European Journal of Human Genetics (1999) 7, 243–246

http://www.stockton-press.co.uk/ejhg

SHORT REPORT

DFNB20: a novel locus for autosomal recessive, non-syndromal sensorineural hearing loss maps to chromosome 11q25–qter

Leanne Moynihan[1], Mark Houseman[1], Valerie Newton[2], Robert Mueller[3] and Nicholas Lench[1]

[1]*Molecular Medicine Unit, University of Leeds, St James's University Hospital, Leeds*
[2]*Centre for Audiology, University of Manchester, Oxford Road, Manchester*
[3]*Department of Clinical Genetics, St James's University Hospital, Leeds, UK*

Autosomal recessive non-syndromal deafness is an extremely heterogeneous condition with at least 19 loci (DFNB1–19) already described. We have used autozygosity mapping to localise a further novel locus, DFNB20, to chromosome 11q25–qter in a consanguineous family originating from Pakistan. A region of homozygosity was observed in affected individuals spanning the interval D11S969–qter.

Keywords: autosomal recessive; sensorineural deafness; autozygosity mapping; 11q25; DFNB20

Hearing loss represents an important social and medical phenomenon affecting a significant proportion of the population. Prelingual deafness is particularly important since it impedes oral language acquisition. Congenital or childhood-onset deafness affects approximately 1/1000 children with the majority of cases (approx. 70%) having no associated syndromal features.[1] A genetic cause is responsible in up to 60% of cases, most of which display an autosomal recessive mode of inheritance. Non-syndromal sensorineural deafness is genetically extremely heterogeneous, and to date 19 autosomal recessive (DFNB1–19) and 15 autosomal dominant (DFNA1–15) loci have been reported.[2] Recently, studies have shown that approximately 50% of all recessive deafness is caused by mutations in the gene for the gap junction protein connexin 26 throughout different populations including families originating from the UK, France, Italy, Spain, Tunisia, Lebanon, Pakistan, Australia and New Zealand.[3,4]

We recently ascertained a consanguineous Pakistani family segregating a novel autosomal recessive form of histiocytosis with associated features of joint contractures and sensorineural deafness and showed it to be linked to a locus on chromosome 11q25.[5] Therefore, to determine if a novel locus for non-syndromal sensorineural deafness also maps to this region, we tested 35 consanguineous families (segregating non-syndromal autosomal recessive sensorineural deafness) originating from Pakistan and the Middle East for linkage to 11q25–qter using 16 microsatellite markers from the region. Previous studies in these 35 consanguineous families had failed to detect linkage to any existing DFNB loci.

In all families studied, detailed clinical histories and physical examinations excluded obvious syndromal or

Correspondence: NJ Lench, Molecular Medicine Unit, Clinical Sciences Building, St James's University Hospital, Leeds, LS9 7TF, UK. Tel: (44) 113 206 5681; Fax: (44) 113 244 4475; E-mail: desnl@stjames.leeds.ac.uk
Received 30 December 1997; revised 10 September 1998; accepted 14 September 1998

acquired causes of hearing loss. DNA was prepared from whole blood or buccal samples using a standard non-organic method. Sixteen fluorescently-labelled microsatellite DNA markers from chromosome 11q25–qter spanning cen–D11S898–D11S439–qter (Figure 1) were amplified by the polymerase chain

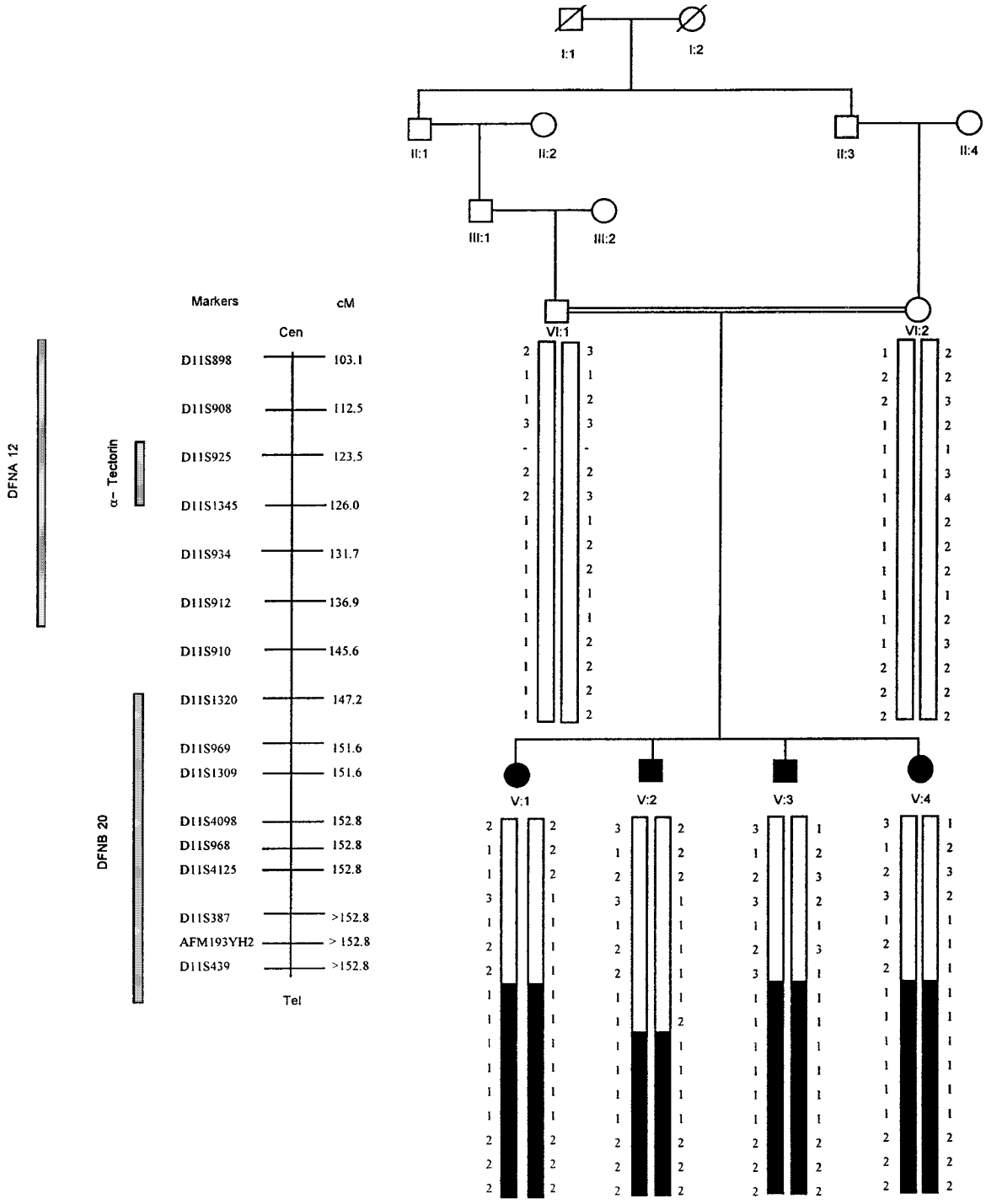

Figure 1 *Pedigree of a consanguineous family with autosomal recessive non syndromal hearing loss. The homozygous intervals are represented by solid bars. The intervals containing DFNA12,[8], α-tectorin[10] and DFNB20 (this report) are also shown. Filled symbols represent affected status, open symbols represent unaffected status. Double lines indicate consanguineous marriages.*

reaction (PCR). Marker heterozygosity values and amplification conditions were obtained from the Genome Database (http://www.gdb.org). PCR was performed in a 10 μl reaction volume containing 40 ng genomic DNA, 3 picomoles of each oligonucleotide primer, 1 U of Taq DNA polymerase (Promega, UK), 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2 mM dNTPs and $MgCl_2$ (1.5 mM). PCR was performed as follows: initial denaturation step 5 min/95°C, followed by 35 cycles of 92°C/30 s, annealing temperature/30 s, 72°C/30 s, with a final extension of 72°C/5 min. PCR products were electrophoresed at 800 volts in 1 × TBE/6% acrylamide gels for 3.5 h on an ABI 373 DNA Sequencer. Gels were processed using the Genescan analysis software Version 2.0.2 (Applied Biosystems). Genotypes were generated using the Genotyper software Version 1.1 (Applied Biosystems).

Linkage analysis was performed using the LIPED program for two point analysis[6] and HOMOZ for multipoint analysis.[7] A fully penetrant autosomal recessive mode of inheritance was assumed with a disease gene frequency of 0.0001. Equal recombination frequencies between males and females were used. Allele frequencies were generated from a panel of 80 unrelated, control individuals from the Pakistani community of West Yorkshire, UK.

In a single family of Pakistani origin, a region of homozygosity was observed in all four affected individuals (Figure 1). All four affected offspring were diagnosed with hearing impairment between the ages of 3 months and 1 year following normal births. Audiological testing revealed that individuals V_2 and V_4 had a moderate hearing impairment (range 50–70 dB), whilst individuals V_1 and V_3 had a profound hearing loss (range > 100 dB). Using two point linkage analysis, a maximum lod score was observed with marker D11S4125 (Z_{max} = 2.82; θ = 0) (Table 1). Multipoint analysis using the HOMOZ program generated a Z_{max} = 3.3 over an interval spanning D11S969–qter. It was not possible to define the telomeric boundary of the interval containing DFNB20 since none of the affected individuals were recombinant with markers close to the telomere and, at present, no polymorphic markers have been reported distal of D11S439. Currently, there are no obvious candidate genes that map to this region, 34 cDNA markers are known to map to the interval D11S969–qter (http://www.ncbi.nlm.nih-.gov/SCIENCE96). A locus for a dominant form of deafness (DFNA12) maps to 11q22–q24[8] and recently, it has been shown that mutations in the α-tectorin gene are responsible for deafness in these families.[9] The α-tectorin gene maps close to the marker D11S925;[10] the fact that all four affected individuals in the consanguineous Pakistani family studied are heterozygous for markers D11S1345, D11S912, D11S910 (D11S934 was uninformative) over an interval of approximately 22 cM distal to D11S925 effectively excludes α-tectorin as the *DFNB20* gene.

In conclusion, we have mapped a further novel locus for non-syndromal autosomal recessive sensorineural

Table 1 Pairwise linkage analysis between the disease locus and chromosome 11q25 DNA markers. Marker order is cen–D11S898–D11S439–qter. Maximum lod score displayed in bold

	lod score at θ =						
Marker	*0.00*	*0.010*	*0.050*	*0.1*	*0.2*	*0.3*	*0.4*
D11S898	–18.789	–9.006	–2.546	–1.499	–0.616	–0.238	–0.064
D11S908	–2.539	–2.067	–0.590	–0.341	–0.138	–0.056	–0.018
D11S925	–15.509	–6.511	–1.586	–0.855	–0.288	–0.077	–0.002
D11S1345	–13.335	–6.863	–2.033	–1.228	–0.526	–0.207	–0.053
D11S934	1.076	1.073	0.921	0.770	0.490	0.258	0.090
D11S912	–7.636	–4.164	–1.054	–0.570	–0.198	–0.067	–0.019
D11S910	–6.733	–3.263	–0.240	0.150	0.319	0.231	0.075
D11S1320	1.356	1.352	1.196	1.038	0.728	0.430	0.162
D11S969	–3.861	–0.865	0.612	0.684	0.529	0.267	0.033
D11S1309	2.671	2.665	2.395	2.111	1.524	0.922	0.343
D11S4098	0.363	0.361	0.295	0.234	0.133	0.063	0.022
D11S968	1.709	1.705	1.523	1.335	0.957	0.588	0.250
D11S4125	**2.823**	2.817	2.544	2.257	1.662	1.048	0.445
D11S387	1.453	1.450	1.285	1.116	0.785	0.467	0.181
AFM193YH2	1.453	1.450	1.285	1.116	0.785	0.467	0.181
D11S439	1.453	1.450	1.285	1.116	0.785	0.467	0.181

deafness to a region of chromosome 11q25 extending from the D11S969 to the telomere, thus demonstrating the extensive genetic heterogeneity of this condition. In previous studies, we have also observed a family segregating an unusual form of histiocytosis with associated features of joint contractures and sensorineural deafness that is linked to this region of chromosome 11.[5] It is possible that a single gene at this region is causal in both syndromal and non-syndromal forms of sensorineural deafness as it has been shown recently that different mutations in the unconventional myosin VIIa gene (*MYO7A*) can result in either syndromic (Usher syndrome type 1b)[11] or non-syndromic forms of dominant and recessive hearing loss.[12,13] This could be explained by allelic heterogeneity or differences in genetic background. In addition, it will be important to determine the relative contribution to non-syndromal deafness of the various loci in different ethnic populations. This will facilitate the provision of accurate recurrence risk estimates in individual families.

Acknowledgements

We thank L Al-Gazali, KA Brown, AH Janjua, G Karbani and G Parry for their contribution to this research. Work in the authors' laboratories is supported by the Wellcome Trust, Medical Research Council, Hearing Research Trust, European Community, Northern and Yorkshire Regional Health Authority, and the West Riding Medical Research Trust.

References

1 Petit C: Genes responsible for human hereditary deafness: symphony of a thousand. *Nat Genet* 1996; **14**: 385–391.
2 Van Camp G, Willems PJ, Smith RJH: Nonsyndromic hearing impairment: unparalleled heterogeneity. *Am J Hum Genet* 1997; **60**: 758–764.
3 Denoyelle F, Weil D, Maw MA *et al*: Prelingual deafness: high prevalence of a 30delG mutation in the connexin 26 gene. *Hum Mol Genet* 1997; **6**: 2173–2177.
4 Zelante L, Gasparini P, Estivill X *et al*: Connexin26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. *Hum Mol Genet* 1997; **6**: 1605–1609.
5 Moynihan LM, Bundey SE, Heath D *et al*: Autozygosity mapping, to chromosome 11q25, of a rare autosomal recessive syndrome causing histiocytosis, joint contractures, and sensorineural deafness. *Am J Hum Genet* 1998; **62**: 1123–1128.
6 Ott J: Estimation of the recombination fraction in human pedigrees: efficient computation of the likelihood for human linkage studies. *Am J Hum Genet* 1974; **26**: 773–775.
7 Kruglyak L, Daly MJ, Lander ES: Rapid multipoint linkage analysis of recessive traits in nuclear families including homozygosity mapping. *Am J Hum Genet* 1995; **56**: 519–527.
8 Verhoeven K, Van Camp G, Govaerts PJ *et al*: A gene for autosomal dominant nonsyndromic hearing loss (DFNA12) maps to chromosome 11q22–24. *Am J Hum Genet* 1997; **60**: 1168–1173.
9 Verhoeven K, Van Laer L, Kirschhofer K *et al*: Mutations in the human alpha-tectorin gene cause autosomal dominant non-syndromic hearing impairment. *Nat Genet* 1998; **19**: 60–62.
10 Hughes DC, Legan PK, Steel KP, Richardson GP: Mapping of the alpha-tectorin gene (TECTA) to mouse chromosome 9 and human chromosome 11: a candidate for human autosomal dominant nonsyndromic deafness. *Genomics* 1998; **48**: 46–51.
11 Weil D, Blanchard S, Kaplan J *et al*: Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* 1995; **374**: 60–61.
12 Liu X-Z, Walsh J, Mburu P *et al*: Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat Genet* 1997; **16**: 188–193.
13 Liu X-Z, Walsh J, Tamagawa Y *et al*: Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. *Nat Genet* 1997; **17**: 268–269.