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Report

Localization of a Gene (*MCUL1*) for Multiple Cutaneous Leiomyomata and Uterine Fibroids to Chromosome 1q42.3-q43

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Dominant transmission of multiple uterine and cutaneous smooth-muscle tumors is seen in the disorder multiple leiomyomatosis (ML). We undertook a genomewide screen of 11 families segregating ML and found evidence for linkage to chromosome 1q42.3-q43 (maximum multipoint LOD score 5.40). Haplotype construction and analysis of recombinations permitted the minimal interval containing the locus, which we have designated "*MCUL1*," to be refined to an ~14-cM region flanked by markers D1S517 and D1S2842. Allelic-loss studies of tumors indicated that *MCUL1* may act as a tumor suppressor. Identification of *MCUL1* should have wide interest, since this gene may harbor low-penetrance variants predisposing to the common form of uterine fibroids and/or may undergo somatic mutation in sporadic leiomyomata.

Uterine fibroids, or leiomyomata, are the most common tumors in women during the reproductive years, yet little is known about their etiology. In most countries, they are the most frequent indication for hysterectomy in premenopausal women and therefore present a major public health issue. In addition to environmental and lifestyle factors, there is evidence that inherited factors play a role. Evidence supporting genetic predisposition to leiomyomata comes both from case reports of families with disease (Vikhlyaeva et al. 1995) and from population-based studies (Kurbanova et al. 1989*a*). Average estimates of familial risk are ~25% in the first-degree relatives of affected probands, and, on the basis of the recurrence rate in siblings, estimates of the heritability of uterine leiomyomata are .26–.80 (Kurbanova et al. 1989*b;* Snieder et al. 1998; Luoto et al. 2000).

Genetic predisposition to fibroids is undoubtedly heterogeneous. Dominant transmission of uterine myomata is, however, seen in the rare disorder multiple leiomyomatosis (ML [MIM 150800]) (Mezzadra 1965; Rudner et al. 1972; Reed et al. 1973; Engelke and Christophers 1979; Guillet et al. 1987); ~15 such families have been reported in the world literature. In this disorder, females develop both multiple skin leiomyomata and multiple uterine fibroids; males develop the skin lesions. The fibroids in patients with ML tend to be of variable size but are usually of early onset and are reportedly similar to the very common sporadic lesions. They may be complicated by anemia and/or subfertility, requiring myomectomy or hysterectomy. Although uncommon, ma-

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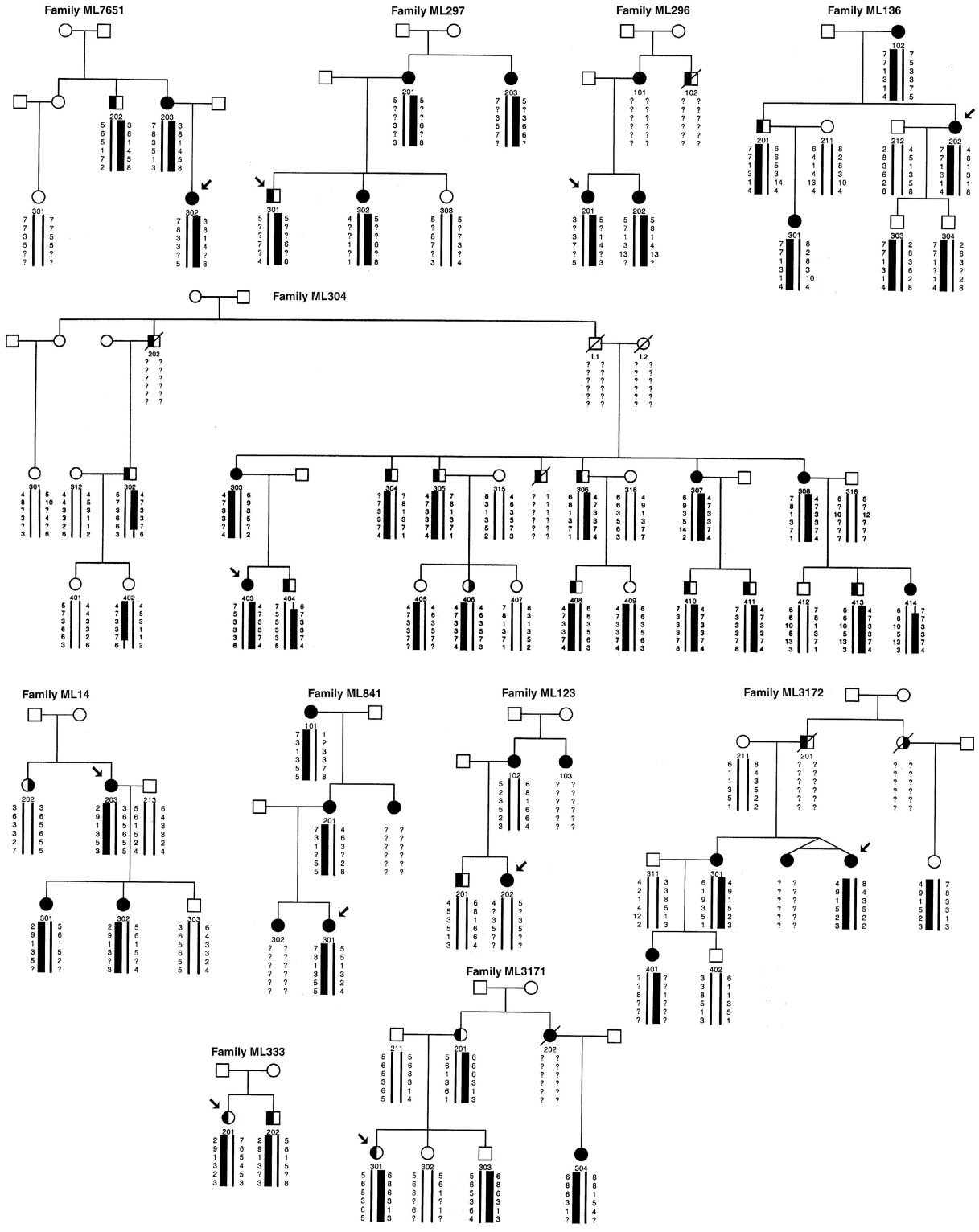

Figure 1 Pedigrees of families studied. Left-half-blackened symbols indicate individuals with cutaneous leiomyomata; right-half-blackened symbols indicate individuals with uterine fibroids. Unaffected, untyped individuals (except spouses) are not shown. Numbered genotypes are shown at markers D1S517, D1S2785, D1S547, D1S404, D1S180, and D1S2842 (listed from top to bottom, in 1cen-qter order). A question mark (?) denotes untyped or failed typing; the thicker black bars indicate the chromosome 1q42.3-q43 haplotype shared by affected individuals. In family 123, note that, although the affected siblings could share alleles at untyped markers D1S180 and D1S2842 (if a recombination has occurred within this region), haplotypes below D1S2842 (not shown) make this unlikely.

Table 1

Two-Point LOD Scores in Families in the Present Study

A. LOD Scores at $\theta = .001$

	LOD Score for					
Family	D1S517	D1S2785	D1S547	D1S404	D1S180	D1S2842
14	.30	.30	.30	.30	.00	.00
123	−2.15	.00	−0.45	−1.01	.00	.00
136	.30	.60	.60	.00	.60	.30
296	−0.05	.00	−0.10	−0.14	.00	.00
297	−0.05	.00	.00	.56	.00	−2.04
304	−2.65	.34	1.30	1.69	1.32	1.02
333	−.10	−.09	−.10	−.08	.00	−.05
841	.30	.30	.00	.26	.00	.00
3171	.36	.49	.39	.13	.14	−2.28
3172	.23	.23	.09	.22	.20	.22
7651	−.10	−.05	−.19	.15	−.09	.21
Total	−3.61	2.12	1.84	2.08	2.17	−2.62

B. LOD Scores at D1S2785, for Various Values of θ

	LOD Score for θ =						
Family	.001	.01	.05	.10	.20	.30	.40
14	.30	.29	.26	.21	.13	.06	.02
123	.00	.00	.00	.00	.00	.00	.00
136	.60	.59	.53	.47	.34	.21	.10
296	.00	.00	.00	.00	.00	.00	.00
297	.00	.00	.00	.00	.00	.00	.00
304	.34	1.03	1.41	1.38	1.04	.61	.23
333	−.09	−.09	−.07	−.06	−.03	−.01	−.00
841	.30	.30	.28	.25	.20	.15	.08
3171	.49	.47	.41	.33	.19	.08	.02
3172	.23	.22	.20	.16	.10	.05	.01
7651	−.05	−.05	−.04	−.03	−.02	−.01	−.00
Total	2.12	2.76	2.98	2.71	1.95	1.14	.46

lignant transformation of uterine lesions has been reported (Reed et al. 1973). The skin leiomyomata occur superficially in the dermis and are thought to arise from the erector pili muscles of the hair follicle. The skin lesions are smooth and shiny, tend to be 0.2–2 cm in diameter, and may be painful, especially in heat or cold or when subjected to pressure.

A small number of candidate loci for ML exist. Alport syndrome (AS [MIM 301050, MIM 104200, MIM 203780, and MIM 308940]) can be caused by mutations of collagen genes, including loci on Xq22.3 and 2q36-2q37. AS is associated not only with multiple leiomyomata, particularly of the esophagus, but also with other specific clinical features (see the Online Mendelian Inheritance in Man website). A patient without AS but with multiple cutaneous leiomyomata has been reported (Fryns et al. 1985) to carry constitutional 9p trisomy/18p distal monosomy.

We have ascertained 11 families with dominantly inherited cutaneous and uterine leiomyomata and have performed a genomewide screen to detect the location of the ML gene. Pedigrees were ascertained through dermatologists throughout the United Kingdom. Patient information, histology reports, and samples were obtained with full informed consent and ethical-review-board approval. Details of affection status were derived either from personal examination or from histopathology reports and medical records and, wherever possible, were confirmed by histological review of tumors. Fresh-frozen samples were obtained from eight cutaneous leiomyomata from the families. DNA was extracted from peripheral blood and tumor samples by standard methods.

To map the position of the ML gene, an initial 10–15-cM genomewide linkage search was undertaken, using fluorescence-labeled oligonucleotide markers taken from the Weber 9a set (Research Genetics). Dye-labeled PCR products were detected on ABI 377 DNA sequencers and were analyzed by GENESCAN and GENOTYPER software (Applied Biosystems). Additional polymorphic markers were taken from The Genetic Location Database map.

ML was modeled as a dominant trait with a gene frequency of .001. Only individuals with skin lesions were classified as affected. Individuals with uterine fibroids but without skin lesions were classified as unknown, to take account of the population prevalence of uterine fibroids and, hence, the relatively high probability of phenocopies from this source. Individuals without skin lesions who married into the family were classified as unaffected. All other family members were classified as unknown. A .001 phenocopy rate for multiple cutaneous leiomyomata was assumed. Two-point LOD scores were calculated for each marker, by the subprogram MLINK (version 5.1) of the LINKAGE program package (Lathrop et al. 1984) as implemented in FASTLINK (version 4.1) (O'Connell and Weeks 1995). Multipoint analyses were undertaken, by means of the VITESSE program (Cottingham et al. 1993). Marker-allele frequencies were either taken from The Genome Database or based on the genotyping of pedigree founders. The map order and distances between markers were based on the integrated map of The Genetic Location Database.

The 11 families (fig. 1) consisted of 54 affected individuals, and DNA samples were obtained from 44 of these. In brief, skin lesions presented at an age between the early teens and the 4th decade and tended to occur in crops on the trunk and limbs. All patients studied were >18 years old (median 47 years, range 28–91 years). Most patients reported that one or more lesions, especially larger ones, were painful in heat or cold. Between individuals, there was notable variation in the number of skin lesions. Of the 36 females affected by skin leiomyomata, 33 reported a history of fibroids (fig. 1); in all cases, these fibroids were confirmed as multiple lesions, some of which measured only a few millimeters in diameter.

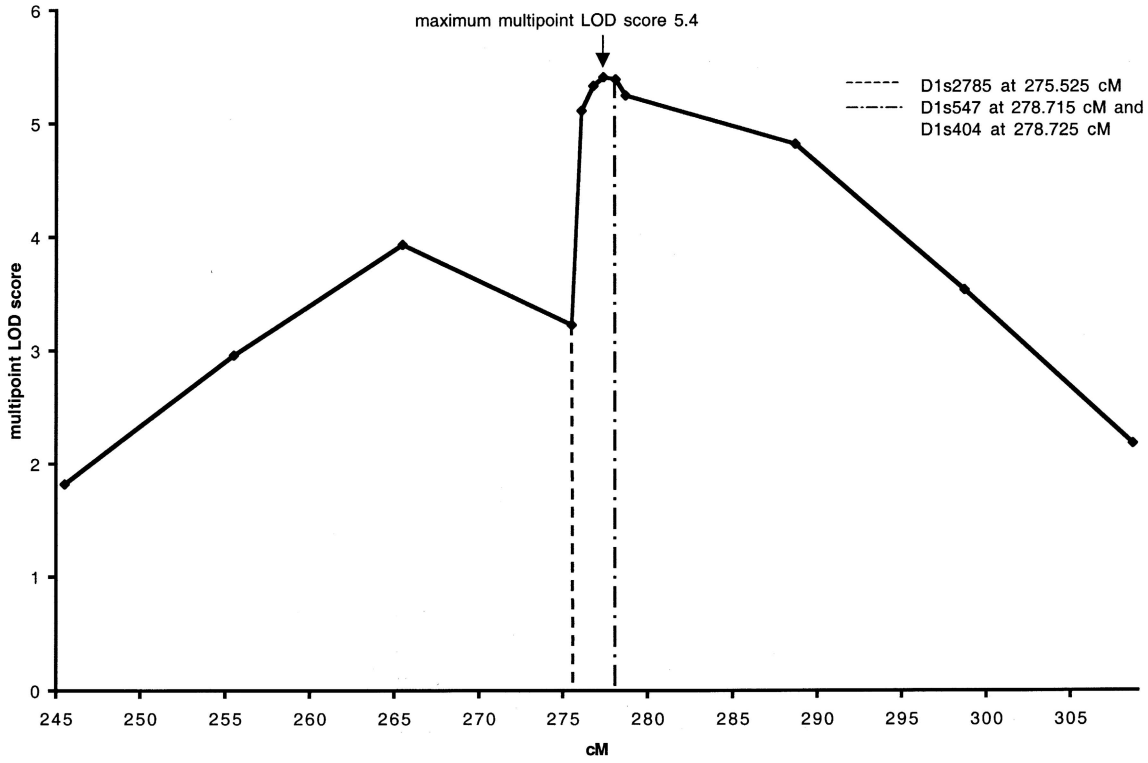

Figure 2 Multipoint linkage analysis with 1q42.3-q43 markers. The *X*-axis shows the marker location on chromosome 1, and the *Y*-axis shows the LOD score.

The initial genome screen detected eight regions with two-point LOD scores >1, with a maximum score of 1.84 (recombination fraction [θ] .001) at marker D1S547 (1q42.3-q43). Additional typing of markers in these eight regions formally excluded all but the region near D1S547 (details not shown). A maximum two-point LOD score of 2.99 (table 1) was obtained with marker D1S2785 (θ = .04). By means of a sliding map of three markers, multipoint LOD scores were calculated across the region around D1S547 and D1S2785. The maximum multipoint LOD score obtained was 5.40, for the markers D1S2785–3.2 cM–D1S547–0.01 cM–D1S404 (fig. 2). In family 304 alone, the maximum multipoint LOD score obtained was 3.97 at D1S404, for these same markers.

Haplotype construction showed that, except for pedigree 123 (fig. 1), all the families studied were compatible with linkage of ML to chromosome 1q42.3-q43. When data from this family were excluded, haplotype analysis placed the predisposition gene, which we have termed "*MCUL1*" (multiple cutaneous and uterine leiomyomata 1), within an ~14-cM interval bounded, on the centromeric side, by D1S517 and, on the telomeric side, by D1S2842. Both the critical recombinations defining this region occurred in the large family, 304. As expected, we found in the 14-cM region no good evidence of haplotype sharing between families.

Of 33 females with the disease-associated haplotype, 25 had both skin leiomyomata and uterine fibroids, 3 had skin leiomyomata only (although occult fibroids were not excluded), 1 (a member of family 304, who was age 33 years at the time of the study) had uterine fibroids only, and 4 (who were ages 28, 34, 35, and 67 years at the time of the study) were unaffected. One

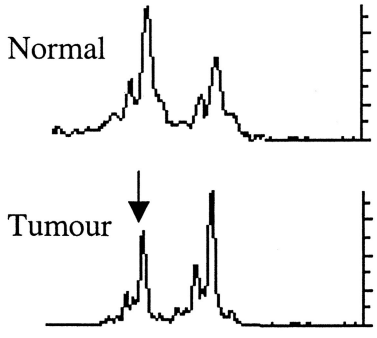

Figure 3 Loss of wild-type allele (*arrow*) at D1S2842 in skin leiomyoma from individual 301 from family 297.

female (a member of family 14) with uterine fibroids did not share the disease-associated haplotype and had developed an unknown number of fibroids during her late 40s; she presumably represents a sporadic case. Of 16 males who had the disease-associated haplotype, 13 had developed skin leiomyomata; the unaffected carriers were ages 33, 35, and 36 years. The disease gene appeared, therefore, to have high penetrance.

Genotyping for allelic loss in the skin leiomyomata was performed by means of the microsatellite D1S2842. Allelic loss was scored if the area under one of the two allelic peaks in the tumor was reduced to $\leqslant 50\%$, relative to its value in the normal tissue, thus making allowance for any contaminating normal tissue in the sample. Loss of the wild-type allele was found in two skin leiomyomata, from families 297 and 14; an example is shown in figure 3. *MCUL1* appears, therefore, to act as a tumor suppressor, and further mapping of allelic loss may help to refine the gene's location.

Genetic alterations in sporadic uterine leiomyomata appear to be few, with most studies concentrating on deletion of chromosome 7q (Ishwad et al. 1995, 1997; Sourla et al. 1996; Zeng et al. 1997; Sell et al. 1998; van der Heijden et al. 1998; Vanni et al. 1999) and on changes in the high-mobility-group genes (Hennig et al. 1996; Klotzbucher et al. 1999; Polito et al. 1999). There are few reports of chromosome 1 alterations in sporadic leiomyomata at any site (Polito et al. 1999), and none of these studies has included markers in the 1q42.3-q43 region (van der Heijden et al. 1998). Gains and losses of 1q are, however, among the most common cytogenetic changes observed in tumors.

Multiple leiomyomatosis is a disease of benign tumors arising from the erector pili muscles and of uterine fibroids. Its chief importance lies in the subfertility and blood loss caused by the fibroids and in its role as a model for sporadic fibroid development. We have provided evidence to show that a high-penetrance, dominant gene, which we have designated "*MCUL1,*" predisposing to multiple leiomyomata of the skin and uterus, is located within a 14-cM interval on chromosome 1q42.3-q43. In this study, data for all the families with ML were consistent with linkage to *MCUL1,* with the exception of one small pedigree. It is possible that genetic predisposition to ML is heterogeneous, but we cannot preclude the possibility of phenocopies or diagnostic error in this family. Several known genes located in the minimal *MCUL1* region may be considered candidates. These include zinc-finger protein ZNF124, nidogen, ryanodine receptor R2, and α2-actinin. Further studies are under way to refine the localization of and to identify *MCUL1*. Identification of *MCUL1* should have wide biological interest because of its possible involvement in both non-Mendelian predisposition to uterine leiomyomata and somatic mutation in sporadic leiomyomata.

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Electronic-Database Information

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

Genetic Location Database, The, http://cedar.genetics.soton.ac.uk/public_html/ldb.html (for map order and distances of markers)

Genome Database, The, http://gdbwww.gdb.org/ (for marker-allele frequencies)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ML [MIM 150800] and AS [MIM 301050, MIM 104200, MIM 203780, and MIM 308940])

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