Germline fumarate hydratase mutations and evidence for a founder mutation underlying multiple cutaneous and uterine leiomyomata

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Multiple cutaneous and uterine leiomyomata syndrome (MCL) is an autosomal dominant disease characterized by the presence of concurrent benign tumors of smooth muscle origin (leiomyoma) in the skin and uterus of affected females, and in the skin of affected males. MCL can also be associated with type II papillary renal cell cancer (HLRCC). The genetic locus for MCL and HLRCC was recently mapped to chromosome 1q42.3-43 and subsequently, dominantly inherited mutations in the fumarate hydratase gene (*FH*) were identified. Importantly, analysis of the *FH* gene in tumors of MCL patients revealed a second mutation inactivating the wild-type allele in some tumors. Based on these findings, it has been suggested that *FH* may function as a tumor suppressor gene in MCL. Here, we report the analysis of the *FH* gene in a group of 11 MCL families, with the identification of 8 different mutations accounting for the disease in all families. One of the mutations, 905-1G>A, has been identified in 4 families of Iranian origin. The analysis of highly polymorphic markers in the vicinity of the *FH* gene showed a shared haplotype in these 4 families, suggesting that 905-1G>A represents a founder mutation. Collectively, identification of 5 novel and 3 recurrent mutations further supports the role of *FH* in the pathogenesis of MCL. (J Am Acad Dermatol 2005;52:410-6.)

Cutaneous leiomyomas are rare, benign tumors arising from the arrector pili muscle of the hair follicle. They can be found in association with uterine fibroids in multiple cutaneous and uterine leiomyomata syndrome (MCL; Online Mendelian Inheritance in Man [OMIM] 150800), inherited as an autosomal dominant trait.[1-3] Association of MCL with familial development of type II renal cell cancer has been characterized in a syndrome called hereditary leiomyomatosis and renal cell cancer (HLRCC; OMIM 605839).[4,5]

Recently, the genetic locus for MCL and HLRCC was mapped to chromosome 1q42.3-43.[3-6] Subsequently, germline mutations in the fumarate hydratase gene (*FH*) were found in MCL and HLRCC.[7-10] Fumarate hydratase is an enzyme that

Abbreviations used:

EDTA:	ethylenediamine tetraacetic acid
FH/FH:	fumarate hydratase gene/protein
FHD:	fumarate hydratase deficiency
HLRCC:	hereditary leiomyomatosis and renal cell cancer
MCL:	multiple cutaneous and uterine leiomyomata syndrome
mRNA:	messenger RNA
OMIM:	Online Mendelian Inheritance In Man
PCR:	polymerase chain reaction

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functions as part of the Krebs cycle, responsible for cellular energy production and amino acid metabolism. *FH* has been predicted to act as a tumor suppressor gene, since loss of the wild-type allele has been found in cutaneous, uterine, and renal tumor biopsies of MCL patients. Moreover, the FH enzymatic activity is low or absent in tumors from individuals with MCL.[7]

While the tumorigenic mechanism of *FH* mutations remains elusive, evidence is clear that dominant mutations in succinate dehydrogenase, also an enzyme of the Krebs cycle, cause tumors of the carotid body and adrenal gland, paraganglioma and pheochromocytoma, respectively.[11] In addition, recessive mutations in the *FH* gene cause fumarate hydratase deficiency (FHD; OMIM 606812), characterized by neurological impairment, encephalopathy, and premature death in infants. Interestingly, MCL has been reported in the carrier mother of one infant with FHD.[7] However, a recent study has identified other carrier parents of FHD infants who are asymptomatic. Thus, it remains controversial whether all heterozygous *FH* mutations predispose to MCL.[8]

We previously reported 5 *FH* mutations in patients affected with MCL, including missense, nonsense, and frameshift mutations.[9] Here, we have identified 4 novel and 3 recurrent mutations in 7 MCL families, in addition to a recurrent novel splicing mutation, 905-1G>A, in 4 MCL families. Haplotype analysis of these 4 families using polymorphic markers surrounding the *FH* gene provides evidence for a founder effect.

MATERIAL AND METHODS

Human subjects

We have identified 11 families (MCL-6-16) with dominantly inherited MCL, comprising 35 affected individuals and 88 unaffected family members, of which 17 affected and 13 unaffected individuals have been available for this study. Blood samples were collected following informed consent. Families were recruited from different areas of Israel (MCL-6-9), the United States (MCL-10-12, 14-16), and Spain (MCL-13). Of these, MCL 6-9 are Jewish families who report ancestors originating from Iran, MCL-11, 12, 13 and 14 are white families, MCL-10 originated from Ecuador, and MCL-15 and 16 originated from the Dominican Republic.

Mutation analysis

Genomic DNA was isolated from peripheral blood collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes using the PureGene DNA Isolation Kit (Gentra Systems, Minneapolis, Minn). To screen for mutations in the human *FH* gene, all exons and splice junctions were polymerase chain reaction (PCR)-amplified from genomic DNA. PCR primers have been described previously.[9] PCR products were sequenced in an ABI Prism 310 Automated Sequencer, using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, Calif), following purification in Centriflex Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, Md). Mutations were identified by visual inspection and comparison with control sequences generated from unrelated, unaffected individuals.

To confirm the mutations identified, direct or mismatched PCR followed by restriction enzyme digestion and/or PCR direct sequencing were used. DNA variants H92R and R190C were confirmed by digestion of the corresponding PCR-amplified products with *Bcl*I and *Mae*II, respectively. In the case of P149L, a forward primer (5′-GCTGGGTTTTGAGTAGTTAGTTGG-3′) and a reverse mismatched primer (5′-GCAGCAGCAATGTGCATTGCTCTG-3′) were used to introduce a *Dde*I restriction site. Mutation 905-1G>A was tested by PCR direct sequencing. Finally, controls for 1176del6 mutation were run on a 6% non-denaturing polyacrylamide gel and visualized by ethidium bromide staining. These mutations were tested in a mixed control population of 142-152 chromosomes.

Haplotype analysis

Microsatellite markers covering the *FH* locus were selected from the Human Genome Working Draft at the University of California Santa Cruz (www.genome.ucsc.edu). Eight polymorphic microsatellite markers, *D1S517*, *D1S2785*, *D1S304*, *D1S180*, *D1S204*, *D1S547*, *D1S1634*, and *D1S1609*, spanning an interval of 4.81 Mb (11.57 cM; Fig 1) surrounding the *FH* gene on chromosome 1 were chosen. The PCR-amplified markers were electrophoresed in 6% non-denaturing polyacrylamide gels and visualized by ethidium bromide staining.

RESULTS

Clinical findings

The patients in the 11 families with MCL originated in 5 different countries, namely, 4 Jewish families from Israel (MCL-6-9), 1 family from Ecuador (MCL-10), one from Spain (MCL-13), 2 from the Dominican Republic (MCL-15, 16), and 3 from the United States (MCL-11, 12 and 14; Fig 1). These families comprised a total of 11 affected women and 6 affected men available for this study. Of 96 unaffected members in these families, 13 were available for this study. Skin leiomyomas were found in all of the 17 affected individuals and none of the unaffected family members. The patients reported