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A human recessive neurosensory nonsyndromic hearing impairment locus is a potential homologue of the murine deafness (*dn*) locus

Pawan K.Jain, Kunihiro Fukushima[1], Dilip Deshmukh[2], Arabandi Ramesh[3], Elizabeth Thomas[4], Anil K.Lalwani[5], Subrinder Kumar[4], Barbara Ploplis, Hana Skarka, C.R.Srikumari Srisailapathy[3], Sigrid Wayne[1], Ross I.S.Zbar[1], Ishwar C.Verma[2], Richard J.H.Smith[1] and Edward R.Wilcox*

Laboratory of Molecular Genetics, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, MD 20850-3227, [1]Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA 52242, USA, [2]Rotary Deaf School, Ichalkaranji-Tilawani, 416 115, Dist. Kolhapur, Maharashtra State, [3]Department of Genetics, University of Madras, Madras, [4]All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India and [5]Laboratory of Molecular Otology, Epstein Laboratories, 533 Parnassus Ave, U490A, San Francisco, CA 94143-0526, USA

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A locus for recessive neurosensory nonsyndromic hearing impairment maps to chromosome 9q13–q21 in two regionally separate consanguineous families from India. Each family demonstrates a LOD score greater than 4.5 to this region. D9S15, tightly linked to the Friedreich's ataxia locus, a region that has been defined with over 1 Mb of YAC contig information and several expressed sequences, is one of the flanking markers. In mice, the deafness (*dn*) locus maps to mouse chromosome 19 and flanking loci are syntenic to human chromosome 9q11–q21. The *dn* mouse is a potential model for the hearing impairment found in both these families.

INTRODUCTION

Congenital hearing impairment occurs in approximately 0.1% of all children born in the United States (1). In more than 60% of the cases there are genetic causative factors, the majority of which probably involve single gene mutations (2). In most cases, there is no additional clinical anomaly and an autosomal recessive mode of inheritance predominates (3). Nonsyndromic hereditary hearing impairment (NSHHI) is considered to be highly heterogeneous; it has been estimated that as many as 100 different genes may be involved in hearing (4).

Single families suitable in size for conventional linkage analysis are not common. At the same time, NSHHI cannot be subclassified reliably by audiometric criteria. Thus, pooling of multiple small families has not been a feasible alternative (5). The strategy of examining consanguineous families from geographically isolated populations has proven effective for homozygosity mapping (6) of several different genes causing recessive NSHHI (Table 1).

A complementary strategy for identifying hearing-related human genes is the localization and cloning of these genes in mice. Genetic studies of the shaker-1 (*sh-1*) mice have identified a novel myosin VII-like gene associated with deafness. Mutations in the human homologue of this gene are associated with Usher syndrome type 1B (7,8).

The purpose of this paper is to describe two multiply inbred families with NSHHI mapping to chromosome 9, defining the locus DFNB7 to an approximate 4 cM interval flanked by D9S50 and D9S15.

RESULTS

Some of the known loci associated with recessive hereditary hearing impairment were excluded by typing markers in the region of 13q12, 11q13.5 and the pericentromeric region of chromosome 17 (DFNB1–3, see Table 1) in the three NIH families. A genome search was then undertaken with CHLC marker set, version 5, in family 1A (Fig. 1A; 9). After exclusion of all chromosomes but 3, 8 and 9, an indication of linkage was found with D9S319. Subsequent testing led to full linkage for markers D9S166, D9S273 and D9S15. The LOD score for marker D9S166 in family 1A is 4.63 at $\theta = 0.0$, using the allele frequencies found in Table 2. The LOD score for family 1B is 5.36 for D9S301 at $\theta = 0.0$, using the allele frequencies found in Table 2. Haplotypes of family members were constructed from these and additional flanking markers (Fig. 1).

Three consanguineous multiplex families from western India and nine families from Tamil Nadu capable of generating LOD scores greater than 3 were screened for linkage to the DFNB7 locus. The two families herein described demonstrated homozygosity by descent and linkage to 9q13–q21.

DISCUSSION

The DFNB7 locus maps to the interval between D9S50 and D9S15 as seen by obligate crossovers in family 1B at those two loci. This defines a region of linkage of approximately 4 cM (Fig. 2). The D9S15 marker, closely linked to Friedreich's ataxia, is placed cytogenetically at 9q13–q21 (10).

Friedreich's ataxia (FRDA; MIM 229300; 11) is a progressive neurodegenerative disorder affecting the sensory afferents of both the central and peripheral nervous system. Onset is common between the ages of 8 and 15 years. Although brainstem evoked response audiometry is abnormal in approximately 50% of patients with FRDA (12) and reflects degenera-

*To whom correspondence should be addressed

Table 1. Loci causing recessive nonsyndromic hereditary hearing impairment

Locus	Chromosomal assignment	Mapping strategy
DFNB1 (28)	13q	Consanguineous unions
DFNB2 (29)	11q	Consanguineous unions
DFNB3 (30)	17	Population isolate
DFNB4 (31)	7q	Consanguineous unions
DFNB5 (5)	14q	Consanguineous unions
DFNB6 (32)	3p	Consanguineous unions
DFNB7 (present work)	9q	Consanguineous unions

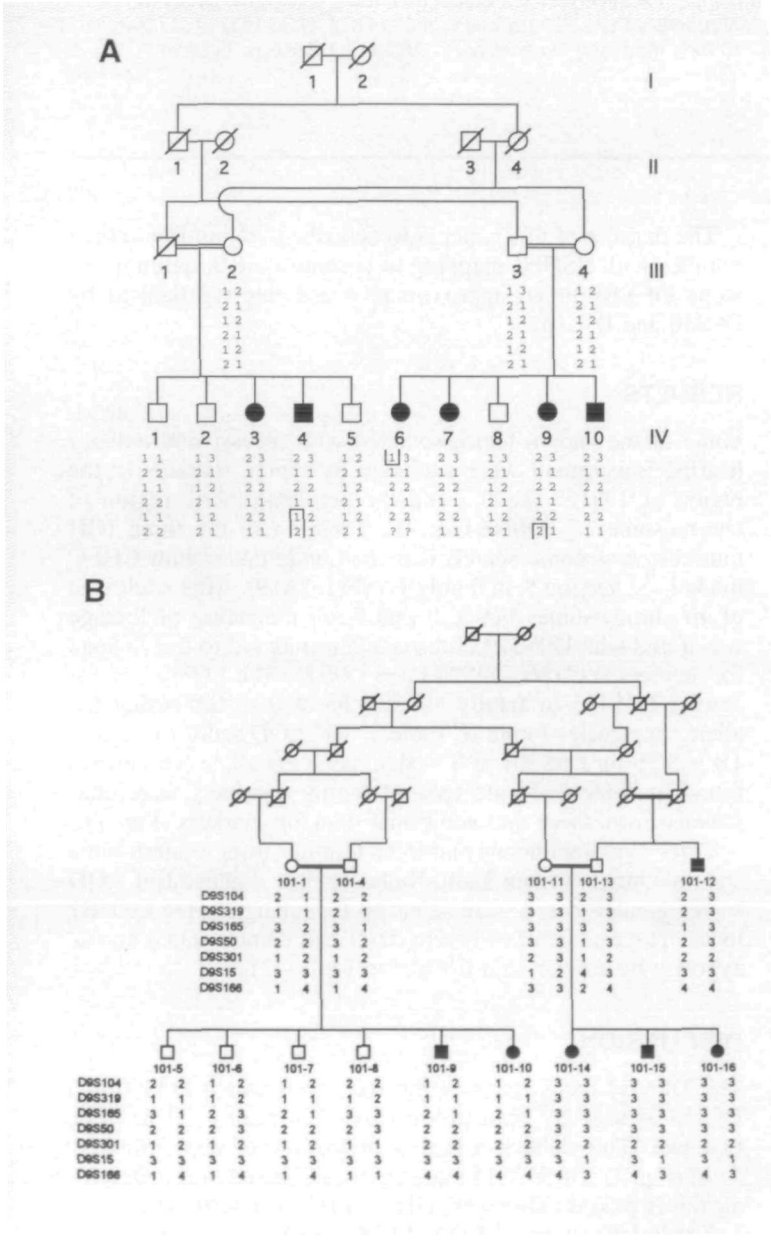

Figure 1. (**A**) Family pedigree with haplotypes drawn below. The markers are listed in the following order: D9S319, D9S15, D9S273, D9S166, D9S276 and D9S153. D9S15, D9S273 and D9S166 all show homozygosity by descent in all affected persons. For D9S166 allele 1 is 249 bp and allele 2 is 233 bp. (**B**) Family pedigree localizing the DFNB7 gene to the D9S50–D9S15 interval containing D9S301. In this family, D9S301 is the only marker showing homozygosity by descent in all affected persons. For D9S301, allele 1 is 228 bp, allele 2 is 224 bp and allele 3 is 220 bp.

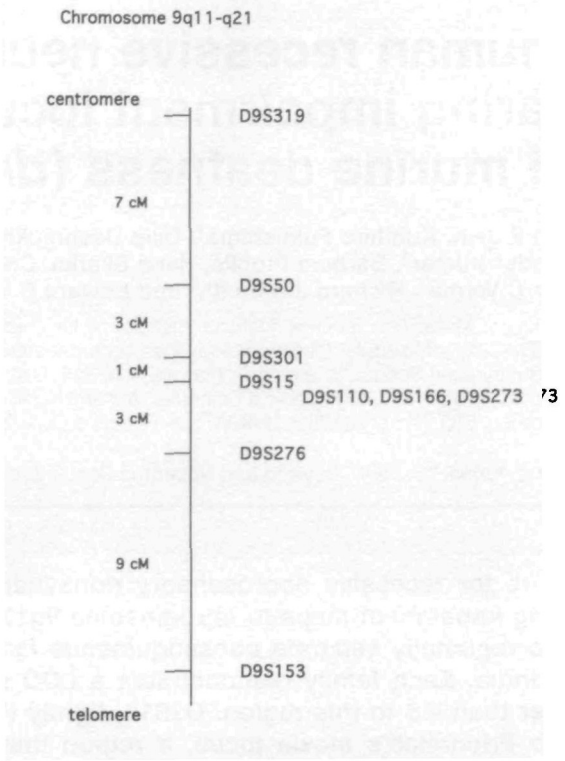

Figure 2. A linkage map of the chromosome 9q11–q21 region pooled from Dr J.White's data presented at the 4th International Chromosome 9 Workshop (25), Gyapay *et al.* (26) and Rodius *et al.* (27). D9S301 is 10 cM from D9S319 (9), placing it within 1 cM of D9S15. D9S110 is physically 80 kb from D9S15 on a YAC contig map. D9S166 and D9S273 belong to the same linkage group as D9S110 (27). D9S276 belongs to the same linkage group as D9S175 and D9S284 (26), both at 3 cM from D9S15.

tion within the brainstem (13,14), clinically significant hearing loss occurs in less than 10% of affected persons and is usually mild-to-moderate in degree. Severe-to-profound hearing loss is extremely rare (15). The FRDA phenotype therefore makes the FRDA gene an extremely unlikely candidate for DFNB7. However, physical maps in the region of FRDA could be useful in cloning the DFNB7 gene (16).

Mice have made valuable contributions to developmental genetic studies of the biological basis of hereditary hearing impairment (17,18). Described herein is a potential human homologue of the *dn* locus in mice, first identified by Deol and Kocher (19). This is a profoundly deaf recessive mutant, with degeneration of the organ of Corti, stria vascularis and occasionally the saccular macula, starting at about 10 days after birth. Recently, the *dn* locus was mapped to chromosome 19 in a region syntenic to human chromosome 9q11–21 (20; J.Battey, personal communication).

Identification of this gene and its protein product will provide appreciable insight into the development of recessive nonsyndromic hearing impairment.

MATERIALS AND METHODS

Families with probable hereditary hearing impairment (National Institute on Deafness and Other Communication Disorders) were ascertained by identification of probands in schools for the deaf in the district of Kolhapur in western India. A complete medical history was obtained to exclude

Table 2. Allele frequencies for D9S301 in Tamil Indians and for D9S166 in the Kolhapur district of western India

D9S166		D9S301	
Allele size (bp)	Frequency	Allele size (bp)	Frequency
233	0.500	232	0.042
243	0.060	230	0.194
245	0.040	228	0.250
247	0.020	226	0.097
249	0.140	224	0.153
251	0.080	222	0.028
253	0.060	220	0.056
255	0.040	218	0.181
257	0.060		

environmental causes of hearing impairment. Physical examination was performed to eliminate syndromic hearing impairments. Audiological testing was performed on all family members. Affected children from these families have a profound prelingual sensorineural hearing impairment. Pure tone audiometry was performed on all family members. Affected individuals demonstrated profound sensorineural hearing impairment. Obligate heterozygote individuals had normal hearing. Neurological evaluation including examination of the cranial nerves cerebellar exam and gate were normal in affected individuals. There were no other associated stigmata of syndromic hearing impairment including pigmentary, retinal or physical abnormality.

Families with probable NSHHI (University of Iowa) were ascertained by identifying probands from four schools for the deaf (St Louis Institute for the Deaf and the Blind, CSI School for the Deaf, Little Flower Covenant School for the Deaf and Bala Vidyalaya School for the Deaf) and from The Institute of Basic Medical Sciences in Madras, India, the capital city of Tamil Nadu, one of the original populations of India. Data on the hearing status of each person were gathered through repeated household visits. Audiograms were obtained to confirm severe-to-profound NSHHI and correlated with data on age-at-onset of hearing loss. Only families with congenital prelingual severe-to-profound NSHHI were included in this study. Conditions such as rubella, prematurity, drug use during pregnancy, perinatal trauma and meningitis were eliminated by history. The biological relationship between spouses was determined by extensive questioning and verified by elderly members of the household. Nuclear families were extended if other family members resided in the vicinity.

Genomic DNA was prepared from blood samples (21). Microsatellite markers were amplified by polymerase chain reaction (PCR) and analyzed on polyacrylamide gels, according to the methods described previously (5,22). With some primer pairs, reaction conditions were modified to optimize amplification. The microsatellite markers were purchased from Research Genetics or synthesized on an Applied Biosystems 394 DNA synthesizer. Sequences for primer pairs are listed in Genome Data Base (GDB).

LOD scores were calculated using the LINKAGE Program Package 5.1 (23). The gene frequency was set at 0.005 (24) and the disease was coded as fully penetrant. Allele sizes were assigned in reference to an M13mp18 sequencing ladder. Genomic DNA from CEPH individual 1347–02 with known allele sizes for each STR was typed with all DNA samples. The allele frequencies for polymorphic markers are unknown in this Indian population and would likely vary in different subpopulations. Allele frequencies for D9S301 and D9S166 were determined by screening respectively 36 and 25 unrelated individuals from the same ethnic and geographic background (Table 2).

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ABBREVIATIONS

dn, deafness locus on mouse chromosome 19; NSHHI, nonsyndromic hereditary hearing impairment; *sh-1*, shaker-1 locus on mouse chromosome 7; DFNB1–3, recessive deafness loci numbers 1–3; DFNB7, recessive nonsyndromic hearing impairment locus number 7; FRDA, Friedreich's ataxia, a progressive neurodegenerative disorder affecting the sensory afferents of both the central and peripheral nervous system.

REFERENCES

1. Fraser,G.R. (1976) *The Causes of Profound Deafness in Childhood*. Johns Hopkins University Press, Baltimore.
2. Marazita,M.L., Ploughman,L.M., Rawling,B., Remington,E., Arnos,K.S. and US school-age population. *Am. J. Med. Genet.*, **46**, 486–491.
3. Rose,S.P., Conneally,P.M. and Nance,W.E. (1977) In Bess,F.H. (ed.), *Childhood Deafness: Causation, Assessment and Management*. Grune and Stratton, New York, pp. 19–35.
4. Chung,C.S. and Brown,K.S. (1970) Family studies of early childhood deafness ascertained through the Clarke School for the Deaf. *Am. J. Hum. Genet.*, **22**, 630–644.
5. Fukushima,K., Ramesh,A., Srisailapathy, C.R.S., Ni,L., Chen,A., O'Neill,M., VanCamp,G., Coucke,P., Smith,S.D., Kenyon,J.B., Jain,P., Wilcox,E.R., Zbar, R.I.S. and Smith,R.J.H. (1995) Consanguineous nuclear families used to identify a new locus for recessive non-syndromic hearing loss on 14q. *Hum. Mol. Genet.* **4**, 1643–1648.
6. Lander,E.S. and Botstein,D. (1987) Homozygosity mapping: A way to map human recessive traits with the DNA of inbred children. *Science*, **236**, 1567–1570.
7. Gibson,F., Walsh,J., Mburu,P., Varela,A., Brown,K.A., Antonio,M., Beisel, K.W., Steel,K.P. and Brown,S.D.M. (1995) A type VII myosin encoded by the mouse deafness gene *shaker-1*. *Nature*, **374**, 62–64.
8. Weil,D., Blanchard,S., Kaplan,J., Guilford,P., Gibson,F., Walsh,J., Mburu,P., Varela,A., Levilliers,J., Weston,M.D., Kelley,P.M., Kimberling, W.J., Wagenaar,M., Levi-Acobas,F., Larget-Piet,D., Munnich,A., Steel, K.P., Brown,S.D.M. and Petit,C. (1995) Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature*, **374**, 60–61.
9. Buetow,K.H., Weber,J.L., Ludwigsen,S., Scherpbier-Heddema,T., Duyk, G.M., Sheffield,V.C., Wang,Z. and Murray,J.C. (1994) Integrated human genome-wide maps constructed using the CEPH reference panel. *Nature Genet.*, **6**, 391–393.
10. Hanauer,A., Chery,M., Fujita,R., Driesel,A.J., Gilgenkrantz,S. and Mandel,J.L., (1990) The Friedreich ataxia gene is assigned to chromosome 9q13–q21 by mapping of tightly linked markers and shows linkage disequilibrium with D9S15. *Am. J. Hum. Genet.*, **46**, 133–137.
11. Chamberlain,S., Farrall,M., Shaw,J., Wilkes,D., Carvajal,J., Hillerman,R., Doudney,K., Harding,A.E., Williamson,R., Sirugo,G., Fujita,R., Koenig, M., Mandel,J-L., Palau,F., Monros,E., Vilchez,J., Prieto,F., Richter,A., Vanasse,M., Melancon,S., Cocozza,S., Redolfi,E., Cavalcanti,F., Pianese, L., Filla,A., DiDonato,S. and Pandolfo,M. (1993) Genetic recombination events which position the Friedreich ataxia locus proximal to the D9S15/D9S5 linkage group on chromosome 9q. *Am. J. Hum. Genet.*, **52**, 99–109.
12. De Pablos,C., Berciano,J. and Calleja,J. (1991) Brain-stem auditory evoked potentials and blink reflex in Friedreich's ataxia. *J. Neurol.*, **238**, 212–216.
13. Ell,J., Prasher,D. and Rudge,P. (1984) Neuro-otological abnormalities in Friedreich's ataxia. *J. Neurol. Neurosurg. Psychiatry*, **47**, 26–32.
14. Jabbari,B., Schwartz,D.M., MacNeil,D.M. and Coker,S.B. (1983) Early abnormalities of brainstem auditory evoked potentials in Friedreich's ataxia: evidence of primary brainstem dysfunction. *Neurology*, **33**, 1071–1074.
15. Harding,A.E. (1981) Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain*, **104**, 589–620.
16. Carvajal,J.J., Pook,M.A., Doudney,K., Hillermann,R., Wilkes,D., Al-Mahdawi,S., Williamson,R. and Chamberlain,S. (1995) Friedreich's ataxia: a defect in signal transduction? *Hum. Mol. Genet.*, **4**, 1411–1419.
17. Steel,K.P. (1991) Similarities between mice and humans with hereditary deafness. *Ann. N. Y. Acad. Sci.*, **630**, 68–79.
18. Steel,K.P. and Brown,S.D. (1994) Genes and deafness. *Trends Genet.*, **10**, 428–435.
19. Deol,M.S. and Kocher,W. (1958) A new gene for deafness in the mouse. *Heredity*, **12**, 463–466.

20. Keats,B.J.B., Nouri,N., Huang,J-M., Money,M., Webster,D.B. and Berlin,C.I. (1995) The deafness locus (*dn*) maps to mouse chromosome 19. *Mamm. Genome*, **6,** 8–10.
21. Grimberg,J., Nawoschik,S., Belluscio,L., McKee,R., Turck,A. and Eisenberg,A. (1989) A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res.*, **17,** 8390.
22. Hughes,A.E. (1993) Optimization of microsatellite analysis for genetic mapping. *Genomics*, **15,** 433–434.
23. Lathrop,G.M., Lalouel,J.M., Julier,C. and Ott,J. (1984) Strategies for multilocus linkage analysis in humans. *Proc. Natl Acad. Sci. USA*, **81,** 3443–3446.
24. Morton,N.E. (1991) Genetic epidemiology of hearing impairment. *Ann. N. Y. Acad. Sci.*, **630,** 16–31.
25. Pericak-Vance,M.A., Bale,A.E., Haines,J.L., Kwiatkowski,D.J., Pilz,S., Slaugenhaupt,S., White,J.A., Edwards,J., Marchuk,D. and Povey,S. (1995) Report on the Fourth International Workshop on Chromosome 9. *Ann. Hum. Genet.*, **59** (in press).
26. Gyapay,G., Morissette,J., Vignal,A., Dib,C., Fizames,C., Millasseau,P., Marc,S., Bernardi,G., Lathrop,M. and Weissenbach,J. (1994) The 1993–94 Genethon human genetic linkage map. *Nature Genet.*, **7,** 246–339.
27. Rodius,F., Duclos,F., Wrogemann,K., Le Paslier,D., Ougen,P., Billault,A., Belal,S., Musenger,C., Brice,A., Durr,A., Mignard,C., Sirugo,G., Weissenbach,J., Cohen,D., Hentati,F., Hamida,M.B., Mandel,J-L. and Koenig,M. (1994) Recombinations in individuals homozygous by descent localize the Friedreich ataxia locus in a cloned 450-kb interval. *Am. J. Hum. Genet.*, **54,** 1050–1059.
28. Guilford,P., Ben Arab,S., Blanchard,S., Levilliers,J., Weissenbach,J., Belkahia,A. and Petit,C. (1994) A non-syndromic form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. *Nature Genet.*, **6,** 24–28.
29. Guilford,P., Ayadi,H., Blanchard,S., Chaib,H., Le Paslier,D., Weissenbach,J. Drira,M. and Petit,C. (1994) A human gene responsible for neurosensory, non-syndromic recessive deafness is a candidate homologue of the mouse *sh-1* gene. *Hum. Mol. Genet.*, **3,** 989–993.
30. Friedman,T.B., Liang,Y., Weber,J.L., Hinnant,J.T., Barber,T.D., Winata,S., Arhya,I.N. and Asher,J.H. (1995) A gene for congenital, recessive deafness *DFNB3* maps to the pericentromeric region of chromosome 17. *Nature Genet.*, **9,** 86–91.
31. Baldwin,C.T., Weiss,S., Farrer,L.A., DeStefano,A.L., Adair,R., Franklyn,B., Kidd,K.K., Korostishevsky,M. and Bonne-Tamir,B. (1995) Linkage of congenital, recessive deafness (DFNB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. *Hum. Mol. Genet.* **4,** 1637–1642.
32. Fukushima,K., Ramesh,A., Srisailapathy,C.R.S., Ni.,L., O'Neill,M.E., VanCamp,G., Coucke,P., Pawan,J., Wilcox,E.R., Zbar,R.I.S. and Smith,R.J.H. (1995) A non-syndromic form of autosomal recessive sensorineural hearing loss maps to 3p. *Genome Res.* (in press).