HHS Public Access

Author manuscript

J Hum Genet. Author manuscript; available in PMC 2012 June 01.

Published in final edited form as:

J Hum Genet. 2011 December ; 56(12): 866–868. doi:10.1038/jhg.2011.110.

A new autosomal recessive nonsyndromic hearing impairment locus DFNB96 on chromosome 1p36.31-p36.13

Muhammad Ansar[1], **Kwanghyuk Lee**[2], **Syed Kamran-ul-Hassan Naqvi**[1], **Paula B. Andrade**[2], **Sulman Basit**[1], **Regie Lyn P. Santos-Cortez**[2], **Wasim Ahmad**[1], and **Suzanne M. Leal**[2]

[1]Department of Biochemistry, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan

[2]Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA

Abstract

A novel locus for autosomal recessive nonsyndromic hearing impairment (ARNSHI), DFNB96, was mapped to 1p36.31-p36.13. A whole genome linkage scan was performed using DNA samples from a consanguineous family from Pakistan with ARNSHI. A maximum two-point LOD score of 3.2 was obtained at marker rs8627 (chr1:8.34Mb) at θ=0 and a significant maximum multipoint LOD score of 3.8 was achieved at 15 contiguous markers from rs630075 (9.3 Mb) through rs10927583 (15.13 Mb). The 3-unit support interval and the region of homozygosity were both delimited by markers rs3817914 (6.42 Mb) and rs477558 (18.09 Mb) and contain 11.67 Mb. Of the 125 genes within the DFNB96 interval, the previously identified ARNSHI gene for DFNB36, *ESPN* and two genes that cause Bartter syndrome, *CLCNKA* and *CLCNKB*, were sequenced, but no potentially causal variants were identified.

Keywords

1p36.31-p36.13; autosomal recessive nonsyndromic hearing impairment; *CLCNKA*; *CLCNKB*; DFNB96; *ESPN*

Description

Although >90 autosomal recessive nonsyndromic hearing impairment (ARNSHI) loci have been mapped and 41 ARNSHI genes have been identified, hundreds of ARNSHI genes remain to be discovered, and the knowledge on the functionality of these genes should aid in improving current diagnostic and treatment protocols for hearing impairment (HI). Here a new locus is reported, DFNB96 which maps to 1p36.31-p36.13 region with a maximum multipoint LOD score of 3.8. The DFNB96 locus was mapped to a region containing 11.67 Mb using DNA samples from a consanguineous Pakistani family which segregates

Corresponding Author: Suzanne M. Leal, Tel: 1-713-798-4011, Fax: 1-713-798-4012, sleal@bcm.edu.

Electronic Database Information The following URLs were accessed for data in this article: Hereditary Hearing Loss Homepage (http://hereditaryhearingloss.org) UCSC Genome Browser (http://genome.ucsc.edu) OMIM (http://www.omim.org)

ARNSHI. Upon study approval from the Institutional Review Boards of Quaid-I-Azam University and the Baylor College of Medicine and Affiliated Hospitals, informed consent was obtained from participating family members. Family 4514, consanguineous kindred from Sindh province, Pakistan, clearly segregates ARNSHI (Figure 1a). No possible cause of environmental HI such as perinatal events, infections, ototoxic drug use and trauma was elucidated. Careful physical examination was performed to rule out syndromic or vestibular disease. Audiograms from two HI individuals IV-1 and IV-6 revealed bilateral severe-to-profound HI that is pre-lingual by clinical history (Figure 2).

Standard DNA extraction from venous blood was performed for nine family members, four of whom have HI (Figure 1a). The *GJB2* gene (MIM 121011) was sequenced in HI individuals and was negative for *GJB2* variants. DNA samples from the nine family members were used to perform a whole genome linkage scan at the Center for Inherited Disease Research (CIDR) using the Infinium iSelect array which has ~6,000 SNP markers. No Mendelian inconsistencies in the genotype data were identified through PEDCHECK[1]. Likewise double recombination events over short genetic distances, which are most likely due to genotyping error, were not detected with MERLIN[2] software.

Linkage analysis was performed using a completely penetrant autosomal recessive mode inheritance with a disease allele frequency of 0.001. Marker allele frequencies were estimated using observed and reconstructed genotypes of founders from 60 Pakistani families that underwent a genome scan at the same time. Using MLINK of the FASTLINK package[3], the maximum two-point LOD score of 3.2 was achieved at marker rs8627 (chr1:8.34Mb) at θ=0 (Table 1). Genetic map distances according to the Rutgers combined linkage-physical map of the human genome Build 36 version[4] were used to carry out the multipoint analysis. For markers which are not on the Rutgers map, the physical map position from the human reference sequence (Build 36) was used to interpolate the genetic map position. Multipoint linkage analysis was performed using ALLEGRO1.2c[5] on chromosome 1p36 region. A significant maximum LOD score of 3.8 was obtained at 15 adjacent markers from rs630075 (9.29 Mb) to rs10927583 (15.13 Mb). The observed LOD score of 3.8 is greater than a LOD of 3.3 which is the criterion for genome-wide significance for parametric linkage studies[6]. The 3-unit support interval lies between SNP marker loci rs3817914 (6.42Mb) and rs477558 (18.09Mb) (Table 1). When haplotypes were reconstructed using SimWalk2[7], the region of homozygosity was found to be bounded by the same markers that flank the 3-unit support interval (Figure 1a). The upper and lower boundary of homozygosity was delimited by historic recombination events between the markers rs3817914 and rs8627 and markers rs10927583 and rs477558, respectively.

The linkage interval spans 17.53 cM region, which contains 11.67 Mb and 125 known genes. Nine hearing impairment loci involved in syndromic or nonsyndromic HI have been mapped to the short arm of chromosome 1 (1p). The syndromic loci include (a) STL2 (Stickler syndrome) at 1p21.1 which is due to mutations in the *COL11A1*[8] (MIM 120280) gene, (b) WS2B[9] (Waardenburg syndrome type 2B; MIM 600193) at 1p21-p13.3, and (c) Bartter syndrome due to three genes, *BSND*[10] (MIM 606412) at 1p32.3, and *CLCNKA*[11] (MIM 602024) and *CLCNKB*[11] (MIM 602023) both at 1p36.13. For autosomal dominant NSHI three loci have been mapped, (a) DFNA2A at 1p34.2 which is due to mutations in the

KCNQ4[12] (MIM 603537) gene, (b) DFNA2B at 1p34.3 due to *GJB3*[13] (MIM 603324) mutations, and (c) DFNA37[14] at 1p21 for which the gene is unknown. For ARNSHI only DFNB36 at 1p36.31 which is due to mutations in *ESPN*[15] (MIM 606351) gene (Figure 1b) has been identified. Of these loci, the DFNB96 interval partially overlaps with the *ESPN* gene and also contains two Bartter syndrome genes, *CLCNKA* and *CLCNKB*. The genes *ESPN*, *CLCNKA* and *CLCNKB* were sequenced in hearing individual III-2 and two HI individuals IV-1 and IV-6 (Figure 1a). After sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit and Applied Biosystems 3730 DNA Analyzer, no potentially causal variants were found to segregate with HI in family 4514, thus excluding the three genes as the cause of HI in family 4514. The linkage region at 1p36.31-p36.13 was therefore assigned as the interval for the novel ARNSHI locus DFNB96. The identification of the gene for DFNB96 will provide us with additional insight into the genetic etiology of HI.

Acknowledgements

We are highly grateful to the family members who participated in this study. The study was supported by the Higher Education Commission, Islamabad, Pakistan (to W.A.) and the National Institutes of Health (NIH) – National Institute of Deafness and other Communication Disorders grant R01-DC03594 and R01-DC011651 (to S.M.L.). Genotyping services were provided by CIDR and were funded through a federal contract from the NIH to the Johns Hopkins University (Contract No N01-HG-65403).

References

1. 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]
2. 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]
3. Cottingham RW Jr, Idury RM, Schäffer AA. Faster sequential genetic linkage computations. Am. J. Hum. Genet. 1993; 53:252–263. [PubMed: 8317490]
4. Matise TC, Chen F, Chen W, De La Vega FM, Hansen M, He C, et al. A second-generation combined linkage physical map of the human genome. Genome Res. 2007; 17:1783–1786. [PubMed: 17989245]
5. Gudbjartsson DF, Jonasson K, Frigge ML, Kong A. Allegro, a new computer program for multipoint linkage analysis. Nat. Genet. 2000; 25:12–13. [PubMed: 10802644]
6. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. 1995; 11:241–247. [PubMed: 7581446]
7. 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]
8. Richards AJ, Yates JRW, Williams R, Payne SJ, Pope FM, Scott JD, et al. A family with Stickler syndrome type 2 has a mutation in the *COL11A1* gene resulting in the substitution of glycine 97 by valine in alpha-1(XI) collagen. Hum. Mol. Genet. 1996; 5:1339–1343. [PubMed: 8872475]
9. Lalwani AK, Baldwin CT, Morell R, Friedman TB, San Agustin TB, Milunsky A, et al. A locus for Waardenburg syndrome type II maps to chromosome 1p13.3-2.1. Am. J. Hum. Genet. 1994; 55(suppl):A14.
10. Birkenhager R, Otto E, Schurmann MJ, Vollmer M, Ruf E-M, Maier-Lutz I, et al. Mutation of *BSND* causes Bartter syndrome with sensorineural deafness and kidney failure. Nat. Genet. 2001; 29:310–314. [PubMed: 11687798]
11. Schlingmann KP, Konrad M, Jeck N, Waldegger P, Reinalter SC, Holder M, et al. Salt wasting and deafness resulting from mutations in two chloride channels. New Eng. J. Med. 2004; 350:1314–1319. [PubMed: 15044642]

12. Kubisch C, Schroeder BC, Friedrich T, Lutjohann B, El-Amraoui A, Marlin S, et al. *KCNQ4*, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell. 1999; 96:437–446. [PubMed: 10025409]

13. Xia J, Liu C, Tang B, Pan Q, Huang L, Dai H, et al. Mutations in the gene encoding gap junction protein beta-3 associated with autosomal dominant hearing impairment. Nat. Genet. 1998; 20:370–373. [PubMed: 9843210]

14. Talebizadeh Z, Kenyon JB, Askew JW, Smith SD. A new locus for dominant progressive hearing loss *DFNA37* mapped to chromosome 1p21. Am. J. Hum. Genet. 2000; 67(suppl 2):314.

15. Naz S, Griffith AJ, Riazuddin S, Hampton LL, Battey JF Jr. Khan SN, et al. Mutations of *ESPN* cause autosomal recessive deafness and vestibular dysfunction. J. Med. Genet. 2004; 41:591–595. [PubMed: 15286153]

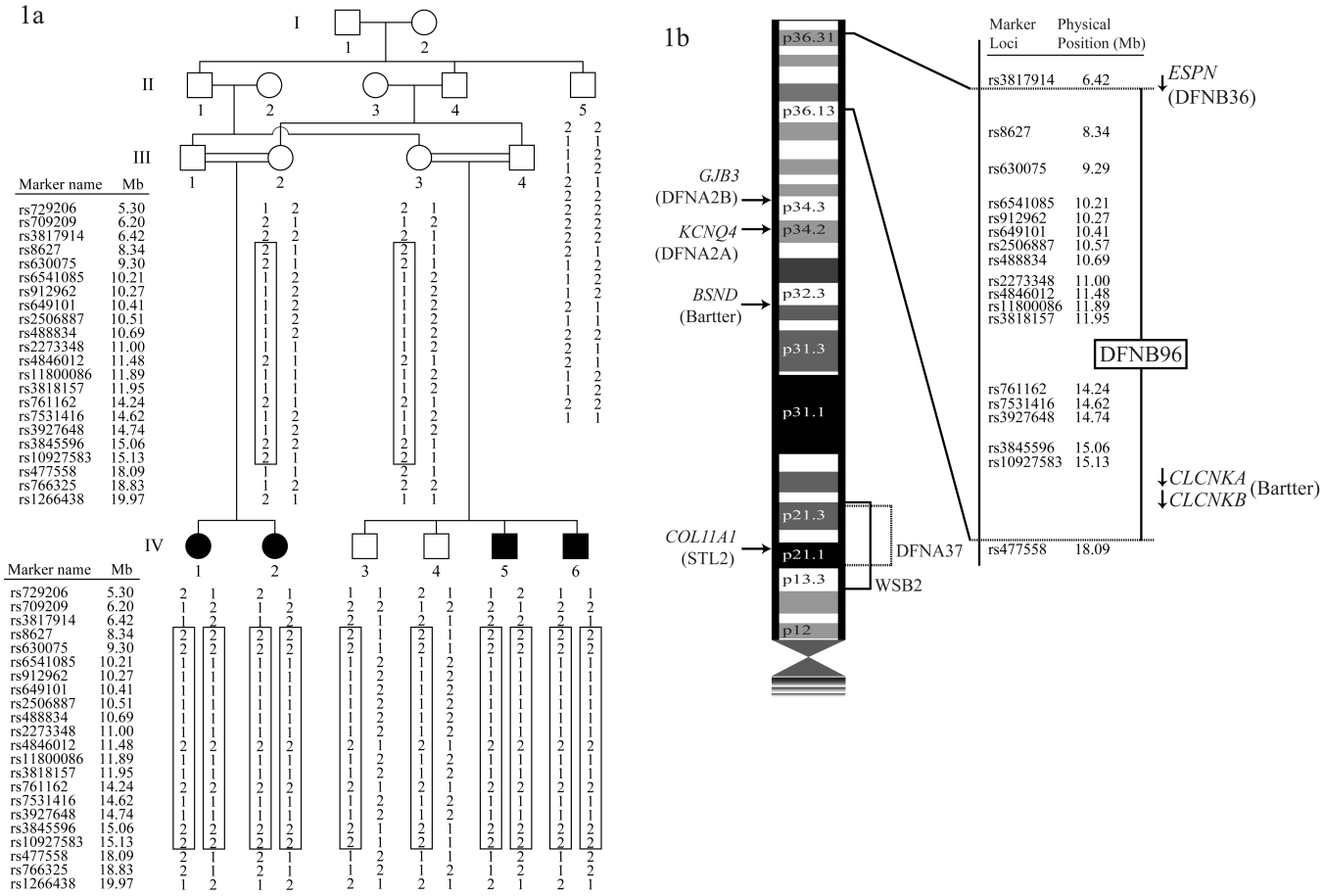

Figure1.

Panel 1a. Pedigree drawing and haplotype of family 4514. *Filled* symbols denote individuals with ARNSHI, while *clear* symbols represent hearing individuals. The haplotype segregating with ARNSHI is shown in a *box*, with paternal haplotypes shown on the left-side and materal haplotypes to the right. The region of homozygosity in individuals with ARNSHI is delimited by markers rs3817914 (chr1:6.42Mb) and rs477558 (chr1:18.09Mb). Panel 1b. Chromosome 1p displaying the genetic interval for DFNB96. The locations of NSHI gene *ESPN* and syndromic genes *CLCNKA* and *CLCKNB* with their direction of transcription denoted by an arrow are also displayed.

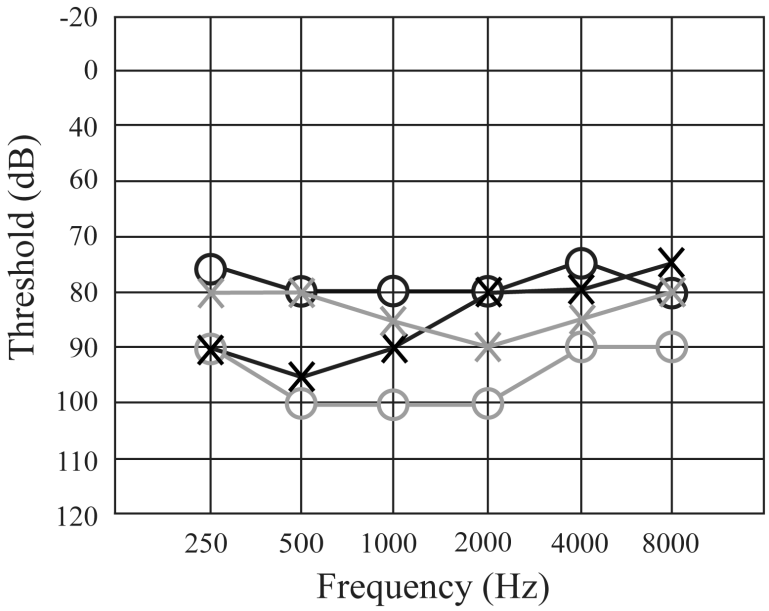

Figure2. Audiograms of individuals IV-1 and IV-6 of family 4514

Air conduction testing is marked using *circles* for the right ear and *crosses* for the left ear. *Black* markings are for individual IV-1 while *gray* markings are for individual IV-6. Testing was performed for individual IV-1 at age 24 and for IV-6 at age 38. Hearing impairment for both individuals was bilateral and severe-to-profound involving all frequencies.

Table1

Two-point and multipoint LOD scores for family 4514 at chromosome 1p36.31-p36.13

Marker[1]	Physical map position[2]	Genetic map position[3]	Multipoint LOD score	Two-point LOD score at θ =						
				0.00	0.01	0.05	0.10	0.20	0.30	0.40
rs729206	5,295,728	13.90	−∞	−∞	−2.4	−1.1	−0.6	−0.3	−0.1	−0.1
rs709209	6,201,001	15.76	−∞	−∞	−2.4	−1.1	−0.6	−0.2	−0.1	−0.1
rs3817914	**6,423,619**	**16.21**	−∞	−∞	−2.5	−1.2	−0.7	−0.2	−0.1	−0.1
rs8627	8,335,522	19.58	3.7	3.2	3.1	2.8	2.5	1.8	1.1	0.5
rs630075	9,293,498	21.16	3.8	2.5	2.4	2.2	1.9	1.3	0.7	0.3
rs6541085	10,208,296	22.24	3.8	1.6	1.5	1.3	1.0	0.4	−0.0	−0.2
rs912962	10,271,299	22.33	3.8	1.6	1.5	1.3	1.0	0.4	−0.0	−0.2
rs649101	10,412,698	22.54	3.8	1.9	1.8	1.6	1.3	0.7	0.2	−0.1
rs2506887	10,507,259	22.68	3.8	1.1	1.0	0.8	0.6	0.0	−0.3	−0.3
rs488834	10,690,489	23.03	3.8	2.1	2.1	1.9	1.6	1.1	0.5	0.2
rs2273348	11,001,664	23.68	3.8	1.7	1.7	1.5	1.3	0.9	0.5	0.2
rs4846012	11,480,513	24.86	3.8	2.7	2.6	2.6	2.0	1.4	0.8	0.3
rs11800086	11,894,336	26.07	3.8	1.7	1.6	1.5	1.3	0.9	0.5	0.2
rs3818157	11,949,895	26.22	3.8	1.4	1.4	1.2	1.6	0.7	0.4	0.1
rs761162	14,243,724	31.08	3.8	1.4	1.3	1.1	0.8	0.3	−0.1	−0.2
rs7531416	14,621,347	32.11	3.8	2.6	2.5	2.3	1.9	1.3	0.6	0.1
rs3927648	14,742,313	32.49	3.8	2.4	2.3	2.1	1.8	1.2	0.7	0.2
rs3845596	15,061,886	33.52	3.8	2.2	2.1	1.9	1.7	1.2	0.7	0.3
rs10927583	15,131,012	33.74	3.8	2.0	1.9	1.7	1.5	0.9	0.5	0.1
rs477558	**18,092,414**	**39.60**	−3.3	−2.1	−0.6	0.1	0.2	0.3	0.2	0.1
rs766325	18,829,045	42.28	−3.0	−2.2	−1.0	−0.4	−0.2	−0.1	−0.1	−0.1
rs1266438	19,968,763	44.67	−2.7	−2.4	−1.1	−0.5	−0.3	−0.2	−0.1	−0.1

[1] Markers in bold denote marker limits based on the 3-unit support interval and the homozygous region

[2] Physical map positions in base pairs from Build 36 of the human reference sequence

[3] Genetic map positions in cM from Rutgers combined linkage-physical map of the human genome Build 36 version