Am. J. Hum. Genet. 63:786–793, 1998

Evidence for Digenic Inheritance of Nonsyndromic Hereditary Hearing Loss in a Swedish Family

Jorune Balciuniene,[1] Niklas Dahl,[2] Erik Borg,[3] Eva Samuelsson,[4] Markus J. Koisti,[2] Ulf Pettersson,[1] and Elena E. Jazin[1]

Units of [1]Medical Genetics and [2]Clinical Genetics, Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden; [3]Department of Physiology and Pharmacology, Karolinska Institute, Stockholm; and [4]Research Unit, Audiology Clinic, Örebro Hospital, Örebro, Sweden

Summary

We investigated a Swedish family with nonsyndromic progressive bilateral sensorineural hearing loss. Thirteen candidate loci for autosomal dominant nonsyndromic hearing loss were tested for linkage in this family. We found significant LOD scores (>3) for markers at candidate locus DFNA12 (11q22-q24) and suggestive LOD scores (>2) for markers at locus DFNA2 (1p32). Our results for markers on chromosome 11 narrowed down the candidate region for the DFNA12 locus. A detailed analysis of the phenotypes and haplotypes shared by the affected individuals supported the notion that two genes segregated together with hearing impairment in the family. Severely affected family members had haplotypes linked to the disease allele on both chromosomes 1 and 11, whereas individuals with milder hearing loss had haplotypes linked to the disease allele on either chromosome 1 or chromosome 11. These observations suggest an additive effect of two genes, each gene resulting in a mild and sometimes undiagnosed phenotype, but both together resulting in a more severe phenotype.

Introduction

Hearing loss is one of the most frequent sensory disorders in humans (Petit 1996) and may result in an alteration of language, speech, cognition, and psychosocial development. Genetic as well as environmental factors are implicated in the etiology of the illness. On the basis of age at onset, hearing deterioration is classified as a prelingual (congenital or developing in early childhood) or postlingual type (age at onset is after speech has developed). The incidence of prelingual deafness is 1/1,000 newborns. The prevalence of postlingual hearing loss increases with age: it affects 1% of young adults, 10% of persons by age 60 years, and 50% of persons at age $\geqslant$75 years (Morton 1991). It has been postulated that postlingual nonsyndromic hearing loss (NSHL) more frequently segregates as an autosomal dominant trait (Gorlin 1995; Van Camp et al. 1997*b*).

Genetic studies of NSHL are hampered by a high degree of genetic heterogeneity, multiple phenocopies, and assortative marriages between deaf persons. To date, 15 autosomal dominant, 19 autosomal recessive, 5 X-linked, and 2 mitochondrial loci have been reported for NSHL (see the Hereditary Hearing Loss home page). Moreover, five nuclear genes (de Kok et al. 1995; Kelsell et al. 1997; Liu et al. 1997*a*, 1997*b*; Lynch et al. 1997; Vahava et al. 1998) implicated in NSHL have been identified, and mutations in three more genes have been associated with hearing loss (Hollway et al. 1998; Li et al. 1998; Verhoeven et al. 1998).

We performed a linkage analysis of an extended Swedish pedigree that segregates, in an autosomal dominant mode, for postlingual progressive deafness. The family initially was tested for linkage to loci previously reported to be candidates for autosomal dominant forms of NSHL. Markers selected for two loci, DFNA2 (MIM 600101) on chromosome 1p and DFNA12 (MIM 601842) on chromosome 11q, provided strong indications for linkage, suggesting that both genes contribute to the etiology of hearing impairment in this Swedish family.

Methods

Clinical Diagnosis

The family was ascertained at Örebro Regional Hospital, Örebro, Sweden. Otoscopic and audiometric examinations were performed on all cooperative family members. Pure-tone audiometry was performed with air

Received February 26, 1998; accepted for publication June 18, 1998; electronically published August 14, 1998.

Address for correspondence and reprints: Dr. Elena E. Jazin, Unit of Medical Genetics, Department of Genetics and Pathology, Box 589, Uppsala University, S-751 23 Uppsala, Sweden. E-mail: elena.jazin@medgen.uu.se

0002-9297/98/6303-0019$02.00

conduction at 125, 250, 500, 1,000, 1,500, 2,000, 3,000, 4,000, 6,000, and 8,000 Hz and with bone conduction at 250, 500, 1,000, 1,500, 2,000, 3,000, and 4,000 Hz. In total, audiograms were available for 27 members of the family. For a few family members, audiograms were available for different ages. Thus, we were able to follow the progression of the hearing impairment. A detailed description of audiometric features is in progress (E. Borg, unpublished data). All individuals included in this study gave informed consent for the use of their clinical data for research purposes.

DNA Analysis

Genomic DNA was prepared from peripheral blood lymphocytes, by phenol-chloroform extraction (Lindqvist et al. 1996). Microsatellite polymorphisms were amplified by PCR. Most of the microsatellite markers were analyzed by use of a multiplex fluorescent-detection method, described elsewhere (Lindqvist et al. 1996), with fluorescently labeled markers ordered from Genset or Research Genetics. PCR reactions had a total volume of 10 μl containing 50 ng DNA, 2.5 pmol each primer, 12.5 mM each dNTP, and 0.5 U AmpliTaq polymerase (Perkin Elmer). After an initial step of 95°C for 5 min, PCR conditions were as follows: 10 cycles of denaturation at 94°C for 30 s, annealing at 47°C–62°C for 1 min, and elongation at 72°C for 1 min; 20 cycles of 89°C for 30 s, 47°C–62°C for 1 min, and 72°C for 1 min; and a final extension step at 72°C for 10 min. Amplification reactions were performed in a PTC-225 thermocycler (MJ Research). Fluorescent PCR products were resolved on 4% polyacrylamide gels and were detected by use of an ABI 377 DNA Sequencer (Applied Biosystems). Sizes of marker alleles were defined by use of GENESCAN software, version 2.0.1 (Applied Biosystems). Analyzed data were imported to the GENOTYPER software package, version 1.1 (Applied Biosystems), for an allele-calling procedure and to make a final table of genotypes.

Overall, we genotyped 52 microsatellite markers. Initially, one marker per candidate locus was tested. The two candidate regions DFNA2 (1p32) and DFNA12 (11q22-24), for which linkage was detected, were tested more thoroughly by typing 25 and 16 microsatellite markers, respectively. All microsatellite markers used are listed in the Généthon map (Dib et al. 1996) and/or the Genetic Location Database (LDB) (Collins et al. 1996). In some cases, the order of the markers on DFNA2 and DFNA12 differed in the different maps.

Simulation Analysis

Simulation analysis was performed by use of the SLINK option of the LINKAGE software package (Cottingham et al. 1993; Schaffer et al. 1994), to determine the probability of detection of linkage in our family. The same genetic models and phenotype definition described in the Linkage Analysis and Results sections, respectively, were used for this analysis. Genotypes were simulated, for 500 replicates, for a marker with five alleles of equal frequencies. The marker was assumed to lie at a recombination distance of .0 (linked) or .5 (unlinked) from the disease gene. Simulation analysis was performed for two models, with age at onset set to 20 years for one and 30 years for the other. When the age at onset of the disease was assumed to be 20 years, a maximum LOD score of 3.8 and an average LOD score of 2.74 were obtained. The probability that a LOD score >3 would be obtained by chance was $< 1 \times 10^{-8}$. For the model with a disease age at onset of 30 years, the maximum LOD score was 2.69, and the average LOD score was 1.89. The probability that a LOD score >2.5 would be obtained by chance was .002.

Linkage Analysis

Pairwise linkage analysis was performed by use of the MLINK option of the LINKAGE software package (FASTLINK 3.0P) (Cottingham et al. 1993; Schaffer et al. 1994). Multipoint linkage analysis was performed by use of the LINKMAP option of the same software package.

Affection status was defined as described in Results. Hearing loss in this family was considered to be an autosomal dominant trait with a maximum penetrance of 98% and a disease-allele frequency of 0.1%. For the analysis of candidate loci previously reported to be linked to autosomal dominant NSHL, we used the same age at onset used in the original studies, except for loci linked to congenital hearing loss, for which the age at onset was set to 20 years. The haplotypes were assigned manually.

Results

Two Different Affected Phenotypes Segregate in a Swedish Pedigree

The pathophysiological analysis of affected family members (E. Borg, unpublished data) showed outer hair-cell damage, without signs of brain-stem and eighth-nerve involvement. According to age at onset and degree of impairment, the affected family members were classified into two phenotypic classes (I and II; fig. 1). The two phenotypes differ in age at onset, progression, and degree of hearing impairment. Phenotype I comprised a group of nine patients (fig. 2) with severe hearing loss, with a mean age at onset of 9 ± 2.2 years. The audiograms of these patients showed decreased hearing, particularly at high frequencies (6–8 kHz), with a $\leqslant$80 dB drop at age 50 years. These patients usually did not show hearing impairment at frequencies <500 Hz. Phenotype

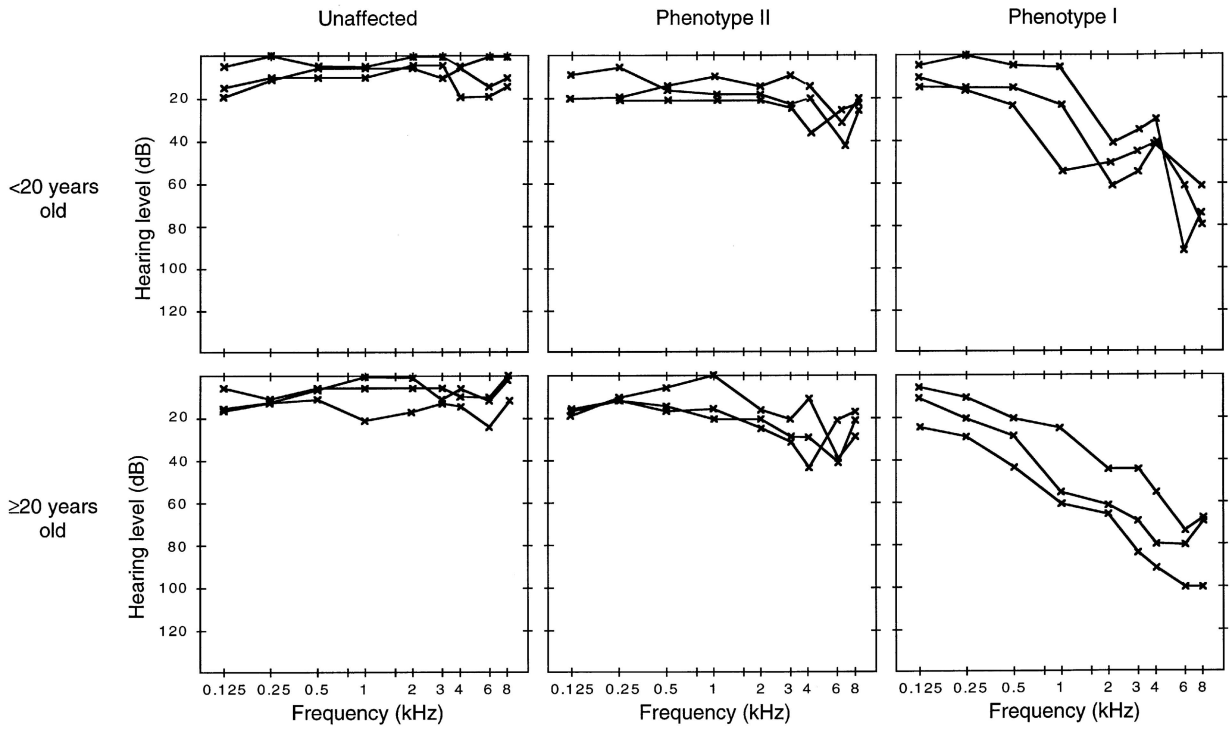

Figure 1 Schematic representation of audiograms for three phenotypic classes: one unaffected phenotype and two affected phenotypes (I and II). Each plot represents audiometric data for the left ear of three random persons belonging to each specific phenotypic class. Individuals from each class were divided into two age groups: <20 years of age (*top row*) and ⩾20 years of age (*bottom row*). The two phenotypic classes differ in age at onset, progression (compare top and bottom plots for each class), and severity of the hearing impairment.

II was observed in four persons (fig. 2) with mild hearing loss at only high frequencies (4–6 kHz), with a mean age at onset of 19 ± 5.5 years. At high frequencies (4–8 kHz), the patients with phenotype I had a mean level of hearing impairment of 54.6 ± 20 dB, at mean age 12.3 ± 3.6 years, whereas those with phenotype II had a mean level of hearing loss of 24.2 ± 9.5 dB, at mean age 19.75 ± 5 years (data for the hearing impairment was calculated for the left ear). All individuals with phenotype I and two individuals (III:9 and III:14) with phenotype II lacked a history of exposure to environmental factors that could explain their hearing disability. Two individuals (III:7 and III:10) with phenotype II had a history of head trauma or potential ototoxic antibiotics. For linkage analysis, only individuals with phenotype I were considered to be affected, whereas individuals with phenotype II were specified as "unknown." Individual III:1 was specified as "unknown," because he did not fulfill completely the criteria for phenotype I and because his in-married father suffered from a mild hearing impairment. Individuals with normal hearing were considered to be unaffected if they were older than the selected age at onset, whereas younger individuals were specified as "unknown."

Linkage-Analysis Results

We obtained negative LOD scores for 11 of 13 candidate loci tested. However, markers positioned around the two candidate loci DFNA2 (1p32) and DFNA12 (11q22-24) gave indications for linkage. Results for these markers are shown in table 1. For markers D11S4171 and D11S4094 on chromosome 11, we obtained maximum LOD scores of 3.87 and 3.79, respectively. For chromosome 1, analysis with markers D1S472 and D1S186 resulted in a maximum LOD score of 2.69 for both markers.

Multipoint analysis of chromosome 11 markers revealed a peak LOD score of 3.86, which was obtained at a map position corresponding to D11S4171 on chromosome 11. For markers on chromosome 1, the multipoint analysis resulted in a peak LOD score of 2.69 at 0.1 cM centromeric to marker D1S2723 and at 0.5 cM telomeric to marker D1S255 (data not shown).

Haplotype Analysis

The most-likely haplotypes for all available family members were constructed (fig. 2). Fine mapping of chromosome 11 revealed two key recombinants (indi-

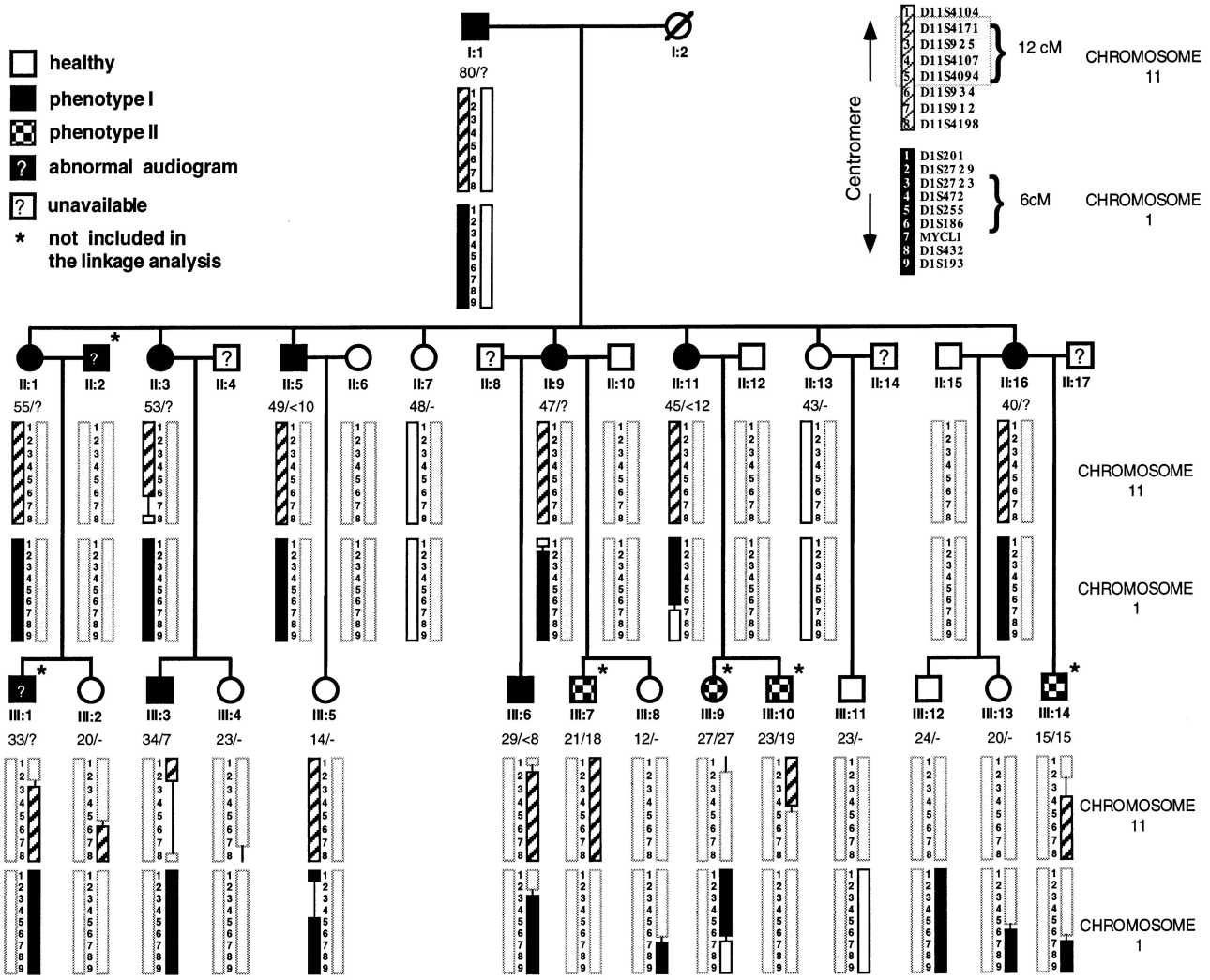

Figure 2 Most-likely haplotypes. The definitions of the symbols are indicated in the upper-left corner of the figure. The numbers between the bars correspond to the markers shown in the upper-right corner of the figure. Blackened and hatched bars represent haplotypes segregating with the disease allele, and unblackened bars represent haplotypes segregating with a healthy allele. A vertical line indicates a region that was not informative. Dotted, gray bars represent haplotypes from in-married individuals. The left haplotypes are from fathers, and the right haplotypes are from mothers. Below the individual designations we have indicated current age/age at onset. A question mark (?) indicates that the age at onset was not known. All individuals that belong to the family are shown in the figure. The order of the markers is according to the LDB, in which MYCL1 is placed telomeric to D1S432.

viduals III:6 and III:2) that narrowed the previously reported region of the DFNA12 locus, from 36 cM to 12 cM interval between markers D11S4104 and D11S934. In affected individual III:6, a recombination event had occurred between markers D11S4104 and D11S4171, which positions the disease allele telomeric to marker D11S4104. In addition, in healthy individual III:2, a recombination event had occurred between markers D11S934 and D11S4094, which localizes the disease gene centromeric to marker D11S934. On chromosome 1, a crossover event between markers D1S2729 and D1S2723 in patient III:6 positions a possible disease-allele region centromeric to marker D1S2729, while a recombination event between markers D1S186 and MYCL1 in patient II:11 excludes the region centromeric to marker MYCL1. The genetic distance between markers MYCL1 and D1S2729 is ~6 cM (Collins et al. 1996). All individuals with severe hearing impairment (phenotype I) share haplotypes linked to the disease allele on both chromosomes (11q22-24 and 1p32). Individuals showing a milder hearing loss (phenotype II) carry only one of the disease haplotypes, on chromosome 1 or chro-

Table 1

Results of Two-Point Linkage Analysis

Locus and Marker	LOD Score, at Recombination Fraction of				
	.00	.01	.05	.1	.2
DFNA12:					
D11S4104	−2.29	−.31	.30	.49	.55
D11S4171	3.87	3.81	3.56	3.23	2.52
D11S925	2.62	2.62	2.57	2.43	1.98
D11S4107	2.47	2.46	2.38	2.22	1.79
D11S4094	3.79	3.73	3.48	3.16	2.46
D11S934	.90	1.09	1.43	1.54	1.40
D11S912	.98	1.15	1.47	1.56	1.41
D11S4198	−8.67	−2.55	−.88	−.2	.29
DFNA2:					
D1S201	−1.92	−1.24	−.08	.33	.52
D1S2729	−1.21	.66	1.19	1.28	1.13
D1S472	2.69	2.65	2.47	2.24	1.73
D1S255	2.12	2.08	1.94	1.75	1.34
D1S186	2.69	2.65	2.47	2.24	1.73
MYCL1	−1.31	.66	1.19	1.28	1.12
D1S432	−1.91	.06	.63	.77	.72
D1S193	−1.91	.06	.63	.77	.72

mosome 11. Healthy persons who were older than the selected age at onset did not have either of the two haplotypes that segregate with the disease.

Discussion

We studied an extended Swedish family with progressive bilateral sensorineural NSHL, and our results indicate linkage of markers at two candidate loci, DFNA2 (1p32) and DFNA12 (11q22-24). The hearing phenotype in the family is progressive, and the age at onset varied within the range ~7–30 years. Thus, to test candidate loci for linkage to progressive hearing loss (e.g., DFNA2), we used the age at onset proposed in the original studies. To test loci linked to congenital hearing loss (e.g., DFNA12), we set the age at onset to 20 years, since the age at onset for the hearing defects is clearly postlingual in the Swedish family.

Maximum LOD scores of 3.8 and 2.67 were obtained for markers at DFNA12 and DFNA2, respectively. As was suggested by simulation data from two models, for which age at onset was set to 20 years for one and 30 years for the other, these LOD scores correspond to the maximum expected (see Methods).

The possibility of having two loci involved in hearing deterioration in the Swedish family was unexpected, since the disease segregates as an apparent monogenic autosomal dominant trait. However, it has been postulated that, under certain circumstances, digenically inherited phenotypes may resemble monogenic autosomal dominant traits (Johnson 1980). The hypothesis of digenic inheritance in the Swedish pedigree is supported by a detailed analysis of the haplotypes and the associated phenotypes of all the family members. We noticed that the subjects with the severe phenotype I have inherited haplotypes linked to the disease allele on both chromosomes 1 and 11, whereas individuals with the milder phenotype II have inherited either the haplotype of chromosome 1 or the haplotype of chromosome 11. Individual III:1 has not been included in the analysis, because of an atypical phenotype and because his inmarried father had hearing impairment. However, this individual also carried the two haplotypes linked to the disease. Finally, the unaffected persons who are >30 years of age have not inherited any of the disease haplotypes. However, the haplotypes linked to the disease were detected in two young unaffected individuals (III:12 and III:5). First, individual III:12 received only the haplotype on chromosome 1. He is 24 years old, and he still has not passed the critical age at onset for the milder form of hearing loss. Individual III:5, who is 14 years old, seems to have inherited both of the disease-linked haplotypes from her affected father. However, markers in the region between D1S201 and D1S255 were not informative. If she carries the two haplotypes, then she already should have presented symptoms of hearing loss, according to our model. Reduced penetrance or a double recombination event may explain the absence of symptoms.

There have been several reports indicating a digenic inheritance pattern in humans. For example, digenic segregation has been demonstrated in families with retinitis pigmentosa, in which the affected phenotype was caused by mutations in two independent genes, peripherin/RDS and ROM1 (Kajiwara et al. 1994) on 6p and 11q, respectively. Electroretinograms of individuals heterozygous for either mutation were very similar, although clinical symptoms appeared only in double heterozygotes. Digenic inheritance also was suggested for a family segregating for Waardenburg syndrome type 2 with ocular albinism. In this case, affected persons were heterozygous for a mutation in a transcription factor and were heterozygous or homozygous for a functionally significant polymorphism of a tyrosinase gene (Morell et al. 1997). Aggravated hyperchlolesterolemia is caused by nonallelic heterozygous mutations in two independent loci implicated in the etiology of monogenic lipid disorders—namely, the LDL receptor and the apolipoprotein B-100 locus, which encodes the ligand for the LDL receptor (Benlian et al. 1996). Digenic inheritance also has been postulated for families with limb-girdle muscular dystrophy (LGMD) (Richard et al. 1995; van Ommen 1995; Beckmann 1996), LGMD and Miyoshi myopathy (Weiler et al. 1996), recessive NSHL (Chen et al. 1997), and myelomeningocele and Waardenburg syndrome type 3 (Nye et al. 1998).

Although our results suggest that two loci segregate with the more severe form of the disease, it is possible

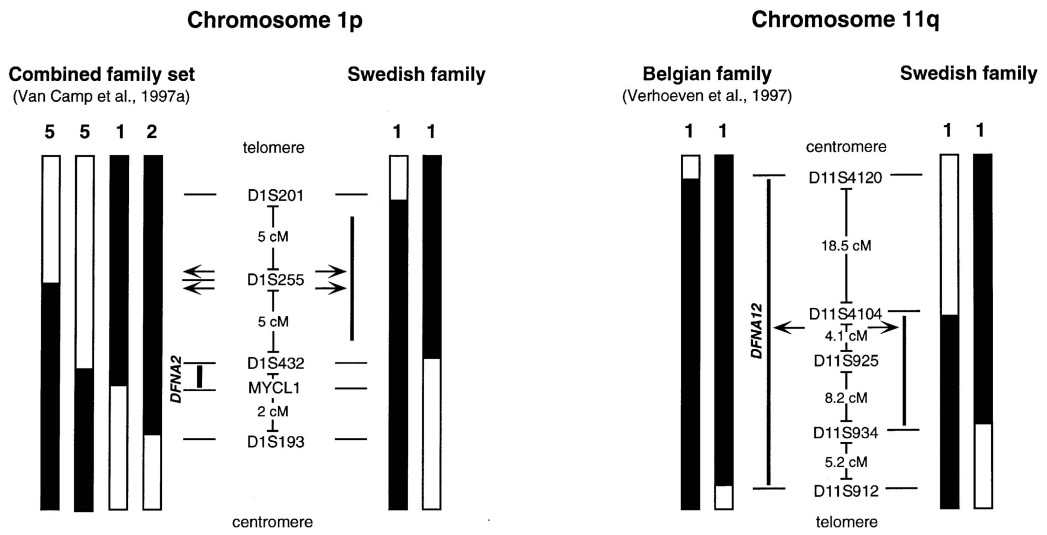

Figure 3 Comparison of the candidate regions for DFNA2 and DFNA12 on chromosomes 1p and 11q, respectively. Schematic representations of chromosomes for key recombinants identified by other studies are illustrated to the left of the markers. Schematic representations of chromosomes for key recombinants obtained in this study are illustrated to the right of the markers. Blackened bars indicate regions linked to the disease allele, unblackened bars indicate regions that recombined with the disease allele, and boldface vertical lines indicate consensus regions based on the combined information from the key recombinants. The numbers above the bars indicate the number of chromosomes for each type of recombination. Arrows indicate possible locations for marker D1S472 on chromosome 1 and for marker D11S4171 on chromosome 11. For comparison purposes, the order of the markers on chromosome 1 was taken from the study by Van Camp et al. (1997*a*). Therefore, the position of MYCL1 does not agree with the position according to the LDB, which we used for haplotype assignment and multipoint analysis.

that only one locus causes the hearing loss, while the other segregates by chance. If this is the case, then the locus on chromosome 11 is a stronger candidate than the locus on chromosome 1, because the chromosome 11 locus is within a 12-cM region included within the previously reported DFNA12 locus and because the statistical evidence is stronger (LOD score >3) for this locus than for the chromosome 1 locus. If this is the case, individual III:9 could be a phenocopy. On the other hand, stronger statistical evidence for chromosome 11 was obtained only because the age at onset for the disease linked to this locus was assumed to be lower, and, thus, more unaffected individuals were included in the linkage analysis.

Another possible interpretation for the presence of two phenotypic classes in the Swedish family might be that the milder phenotype II is caused by a defective nuclear gene and a mitochondrial mutation. This is supported by the observation that all four individuals with phenotype II are offspring of affected mothers. However, the absence of other clinical manifestations, such as diabetes or other neurological symptoms that usually cooccur with hearing loss due to a mitochondrial mutation (Jacobs 1997), as well as a negative history of treatment with aminoglycosides (Estivill et al. 1998), do not support a mitochondrial etiology.

Comparisons of the results from our study with results from previous studies of DFNA2 and DFNA12 are shown in figure 3. The original work on DFNA2 (Coucke et al. 1994) described a region of 6 cM that overlaps with our locus. This work was followed by another study (Van Camp et al. 1997*a*) investigating new families linked to DFNA2. In total, five families (one from Indonesia, one from the United States, one from Belgium, and two from The Netherlands) with similar phenotypes linked to DFNA2 now have been analyzed. The age at onset of the disease and the shape of audiograms varied substantially between and within the families. The combination of data from all these families defined a candidate region of <1.25 Mb for the disease gene, flanked by markers D1S432 and MYCL1. In our family, a recombination event between markers MYCL1 and D1S432 in affected person II:11 excluded the 1.25-Mb region. The differences between the localization of DFNA2 by Van Camp et al. (1997*a*) and by us may have different explanations. In the Swedish family, the chromosome 1 haplotype may segregate with the disease phenotype just by chance, as indicated above. Another possibility is that a second gene located on the same chromosome might be implicated in the hearing loss. A third possibility is the presence of other sequence elements, involved in transcriptional regulation of the DFNA2 gene, that are located upstream of the DFNA2 locus, as has been suggested for the DFN3 locus (de Kok et al. 1996). A final possibility is that there might be

heterogeneity among the families described by Van Camp et al. (1997*a*).

Another source of disagreement between the studies is the order of the markers used. According to the marker order from the LDB, MYCL1 is telomeric to D1S432, whereas the opposite order is postulated by Van Camp et al. (1997*a*). In their study, the order of the markers was determined by recombination analysis of the individuals in the families studied. The finding that one individual was recombinant for marker MYCL1 indicated that the disease gene was telomeric to MYCL1. Furthermore, five recombinations were identified for marker D1S432, which placed the disease gene centromeric to this marker (fig. 3). As a result, these six recombinations confined the region to markers MYCL1 and D1S432. However, if marker MYCL1 resides telomeric to D1S432 (as indicated in the LBD), then the linked region would comprise two nonoverlapping regions, one that is centromeric to marker D1S432 and another that is telomeric to marker MYCL1. The latter region would be a candidate in at least one of their families and would coincide with the data from our family.

The linkage to locus DFNA12 is easier to analyze. Congenital and stable hearing loss previously have been shown to be linked to the 36-cM DFNA12 region restricted by markers D11S4120 and D11S912 (Verhoeven et al. 1997). Hearing loss in our family is linked to a 12-cM interval between markers D11S4104 and D11S934, which is included within the 36-cM region. Interestingly, the phenotype in our family is different from that described by Verhoeven et al. (1997) (i.e., it is progressive, with a later onset), which may indicate allelic forms of hearing impairment at DFNA12 that are analogous to other DFN loci (Liu et al. 1997*a,* 1997*b;* Weil et al. 1997).

In summary, we have reported a family with progressive bilateral sensorineural NSHL with indications of linkage to two independent loci, DFNA12 and DFNA2. Our combined results from the analysis of haplotypes and phenotypes support digenic inheritance of the severe form of hearing loss observed in the family. An additive effect of two independently segregating genes is postulated, in which the contribution of each gene results in a milder or subclinical phenotype.

Acknowledgments

We thank all the members of the family for their participation in this study. This work was supported by grants from the Beijer Foundation, the Swedish Medical Research Council, the Söderberg Foundation, and the Örebro County Council.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr (for microsatellite markers used)

Hereditary Hearing Loss home page, http://dnalab-www.uia.ac.be/dnalab/hhh (for NSHL loci)

Genetic Location Database, ftp://cedar.genetics.soton.ac.uk/public_html/index.html (for microsatellite markers used)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for autosomal dominant NSHL linked to DFNA2 [MIM 600101] and to DFNA12 [MIM 601842])

References

Beckmann JS (1996) The Réunion paradox and the digenic model. Am J Hum Genet 59:1400–1402

Benlian P, de Gennes JL, Dairou F, Hermelin B, Ginon I, Villain E, Lagarde JP, et al (1996) Phenotypic expression in double heterozygotes for familial hypercholesterolemia and familial defective apolipoprotein B-100. Hum Mutat 7:340–345

Chen A, Wayne S, Bell A, Ramesh A, Srisailapathy CR, Scott DA, Sheffield VC, et al (1997) New gene for autosomal recessive non-syndromic hearing loss maps to either chromosome 3q or 19p. Am J Med Genet 71:467–471

Collins A, Frezal J, Teague J, Morton NE (1996) A metric map of humans: 23,500 loci in 850 bands. Proc Natl Acad Sci USA 93:14771–14775

Cottingham RW Jr, Idury RM, Schäffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–263

Coucke P, Van Camp G, Djoyodiharjo B, Smith SD, Frants RR, Padberg GW, Darby JK, et al (1994) Linkage of autosomal dominant hearing loss to the short arm of chromosome 1 in two families. N Engl J Med 331:425–431

de Kok YJ, van der Maarel SM, Bitner-Glindzicz M, Huber I, Monaco AP, Malcolm S, Pembrey ME, et al (1995) Association between X-linked mixed deafness and mutations in the POU domain gene POU3F4. Science 267:685–688

de Kok YJ, Vossenaar ER, Cremers CW, Dahl N, Laporte J, Hu LJ, Lacombe D, et al (1996) Identification of a hot spot for microdeletions in patients with X-linked deafness type 3 (DFN3) 900 kb proximal to the DFN3 gene POU3F4. Hum Mol Genet 5:1229–1235

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Estivill X, Govea N, Barceló E, Perelló E, Badenas C, Romero E, Moral L, et al (1998) Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment of aminoglycosides. Am J Hum Genet 62:27–35

Gorlin RJ (1995) Genetic hearing loss with no associated abnormalities. In: Gorlin RJ, Toriello HV, Cohen MM (eds) Hereditary hearing loss and its syndromes. Oxford University Press, Oxford, pp 43–61

Hollway GE, Suthers GK, Battese KM, Turner AM, David DJ, Mulley JC (1998) Deafness due to Pro250Arg mutation of FGFR3. Lancet 351:877–878

Jacobs HT (1997) Mitochondrial deafness. Ann Med 29: 483–491

Johnson WG (1980) Metabolic interference and the +-heterozygote: a hypothetical form of simple inheritance which is neither dominant nor recessive. Am J Hum Genet 32: 374–386

Kajiwara K, Berson EL, Dryja TP (1994) Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 264:1604–1608

Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, et al (1997) Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature 387: 80–83

Li XC, Everett LA, Lalwani AK, Desmukh D, Friedman TB, Green ED, Wilcox ER (1998) A mutation in PDS causes non-syndromic recessive deafness. Nat Genet 18:215–217

Lindqvist AK, Magnusson PK, Balciuniene J, Wadelius C, Lindholm E, Alarcon-Riquelme ME, Gyllensten UB (1996) Chromosome-specific panels of tri- and tetranucleotide microsatellite markers for multiplex fluorescent detection and automated genotyping: evaluation of their utility in pathology and forensics. Genome Res 6:1170–1176

Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJ, Steel KP, Brown SD (1997*a*) Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. Nat Genet 16: 188–190

Liu XZ, Walsh J, Tamagawa Y, Kitamura K, Nishizawa M, Steel KP, Brown SD (1997*b*) Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. Nat Genet 17:268–269

Lynch ED, Lee MK, Morrow JE, Welcsh PL, Leon PE, King MC (1997) Nonsyndromic deafness DFNA1 associated with mutation of a human homolog of the drosophila gene diaphanous. Science 278:1315–1318

Morell R, Spritz RA, Ho L, Pierpont J, Guo W, Friedman TB, Asher JH Jr (1997) Apparent digenic inheritance of Waardenburg syndrome type 2 (WS2) and autosomal recessive ocular albinism (AROA). Hum Mol Genet 6:659–664

Morton NE (1991) Genetic epidemiology of hearing impairment. Ann N Y Acad Sci 630:16–31

Nye JS, Balkin N, Lucas H, Knepper PA, McLone DG, Charrow J (1998) Myelomeningocele and Waardenburg syndrome (type 3) in patients with interstitial deletions of 2q35 and the PAX3 gene: possible digenic inheritance of a neural tube defect. Am J Med Genet 75:401–408

Petit C (1996) Genes responsible for human hereditary deafness: symphony of a thousand. Nat Genet 14:385–391

Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, Brenguier L, et al (1995) Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. Cell 81:27–40

Schaffer AA, Gupta SK, Shriram K, Cottingham RW Jr (1994) Avoiding recomputation in linkage analysis. Hum Hered 44: 225–237

Vahava O, Morell R, Lynch ED, Weiss S, Kagan ME, Ahituv N, Morrow JE, et al (1998) Mutation in transcription factor POU4F3 associated with inherited progressive hearing loss in humans. Science 279:1950–1954

Van Camp G, Coucke PJ, Kunst H, Schatteman I, Van Velzen D, Marres H, van Ewijk M, et al (1997*a*) Linkage analysis of progressive hearing loss in five extended families maps the DFNA2 gene to a 1.25-Mb region on chromosome 1p. Genomics 41:70–74

Van Camp G, Willems PJ, Smith RJH (1997*b*) Nonsyndromic hearing impairment: unparalleled heterogeneity. Am J Hum Genet 60:758–764

van Ommen GJ (1995) A foundation for limb-girdle muscular dystrophy. Nat Med 1:412–414

Verhoeven K, Van Camp G, Govaerts PJ, Balemans W, Schatteman I, Verstreken M, Van Laer L, et al (1997) A gene for autosomal dominant nonsyndromic hearing loss (DFNA12) maps to chromosome 11q22-24. Am J Hum Genet 60: 1168–1173

Verhoeven K, Van Laer L, Kirschhofer K, Legan PK, Hughes DC, Schatteman I, Verstreken M, et al (1998) Mutations in the human alpha-tectorin gene cause autosomal dominant non-syndromic hearing impairment. Nat Genet 19:60–62

Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Drira M, Ayadi H, et al (1997) The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. Nat Genet 16:191–193

Weiler T, Greenberg CR, Nylen E, Halliday W, Morgan K, Eggertson D, Wrogemann K (1996) Limb-girdle muscular dystrophy and Miyoshi myopathy in an aboriginal Canadian kindred map to *LGMD2B* and segregate with the same haplotype. Am J Hum Genet 59:872–878