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LETTER TO JMG

A gene responsible for autosomal dominant auditory neuropathy (AUNA1) maps to 13q14–21

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Hearing loss is most commonly defined as either conductive, affecting the sound conduction mechanism comprised of the external auditory canal, tympanic membrane, and middle ear ossicles, or sensorineural (SNHL), affecting the cochlea, the auditory nerve, or the central auditory pathway. However, the recent discovery that outer hair cells (OHC) generate otoacoustic emissions (OAEs) has allowed differentiation of sensory hearing loss (in which OAEs are absent) from neural hearing loss, which is caused by a lesion of inner hair cells and/or the auditory nerve. The hallmark of auditory neuropathy (AN), a neural type of hearing loss, is preservation of OAEs and abnormal or absent auditory brainstem responses.[1] Most patients with SNHL are found to have a sensory type of hearing loss, and numerous genes for both syndromic and non-syndromic forms have been identified (Hereditary Hearing Loss Homepage, http://www.uia.ac.be/dnalab/hhh/). However, none of the approximately 50 dominant (DFNA) loci are known to represent an auditory neuropathy phenotype.

AN may accompany peripheral neuropathy in a variety of dominant syndromes such as Charcot-Marie-Tooth disease[2] and Freidreich's ataxia.[3] AN unassociated with peripheral neuropathy most commonly occurs as a sporadic or recessive trait,[4–6] but X linked recessive[6] and autosomal dominant[7] forms have also been described. We have mapped a gene responsible for autosomal dominant auditory neuropathy in a multigenerational family from the United States to a novel locus, AUNA1 (auditory neuropathy, dominant, 1) on 13q14–21.

Key points

- Auditory neuropathy (AN) is a type of hearing loss defined by the preservation of outer hair cell function and abnormal or absent auditory brainstem responses. We studied 47 members of a family of European descent from the United States segregating autosomal dominant non-syndromic AN.
- AUNA1 maps to a 5.47 cM interval on chromosome 13q14–21 between D13S153 (centromeric) and D13S1317 (telomeric). Two individuals homozygous for the haplotype common to affected family members did not appear to be more severely affected than the heterozygotes. The maximum two point LOD score was 9.87 at $\theta = 0.019$ for D13S153.
- We conclude that AUNA1 is the first locus found responsible for autosomal dominant AN. Assessment of outer hair cell function by otoacoustic emissions or cochlear microphonics will clarify the prevalence of AN in non-syndromic deafness families.

METHODS

The family is of European descent and was ascertained through two different probands by both the University of Michigan and the University of California at Irvine. The Institutional Review Boards of the University of Michigan Medical School, Louisiana State University Health Sciences Center, and the University of California at Irvine approved the study, and informed consent was obtained from all subjects. Four generations were available for study, including 47 family members informative for genetic analysis of whom 33 were affected, four were unrelated spouses, and 10 were unaffected (fig 1). All unaffected members were at least 18 years old. Two individuals (VI:9 and VI:13) had isolated high frequency sensorineural hearing loss consistent with their sex and age and were characterised as unaffected prior to linkage analysis. Information was obtained from questionnaires and interviews with family members. Standard pure tone audiometry was performed for all participants, and peripheral blood or buccal cell samples were obtained. The phenotype was extensively characterised by otologic and neurologic examination and by audiological, psychoacoustic, and neurophysiological testing.[8] SLINK analysis predicted an average maximum LOD score of 7.90, with 100% of replicates greater than 3.0.[9 10]

Genomic DNA was isolated from peripheral blood lymphocytes and buccal epithelial cell samples using standard methodology. A genome scan was performed by the Center for Inherited Disease Research (CIDR, http://www.cidr.jhmi.edu/) as an automated fluorescent microsatellite analysis using a marker set of approximately 400 primer pairs with average spacing of 10 cM. Two point LOD scores assuming complete penetrance, gene frequency of 0.00001, and 0% phenocopy rate were calculated using the MLINK and ILINK programs from the LINKAGE package.[11]

Fine mapping was performed by genotyping additional markers on chromosome 13 (fig 1) through the University of Michigan Sequencing Core. Allele frequencies and sizes were calculated by comparing the genotypes of CEPH individual 1347-02 to the CEPH genotype database (http://www.cephb.fr). To avoid overstated evidence for linkage caused by underestimation of marker allele frequencies, the allele frequency for the linked allele was not allowed to be less than 0.1.

RESULTS

The hearing loss was inherited as an autosomal dominant trait with an average age of onset of 18.6 years. There were

Abbreviations: ABR, auditory brainstem responses; AN, auditory neuropathy; CIDR, Center for Inherited Disease Research; CMs, cochlear microphonics; ENU, *N*-ethyl-*N*-nitrosurea; OAEs, otoacoustic emissions; OHC, outer hair cell; SNHL, sensorineural hearing loss; WS, Wolfram syndrome

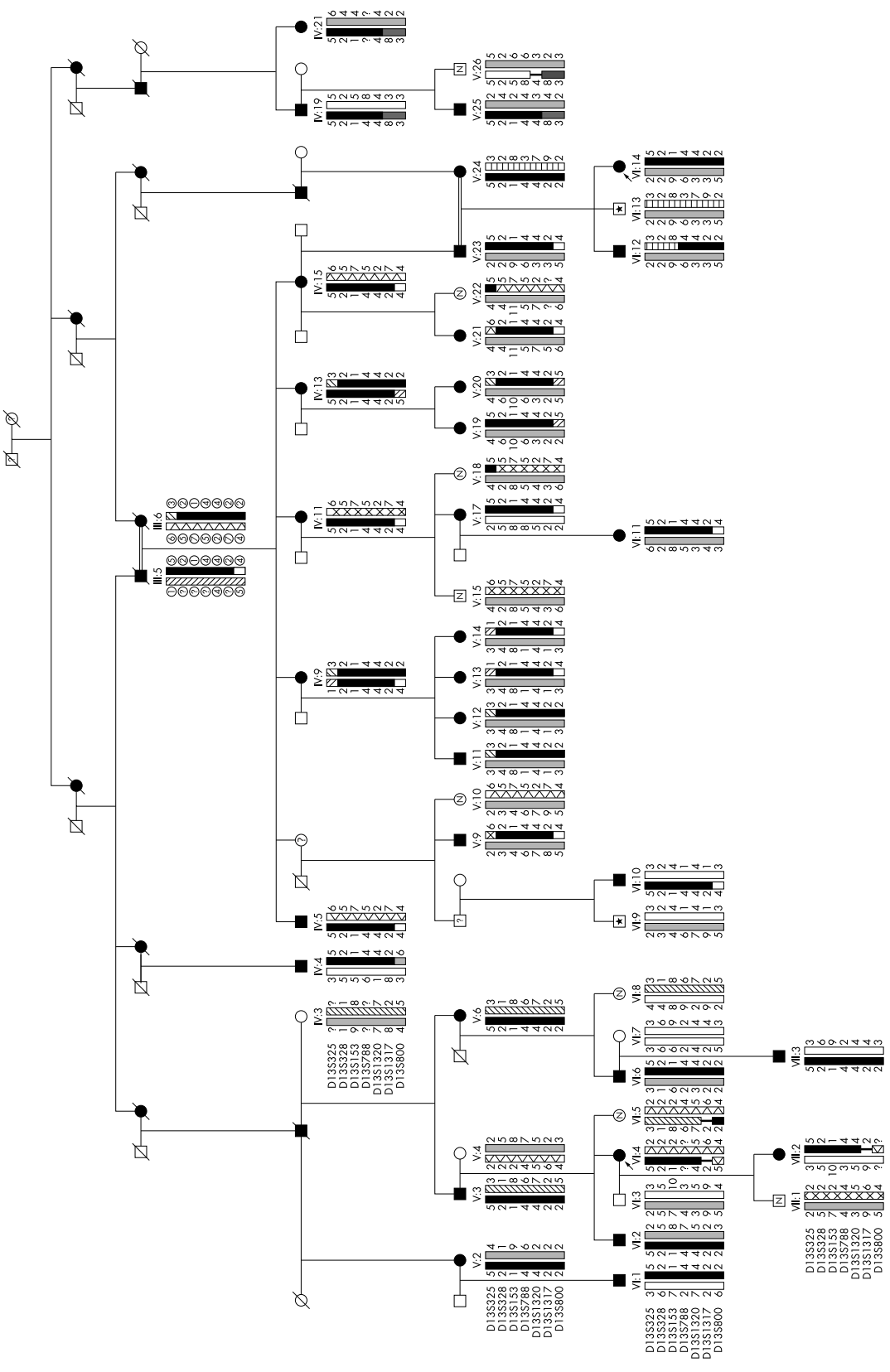

Figure 1 Haplotype analysis of the AUNA1 family. Filled in symbols indicate affected persons, open symbols indicate unrelated spouses, N indicates unaffected person, asterisk indicates unaffected person with high frequency sensorineural hearing loss, and ? indicates hearing test not available. Markers are indicated at the left ordered from centromeric (top) to telomeric (bottom), with obligate recombination events indicated. The haplotype assumed to carry the disease allele is indicated by the black bar, with other shading and hatching representing other haplotypes. Inferred alleles are indicated in parentheses. Solid lines in bars indicate phase unknown genotypes.

two consanguineous marriages in the family, one between affected first cousins (III:5 and III:6) and the other between affected second cousins (V:23 and V:24). Of seven affected offspring available for study from these consanguineous marriages, two (IV:9 and IV:13) were found to be homozygous for the haplotype common to the affected family members. However, with the exception of an age of onset at the lower end of the range (8 and 9 years for IV:9 and IV:13, respectively), there were no apparent clinical features differentiating their phenotype from that of the heterozygotes.

The youngest affected family members presented with auditory neuropathy, defined as preserved OHC function (as documented by normal distortion product OAE responses), and hearing loss documented by pure tone audiometry and/or auditory brainstem response (ABR). Over time, OAEs disappeared and thresholds increased, diagnostic of profound sensorineural hearing loss. No evidence of cranial or peripheral neuropathies was found. The results of the haplotype analysis were consistent with our assumption of complete penetrance after age 18, even though some individuals reported onset as late as 45 years of age. However, for most participants, the age of onset was estimated based on that individual's recollection. Affected family members presented with a range of phenotypes which will be described elsewhere.[8] Intrafamilial variability is quite common even for hereditary hearing loss segregating as a simple Mendelian trait, most likely due to environmental and secondary genetic factors.[12]

The maximum two point LOD score was 9.87 at $\theta = 0.019$ for D13S153 (table 1). No recombination events were observed for D13S788 (LOD = 7.91 at $\theta = 0$) or D13S1320 (LOD = 9.33 at $\theta = 0$). A recombination event between D13S153 and D13S788 in individual VI:12 defines the centromeric end of the interval (fig 1). The telomeric end of the interval is defined by an obligate recombination event that occurred between D13S1320 and D13S1317, transmitted to individuals IV:19, IV:21, and V:25. The interval between D13S153 (centromeric) and D13S1317 (telomeric) spans 5.47 cM in 13q14–21 (fig 2) as defined by recombination events in affected individuals only. The physical distance is approximately 18 Mb (UCSC Genome Browser: http://genome.ucsc.edu). The locus was designated as AUNA1 (http://www.gene.ucl.ac.uk/nomenclature), and the interval does not overlap with other known human or murine deafness loci, corresponding to mouse chromosome 14 and a small portion of mouse 8.

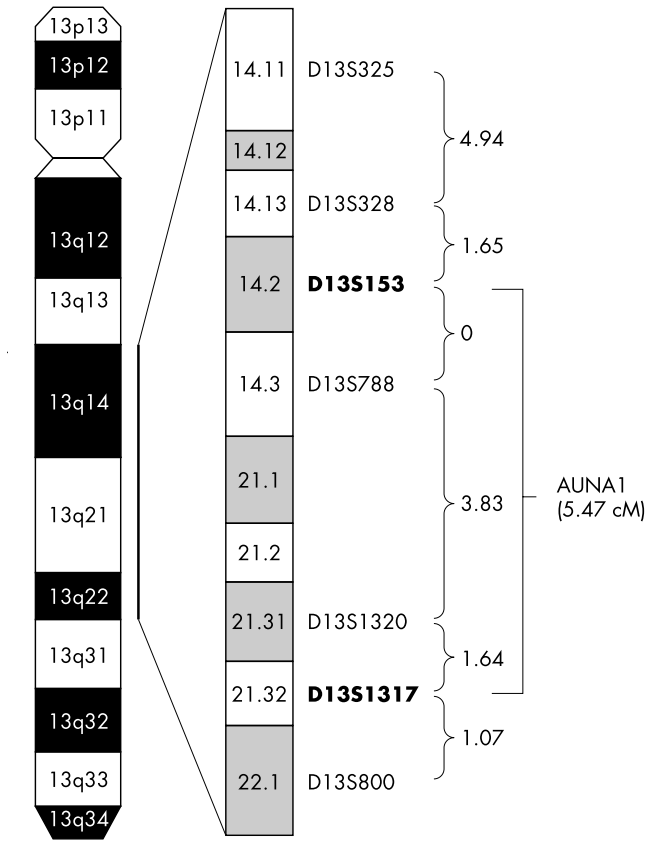

Figure 2 Ideogram of chromosome 13 indicating genotyped markers on 13q14–21 defining AUNA1 interval. Distances between markers are in centimorgans (cM) and were obtained from the Marshfield Center for Medical Genetics (http://research.marshfieldclinic.org/genetics/).

DISCUSSION

Great progress has been made in identifying genes responsible for non-syndromic hearing impairment, with at least 40 genes cloned and an equivalent number mapped for dominant, recessive, or X linked hearing loss (Hereditary Hearing Loss Homepage, http://www.uia.ac.be/dnalab/hhh/). Routine pure tone audiometry testing of both air and bone conduction can distinguish conductive from sensorineural hearing loss. However, very few studies of hereditary hearing impairment have measured OAEs or cochlear microphonics (CMs) to assess OHC function in affected subjects. Such testing is necessary to differentiate sensory hearing loss (caused by disorders of the cochlear outer hair cells) from neural hearing loss in which cochlear inner hair cells, the auditory nerve, and/or the synapses between inner hair cells and the auditory nerve are affected.

Patients with AN have absent or abnormal ABRs, absent middle ear reflexes, normal OAEs, and cochlear microphonic responses that invert with stimulus polarity.[1 13 14] Thresholds on pure tone audiometry may be normal or elevated to levels ranging from mild to profound hearing loss. Evidence suggests that some patients with AN are unlikely to benefit from hearing aids.[14 15] Approximately one third of patients with AN will ultimately demonstrate loss of OHC function to develop a true SNHL.[16] Thus, OAEs, CMs, and ABRs must be tested early in life to recognise a hearing loss as AN.

Animal models of auditory neuropathy have been induced by carboplatin treatment of chinchillas[17 18] and ouabain infusion in gerbil cochleas.[19] Both Bronx waltzer (bv), a spontaneous mutant mapping to mouse chromosome 5,[20] and Beethoven (Bth), arising from *N*-ethyl-*N*-nitrosurea (ENU) mutagenesis of the tmc1 gene,[21] demonstrate inner hair cell loss preceding OHC loss. Patients with DFNA36 (MIM 606705) (Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim) or DFNB7/11 (MIM 600974) deafness caused by mutations of the human ortholog TMC1 (MIM 606706) are not reported to have

Table 1 Two point LOD scores for chromosome 13 markers

	LOD score at θ =						
Marker	0	0.1	0.2	0.3	0.4	θ	Z_{max}
D13S325	−∞	1.31	1.68	1.24	0.63	0.176	1.71
D13S328	7.57	5.84	4.16	2.54	1.07	0	7.57
D13S153	−∞	8.69	6.49	4.09	1.78	0.019	9.87
D13S788	7.91	6.11	4.27	2.46	0.87	0	7.91
D13S1320	9.33	7.33	5.29	3.27	1.42	0	9.33
D13S1317	−∞	4.66	3.50	2.12	0.85	0.05	4.93
D13S800	−∞	−0.68	0.04	0.15	0.13	0.324	0.15

auditory neuropathy,[22] but neither OAEs nor CMs were tested in these families (A Griffith, personal communication).

Mutations in the otoferlin (OTOF, MIM 603681) gene, first identified as the cause of DFNB9 (MIM 601071), appear to be a common cause of non-syndromic autosomal recessive AN.[5 23] AN in conjunction with Charcot-Marie-Tooth disease has been attributed to mutations in the myelin protein zero (MPZ, MIM 159440), peripheral myelin protein 22 (PMP22, MIM 601097), gap junction protein, beta 1 (GJB1, MIM 304040), and early growth response 2 (EGR2, MIM 129010) genes.[24] In addition, mutations in the N-myc downstream regulated gene (NDRG1, MIM 605262) gene are associated with the autosomal recessive disorder known as hereditary motor and sensory neuropathy-Lom. Acquired or environmental causes of AN include neonatal hyperbilirubinemia,[25] anoxia, and prematurity.

True dominance is exceedingly rare, as there is usually a more severe phenotype (for example, embryonic lethality) associated with homozygosity as compared to heterozygosity for a mutated allele.[26] The notable exception is Huntington's disease (HD, MIM 143100),[27] caused by abnormal expansion of triplet repeats. Non-syndromic hearing loss offers another example of an individual homozygous for a mutated allele that causes dominant hearing loss in both of his consanguineous parents. While Wolfram syndrome (WS, MIM 222300) is caused by homozygous and usually inactivating mutations in the WFS1 (MIM 606201) gene, one patient homozygous for the A716T mutation in WFS1 had only juvenile onset insulin dependent diabetes mellitus and cataracts[28] without the optic atrophy necessary for a diagnosis of WS. A716T is one of many heterozygous missense mutations shown to cause non-syndromic dominant low frequency sensorineural hearing loss, DFNA6/14/38 (MIM 600965).[29]

We would predict that the mutation in this family will be found to be non-inactivating, for example, a missense mutation, rather than a null mutation resulting in haploinsufficiency. In the latter case, the complete lack of functional protein in the homozygotes would be expected to result in a more severe phenotype. Prospective clinical studies would be necessary to determine whether the homozygotes have an earlier age of onset. Although we could not identify any clinical features unique to the homozygous individuals in this study, identification of the AUNA1 gene may suggest targets for clinical testing based on knowledge of the gene's function.

Numerous candidate genes of interest map to the AUNA1 interval (UCSC Genome Browser: http://genome.ucsc.edu) including diaphanous homolog 3 (DIAPH3), several protocadherins (PCDH9 (MIM 603581), PCDH8 (MIM 603580), PCDH17, and PCHD20), and genes implicated in protein and/or ion transport (WD repeat and FYVE domain containing 2 (WDFY2) and potassium channel regulator gene, KCNRG (MIM 607947)).

We report here a novel locus, AUNA1, which is responsible for progressive autosomal dominant auditory neuropathy in a large kindred from the United States. Our strategies to identify the AUNA1 gene will include recruiting additional family members, genotyping additional markers in DNA samples from existing and new family members to look for new recombination events to further narrow the interval, and testing candidate genes within the interval. Identification of a gene responsible for auditory neuropathy will allow for genetic screening in sporadic cases or in families too small for genetic linkage analysis. In addition, assessing OHC function by OAE or CM testing in patients and families with non-syndromic hearing loss will lead to a better understanding of the variability among phenotypes.

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