Immunohistochemical Characterization of Fumarate Hydratase (FH) and Succinate Dehydrogenase (SDH) in Cutaneous Leiomyomas for Detection of Familial Cancer Syndromes

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Abstract

Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC) is caused by germline mutations in the *FH* gene, and is associated with increased incidence of leiomyomas and a potentially aggressive variant of renal cell carcinoma (HLRCC-associated RCC). Absent immunohistochemical expression of fumarate hydratase (FH) has previously been employed to diagnose HLRCC-associated RCC, but immunohistochemical staining of leiomyomas is not standard practice. We performed immunohistochemistry (IHC) on whole sections from consecutive cutaneous leiomyomas from our archives to evaluate for both FH and SDH-B expression, in addition to clinicopathologic data collection and review of all H&E slides for blinded morphologic evaluation of features reported to be seen in HLRCC-associated uterine leiomyomas. Ninety-six cutaneous leiomyomas from 87 patients were identified; 12 of these specimens were from 7 patients with documented HLRCC. FH expression by IHC was negative in 9 specimens and retained in 85 specimens; 2 cases were equivocal with minimal FH expression. Seven of the 9 negative specimens were from patients with HLRCC, as were both of the equivocal specimens. The overall sensitivity and specificity of negative FH expression in leiomyomas for detection of patients with HLRCC were 70.0% and 97.6%, respectively. Inclusion of cases classified as equivocal increased sensitivity to 75.0%. SDH-B expression was retained in 95 specimens and equivocal in 1 specimen. None of the evaluated morphologic features showed any association with leiomyomas in HLRCC. Loss of FH immunohistochemical expression in cutaneous leiomyomas is a sensitive and specific marker for detection of HLRCC, thus suggesting a role for prospective FH IHC in patients with these tumors to screen for HLRCC.

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Introduction

Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC), also known as Reed Syndrome, is caused by a heterogeneous collection of germline heterozygous mutations in the fumarate hydratase (*FH*) gene on chromosome 1q43 [1–2]. The fumarate hydratase enzyme is an integral component of the citric acid (Krebs) cycle, and normally catalyzes the conversion of fumarate into malate. Alterations in FH enzyme lead to intracellular fumarate accumulation, resulting in marked disturbances of downstream cellular metabolism, epithelial to mesenchymal transition, and epigenetic modifications [3]. Phenotypically, HLRCC is associated with multiple cutaneous leiomyomas, uterine leiomyomas, and a potentially aggressive variant of renal cell carcinoma (RCC) [4–5]. This constellation of findings shows variable penetrance and was first described by Reed *et al.*, in 1973 [6]. Several studies characterizing HLRCC-associated renal cell carcinoma (HLRCC-associated RCC) show these tumors to portend a poor prognosis with a high mortality rate and high stage at presentation, possibly representing a more aggressive disease course when compared with conventional clear cell renal cell carcinoma and other RCC [7–9]. The lifetime risk of renal cell carcinoma in HLRCC has been suggested to be as high as 20–30% [10–12], however, when selection bias is removed, the actual penetrance of renal cancer in HLRCC may be closer to 10–15% [13–15]. Regardless of the discrepancies in reported incidence, the aggressive course of HLRCC-associated RCC has led to efforts to diagnose the syndrome early in life so that serial renal imaging can be used to detect renal cancers at an early stage [14].

Most often, the initial presenting neoplasm in a person with HLRCC is a leiomyoma of the uterus or skin, rather than renal cell carcinoma. In fact, the presence of multiple cutaneous leiomyomas is the most sensitive and specific phenotypic feature of HLRCC, and this finding justifies molecular genetic testing of an affected individual or family to detect germline *FH* mutations [16–17]. Recently, the use of absent immunohistochemical expression of FH has been employed to diagnose HLRCC-associated RCC [9,18], but immunohistochemical staining of leiomyomas of cutaneous, uterine, or other anatomic sites, is not yet standard practice. Furthermore, succinate dehydrogenase (SDH) is another citric acid cycle enzyme for which deficiency is associated with oncocytic renal tumors [19–21], and its expression has not been described in cutaneous leiomyomas (with or without association with HLRCC). Therefore, we investigated the use of FH and SDH-B IHC staining of cutaneous leiomyomas to evaluate for the presence of familial cancer syndromes.

Some specific histopathologic features have been previously reported to be observed in HLRCC-associated RCC, such as prominent orangeophilic/eosinophilic nucleoli and perinucleolar halos [7,10,18]. These and other features such as increased cellularity, nuclear pleomorphism, and the presence of eosinophilic globular cytoplasmic inclusions, have been

reported in uterine leiomyomas in HLRCC, and are shown in Figure 1 [22–25]. These findings have not been similarly observed in cutaneous leiomyomas in HLRCC [26–27], but no blinded systematic evaluation of these morphologic features in a large cohort has been previously described. Therefore, we also sought to evaluate if these reported morphologic features or any other are present in cutaneous leiomyomas in HLRCC.

Materials and Methods

Case Selection

With approval by the University of Michigan Institutional Review Board, the University of Michigan pathology database was searched for cutaneous leiomyomas diagnosed between 1995 and 2015, by keyword search of “cutaneous leiomyoma,” “pillar leiomyoma,” “piloleiomyoma,” “angioliomyoma,” and “leiomyoma,” with the last term restricted to assignment to the dermatopathology division. All available hematoxylin and eosin stained slides with available paraffin-embedded blocks were re-examined by sub-specialty trained pathologists (with expertise in genitourinary pathology, gynecologic pathology and dermatopathology) and relevant clinicopathological data was recorded for each patient. Multiple specimens from the same patient were not excluded in an effort to perform blinded immunohistochemical and morphologic analysis of each cutaneous leiomyoma specimen without the introduction of bias from clinical data (data selection bias).

Immunohistochemistry

Since both FH and SDH are citric acid (Krebs) cycle enzymes and renal tumors of patients with HLRCC might uncommonly demonstrate oncocytic morphology [28], we performed immunohistochemistry (IHC) on consecutive whole tissue sections from each case to evaluate for both FH and SDH-B expression. Slides were stained on the Ventana Benchmark XT platform with the following commercially available antibodies: FH mouse monoclonal antibody, clone J-13 (1:100 dilution; Santa Cruz Biotechnology, Dallas, TX), and SDH-B mouse monoclonal antibody, clone 21A11AE7 (1:100 dilution; Abcam, Cambridge, MA). IHC slides were visually scored as positive, negative, or equivocal, based on the expression of any cytoplasmic granular staining within the smooth muscle cells of the leiomyomas. External (positive and negative) immunohistochemical controls for both biomarkers were evaluated to ensure stain quality, and internal controls for each slide were similarly evaluated using skin adnexal or vascular structures as positive controls, and confirmed to be present in all negative cases. FH-deficient leiomyomas were defined as neoplasms which demonstrated completely negative FH expression with positive internal controls. IHC data was recorded in a blinded manner by three independent sources (CC, SS and RM), with consensus reached in cases of discordance by blinded re-evaluation performed by the same pathologists in concert.

Morphologic Evaluation

The leiomyomas were also evaluated for the presence of histologic features that have been previously proposed to be features of HLRCC-associated RCC, as well as HLRCC-associated uterine leiomyomas. These included prominent orangeophilic or eosinophilic nucleoli, perinucleolar halos, increased cellularity, nuclear pleomorphism defined by multinucleation, or large, hyperchromatic or otherwise bizarre nuclear forms, and the

presence of eosinophilic cytoplasmic globules (Figure 1). With the exception of cellularity, which was evaluated using a binary score (cellular vs. not cellular), each histologic feature was scored on a 3-point scale: absent to very rare (present in 0–1% of the tumor), focal (present in 1–10% of the tumor), and occasional to frequent (present in greater than 10% of the tumor). The scoring criteria were provided to each evaluating pathologist (AL and MC, with expertise in gynecologic pathology and dermatopathology, respectively) with photomicrograph examples of each. The evaluators were then asked to give an overall suspicion based on these criteria, also on a 3-point scale: no suspicion, low suspicion, and moderate to high suspicion for a cutaneous leiomyoma associated with HLRCC. The 3-point scale criteria were given a value from 0–2, and cellularity was given a value from 0–1. The overall tumor was ultimately assigned a morphologic suspicion score between 0 and 11 by summation of all scores.

FH Mutational Status

Mutational analysis was based on mutation testing ordered and interpreted during clinical practice, available within the patient's medical record. As such, mutational analysis methods slightly varied depending upon the independent laboratory at which they were performed, but analysis was subject to the quality control measures required for clinical genetic testing. While the laboratory developed tests had slightly different characteristics, mutation analysis in each case included extraction of genomic DNA from peripheral whole blood samples followed by amplification of exons spanning the coding region of the fumarate hydratase gene (exons 0–9) by polymerase chain reaction (PCR), and then sequencing via an automated DNA sequencer and comparison to normal sequence. These were performed on peripheral blood samples in order to ensure that germline mutation status could be assessed, as a small but not insignificant proportion of uterine leiomyoma cases have been reported to acquire somatic FH mutations [29–31].

Statistical Analysis

Association between clinical features such as the presence of multiple cutaneous leiomyomas and *FH* mutations was examined by the Fisher's exact test. Association between FH and SDH-B IHC staining and *FH* mutational status was similarly examined by Fisher's exact test. Difference in age at cutaneous leiomyoma diagnosis between patients with HLRCC and without HLRCC was calculated using a two-tailed t-test. A cutoff of $p < 0.05$ for statistical significance was used in all analyses. IHC sensitivities and specificities were calculated from data based on evaluation of each specimen as an independent event.

Results

Clinical Features

Ninety-six consecutive specimens of cutaneous leiomyomas from 87 patients diagnosed between 1995 and 2015 were identified. Twelve of these specimens were from 7 patients (3 male and 4 female) with documented HLRCC; the remaining 84 specimens were from 80 patients without known HLRCC, including 4 re-excision specimens after biopsy. Syndrome diagnosis was proven by mutational analysis of the affected individual with associated clinicopathologic features in 6 cases (Table 1). The remaining patient deferred genetic

testing but had a strong family history of HLRCC, though the precise germline mutation for that family was unavailable. Of clinical features, multiple leiomyomas were present in 6 of 7 patients with HLRCC (86%), compared to none of the 80 patients without known HLRCC ($p < 0.0001$). Patients with HLRCC were significantly younger at diagnosis of cutaneous leiomyoma compared to patients without known HLRCC (30.2 years vs 51.8 years, $p = 0.0011$). A reported history of RCC in an immediate family member was present in 2 of 7 patients with HLRCC (28.6%) and none among the 80 individuals without known HLRCC ($p = 0.0056$). Hysterectomy or uterine myomectomy was performed for symptomatic leiomyomas in 3 of 4 women with HLRCC (28.6%) and 2 of 53 women without known HLRCC ($p = 0.0013$). The remaining woman with HLRCC was treated medically for symptomatic fibroids due to her young age (21 years). Renal imaging during initial work up for HLRCC showed renal cysts in 4 of 7 patients with HLRCC (57.1%), and none among patients without known HLRCC, though one patient did have a solid renal mass suspicious for RCC ($p < 0.0001$). These and additional clinicopathologic features of patients with HLRCC are shown in Table 2.

Immunohistochemistry results

FH expression was absent in 9 specimens and retained in 85 specimens (Figures 2 and 3); 2 specimens were classified as equivocal with minimal FH expression (Figure 3). Seven of the 9 negative specimens were from patients with HLRCC, as were both of the equivocal specimens (Table 1). The overall sensitivity and specificity of negative FH expression in cutaneous leiomyoma specimens for patients with HLRCC in this cohort were 70.0% and 97.6%, respectively, when excluding equivocal staining. Inclusion of specimens classified as equivocal with those classified as negative increased sensitivity to 75.0%. SDH-B expression was retained in 95 specimens and was equivocal in 1 specimen. In leiomyomas with retained FH and SDH-B, immunohistochemical expression for these proteins assessed on whole tissue sections was found to be homogeneous in neoplastic areas.

Morphologic results

Of the histopathologic features evaluated, including prominent orangeophilic or eosinophilic nucleoli, perinucleolar halos, increased cellularity, nuclear pleomorphism characterized by multinucleation, or large, hyperchromatic or otherwise bizarre nuclear forms, and the presence of eosinophilic globules, none showed a significant correlation with patients with known HLRCC (Table 3). The morphologic suspicion score for lesions in patients with HLRCC, when averaged between the 2 reviewers, ranged from 0 to 6, with an overall average of 0.63. Only 2 of 12 cases (16.6%) were selected by either reviewer to be of low suspicion, and none were selected as moderate to high suspicion. In contrast, 32 of 84 cases (38%) from patients with no known HLRCC were selected as at least low suspicion, with overall scores in this cohort ranging from 0 to as high as 9. Overall, there was no correlation between the morphologic suspicion score and HLRCC, nor any individual morphologic feature. In fact, average scores for lesions in patients without known HLRCC were lower on average than those of patients with known HLRCC, but not to a significant degree.

FH Mutations

Of the 6 patients with identified specific mutations, 4 were missense mutations, 1 was a nonsense mutation, and 1 was a 7 base-pair deletion leading to a frameshift (Table 1). These mutations were distributed broadly across the exon regions of the gene. Of those HLRCC cases in this cohort with either equivocal or positive FH staining, all were in patients with missense mutations rather than nonsense or frameshift mutations.

Discussion

Our study demonstrates that complete loss of FH IHC expression in cutaneous leiomyomas is a sensitive and highly specific marker for HLRCC in this cohort. Our study showed similar rates of complete loss of FH IHC expression in HLRCC-associated cutaneous leiomyomas compared to previous studies [26–27], and overall showed similar sensitivity, but much higher specificity. To our knowledge, no other study to date has examined the effectiveness of FH IHC in consecutive cases within a large cohort from a single institution. Our cohort much more closely simulates a prospective patient population, which could be triaged towards germline mutation genetic testing. While the incidence of HLRCC is likely to be higher in the setting of in-house cases from a large tertiary care center with clinical referral services, one could reasonably expect to encounter biopsies of these tumors in this practice setting. While it could be argued that the presence of multiple cutaneous leiomyomas in a single patient is sensitive enough to proceed directly to genetic testing, the privilege of an accurate and pertinent clinical history is not always provided, especially prior to the initial diagnosis of a cutaneous leiomyoma, and in situations where clinicians without extensive clinical experience with a rare genetic syndrome like HLRCC are performing the biopsy. This likely scenario further supports the prospective use of a routine, commercially available IHC stain in triaging all cutaneous leiomyomas, whether solitary or multiple. Furthermore, all 7 patients in our cohort did not carry a genetically confirmed nor a clinical diagnosis of HLRCC prior to their initial cutaneous leiomyoma biopsy or excision.

Further investigation of the cases of cutaneous leiomyomas with weak but retained expression of FH by IHC demonstrates that expression appears to correlate with the type of *FH* mutation, as was observed in a previous study of uterine leiomyomas [25]. Some cases with missense mutations demonstrated equivocal or weak staining, while those cases from patients with nonsense or frameshift mutations showed definitive absence of FH expression. The observation of weak or equivocal staining in patients with missense mutations may be due to only slight alteration of the protein-antibody interaction. With the proposed use of FH IHC as a screening tool for triage to further genetic testing, it may be prudent to report cases with very weak or equivocal expression to clinicians for consideration of further clinical work up and/or germline testing, given the clinical implications of these findings.

SDH-B immunohistochemical expression was retained in all but 1 leiomyoma specimen, which was interpreted as equivocal staining due to the very weak, granular cytoplasmic staining identified. This finding demonstrates that the known *FH* mutations identified by germline testing do not alter SDH-B protein expression, which is an intuitive finding since succinate dehydrogenase lies directly upstream of fumarate hydratase in the citric acid cycle. However, it is interesting to note that mutations in both FH and SDH are associated with

potentially aggressive renal tumors, presumably because of similar metabolic shifts from oxidative phosphorylation to aerobic glycolysis caused by the disruption of the citric acid cycle. The downstream effects of these shifts might promote renal oncogenesis by way of activation of the cellular hypoxia response pathway in the presence of adequate oxygen [32]. Also, *FH* mutations (linked to HLRCC-associated RCC) might generate renal tumors which uncommonly demonstrate a morphologic overlap with SDH deficient RCC [28]. Despite these shared/convergent metabolic cellular insults and possible morphologic overlap generated by FH and SDH deficiency, the association of cutaneous and uterine leiomyomas seems to be limited to *FH* mutations in our study, suggesting that widespread succination caused by reduced FH enzymatic activity could potentially play a role in the formation of leiomyomas in these patients [33]. Similar to previous studies [26–27], no significant correlation was found between the morphologic features evaluated (prominent orangeophilic/eosinophilic nucleoli, perinucleolar halos, increased cellularity, nuclear pleomorphism characterized by multinucleation, or large, hyperchromatic or otherwise bizarre nuclear forms, and the presence of eosinophilic globules) and the presence of known HLRCC or FH immunohistochemical expression status in our current cohort of patients.

The higher proportion of renal cyst abnormalities on renal imaging is potentially impacted by a difference in imaging frequency between patients with known HLRCC and those without known HLRCC, as many patients had no record of renal imaging. However, the proportion of patients in our study (4 of 7 patients, 57.1%) is much higher than the recorded prevalence of the general population, which varies between 11.9%–17.6% by ultrasound and 21–41% by CT scan [34–39]. An association between HLRCC and increased incidence of renal cysts has been suggested previously, but the serial nature of renal imaging in patients with HLRCC potentially might introduce some reporting bias [34]. One of the primary purposes of confirming an early diagnosis of HLRCC in a proband is to initiate serial renal imaging in order to diagnose associated renal carcinomas at an early stage, and to initiate molecular genetic testing of asymptomatic at-risk relatives to allow for improved diagnostic certainty and early surveillance and treatment. The potential clinical significance for an early diagnosis of HLRCC cannot be over-emphasized, considering the possibility of rapid evolution of renal tumors with a dismal prognosis in such patients [18]. We speculate that testing of cutaneous leiomyomas for FH immunohistochemical expression can provide an early trigger for further clinical workup and/or confirmatory molecular genetic testing in patients with potential HLRCC. This, in turn, is presumed to provide a survival benefit in such patients, though a real-time study to assess this benefit is difficult to design and carry out based on the rarity of this disease.

Strengths of this study include the consecutive nature of the clinical cohort at a large tertiary care academic center, allowing for a much broader and more comprehensive availability of clinical data than is usually available with consult material. Another strength of the study is the use of whole tissue sections, as well as the use of a currently commercially available antibody with easy potential applicability into a clinical immunohistochemical practice. Whole tissue sections permit a more robust protein expression assessment compared to tissue microarrays, especially when evaluating for the absence of immunohistochemical expression. Morphologic analysis in the current clinical cohort was blinded and systematic, based on pre-defined evaluation criteria, and our conclusions further support the previous

assertions that no known morphologic features can be relied upon to suggest an underlying HLRCC in cutaneous leiomyomas.

Our patients with HLRCC in this cohort were documented to have germline mutations through direct blood samples or other appropriate germline mutation testing samples. A limitation of our study is that there was insufficient clinical suspicion for HLRCC for genetic testing to be performed in most patients in our cohort; therefore, they have unknown *FH* mutational status. This reduces the confidence of the definitive accuracy of our specificity, as the performance characteristics of the antibody are based on the assumption that those patients without multiple cutaneous or uterine leiomyomas are negative for *FH* mutations due to absence of other clinical stigmata coupled with the low prevalence of the disease. As such, since *FH* mutational status was not performed in many of the cases, and since little data is available regarding the effectiveness of serial renal imaging on mortality reduction in patients with HLRCC, we were not able to analyze cost-effectiveness of *FH* immunohistochemistry in this study. These are important factors to investigate in future studies. We were not able to evaluate the genomic cause of *FH* IHC expression loss in the two specimens from patients without known HLRCC (because of tissue unavailability), though the IHC stains on these specimens were repeated and results remained consistent. The observed sensitivity and specificity of loss of *FH* IHC expression for HLRCC in this cohort is based upon the assumption that these two patients without known HLRCC have a somatic and not germline *FH* loss. Another possible limitation is a likely reporting bias for clinical criteria such as family history of renal cancer or personal history of renal mass, as renal imaging and family cancer history is much more likely to be performed and documented in patients with HLRCC. However, despite this limitation, the use of almost entirely in-house cases in our study allowed for adequate family history documentation in the vast majority of cases.

In conclusion, *FH* immunohistochemical expression on whole tissue sections of our consecutive clinical cohort of cutaneous leiomyoma cases was fairly sensitive and highly specific for association with HLRCC. This immunohistochemical stain has potential to be used prospectively as a triage tool to prompt additional clinical work up and/or germline testing in patients with cutaneous leiomyomas, including those with first-time diagnosis of cutaneous leiomyomas, or in the common situation where significant clinical history is lacking. Performance of the antibody is better than previously reported [26] when applied to a more realistic patient cohort, such as the one in our study using consecutive non-consult cases. *SDH-B* immunohistochemical expression is unaltered in cutaneous leiomyomas associated with HLRCC in our study. Morphologic criteria observed in some uterine leiomyomas associated with HLRCC did not demonstrate any role in triaging further testing when applied to patients with cutaneous leiomyomas in this clinical cohort.

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Figure 1.

Images of H&E stained sections and corresponding fumarate hydratase (FH) immunohistochemistry (IHC) from a uterine leiomyoma of a 22-year-old patient where the leiomyoma was found to lack FH expression by IHC. A, Low-power magnification (40x) highlights the cellular nature of this leiomyoma. B and C, High-power magnifications (400x and 600x, respectively) highlight perinucleolar clearing with enlarged eosinophilic nucleoli (thin arrows), and cytoplasmic eosinophilic globules (thick arrows); these morphologic features are likely to be associated with uterine leiomyomas which lack FH expression. D, Cytoplasmic FH expression loss in the same uterine leiomyoma (600x). Note the retained FH expression within vessels (thin arrow) serving as positive internal controls.

Figure 2.

Images of H&E stained sections and corresponding negative fumarate hydratase (FH) immunohistochemistry (IHC) from two patients with hereditary leiomyomatosis and renal cell carcinoma (HLRCC). A and C, Example of a piloleiomyoma (40x and 400x magnification), with short, interlacing bundles of smooth muscle fibers resembling arrector pili muscle (thin arrow in A and B), with less defined borders than typical cutaneous leiomyomas. The interlacing bundles can be easily appreciated at high-power magnification (C). B and D, Absent cytoplasmic FH IHC expression (40x and 400x) in the same lesion depicted in A. Note the accompanying eccrine duct (thick arrow in C and D) serving as a

positive internal control for FH IHC. E and G, Example of a conventional cutaneous leiomyoma (40x and 400x) with a well circumscribed border (thin arrow in E and F). F and H, Absent cytoplasmic FH IHC expression (40x and 400x) in the same lesion. Note the accompanying arteriole (thick arrow in G and H) serving as a positive internal control for FH IHC.

Figure 3.

Images of H&E stained sections and corresponding fumarate hydratase (FH) immunohistochemistry (IHC), each depicted at 40x magnification with inset 400x magnification. A, Example of an angioleiomyoma, with many small, slit-like vessels (thin arrow in A and B) intersecting with compact smooth muscle bundles, with a well circumscribed border. B, Positive FH IHC expression in the same lesion depicted in A, demonstrating prominent granular cytoplasmic staining. This individual did not have hereditary leiomyomatosis and renal cell carcinoma (HLRCC). C, Example of a conventional cutaneous leiomyoma, with a circumscribed border (thin arrow in C and D), and without features of piloleiomyoma or angioleiomyoma. D, Equivocal FH IHC expression, with a faint blush of granular cytoplasmic staining in some of the smooth muscle fibers. This lesion was from a patient with HLRCC, with an *FH* missense mutation.

Table 1

Clinicopathologic features, mutational status, and immunohistochemical staining characteristics of cutaneous leiomyomas from patients with HLRCC.

Patient Number	Age at Excision	Gender	Family history of Renal Cancer or HLRCC	Multiple cutaneous lesions	Renal imaging*	FH Gene Variant	FH IHC	SDH-B IHC
1	27	Male	Father: Papillary RCC	Yes	No imaging	Nonsense mutation	Neg	Pos
2	25	Male	No	Yes	Simple cyst in right kidney	Missense mutation	Equiv -2, Neg -3 (5 total)	Pos
3	28	Male	No	Yes	Negative	Missense mutation	Pos	Pos
4	15	Female	Father, brother: HLRCC; Paternal grandfather: RCC	Yes	Negative	Frameshift, protein truncation	Neg	Pos
5	39	Female	Mother: HLRCC	Yes	Simple cyst in left kidney	Deferred personal testing	Neg	Pos
6	38	Female	No	Yes	Simple cyst in left kidney	Missense mutation	Pos -1, Neg -1 (2 total)	Pos
7	40	Female	No	No	Bilateral simple renal cysts	Missense mutation	Pos	Pos

Abbreviations: HLRCC: Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC); RCC: Renal cell carcinoma; FH: Fumarate hydratase; Neg: negative; Pos: positive; Equiv: equivocal

* Renal imaging was performed after patients were confirmed with diagnosis of HLRCC.

Table 2

Comparison of clinicopathologic features of patients with cutaneous leiomyomas with HLRCC vs. no known HLRCC.

	HLRCC	No known HLRCC	Difference between groups (p-value)
Number of individuals	7	80	
Average age at diagnosis of leiomyoma	30.2 years	50.1 years	0.0011
Renal mass or cyst	4 of 7 (57.1%)	1 of 80 (1.3%)	0.0001
Family history of RCC	2 of 7 (28.6%)	0 of 80 (0%)	0.0056
Number of women	4 of 7 (57.1%)	53 of 80 (66.3%)	0.69
Women with history of excision/resection of uterine leiomyomas	3 of 4 (75.0%)	2 of 53 (3.8%)	0.0013
Proportion of individuals with multiple cutaneous lesions	6 of 7 (85.7%)	0 of 80 (0%)	0.0001

Abbreviations: HLRCC: Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC).

Table 3

Results of blinded cutaneous leiomyoma morphologic analysis.

	HLRCC (n=12)	No known HLRCC (n=84)
Average overall score	0.63	1.40
Range of overall score	0–6	0–9
Number of cases with any degree of suspicion	2 of 12 (16.6%)	32 of 84 (38%)
Average suspicion score	0.08	0.29
Average perinuclear halo score	0.04	0.25
Average eosinophilic nucleoli score	0.13	0.30
Average increased cellularity score	0.0	0.07
Average nuclear pleomorphism score	0.13	0.26
Average eosinophilic globules score	0.25	0.25

Abbreviations: HLRCC: Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC).