Association of Germline Mutations in the Fumarate Hydratase Gene and Uterine Fibroids in Women With Hereditary Leiomyomatosis and Renal Cell Cancer

Laveta Stewart, MS; Gladys M. Glenn, MD, PhD; Pamela Stratton, MD; Alisa M. Goldstein, PhD; Maria J. Merino, MD; Margaret A. Tucker, MD; W. Marston Linehan, MD; Jorge R. Toro, MD

Objective: To investigate the risk of uterine fibroids and other reproductive risk factors in women with hereditary leiomyomatosis and renal cell cancer (HLRCC).

Design: Case-control study.

Setting: National Institutes of Health, Rockville, Maryland.

Patients: A family-based case-control study was conducted between July 1, 2004, and June 30, 2006, including 105 women from families with HLRCC ascertained throughout North America. A telephone interview was conducted with all participants using a standardized questionnaire that elicited information about their menstrual, pregnancy, uterine fibroid, and hormonal contraceptive use history. Diagnosis of uterine fibroids was confirmed by pathologic diagnosis and by medical record review. DNA was extracted from blood samples and was screened for germline mutations in the fumarate hydratase (FH) gene.

Main Outcome Measures: FH germline mutation status, presence of uterine fibroids, age at diagnosis, and symptoms and treatment of uterine fibroids.

Results: Of 105 women, 77 reported a history of uterine fibroids. Regardless of uterine fibroid status, 75 of 105 women had a germline mutation in FH (FHmut positive). The risk of uterine fibroids in FHmut-positive women was statistically significantly increased compared with that in FHmut-negative women (odds ratio [OR], 7.6; 95% confidence interval [CI], 2.9-20.0), as it was among women clinically affected with HLRCC compared with those clinically unaffected with HLRCC (8.6; 3.1-24.0). The median age at uterine fibroid diagnosis for FHmut-positive women (28 years) was significantly younger than that for FHmut-negative women (38 years) (P = .03). Women with a germline mutation in FH or clinically affected with HLRCC reported younger age at menarche (P < .004) compared with FHmut-negative women (P = .02) or women who were clinically unaffected with HLRCC. Women with HLRCC were more likely to have had treatment for uterine fibroids (OR, 4.6; 95% CI, 1.4-15.8), including hysterectomy (P = .02) at an earlier age compared with women who were clinically unaffected with HLRCC.

Conclusions: This study provides the first evidence (to our knowledge) that women with germline mutations in FH and with clinical HLRCC have an increased risk of developing uterine fibroids. These women also have a younger age at uterine fibroid diagnosis and are more likely to have treatment for uterine fibroids at a younger age than women without HLRCC in their families.

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TERINE LEIOMYOMAS (FI-

broids), benign smooth muscle tumors of the uterus, are the most common gynecologic tumors in reproductive-aged women, reported in up to 77%.1-3 Approximately 25% of women with uterine fibroids report severe pain, menorrhagia, infertility, and pregnancy complications.1,4,5 As a result, uterine fibroids are the most frequent indication for hysterectomy in the United States and are a major public health problem for women of reproductive age.3,6,7 Uterine fibroids increase in prevalence with age, such that women aged 40 to 44 years are at highest risk.8 The etiology of uterine fibroids is largely unknown. However, uterine fibroids are more common among women who are obese, nulliparous, or African American or who experience menarche before age 11 years.9-12 Smoking and oral contraceptive exposure have been inconsistently reported as risk factors.8,11-18

In 1854, Virchow10 first described cutaneous leiomyomas as rare smooth muscle neoplasms of the skin. Cutaneous leiomyomas are benign mesenchymal tumors with smooth muscle differentiation. Multiple piloleiomyomas are the most common type. The other 2 main types of cutaneous leiomyomas are angioleiomyomas, which occur most frequently in the subcutis and less often in the deep dermis, and genital leiomyomas, which oc-
Cutaneous leiomyomas may manifest as a solitary lesion; as a clustered, linear, segmental arrangement sometimes involving a whole extremity; or as bilateral, symmetrical, or disseminated lesions.\(^{10,21}\) Cutaneous leiomyomas are found most frequently on the extremities, followed by the trunk.\(^{22}\) Most solitary lesions are sporadic, but in patients with a family history of cutaneous leiomyomas, this tumor represents a manifestation of an inherited predisposition. Most leiomyomas do not spontaneously regress; they may gradually increase in size, and new lesions usually develop over time.

About a century after the first description by Virchow,\(^{19}\) Kloepfer et al\(^{23}\) proposed that cutaneous leiomyomas occur in individuals from families exhibiting an autosomal dominant inheritance pattern with incomplete penetrance. In 1973, Reed et al\(^{24}\) described 2 kindreds in which cutaneous and uterine leiomyomas cosegregated in multiple members during 3 generations in an autosomal dominant pattern. Since then, the association between cutaneous and uterine leiomyomas has been known as Reed syndrome. Other names commonly used to describe this association include multiple cutaneous and uterine leiomyomatosis, leiomyomatosis cutis et uteri, and multiple leiomyomatosis.\(^{25-28}\) Numerous unrelated families having 2 or more individuals with cutaneous leiomyomas have been described worldwide. In 2001, Launonen et al\(^{25}\) described 2 families in whom cutaneous and uterine leiomyomas and a form of papillary renal cell cancer (RCC) segregated together, which they named hereditary leiomyomatosis and renal cell cancer (HLRCC [OMIM 605839]). Hereditary leiomyomatosis and RCC is recognized as the autosomal dominant predisposition to the development of cutaneous and/or uterine leiomyomas and RCC.\(^{25-27}\)

The susceptibility gene for HLRCC was mapped to the long arm of chromosome 1 in band q42 and was subsequently identified as the floretate hydratase (\(FH\)) gene (Genbank NM000143), which encodes the \(FH\) enzyme.\(^{25-28}\) In the identification of \(FH\) as the HLRCC susceptibility gene, cutaneous leiomyomas were clinically used as a marker of disease status, as these cutaneous lesions are rare in the general population but are the most sensitive and specific clinical marker of the disease among the clinical manifestations of HLRCC.\(^{25-28}\) In 2003, investigators at the National Cancer Institute described the first 35 families in North America affected with HLRCC,\(^{27}\) and 55 families have been described by the National Cancer Institute group.\(^{29,30}\) Approximately 76% of families with HLRCC have individuals with cutaneous leiomyoma (1 to \(>100\) lesions).\(^{27,30}\) Therefore, there is variable expression of cutaneous manifestations in HLRCC, ranging from absent to severe involvement. However, cutaneous leiomyomas are pathognomonic for HLRCC. Previous studies\(^{27,30}\) showed that 98% to 100% of women with cutaneous leiomyomas had uterine leiomyomas. Most important, women with HLRCC were diagnosed as having uterine fibroids at a mean age of 30 years, and more than 50% underwent hysterectomy for symptomatic uterine fibroids at or before age 30 years.\(^{27}\)

Previously, the association of cutaneous and uterine leiomyomas was assumed based on descriptions of families with cosegregation of both traits, but the presumed association was never evaluated formally.\(^{24-28}\) Therefore, we designed a family-based case-control study to investigate whether women with a germline mutation in \(FH\) have an increased risk of uterine fibroids, report a younger age at uterine fibroid diagnosis and treatment, and have more treatments, as well as whether they have more uterine fibroid symptoms compared with women in their families without HLRCC or without a germline mutation in \(FH\). Assessing the risk of uterine leiomyomas in women with a germline mutation in \(FH\) is important because the prevalence of uterine fibroids is high among the general population. To our knowledge, this is the first epidemiologic study to evaluate the risk and severity of uterine fibroids in women with HLRCC.

**METHODS**

**PATIENT ASCERTAINMENT AND STUDY DESIGN**

Fifty-six families with HLRCC from the United States and Canada were ascertained on the basis of the presence of cutaneous leiomyomas (52 families) or a history of RCC (4 families). All subjects were enrolled in a protocol studying HLRCC approved by the institutional review board of the National Cancer Institute, Rockville, Maryland. Each patient provided written informed consent before participation. From October 26, 2004, to June 1, 2006, women from these families were invited by mail to participate in a reproductive health study that included a telephone interview, medical record review, and blood drawing. If a woman had not responded after 2 weeks, she was contacted by telephone. If a woman declined participation, no further contact occurred. If she agreed to participate, a telephone interview was scheduled. Of 131 women identified through review of the family medical histories, 12 women had died (10 of RCC and 2 of other causes), and 2 women could not be located. Of 117 eligible women, 10 declined participation, and 107 agreed to participate (initial response rate, 91.5%). Ultimately, 105 women were interviewed (final response rate, 89.7%).

**DEFINITIONS**

A family with HLRCC was defined as a family of 2 or more individuals (first- and second-degree relatives) having at least 1 individual with clinical HLRCC. A case was defined as a woman from a family with at least 1 blood relative with HLRCC who reported having a diagnosis of uterine fibroids, whereas a control was defined as a woman from the same family who did not report a history of uterine fibroids. Exposure groups were based on \(FH\) germline mutation status: exposed had a germline mutation in \(FH\) (\(FH_{mut}\)-positive women), while unexposed did not (\(FH_{mut}\)-negative women). In addition, each woman was classified as having or as not having a clinical diagnosis of HLRCC. The clinical diagnosis of HLRCC was defined by the presence of pathologically confirmed cutaneous leiomyomas or RCC with specific histologically confirmed HLRCC.\(^{27}\) All women having a clinical diagnosis of HLRCC had an \(FH\) germline mutation.

**SEQUENCING OF THE \(FH\) GENE**

Blood samples were obtained from interviewed women, and DNA was extracted from peripheral blood leukocytes according to standard procedures.\(^{27}\) The genomic sequence containing \(FH\) was determined by BLAT (BLAST [basic local alignment search tool]-like alignment tool) alignment of the mitochondrial \(FH\) precursor complementary DNA (with the assembled genomic
sequence (Building 34; National Center for Biotechnology Information, Bethesda, Maryland). Methods for identification of exon and intron boundaries and for high-throughput DNA sequencing were as previously described.27

TELEPHONE INTERVIEW

A telephone interview was conducted using a standardized questionnaire that elicited information regarding various aspects of reproductive history, including pregnancy, uterine fibroids, uterine surgery, hormonal contraceptive use, and menstruation (age at onset, duration, and symptoms). The menstrual history section asked women about their early reproductive life before pregnancy or hormonal contraceptive use. Questions were also asked about demographics, smoking, hypertension, and cutaneous leiomyoma and RCC history. To evaluate the morbidity associated with uterine fibroids, women were asked about their age at diagnosis, uterine fibroid symptoms, age at symptom manifestation, uterine fibroid treatment and age at treatment, and the presence of single or multiple uterine fibroids at diagnosis. Two interviewers (L.S. and an experienced assistant) blinded to the patient’s germline mutation and uterine fibroid status administered the questionnaire, which took approximately 30 to 45 minutes. Medical records were obtained for review of radiologic, surgical, or pathologic reports, and histologic slides were obtained to confirm uterine fibroid diagnosis.

STATISTICAL ANALYSIS

Questionnaire data were coded and double-keyed by Westat, Inc (Rockville, Maryland). Analyses were conducted using 2-sided
of 30 women without germline mutations in FH had cutaneous leiomyomas or HLRCC-associated RCC. Of 60 women clinically affected with HLRCC, 59 had cutaneous leiomyomas (9 of whom also had HLRCC-associated RCC), and 1 had HLRCC-associated RCC but no cutaneous leiomyomas. Forty-five women (median age, 42.5 years), including all 30 FHmut-negative women, were clinically unaffected with cutaneous leiomyomas or RCC.

Of 105 women, 77 reported a history of uterine fibroids, and 28 did not. The diagnosis of uterine fibroids was confirmed in 97.4% (75 of 77) by medical records and in 89.6% (69 of 77) by pathologic review of slides obtained after hysterectomy or myomectomy (Figure). Medical records were unavailable for the 2 women whose uterine fibroid diagnosis was not confirmed. Mild histologic atypia was reported in 6 uterine fibroid cases, but there were no reports of leiomyosarcoma of the uterus. Renal cell cancer was present in 10 women; all cases occurred before ascertainment of the families and were histologically confirmed. The diagnosis of cutaneous leiomyoma was histologically confirmed in all 59 women who reported having cutaneous leiomyomas. Table 1 gives the demographic characteristics for all participants. More than 90% of women were white, and the age at interview ranged from 19 to 81 years.

## COMPARISON BY FH GERMLINE MUTATION STATUS

### Smoking, Menstrual, Pregnancy, and Hormonal Contraceptive Use History

Smoking history among FHmut-positive and FHmut-negative women did not differ for ever or never smoking, number of cigarettes per day, smoking regularly for

## MUTATION SCREENING

We identified FH germline mutations in 100.0% (56 of 56) of families with HLRCC. Each missense mutation cosegregated with disease and was absent in more than 160 controls. Seventy-five were FHmut-positive women, while 30 were FHmut-negative women.

## GENERAL PATIENT CHARACTERISTICS

Regardless of uterine fibroid status, 75 women were FHmut positive; 60 of them had clinical features of HLRCC, and the other 15 had no clinical evidence of HLRCC. None of 30 women without germline mutations in FH had cutaneous leiomyomas or HLRCC-associated RCC. Of 60 women clinically affected with HLRCC, 59 had cutaneous leiomyomas (9 of whom also had HLRCC-associated RCC), and 1 had HLRCC-associated RCC but no cutaneous leiomyomas. Forty-five women (median age, 42.5 years), including all 30 FHmut-negative women, were clinically unaffected with cutaneous leiomyomas or RCC.

Of 105 women, 77 reported a history of uterine fibroids, and 28 did not. The diagnosis of uterine fibroids was confirmed in 97.4% (75 of 77) by medical records and in 89.6% (69 of 77) by pathologic review of slides obtained after hysterectomy or myomectomy (Figure). Medical records were unavailable for the 2 women whose uterine fibroid diagnosis was not confirmed. Mild histologic atypia was reported in 6 uterine fibroid cases, but there were no reports of leiomyosarcoma of the uterus. Renal cell cancer was present in 10 women; all cases occurred before ascertainment of the families and were histologically confirmed. The diagnosis of cutaneous leiomyoma was histologically confirmed in all 59 women who reported having cutaneous leiomyomas. Table 1 gives the demographic characteristics for all participants. More than 90% of women were white, and the age at interview ranged from 19 to 81 years.

## COMPARISON BY FH GERMLINE MUTATION STATUS

### Smoking, Menstrual, Pregnancy, and Hormonal Contraceptive Use History

Smoking history among FHmut-positive and FHmut-negative women did not differ for ever or never smoking, number of cigarettes per day, smoking regularly for
at least 1 year, total number of years smoking (Table 1), or pack-years (data not shown).

Menstrual, pregnancy, and hormonal contraceptive use history are given in Table 2. The age of onset of menarche was significantly younger in FHmut-positive women compared with FHmut-negative women (P = .02). Similarly, the age of onset of menarche was significantly younger in women clinically affected with HLRCC compared with those clinically unaffected with HLRCC (P = .004). However, no differences were observed in menstrual cycle pattern or symptoms or in contraceptive use or duration between comparison groups. Approximately 75% of women in each comparison group used hormonal contraceptives for at least 1 year. Most of these women used a combination of estrogen and progesterone.

### Table 2. Distribution and Significance Testing of Menstrual, Contraceptive Use, and Pregnancy History Characteristics by FH Germline Mutation Status and by Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) Clinical Status Among 105 Women

<table>
<thead>
<tr>
<th>Variable</th>
<th>FH Positive (n=75)</th>
<th>FH Negative (n=30)</th>
<th>P Value</th>
<th>Affected (n=60)</th>
<th>Unaffected (n=45)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche, No. (%), y</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>≤12</td>
<td>23 (46.9)</td>
<td>6 (23.7)</td>
<td>.02</td>
<td>20 (52.6)</td>
<td>9 (27.3)</td>
<td>.004</td>
</tr>
<tr>
<td>13-15</td>
<td>19 (38.8)</td>
<td>16 (72.7)</td>
<td></td>
<td>12 (31.6)</td>
<td>23 (69.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;15</td>
<td>7 (14.3)</td>
<td>0</td>
<td></td>
<td>6 (15.8)</td>
<td>1 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Self-reported period flow, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>7 (9.5)</td>
<td>4 (13.3)</td>
<td>.74</td>
<td>5 (8.5)</td>
<td>6 (13.3)</td>
<td>.57</td>
</tr>
<tr>
<td>Medium</td>
<td>45 (60.8)</td>
<td>16 (53.3)</td>
<td></td>
<td>37 (62.7)</td>
<td>24 (53.3)</td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td>22 (29.7)</td>
<td>10 (33.3)</td>
<td></td>
<td>17 (28.8)</td>
<td>15 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Self-reported pain during menstruation, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19 (25.3)</td>
<td>5 (16.7)</td>
<td>.02</td>
<td>16 (26.7)</td>
<td>8 (17.8)</td>
<td>.47</td>
</tr>
<tr>
<td>Mild</td>
<td>15 (20.0)</td>
<td>11 (36.7)</td>
<td></td>
<td>13 (21.7)</td>
<td>13 (28.9)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>20 (26.7)</td>
<td>10 (33.3)</td>
<td>.15</td>
<td>15 (25.0)</td>
<td>15 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>21 (28.0)</td>
<td>10 (33.3)</td>
<td></td>
<td>16 (26.7)</td>
<td>9 (20.0)</td>
<td></td>
</tr>
<tr>
<td>Irregular cycles, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always regular</td>
<td>58 (77.3)</td>
<td>20 (66.7)</td>
<td></td>
<td>49 (81.7)</td>
<td>29 (64.4)</td>
<td>.14</td>
</tr>
<tr>
<td>Sometimes irregular</td>
<td>7 (9.3)</td>
<td>3 (10.0)</td>
<td>.44</td>
<td>4 (6.7)</td>
<td>6 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Always irregular</td>
<td>10 (13.3)</td>
<td>7 (23.3)</td>
<td></td>
<td>7 (11.7)</td>
<td>10 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Used contraceptives ≥1 y, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>62 (82.7)</td>
<td>26 (86.7)</td>
<td>.62</td>
<td>50 (83.3)</td>
<td>38 (84.4)</td>
<td>.88</td>
</tr>
<tr>
<td>No</td>
<td>13 (17.3)</td>
<td>4 (13.3)</td>
<td></td>
<td>10 (16.7)</td>
<td>7 (15.6)</td>
<td></td>
</tr>
<tr>
<td>Contraceptive type, No. (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hormonal</td>
<td>57 (91.9)</td>
<td>22 (84.6)</td>
<td>.30</td>
<td>45 (90.0)</td>
<td>34 (89.5)</td>
<td>.94</td>
</tr>
<tr>
<td>Nonhormonal</td>
<td>5 (8.1)</td>
<td>4 (15.4)</td>
<td></td>
<td>5 (10.0)</td>
<td>4 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Used contraceptives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hormonal</td>
<td>57 (76.0)</td>
<td>22 (73.3)</td>
<td>.78</td>
<td>45 (75.0)</td>
<td>34 (75.6)</td>
<td>.95</td>
</tr>
<tr>
<td>Never used hormonal or nonhormonal contraceptives</td>
<td>18 (24.0)</td>
<td>8 (26.7)</td>
<td></td>
<td>15 (25.0)</td>
<td>11 (24.4)</td>
<td></td>
</tr>
<tr>
<td>Duration of contraceptive use, y</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.5</td>
<td>6.0</td>
<td>.60</td>
<td>6.0</td>
<td>6.5</td>
<td></td>
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<tr>
<td>Mean (SD)</td>
<td>7.2 (5.6)</td>
<td>7.4 (5.5)</td>
<td></td>
<td>7.3 (5.8)</td>
<td>7.2 (5.1)</td>
<td></td>
</tr>
<tr>
<td>Minimum/maximum</td>
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<td>1/20</td>
<td></td>
<td>1/23</td>
<td>1/20</td>
<td></td>
</tr>
<tr>
<td>Duration of hormone use, No. (%), y</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤6.5</td>
<td>25 (50.0)</td>
<td>10 (52.6)</td>
<td>.85</td>
<td>20 (51.3)</td>
<td>15 (50.0)</td>
<td>.92</td>
</tr>
<tr>
<td>&gt;6.5</td>
<td>25 (50.0)</td>
<td>9 (47.4)</td>
<td></td>
<td>19 (48.7)</td>
<td>15 (50.0)</td>
<td></td>
</tr>
<tr>
<td>History of pregnancy, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>58 (77.3)</td>
<td>20 (66.7)</td>
<td>.26</td>
<td>44 (73.2)</td>
<td>34 (75.6)</td>
<td>.80</td>
</tr>
<tr>
<td>No</td>
<td>17 (22.7)</td>
<td>10 (33.3)</td>
<td></td>
<td>16 (26.7)</td>
<td>11 (24.4)</td>
<td></td>
</tr>
<tr>
<td>Trouble getting pregnant among those trying to get pregnant, No. (%)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>15 (23.8)</td>
<td>6 (21.4)</td>
<td>.80</td>
<td>12 (25.0)</td>
<td>9 (20.9)</td>
<td>.65</td>
</tr>
<tr>
<td>No</td>
<td>48 (76.2)</td>
<td>22 (78.6)</td>
<td></td>
<td>36 (75.0)</td>
<td>34 (79.1)</td>
<td></td>
</tr>
<tr>
<td>Parity, No. (%)</td>
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<tr>
<td>Nulliparous</td>
<td>27 (36.0)</td>
<td>10 (33.3)</td>
<td>.79</td>
<td>22 (36.7)</td>
<td>15 (33.3)</td>
<td>.72</td>
</tr>
<tr>
<td>Parous</td>
<td>48 (64.0)</td>
<td>20 (66.7)</td>
<td></td>
<td>38 (63.3)</td>
<td>30 (66.7)</td>
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<tr>
<td>No. of full-term or near-full-term live births</td>
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<td></td>
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</tr>
<tr>
<td>Median</td>
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<td>2.5</td>
<td>.29</td>
<td>2.0</td>
<td>2.0</td>
<td>.15</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>2.0 (1.3)</td>
<td>2.7 (1.5)</td>
<td></td>
<td>2.1 (1.3)</td>
<td>2.2 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Minimum/maximum</td>
<td>0/5</td>
<td>1/7</td>
<td></td>
<td>0/5</td>
<td>0/7</td>
<td></td>
</tr>
<tr>
<td>No. of live births delivered by cesarean section, No. (%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>33 (68.8)</td>
<td>15 (75.0)</td>
<td>.61</td>
<td>26 (68.4)</td>
<td>22 (73.3)</td>
<td>.66</td>
</tr>
<tr>
<td>≥1</td>
<td>15 (31.3)</td>
<td>5 (25.0)</td>
<td></td>
<td>12 (31.6)</td>
<td>8 (26.7)</td>
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<tr>
<td>Pregnancy complications, No. (%)</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>22 (38.6)</td>
<td>10 (52.6)</td>
<td></td>
<td>15 (34.9)</td>
<td>17 (51.5)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35 (61.4)</td>
<td>9 (47.4)</td>
<td>.29</td>
<td>28 (65.1)</td>
<td>16 (48.5)</td>
<td></td>
</tr>
</tbody>
</table>

*Some variable totals do not sum to column totals because of missing data. Percentages are based on the variable totals.
No differences were observed in parity, pregnancy history, trouble getting pregnant, or pregnancy complications between comparison groups. These results are summarized in Table 2.

**Uterine Fibroid Risk Assessment**

FHmut-positive women had a significantly increased risk of uterine fibroids compared with FHmut-negative women (odds ratio [OR], 7.6; 95% confidence interval [CI], 2.9-20.0) (Table 3). When stepwise logistic regression analysis was conducted for uterine fibroid risk among FHmut-negative vs FHmut-positive women incorporating age at interview, smoking duration, hormonal contraceptive use, and age or school grade at menarche in the model, only age at interview remained, altering the previous OR slightly (OR, 7.9; 95% CI, 2.9-22.0).

In addition, FHmut-negative women who used hormonal contraceptives showed a reduced risk of uterine fibroids. However, this finding was not statistically significant (OR, 0.3; 95% CI, 0.1-1.8) (data not shown, P = .02). These results should be viewed with caution, and this area needs to be studied further because sufficiently detailed information was unavailable to determine whether hormonal contraceptives were used for treatment of uterine fibroids.

**Uterine Fibroid Outcomes, Including Age at Diagnosis, Symptoms, and Treatment**

The median age at uterine fibroid diagnosis for FHmut-positive women was significantly younger compared with that for FHmut-negative women (28 vs 38 years, P = .03). Moderate or severe pain was more commonly reported by FHmut-positive women than by FHmut-negative women who underwent myomectomy and hysterectomy (61% vs 39% and 60% vs 25%, respectively) (data not shown). These symptoms may have contributed to the age at uterine fibroid diagnosis. Knowledge of the number of uterine fibroids was unavailable for many women and could not be considered for its effect on risk of treatment.

**COMPARISON BY HLRCC CLINICAL STATUS**

Women clinically affected with HLRCC were significantly older (P = .001) and reported smoking longer (≤11 vs >11 years, P = .04) compared with women clinically unaffected with HLRCC (Table 1). Women clinically affected with HLRCC reported younger age at menarche than women clinically unaffected with HLRCC (P = .004) (Table 2). Women with clinical HLRCC who used hormonal contraceptives had a significantly increased risk of uterine fibroids (OR, 7.8; 95% CI, 1.3-48.3). Women without clinical HLRCC who used hormonal contraceptives showed a reduced risk of uterine fibroids, which was not statistically significant (OR, 0.3; 95% CI, 0.05-2.4) (data not shown, P = .20). These results need to be studied further because sufficiently detailed information was unavailable to determine whether hormonal contraceptives were used for treatment of uterine fibroids.

The risk of uterine fibroids in women with a clinical diagnosis of HLRCC was significantly elevated compared with that in women without a clinical diagnosis of HLRCC (OR, 8.6; 95% CI, 3.1-24.0) (Table 3). Compared with women who were HLRCC unaffected and FHmut-positive and with women who were HLRCC unaffected and FHmut-negative, women who were HLRCC affected and FHmut-positive showed a significantly increased risk of uterine fibroids (OR, 4.5; 95% CI, 1.0-21.6; and 11.8; 3.5-42.1, respectively). When stepwise logistic regression analysis was conducted for uterine fibroid risk among women affected with HLRCC vs women unaffected with HLRCC, none of the potential confounding variables contributed to clinical status.

Women affected clinically with HLRCC were significantly more likely to have had surgical treatment for uterine fibroids (OR, 4.6; 95% CI, 1.2-17.8; P = .02) compared with women who were unaffected clinically with HLRCC (Table 4). Women clinically affected with HLRCC were first treated for uterine fibroids at a younger median age than women clinically unaffected with HLRCC (31.5 vs 37.0 years, P = .03), reporting a younger age at hysterectomy (≤42.5 vs >42.5 years, P = .02).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Uterine Fibroid Positive (n=77)</th>
<th>Uterine Fibroid Negative (n=28)</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germline mutation status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH positive</td>
<td>64 (83.1)</td>
<td>11 (39.3)</td>
<td>7.6 (2.9-20.0)</td>
</tr>
<tr>
<td>FH negative</td>
<td>13 (16.9)</td>
<td>17 (60.7)</td>
<td></td>
</tr>
<tr>
<td>HLRCC clinical status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected</td>
<td>54 (70.1)</td>
<td>6 (21.4)</td>
<td>8.6 (3.1-24.0)</td>
</tr>
<tr>
<td>Unaffected</td>
<td>23 (29.9)</td>
<td>22 (78.6)</td>
<td></td>
</tr>
<tr>
<td>Combined FH germline mutation status and HLRCC clinical status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH positive and HLRCC unaffected</td>
<td>10 (13.0)</td>
<td>5 (17.9)</td>
<td>4.5 (1.0-21.6)</td>
</tr>
<tr>
<td>FH positive and HLRCC affected</td>
<td>54 (70.1)</td>
<td>6 (21.4)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>FH negative and HLRCC unaffected</td>
<td>13 (16.9)</td>
<td>17 (60.7)</td>
<td>11.8 (3.5-42.1)</td>
</tr>
</tbody>
</table>

To our knowledge, this is the first epidemiologic study to investigate reproductive health risk factors in women with HLRCC. We showed that women with an FH germline mutation and women with clinical HLRCC (cutaneous leiomyomas or HLRCC kidney cancer) have an 8- to 9-fold increased risk of developing uterine fibroids. Since the original description in 1973 by Reed et al,24 the association of cutaneous and uterine leiomyomas was assumed based on descriptions of families with cosegregation of both traits, but the presumed association was never formally evaluated.25-28

In families with HLRCC, the risk assessment, baseline screening, surveillance, and clinical management de-
Table 4. Odds Ratios for Uterine Leiomyoma According to Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) Clinical Status Among 77 Women With a History of Uterine Fibroids

<table>
<thead>
<tr>
<th>Variable</th>
<th>HLRCC Clinical Status, No.</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, y≤38</td>
<td>44</td>
<td>3.1 (0.9-10.9)</td>
</tr>
<tr>
<td>Age at diagnosis, y&gt;38</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45</td>
<td>4.6 (1.4-15.8)</td>
</tr>
<tr>
<td>No</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Surgery</td>
<td>30</td>
<td>4.6 (1.2-17.8)</td>
</tr>
<tr>
<td>No surgery</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>≥1 Surgery</td>
<td>14</td>
<td>5.7 (1.0-35.6)</td>
</tr>
<tr>
<td>No surgery</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Myomectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>0.8 (0.2-3.4)</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age at myomectomy, y≤35.5</td>
<td>19</td>
<td>19.0 (1.1-709.5)</td>
</tr>
<tr>
<td>Age at myomectomy, y&gt;35.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hysterectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38</td>
<td>2.7 (0.5-14.3)</td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age at hysterectomy, y≤42.5</td>
<td>35</td>
<td>11.7 (1.4-111.7)</td>
</tr>
<tr>
<td>Age at hysterectomy, y&gt;42.5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

*Only 76 responses were received.*

The prevalence of uterine fibroids increases with age, becoming most prevalent in the general population among women aged 40 to 44 years. However, in this study, uterine fibroids were diagnosed at a significantly younger age in women with a germline mutation in FH compared with those without a germline mutation in FH (median, 28 vs 38 years). This is also significantly younger than the age in the general population. Women with HLRCC had a 5-fold increased risk of having treatment for uterine fibroids and were younger than women without HLRCC at myomectomy or hysterectomy. Almost two-thirds of women who had surgical treatment reported moderate to severe pain before surgery. In our study overall, 73.3% of HLRCC-affected women and 74.4% of FH*mut*-positive women reported dysmenorrhea and pain, symptoms previously described with uterine fibroids and at much higher rates than the 20% to 50% reported in the general population.

In this study, we also found that women with an FH germline mutation, and specifically women with clinical HLRCC, experienced menarche at a younger age than women without an FH germline mutation or women without clinical HLRCC. Early menarche has been previously reported to increase the risk of developing uterine fibroids. Menorrhagia, the most frequently reported symptom in women with uterine fibroids, was common among our study population but did not differ between the comparison groups. This is not surprising because uterine bleeding is difficult to quantify accurately by verbal reports. Other menstrual cycle characteristics did not differ between the comparison groups.

Women with HLRCC who used hormonal contraceptives seemed to be at a significantly increased risk of developing uterine fibroids. Given the early age at uterine fibroid diagnosis, the high proportion with symptoms and treatment, and the small sample size of affected with HLRCC, it is difficult to know whether the use of hormonal contraceptives led to the development of uterine fibroids or was merely a treatment for symptoms. Women without an FH germline mutation and women who were clinically unaffected by HLRCC had a lower risk of having uterine fibroids associated with hormonal contraceptive use, an observation similar to that in the general population. These findings could be better assessed and confirmed with a longitudinal study rather than a cross-sectional study.

Some evidence suggests that uterine fibroids may cause infertility. Neither parity nor infertility was associated with uterine fibroids in this study. Pregnancy has been postulated to be protective against developing uterine fibroids, as increasing parity is inversely associated with uterine fibroids. The high proportion of women with clinical HLRCC reporting that they were not trying to get pregnant and the small sample size may have hampered our ability to detect an increased risk of infertility. Consistent with previous findings but in contrast to others, women with HLRCC in this study did not develop uterine leiomyosarcoma. This issue requires further study because the risk of leiomyosarcoma has important surveillance and management implications for women considering hysterectomy for uterine fibroids. The sample size and cross-sectional nature of our study precluded further evaluation of this question.

In this study of women with HLRCC, a germline mutation in FH seems to increase susceptibility for uterine fibroids. Familial clustering of uterine fibroids in the general population suggests that uterine fibroids may have a significant genetic component. In 2004, genetic linkage analysis of 123 families with uterine fibroids (but not HLRCC) and with at least 1 affected sib pair suggested FH locus linkage to the long arm of chromosome 1 in band q42 among women younger than 40 years at diag-
nosis. Using fluorescent in situ hybridization, a copy of FH was absent in 9 of 11 uterine fibroid samples from women in these families. In another study, single and biallelic somatic FH inactivation was reported in some sporadic uterine fibroids. Overall, laboratory investigations demonstrate a loss of heterozygosity mapped to the long arm of chromosome 1 in band q42 around the FH locus in HLRCC-associated uterine fibroids, suggesting that FH acts as a tumor suppressor gene. Hypoxia pathways may also be involved in the development of uterine fibroids, as fibroids exhibit upregulation of HIF1 targets such as VEGF (vascular endothelial growth factor) and BNIP1 (BCL2/adenovirus E1B 19-kDa interacting protein 1) and downregulation of TSP1. In addition, in vitro investigations indicate that increased intracellular fumarate directly impairs HIF prolyl hydroxylase and leads to accumulation of HIF. These data suggest that loss of FH might be an important event in the pathogenesis of a subset of familial uterine fibroids.

Hereditary leiomyomatosis and renal cell cancer is a rare syndrome, which limited the sample size in this study. However, the high response rate (89.7%) allowed us to identify that women with HLRCC have an increased risk of developing uterine fibroids. The small sample size did not allow sufficient power to detect effects by stratification or condition on family in the analyses. Because participants were selected from families with HLRCC evaluated at the National Institutes of Health, our study could be biased toward women who sought medical care or who had symptoms. Furthermore, recall bias is a limitation in studies requiring women to remember specific dates and symptoms of major reproductive events such as menstrual, pregnancy, and contraceptive use history. This was a cross-sectional study of families, and young FH germ-line mutation carriers may develop uterine fibroids in the future. Prospective follow-up of families with HLRCC will be required to evaluate these issues further and to determine their effects on the results.

CONCLUSIONS

To our knowledge, this study provides the first statistical evidence that women with germline mutations in FH and women with clinical HLRCC have an increased risk of developing uterine fibroids, are diagnosed as having uterine fibroids at an earlier age, and are more likely to have treatment for uterine fibroids at a younger age than their family members without clinical HLRCC or without germline mutations in FH. Therefore, HLRCC-associated uterine fibroids may represent a distinct gynecologic entity. Cutaneous leiomyoma is the most sensitive and specific clinical marker of HLRCC. Therefore, if genetic testing is unavailable, a dermatologic examination for cutaneous leiomyomas can be used to identify high-risk women with HLRCC. Women with cutaneous leiomyomas may be informed of their uterine fibroid risk. In addition, if genetic testing is available, it may be used to detect high-risk women in these families, especially women without cutaneous manifestations. Awareness of the diagnosis of HLRCC also benefits patients as an alert to the need to screen for renal tumors, as patients with HLRCC are at risk of aggressive renal cell cancer. Our study contributes to the understanding of the genetic basis of hereditary uterine fibroids. Future investigation of HLRCC will help to elucidate the etiology of HLRCC-associated uterine fibroids and the role of FH germline mutations among women in the general population.

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Correspondence: Jorge R. Toro, MD, Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, 6120 Executive Blvd, Executive Plaza South, Room 7012, Rockville, MD 20892-7231 (toroj@mail.nih.gov).

Author Contributions: Dr Toro had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Stewart, Glenn, Stratton, Tucker, Linehan, and Toro. Acquisition of data: Stewart, Glenn, Linehan, and Toro. Analysis and interpretation of data: Stewart, Glenn, Stratton, Goldstein, Merino, Tucker, and Toro. Drafting of the manuscript: Stewart, Glenn, Stratton, and Toro. Critical revision of the manuscript for important intellectual content: Stewart, Glenn, Stratton, Goldstein, Merino, Tucker, and Toro. Statistical analysis: Stewart and Goldstein. Obtained funding: Tucker and Toro. Administrative, technical, and material support: Stewart, Merino, and Toro. Study supervision: Glenn, Stratton, Tucker, Linehan, and Toro.

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REFERENCES


