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A gene for autosomal dominant nonsyndromic hereditary hearing impairment maps to 4p16.3

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Mapping genes for nonsyndromic hereditary hearing impairment may lead to identification of genes that are essential for the development and preservation of hearing. We studied a family with autosomal dominant, progressive, low frequency sensorineural hearing loss. Linkage analysis employing microsatellite polymorphic markers revealed a fully linked marker (D4S126) at 4p16.3, a gene-rich region containing IT15, the gene for Huntington's disease (HD). For D4S126, the logarithm-of-odds (lod) score was 3.64 at $\theta = 0$, and the overall maximum lod score was 5.05 at $\theta = 0.05$ for D4S412. Analysis of recombinant individuals maps the disease gene to a 1.7 million base pair (Mb) region between D4S412 and D4S432. Genes for two types of mutant mice with abnormal cochleovestibular function, tilted (*tlt*) and Bronx waltzer (*bv*), have been mapped to the syntenic region of human 4p16.3 on mouse chromosome 5. Further studies with the goals of cloning a gene for autosomal nonsyndromic hearing impairment and identifying the murine homologue may explain the role of this gene in the development and function of the cochlea.

INTRODUCTION

To date, several genes associated with syndromic hereditary hearing impairment have been cloned, and still more have been mapped (1,2). Yet, approximately two-thirds of all cases of hereditary hearing impairment are non-syndromic (3). Several genes for nonsyndromic hearing loss have been mapped, but only X-linked and mitochondrial genes have been cloned (4–12). The discovery of increasing numbers of loci linked to hereditary hearing impairment attests to the marked genetic heterogeneity of nonsyndromic deafness. Furthermore, there are families with hereditary hearing loss that are not linked to any known loci for hereditary hearing impairment (E.Wilcox and R.J.H.Smith, pers. comm.).

Based on the Fay survey of deaf by deaf marriages, it was estimated that there are at least 10 loci for recessive deafness, assuming equal allele frequencies of the mutant genes (13). Sixty autosomal loci for prelingual deafness and another 60 autosomal loci for postlingual deafness were predicted by multiplying 20-fold the number of X-linked loci (14). The number of genes involved in nonsyndromic deafness and the interactions between them may be quite complex. It has been suggested that high frequency hearing loss linked to 13q12 may be inherited either as a recessive or a dominant disorder, resulting from different mutations in the same gene (8,11). Allelic mutations can also cause clinically distinct phenotypes, as in Jackson–Weiss and Crouzon syndromes (15).

Previously described loci for dominant nonsyndromic hereditary hearing impairment include 5q31, 1p32 and 13q12 (6,7,11). The 5q31 locus was discovered through linkage analysis of a Costa Rican family with low frequency hearing loss. The hearing loss in these individuals invariably progresses to bilateral profound hearing loss, distinguishing it from the hearing loss in the present study. Hereditary high frequency hearing loss progressing to profound hearing loss in an Indonesian family and a United States family maps to 1p32, and a French family with predominantly high frequency hearing loss was linked to 13q12, a locus first identified as one for recessive hereditary hearing impairment.

The present study involves a large multi-generational family from the southeastern USA with nonsyndromic, progressive, dominant low frequency hearing loss. This family was first described in 1968 by the Vanderbilt University Hereditary Deafness Study Group (16). The two sets of audiograms obtained 25 years apart were useful in assessing the progression of the hearing loss. Linkage analysis at that time revealed no linkage to ABO, Rh or MNS.

In the majority of patients the hearing loss is bilateral and symmetric, involving frequencies of 250, 500 and 1000 Hz at onset. Speech discrimination scores were consistent with the pure tone averages. The progression of the hearing loss was quite variable but generally followed one of three patterns: (i) remaining confined to the low frequencies; (ii) involving all frequencies producing a flat audiogram; or (iii) involving low and high frequencies with sparing of the middle frequencies (2000 Hz). In the latter case, presbycusis or noise-induced hearing loss accompanying the hereditary component cannot be ruled out. The severity of hearing loss at a young age did not invariably imply a progression to profound deafness, as

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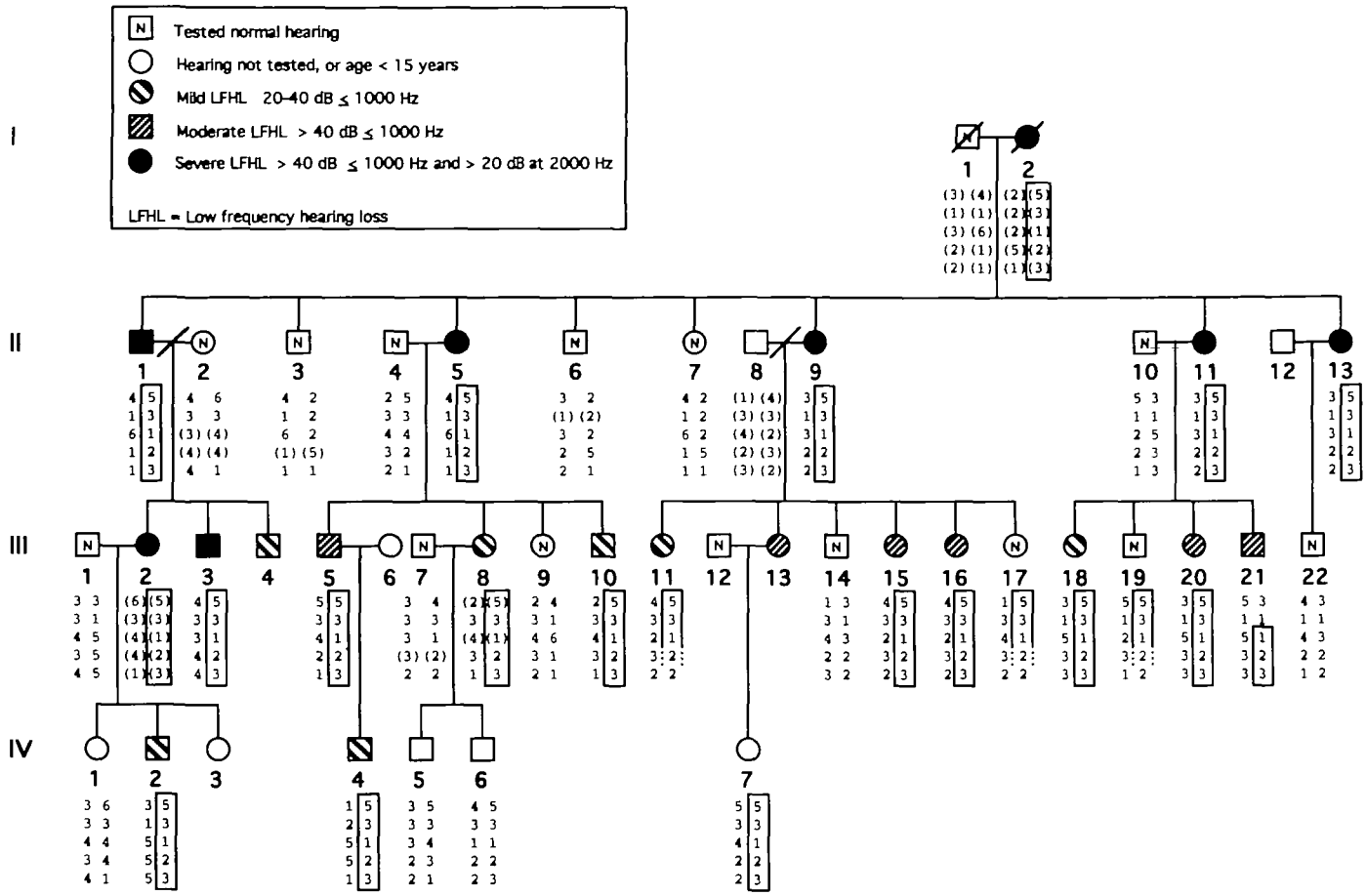

Figure 1. Haplotype analysis of family with nonsyndromic hereditary hearing impairment. Key to affected status is given in upper left corner. Parentheses indicate inferred genotype. Question marks indicate genotype unknown. Markers genotyped are displayed vertically, from top to bottom: D4S43, D4S127, D4S412, D4S126 and D4S432. Boxes indicate inheritance of the chromosome linked to the disease. Dashed lines indicate that the affected parent is uninformative for that marker.

some hearing levels remained stable over decades. Most of the family members do not use hearing aids, and none have cochlear implants.

The age of onset is generally in the second decade of life, with age-dependent penetrance. 'Borderline affected' individuals in the 1968 study had thresholds of 20–30 decibels at frequencies below 2000 Hz. This group consisted of four children ranging in age from 3 to 10 years. Although the 1968 thresholds were considered to be within normal limits, audiograms obtained recently on two of these individuals were consistent with those of affected family members. Thus, over time, at least some of the individuals with minimal (<30 decibels) low frequency hearing loss will become affected. In both studies, there were patients between the ages of 6 and 10 who could be clearly identified as affected; some had low frequency thresholds as high as 40–50 decibels. No family members presented with hearing loss before age 5, and all affected members had developed hearing loss by age 15. To allow for the change in penetrance from 0% before age 5 and 100% after age 15, individuals in this age group were assigned to a liability class with an intermediate penetrance value of 60%. The complete clinical description of this family will be reported elsewhere (J.W.Hall, M.M.Lesperance, E.R.Wilcox, T.B.San Agustin and F.H.Bess, in preparation).

RESULTS

After exclusion of the known loci for autosomal dominant hereditary hearing impairment [5p31, 1p32 and 13q12; (6,7,11)], we performed a genome-wide search using published maps of polymorphic microsatellite markers (17–19). Linkage analysis was initially detected with marker D4S432 after testing over 300 primer pairs. The commonly accepted criterion for significant linkage holds true even when multiple markers are tested. As stated by Terwilliger and Ott, 'In a normal, well-characterized mendelian disease, the critical value of $Z_{max}>3$ as a test for linkage is robust to multiple testing, because as we find negative test results with more markers, the prior probability of linkage to the remaining markers increases sufficiently to offset the increased probability of finding a significant result by chance'. It should be noted that only one model, dominant inheritance with age-dependent penetrance, has been tested (for further discussion, see refs 27,58). Detailed high resolution maps of the 4p telomere were

Table 1. Nonsyndromic hereditary hearing impairment: pairwise lod scores

Locus	Lod score at θ =								
	0	0.025	0.05	0.1	0.2	0.3	0.4	θ_{max}	Max lod score
D4S43	−4E + 19	3.97	4.04	3.88	3.15	2.15	0.93	0.05	4.04
D4S127	−4E + 19	2.46	2.85	3.00	2.62	1.84	0.79	0.1	3.00
D4S412	−4E + 19	5.01	5.05	4.81	3.91	2.70	1.23	0.05	5.05
D4S126	3.64	3.49	3.34	3.01	2.30	1.48	0.57	0	3.64
D4S432	−4E + 19	3.72	3.93	3.92	3.30	2.30	1.03	0.075	3.97

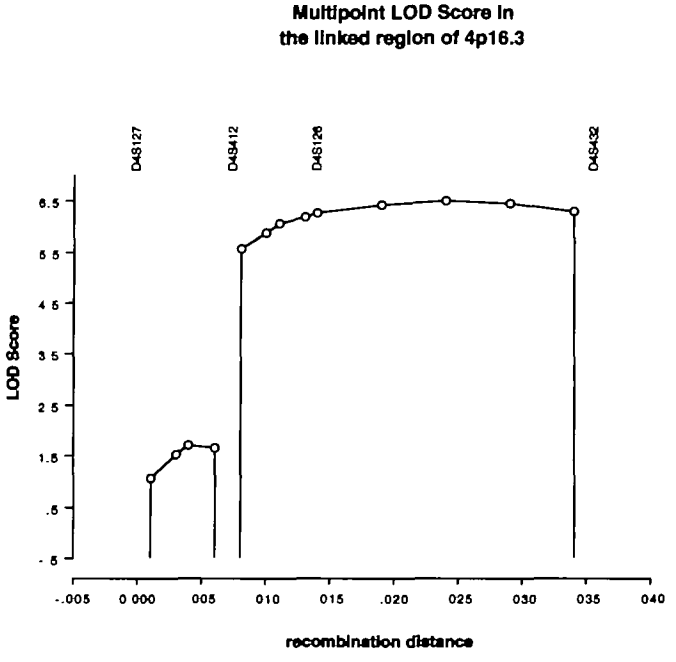

Figure 2. Multipoint LOD score in the linked region of 4p16.3 for nonsyndromic hereditary hearing impairment. A five-point analysis was performed between the disease locus and four markers in the order as shown. Map distances are given in recombination units. The zero point was arbitrarily set at D4S127. The curve asymptotically approaches infinity at marker loci at which recombinants occurred. The curve reaches a maximum lod score of 6.5 in the interval between D4S126 and D4S432.

then used to select other markers in the region (20–22). The complete family set was then genotyped for D4S432 (17), D4S412 (17), D4S127 (23), D4S43 (24) and D4S126 (25). These five markers span a genetic distance of ~5 cM (22). The region has a much higher rate of recombination (26) than would be expected for its physical size (<3 Mb).

Four recombinants were identified by haplotype analysis (Fig. 1). Individual III-21 has a recombination between D4S127 and D4S412. Individuals III-17 and III-19 are unaffected with recombinations between D4S126 and D4S432. Since III-11 is an affected individual with the same recombinant haplotype, we postulate that the breakpoints for III-11 versus III-17 and III-19 lie on opposite sides of the gene for hearing loss. D4S126 is not a fully informative marker; the affected parents of the recombinants are homozygous at this locus, a state which prevents detection of recombinants. Thus, the most likely interval for the gene is between D4S412 and D4S432, a distance of 1.7 Mb (20).

The logarithm-of-odds table for the family versus each marker is shown in Table 1. The maximum lod score was 5.05 at θ = 0.05 for D4S412. As noted above, complete linkage was noted for D4S126, with a lod score of 3.64 at θ = 0. Given the marker order of D4S412–D4S126–D4S432, multipoint mapping yielded a maximum lod score of 6.5 when the disease gene was placed in the interval between D4S126 and D4S432 (Fig. 2).

Individuals III-17 and III-19 may be unaffected because of incomplete penetrance, rather than because of recombination. To test for the effects of very low penetrance, that is 0.1%, an affected-only analysis was performed, with the maximum lod scores for each marker as follows: D4S43, 3.83; D4S127, 2.46; D4S412, 5.00; D4S126, 2.64; D4S432, 2.93. The lod scores decreased over all markers, as expected, because of the loss of information from unaffected individuals (27). In general, the recombination frequency yielding the maximum lod score decreased, because the unaffected individuals were no longer seen as recombinants. If only affected recombinants (III-11 and III-21) are considered, the most likely interval for the gene is between D4S127 and D4S432.

DISCUSSION

The interval 4p16.3 has been well mapped, with over 20 genes identified in the course of the search for the Huntington's disease gene, known as HD or IT15 (28,29). The 4p telomere is a member of isochore family H3, a group of chromosomal regions which are highly recombinogenic and known to have a gene concentration 8–16-fold higher than the rest of the genome (30). Isochores are defined by Bernardi as large DNA regions (average >300 kb) that are compositionally homogeneous characterized by different G+C levels and distinct chromatin structure (31). The number of recombinant events and the existence of many physical maps of the region have allowed us to map the gene to an interval of ~2 Mb. This discussion will highlight some of the potential candidate genes in the region rather than attempting a comprehensive review (29).

The gene for nonsyndromic hereditary hearing impairment is fully linked to a marker, D4S126, that is 520 kb from the 3′ end of IT15, the gene for HD (28). Expression of IT15 has been detected in all areas of the cerebral cortex, predominantly in neurons (32). Hearing loss has not been described as a clinical feature of HD; however, to our knowledge there has been no study specifically screening hearing in patients with HD. Our multipoint data suggest a location proximal to IT15 and D4S126, a region in which the gene for the α2C-adrenergic

receptor (ADRA2C) maps (33). Alpha-2-adrenergic receptors are widely expressed in the brain, especially in regions with high dopamine content. Another candidate gene in this region is the α2-macroglobulin receptor-associated protein, A2MRAP, also known as LRPAP1, the low-density lipoprotein receptor-related protein-associated protein 1 (34,35). Although the function of this protein is unknown, its corresponding receptor is important in proteinase inhibition and lipoprotein metabolism.

Other genes in the region have been implicated in hereditary hearing loss. Mutations in the α-L-iduronidase gene (IDUA) cause mucopolysaccharidosis type I, an autosomal recessive disorder with different clinical subtypes known as Hurler and Scheie syndromes (36). Glycosaminoglycans accumulate in the middle ear and eventually infiltrate the cochlear nerve, leading to refractory chronic serous otitis media and mixed hearing loss (37,38). Achondroplasia is caused by mutations in FGFR3, the fibroblast growth factor-3 gene (39). Chronic otitis media is common in achondroplasia because the abnormalities of the cranial base lead to eustachian tube dysfunction (40). However, a significant incidence of sensorineural hearing loss has also been reported (41). The homologous gene in the mouse, *fgfr3*, is expressed at high levels in the hair cells and supporting cells of the developing cochlea (42).

Distal 4p also contains a regulatory myosin light chain gene, MYL5 (43). Myosins are attractive candidate genes for neuroepithelial defects because of a possible role in signal transduction (1). MYL5 is expressed in adult retina, basal ganglia and cerebellum at very low levels consistent with either low levels of cellular expression or a higher level of tissue-specific expression (43). Mutations in myosin VIIA, a myosin heavy chain gene, cause Usher syndrome type IB (2).

The Wolf–Hirschhorn syndrome is a contiguous gene syndrome involving deletions of 4p. The syndrome is characterized by growth retardation, mental retardation, microcephaly, dysmorphic facies and other features, including cleft lip and palate (44). The critical region for Wolf–Hirschhorn syndrome has been defined as a ~2 Mb region between D4S43 and D4S142 (45). However, a deletion of ~8 Mb extending from the p telomere to D4S62 was associated with hearing loss, external ear deformity and cleft palate (46). A preliminary phenotypic map of 4p, based on this patient and other cases of Wolf–Hirschhorn syndrome, has been developed (47). Although phenotypic maps must be interpreted with caution (48), it is interesting to note that our map location for nonsyndromic hereditary hearing impairment (between D4S412 and D4S432) contains the interval for hearing loss on the 4p phenotypic map (between D4S10 and D4S240). Ideally, all patients with Wolf–Hirschhorn syndrome would undergo a complete auditory work-up to verify these findings, but testing mentally retarded infants can be very difficult. Precise mapping of deletions using fluorescent *in situ* hybridization (FISH) may provide further evidence of the existence of a gene involved in hearing in this interval.

The murine homologues of many genes from human chromosome 4p16.3 have been mapped to two regions of synteny conservation on mouse chromosome 5 (49). The mouse gene tilted (*tlt*) maps to 5.21, at the same locus as D5H4S43, which corresponds to the human D4S43 (50). Tilted mice cannot swim, and the Preyer reflex is absent. The mutation is known to be recessive but is otherwise not well characterized. Further investigation of the development, anatomy and function of the inner ear in this mouse may reveal the role of the gene for nonsyndromic hereditary hearing impairment herein described.

The locus order in human 4p16.3 has been conserved in the mouse chromosome 5 except for the displacement of *rd1*, the murine homologue of the human β-subunit of rod cGMP phosphodiesterase (PDEB), and *idua*, which map to mouse 5.49 (49). The mutation that causes the Bronx waltzer (*bv*) mouse phenotype (deafness and circling) has been mapped to 5.50 (K.Steel and T.Bussoli, pers. comm.). The Bronx waltzer mouse has loss of inner hair cells and spiral ganglion cell degeneration with preservation of outer hair cells (50). Since IDUA and PDEB are ~3 Mb distal to our locus, *bv* is a less likely candidate gene.

We have mapped a gene for nonsyndromic hereditary hearing impairment to 4p16.3. Although this is a well-studied, gene-rich region, the interval between D4S125 and D4S10 has been difficult to clone in YACs and cosmids (51). Further efforts using positional cloning techniques to improve the resolution of the map in this region, clone the gene and identify the mutation responsible for the hearing loss may provide insight into the mechanisms of genetic hearing loss, presbycusis and even hearing itself.

Table 2. Specific allele sizes in base pairs

D4S43	D4S127	D4S412	D4S126	D4S432
1 = 161	1 = 196	1 = 253	1 = 175	1 = 254
2 = 159	2 = 190	2 = 249	2 = 167	2 = 244
3 = 155	3 = 186	3 = 247	3 = 173	3 = 232
4 = 184		4 = 245	4 = 171	4 = 224
5 = 180		5 = 243	5 = 169	5 = 258
6 = 194		6 = 237		

MATERIALS AND METHODS

Family data

Thirty-four family members of three generations were available for the analysis. Of the 17 affected family members, 10 were female and seven were male. Seventeen unaffected family members included five unaffected spouses (four females, one male), four unaffected children under 15 years old (two females, two males) and eight unaffected adult family members (three female, five male). Six additional spouses otherwise uninformative for linkage were genotyped to obtain population allele frequencies (data not shown in Fig. 1).

Participating family members underwent a complete history and physical examination, pure tone audiometry, speech audiometry, immittance testing and distortion product otoacoustic emissions (DPOAE). The history revealed progressive hearing loss without fluctuation. Tinnitus and vestibular symptoms were uncommon. Selected patients underwent auditory brainstem response (ABR), electronystagmography (ENG) and/or high resolution computerized tomography (CT) of the temporal bone. Conductive hearing loss, retrocochlear pathology and acquired causes of hearing loss (mumps, rubella, meningitis, ototoxic drugs chronic otitis media) were ruled out. The family members were generally in good health, with no features suggestive of syndromic hearing loss such as vestibular, visual or pigmentary abnormalities. Two members (III-18 and III-19) are mentally retarded in association with Down's syndrome; nonetheless, they were able to complete the same evaluation as other family members. The complete clinical description of this family will be reported elsewhere (J.W.Hall, M.M.Lesperance, E.R.Wilcox, T.B.San Agustin and F.H.Bess, in preparation).

PCR

Venous blood was collected from each consenting family member Standard methodology was used to prepare genomic DNA from leukocytes. The genome

search utilized commercially available primer pairs (Research Genetics) to amplify microsatellite polymorphisms covering the 22 autosomal chromosomes to an average density of 20–30 cM. The markers were screened with a subset of 21 patients. For primer pairs not commercially available, oligonucleotides were prepared on an Applied Biosystems 394 DNA/RNA synthesizer (Applied Biosystems). Genetic maps from published literature covering the entire genome (17–19) and those covering in detail 4p16.3 (20–22) were used to determine the physical distance and recombination frequency between loci. The complete family set was then genotyped for D4S432 (17), D4S412 (17), D4S127 (23), D4S43 (24) and D4S126 (25).

The PCR reactions had a total volume of 10 µl containing 40 ng of genomic DNA, 2.5 pmol of each primer, 0.2 pmol of one primer end-labeled with [γ-^{32P}]ATP (ICN Pharmaceuticals), 200 µM of each dNTP, 0.25 U Ampli*Taq* DNA Polymerase (Perkin Elmer Cetus), 0.055 µg of TaqStart™ Antibody (Clontech), and 1×Perkin Elmer Cetus PCR buffer. After an initial step at 95°C for 2 min to remove the monoclonal antibody and to denature, the samples underwent 35 cycles of amplification (95°C for 30 s, 55°C for 30 s and 72°C for 30 s) with a final extension step at 72°C for 3 min. After PCR, 5 µl of loading buffer (95% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol and 10 mM EDTA) was added to each sample. The samples were denatured at 95°C for 5 min prior to loading. Gel electrophoresis was carried out on a 6% denaturing polyacrylamide/urea gel. The gels were dried for 1 h at 80°C prior to autoradiography with a film exposure between 2 and 12 h at room temperature.

Linkage analysis

Pairwise and multipoint linkage analysis was performed on LINKAGE software version 5.01 (52). SLINK simulation determined a maximum possible logarithm-of-odds (lod) score of 7.53 at a recombination frequency $\theta = 0$ for a fully informative marker with five equally frequent alleles (53,54). Three liability classes were assigned: age under 5 years, 0% penetrance; age between 5 and 15 years, 60% penetrance; age over 15 years, 100% penetrance. Children under 15 years of age without hearing impairment were considered to have a phenotype of unaffected.

MLINK two-point linkage analysis was performed between the disease gene and each marker. The disease allele frequency was set at 0.0001. Equal recombination frequencies for males and females were assumed. Allele sizes were estimated by comparison to a M13/pUC18 sequencing ladder [dsDNA Cycle Sequencing System (BRL, Gaithersburg, MD)] for all markers as given in Table 2.

For markers D4S432 and D4S412, allele sizes were confirmed by genotyping individual 1347-02 from the CEPH pedigrees (17). Allele frequencies were determined by genotyping 11 spouses in the pedigree. We compared the allele sizes and frequencies with those based on genotyping of CEPH families, as reported in the Genome Data Base (55). Varying the allele frequencies did not significantly change the lod scores, probably due to the small number of untyped individuals in the pedigree. It is known that allele sizes may vary, depending on which primer is kinased and other factors, and it is not unusual to find new alleles (56). Therefore, we feel it is most accurate to report the lod scores based on the spouse allele frequencies (Table 2).

Five-point multipoint analysis was performed using LINKMAP software using the marker order D4S127-D4S412-D4S126-D4S432 (Fig. 2). Due to computational constraints, it was necessary to down-code alleles to a maximum of five for D4S412 and D4S43 (57).

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ABBREVIATIONS

θ, the recombination frequency; ABR, auditory brainstem response; ADRA2C, α2C-adrenergic receptor; *bv*, Bronx waltzer; CT, computerized tomography; DPOAE, distortion product otoacoustic emissions; ENG, electronystagmography; FGFR3, fibroblast growth factor-3; IT15, gene for Huntington's disease; HD, Huntington's disease; IDUA, α-L-iduronidase; lod, logarithm-of-odds; *tlt*, tilted mouse; PDEB, β-subunit of rod cGMP phosphodiesterase.

REFERENCES

1. Steel,K.P. and Brown,S.D.M. (1994) Genes and deafness. *Trends Genet.*, **10**, 428–435.
2. 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.
3. Cremers,C.W., Marres,H.A. and van Rijn,P.M. (1991) Nonsyndromal profound genetic deafness in childhood. *Ann. NY Acad. Sci.*, **630**, 191–196.
4. de Kok,Y.J.M., van der Maarel,S.M., Bitner-Glindzicz,M., Huber,I., Monaco,A.P., Malcolm,S., Pembrey,M.E., Ropers,H.-H. and Cremers,F.P.M. (1995) Association between X-linked mixed deafness and mutations in the POU domain gene POU3F4. *Science*, **267**, 685–688.
5. Prezant,T.R., Agapian,J.V., Bohlman,M.C., Bu,X., Öztas,S., Qiu,W.-Q., Arnos,K.S., Cortopassi,G.A., Jaber,L., Rotter,J.I., Shohat,M. and Fischel-Ghodsian,N. (1993) Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nature Genet.*, **4**, 289–294.
6. Léon,P.E., Raventos,H., Lynch,E., Morrow,J. and King,M.-C. (1992) The gene for an inherited form of deafness maps to chromosome 5q31. *Proc. Natl Acad. Sci. USA*, **89**, 5181–5184.
7. Coucke,P., Van Camp,G., Djoyodiharjo,B., Smith,S.D., Frants,R.R., Padberg,G.W., Darby,J K., Huizing,E.H., Cremers,C.W.R.J., Kimberling,W.J., Oostra,B.A., Van de Heyning,P.H. and Willems,P.J. (1994) Linkage of autosomal dominant hearing loss to the short arm of chromosome 1 in two families. *N. Engl. J. Med.*, **331**, 425–431.
8. 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.
9. Lalwani,A.K., Brister,J.R., Fex,J., Grundfast,K.M., Pikus,A.T., Ploplis,B., San Agustin,T., Skarka,H. and Wilcox,E.R. (1994) A new nonsyndromic X-linked sensorineural hearing impairment linked to Xp21.2. *Am. J. Hum. Genet.*, **55**, 685–694.
10. Guilford,P., Ayadi,H., Blanchard,S., Chaïb,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.
11. Chaïb,H., Lina-Granade,G., Guilford,P., Plauchu,H., Levilliers,J., Morgon,A. and Petit,C. (1994) A gene responsible for a dominant form of neurosensory non-syndromic deafness maps to the NSRD1 recessive deafness gene interval. *Hum. Mol. Genet.*, **3**, 2219–2222.
12. 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.
13. 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.
14. Morton,N.E. (1991) Genetic epidemiology of hearing impairment. *Ann. NY Acad. Sci.*, **630**, 16–31.
15. Jabs,E.W., Li,X., Scott,A.F., Meyers,G., Chen,W., Eccles,M., Mao,J., Charnas,L.R., Jackson,C.E. and Jaye,M. (1994) Jackson–Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2. *Nature Genet.*, **8**, 275–279.
16. The Vanderbilt University Hereditary Deafness Study Group. (1968) Dominantly inherited low-frequency hearing loss. *Arch. Otolaryngol.*, **88**, 40–48.
17. Gyapay,G., Morissette,J., Vignal,A., Dib,C., Fizames,C., Millasseau,P., Marc,S., Bernardi,G., Lathrop,M. and Weissenbach,J. (1994) The 1993–4 Généthon human genetic linkage map. *Nature Genet.*, **7**, 246–339.
18. Matise,T.C., Perlin,M. and Chakravati,A. (1994) Automated construction of genetic linkage maps using an expert system (MultiMap): a human genome linkage map. *Nature Genet.*, **6**, 384–390.
19. Murray,J.C., Buetow,K.H., Weber,J.L., Ludwigsen,S., Scherpbier-Heddema,T., Manion,F., Quillen,J.C.S.V., Sheffield,V., Sunden,S., Duyk,G.M., Weissenbach,J., Gyapay,G., Dib,C., Morrissette,J., Lathrop,G.M., Vignal,A., White,R., Matsunami,N., Gerken,S., Melis,R., Albertsen,H., Plaetke,R., Oldelberg,S., Ward,D., Dausset,J., Cohen,D. and Cann,H. (1994) A comprehensive human linkage map with centimorgan density. *Science*, **265**, 2049–2054.
20. Fan,J.-B., DeYoung,J., Lagacé,R., Lina,R.A., Xu,Z., Murray,J.C., Buetow,K.H., Weissenbach,J., Goold,R.D., Cox,D.R. and Myers,R.M. (1994) Isolation of yeast artificial chromosome clones from 54 polymorphic loci mapped with high odds on human chromosome 4. *Hum. Mol. Genet.*, **3**, 243–246.
21. Goold,R.D., diSibio,G.L., Xu,H., Lang,D.B., Dadgar,J., Magrane,G.G.,

Dugaiczyk,A., Smith,K.A., Cox,D.R., Masters,S.B. and Myers,R.M. (1993) The development of sequence-tagged sites for human chromosome 4. *Hum. Mol. Genet.*, **2**, 1271–1288.
22. Baxendale,S., MacDonald,M.E., Mott,R., Francis,F., Lin,C., Kirby,S.F., James,M., Zehetner,G., Hummerich,H., Valdes,J., Collins,F.S., Deaven,L.J., Gusella,J.F., Lehrach, H. and Bates,G.P. (1993) A cosmid contig and high resolution restriction map of the 2 megabase region containing the Huntington's disease gene. *Nature Genet.*, **4**, 181–186.
23. Taylor,S.A., Barnes,G.T., MacDonald,M.E. and Gusella,J.F. (1992) A dinucleotide repeat polymorphism at the D4S127 locus. *Hum. Mol. Genet.*, **1**, 142.
24. Tagle,D.A., Blanchard-McQuate,K.L. and Collins,F.S. (1992) Dinucleotide repeat polymorphism in the Huntington's disease region at the D4S43 locus. *Hum. Mol. Genet.*, **1**, 215.
25. Tagle,D.A., Blanchard-McQuate,K.L., Valdes,L., Castilla,L. and Collins,F.S. (1993) Dinucleotide repeat polymorphisms at the D4S126 and D4S114 loci. *Hum. Mol. Genet.*, **2**, 1077.
26. Allitto,B.A., MacDonald,M.E., Bucan,M., Richards,J., Romano,D., Whaley,W.L., Falcone,B., Ianazzi,J., Wexler,N.S., Wasmuth,J.J., Collins,F.S., Lehrach,H., Haines,J.L. and Gusella,J. (1991) Increased recombination adjacent to the Huntington disease-linked D4S10 marker. *Genomics*, **9**, 104–112.
27. Terwilliger,J.D. and Ott,J. (1994) *Handbook of Human Genetic Linkage*. Johns Hopkins University Press, Baltimore, MD, pp. 220–226.
28. The Huntington's Disease Collaborative Research Group. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, **72**, 971–983.
29. Riess,O., Winkelmann,B. and Epplen,J.T. (1994) Toward the complete genomic map and molecular pathology of human chromosome 4. *Hum. Genet.*, **94**, 1–18.
30. Mouchiroud,D., D'Onofrio,G., Aïssani,B., Macaya,G., Gautier,C. and Bernardi,G. (1991) The distribution of genes in the human genome. *Gene*, **100**, 181–187.
31. Saccone,S., DeSario,A., Della Valle,G. and Bernardi,G. (1992) The highest gene concentrations in the human genome are in telomeric bands of metaphase chromosomes. *Proc. Natl Acad. Sci. USA*, **89**, 4913–4917.
32. Landwehrmeyer,G.B., McNeil,S.M., Dure,L.S., Ge,P., Aizawa,H., Huang,Q., Ambrose,C.M., Duyao,M.P., Bird,E.D., Bonilla,E., deYoung,M., Avila-Gonzales, A.J., Wexler,N.S., DiFiglia,M., Gusella,J.F., MacDonald,M.E., Penney,J.B., Young,A.B. and Vonsattel,J.-P. (1995) Huntington's disease gene: regional and cellular expression in brain of normal and affected individuals. *Ann. Neurol.*, **37**, 218–230.
33. Riess,O., Thies,U., Siedlaczck,I., Potisek,S., Graham,R., Theilmann,J., Grimm,T., Epplen,J.T. and Hayden,M.R. (1994) Precise mapping of the brain α_2-adrenergic receptor gene within chromosome 4p16. *Genomics*, **19**, 298–302.
34. Jou,Y.-S., Goold,R.D. and Myers,R.M. (1994) Localization of the α2-macroglobulin receptor-associated protein 1 gene (LRPAP1) and other gene fragments to human chromosome 4p16.3 by direct cDNA selection. *Genomics*, **24**, 410–413.
35. Van Leuven,F., Hilliker,C., Serneels,L., Umans,L., Overbergh,L., De Strooper,B., Fryns,J.P. and Van den Berghe,H. (1995) Cloning, characterization, and chromosomal localization to 4p16 of the human gene (LRPAP1) coding for the α2-macroglobulin receptor-associated protein and structural comparison with the murine gene coding for the 44-kDa heparin-binding protein. *Genomics*, **25**, 492–500.
36. Scott,H.S., Litjens,T., Nelson,P.V., Thompson,P.R., Brooks,D.A., Hopwood,J.J. and Morris,C.P. (1993) Identification of mutations in the α-L-iduronidase gene (*IDUA*) that cause Hurler and Scheie syndromes. *Am. J. Hum. Genet.*, **53**, 973–986.
37. Ruckenstein,M.J., Macdonald,R.E., Clarke, J.T.R. and Forte,V. (1991) The management of otolaryngological problems in the mucopolysaccharidoses: a retrospective review. *J. Otolaryngol.*, **20**, 177–183.
38. Friedmann,I., Spellacy,E., Crow,J. and Watts,R.W.E. (1985) Histopathological studies of the temporal bones in Hurler's disease [mucopolysaccharidosis (MPS) IH]. *J. Laryngol. Otol.*, **99**, 29–41.
39. Shiang,R., Thompson,L.M., Zhu,Y-Z., Church,D.M., Fielder,T.J., Bocian,M., Winokur,S.T. and Wasmuth,J.J. (1994) Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell*, **78**, 335–342.
40. Hall,J.G. (1988) The natural history of achondroplasia. *Basic Life Sci.*, **48**, 3–9.
41. Glass,L., Shapiro,I., Hodge,S.E., Bergstrom,L. and Rimoin,D.L. (1981) Audiological findings of patients with achondroplasia. *Int. J. Pediatr. Otorhinolaryngol.*, **3**, 129–135.
42. Peters,K., Ornitz,D., Werner,S. and Williams,L. (1993) Unique expression pattern of the FGF receptor 3 gene during mouse organogenesis. *Dev. Biol.*, **155**, 423–430
43. Collins,C., Schappert,K. and Hayden,M.R. (1992) The genomic organization of a novel regulatory myosin light chain gene (MYL5) that maps to chromosome 4p16.3 and shows different patterns of expression between primates. *Hum. Mol. Genet.*, **1**, 727–733.
44. Estabrooks,L.L., Breg,W.R., Hayden,M.R., Ledbetter,D.H., Myers,R.M., Wyandt,H.E., Yang-Feng,T.L. and Hirschhorn,K. (1995) Summary of the 1993 ASHG ancillary meeting 'Recent research on chromosome 4p syndromes and genes.' *Am. J. Med. Genet.*, **55**, 453–458.
45. Gandelman,K.-Y., Gibson,L., Meyn,M.S. and Yang-Feng,T. (1992) Molecular definition of the smallest region of deletion overlap in the Wolf–Hirschhorn syndrome. *Am. J. Hum. Genet.*, **51**, 571–578.
46. Estabrooks,L.L., Lamb,A.N., Aylsworth,A.S., Callanan,N.P. and Rao,K.W. (1994) Molecular characterisation of chromosome 4p deletions resulting in Wolf–Hirschhorn syndrome. *J. Med. Genet.*, **31**, 103–107.
47 Estabrooks,L.L., Rao,K.W., Driscoll,D.A., Crandall,B.F., Dean,J.C.S., Ikonen,E., Korf,B. and Aylsworth,A.S. (1995) *Am. J. Med. Genet.*, in press.
48. Epstein,C.J. (1993) The conceptual bases for the phenotypic mapping of conditions resulting from aneuploidy. *Prog. Clin. Biol. Res.*, **384**, 1–18.
49. Grosson,C.L.S., MacDonald,M.E., Duyao,M.P., Ambrose,C.M., Roffler-Tarlov,S. and Gusella,J.F. (1994) Synteny conservation of the Huntington's disease gene and surrounding loci on mouse chromosome 5. *Mamm. Genome*, **5**, 424–428.
50. Lyon,M.F. and Searle,A.G. (1989) *Genetic Variants and Strains of the Laboratory Mouse*. 2nd edn, Oxford University Press, Oxford.
51. Myers,R.M., Goold,R.D. and van Ommen,G.-J. (1994) Report of the third international workshop on human chromosome 4 mapping 1993. *Cytogenet. Cell Genet.*, **66**, 218–236.
52. 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.
53. Weeks,D.E., Ott,J. and Lathrop,G.M. (1990) SLINK: a general simulation program for linkage analysis. *Am. J. Hum. Genet.*, **47** (suppl.), A204.
54. Ott,J. (1989) Computer-simulation methods in human linkage analysis. *Proc. Natl Acad. Sci. USA*, **86**, 4175–4178.
55. Cuticchia,A.J., Fasman,K.H., Kingsbury,D.T., Robbins,R.J. and Pearson,P.L. (1993) The GDB human genome data base anno 1993. *Nucleic Acids Res.*, **21**, 3003–3006.
56. Knowles,J.A., Vieland,V.J. and Gilliam,T.C. (1992) Perils of gene mapping with microsatellite markers. *Am. J. Hum. Genet.*, **51**, 905–909.
57. Ott,J. (1978) A simple scheme for analysis of HLA linkages in pedigrees. *Am. J. Hum. Genet.*, **42**, 225–257.
58. Hodge,S.E. and Elston,R.C. (1994) Lods, wrods, and mods: the interpretation of lod scores calculated under different models. *Genet. Epidemiol.*, **11**, 329–342.