more than 60% of the mapped NSHI loci a gene has yet to be identified. Many of these loci with an unidentified gene were mapped in single families, some of which are consanguineous. The size of the mapped interval plus the number of candidate genes within the interval, if large, may make the task of finding the causative gene daunting. The identification of linkage of multiple NSHI families to a single locus validates the linkage finding and can make gene identification more attainable due to a smaller physical interval for the NSHI locus.

The *DFNB68* locus is within the chromosome 19 region for *DFNB15*. The *DFNB15* locus interval was previously mapped in a consanguineous Indian family to two chromosomal locations, 19p13.3–p13.1 and 3q21.3–q25.2. Linkage to both of these regions was based on a non-significant LOD score of 2.8 with a genetic region of ≥ 32 cM intervals on both chromosomes 3 and 19 (Chen et al. 1997). Presented here are two Pakistani families with autosomal recessive NSHI that exclusively maps to chromosome 19p13.2, with highly significant multipoint LOD scores of 4.8 and 4.6. The overlapping region of homozygosity for these two families is 1.9 cM. Since it is not clear whether the *DFNB15* locus is on chromosome 3 or 19, the NSHI locus segregating in these two Pakistani families has been designated as novel NSHI locus *DFNB68*.

Materials and methods

Family history

Prior to onset the study was approved by the Institutional Review Boards of the Quaid-I-Azam University and Baylor College of Medicine and Affiliated Hospitals. Informed consent was secured from all individuals who agreed to participate in the research. Family 4100 resides in the southern part of Punjab province, while family 4154 hails from Sind province. For both kindreds, family members rarely marry outside the community, and consequently, consanguineous unions are common. The pedigree drawings (Fig. 1) provided convincing evidence of autosomal recessive mode of inheritance in both families, and consanguineous loops accounted for all the affected persons being homozygous for the mutant allele. Clinical findings in these families are consistent with the diagnosis of autosomal recessive NSHI. All affected individuals have a history of prelingual profound HI and communicate by sign language. Affected individuals underwent examination for defects in ear morphology, dysmorphic facial features, eye disorders including night blindness and tunnel vision, limb deformities, mental retardation and other clinical features that could indicate that HI was syndromic, and were all negative for findings. No gross vestibular involvement was noted from the clinical history and physical examination.

Extraction of genomic DNA and genotyping

Peripheral blood samples were taken from ten members in family 4100 including five hearing-impaired individuals, and eleven members from family 4154, six of whom had HI. Genomic DNA was extracted from whole blood following a standard protocol (Grimberg et al. 1999), quantified by spectrophotometric reading at optical density 260, and diluted to 40 $\mu\text{g}/\mu\text{l}$ for amplification by polymerase chain reaction (PCR). Genome scans were carried out at the Center for Inherited Disease Research (CIDR) and at the National Heart, Lung and

Blood Institute (NHLBI) Mammalian Genotyping Service (Marshfield, WI, USA). An average of 399 fluorescently labeled, short tandem repeat markers were genotyped across the 22 autosomes and X and Y chromosomes at approximately 10 cM apart. For fine mapping, microsatellite markers were PCR amplified according to standard procedure in a total volume of 25 μ l with 40 ng genomic DNA, 240 η M of primer (Invitrogen Corp., Carlsbad, CA, USA), 200 μ M dNTP, and 1 unit of Taq DNA Polymerase (Fermentas Life Sciences, Burlington, ON, Canada) in GeneAmp[®] PCR System 9700 (Applied Biosystems, Applera Corp., Foster City, CA, USA). PCR products were resolved on 8% non-denaturing polyacrylamide gel and genotypes were assigned by visual inspection.

Linkage analysis

PEDCHECK (O'Connell and Weeks 1998) was used to identify Mendelian inconsistencies while the MERLIN (Abecasis et al. 2002) program was utilized to detect potential genotyping errors that did not produce a Mendelian inconsistency. For both genome scan and fine-mapping markers, two-point linkage analysis was carried out with the MLINK program of the FAST-LINK computer package (Cottingham et al. 1993) and multipoint linkage analysis was performed using ALLEGRO (Gudbjartsson et al. 2002). An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were assumed. Genome scan marker allele frequencies were estimated from the founders and reconstructed genotypes of founders from these pedigrees and additional pedigrees that underwent a genome scan at the same time. For the fine-mapping markers, it was not possible to estimate allele frequencies from the founders because these markers were only genotyped in these two families, so equal allele frequencies were applied initially. Since false positive results can be obtained when analyzing the data using too low of an allele frequency for an 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 0.6 for the fine-mapping markers. In order to determine the order of fine-mapping and genome scan markers, the physical position of each marker was determined from the National Center for Biotechnology Information (NCBI) Build 34 sequence-based physical map (International Human Genome Sequence Consortium 2001). Genetic map distances were then derived from the Rutgers combined linkage-physical map of the human genome (Kong et al. 2004), either directly or by interpolation. After linkage analyses, haplotypes were constructed via SIMWALK2 (Weeks et al. 1995; Sobel and Lange 1996).

Candidate gene sequencing

Using Primer3 software (Rozen and Skaletsky 2000), primers were designed for the exons of the following genes: *MYO1F* (MIM 601480; NM_012335); *KEAP1* (MIM 606016; NM_012289); *SLC44A2/CTL2* (MIM 606106; NM_020428); and *CDKN2D* (MIM 600927; NM_001800). From each family, DNA from one unaffected and two hearing-impaired individuals were diluted to 5 μ g/ η l, amplified by PCR under standard conditions, and purified with ExoSAP-IT[®] (USB Corp., Cleveland, OH, USA). Sequencing was performed with the BigDye[®] Terminator v3.1 Cycle Sequencing Kit together with an Applied Biosystems 3700 DNA Analyzer (Applera Corp., Foster City). Sequence variants were identified via Sequencher[™] Version 4.1.4 software (Gene Codes Corp., Ann Arbor, MI, USA).

Results

Two-point linkage analysis of the genome scan markers generated a maximum LOD score at $\theta=0$ of 2.2 for family 4100 and 3.3 for family 4154; both LOD scores were derived at marker D19S586. For the multipoint analysis, the maximum LOD score was obtained also at

genome scan marker D19S586 for both families, with a score of 3.0 for family 4100 and 3.2 for family 4154.

In order to fine map the region, 23 additional markers from the Marshfield genetic map (Broman et al. 1998) plus five markers from the Rutgers combined linkage-physical map (Kong et al. 2004) were selected and genotyped in both families. The results of the two-point linkage analyses are presented in Table 1, which shows the LOD scores that were derived at each marker at $\theta=0$. The highest two-point LOD score for family 4100 was 2.3 at markers D19S583 ~ D19S581 and D19S558, and for family 4154 it was 3.3 at D19S586. Note that some of the markers were uninformative for one of the two families (Table 1); these markers were removed while performing multipoint linkage analyses and haplotype reconstruction for the family for which the markers were uninformative.

For family 4100, two peaks in the multipoint LOD scores can be seen: one with a maximum LOD score of 4.8 at D19S581, and a second peak with a value of 4.7 at marker D19S558. For the first peak, the three-unit support interval was delimited by markers D19S884 and D19S906. The second peak was bordered by D19S914 and D19S432. When sensitivity analysis was carried out by varying the frequencies of the fine-mapping marker alleles which segregate with the disease phenotype between 0.2 and 0.6, the maximum LOD score fluctuated from 5.1 to 3.2 but remained at the same markers D19S581 and D19S558.

On the other hand, for family 4154, the maximum LOD score after multipoint linkage analysis was 4.6 at markers D19S432 ~ D19S714 ~ D19S252, with the three-unit support interval between markers D19S1034 and D19S199. When sensitivity analyses were performed at marker allele frequencies 0.2–0.6, the maximum multipoint LOD score was maintained at 4.5–4.6, not only at markers D19S432 ~ D19S714 ~ D19S252 but also at markers D19S581 ~ D19S584.

The three-unit support interval for family 4154 contains 10.8 Mb according to the human reference sequence and has a length of 21.8 cM based on the Rutgers combined linkage-physical map. In comparison, the 3-unit support interval for family 4100 is smaller, with 8.2 cM/3.7 Mb for the first peak and 5.8 cM/2.7 Mb for the second peak, both of which are completely contained within the interval for family 4154.

To further delineate the boundaries of the *DFNB68* locus, haplotypes were reconstructed for each family (Fig. 1). In four hearing-impaired siblings from family 4100, the region of homozygosity is bounded by markers D19S884 and D19S432 (Fig. 1a), and therefore spans the two 3-unit support intervals (includes 7.5 Mb and is 14.3 cM long). The region of homozygosity in family 4154 is the same as the 3-unit support interval, and is delimited at the proximal end by D19S1034 in all affected family members, and at the distal end by marker D19S199 due to a historic recombination event in individual V-6 (Fig. 1b). Upon closer inspection, however, the unaffected individuals from both families are also homozygous for several of the markers within the region of homozygosity. If we take into consideration solely those markers that are homozygous only in the hearing-impaired but not among unaffected relatives, the true region of homozygosity in family 4100 is bounded by D19S586 and D19S906, while in family 4154 the interval is bordered by D19S905 and D19S584. The overlap between these two regions from the two families is flanked by markers D19S586 and D19S584, which can now be assigned as the limits of the *DFNB68* locus. The genetic interval for the *DFNB68* locus is 1.9 cM and contains 1.4 Mb.

Within the 1.4 Mb region of homozygosity, there are 40 known genes, of which four encode hypothetical proteins (Build 34 of the human reference sequence as seen from the University of California Santa Cruz Genome Browser). Five genes were found within published inner ear databases (The Hearing Research Group at Brigham and Women's Hospital 2002;

Holme et al. 2002), namely, *DNMT1* (MIM 126375), *ICAM1* (MIM 147840), *KEAP1*, *CTL2*, and *SMARCA4* (MIM 603254) (Fig. 2). The genes *KEAP1* and *CTL2* were screened by sequencing in the two families, but no functional sequence variants were identified. The *CDKN2D* gene, which is homologous to hearing loss gene *Ink4d* in mice (Chen et al. 2003), was also sequenced; however no functional variants were found in the coding exons of *CDKN2D*.

Discussion

The *DFNB15* locus was previously mapped to two regions, 3q21.3–q25.2 and 19p13.3–p13.1 (Chen et al. 1997). However, it has not been established which one of these regions actually holds the causative HI gene, or if there is digenic inheritance for the family involved. One caveat was that the maximum LOD score that could ever be derived for the *DFNB15* family was less than 3.0, and analyses of both regions on chromosomes 3 and 19 both resulted in a LOD score of 2.8. The new locus described here is contained within the *DFNB15* interval on chromosome 19 (Fig. 2). Additionally, for the two families reported here, there is no evidence of linkage to chromosome 3q or to any other chromosome, discounting any possibility of digenic inheritance. Nevertheless, this does not bolster or refute the hypothesis that an HI gene in the 19p13 region caused the HI in the *DFNB15* family. Due to the lack of evidence that the HI locus in this article is the same as *DFNB15*, this novel locus on 19p13.2 was assigned as *DFNB68*. Only one candidate gene, *MYO1F*, a member of the unconventional myosin family (which includes HI genes *MYO1A* (MIM 601478), *MYO6* (MIM 600970), *MYO7A* (MIM 276903), and *MYO15* (MIM 602666)), was reported to have been sequenced in the *DFNB15* family, but no mutation was found (Chen et al. 2001). Likewise, the *MYO1F* gene had no functional sequence variants in families 4100 and 4154. This is not surprising given that *MYO1F* lies outside the 1.9 cM region of *DFNB68* (Fig. 2).

Although high LOD scores were obtained for the two families at the more proximal marker D19S391 and at distal markers D19S558 and D19S840 (Table 1), it was discovered that at these markers the families were dissimilar in terms of the allele that was homozygous among affected individuals in each family. On the other hand, hearing-impaired individuals in both families are homozygous for the same alleles at contiguous markers from D19S586 to D19S914, though some of these markers were non-informative for each family. Based on the segregation of alleles among hearing impaired and hearing members of each pedigree, the assigned region of homozygosity for each pedigree decreased in length (Fig. 1) and from these smaller intervals the region of overlap was designated as the *DFNB68* locus. Currently we are concentrating our search for candidate genes within the 1.4 Mb region of overlap seen in the two families, which is between markers D19S586 and D19S584 (Fig 2). It is not possible to conclude if both families have HI due to the same functional variant, due to different functional variants within the same gene, or even due to two different genes. Nevertheless, the observation of the same haplotypes in both families within the 1.9 cM region of homozygosity suggests that they may segregate the same variant.

Of the 40 genes within the overlapping regions of homozygosity, two candidate genes in the inner ear databases, *KEAP1* and *CTL2*, were selected for sequencing in the *DFNB68* families. *KEAP1* (Kelch-like Ech-associated protein 1) is a functional repressor of transcription factor *NRF2* (MIM 600492), which is a regulator of detoxifying and antioxidant genes. It was discovered that the Kelch repeat domain of the *KEAP1* protein associates with the SH3 domain of *MYO7A* in specialized adhesion junctions in the testis (Velichkova et al. 2002). Moreover, both *KEAP1* and *MYO7A* are expressed in cell bodies of cochlear inner hair cells, and it was proposed that *KEAP1* facilitates the association of *MYO7A* with the actin cytoskeleton of hair cell stereocilia, thus promoting cell adhesion that

is important for stereocilia bundle organization. Screened family members of 4100 and 4154 were negative for functional sequence variants in the coding regions of the *KEAP1* gene.

The *CTL2* (choline-transporter like protein 2) gene encodes a highly conserved transmembrane protein which is the target of antibody-mediated hair cell loss (Nair et al. 2004). The protein is expressed in cochlear and vestibular supporting cells, and is purported to function in these cells as a choline transporter. However, DNA sequencing in families that were linked to *DFNB68* revealed no functional variants in the *CTL2* gene.

The *CDKN2D* (cyclic-dependent kinase inhibitor 2D) gene is the human homolog of mouse gene *Ink4d*, which is required to maintain the inner hair cells in the post-mitotic state (Chen et al. 2003). In *Ink4d* knockout mice, re-entry of the inner hair cells into the cell cycle resulted in apoptotic cell death and subsequently progressive hearing loss. Although *CDKN2D* was not identified in the inner ear databases, other kinase inhibitors (*CDKN1A*, *CDKN1B*) were also found to be expressed in the inner ear (The Hearing Research Group at Brigham and Women's Hospital 2002). Members of families 4100 and 4154 did not carry functional variants within the coding exons of *CDKN2D*.

Three other genes within the *DFNB68* interval, *DNMT1*, *ICAM1*, and *SMARCA4*, were found in the inner ear databases. For these genes, there is currently no additional information that is linked to hearing physiology. There is, however, a report of the *ICAM1* mRNA expression by cultured spiral ligament fibro-cytes after cytokine (TNF- α) stimulation (Ichimiya et al. 2003), which may suggest a role for *ICAM1* in immune-mediated inner ear disease. Based on a positional cloning approach, the genes within the *DFNB68* interval will be investigated further for a possible role in HI causation.

Acknowledgments

We thank the two families who participated in this research. We are also grateful to Xuan Zhang for assistance with the figures. This work was supported by the Higher Education Commission, Pakistan, and National Institutes of Health, National Institute of Deafness and other Communication Disorders grant R01-DC03594. Genotyping services were provided by the CIDR and the NHLBI Mammalian Genotyping Service (Marshfield, WI, USA). 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

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin: rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002;30:97–101. [PubMed: 11731797]
- Broman K, Murray JC, Scheffield 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 CRS, Scott DA, Sheffield VC, Van Hauwe P, Zbar RIS, Ashley J, Lovett M, Van Camp G, Smith RJH. 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]
- Chen AH, Stephan DA, Hasson T, Fukushima K, Nelissen CM, Chen AF, Jun AI, Ramesh A, Van Camp G, Smith RJ. MYO1F as a candidate gene for nonsyndromic deafness, DFNB15. *Arch Otolaryngol Head Neck Surg* 2001;127:921–925. [PubMed: 11493199]
- Chen P, Zindy F, Abdala C, Liu F, Li X, Roussel MF, Segil N. Progressive hearing loss in mice lacking the cyclin-dependent kinase inhibitor Ink4d. *Nat Cell Biol* 2003;5:422–426. [PubMed: 12717441]
- Cottingham R, Indury RM, Schaffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;53:252–263. [PubMed: 8317490]

- 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]
- 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* 1999;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]
- Holme, RH.; Bussoli, TJ.; Steel, KP. Table of gene expression in the developing ear. 2002. URL: <http://www.ihr.mrc.ac.uk/Hereditary/genetable/index.shtml>
- Ichimiya I, Yoshida K, Suzuki M, Mogi G. Expression of adhesion molecules by cultured spiral ligament fibrocytes stimulated with proinflammatory cytokines. *Ann Otol Rhinol Laryngol* 2003;112:722–728. [PubMed: 12940672]
- International Human Genome Sequence Consortium. Initial sequence and analysis of the human genome. *Nature* 2001;409:860–921. [PubMed: 11237011]
- Kong X, Murphy K, Raj T, He C, White PS, Matise TC. A combined linkage-physical map of the human genome. *Am J Hum Genet* 2004;75:1143–1148. [PubMed: 15486828]
- Nair TS, Kozma KE, Hoefling NL, Kommareddi PK, Ueda Y, Gong TW, Lomax MI, Lansford CD, Telian SA, Satar B, Arts HA, El-Kashlan HK, Berryhill WE, Raphael Y, Carey TE. Identification and characterization of choline transporter-like protein 2, an inner ear glycoprotein of 68 and 72 kDa that is the target of antibody-induced hearing loss. *J Neurosci* 2004;24:1772–1779. [PubMed: 14973250]
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;63:259–266. [PubMed: 9634505]
- Rozen, S.; Skaletsky, HJ. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S.; Misener, S., editors. *Bioinformatics methods and protocols: methods in molecular biology*. Humana Press; NJ: 2000. p. 365–386.
- 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]
- The Hearing Research Group at Brigham and Women's Hospital. Human Cochlear cDNA library and EST database. 2002. <http://www.hearing.bwh.harvard.edu/estinfo.HTM>
- Van Camp, G.; Smith, RJH. Hereditary hearing loss. 2005. Homepage: <http://www.webhost.ua.ac.be/hhh/>
- Velichkova M, Guttman J, Warren C, Eng L, Kline K, Vogl AW, Hasson T. A human homologue of *Drosophila* kelch associates with myosin-VIIa in specialized adhesion junctions. *Cell Motil Cytoskeleton* 2002;51:147–164. [PubMed: 11921171]
- 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.

Drawing of pedigrees 4100 (a) and 4154 (b). Filled symbols represent individuals with hearing impairment, while clear symbols are for unaffected family members. Haplotypes are shown beneath each genotyped individual with maternal haplotypes on the left-hand side and paternal haplotypes on the right. The boxed area indicates the region of homozygosity for each individual. Shaded genotypes are for markers that completely segregate with the hearing impairment status. Genotypes marked as "O O" are unknown for the individual

Fig. 2.

Schematic representation of the three-unit support interval (3-u SI) and region of homozygosity (ROH) for the *DFNB68* locus for families 4100 and 4154. Markers are scaled according to the genetic map position in the Rutgers map. The *DFNB15* interval at chromosome 19 is also shown for comparison. Candidate genes that are found in inner ear expression databases are listed. The region of overlap between the ROH of the two families that completely segregate with hearing impairment (*thick line* on 4100 ROH) is flanked by D19S586 and D19S584, and includes the genes *DNMT1*, *ICAM1*, *KEAP1*, *CDKN2D*, *CTL2*, and *SMARCA4*.

Table 1Two-point LOD score results between *DFNB68* and chromosome 19 fine-mapping markers

Marker ^a	Genetic map position ^b	Physical map position ^c	LOD score at $\theta = 0^d$	
			4100	4154
<i>D19S591</i>	9.26	3,026,844	-0.48	-1.22
D19S894	14.64	4,343,406	-∞	-∞
D19S216	16.55	4,900,357	-2.14	-∞
<i>D19S1034</i>	18.80	6,064,253	-∞	-1.05
D19S869	22.31	7,025,187	-2.44	-
D19S905	23.56	7,575,847	-0.85	1.73
D19S922	24.43	8,043,696	-0.85	-
D19S884	24.43	8,055,955	-0.87	-
D19S391	26.21	8,524,537	1.73	3.15
D19S916	27.99	8,978,841	1.73	-
D19S865	28.79	9,039,342	-	3.15
<i>D19S586</i>	29.67	9,665,793	2.29	3.28
D19S583	29.67	9,919,343	2.30	-
D19S581	31.59	10,556,822	2.30	3.02
D19S584	31.59	11,063,672	-	2.88
D19S906	32.66	11,787,026	-∞	-
D19S221	32.91	12,573,742	-∞	2.62
D19S914	32.91	12,802,614	-∞	2.62
D19S558	33.79	13,174,061	2.30	-
D19S840	34.62	13,701,918	2.08	2.88
D19S929	37.99	15,021,440	-	1.88
D19S432	38.71	15,542,990	-0.85	2.63
<i>D19S714</i>	<i>38.71</i>	<i>15,589,133</i>	<i>0.09</i>	<i>1.32</i>
D19S588	38.71	15,589,156	-0.5	-
D19S252	38.71	15,598,394	-0.85	1.88
D19S917	39.72	16,222,017	1.73	2.63
D19S199	40.56	16,829,262	-2.12	-∞
D19S899	41.58	17,095,083	-1.07	-∞
D19S895	45.68	18,614,110	-0.87	-∞
D19S931	48.74	33,315,122	-	-∞
D19S919	49.31	34,602,836	0.54	-∞
<i>D19S433</i>	<i>50.73</i>	<i>35,108,867</i>	-∞	-∞
D19S396	51.01	35,447,110	-	-∞

^a Genome scan markers are shown in italics. Markers in bold flank the haplotype. Five markers, D19S583, D19S581, D19S906, D19S221 and D19S914, were chosen from the Rutgers combined linkage-physical map (Kong et al. 2004). The rest of the fine-mapping markers were selected from the Marshfield genetic map (Broman et al. 1998)

^b Sex-averaged Kosambi map distance (cM) from the Rutgers map (Kong et al. 2004)

^cSequence-based physical map distance in bases according to Build 34 of the human reference sequence (International Human Genome Sequence Consortium 2001)

^dLOD scores are not reported for markers that were non-informative for the family