Am. J. Hum. Genet. 66:733–739, 2000

Report

Vacuoliting Megalencephalic Leukoencephalopathy with Subcortical Cysts, Mapped to Chromosome $22q_{tel}$

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Summary

The leukodystrophies form a complex group of orphan genetic disorders that primarily affect myelin, the main constituent of the brain white matter. Among the leukodystrophies of undetermined etiology, a new clinical entity called "vacuoliting megalencephalic leukoencephalopathy" (VL) was recently recognized. VL is characterized by diffuse swelling of the white matter, large subcortical cysts, and megalencephaly with infantile onset. Family studies in several ethnic groups have suggested an autosomal recessive mode of inheritance. We mapped the *VL* gene to chromosome $22q_{tel}$, within a 3-cM linkage interval between markers D22S1161 and n66c4 (maximum LOD score 10.12 at recombination fraction .0, for marker n66c4; maximum multipoint LOD score 17 for this interval) by genome scan of 13 Turkish families. Linkage analysis under the genetic-heterogeneity hypothesis showed no genetic heterogeneity. No abnormalities were found in three tested candidate genes (fibulin-1 and glutathione S-transferases 1 and 2).

The leukodystrophies are a heterogeneous group of orphan genetic disorders that primarily affect the white matter of the brain and its main constituent, myelin. Among the leukodystrophies of undetermined etiology, a new clinical entity called "vacuoliting megalencephalic leukoencephalopathy" (VL) [MIM 604004] recently has been recognized. This disorder is distinct from the other leukodystrophies (i.e., Canavan disease [MIM 271900], Alexander disease [MIM 203450], and GM1 gangliosidosis [MIM 230500]) that exhibit progressive megalencephaly. VL is characterized by a milder clinical course, which contrasts with severe abnormalities on magnetic-resonance-imaging analysis of the brain. The supratentorial hemispherical white matter appears diffusely swelled, with bilateral subcortical cysts of typical temporal location, whereas the gray-matter structures are preserved (van der Knaap et al. 1995; Goutieres et al. 1996; Singhal et al. 1996; Mejaski-Bosnjak et al. 1997; Topçu et al. 1998). Consanguinity between the parents of VL-affected children, which is suggestive of an autosomal recessive mode of inheritance, has been observed in several ethnic groups. We report the localization of the *VL* gene to chromosome $22q_{tel}$, by genome scan of 10 consanguineous families and 3 families with no known relationship that originated from rural areas of central and southeastern Anatolia, in Turkey.

The study was approved by the review board of Hacettepe University, Faculty of Medicine (Ankara). Informed consent was obtained from family members. Twenty-one patients (9 boys and 12 girls) from 13 different families were analyzed. Consanguinity was known to have occurred in 10 families. Clinical and neurobiological features of 12 patients (from families MEG1, MEG3, MEG 4, MEG 6, MEG8, and MEG10) have been reported elsewhere (Topçu et al. 1998). The diagnostic criteria for selection of the other families were identical. All other causes of leukodystrophies have been ruled out by an extensive biochemical analysis, including quantitation of lysosomal activities (of β-hexosamini-

Received July 26, 1999; accepted for publication November 29, 1999; electronically published February 1, 2000.

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0002-9297/2000/6602-0043$02.00

dase, arylsulfatase A, β-galactosidase, and galactocerebrosidase β-galactoside).

Because biochemical and enzymatic studies revealed no known abnormalities, and because the high degree of consanguinity between the parents of affected children suggested an autosomal recessive mode of inheritance, we undertook a genome scan to map the *VL* gene. DNA was isolated and purified from peripheral blood, by use of standard protocols. Genotyping was performed with fluorescent end-labeled microsatellite markers from the Généthon map (Vignal et al. 1993). Alleles were separated by electrophoresis performed with ABI 373 sequencers. Genotyping data were processed with GENOTYPER (ABI), GENSCAN (ABI), and MARKSYN (Généthon) software. Two-point LOD-score analysis was conducted with the FASTLINK program (Schaffer 1996), and multipoint LOD-score analysis was conducted with the GENEHUNTER program (Kruglyak et al. 1996); and an autosomal recessive mode of inheritance with full penetrance and true marker-allele frequencies was assumed. The disease-allele frequency was set to .0001. This initial genome screening with the nine initially collected families (Topçu et al. 1998) genotyped with 257 fluorescent end-labeled microsatellite Généthon markers spaced at an average distance of 14 cM (Weissenbach et al. 1992; Dib et al. 1996) failed to reveal linkage. A second genome search, with 300 additional microsatellite markers (average spacing for the combined set of markers was 6.5 cM), was therefore performed. Linkage analysis of D22S1160 and D22S1169, two markers separated by 11 cM, yielded a multipoint LOD score of 4. Four additional Turkish families (MEG13, MEG14, MEG15, and MEG16) were collected, and the complete set of families was genotyped with four new Généthon markers. Multipoint linkage analysis yielded a Z_{max} of 15. However, although markers showed homozygosity (Lander and Botstein 1987) with fully informative families, several families remained partially or totally uninformative, raising the possibility of genetic heterogeneity.

To increase informativity of these families, new microsatellite markers were developed. Sequences of genomic clones spanning the telomeric region that were downloaded from The Sanger Center were computer scanned for short tandem repeats. Three microsatellite sequences were identified, two in the vicinity of the genes encoding fibulin (*FBLN-1*) and arylsulfatase A (*ARSA*) and one in the genomic clone n66c4. New markers were developed, and the 13 families were genotyped. Primer sets flanking the microsatellite motifs were developed for FBLN1D/$(CA)_n$ (*FBLN-1* [GenBank accession number Z95331] [motif: CA repeat, primer set GACCTACTATGTGCCAGGAAGG and GGCTGAGGCACAAGAGTTTC, size 300–300 bp]), ARSA$(CT)_n$ (*ARSA* [Genbank accession number U62317] [motif: CT repeat, primer set CCGGCCAAAAATGACTTTTA and CTGGAAAGAGCAAGACCCTG, size 200–204 bp]), and n66c4 clone [Genbank accession number AC000050] [motif: CA repeat, primer set ACTCATCGCATACACTGTGTCC and CAGGGTGGATCACCAAGC, size: 266–274 bp]). The allele size was estimated by use of individual 134702 from the CEPH reference family. Two-point linkage analysis yielded a maximum LOD score (Z_{max}) of 10.12 at a recombination fraction (θ) of 0, for marker n66c4 (table 1). This result is in agreement with the results of two-point linkage simulation (autosomal recessive mode of inheritance, full penetrance, five isofrequent marker alleles, and disease-allele frequency .0001), performed with SLINK (Weeks et al. 1990), which yielded an average LOD score of 10 at $\theta = .0$ for this family set.

Multipoint linkage analysis performed with GENEHUNTER (Kruglyak et al. 1996) yielded a Z_{max} of 17 (fig. 1). This result was confirmed by haplotype construction with D22S294, D22S1170, D22S1161, D22S922, D22S1169, ARSA/$(CT)_n$, and n66c4/$(CA)_n$, which places the *VL* gene within a 3-cM linkage interval defined by D22S1161 (proximal) and n66c4 (distal) (fig. 2). The map is based on the recently published sequence of chromosome 22 (Dunham et al. 1999). Multipoint linkage analysis performed under the heterogeneity hypothesis revealed no genetic heterogeneity (data not shown).

Three affected children were born from three marriages that had no known consanguinity (i.e., families MEG6, MEG11, and MEG16). Haplotype analysis of family MEG11 showed an 11-cM homozygosity region, suggesting that the parents were likely to be related. Results showed no homozygosity for the affected child in each of families MEG6 and MEG16. However, analysis of the extended haplotypes showed that the unaffected siblings had inherited different combinations of recombinant haplotypes (fig. 2). Taken together, these results show that VL is linked to a single locus that spans a 3-cM region located on chromosome $22q_{tel}$. Whereas this rare disorder was mapped in a set of Turkish families originating from small villages in a rural area, a founder

Table 1

Two-Point Linkage-Analysis Data, from an Autosomal Recessive Model with Full Penetrance

	LOD Score at θ =						
Marker	.00	.1	.2	.3	.4	Z_{max}	θ_{max}
D22S294	$-\infty$	1.50	1.41	.93	.41	1.55	.132
D22S1170	$-\infty$	2.99	2.00	1.00	.32	3.10	.069
D22S1161	$-\infty$	6.01	4.05	2.30	.92	7.04	.026
D22S922	5.17	4.54	3.12	1.83	.76	5.47	.017
D22S1169	8.57	7.03	4.67	2.61	1.04	8.76	.010
ARSA	6.34	4.48	2.83	1.52	.58	6.34	.000
n66c4	10.12	7.41	4.84	2.64	.97	10.12	.000

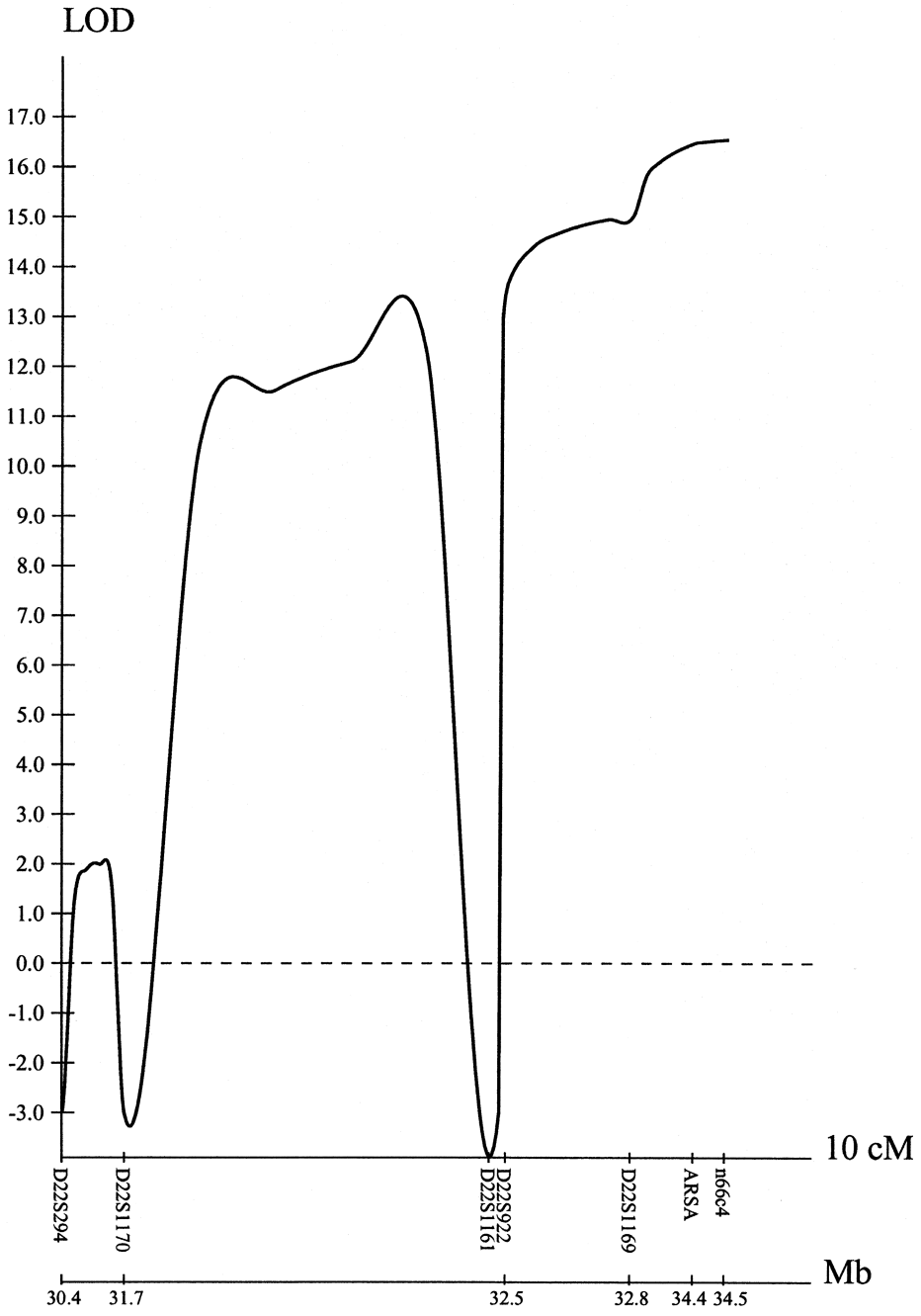
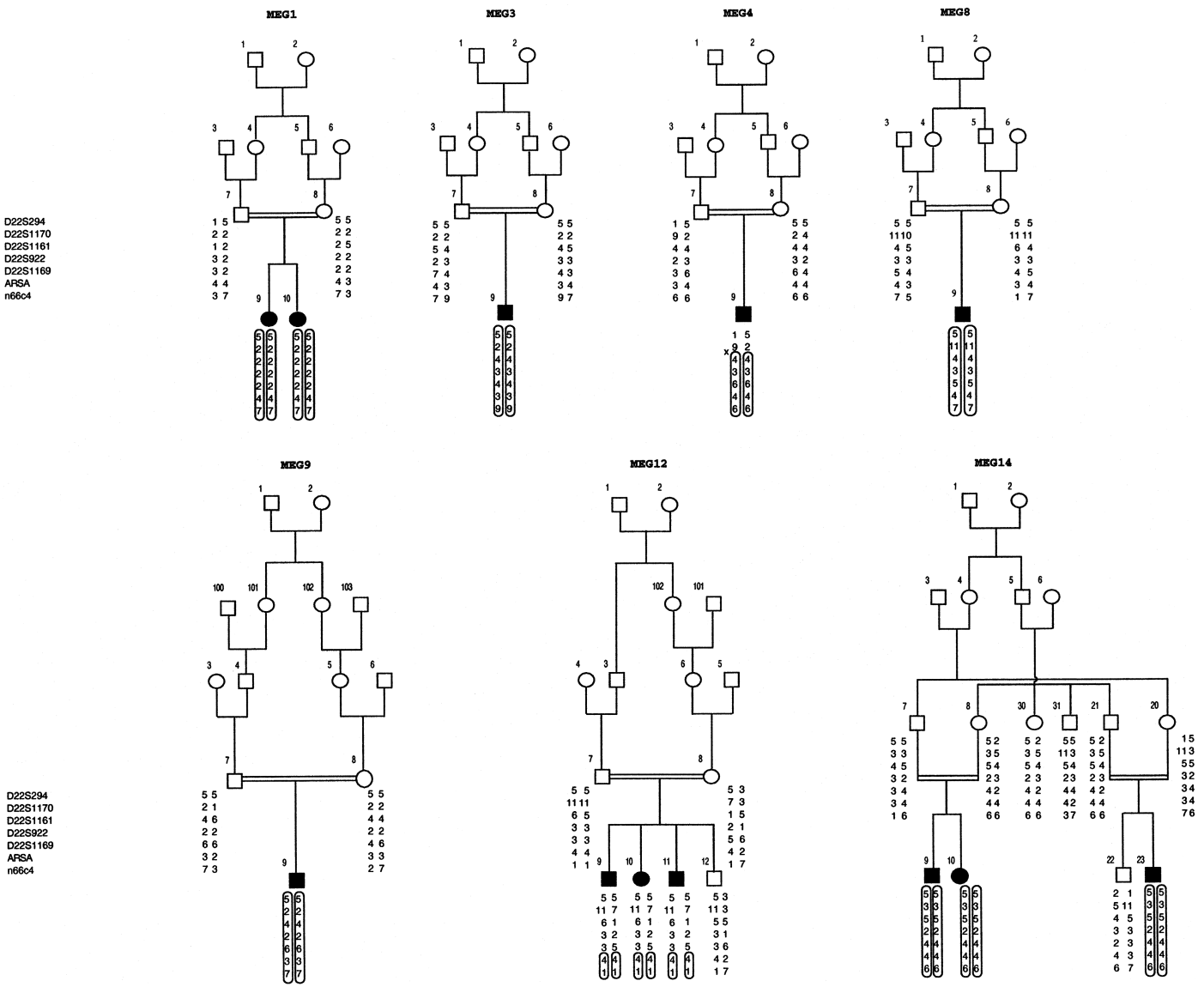


Figure 1 Graphic representation of multipoint LOD-score analysis of the $22q_{tel}$ chromosomal region linked to the *VL* gene. The multipoint LOD score was computed with GENEHUNTER (Kruglyak et al. 1996) by use of a recessive mode of inheritance, complete penetrance, and true marker-allele frequencies. The disease-allele frequency was set to .0001.

effect that would be revealed by a common haplotype was anticipated. However, within the limits of the analyzed markers, haplotype analysis did not confirm this hypothesis.

Three candidate genes—fibulin-1 (Argraves et al. 1989) and glutathione S-transferases 1 GSTT1 (Pemble et al. 1994) and 2 GSTT2 (Tan et al. 1995)—that mapped within the initial linkage interval were analyzed for their potential involvement in the disease etiology. FBLN1D/$(CA)_n$, physically mapped between D22S1141 and D22S1170, revealed three recombination events. Sequence analysis of GSTT1 and GSTT2 revealed no sequence variations. Therefore, *FBLN1*, *GSTT1*, and *GSTT2* were excluded.


MEG1
MEG3
MEG4
MEG8
D22S294
D22S1170
D22S1161
D22S922
D22S1169
ARSA
n66c4
MEG9
MEG12
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D22S922
D22S1169
ARSA
n66c4

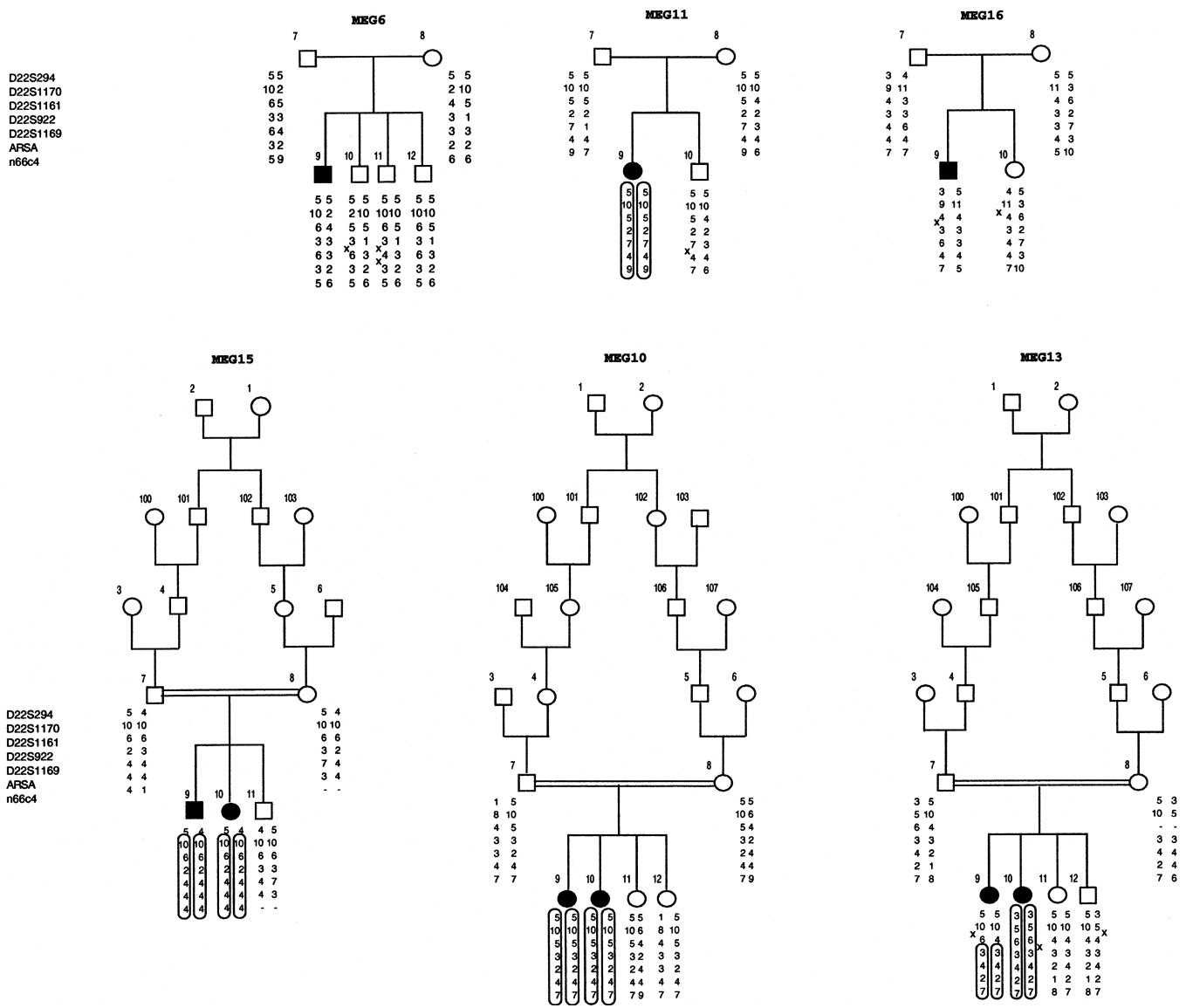

Figure 2 Haplotypes of seven $22q_{tel}$ DNA markers defining the shortest linkage interval derived from 10 consanguineous families and from 3 families with no known relationship. Haplotypes were inferred by use of GENEHUNTER (Kruglyak et al. 1996). Disease haplotypes are enclosed within ovals. Missing genotypes are indicated by hyphens (-), and recombination events are indicated by "×". Physical position (in Mb) of the markers along the chromosome is indicated next to the marker names (Dunham et al. 1999).

We mapped the *VL* gene to chromosome $22q_{tel}$, within a 3-cM linkage interval between D22S1161 and n66c4, by genome scan and fine mapping. This result was supported by a two-point LOD score ($Z_{max} = 10.12$ at maximum θ [θ] = .0, for marker n66c4) and by a multipoint LOD score of 17 for the interval between D22S1161 and n66c4. Haplotype analysis of the three affected children born from marriages between parents of unknown relationship (families MEG6, MEG11, and MEG16) showed extended homozygosity for family MEG11, suggesting that the parents are likely to be related.

Although consanguinity is expected to permit gene mapping with a limited number of families, the mapping of the *VL* gene proved to be difficult. To reveal linkage, we had to increase the marker density of our genome scan. This was probably because of lack of informativity of the markers, rather than because of clinical heterogeneity. In contrast, several factors are likely to be responsible for the difficulties that we encountered. First, approximately half of the parents were distant cousins. Therefore, the chromosomal region homozygous by descent was expected be small. Second, these families originated from a population in which ~30% of the marriages are consanguineous. The number of observed heterozygotes was approximately half of what would be expected on the basis of marker PIC values. Therefore, consanguinity reduces marker informativity by limiting the allele number. Third, the *VL* gene mapped to the telomeric end of the long arm of chromosome 22, a region with poor marker coverage. Fourth, the size of the chromosomal region shared by the affected children appeared to be small (i.e., 3 cM).

Although the families originated from rural areas of central and southeastern Anatolia, in Turkey, no shared allele or shared haplotype was detected. This suggests either that the genetic markers are still too far from the disease gene to allow detection of an ancient founder effect or the existence of allelic heterogeneity.

Although mild and severe forms of the disease were noted, both forms appeared to be linked to the same locus. The clinical variations could actually be due either to nongenetic factors or to modifier genes that affect the phenotype and the course of the disease.

In an attempt to identify the disease gene, we analyzed three candidate genes—*FBLN-1, GSTT1,* and *GSTT2*. A microsatellite marker located 3′ to *FBLN-1* was developed. Three families showed recombination events that excluded this gene. *GSTT1* and *GSTT2* were analyzed by PCR amplification and exon sequencing. PCR analysis showed a higher frequency of deletion of the *GSTT1* gene than previously had been reported (59% vs. 38%). However, sequence variations in the two *GSTT* genes were not observed.

At present, we are attempting the positional cloning of the *VL* gene, to determine the underlying mechanism of this disorder. A search of the GeneMap'99 database for the D22S1161-$22q_{tel}$ region revealed 30 expressed sequence tags (ESTs). Development of new microsatellites or single-nucleotide-polymorphism markers would help to further define both the linkage interval and the number of ESTs necessary to screen for mutations.

Acknowledgments

We would like to thank Dr. Odile Boespflug-Tanguy, for clinical-diagnosis protocols, and Dr. Jean-François Prud'homme, for helpful comments on this report. We also thank the patients and their families for collaborating in this study. This study was supported by grants from the Association Française contre les Myopathies (to E.S.).

Electronic-Database Information

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

GeneMap'99, http://www.ncbi.nlm.nih.gov/genemap

GenBank Overview, http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html (for *FBLN-1* [accession number Z95331], *ARSA* [accession number U62317], and n66c4 clone [accession number AC000050])

Online Mendelian Inheritance In Man Database (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Alexander disease [MIM 203450], GM1 gangliosidosis [MIM 230500], Canavan disease [MIM 271900], and VL [MIM 604004])

Sanger Centre, The, http://www.sanger.ac.uk/HGP/Chr22

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