Candidate Locus for a Nuclear Modifier Gene for Maternally Inherited Deafness

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Maternally inherited deafness associated with the A1555G mutation in the mitochondrial 12S ribosomal RNA (rRNA) gene appears to require additional environmental or genetic changes for phenotypic expression. Aminoglycosides have been identified as one such environmental factor. In one large Arab-Israeli pedigree with congenital hearing loss in some of the family members with the A1555G mutation and with no exposure to aminoglycosides, biochemical evidence has suggested the role of nuclear modifier gene(s), but a genomewide search has indicated the absence of a single major locus having such an effect. Thus it has been concluded that the penetrance of the mitochondrial mutation appears to depend on additive effects of several nuclear genes. We have now investigated 10 multiplex Spanish and Italian families with 35 members with the A1555G mutation and sensorineural deafness. Parametric analysis of a genomewide screen again failed to identify significant evidence for linkage to a single autosomal locus. However, nonparametric analysis supported the role of the chromosomal region around marker D8S277. The combined maximized allele-sharing LOD score of 3.1 in Arab-Israeli/Spanish/Italian families represents a highly suggestive linkage result. We suggest that this region should be considered a candidate for containing the first human nuclear modifier gene for a mitochondrial DNA disorder. The locus operates in Arab-Israeli, Spanish, and Italian families, resulting in the deafness phenotype on a background of the mitochondrial A1555G mutation. No obvious candidate genes are located in this region.

Introduction

The phenotypic expression of mitochondrial DNA mutations is poorly understood. In most mitochondrial diseases, the tissue-specific expression and severity of the disease is attributed to the distribution of the mutated mitochondrial chromosomes. However, even in the homoplasmic mitochondrial diseases, tissue specificity and great variation in expressivity are the norm (reviewed in Fischel-Ghodsian 1998). For example, the mitochondrial A1555G mutation in the 12S rRNA gene has been associated with hearing impairment, ranging from profound congenital deafness, through progressive moderate hearing loss starting in adult life, to completely normal hearing. These differences exist in the absence of exposure to aminoglycosides, which have been shown to trigger hearing loss in individuals with the A1555G mutation (Prezant et al. 1993; Estivill et al. 1998). Mitochondrial haplotypes may explain some of the differences between families and ethnic groups, whereas biochemical and genetic analyses have implicated nuclear factors as the main explanation for the phenotypic differences within families (Guan et al. 1996; Fischel-Ghodsian 1998). An extensive genomewide search undertaken by us in one large Arab-Israeli kindred with the homoplasmic A1555G mutation revealed that the nuclear modifying factors are likely to be multiple (Bykhovskaya et al. 1998).

Recently, a total of 19 families with matrilineal hearing loss with and without aminoglycoside exposure, and with the mitochondrial A1555G mutation, were identified in Spain (Estivill et al. 1998). Phylogenetic analysis of the mtDNA haplotypes revealed that the A1555G mutation in these families is the product of several independent mutational events (Torroni et al. 1999), which supports the observation in the Arab-Israeli pedigree that mitochondrial genetic background does not play a major role in disease expression (Fischel-Ghod-

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sian 1998). This conclusion was further strengthened by identification of different mitochondrial haplotypes in two A1555G-positive families from Italy (Casano et al. 1998).

To identify the chromosomal location(s) of putative nuclear modifier gene(s), we performed a genomewide search in the Spanish families. Nonparametric linkage (NPL) analysis of the data led to the identification of a locus, D8S277, that appears to be common in the Arab-Israeli, Spanish, and Italian families, with a highly suggestive combined allele-sharing LOD score of 3.1.

Subjects and Methods

Pedigrees

Spanish Families.—We studied eight multiplex Spanish families with severe sensorineural matrilineal hearing loss (fig. 1). The clinical phenotype of these families was described by Estivill et al. (1998). In three of the eight families (S6, S11, and S16), several members received aminoglycosides (in fig. 1, individuals marked by blackened symbols with white centers) and became deaf. In the other five families (S1, S3, S5, S12, and S15) there was no record of aminoglycosides exposure.

Italian Families.—We investigated two Italian families with matrilineal nonsyndromic hearing loss, previously described by us and found to carry the homoplasmic A1555G mutation (Casano et al. 1998) (fig. 2). Each of these families has at least one hearing-impaired sib pair who had not been exposed to aminoglycosides.

Arab-Israeli Family.—The Arab-Israeli family was described by us elsewhere (Bykhovskaya et al. 1998).

Informed consent for analysis was obtained from all members of the Spanish, Italian, and Arab-Israeli families who participated in the study.

Genotyping

Genomewide genotyping was performed using fluorescence-labeled primers from the ABI PRISM Linkage Mapping Set (Applied Biosystems). PCR, pooling, and electrophoresis of amplified DNA were done according to the manufacture's protocol, using the GeneAmp PCR system 9600 (Perkin-Elmer) and a 373 automated DNA Sequencer (Applied Biosystems). Semiautomated DNA fragment sizing was performed using GENESCAN 672 (version 2.0.2) software, and genotyping was performed using GENOTYPER version 1.1 software (Applied Biosystems).

Linkage Analysis

Two-Point Parametric LOD Score Linkage Analysis.—Pairwise LOD scores between the disease phenotype and the genetic markers were calculated using the MLINK program from the LINKAGE computer package (Ott 1991; Terwilliger and Ott 1994) for recombination fractions equal to 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.40, using equal marker allele frequencies. Individuals who received aminoglycosides were coded as having "unknown" phenotype, which allowed them to remain neutral with respect to deafness phenotype but provided marker genotype information for parental inference.

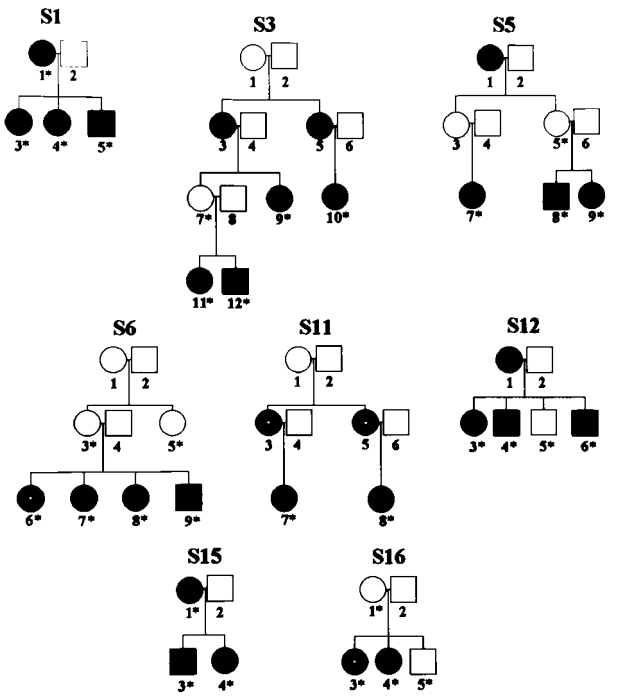

Figure 1 Pedigree structures of eight Spanish families. Hearing-impaired individuals are indicated by blackened symbols. Asterisks (*) indicate individuals from whom DNA samples were obtained. Blackened symbols with white centers denote individuals who became deaf after aminoglycoside exposure.

Multipoint Parametric LOD Score Linkage Analysis.—Multipoint parametric linkage LOD scores based on the user-specified model were calculated by means of the GENEHUNTER computer program (Kruglyak et al. 1996).

Multipoint Affected Sib–Pair Linkage Analysis.—Multipoint sib-pair analyses were conducted using the MAPMAKER/SIBS computer program (Kruglyak and Lander 1995). Twelve available pairs of affected siblings were included in the analysis of the Spanish families' genome screen data; seven pairs of affected sibs from Italian families were added for the analysis of the candidate loci, and 65 affected sib-pairs from Arab-Israeli kindred were incorporated into the combined analyses.

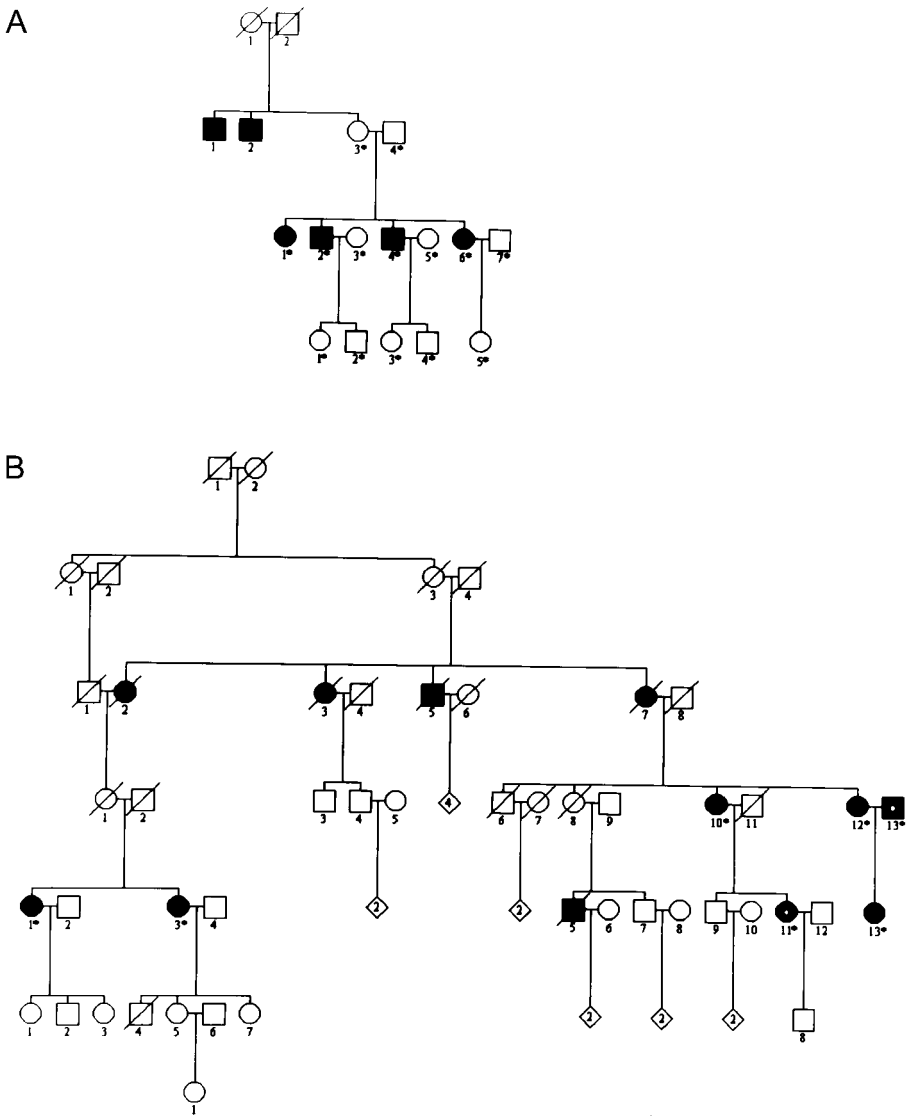

Figure 2 Pedigree structures of two Italian families. Hearing-impaired individuals are indicated by blackened symbols. Asterisks (*) indicate individuals from whom DNA samples were obtained. Blackened symbols with white centers denote individuals who became deaf after aminoglycoside exposure.

To maximize use of available information in a large sibship of 10 affected siblings in the Arab-Israeli kindred and to calculate LOD scores in addition to the NPL scores and *P* values, we used the GENEHUNTER-PLUS (ASM 1.0) program (Kong and Cox 1997) in all combined families. All the pedigrees included in the analysis were divided into basic nuclear families, and the scoring function was chosen to be "pairs."

Marker allele frequencies were considered to be equal, and the sex-averaged map distances reported by the Marshfield Medical Research Foundation were used in all initial analyses. For regions of interest, allele frequencies from the CEPH families were used for the Spanish families, and the marry-in allele frequencies were used in the Arab-Israeli pedigree.

Results

Model-Based Analysis of the Genome Screen Data in the Spanish Families Reveals That Inheritance Pattern Is Likely to Be Complex and/or Heterogeneous

We typed 331 markers from the ABI PRISM Linkage Mapping Set, using DNAs of all 32 available members of the Spanish families. The marker data were analyzed under dominant and recessive models with reduced pen-

Table 1

Results of Parametric Linkage Analysis of Genome Screen Data of the Spanish Families: Comparison with Arab-Israeli Kindred

	LOD Score in Families		
	Spanish		Arab-Israeli
Model and Marker	Two-point	Multipoint	Two-point
Autosomal dominant:			
D7S519	1.8	1.1	−8.3
D3S1271	1.4	.9	.0
D15S128	1.4	1.1	−1.0
D15S127	1.4	.2	−4.9
D3S1266	1.3	1.9	−2.2
D7S636	1.1	1.3	−1.8
D16S411	1.0	1.0	−5.3
Autosomal recessive:			
D8S277	1.5	1.3	.8
D9S175	1.2	1.8	−4.1

etrance. Under autosomal dominant inheritance, disease-allele frequency varied from .01 to .4 at reduced penetrance of .8. Under autosomal recessive inheritance, a common disease-allele frequency of .5 and reduced penetrance of .8 were assumed. The high disease-allele frequency in the autosomal recessive model was chosen, since, in the Spanish and Italian families, hearing loss is quite common in the different branches of the family, despite all the marry-in males. Formal segregation analysis in the Arab-Israeli family supported an autosomal recessive model with disease-allele frequency of .52 (Bu et al. 1993). Similarly, for an autosomal dominant model, a common allele is much more likely, but a rare allele with significantly reduced penetrance is possible. Use of a high allele frequency does not preclude successful identification of a disease locus (Hanis et al. 1996).

Table 2

Results of Multipoint Sib-Pair Analysis of Spanish Families' Genome Screen Data: Comparison with Arab-Israeli Kindred

	Maximum Multipoint Nonparametric LOD Score in Families	
Marker	Spanish	Arab-Israeli
D17S949	1.7	.4
D8S277	1.4	1.6
D1S213	1.3	.0
D9S175	1.3	.0
D6S305	1.1	.1
D3S1300	.9	.0
D7S550	.9	.6
D2S347	.7	.3
D10S597	.6	.0
D18S64	.5	.2

Note. The shared locus is underlined.

Two-point LOD score analysis did not reveal any significant LOD scores, but a total of nine loci showed suggestive results, with LOD scores ranging from 1.0 to 1.8 (table 1). Subsequently, we performed linkage analysis with GENEHUNTER under the models specified above. The values of multipoint LOD scores were not dramatically different from two-point LOD scores and did not become significant in any of the regions. In addition, when the same loci were compared with data from the Arab-Israeli family, none of the loci showed a positive LOD score except for the marker D8S277. At D8S277, the combined maximized LOD scores in the Spanish and Italian families were 2.7 and 1.7 for two-point and multipoint analyses, respectively, using autosomal recessive inheritance, 100% penetrance, 5% phenocopies, and a .1 disease-allele frequency (data not shown).

Affected Sib–Pair NPL Analysis Leads to the Identification, in Spanish and Italian Families, of a Region Previously Described in the Arab-Israeli Kindred

To identify potential chromosomal regions linked to the deafness phenotype in the Spanish families without assuming a specific inheritance model, we performed multipoint NPL analysis for all autosomal chromosomes. A total of 10 chromosomal regions showed greater allele sharing between affected sibs, with multipoint LOD scores ranging from 0.5 to 1.7 (table 2).

Of interest, the marker D8S277 showed the second-highest LOD score (1.4) in Spanish families and is ~4 cM from D8S262, which was previously described by us to have the second-highest nonparametric multipoint LOD score (1.6) in the Arab-Israeli pedigree (Bykhovskaya et al. 1998).

To evaluate further the possible involvement of this candidate region, we genotyped the DNAs from the Italian and Arab-Israeli family members (table 3). The value of multipoint nonparametric LOD score in the Spanish and Italian families was maximal at D8S277, with a value of 2.1, and in the Arab-Israeli family the maximum multipoint LOD score of 1.6 was also at D8S277. Thus in both populations the maximal multipoint LOD score occurred at the same marker.

We did also use varying allele frequencies, especially in the region around D8S277, using the allele frequencies in the CEPH families for the analysis of the Spanish families and the parental allele frequencies for the analysis of the Arab-Israeli pedigree, and we did not see a change in LOD score of >0.1. For the markers around D8S277, there were also no significant differences in allele frequencies between the two populations.

We subsequently tested the other nine loci with multipoint nonparametric LOD scores of ≥ 0.5, which we

Table 3

Distances and Multipoint Nonparametric LOD Scores for the Markers in the Candidate Region around D8S277 in Spanish/Italian and Arab-Israeli Families

Marker	Distance (cM)	Multipoint Nonparametric LOD Score in Families	
		Spanish/Italian	Arab-Israeli
8pter	0		
D8S264	1	1.5	1.1
D8S262	5	1.2	1.1
D8S277	9	2.1	1.6
D8S503	17	.9	1.3
D8S265	23	.6	1.0
D8S552	28	.4	.7
D8S1145	39	.1	.2

Note. The shared locus is underlined.

had described previously in the Arab-Israeli pedigree. Results were negative for the Spanish data set alone (data not shown) as well as for the combined Spanish/Italian data set (table 4).

Calculation of the Allele-Sharing LOD Score in the Combined Data Set for D8S277

To calculate the resulting multipoint allele-sharing LOD score for the combined set of families, we used two linkage programs. The LOD score from MAPMAKER/SIBS reached 2.8 for all the families combined. To maximize the use of information on siblings in the large nuclear family with 10 affected sibs in the Arab-Israeli kindred, we used GENEHUNTER-PLUS to reanalyze the combined Arab-Israeli/Spanish/Italian data set. The peak value of the NPL score reached 3.5, with a *P* value of .0004 and corresponding LOD score of 3.1.

Table 4

Testing of Arab-Israeli Candidate Nonparametric Loci in Spanish/Italian Data Set

Marker	Maximum Multipoint Nonparametric LOD Score in Families	
	Arab-Israeli	Spanish/Italian
D14S280	2.1	.4
D8S277	1.6	2.1
D15S107/D15S130	.9	.0
D18S462	.7	.2
D1S234	.7	.3
D4S1627	.6	.0
D12S269/D12S336	.6	.0
D7S507	.5	.0
D20S470/D20S118	.5	.0
D5S471	.5	.1

Note. The shared locus is underlined.

Discussion

Hearing impairment associated with the mitochondrial A1555G mutation represents the best paradigm to understand the phenotypic expression of mitochondrial DNA mutations in general, since the mutation is homoplasmic (or nearly homoplasmic) and does not interfere with reproduction. Although environmental and mitochondrial haplotype can have an effect on the phenotypic expression (Fischel-Ghodsian 1998; Pandya et al. 1999), nuclear factors appear to be involved in most nonaminoglycoside cases. On the basis of results of a genomewide search in a large Arab-Israeli kindred, a model of a complex inheritance was proposed as being responsible for clinical penetrance of the disease (Bykhovskaya et al. 1998).

Our first aim was to test the possibility that a single modifier gene was missed in the Arab-Israeli screen, for reasons ranging from errors in sample labeling to biological problems such as phenocopies. The repeated failure to identify a single major modifier locus in the Spanish families after extensive linkage analysis under various Mendelian inheritance models could have several explanations. For instance, it has been shown that parametric linkage analysis can be highly sensitive to misspecification of the model (Clerget-Darpoux et al. 1986). Alternatively, given the known aminoglycoside interaction with the A1555G mutation, other as yet unidentified environmental factors could modulate the phenotypic expression. A third possibility is that the genetic inheritance of the nuclear locus (loci) responsible for phenotypic expression of the A1555G mutation in these families might be more complex and/or heterogeneous. This possibility is supported by our original findings in the Arab-Israeli pedigree and by the results described here. In particular, when we tested in the Arab-Israeli family the best parametric loci from the Spanish families, the two-point linkage LOD scores were negative for all but D8S277, for which a nonsignificant positive two-point LOD score of 0.8 was detected (table 1). Similarly, when we tested the eight best parametric loci identified in the Arab-Israeli pedigree in the Spanish and Italian families, no significant LOD score was obtained (data not shown).

Multipoint affected sib–pair analysis is widely considered to be a robust and sensitive statistical method of choice for genetic analysis of all but the simplest of traits. Application of this analysis to the genomewide genotyping data of Spanish families led to the independent identification, in a different data set, of a candidate region previously described in the Arab-Israeli kindred. We found that the second-best nonparametric locus, D8S277, with a LOD score of 1.6 in the Arab-Israeli kindred, is the second-best one in Spanish families, with a LOD score of 1.4. It is also the only candidate locus

for which the LOD score calculated in the Spanish-Italian data set becomes more significant (LOD score 2.1) than in the Spanish data set. The resulting allele-sharing LOD score for the combined data set varies from 2.8 to 3.1, depending on the statistical program implemented for the calculations. Direct comparison of these results with the guidelines for the magnitude of significance suggested by Lander and Kruglyak (1995) show that the established values of linkage LOD score are very close to the threshold of significant linkage (LOD score 3.6) and are much higher than the proposed threshold of suggestive linkage (LOD score 2.2).

These linkage results in the Arab-Israeli, Spanish, and Italian families with the A1555G mutation strongly suggest our having identified the first nuclear modifier gene for a mitochondrial DNA disease mutation. However, ultimate proof of this linkage, and eventual identification of the responsible gene within this chromosomal region, will require additional data. We suggest that this region should be considered a high-priority location both for follow-up linkage studies in additional families with maternally inherited nonsyndromic hearing loss and the mitochondrial A1555G mutation and for candidate-gene screening. The eventual identification of the modifier gene within this region could shed light on the pathophysiological pathways involved in the clinical expression of the A1555G mutation.

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Electronic-Database Information

URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/ (for sex-averaged map distances used in the analyses)

Centre d'Étude du Polymorphisme Humain (CEPH), http://www.cephb.fr/cgi-bin/wdb/ceph/systeme/form (for CEPH reference families allele frequencies searchable index)

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