ORIGINAL ARTICLE
See related Editorials on pages iv and v and Commentary on page vii

Germline Fumarate Hydratase Mutations in Families with Multiple Cutaneous and Uterine Leiomyomata

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Germline mutations in the fumarate hydratase gene (*FH*) predispose to multiple cutaneous and uterine leiomyoma syndrome (MCL) and MCL associated with renal cell cancer. MCL is inherited in an autosomal dominant pattern, manifesting as skin leiomyoma and uterine fibroids in affected individuals. Fumarate hydratase, a component of the tricarboxylic acid cycle, acts as a tumor suppressor gene in the development of cutaneous and uterine leiomyoma and renal cell cancer in this syndrome. Here we report the clinical and mutational analysis of five families with MCL, with the identification of five new mutations affecting highly conserved residues of the FH protein. These results provide further evidence for the role of the *FH* gene in the pathogenesis of MCL. ***Keywords: leiomyoma/renal cell cancer. J Invest Dermatol 121:741–744, 2003***

Cutaneous leiomyomas are rare, benign tumors arising from the arrector pili muscle of hair follicles. Multiple cutaneous and uterine leiomyoma syndrome (MCL; MIM #150800), in which affected individuals develop associated skin and uterine leiomyomas, is a disorder inherited in an autosomal dominant pattern. The skin lesions usually appear between the early teens and the fourth decade of life. For the majority of affected women, uterine fibroids of early onset usually require hysterectomy or myomectomy (Stewart, 2001). In some families with MCL, the disease is associated with an additional predisposition to type II papillary renal cell carcinoma (Launonen *et al*, 2001).

The genetic locus for MCL was recently mapped to chromosome 1q42.3-43 (Alam *et al*, 2001; Kiuru *et al*, 2001; Martinez-Mir *et al*, 2002) and subsequently, mutations in the fumarate hydratase gene (*FH*) were identified (Tomlinson *et al*, 2002). The gene product for *FH* is fumarate hydratase, part of the tricarboxylic acid cycle involved in energy production for the cell. Dominant mutations in *FH* cause MCL in approximately 59.5% of affected individuals (Tomlinson *et al*, 2002). Based on its role in MCL, *FH* has been hypothesized to act as a tumor suppressor gene in sporadic tumors as well. However, two recent reports show a very low frequency of *FH* mutations in different types of tumors, including uterine and cutaneous leiomyomas, leiomyosarcoma, and renal cell carcinoma (Barker *et al*, 2002; Kiuru *et al*, 2002). Whether *FH* plays a role in sporadic tumorigenesis remains to be determined. Mutations in three different subunits of succinate dehydrogenase, another enzyme of the tricarboxylic acid cycle, also correlate with a high incidence of tumors, in particular, hereditary paragangliomas (Baysal *et al*, 2001). Interestingly, autosomal recessive mutations in the *FH* gene have been implicated in fumarate hydratase deficiency, which presents with progressive encephalopathy and developmental delay (MIM #606812). Accordingly, Tomlinson *et al* (2002) reported a patient with fumarase deficiency whose mother, a carrier for an *FH* mutation, developed skin leiomyoma. How these two phenotypically distinct disorders are linked to different types and combinations of mutations in *FH* calls for further investigation.

In this study, we report the clinical and mutational analysis of five families with MCL. We have identified five new mutations in the *FH* gene, further supporting the role of *FH* in MCL.

MATERIALS AND METHODS

Human subjects We have identified five families with dominantly inherited MCL, comprising 16 affected individuals available for this study. Blood samples were collected according to the local Institutional Review Board. Clinical features and pedigrees of four families were previously published in case reports (MCL-1, MCL-2, MCL-3, and MCL-5) (Lun and Spelman, 2000; Tsoitis *et al*, 2001; Alam *et al*, 2002; Martinez-Mir *et al*, 2002).

Mutation analysis Genomic DNA was isolated from peripheral blood collected in EDTA-containing tubes using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). To screen for mutations in the human *FH* gene, all exons and splice junctions were PCR-amplified from genomic DNA. PCR primers are shown in **Table I**. PCR products were sequenced in an ABI Prism 310 automated sequencer, using the ABI Prism BigDye terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Foster City, CA), following purification in Centriflex gel filtration cartridges (Edge Biosystems, Gaitherburg, MD).

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Abbreviations: *FH*, fumarate hydratase gene; MCL, multiple cutaneous and uterine leiomyoma syndrome; MIM, Mendelian inheritance in man.

Table I. PCR primers for amplification of the *FH* gene

Exon	Forward primer	Reverse primer	Size (bp)
1	CCCAGAAATTCTACCCAAGC	AGGGCTGAAGGTCACTGC	214
2	TGATCCTGGGTTTCTTTTCAAC	ATGAATACAGCCTACTTCATCC	240
3	CCAAAATAATAAACTTCCATGC	ATGGGTCTGAGGTTATTAAG	221
4	CTGTATTCAAACTCTGTGGC	TTATAACCAAAAAACAGCAAAGC	288
5	GTTTTTGTTGCCTCTGATTTAAC	TGGCCATTTGTACCAAGCTC	290
6	GAGTAACTTGTAAGCTATTAGG	AATGTACAGACCACGTA	285
7	TAACTTGTTCACCCATCTAGG	CTAGTCAAGTTTTAGCTCCAAC	287
8	TTAGTCTTTACTCTGTCATTGG	TAATAAGCCTTTGGTCAAAAAAC	212
9	ATTGTATATTTACTGTCAACCAG	AAACACTGATCCACTTGTCTCT	356
10	CTGCTAACCCATATGTCGTC	CGTTTTTAAGAAATGGGAGTCTG	252

Mutations were identified by visual inspection and comparison with control sequences generated from unrelated unaffected individuals.

To confirm the mutations identified, direct and mismatched PCR were used. The nonsense mutation Q142X and the missense H275Y and V351L were confirmed by digestion of the corresponding PCR products with *Bst*NI, *Bsp*HI, and *Mae*III, respectively. In the case of S115I, a reverse mismatched primer (5′-CCT AAC ATT TCA ATT GCT CTA T**A**G-3′) was used to introduce an *Alu* I restriction site. Finally, the PCR product corresponding to frameshift mutation 1081del4 was run on a polyacrylamide gel and visualized by ethidium bromide staining.

All five DNA variants were tested in a mixed control population of 46–49 individuals. The missense variants S115I, H275Y, and V351L were tested in an additional sample of 32–43 control individuals.

RESULTS

Clinical findings The patients in the five families with MCL (**Fig 1**) originated in five different countries, namely, two Jewish families from Tunisia (MCL-1) and Ethiopia (MCL-4), Greece (MCL-2), Australia (MCL-3), and Puerto Rico (MCL-5). They comprised a total of 11 and 5 affected women and men, respectively. Skin leiomyomas were found in 14 individuals, 9 women and 5 men, ranging in age from 14 to 55 y. The affected patients reported sensitivity of the lesions to cold temperature and touch. Two of the female patients exhibited skin leiomyomas alone, without any uterine abnormality. The typical skin lesions are firm, skin colored to red, ranging from a few in number to approximately 100 in older individuals (**Fig 2**). The lesions range between 0.2 and 1.0 cm in diameter and cluster with time.

Of the 9 women with uterine leiomyoma, 7 had coexisting skin leiomyoma and 2 had uterine leiomyoma alone. Six of these patients with affected uteri eventually underwent hysterectomy or myomectomy.

Mutation analysis in the *FH* gene We have analyzed five families with dominantly inherited MCL for *FH* mutations. All were found to carry heterozygous mutations in the *FH* gene (**Table II**). None of these mutations have been previously reported in patients with MCL or fumarate hydratase deficiency, and each of them was unique to a single family. The fact that they have not been detected in the control population excludes them as common polymorphisms.

A C > T transition at nucleotide position 553, which results in the nonsense mutation Q142X (amino acid residue 185 in the mitochondrial isoform), was identified in family MCL-2, and a deletion of four nucleotides in exon 7 was found in family MCL-

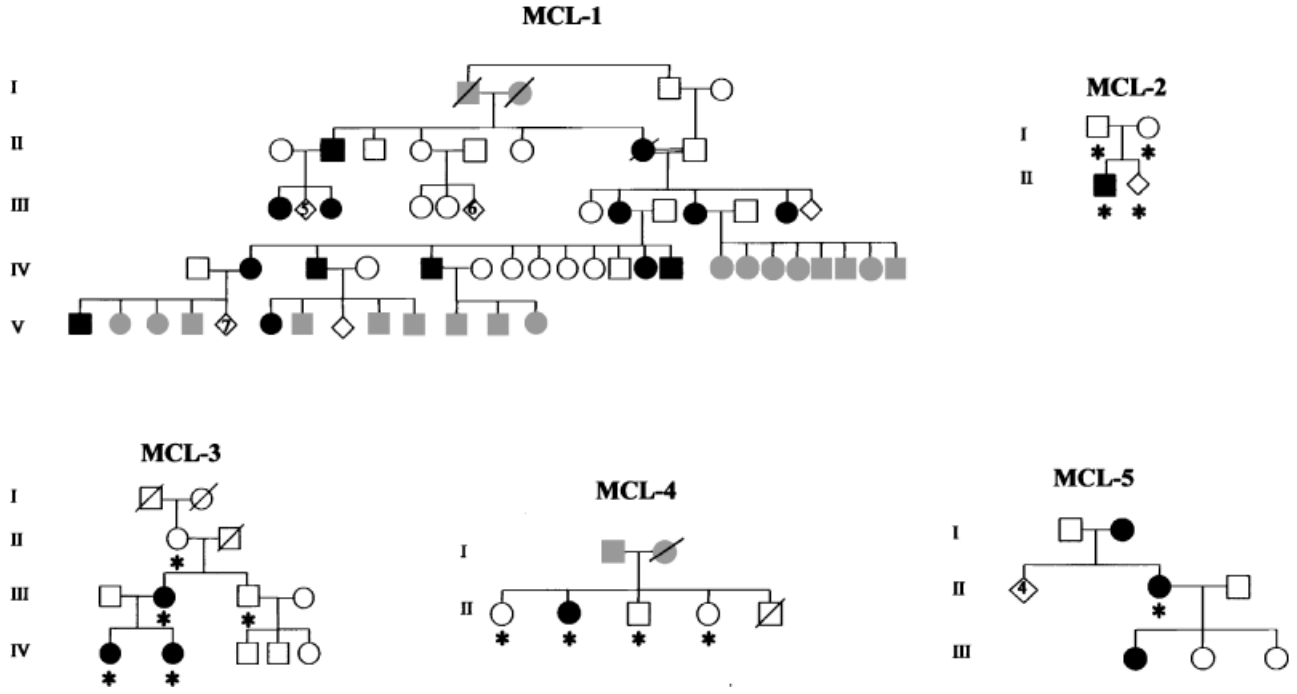

Figure 1. Pedigrees with MCL. *Black, gray, and empty symbols,* affected, unknown, and unaffected family members, respectively. *Asterisks,* individuals whose blood samples were available, except in MCL-1 where blood samples for most family members were available.

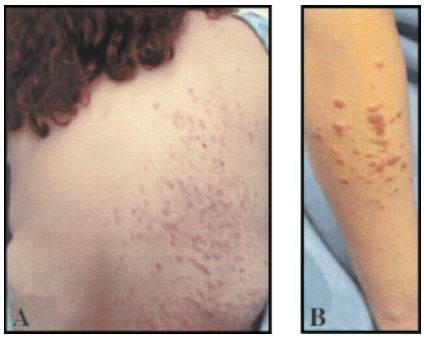

Figure 2. Clinical picture of cutaneous leiomyoma. Clustering of skin leiomyoma lesions can be observed on upper back (*A*) and arm (*B*).

Table II. Germline *FH* mutations in MCL families

Family	Wild-type sequence	Mutant sequence	Amino-acid change[a]	Exon
MCL-4	AGC	ATC	S115I	4
MCL-2	CAG	TAG	Q142X	4
MCL-1	CAT	TAT	H275Y	7
MCL-5	GAAAATG-AACCA	GAAAACCA	4-bp del between amino acids 318 and 319	7
MCL-3	GTC	CTC	V351L	8

[a]Amino-acid positions are derived from the cytosolic enzyme sequence (468 amino acids). The exon number corresponds to the entire gene; exon 1 encodes the 43 amino acids that form the mitochondrial signal peptide and the first amino acid of the cytosolic protein.

5. This frameshift mutant allele is the result of a 4-bp deletion starting at any position between nucleotides 1081–1083, creating a premature termination codon 12 amino acids downstream of the deletion site. Three missense mutations were also identified: a G>T transversion at nucleotide position 473 in exon 4, leading to the missense mutation S115I (mitochondrial amino acid residue 158; family MCL-4), a C>T transition at position 952 in exon 7 resulting in the missense mutation H275Y (mitochondrial amino acid residue 318; family MCL-1), and a G>C transversion at position 1180 in exon 8, creating the missense mutation V351L (mitochondrial amino acid residue 394; family MCL-3). Exon number and nucleotide positions are derived from the *FH* sequence (Accession No. 18549070). The amino acid position for the cytosolic isoform is given, for comparison with the previously reported mutations (Tomlinson *et al*, 2002; **Table II**).

DISCUSSION

In this study, we have identified five new *FH* "mutations" in MCL families, further supporting the role of this housekeeping gene in tumorigenesis. The nature of these mutations, including non-sense, frameshift, and missense mutations, clearly supports the role of the *FH* gene in MCL. Nonetheless, the involvement of a housekeeping gene such as *FH* in tumorigenesis unveils new questions about the etiologic mechanism underlying MCL.

Our study has identified a heterozygous nonsense (Q142X) and a frameshift mutation (1081del4) in two of the families. The most likely fate of the mRNA of these mutant alleles would be nonsense-mediated mRNA decay, where the nonsense-bearing mRNA is recognized and degraded soon after transcription (Frischmeyer and Dietz, 1999). Unfortunately, mRNA samples from these families were not available for further testing and therefore other possible mechanisms such as translation into a truncated protein or exon skipping cannot be ruled out.

Regarding the three missense changes identified in our study, S115I (MCL-4), H275Y (MCL-1), and V351L (MCL-3), the possibility of these being rare polymorphisms cannot be completely excluded, because a mixed control population was used owing to unavailability of a sufficient number of ethnically matched controls. This is especially true for V531L, where both amino acids consist of hydrophobic side chains with only one carbon difference. It is noteworthy, however, that 7 of the 17 mutations reported by Tomlinson *et al* (2002) were also missense mutations. The affected amino acid residues S115, H275, and V351 are conserved among human, pig, rat, and mouse FH.

Cosegregation between the mutations and the MCL phenotype was confirmed for families MCL-1 (H275Y) and MCL-3 (V351L), for which blood samples from several affected family members were available. These two missense variants show reduced penetrance for the skin and uterine lesions. H275Y was identified in family MCL-1 (**Fig 1**), in which we had previously performed linkage studies (Martinez-Mir *et al*, 2002). Restriction analysis in all family members showed that 12 individuals, 7 women and 5 men, were heterozygous for the mutation. Of the 7 carrier women, 4 presented with both skin and diagnosed or suspected uterine lesions (hysterectomy in III-17 and III-19, diagnosed myoma in IV-12, and menorrhagia in V-12), 2 presented with skin lesions alone (III-21 and IV-2), and 1 was an asymptomatic female (V-2). With the exception of V-17, all carrier men suffered from skin leiomyomata. Thus, only two young asymptomatic carriers were identified, V-2 (age 19) and V-17 (age 18). V-17 was reported to carry the disease-associated haplotype during our studies to refine the *MCUL*1 locus (Martinez-Mir *et al*, 2002). V-2, on the other hand, showed a key recombination event within the disease-associated haplotype (Martinez-Mir *et al*, 2002). Owing to the fact that V-2 was an unaffected individual and that the location and identity of the causative gene was still unknown at the time these studies were performed, her carrier status as an asymptomatic carrier or unaffected could not be established. With the identification of the *FH* gene by Tomlinson *et al* (2002), we confirmed that V-2 did indeed carry the disease-associated haplotype and, as shown here, an *FH* mutation.

In family MCL-3 (**Fig 1**), on the other hand, the V351L missense substitution was present in three women, one of them with both uterine and skin involvement and the remaining two with only uterine myoma. The grandmother in this family, reported to have uterine polyps on a biopsy report, did not carry the mutation. For family MCL-2 (Q142X; **Fig 1**) the mutation was only present in the affected individual and his mother and absent in the unaffected father and sibling. Since no family history of MCL was reported for the affected member of this family, the carrier mother will be reexamined for skin and uterine lesions. The missense variant S115I in family MCL-4 was present in the affected family member only and absent in the remaining three unaffected siblings (**Fig 1**). The parents of these individuals were not available for the study. Finally, only one individual from family MCL-5 (**Figs 1, 2**), an affected woman, was available for examination and was heterozygous for the frameshift mutation. None of the families studied here suffered from any other type of tumor, including papillary renal cell cancer.

In summary, of 11 female patients, 7 exhibited concurrent skin and uterine leiomyoma involvement. Of the remaining 4 affected female patients, 2 had skin lesions only and 2 had uterine leiomyoma alone. Patients from the same family may display different phenotypic expression of the disease. For example, in family MCL-3, a mother with uterine leiomyoma and no skin lesions had two daughters, one with both skin and uterine lesions and the other with uterine involvement only.

Seventeen of 42 families reported by Tomlinson *et al* (2002) did not display mutations in the *FH* gene. The rate of mutation detection in our study, on the other hand, reached 100%. Tomlinson *et al* (2002) performed single-strand conformation polymorphism and conformation-specific gel electrophoresis as mutation detection methods in some of their families versus direct sequencing in

our study. This could account for the lower mutation rate in the original report. Overall, it still remains possible that the predisposition to MCL is genetically heterogeneous.

One of the implications of gene identification for inherited susceptibility to tumorigenesis is the possible role they may play in the more common sporadic presentation of the same type of tumors. Families with MCL present with benign skin and uterine leiomyomas, the latter being a major public health issue. In addition, the aggressive papillary renal cell carcinoma can also be associated with MCL, as well as leiomyosarcoma (Launonen *et al*, 2001). Based on this hypothesis, two groups have recently searched for mutations in the *FH* gene in different types of tumors. Kiuru *et al* (2002) have analyzed a total of 200 sporadic tumors, including uterine and cutaneous leiomyomas, leiomyosarcomas, and papillary renal cell carcinomas. However, only five *FH* mutations were identified. Barker *et al* (2002), on the other hand, studied 26 leiomyosarcomas and 129 uterine leiomyomas. In this case, no *FH* mutations were identified, although LOH for 1q and 1q42-43 was detected in 50 and 5% of leiomyosarcomas and leiomyomas, respectively. Collectively, these data suggests either that *FH* does not play a major role in the development of sporadic tumors or that the pathologic mechanism of *FH* in MCL involves events different from somatic mutations.

The identification of the particular *FH* mutation in each MCL family will allow for close follow-up of those individuals at risk for the development of the aggressive papillary renal cell cancer (Kiuru *et al*, 2001; Launonen *et al*, 2001). Establishing the etiologic mechanisms of MCL may facilitate development of new therapeutic options for affected patients.

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