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Assignment of the Mulibrey Nanism Gene to 17q by Linkage and Linkage-Disequilibrium Analysis

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Summary

Mulibrey nanism (MUL) is an autosomal recessive disorder with unknown basic metabolic defect. It is characterized by growth failure of prenatal onset, characteristic dysmorphic features, constrictive pericardium, hepatomegaly as a consequence of constrictive pericardium, yellowish dots in the ocular fundi, and J-shaped sella turcica. Hypoplasia of various endocrine glands, causing hormone deficiencies, is common. Here we report the assignment of the MUL gene, by linkage analysis in Finnish families, to a 7-cM region flanked by D17S1799 and D17S948 on chromosome 17q. Multipoint linkage analysis gave a maximum LOD score of 5.01 at loci D17S1606-D17S1853 and at D17S1604. The estimate of the critical MUL region was further narrowed to within ~250 kb of marker D17S1853 by linkage disequilibrium analysis. Positional candidate genes that belong to the growth hormone and homeobox B gene clusters were excluded. These data confirm the autosomal recessive inheritance of MUL and allow highly focused attempts to clone the gene.

Introduction

Mulibrey nanism (MUL; *mu*scle-*li*ver-*br*ain-*ey*e nanism; MIM 253250) is a rare disorder involving several tissues of mesodermal origin (Perheentupa et al. 1973). Autosomal recessive inheritance implying a highly pleiotropic gene is evident (Perheentupa et al. 1973; Lipsanen-Nyman 1986; Lapunzina et al. 1995). The proportion of affected children in the MUL families is .25 (Lipsanen-Nyman 1986). The basic metabolic defect is not known.

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Mulibrey nanism was first described in Finland in 1970 (Perheentupa et al. 1970). The disorder is characterized by growth failure of prenatal onset and by characteristic dysmorphic features, including a triangular face, broad forehead, low nasal bridge, and telecanthus. Also, constrictive pericardium, hepatomegaly as a consequence of constrictive pericardium, yellowish dots in the ocular fundi, J-shaped sella turcica, enlarged brain ventricles, fibrous cysts of tibia, and nevi flammei are common features (Perheentupa et al. 1970; Lipsanen-Nyman 1986; Lapunzina et al. 1995). Hypoplasia of various endocrine glands, causing hormone deficiencies, is frequent. Deficiencies of growth hormone, ACTH, cortisol, sex hormone, and thyroid hormone have been described (Lenko et al. 1982; Lipsanen-Nyman 1986; Haraldsson et al. 1993). The diagnosis is based on the phenotype. The degree of constriction of the pericardium is the most important factor in determining the quality of life and the prognosis (Perheentupa et al. 1973; Lipsanen-Nyman 1986).

The genetically isolated population of Finland exhibits typical founder effects. Mutations causing several recessive diseases that are rare elsewhere are more prevalent in the Finnish population (Norio 1981). MUL is a typical example of these disorders. Worldwide, some 75 MUL patients have been described, the majority from Finland, where the incidence of MUL is estimated to be 1:40,000 (Lipsanen-Nyman 1986). However, sporadic patients have been reported, from Egypt (Thoren 1973), Canada (Cumming et al. 1976), the United States (Voorhess et al. 1976), Spain, and Argentina (Lapunzina et al. 1995). The unique features of the Finnish population history allow fine mapping of disease genes by linkage disequilibrium (e.g., see Hästbacka et al. 1992, 1994; International Batten Disease Consortium 1995; Mitchison et al. 1995; Pennacchio et al. 1996; Virtaneva et al. 1996).

In this paper results are described that allowed the MUL gene to be localized to a small region of the genome. After a systematic search for linkage in six families, the gene was mapped to a 7-cM interval on chromosome 17q. The localization was then greatly refined by

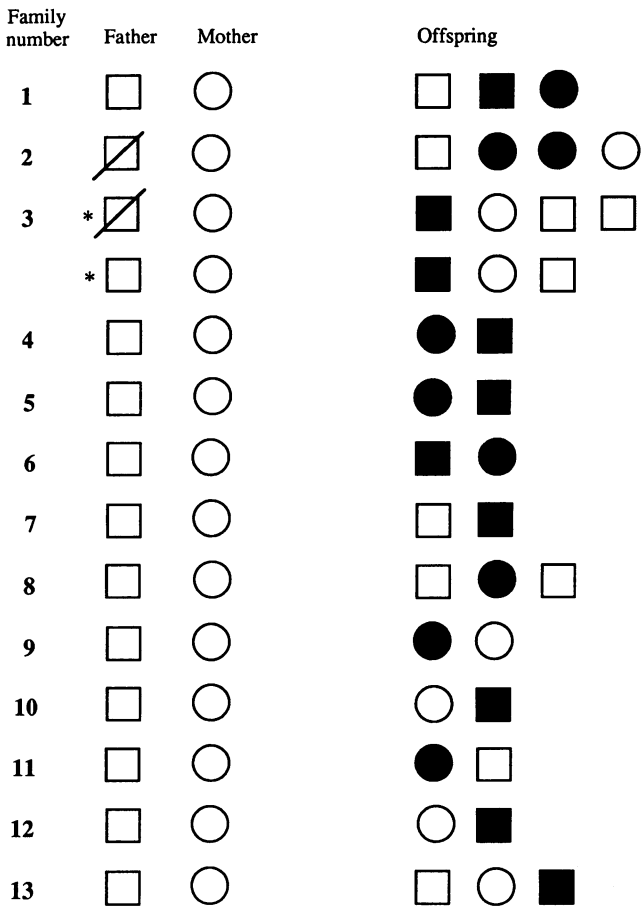

Figure 1 Composition of MUL families studied. Squares denote males; circles denote females; unblackened symbols denote unaffected individuals; and blackened symbols denote affected subjects; a diagonal slash (/) through a symbol denotes that the individual is deceased. The two fathers in pedigree 3, denoted by asterisks (*), were close relatives (uncle and nephew).

linkage-disequilibrium analysis. Moreover, positional candidate genes at the growth-hormone gene (GH) and homeobox gene (HOXB) clusters were excluded.

Subjects and Methods

Families and Samples

We studied a total of 13 Finnish MUL families including 19 affected and 43 unaffected individuals. The pedigree structures are shown in figure 1. The diagnosis was based on the typical phenotype and was established by one of us (M.L.-N. or J.P.). From each consenting individual 20–30 ml of venous blood was drawn, as a source of DNA.

DNA Marker Analysis

A genomewide search for linkage was done with a panel of 240 $(CA)_n$-repeat markers. Most of the DNA markers used in this study were developed at Généthon (Weissenbach et al. 1992; Gyapay et al. 1994; Dib et al. 1996). For the intragenic microsatellite markers at GH and HOX2B, see the work of Polymeropoulos et al. (1991) and Deinard et al. (1992), respectively. PCR was performed as described elsewhere (Weber and May 1989; Vignal et al. 1993). In the initial systematic screening for linkage, we used a nonradioactive genotyping method (Vignal et al. 1993). Several amplification products from the same DNA sample, generated with different primer sets, were pooled and coprecipitated and coelectrophoresed in a single lane of a 6% polyacrylamide, 50% urea sequencing gel. After the products were transfered to Hybond N+™ (Amersham) membranes, hybridization was performed with peroxidase-labeled PCR primers. The alleles were detected by use of a chemiluminescence-based ECL™ (Amersham) detection system. In refining linkage, the chromosome 17 markers were analyzed by radioactively labeled markers as described in detail elsewhere (Sulisalo et al. 1993). After PCR, the amplified samples were electrophoresed in a 6% polyacrylamide, 50% urea sequencing gel and were dried and autoradiographed for 2 h–5 d. In the initial screening for linkage, the films were scored for the presence or absence of recombinations, by familywise comparison of the alleles of affected individuals. If both affected sibs in a family had the same alleles, the family was marked with a plus sign (+); if not, they were marked with a minus sign (−). In family 3 (fig. 1), where the affected children were cousins once removed, the presence of one common allele resulted in a plus sign. This rapid and simple method gave a clear-cut result. Only a single marker, D17S787, gave five plus signs, and subsequent analyses proved it to be linked to MUL. The next class (four plus signs) comprised four markers.

Linkage Analysis

In the primary screening for linkage, a panel of six families including 12 affected individuals was used (families 1–6; fig. 1). By the simulation program SLINK (Ott 1989; Weeks et al. 1990) this panel was estimated to give an average LOD score of 2.7 (recombination fraction [θ] = .0), when a heterozygosity value of .70 for the markers was assumed. Two-point linkage analyses were performed with the MLINK program of the LINKAGE package OS/2-version 5.2 (Lathrop et al. 1984). Multipoint linkage analysis was performed with the novel program VITESSE (O'Connell and Weeks 1995). It uses an algorithm for recoding genotypes that effectively addresses both the memory and computational limitations encountered in the processing of large numbers of marker alleles, thus accelerating the calculation. Multipoint analysis was performed under the assumption of a fixed order and fixed distances of the eight marker loci (Dib et al. 1996). The order of markers and

the θ's between the markers were as follows: centromere–D17S1799–.00–D17S1607–.05–D17S1606–.00–D17S1853–.01–D17S1604–.01–D17S1811/D17S1855–.00–D17S94 8–telomere. Data for loci D17S1811 and D17S1855 were combined for computation with the VITESSE program, while the other loci were considered separately. All calculations were done under the assumption of complete penetrance and equal θ in males and females.

Linkage-Disequilibrium Analysis

To test whether there is a single allele with a significantly higher frequency on disease-bearing chromosomes than on control chromosomes, we used Fisher's exact test with a correction for multiple testing. The non–MUL-bearing chromosomes of the parents were used as control chromosomes.

Subprogram ILINK was used in the estimation of marker-allele frequencies and in testing the null hypothesis of linkage equilibrium between MUL and marker loci (Hellsten et al. 1993). For the markers showing significant deviation from equilibrium, the observed allelic association was incorporated into the haplotype frequencies and was used in the pairwise-LOD-score computations by subprogram ILINK.

Luria-Delbrück–based calculation (Hästbacka et al. 1992; Lehesjoki et al. 1993) was used to estimate the genetic distance (θ) between markers and MUL, under the following assumptions: the number of generations since "founding" (g)—that is, the beginning of spreading of the MUL mutation in the population—was 10–100; the mutation rate (μ) of the MUL locus was 5×10^{-6}, since the disease is rare worldwide; and the overall MUL gene frequency (q) was .005, on the basis of Hardy-Weinberg equilibrium and the actual observed incidence of 1:40,000 for the disease phenotype in Finland (Lipsanen-Nyman 1986). Allelic excess was calculated by the formula $p_{excess} = (p_{affected} - p_{normal})/(1 - p_{normal})$, where p denotes allele frequency. The θ value was calculated by the formula $p_{excess} = (1 - \mu g q^{-1})(1 - \theta)^g$, where $(1 - \mu g q^{-1})$ denotes the proportion of MUL chromosomes carrying the same ancestral mutation.

Results

Linkage Mapping of MUL

In the genomewide search in families 1–6 (fig. 1), only a single marker, D17S787, was fully informative and did not show any recombinations, giving the LOD score of 2.85 at θ = .00. Linkage to chromosome 17 was confirmed by analysis of additional markers. The results are shown in table 1. Recombinations were observed between MUL and D17S1799, on the centromeric side, and between MUL and D17S948, on the telomeric side, defining an interval of ~7 cM. Within this region the highest two-point LOD score, 4.46 at θ = .00, was obtained at locus D17S1604. In the multipoint analysis (fig. 2) an equally high, eight-point maximum LOD score (Z_{max}) of 5.01 was reached at loci D17S1606-D17S1853 and at locus D17S1604. The ±2-LOD-unit interval covers 7 cM on the map.

A microsatellite-repeat polymorphism at the GH locus showed a recombination in family 13, in the maternal meiosis (data not shown). Since the telomeric flanking marker D17S948 also had recombined in the same meiosis, the GH locus is most likely excluded as an MUL candidate. At the HOX2B locus, a microsatellite-repeat marker did not show any recombinations in MUL families (data not shown), but HOXB was excluded as a likely MUL candidate, on the basis of the absence of linkage disequilibrium with the marker (table 2).

Fine Mapping of MUL by Linkage Disequilibrium

Alleles at seven marker loci on the MUL-bearing chromosomes of 13 patients were compared with alleles on the normal chromosomes of the parents. The results are shown in table 2. A highly significant ($P < .001$) allelic association was observed for the markers D17S1606, D17S1853, and D17S1604, the most common allele occurring in 74%, 85%, and 44%, respectively, of the MUL-bearing chromosomes, but in only 15%, 11%, and 4%, respectively, of the normal chromosomes (table 2). When linkage disequilibrium was allowed for in the calculations, the two-point LOD scores rose to 10.26 at D17S1606, 12.71 at D17S1853, and 10.45 at D17S1604 (table 1). Allelic association was not detected between MUL and HOX2B.

Applying strategies described elsewhere (for details, see Subjects and Methods), we used linkage disequilibrium to estimate the genetic distances between the MUL gene and the marker loci that demonstrated significant allelic association. The p_{excess} values for markers D17S1606, D17S1853, and D17S1604 were .70, .83, and .42, respectively (fig. 3). Assuming a single founding mutation, a q of .005, and a μ of 5×10^{-6}, we calculated the genetic distance between MUL and three marker loci, under different assumptions about g. The results are shown in figure 3. By these criteria the MUL gene resides closest to D17S1853. If $g = 50$ is assumed, the distance of MUL from D17S1853 is 0.25 cM; if $g = 25$ generations, 0.65 cM.

Discussion

By conventional recombinational mapping, we localized MUL to an interval ~7 cM in length. To further narrow the region, we took advantage of the unique features of the Finnish population (de la Chapelle 1993) and conducted linkage-disequilibrium studies. Linkage-

Table 1

Pairwise LOD Scores, between MUL and Eight Marker Loci, in 13 Finnish Families

Locus	Pairwise LOD Score at θ =							Z_{max}	Maximum θ	90% Confidence Limits
	.00	.001	.01	.05	.10	.20	.30			
D17S1799	−∞	.28	1.21	1.59	1.49	1.00	.49	1.65	.050	
D17S1607	2.17	2.16	2.11	1.87	1.57	.97	.47	2.17	.000	
D17S1606	4.41	4.39	4.25	3.63	2.90	1.66	.75	4.41	.000	.00–.06
	(10.26)	(10.24)	(10.08)	(9.32)	(8.35)	(6.34)	(4.27)	(10.26)	(.000)	(.00–.05)
D17S1853	4.41	4.39	4.25	3.64	2.93	1.69	.77	4.41	.000	.00–.07
	(12.71)	(12.69)	(12.50)	(11.63)	(10.55)	(8.27)	5.80	(12.71)	(.000)	(.00–.05)
D17S1604	4.46	4.44	4.31	3.72	3.02	1.78	.82	3.48	.000	.00–.07
	(10.45)	(10.43)	(10.30)	(9.67)	(8.79)	(6.86)	(4.76)	(10.45)	(.000)	(.00–.06)
D17S1811	2.78	2.77	2.69	2.36	1.95	1.19	.57	2.78	.000	
D17S1855	2.60	2.59	2.51	2.17	1.76	1.04	.49	2.60	.000	
D17S948	−∞	1.14	.74	1.60	1.58	1.06	.52	1.62	.050	

NOTE.—Results in parentheses include linkage-disequilibrium data.

disequilibrium mapping is a powerful tool in refining disease-gene localizations for Mendelian disorders that are relatively rare in the population (Jorde 1995). In an ideal situation, most disease chromosomes in the population descend from a single ancestral mutation. Moreover, the mutation should be old enough so that recombination has made the region of strongest linkage disequilibrium small but not too small (Hästbacka et al. 1992), which is the case in Finland (de la Chapelle 1993). Linkage-disequilibrium mapping already has been proved to be a crucial step in the positional cloning of several disease genes (e.g., see Hästbacka et al. 1994; International Batten Disease Consortium 1995; Pennacchio et al. 1996). In MUL, significant allelic association was detected with three markers. To estimate the genetic distance of MUL from these loci, we applied Luria-Delbrück–based calculations (Hästbacka et al. 1992; Lehesjoki et al. 1993). Two key assumptions in these calculations are q and g. We used q = .005, on the basis of the actual MUL incidence of 1:40,000 in the Finnish population (Lipsanen-Nyman 1986). Assumptions about the actual age of the MUL mutation in the Finnish population can be made by implications based on the geographical distribution of birthplaces of carriers of the MUL mutation. This distribution is uneven in MUL (Lipsanen-Nyman 1986), since the disease is clustered in the Savo and North Carelia regions, representing partly early- and partly late-settled areas (de la Chapelle 1993). This pattern is interpreted to indicate that $g \geq 25$, and probably more, for the major MUL mutation within the population. Our finding of significant linkage disequilibrium over a very small genomic region suggests a relatively long time since founding. If g = 50 is assumed, the MUL gene resides <0.25 cM from marker locus D17S1853, which showed the most significant linkage disequilibrium. This interval corresponds to a distance of 250 kb in each direction from the marker, a region small enough for physical mapping attempts, assuming the average correspondence of 1 cM to 1 Mb of DNA. The estimate of genetic distance based on linkage disequilibrium is subject to some variation, and one can calculate approximate SDs on the parameters of interest (Hästbacka et al. 1992)—for example, on sampling error, as shown in figure 3. Moreover, the predicted physical distances are dependent on the ratio of physical distance to genetic distance in the region of interest. So

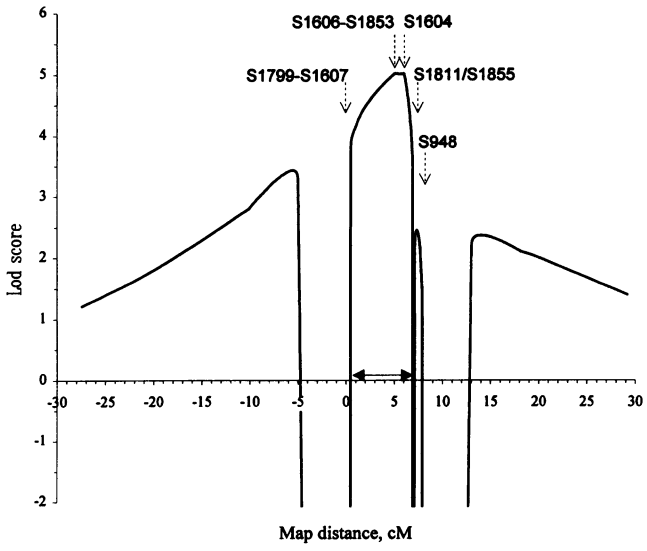

Figure 2 Results of multipoint linkage analysis: eight-point linkage analysis between MUL and eight marker loci in 13 families, with the computer program VITESSE. The Z_{max}, 5.01, was reached at D17S1606/D17S1853. The arrow bar on the X-axis corresponds to Z_{max} ± 2 LOD units. The centromere is to the left.

Table 2

Distribution of Alleles in Normal/Disease Chromosomes, at Six Marker Loci and at the HOX2B Locus

	No. of Normal Chromosomes/No. of Disease Chromosomes for Allele													
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D17S1607	1/1	0/2	7/11	1/1	10/9	3/2	4/1							
D17S1606			1/0	2/0	1/0	4/20*	8/3	3/0		5/3	2/1	1/0		
D17S1853	1/0	1/0	3/23*	8/0	9/0	5/2	0/1	0/1						
D17S1604	3/0		4/0	1/2	9/8	1/12*	0/1	2/0	1/0	0/1		3/0	2/3	1/0
D17S1811	1/0	4/5	1/1	4/4	5/0	2/3	9/12	1/1						
D17S1855	1/0	4/5	1/1	4/4	4/0	3/2	7/12	3/3						
HOX2B	1/1	2/2	2/3	5/2	15/17	1/1								

* $P < .001$.

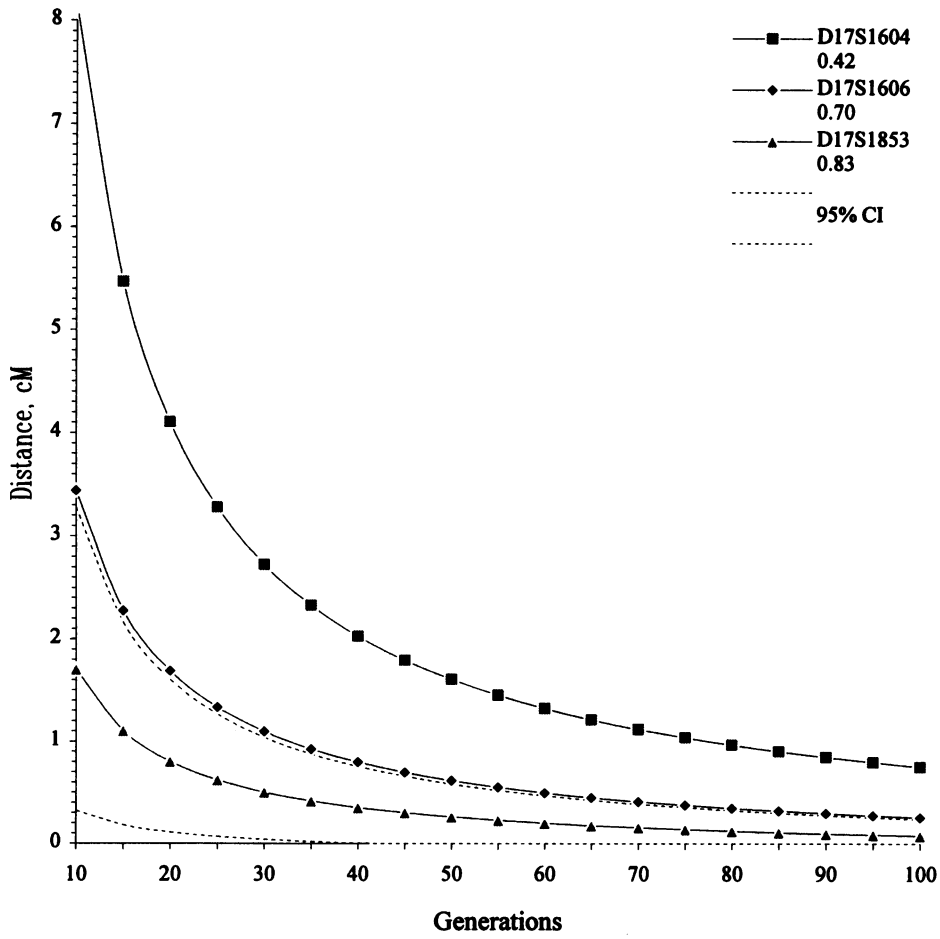

Figure 3 Genetic-distance estimates between MUL and marker loci, shown as a function of g. Symbols for the loci are indicated in the insert, where the value below each locus denotes the respective p_{excess}. The 95% confidence interval (95% CI), based on sampling error for p_{excess}, is shown for locus D17S1853. If it is assumed that $g = 50$, the MUL gene lies <0.25 cM from D17S1853.

far, the predictions have been proved to be surprisingly accurate, as, for example, in diastrophic dysplasia, where the gene has been estimated to lie <0.06 cM (or ~60 kb) from the CSF1R gene (Hästbacka et al. 1992) and has been found ~70 kb proximal to CSF1R (Hästbacka et al. 1994).

The most likely physical location of the markers linked to MUL is 17q21-24 (Black et al. 1993). A Genome Database search of human genes mapped to this interval resulted in a listing of >100 genes. On the basis of available mapping information and the known function of the genes, we studied further two candidate loci, the GH locus and the HOXB gene cluster.

The GH locus, containing five genes and spanning some 50 kb, has been localized to 17q22-q24 (George et al. 1981), with a reported mapping location between marker loci D17S1607 and D17S1604 (Paavola et al. 1995). Mutations involving different genes of the GH gene complex have been observed in growth-hormone deficiency or absence (Phillips and Cogan 1994). In MUL, one of the key findings is short stature, but only some of the patients are growth hormone deficient (Lenko et al. 1982; Lipsanen-Nyman 1986; Haraldsson et al. 1993; Lapunzina et al. 1995). In this study, on the basis of a recombination event between MUL and a microsatellite-repeat polymorphism at the GH locus, we were able to exclude GH as an MUL candidate.

Mammalian HOX genes play a fundamental role in the regulation of vertebrate embryogenesis (McGinnis and Krumlauf 1992). The HOX genes are organized into clusters, of which the HOXB cluster, spanning 180 kb and including nine genes (Acampora et al. 1989), has been mapped to 17q21-22 (Solomon and Baker 1989). We analyzed a dinucleotide-repeat polymorphism at the HOX2B locus and detected no recombinations. Unfortunately, the family in which a recombination had been detected with D17S1799 at the centromeric boundary of the MUL region, where the HOXB cluster maps, was not informative for HOX2B. Therefore we cannot rely on recombination to determine whether it resides outside or within the MUL region. However, the previous linkage-mapping data, localizing HOX2B centromeric to D17S1799 (Willhelmsen et al. 1994), and the absence of linkage disequilibrium at HOX2B (table 2) in the MUL families suggest that none of these HOX genes is the MUL gene.

Human chromosome 17 and mouse chromosome 11 represent one of the most complete and extensive syntenic domains of the two species, in that all genes thus far mapped to human chromosome 17 also map to mouse 11 (Nadeau 1989). A database search of mouse loci mapping to the region between the Hox-2 and the Gh clusters resulted in an additional plausible MUL candidate: the Meox-1 gene (mesoderm/mesenchyme HOX gene) is involved in the regulation of the mesodermal development of the embryo (Candia et al. 1992). The human homologue, MOX1, has been cloned (Futreal et al. 1994) and has been physically mapped to the BRCA1 region (Neuhausen et al. 1994), which, on the genetic map, is proximal of the MUL region. This assignment is likely to exclude MOX1 as the causative locus for MUL.

Our results show that the gene causing MUL in these 13 Finnish families maps to a single locus on chromosome 17 and confirm the recessive mode of inheritance. Moreover, our data suggest genetic homogeneity, at least in Finland, and demonstrate the validity of our diagnostic criteria for MUL. This in turn implicates the MUL gene as the sole cause of this multiorgan phenotype; that is, the gene is highly pleiotropic.

Acknowledgments

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