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Mapping of the Mucolipidosis Type IV Gene to Chromosome 19p and Definition of Founder Haplotypes

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Summary

Mucolipidosis type IV (MLIV) is a lysosomal storage disorder characterized by severe neurologic and ophthalmologic abnormalities. It is a rare autosomal recessive disease, and the majority of patients diagnosed, to date, are of Ashkenazi Jewish descent. We have mapped the MLIV gene to chromosome 19p13.2-13.3 by linkage analysis with 15 markers in 13 families. A maximum LOD score of 5.51 with no recombinants was observed with marker *D19S873*. Several markers in the linked interval also displayed significant linkage disequilibrium with the disorder. We constructed haplotypes in 26 Ashkenazi Jewish families and demonstrate the existence of two founder chromosomes in this population. The localization of MLIV to chromosome 19 will permit genetic prenatal diagnosis in affected families and will aid in the isolation of the disease gene.

Introduction

Mucolipidosis (ML) type IV (MLIV; MIM 252650) belongs to a group of inherited metabolic diseases known as the lysosomal storage disorders. It was first described in 1974 as a new variant of the mucolipidoses and was characterized by corneal clouding and abnormal systemic storage bodies (Berman et al. 1974). The absence of mucopolysaccharide excretion and the lack of identifiable lysosomal-enzyme changes distinguish it from the mucopolysaccharidoses and from the other mucolipidoses (ML type I, type II [I-cell disease], and type III), respectively (Crandall et al. 1982). MLIV is a progressive neurologic disease that usually presents during the first year of life, with mental retardation, corneal opacities, and delayed motor milestones (Amir et al. 1987). Definitive diagnosis of MLIV is made either by electron-microscopic demonstration of inclusions in the lysosomes of different cell types in skin or by conjunctival biopsies. Interestingly, patients with MLIV do not show mucopolysaccharide excretion, skeletal changes, or organomegaly, as do patients with other mucolipidoses. To date, >80 patients with MLIV have been reported, the majority of whom are of Ashkenazi Jewish descent (Schiffman et al. 1998).

The storage materials in MLIV are heterogeneous, having been identified by Bargal and Bach (1997) as sphingolipids, phospholipids, and acid mucopolysaccharides, and accumulate in the lysosomes in cells from every tissue and organ of patients with MLIV (Chen et al. 1998). Lipid-storage disorders typically are a result of defective lysosomal hydrolases or activator proteins, but extensive studies have shown that the lysosomal hydrolases involved in the catabolism of the stored material in MLIV have normal activity (Bach et al. 1999) and that the storage products are normally catabolized and discharged. Consequently, MLIV may be a result of abnormal transport of lipids to the lysosomes rather than of abnormal catabolism. Experiments done in cultured fibroblasts suggest that phospholipids and gangliosides accumulate in MLIV as the result of a defect in the process of endocytosis of membranous components. The defect appears to lead to excessive transport of these macromolecules into the lysosomes, consistent with the heterogeneity of the stored materials in MLIV (Bargal and Bach 1997). Examination of the movement of a lipid analogue along the lysosomal pathway has implicated a defect that affects the late steps in the endocytic pathway in MLIV cells (Chen et al. 1998). A defect in the sorting and transport pathway, rather than a degradation defect, might explain the absence of a massive buildup of material and the resulting organomegaly seen in other lysosomal storage diseases. Recent studies done in patients

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with MLIV have shown that they are also constitutively achlorhydric and have partially activated parietal cells. This finding suggests that the defective protein in MLIV probably also plays a role in parietal cell activation and is critical to a specific type of cellular vacuolar trafficking between the cytoplasm and the apical membrane domain (Schiffman et al. 1998).

Since the majority of the lysosomal storage disorders are due to defects in the catabolism of specific storage products, it is likely that the function of the MLIV gene will be unique in its disease-causing mechanism. The purpose of this study is to determine the chromosomal localization of the MLIV gene by a genomewide search for linkage. Using 13 Ashkenazi Jewish families, we mapped the MLIV gene to chromosome 19p13.2-13.3 and identified two founder haplotypes in the population. The localization of the MLIV gene will permit identification of the defective gene, which will eventually shed light on the exact nature of the transport defect along the late-endocytic pathway that is responsible for the disease.

Families and Methods

MLIV Families

We collected 26 Ashkenazi Jewish families with MLIV through collaboration with the Mucolipidosis IV Foundation. Approval from the institutional review boards at Massachusetts General Hospital, Harvard Medical School, the National Institute of Neurological Disorders and Stroke, and Hadassah University Hospital was obtained prior to the collection of samples. For families included in this study, MLIV was diagnosed according to standard criteria. Patients from 16 families were seen by R.S., and patients from 6 families were seen by G.B. Patients from the other four families were not examined directly by physicians involved in this study; however, their diagnosis was uncomplicated and was made by use of standard criteria. Four of the families had two or more affected members, and nine families had one or more unaffected sibling, making them useful for linkage analysis (fig. 1). The remaining 13 families, which were composed of a single affected child, parents, and, in some cases, grandparents, were used only for haplotype analysis. In total, we studied 52 disease chromosomes, 54 non-MLIV chromosomes (obtained from heterozygote carriers), and 17 control chromosomes (obtained from unaffected individuals married into the MLIV families).

DNA Analysis

Genomic DNA was prepared either from lymphoblast cell lines (Anderson and Gusella 1984) with the SDS-proteinase K method followed by phenol extraction or directly from blood with the Nucleon II kit (Scotlab). PCR was performed in a total volume of 15 μL with ~100 ng genomic DNA; 20 pmol each primer; 0.5 U *Taq* polymerase; 1.5 mM $MgCl_2$; 50 mM KCl; 200 μM dATP, dCTP, and dTTP; 20 μM dGTP; and 0.1 uCi $\alpha[^{32}P]$-dGTP. PCR was carried out in a PTC-100 thermal cycler (MJ Research) with the following cycle: initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 20 s, 53°C–60°C for 20 s, and 72°C for 20 s. The annealing temperature was optimized for each set of primers. PCR products were electrophoresed on a 6% polyacrylamide gel (90W for 2–3 h at constant power), and autoradiography was performed by exposure of the gels to XAR film (Kodak) for 3–4 h.

Two-Tiered Genome Scan

During the initial phase of family collection, we performed a complete genome scan in five individuals from family 1 (fig. 1) by using the Cooperative Human Linkage Center Human Screening Set/Weber (v. 8; Research Genetics). This screening set consists of 366 markers with a mean spacing of 10 cM and an average heterozygosity of .76. We genotyped the parents, the two affected children, and one unaffected child. By evaluating marker-sharing between the siblings, we prioritized the markers to be genotyped in the primary linkage family set, which consisted of families 1–3 and 6. On the basis of the results from our initial scan, we genotyped families 1–3 and 6 for 117 markers. The additional 22 families used in our study were genotyped only for the chromosome 19 markers, after the discovery of linkage.

Statistical Analysis

To determine the power of our families to detect linkage, we performed simulation analysis using version 2.6 of the SLINK program (Ott 1989; Weeks et al. 1990). Five hundred replicates were simulated under a recessive disease model with complete penetrance, with use of a disease-gene frequency of .001. Two-point LOD scores between the disease and individual markers were calculated with the MLINK program of the FASTLINK 3.0P software package (Cottingham et al. 1993; Schaffer et al. 1994; Schaffer 1996), a faster version of the original LINKAGE package (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986). Haplotypes were constructed by visual inspection of the linked markers.

Results

Genome Scan

SLINK analysis of our primary linkage family set (families 1–3 and 6; fig. 1) yielded a maximum LOD score of 4.38 and an average LOD score of 2.98 at recombination fraction (θ) = 0, indicating power to detect linkage in these four families. The initial genome

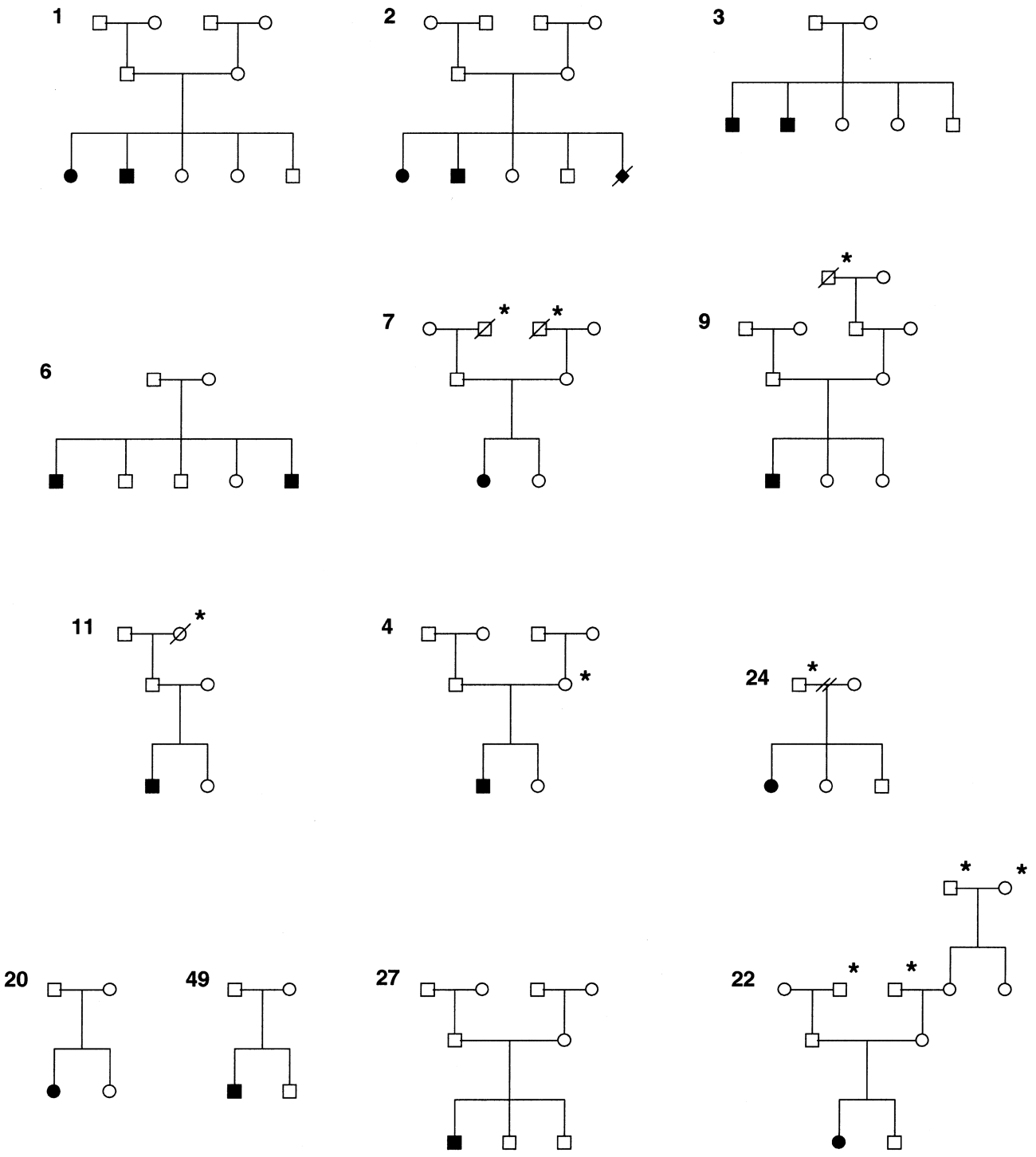

Figure 1 MLIV linkage families. An asterisk (*) indicates individuals not sampled.

scan was done in the parents, two affected children, and one unaffected child from family 1. We evaluated marker sharing between siblings, which resulted in the following prioritization scheme for the genotyping of the primary linkage family set: 14 markers were homozygous, shared in the affected sibs and different in the unaffected sib; 64 markers were heterozygous, shared in the affected sibs and different in the unaffected sib; and 39 markers were uninformative in the family. This reduced to 117 the number of markers that were genotyped in families

1–3 and 6. This is a 69% reduction in the total number of markers, which represents a significant time and cost savings.

Of the 117 markers genotyped in the primary family set, four markers yielded LOD scores >2.0 at $\theta = 0$: *GATA30E06* (chromosome 2), LOD = 2.28; *D3S1265,* LOD = 2.5; *D12S1300,* LOD = 2.28; and *D19S586,* LOD = 2.15. All of these regions were followed up by genotyping the flanking markers in the screening set and, if necessary, by the addition of closely spaced markers. The addition of marker *D19S873* gave a LOD score of 4.38 and, therefore, demonstrated significant linkage to chromosome 19. We then saturated this region by genotyping 15 closely spaced markers that map to the 19p13.2-13.3 interval in the 13 families shown in figure 1. The genetic location of the markers and the LOD scores obtained from our analysis are given in table 1. Recombinants in our families place the MLIV gene within a 5.62-cM interval, between markers *D19S1034* and *D19S884.*

Table 1

Pairwise LOD Scores of Chromosome 19 Markers With the 13 MLIV Families in Figure 1

Marker	Location (in cM)	Recombination Fraction at θ = .00	.01	.05	.1	.2	.3	.4
D19S1034	20.75	−∞	.21	.78	.84	.60	.30	.09
D19S869	22.96	3.47	3.41	3.09	2.65	1.75	.91	.26
INSR	25.17	.98	.99	.98	.89	.60	.29	.08
D19S592	...[a]	4.18	4.03	3.45	2.75	1.52	.63	.14
D19S406	25.17	2.37	2.31	2.07	1.76	1.15	.60	.17
D19S873	25.17	5.51	5.34	4.66	3.83	2.29	1.05	.27
D19S901	25.17	4.98	4.84	4.25	3.53	2.17	1.03	.27
D19S905	25.17	2.22	2.14	1.84	1.49	.90	.44	.12
D19S76	25.17	2.26	2.21	1.98	1.69	1.12	.59	.17
D19S912	26.37	4.55	4.43	3.93	3.30	2.06	.99	.26
D19S884	26.37	−∞	3.73	3.82	3.35	2.15	1.04	.27
D19S922	26.37	−∞	2.05	2.25	1.95	1.19	.54	.13
D19S916	31.29	−∞	2.61	2.84	2.55	1.71	.87	.24
D19S586	32.94	3.05	2.94	2.49	1.96	1.05	.43	.10
D19S906	36.22	−∞	−2.08	−.31	.19	.34	.21	.06

[a] Marker location determined on the basis of Lawrence Livermore National Laboratory physical map of chromosome 19p.

Disequilibrium and Haplotype Analysis

We observed significant linkage disequilibrium with 12 of the 15 markers genotyped across this region: *D19S1034, P* < .002; *D19S869, D19S592, D19S406, D19S873, D19S901, D19S905, D19S76, D19S912,* and D19S884, *P* < .0001; and *INSR* and *D19S922, P* < .0002. We therefore undertook haplotype analysis of MLIV in an attempt to define the founder haplotypes, refine the candidate region on the basis of historical recombinants, and estimate the number of independent mutations represented in the MLIV population. A major founder haplotype was observed for 39 (75%) of the 52 MLIV chromosomes examined. In addition, a second less-frequent founder haplotype was observed for 11 (21%) of the 52 chromosomes (table 2). The two remaining chromosomes shown in table 2 display significant allele differences at multiple markers. In each case, these "odd" haplotypes were present in patients also carrying one of the major haplotypes. These haplotypes suggest that there may be as many as four independent gene mutations that cause MLIV in the Ashkenazi Jewish population. None of the 54 non-MLIV chromosomes from heterozygote carriers, nor of the 17 control chromosomes, displayed a haplotype identical to any of the MLIV chromosomes. However, 3 of the 54 non-MLIV chromosomes shared the same alleles as the major MLIV haplotype and as the two "odd" MLIV haplotypes, respectively, for the core markers *D19S406–D19S873–D19S901–D19S905–D19S76.* The two frequent founder haplotypes are recognizable across the interval from *D19S869–D19S922,* estimated to be ~3.41 cM. There is no genetic evidence distinguishing the order of the markers *INSR, D19S406, D19S873, D19S901, D19S905,* and *D19S76* within this interval. Marker *D19S592* has been localized physically to this region, but it is not on any genetic map. However, the order of *INSR–D19S592–D19S406–D19S76* is supported by the Lawrence Livermore National Laboratory physical map of chromosome 19 (Lawrence Livermore National Library Human Genome Center). The chromosomes that support potential ancestral recombinants within the founder haplotypes are de-

Table 2

Haplotypes Associated with MLIV

Haplotype	Marker *D19S869*	*INSR*	*D19S592*	*D19S406*	*D19S873*	*D19S901*	*D19S905*	*D19S76*	*D19S912*	*D19S884*	*D19S922*	Number
Major	283	3	4	207	120	152	216	3	175	230	236	39[a]
Minor	275	3	5	211	126	161	234	4	179	230	240	11[a]
Odd 1	275	2	5	211	112	157	234	4	175	236	244	1
Odd 2	277	3	4	211	114	159	218	4	175	234	244	1

[a] See figure 2 for details.

D19S869	*INSR*	*D19S592*	*D19S406*	*D19S873*	*D19S901*	*D19S905*	*D19S76*	*D19S912*	*D19S884*	*D19S922*	Number
283	3	4	207	120	152	216	3	175	230	236	23*
285	3	4	207	120	152	216	3	187	232	240	1
277	3	4	207	120	152	216	3	175	230	236	1
275	6	4	207	120	152	216	3	175	230	236	1
271	2	5	207	120	152	216	3	175	234	246	1
277	3	2	211	120	152	216	3	175	230	236	1
283	3	4	207	120	152	216	3	177	236	250	2
283	3	4	207	120	152	216	3	187	232	244	1
283	3	4	207	120	152	216	3	187	234	242	1
283	3	4	207	120	152	216	3	179	232	240	1
283	3	4	207	120	152	216	3	177	234	240	1
283	3	4	207	120	152	216	3	179	236	250	1
283	3	4	207	120	152	216	3	167	222	252	1
283	3	4	207	120	152	216	3	175	234	244	1
283	3	4	207	120	152	216	3	175	234	246	1
283	3	4	207	120	152	216	3	175	230	248	1
275	3	5	211	126	161	234	4	179	230	240	6*
285	3	5	211	126	161	234	4	179	230	240	1
275	3	5	211	126	161	234	4	179	230	236	1
271	3	5	211	126	161	234	4	179	239	149	1
275	3	5	211	126	161	234	4	167	222	256	1
275	3	5	211	126	161	234	4	175	222	248	1

Figure 2 Extended haplotype analysis of 52 MLIV chromosomes with 11 markers. The major haplotype is shown in row 1 (marked with an asterisk [*]), and the minor haplotype is shown in row 17 (marked with asterisk [*]). The other haplotypes, which have probably arisen from ancestral recombination events, are depicted with the identical MLIV core markers outlined.

picted in figure 2. Examination of the potential ancestral recombinants in the founder haplotypes supports the following order of the markers: *INSR–D19S592–D19S406–*[*D19S873, D19S901, D19S905, D19S76*]*–D19S912–D19S884–D19S922* and suggests that the MLIV gene maps between *D19S406* and *D19S912,* estimated at ~1.2 cM. Figure 2 shows that, on the proximal side of the map, we observe one ancestral recombinant with *D19S406,* one additional recombinant with *D19S592,* one additional recombinant with *INSR,* and one additional recombinant with *D19S869.* With the distal markers, we see 10 ancestral recombinants with *D19S912,* four additional recombinants with *D19S884,* and one additional recombinant with *D19S922.* It is important to point out, however, that, until the exact physical order of the markers in this region is determined, the extent of the candidate interval for the MLIV gene remains uncertain.

Discussion

MLIV is a unique member of a family of lysosomal storage disorders. It is a devastating disease that causes motor and mental retardation and severe ophthalmologic abnormalities that usually lead to blindness. The majority of patients diagnosed to date are of Ashkenazi Jewish descent. This study provides strong evidence for localization of the gene causing MLIV to the chromosome 19p13.2-13.3 region. The definition of closely linked, highly informative markers flanking the disease gene provides the basis for prenatal diagnosis and carrier detection in MLIV families. Presently, prenatal diagnosis is made by electron microscopy of amniocytes, a technique that requires a high degree of skill and expertise in the diagnosis of MLIV. The finding of genetic linkage will allow the prenatal test to become more widely available.

The assignment of the MLIV gene to 19p13.2-13.3 also opens an avenue to isolating the disease gene by positional cloning. Haplotype analysis suggests that there are four different haplotypes that span the MLIV region, and, therefore, potentially four independent mutations. However, until the exact physical order of these markers is determined and new markers are isolated in the region, it is difficult to say with certainty that these four haplotypes are completely independent. This region of chromosome 19 is extremely gene rich; in fact, there are >18 other disorders that have been mapped to 19p13. A scan of the public databases also shows that >100 known genes have been mapped to the 19p13.2-13.3 region. Although some of these can be excluded as candidates on the basis of their fine mapping, the putative functions of the others does not reveal any that are likely candidates for MLIV. The human transcript map at the GeneMap'98 website (National Center for Biotechnology Information) maps 13 genes and eight expressed sequence tags to the interval between *D19S216* and *D19S912,* which would include the MLIV

candidate region. None of the known genes are likely candidates, since previous studies suggest that the MLIV gene plays a role in the late-endocytic pathway and in the final stages of parietal cell activation. Although no obvious candidate gene for MLIV maps to this region, the results of this study suggest that it will be possible to narrow the candidate-gene interval by use of linkage disequilibrium. Evaluation of the physical map of the region will determine which of the known genes map to the precise candidate interval and will also permit the identification of new MLIV candidate genes.

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Electronic-Database Information

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

Lawrence Livermore National Laboratory Human Genome Center, http://www-bio.llnl.gov/genome/genome.html (for location of *D19S592* and physical map order)

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/genemap/ (for GeneMap'99 data)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for MLIV [MIM 252650])

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