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# Short Communication

## Familial Cutaneous Leiomyomatosis Is a Two-Hit Condition Associated with Renal Cell Cancer of Characteristic Histopathology

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**Little has been known about the molecular background of familial multiple cutaneous leiomyomatosis (MCL). We report here a clinical, histopathological, and molecular study of a multiple cutaneous leiomyomatosis kindred with seven affected members. This detailed study revealed strong features of a recently described cancer predisposition syndrome, hereditary leiomyomatosis and renal cell cancer (HLRCC). The family was compatible with linkage to the HLRCC locus in 1q. Also, all seven cutaneous leiomyomas derived from the proband and analyzed for loss of heterozygosity displayed loss of the wild-type allele, confirming the association with a susceptibility gene in chromosome 1q. One individual had had renal cell cancer at the age of 35 years. This tumor displayed a rare papillary histopathology, which appears to be characteristic for HLRCC. The derived linkage, loss of heterozygosity, and clinical data suggest that MCL and HLRCC are a single disease with a variable phenotype. The possibility that members of leiomyomatosis families are predisposed to renal cell cancer should be taken into account.** ***(Am J Pathol 2001, 159:825–829)***

Cutaneous leiomyomas are rare benign tumors of the skin, which are thought to originate from the arrectores pilorum muscle. Clinically, cutaneous leiomyomas are manifested as erythematous firm nodules, varying in number from a few to thousands. They usually appear on the face, back, and extensor surfaces of the extremities, and are painful to touch.[1,2] Kloepfler and colleagues[3] described a family with several individuals affected with cutaneous leiomyomas and suggested an autosomal-dominant inheritance with incomplete penetrance (multiple cutaneous leiomyomatosis). The conclusion was supported by reports of additional families with affected individuals in multiple generations. Interestingly, cutaneous leiomyomatosis seemed to be associated with uterine leiomyomas.[4–9]

Uterine leiomyomas are the most common gynecological tumors in women of reproductive age, with prevalence ranging from 20% to as high as 77%.[10,11] Uterine leiomyomas constitute a major health issue for women by accounting for the majority of hysterectomies, being associated with infertility, and causing variable clinical symptoms, including abdominal pain and menorrhagia.[12] The molecular background of cutaneous and uterine leiomyomas has remained relatively obscure. Fryns and co-workers[13] reported a female with 9p trisomy and 18p distal monosomy having cutaneous leiomyomas, in addition to phenotypic features of 9p trisomy. In uterine leiomyomas, the most common somatic cytogenetic alterations are translocations involving chromosomes 12 and 14, and interstitial deletions of chromosome 7q. The presence of a leiomyoma suppressor locus on chromosome 7q22 has been suggested based on molecular and cytogenetic analyses.[14] 7q seems to be the most common site for loss of heterozygosity (LOH) in uterine

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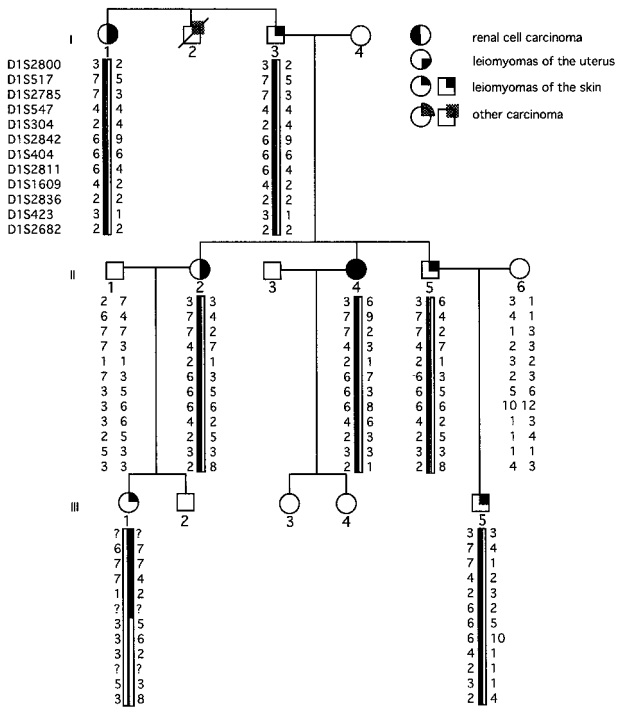


**Figure 1.** Pedigree of the multiple cutaneous leiomyomatosis kindred studied. For reasons of confidentiality, haplotypes of the unaffected individuals are not shown. One healthy individual had the disease-associated haplotype. The observed recombinations fine map the interval containing the disease locus as reported by Launonen and colleagues[17] (between D1S517 and D1S404; **black bar** depicts the shared region in the current family).

leiomyomas, but otherwise LOH is very infrequently detected in these lesions.[15]

A novel syndrome predisposing to renal cell cancer of specific papillary histology, as well as uterine leiomyomas, has been reported recently. Cutaneous leiomyomas were seen in one of the two families described.[16] The condition was named hereditary leiomyomatosis and renal cell cancer (HLRCC), and the work mapped the tumor predisposition locus to chromosome 1q42-q44. The study also reported loss of the wild-type chromosome in several of the studied renal cell cancers, as well as in one uterine leiomyoma, derived from the 1q-linked families.[16] To clarify whether multiple cutaneous leiomyomatosis and HLRCC are in fact one disease with somewhat variable phenotype, and to study the molecular nature of cutaneous leiomyomas, we studied a kindred that had been diagnosed with multiple cutaneous leiomyomatosis in a university hospital (Figure 1). In addition, we examined 26 sporadic uterine leiomyomas and 10 sporadic cutaneous leiomyomas for LOH at the susceptibility locus.

## *Methods and Results*

In addition to physical examination of the proband (Figure 1, III-5), the clinical features of the kindred were studied using patient records, and telephone interview of all of the family members. The cancer status of all individuals was verified from the Finnish Cancer Registry.[17] After deriving informed consent, blood samples for linkage analyses were obtained from 10 individuals (Figure 1). Seven fresh-frozen cutaneous leiomyomas, two paraffin-embedded uterine leiomyomas, and one paraffin-embedded malignant tumor were available for LOH analyses. Detailed histopathological analyses were made on the malignant tumor and two uterine tumors, of which one was suspected malignant.

The family included seven individuals with cutaneous nodules/leiomyomas (Figure 1, Table 1). The proband (III-5) was severely affected, having hundreds of lesions some of which were painful. These were first noticed at 10 years of age in the left arm, in a skin area that had been covered by a plaster. In the present examination, at the age of 22 years, he had hundreds of reddish lesions on his left arm, trunk, and left leg. Several painful lesions have been surgically removed in recent years. In most individuals in the kindred the skin nodules were present from early adulthood. Leiomyomas from patients I-1, II-5, III-1, and III-5 had been histologically evaluated. The number of lesions varied from few to hundreds. Patients II-2 and II-4 were also affected with uterine leiomyomas, ages 23 and 34 years at the time of diagnosis, respectively. Individual I-1 reported that uterine tumors, one of which was suspected malignant, had been removed at age 27 years, with subsequent gynecological follow-up for 5 years. Systematic examination of patient records, as well as Cancer Registry data, revealed that patient I-1 had been diagnosed with uterine leiomyosarcoma at the age of 27 years, I-2 had died from metastasized (liver, bone) cancer of unknown primary site at the age of 61 years, and patient II-4 had been diagnosed with renal cell cancer at the age of 35 years.

Linkage analyses were performed by using the Genehunter[18] and Fastlink[19] programs. Dominant inheritance for the tumor predisposition phenotype was assumed,

**Table 1.** Characteristics of the Affected Individuals of the Family (Individuals with Cutaneous Nodules/Leiomyomas, Uterine Leiomyomas)

| Individual* | Sex | Skin lesions | Uterine tumors | Renal cell cancer |
|---|---|---|---|---|
| I-1 | F | Leiomyomas (31)† | Diagnosis uncertain | No |
| I-3 | M | Cutaneous nodules | — | No |
| II-2 | F | Cutaneous nodules | Leiomyomas (23) | No |
| II-4 | F | Cutaneous nodules | Leiomyomas (34) | Yes (35) |
| II-5 | M | Leiomyomas (35) | — | No |
| III-1 | F | Leiomyomas (28) | No | No |
| III-5 | M | Leiomyomas (10) | — | No |

*The listed individuals shared the disease-associated 1q haplotype. In addition, one unaffected female displayed the linked haplotype.
†Age at diagnosis, when known, is in parentheses.

**Table 2.** Pairwise Lod Scores with 12 Chromosome 1q42-q44 Markers

| Marker | 0.00 | 0.001 | 0.01 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
|---|---|---|---|---|---|---|---|---|
| D1S2800 | 0.63 | 0.63 | 0.62 | 0.59 | 0.53 | 0.38 | 0.22 | 0.08 |
| D1S517 | 0.97 | 0.97 | 0.96 | 0.90 | 0.82 | 0.61 | 0.38 | 0.16 |
| D1S2785 | 0.62 | 0.62 | 0.61 | 0.58 | 0.52 | 0.38 | 0.22 | 0.08 |
| D1S547 | 0.35 | 0.35 | 0.35 | 0.35 | 0.34 | 0.28 | 0.20 | 0.11 |
| D1S304 | 0.95 | 0.95 | 0.94 | 0.88 | 0.80 | 0.60 | 0.37 | 0.16 |
| D1S2842 | 1.05 | 1.05 | 1.03 | 0.94 | 0.82 | 0.57 | 0.33 | 0.12 |
| D1S404 | −2.56 | −2.42 | −1.88 | −1.22 | −0.89 | −0.54 | −0.32 | −0.15 |
| D1S2811 | 0.38 | 0.38 | 0.37 | 0.35 | 0.31 | 0.21 | 0.09 | 0.00 |
| D1S1609 | −1.31 | −1.16 | −0.64 | −0.11 | 0.07 | 0.13 | 0.09 | 0.03 |
| D1S2836 | 0.66 | 0.65 | 0.64 | 0.59 | 0.53 | 0.40 | 0.27 | 0.14 |
| D1S423 | 0.68 | 0.68 | 0.66 | 0.60 | 0.52 | 0.35 | 0.18 | 0.05 |
| D1S2682 | −1.54 | −1.40 | −0.87 | −0.31 | −0.09 | 0.05 | 0.07 | 0.05 |

and individuals with cutaneous and uterine leiomyomas were considered as affected. The penetrance of the trait was set to 0.80. Probabilities for phenocopies were set to 0.20 for uterine leiomyomas and 0.002 for cutaneous leiomyomatosis. Linkage analysis was performed with 12 microsatellite markers on chromosome 1q42-q44, recently reported as the HLRCC locus.[16] The leiomyomatosis phenotype segregated with 1q42-q44 markers. The haplotypes are shown in Figure 1. There is no relationship between the studied family and the HLRCC families reported by Launonen and colleagues.[16] Two-point lod scores are given in Table 2. The maximum two-point lod score, 1.05, was obtained with marker D1S2842 whereas the multipoint maximum, 1.02, was at D1S304. The phenotypes of the individuals segregating the linked haplotype are summarized in Table 1. In addition, LOH at the susceptibility locus (markers D1S517, D1S547, D1S423) was examined from seven cutaneous leiomyomas from individual III-5, and two uterine leiomyomas and a renal cell tumor from individual II-4. All these lesions showed loss of the wild-type chromosome (Figure 2). Furthermore, LOH analysis was performed on 26 sporadic uterine leiomyomas and 10 sporadic cutaneous leiomyomas. Losses of the normal chromosome were detected in 1 out of 24 informative uterine leiomyomas and zero out of seven informative cutaneous leiomyomas.

The renal cell cancer of patient II-4 was histopathologically re-evaluated. The pathologist's gross report, histological slides, and paraffin-embedded tumor tissue were obtained from the archives of the Department of Pathology of the Turku University Hospital. Hematoxylin and eosin staining (Figure 3) and immunohistochemistry for epithelial membrane antigen and vimentin (DAKO, Glostrup, Denmark), cytokeratin 7 (CK7; Boehringer Mannheim, Mannheim, Germany), and human milk fat globule (NCL-HMFG 1 and 2; Novo-Castra, Newcastle on Tyne, UK) were performed.

The solitary tumor affecting the left kidney measured 9 cm × 6 cm. Grossly the tumor was fairly well defined, but histology showed irregular border between the tumor and the kidney parenchyma. The tumor was mainly papillary with some solid areas. The cells had a large amphophilic cytoplasm. The nuclei were large, Fuhrman grade 3 to 4, and often had inclusion-like large eosinophilic nucleoli. Mitoses were moderate, some round cell infiltrates occurred, and apoptotic cell groups were abundant. No necroses and hemorrhages were visible in the available slides. No psammoma bodies or stromal macrophages could be seen. The observed rare histological type is characteristic of HLRCC.[16] In immunohistochemical stainings, the tumor was positive for epithelial membrane

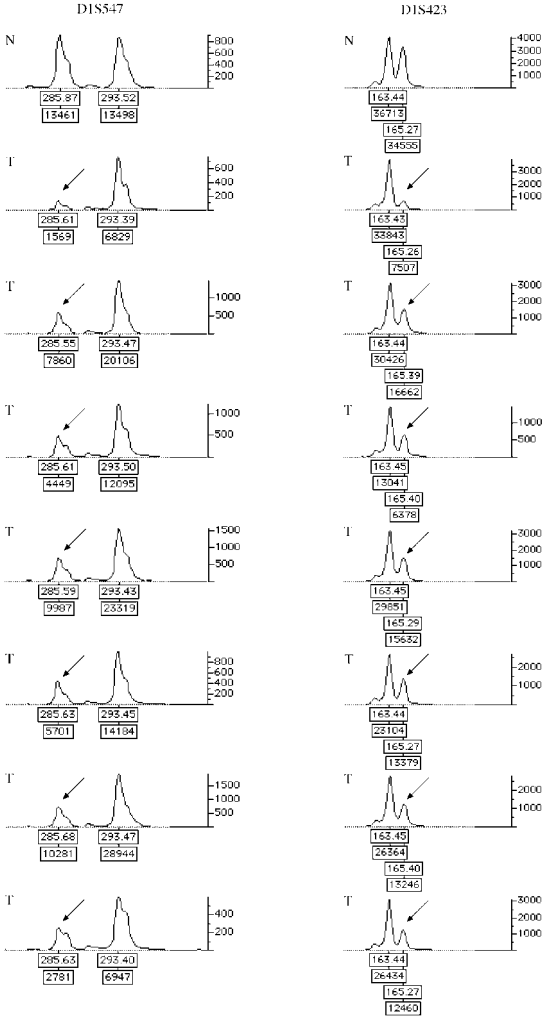


**Figure 2.** LOH analysis of seven cutaneous leiomyomas with markers D1S547 and D1S423. N depicts the normal tissue (blood) DNA-derived germline alleles. All seven lesions displayed LOH (**arrow** depicts the lost allele) at both loci, as well as D1S517 (not shown).

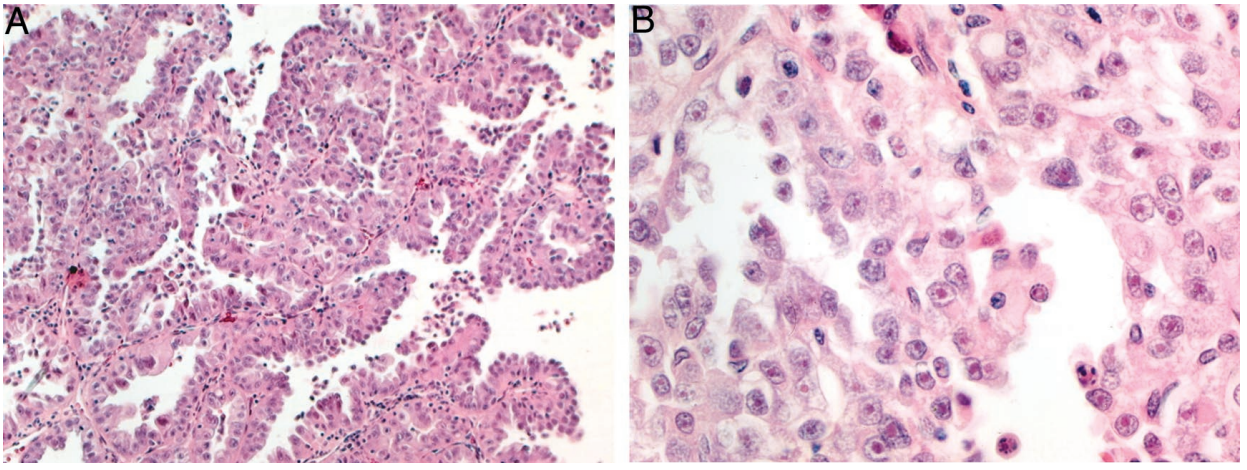


**Figure 3.** Histopathology of the kidney cancer of individual II-4, showing typical HLRCC features. **A:** Papillary structure of the renal cancer (H&E; original magnification, ×100). **B:** Characteristic cytology with prominent eosinophilic nucleoli. Single apoptotic cell are visible (H&E; original magnification, ×400).

antigen, HMFG1, and HMFG2, and negative for vimentin and CK7.

Histopathological analyses of the leiomyosarcoma of individual I-1 revealed that the lesion was a symplastic leiomyoma rather than a malignant tumor. Another lesion from this individual was also available for evaluation, and was a typical leiomyoma.

## *Discussion*

Originally the kindred studied had been diagnosed in a university hospital as having the typical familial leiomyomatosis phenotype; dominantly segregating cutaneous and uterine leiomyomatosis. Although the linkage data from this family is only suggestive of a predisposition gene in 1q, combined with the LOH data obtained from seven benign cutaneous leiomyomas, two uterine leiomyomas, and one renal cell cancer, the molecular analyses unambiguously point toward a predisposition gene in 1q42-q44. The LOH data were not valuable merely in confirming the 1q linkage, but they also established cutaneous leiomyomas as two-hit lesions,[20] similar to uterine leiomyomas and renal cell cancers associated with HLRCC.[16] Together with previous results[16] the observed recombinations fine map the predisposition locus between D1S517 and D1S404 (Figure 1, Table 2).

That the cutaneous lesions of the proband displayed two hits, were patchy, and first appeared after irritation, is of interest. It seems unlikely, although possible, that the irritation caused by the plaster would have been the cause of the loss of the wild-type allele in the progenitor cells. The alternative hypothesis is that the irritation provoked the growth of some of the already existing cells bearing two hits at the 1q tumor suppressor locus, and thus growth potential. That changes in microenvironment may provoke tumor growth in eg, hamartomas has been proposed previously (landscaper effect).[21] The model suggested by the data derived from the proband, irritation-provoked growth of two-hit cells, may in part explain why some benign lesions such as hamartomas display nonneoplastic histopathology, clonality of two-hit cells, and patchy occurrence.

The frequency of LOH detected in sporadic uterine and cutaneous leiomyomas was not high; 1 out of 24 or 4% in uterine leiomyomas and zero out of seven in cutaneous leiomyomas. However, high frequencies of LOH are not expected in these benign lesions, and even such a low degree may reflect a significant phenomenon. The results are consistent with previous studies on uterine leiomyomas. In a genome-wide study of 102 tumors, only 7q21-q31, 1q42, and 16q12-q22 displayed any LOH.[15] Similarly, balanced translocations involving 1q42 have been reported in uterine leiomyomas.[22] Mechanisms of inactivation of the HLRCC predisposition gene other than LOH, most of all transcriptional silencing through promotor hypermethylation, may be involved in sporadic leiomyomas. This should be studied after gene identification.

It is noteworthy that the kindred in the course of this study turned out to have an individual with renal cell cancer at a relatively young age. The features of the lesion were well compatible with the rare HLRCC type histology, strongly suggesting that affected members of the family are also predisposed to renal cell cancer as in HLRCC.[16] A recent work mapped a predisposition locus for multiple cutaneous leiomyomatosis to the HLRCC locus in 1q42-q44.[23] However, susceptibility to renal cell cancer in the families was not reported. It is conceivable that if leiomyomatosis phenotype is the first selection criteria, families with less prominent renal cell cancer patterns are identified. More extensive studies are needed to clarify whether risk for renal cell cancer is increased in all leiomyomatosis kindreds. The data call for attention; a possible risk of renal cell cancer in familial leiomyomatosis should be recognized.

At present we recommend annual screening of at risk HLRCC family members by ultrasound. This should also include transvaginal examination of the uterus because leiomyosarcomas also appear to be associated with the condition.[5,16] Prophylactic hysterectomy may be an op-

tion after an at-risk woman has completed her family. The HLRCC cancer risk, tumor spectrum, incidence, as well as optimal methods for cancer prevention, need to be evaluated urgently.

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