GYNECOLOGY

Clinical and molecular risk factors for repeat interventions due to symptomatic uterine leiomyomas

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BACKGROUND: Repeat leiomyoma occurrence or even reintervention is common after myomectomy. Little is known about the factors related to repeat interventions.

OBJECTIVE: This study aimed to determine the frequency of leiomyoma-related reintervention after an initial laparoscopic or abdominal myomectomy and to analyze both clinical and molecular risk factors for reinterventions. Another objective was to define the frequency of clonally related tumors from repeat operations.

STUDY DESIGN: This retrospective cohort study included 234 women who had undergone laparoscopic or abdominal myomectomy in 2009 to 2014. Information on repeat leiomyoma-related interventions as well as on other clinical factors was collected from medical records after a median follow-up time of 11.4 years (range 7.9—13.8 years) after the index procedure. The effect of clinical risk factors on the risk of reintervention was analyzed by the Kaplan-Meier estimator and the Cox proportional hazards model. For molecular analyses, we examined the mutation profiles of 133 formalin-fixed paraffin-embedded leiomyoma samples from 33 patients with repeat operations. We screened the tumors for the 3 primary leiomyoma driver alterations—mediator complex subunit 12 mutations, high mobility group AT-hook 2 overexpression, and fumarate hydratase-deficiency—utilizing Sanger sequencing and immunohistochemistry. To further assess the clonal relationship of the tumors, we executed whole-exome sequencing for 52 leiomyomas from 21 patients who exhibited the same driver alteration in tumors obtained from multiple procedures.

RESULTS: Reintervention rate at 11.4 years after myomectomy was 20% (46/234). Number of leiomyomas removed at the index myomectomy was a risk factor (hazard ratio 1.21; 95% confidence interval 1.09—1.34). Age at index myomectomy (hazard ratio 0.94; 95% confidence interval 0.89—0.99) and postoperative parity (hazard ratio 0.23; 95% confidence interval 0.09—0.60) were protective factors. Molecular characterization of tumors from index and nonindex operations confirmed a clonal relationship of the tumors in 3/33 (9%) patients. None of the leiomyomas harboring a mediator complex subunit 12 mutation—the most common leiomyoma driver—were confirmed clonally related. Fumarate hydratase-deficiency was detected in repeat leiomyomas from 3/33 (9%) patients. All these patients harbored a germline fumarate hydratase mutation, which is distinctive for the hereditary leiomyomatosis and renal cell cancer syndrome. Finally, we identified 3 (3/33; 9%) patients with 2 tumors each displaying somatic mutations in a recently identified novel leiomyoma driver gene, YEATS domain-containing protein 4. All YEATS domain-containing protein 4 mutations were different and thus the tumors were not clonally related.

CONCLUSION: Our study shows that reintervention is common after surgical myomectomy. Uterine leiomyomas typically develop independently, but some share a clonal origin. Repeat leiomyoma occurrence may be due to genetic predisposition, such as a germline fumarate hydratase mutation. Distinct somatic YEATS domain-containing protein 4 mutations identified in multiple leiomyomas from the same patient indicate a possible role for YEATS domain-containing protein 4 in repeat leiomyomas.

Key words: FH, HLRCC, HMGA2, MED12, myomectomy, YEATS4

Introduction

Uterine leiomyomas, or fibroids, are benign smooth muscle tumors that typically affect women in their reproductive years. Symptoms include abnormal uterine bleeding, pelvic pain, and even reproductive dysfunction.[1] Myomectomy is the recommended treatment option for women wishing to preserve their fertility.[2] Based on ultrasound evaluation, leiomyoma recurrence rate as high as 57% at 5 years after myomectomy has been reported, and 12% to 19% of patients require reintervention.[3–5] In previous studies, the number of leiomyomas removed has been the most consistent clinical risk factor for recurrence.[6,7] Very rarely, leiomyoma recurrence results from a previous myomectomy performed by tissue morcellation that has led to the dissemination of tumor cells into abdominal cavity.[8]

Uterine leiomyomas can be divided into 3 well-established molecular subtypes: tumors exhibiting specific mutations in mediator complex subunit 12 (*MED12*), tumors showing high mobility group AT-hook 2 (HMGA2) overexpression, and tumors with biallelic inactivation of fumarate hydratase (*FH*).[9] FH-deficient leiomyomas are rare (1%—2%) and may occur sporadically or via germline mutations causing hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome.[10–12] These 3 genetic alterations account for over 80% of leiomyomas in both perimenopausal and fertile-aged women.[9,13] Other, recently discovered rare subclasses include tumors with mutations in genes encoding for the members of the Snf2-Related CREBBP Activator Protein (SRCAP) complex and tumors with mutations in genes associated with neddylation of the Cullin 3-RING E3 ligase.[14,15]

Uterine leiomyomas are monoclonal tumors that originate from the uterine wall.[16,17] Their repeat occurrence is poorly understood regarding clonality

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AJOG at a Glance

Why was this study conducted?

Repeat leiomyoma occurrence and reinterventions are common following surgical myomectomy. However, information on the clinical and molecular risk factors for reinterventions is limited. No previous study has systematically investigated the clonal relationship of leiomyomas from repeat operations.

Key findings

Reintervention after myomectomy is common (20% after 11 years). The number of leiomyomas removed increased the risk for a reintervention, and protective factors included age and postoperative parity. We confirmed a clonal relationship of tumors in 3/33 patients (9%). Genetic predisposition with germline fumarate hydratase (*FH*) mutations was found in 3 patients. Another 3 patients harbored tumors with distinct somatic YEATS domain-containing protein 4 (*YEATS4*) mutations.

What does this add to what is known?

Leiomyomas from repeat interventions are usually independent lesions, but some share a clonal origin. Germline *FH* mutations predispose to repeat tumors and thus to multiple leiomyoma-related interventions. Distinct somatic *YEATS4* mutations may be associated with repeat leiomyoma occurrence.

and molecular characteristics. Studies utilizing X chromosome inactivation pattern have shown that multiple concurrent leiomyomas are usually independent lesions.[18,19] Later studies have demonstrated that concurrent leiomyomas with *HMGA2* and *FH* aberrations may present as multiple clonally related tumors.[20,21]

This study focuses on fertile-aged patients who have undergone multiple procedures related to uterine leiomyomas. We aimed to determine the frequency and clinical risk factors for reintervention after an abdominal or laparoscopic myomectomy. Another aim was to determine the molecular features of leiomyomas from repeat operations and to define the frequency of clonally related tumors.

Materials and methods

Patient information and statistical analysis

The study was approved by the ethics review board of the Hospital District of Helsinki and Uusimaa, Finland (24/13/03/03/2015) and carried out in accordance with the Declaration of Helsinki. Tissue samples and clinical data were collected after signed informed consent was obtained from the patients or with the permission from the National Supervisory Authority for Welfare and Health (Valvira; 602/06.01.03.01/2016).

This study is based on a previously described cohort of 234 myomectomy patients aged 17 to 45 years.[13] Open abdominal myomectomy was performed for 51% of the patients, and morcellator was utilized in 97% of laparoscopic myomectomies. The median number of leiomyomas removed was 2 (range 1−13) in open abdominal myomectomies, and 1 (range 1−12) in laparoscopic myomectomies. Myomectomy performed in 2009 to 2014 was considered as the index procedure, and leiomyoma-related reintervention was defined as any surgical or minimally invasive procedure (high-intensity focused ultrasound and uterine artery embolization) to treat symptoms related to uterine leiomyomas. Statistical analyses were performed to investigate clinical risk factors for leiomyoma-related reintervention following index myomectomy. If the patient had several leiomyoma-related procedures during the follow-up time, only the first was included in the statistical analyses. Follow-up was performed from medical records up to December 2022, with a median follow-up time of 11.4 years (range 7.9−13.8 years) (Figure 1). Statistical methods included the Pearson chi-square test, the Fisher's exact test, the Mann-Whitney *U* test, the Kaplan-Meier estimator with log-rank test, and the Cox proportional hazards regression model. For detailed description, see Supplemental Methods.

Sample collection

Molecular status of the leiomyomas removed at the index operations was already available.[13] Here, we collected formalin-fixed paraffin-embedded (FFPE) leiomyoma samples from surgical reinterventions up to August 2019, as well as samples from myomectomies that preceded the index procedure. Patients' medical history and pathology reports were carefully examined to exclude any residual tumors. Altogether 133 leiomyomas were obtained from 33 patients who had undergone multiple leiomyoma-related operations: 62 index and 71 nonindex leiomyomas (Figure 1). In 6 patients, the repeat operation took place before the index operation.

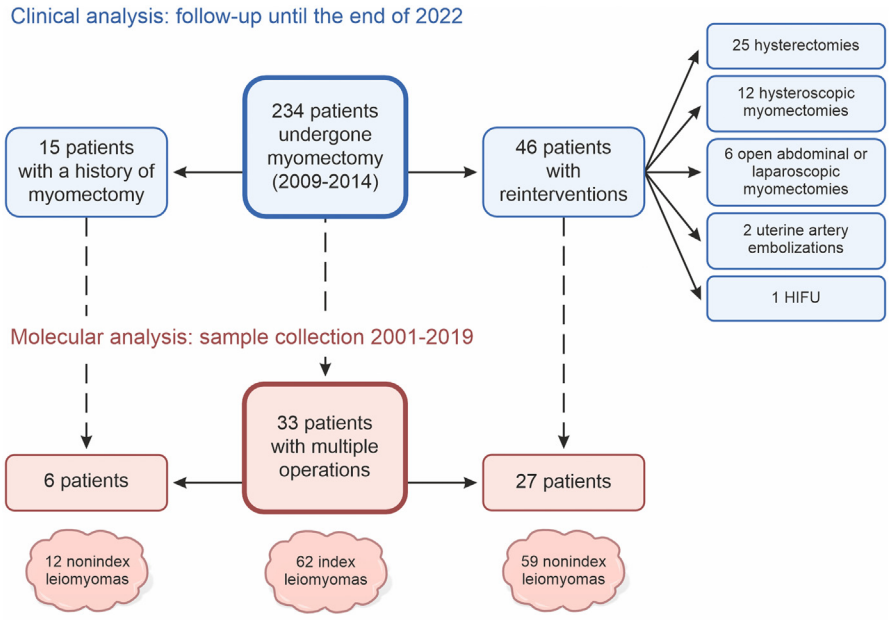
Immunohistochemistry and Sanger sequencing

Immunohistochemical staining with an anti-2-succinylcysteine antibody (1:500, crb2005017d, Cambridge Research Biochemicals, Billingham, UK) was used to detect FH status. HMGA2 expression levels were detected with an anti-HMGA2 antibody (1:2000, 59170AP, Biocheck, South San Francisco, CA). Sanger sequencing was performed for *MED12* exons 1 and 2 and YEATS domain-containing protein 4 (*YEATS4*) exons 2, 3, 4, 5, and 7. For detailed description, see Supplemental Methods.

Whole-exome sequencing

Whole-exome sequencing was performed for 52 tumors and 12 matching normal samples. DNA libraries were generated by either the Twist Library Preparation EF 2.0 kit (Twist Bioscience, San Francisco, CA) or KAPA Hyper Prep kit (Roche NimbleGen, Madison, WI). Sequencing was conducted using the

FIGURE 1
Myomectomy patients and uterine leiomyoma samples included in the study



The myomectomy performed in 2009 to 2014 was defined as the index operation. The clinical analyses included 234 myomectomy patients, of whom 15 had a history of myomectomy before the index operation, and 46 patients who underwent at least 1 leiomyoma-related reintervention during the follow-up (in *blue*). Altogether 133 samples from 33 patients with multiple leiomyoma-related operations entered the molecular analysis (in *red*). These include 62 leiomyomas from the index operations and 71 nonindex leiomyomas from operations preceding (n=12) or following (n=59) the index operation. Samples from 14 patients were not included in the study due to unavailability, poor quality, and postoperative histology other than leiomyoma. Additional 14 surgical reinterventions were performed after the completion of the sample collection.

HIFU, high-intensity focused ultrasound.

Illumina NovaSeq 6000 or NextSeq500 (Illumina, San Diego, CA). For detailed information, refer to Supplemental Table 1. Data preprocessing followed the Genome Analysis ToolKit 4 best practices.[22] Joint somatic variant calling (paired and nonpaired) was performed using Mutect2 with default parameters.[22] Detected variants were filtered against an in-house panel of normals, a panel of normals generated from the 1000 genomes project,[23] and variants present in the Genome Aggregation Database (exomes and genomes v2.0.1 and v3).[24] Somatic copy number alterations (SCNAs) were called using CNVkit with default parameters.[25] SCNAs were compared against a pooled normal derived from 12 normal tissue samples from patients of this study. For detailed description, see Supplemental Methods.

Results

Clinical factors

A fifth of myomectomy patients experience a reintervention, most commonly a hysterectomy

During a median follow-up time of 11.4 years after the index myomectomy, 46/234 patients (20%) had undergone at least 1 leiomyoma-related reintervention (Figure 1). Altogether 36/46 patients had undergone 1 reintervention, 8 patients had undergone 2, and 2 patients had undergone 3 reinterventions. The median age was 34 years at the index myomectomy and 41 years at the first repeat procedure. Hysterectomy was the most common procedure with 25/46 patients (54%). Table 1 shows clinical information and tumor characteristics at the time of the index operation, as well as a comparison between patients with and without a reintervention. The median number of leiomyomas removed at the index operation was significantly higher in women with a reintervention compared to women without a reintervention (P=.027).

Surgical approach of the index myomectomy is not associated with the risk of repeat operation

Comparison of the cumulative rates of reinterventions between open abdominal and laparoscopic myomectomy patients is presented in Figure 2, A. At the end of follow-up, Kaplan-Meier estimate of the rate of reintervention was 26% for an open abdominal myomectomy and 19% for a laparoscopic myomectomy. The difference was not statistically significant (log rank P=.156). Figure 2, B shows the cumulative rate of reintervention according to the molecular status. Here, patients were grouped based on the molecular drivers detected in the index leiomyomas. For instance, *MED12* refers to patients who harbored a *MED12* mutation in all removed leiomyomas, and HMGA2 to patients who only had HMGA2 positive leiomyomas. Patients with multiple leiomyomas with different driver alterations were categorized in the group "Multiple." At the end of follow-up, Kaplan-Meier estimate of the rate of reintervention varied from 12% for patients with only wild type (WT) leiomyomas to 33% for patients with only FH-deficient leiomyomas. There was no statistically significant difference between the groups (log rank P=.445).

Clinical risk factors for leiomyoma-related reintervention

In the Kaplan-Meier analysis, postoperative parity and history of myomectomy produced significant (log rank P=.001) or nearly significant (log rank P=.052) P values, respectively. These factors were further analyzed with the Cox proportional hazards model together with age and number of removed leiomyomas, which both have been previously reported as risk factors for reintervention[6,7] (Table 2). In the multiple Cox model, the number of removed leiomyomas was an independent risk factor for reintervention

TABLE 1
Clinical characteristics of the study participants at the time of index myomectomy

Characteristics	Index myomectomy patients	Patients with reintervention	Patients without reintervention	*P* value
Number of patients	234	46	188	
Ethnicity, n (%)[a]				.576[c]
Finnish and other non-African	211 (91)	41 (89)	170 (91)	
African	21 (9.0)	5 (11)	16 (8.6)	
Prior myomectomy, n (%)	15 (6.4)	6 (13)	9 (4.8)	.084[c]
Infertility, n (%)	57 (24)	16 (35)	41 (22)	.084[d]
Median preoperative number of pregnancies (range)	0 (0–9)	0 (0–4)	0 (0–9)	.920[e]
Median age at operation, y (range)	34 (17–45)	34 (23–41)	34 (17–45)	.479[e]
Median body mass index, kg/m2 (range)[b]	23 (17–45)	24 (19–42)	23 (17–45)	.074[e]
Surgical method, n (%)				.141[d]
Abdominal myomectomy	119 (51)	28 (61)	91 (48)	
Laparoscopic myomectomy	115 (49)	18 (39)	97 (52)	
Morcellator used, n (%)	94 (40)	14 (30)	80 (43)	.179[d]
Median number of leiomyomas removed (range)	1 (1–13)	1.5 (1–12)	1 (1–13)	**.027**[e]
Median diameter of the largest leiomyoma, cm (range)	7 (1.5–20)	6.5 (2–16)	7 (1.5–20)	.387[e]
Molecular drivers[f], n (%)				.461[d]
MED12	123 (53)	27 (59)	96 (51)	
HMGA2	29 (12)	5 (11)	24 (13)	
FH	10 (4.3)	3 (6.5)	7 (3.7)	
WT	46 (20)	5 (11)	41 (22)	
Multiple drivers	26 (11)	6 (13)	20 (11)	

$P<.05$ is considered statistically significant (in bold).

FH, fumarate hydratase; *HMGA2*, high mobility group AT-hook 2; *MED12*, mediator complex subunit 12; *WT*, wild type.

[a] 2 missing values; [b] 15 missing values; [c] Fisher's exact test; [d] Pearson chi-square test; [e] Mann-Whitney *U* test; [f] Patients were grouped based on the molecular drivers detected in the index leiomyomas. Patients with multiple leiomyomas harboring different drivers were categorized in the "Multiple drivers" group.

(hazard ratio 1.21; 95% confidence interval [CI] 1.09–1.34; $P<.001$). Postoperative parity (hazard ratio 0.23; 95% CI 0.09–0.60; $P=.002$) and older age at the index myomectomy (hazard ratio 0.94; 95% CI 0.89–0.99; $P=.028$) reduced the risk for reintervention. Median diameter of the largest leiomyoma or median body mass index were not associated with the risk of reintervention.

Molecular factors

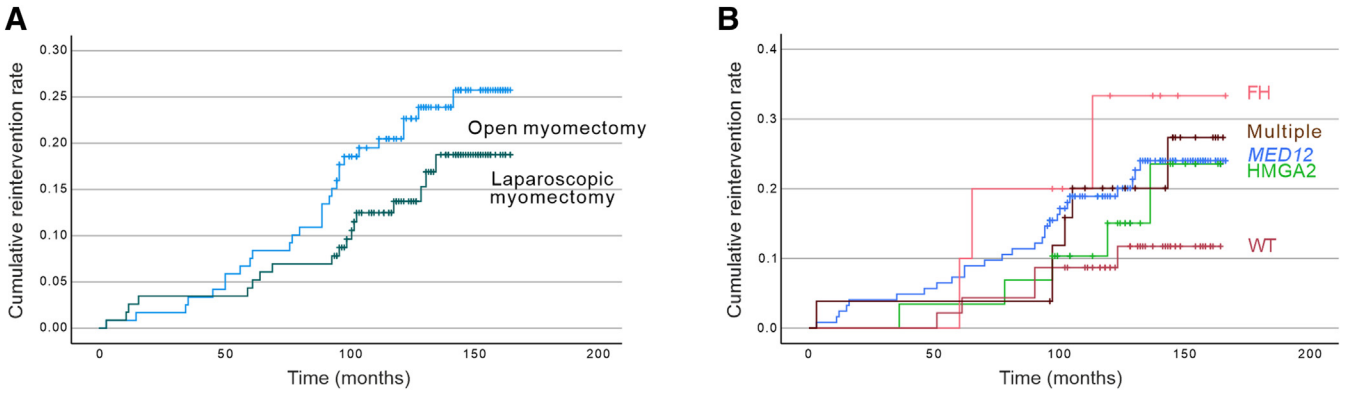
Distribution of molecular drivers in index and nonindex leiomyomas

The frequency of *MED12* mutations, HMGA2 overexpression, and FH-deficiency in 62 index and 71 nonindex leiomyomas is shown in Figure 3, A (Supplemental Table 2). Compared to the index leiomyomas, the frequency of *MED12* mutations was lower among leiomyomas from reinterventions while the frequency of HMGA2 overexpression and FH-deficiency was higher. Twenty-two out of 33 (67%) patients had the same leiomyoma driver alteration in the index and nonindex leiomyomas, indicating potentially clonally related tumors (Figure 3, B). Most patients (27/33) whose samples were included in molecular analyses underwent 1 leiomyoma-related reoperation. Six patients underwent 2 or more reoperations, 4 of whom had the same driver alteration (1 HMGA2, 2 FH, and 1 WT) in all removed leiomyomas (Figure 3, C).

Clonally related uterine leiomyomas confirmed in patients with repeat operations

To further evaluate the clonal relationship of the tumors with the same driver alteration in both the index and nonindex tumors, we conducted whole-exome sequencing on 52 tumors from 21 patients (1 patient was excluded due to unavailability of the index sample). We analyzed SCNAs from all the tumors, while point mutations and indels were

FIGURE 2
Kaplan-Meier curves showing the cumulative rate of reintervention after myomectomy



(**A**) The cumulative rate of reintervention according to the surgical approach at index myomectomy (open/laparoscopic). (**B**) The cumulative rate of reintervention according to the molecular driver alterations of the leiomyomas removed in the index myomectomy. The "Multiple" group refers to patients with multiple leiomyomas with different driver alterations.

FH, fumarate hydratase; *HMGA2*, high mobility group AT-hook 2; *MED12*, mediator complex subunit 12; *WT*, wild type.

analyzed only from 32/52 tumors (12 patients) with available matching normal.

We confirmed the clonal relationship of tumors from 3 patients through shared SCNAs and point mutations (Figure 4). The index operations included 1 open abdominal myomectomy (patient 1038) and 2 laparoscopic myomectomies (patients 1730 and 1224) of which one utilized power morcellation (patient 1224). Two HMGA2-positive tumors were removed from patient 1224 with 9 years between the operations. These tumors exhibited a deletion on chromosome 17q that underlies a known cancer gene, neurofibromin 1 (*NF1*) (Figure 4, A and B). Additionally, the tumors shared 7 point mutations, including c.8204T>G (p.L2735R) in *NF1* leading to biallelic loss (Figure 4, C, Supplemental Table 3). Two FH-deficient tumors were removed from patient 1730 with 5 years between the operations. The SCNA analysis revealed deletions on chromosome 1p and 1q (Figure 4, A and B). Without matching normal, we were unable to perform gene-level clonality analysis. Patient 1038 harbored 2 WT tumors with 5 years between their removal. These tumors displayed a distinct pattern of shared deletions across chromosomes 1 and 14q, along with smaller deleted regions on chromosomes 2p, 7q, and 8q (Figure 4, A and B). Additionally, the tumors shared 6 point mutations, 2 of which involved cancer-related genes according to COSMIC (v99) Cancer Gene Census[26]: c.6155A>G (p.D2052G) in ubiquitin protein ligase E3 component n-recognin 5 (*UBR5*) and c.7087C>T (p.P2363S) in CREB binding protein (*CREBBP*) (Figure 4, C, Supplemental Table 3).

Overall, the tumors displayed relatively stable genomes, with the majority harboring no SCNAs (Figure 4, A). Analysis of gene-level mutations showed that most tumors (28/32) from the same patient had unique point mutations and indels (Figure 4, C). No clonal relationship was observed among the *MED12* tumor pairs (n=10; Figure 4, A and C). Clonality assessment was not possible for 16/52 tumors (7/21 patients) that lacked matching normal tissue and did not exhibit any SCNAs. Large structural rearrangements, for example, those associated with HMGA2 overexpression,

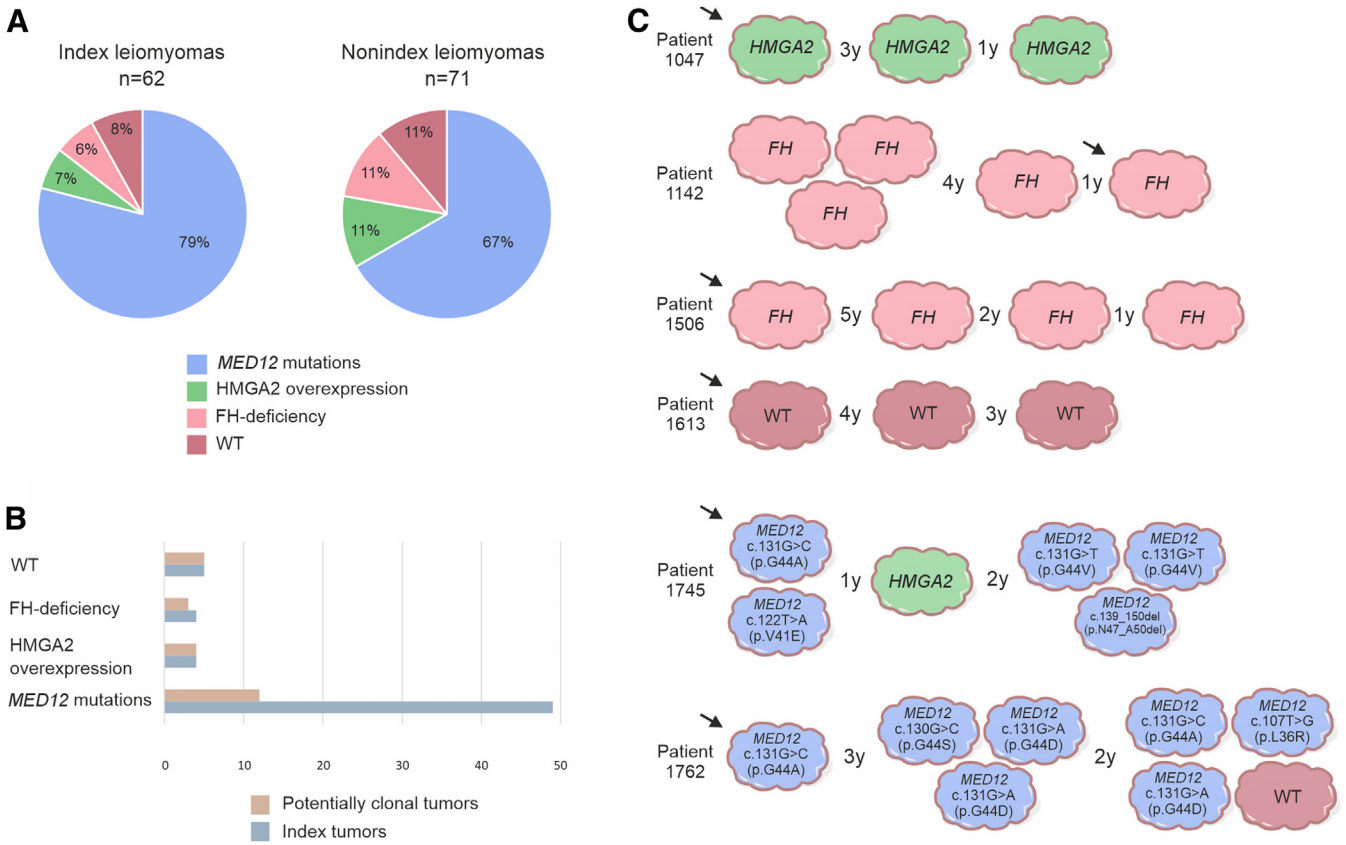
TABLE 2
Multiple Cox proportional hazards analysis of risk factors for a leiomyoma-related reintervention following myomectomy

Covariate	Coefficient	Standard error	*P* value	Hazard ratio	95% CI
Age	−0.061	0.028	**.028**	0.941	0.891−0.994
History of myomectomy	0.863	0.454	.057	2.371	0.974−5.774
Postoperative parity	−1.451	0.476	**.002**	0.234	0.092−0.596
Number of leiomyomas removed	0.187	0.052	**<.001**	1.206	1.088−1.336

P<.05 is considered statistically significant (in bold).

CI, confidence interval.

FIGURE 3
Well-established leiomyoma driver alterations in leiomyomas removed at the index and nonindex operations



(**A**) Frequency of the established leiomyoma driver alterations (*MED12* mutations, HMGA2 overexpression, FH-deficiency) in 62 index and 71 nonindex leiomyomas included in the study. One of the 71 nonindex leiomyomas displayed both FH-deficiency and HMGA2 overexpression. (**B**) Proportion of potentially clonally related tumors in each molecular leiomyoma subgroup. Potential clonal relationship is considered when the tumors from the index (*gray*) and repeat (*brown*) operation share the same driver alteration. (**C**) Six patients had undergone more than 1 leiomyoma-related reintervention. The figure shows the number of leiomyomas removed in each operation and the years between the operations. Driver alterations are shown within the schematic tumors. Four patients displayed the same driver alteration across all leiomyomas removed. Tumors from the index operations are marked with an *arrow*.

FH, fumarate hydratase; *HMGA2*, high mobility group AT-hook 2; *MED12*, mediator complex subunit 12; *WT*, wild type.

could not be detected with this sequencing approach.

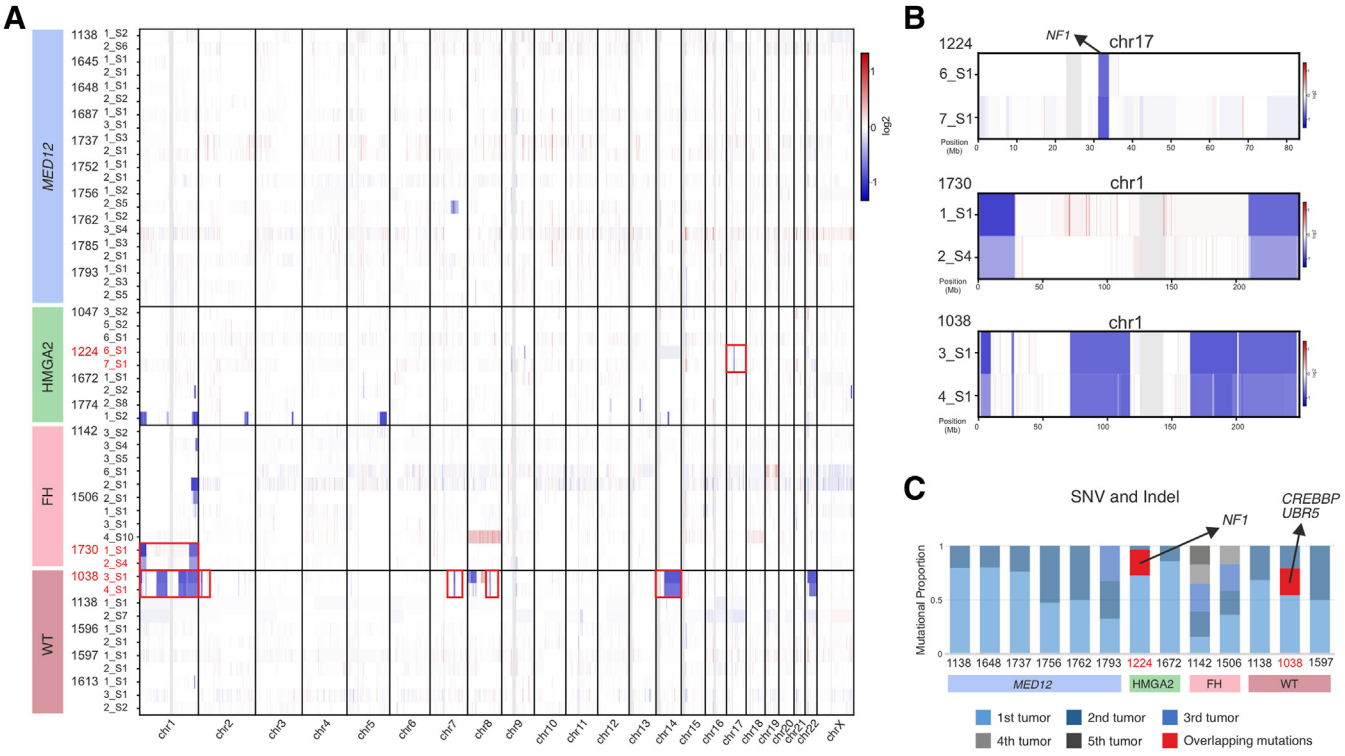
In addition to well-established driver mutations, we identified mutations in 2 leiomyoma-related genes cut like homeobox 1 (*CUX1*) and DEP domain containing 5 (*DEPDC5*). One *MED12* tumor (1756_2_S5) and 2 WT tumors from patient 1038 displayed 7q22 deletions underlying *CUX1* (Figure 4, A). Additionally, 1 *MED12* tumor (1138_1_S2) harbored a somatic *CUX1* nonsense mutation c.124A>T (p.K42*). *CUX1* has been reported as a haploinsufficient tumor suppressor gene related to tumor progression.[27] Two *MED12* tumors from patient 1687 harbored the same *DEPDC5* nonsense mutation c.346C>T (p.R116*). Due to the lack of matching normal, we were unable to confirm the somatic status of the mutation. Both tumors harbored the second hit in *DEPDC5*, a nonsense mutation c.4570C>T (p.Q1524*) in tumor 1687_1_S1 and a frameshift mutation c.4624del (p.E1542Sfs*32) in tumor 1687_3_S1, supporting the notion that biallelic loss of *DEPDC5* is a secondary driver alteration related to tumor progression.[21]

Germline *FH* mutations in patients with repeat interventions

Three patients had undergone multiple operations due to FH-deficient tumors (Figure 5). All patients carried a germline *FH* mutation. A somatic second hit was detected in 9/11 FH-deficient tumors. SCNA indicated deletions in 1q encompassing *FH* in 6 of the 11 tumors (Supplemental Figure 1).

Patient 1142 had undergone 3 operations with altogether 5 tumors removed. Analysis of a normal tissue confirmed a c.911del (p.P304Lfs*25) germline

FIGURE 4
Whole-exome sequencing reveals the somatic mutational profile of leiomyomas with a consistent driver alteration in both the index and nonindex tumor



(**A**) Genome-wide view presenting the somatic copy number profiles of 52 tumors from 21 patients with repeat operations. Tumors from each patient are listed chronologically, starting from the first occurrence. Clonally related tumors and their identical genetic alterations are indicated in red. Patient 1138 harbored both *MED12* and WT tumors. (**B**) A close-up of identical copy number alterations observed in tumors from 3 patients. Patient 1224 had 2 tumors displaying HMGA2 overexpression and identical deletion in chromosome 17 (*top*). Two tumors from patient 1730 showed FH-deficiency and identical deletions in chromosome 1p and 1q (*middle*). Two tumors from patient 1038 were WT for all driver alterations and shared an identical deletion pattern in chromosome 1 (*bottom*). (**C**) The distribution of unique and shared somatic point mutations and indels in tumors from the same patient. The analysis included 32 tumors from 12 patients with available normal tissue sample. Most tumors from the same patient harbored only unique mutations indicating independent origin. Tumors from patients 1224 and 1038 showed also shared mutations referring to clonal origin. Shared mutations included mutations in the known cancer genes *NF1*, *CREBBP*, and *UBR5*.

CREBBP, CREB binding protein; *FH*, fumarate hydratase; *HMGA2*, high mobility group AT-hook 2; *MED12*, mediator complex subunit 12; *NF1*, neurofibromin 1; *SNV*, single-nucleotide variant; *UBR5*, ubiquitin protein ligase E3 component n-recognin 5; *WT*, wild type.

mutation. Patient 1506 underwent 4 operations, with a single tumor removed in each. A c.1256C>T (p.S419L) germline mutation was detected in the normal tissue sample. Patient 1730 had had 2 operations with 1 tumor removed in each. Both tumors displayed the same c.1027C>T (p.R343*) nonsense mutation and a deletion as a second hit. Normal tissue sample was not available, but the patient's medical records confirmed HLRCC and the nonsense mutation has been reported as a germline mutation in a Finnish patient with HLRCC.[28]

Somatic *YEATS4* mutations in leiomyomas from repeat operations

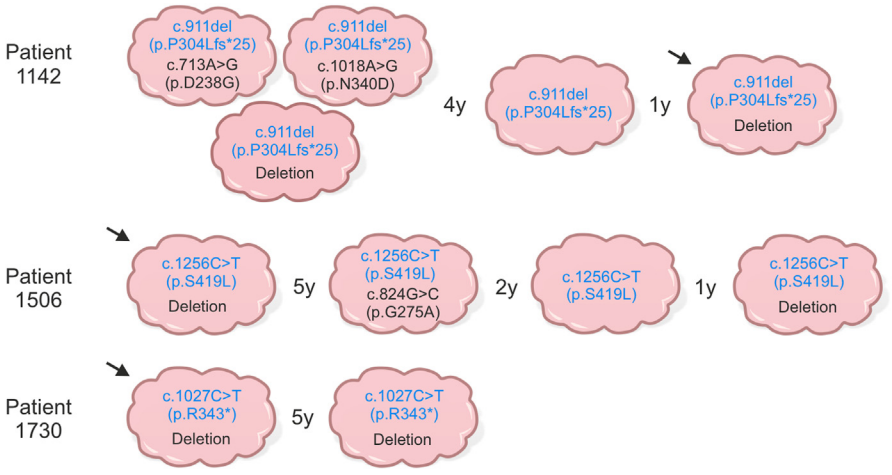
Five patients underwent multiple operations with WT leiomyomas, resulting in the removal of altogether 11 tumors. Whole-exome sequencing data was examined to identify mutations potentially related to tumorigenesis. We analyzed genes that were recurrently mutated by nonsynonymous point mutations and indels. This revealed *YEATS4* as the only recurrently mutated gene, with mutations identified in 6 tumors from 3 patients (Figure 6, A). All *YEATS4* mutations were somatic and distinct, including splice site, frameshift, and missense mutations distributed throughout the gene (Figure 6, B). All mutations were validated using Sanger sequencing (Supplemental Figure 2).

Comments

Principal findings

This retrospective cohort study shows that reintervention after an initial laparoscopic or abdominal myomectomy is common (20% after 11 years). The number of leiomyomas removed during the index procedure was an independent

FIGURE 5
Germline and somatic *FH* mutations in repeat FH-deficient leiomyomas



Three patients had undergone multiple operations due to FH-deficient leiomyomas. All patients harbored a germline *FH* mutation indicative of HLRCC-syndrome. Time between the operations is presented between the tumors (in years). Germline mutations are indicated in *blue* and somatic mutations in *black*. Tumors from the index operations are marked with an *arrow*.

FH, fumarate hydratase; *HLRCC*, hereditary leiomyomatosis and renal cell cancer.

risk factor for reintervention, while increasing age and postoperative parity were protective factors. The surgical approach of the index myomectomy (laparoscopic/abdominal) did not affect the risk of reintervention. Molecular characterization of the index and non-index leiomyomas from 33 patients revealed a clonal relationship of the tumors in 3 patients. Leiomyomas with *MED12* mutations were not among the clonally related tumors. Germline predisposition to leiomyomas was confirmed in all 3 patients with reinterventions due to FH-deficient leiomyomas. Finally, distinct somatic *YEATS4* mutations were identified in nonclonal tumors obtained from multiple operations of 3 patients.

Results in the context of what is known

Clinical factors related to the risk of reintervention

Varying estimates of reintervention rate after myomectomy have been presented. In a systematic review, the reintervention risk was 12% 5 years after laparoscopic or abdominal myomectomy.[5] Even higher reintervention rates were reported in a large U.S. cohort study (19% at 5 years), and in a British multicenter database study (25% after a median follow-up of 2.7 years).[3,30] A reintervention rate of 20% was observed in our study after a considerably longer follow-up (median 11 years). Our findings are in line with previous research showing that higher number of leiomyomas removed in the initial myomectomy increases the risk for repeat leiomyoma occurrence or reintervention.[4,6,7,31] Postoperative gestation has been shown to reduce the risk of leiomyoma recurrence assessed by ultrasound.[4] Comparably, we demonstrate that postoperative parity is associated with a lower risk of reintervention. However, a recent study implied that on cellular level, mechanical stress related to pregnancy might in fact facilitate leiomyoma tumorigenesis.[32] It is likely that the negative correlation between parity and leiomyoma-related reintervention only reflects the adverse effect of leiomyomas on conceiving. The effect of age during the initial myomectomy on leiomyoma recurrence remains controversial. In one study, age at initial surgery of less than 35 years was associated with the lowest risk of recurrence,[31] while in another study an age of 30 to 40 years increased the risk of symptomatic recurrence.[6] In our study, the risk of reintervention was the highest in those who were the youngest during the initial myomectomy. Finally, our results support the earlier findings that leiomyoma-related reintervention rates are comparable between laparoscopic and abdominal myomectomy.[3,33]

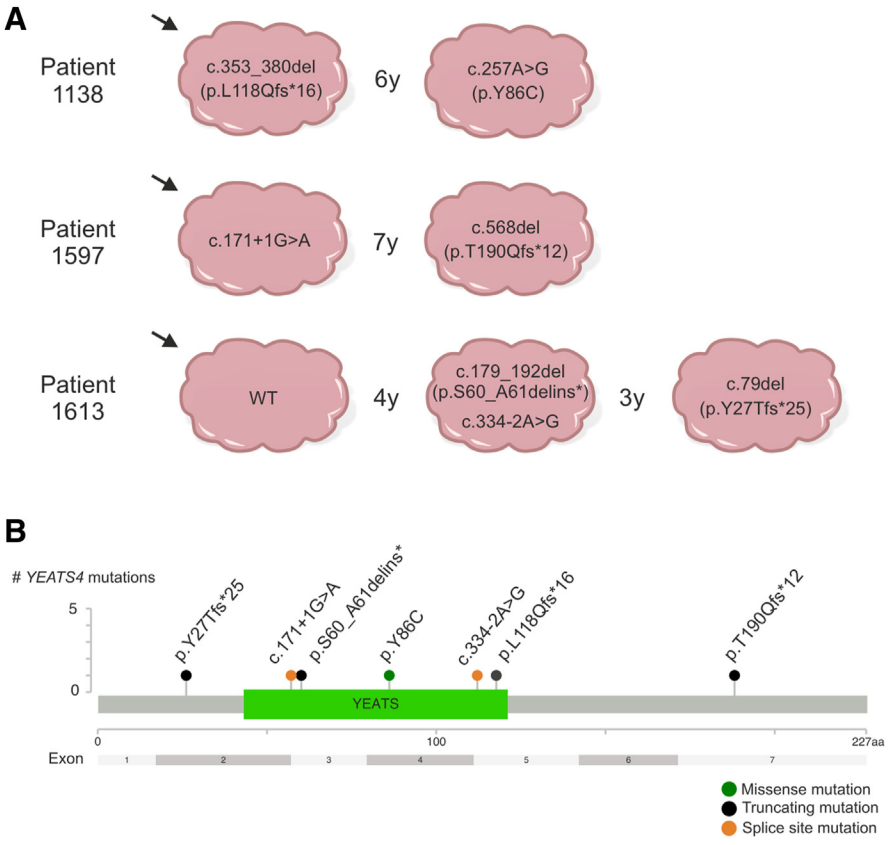
Molecular factors related to the risk of reintervention

Molecular studies on leiomyoma recurrence are scarce. Two studies have offered insights into predictive biomarkers for repeat leiomyoma occurence.[34,35] While these studies primarily focus on repeat likelihood, they do not elucidate the underlying biological mechanisms causing repeat tumors. Here, identical point mutations and SCNA revealed clonal relationships in FH, HMGA2, and WT leiomyomas from 3 patients. Despite the relatively low incidence of clonally related tumors (3/33 patients; 9%), our results align with previous reports that have suggested that concurrent leiomyomas are typically independent lesions.[18,19] We found no clonal associations among tumor pairs with identical *MED12* mutations, which is consistent with earlier studies on concurrent leiomyomas.[21] Overall, the frequency of clonally related tumors may be somewhat larger than reported here, as clonality analysis was not possible in all tumor pairs with identical driver mutations.

FH-deficiency was observed in repeat tumors of 3 patients, all of whom were confirmed to have HLRCC. While FH-deficiency due to somatic mutations is more common than HLRCC,[11] our high cumulative reintervention rate (33%) of patients with only FH-deficient leiomyomas in the index operation was completely explained by germline mutations. Compared to women with sporadic leiomyomas, women with HLRCC have been shown to develop a higher number of leiomyomas that are also diagnosed and operated at a younger age.[28,36,37] Our results suggest that HLRCC is also associated with leiomyoma-related reinterventions.

Somatic mutations in the SRCAP complex genes, including *YEATS4*, have been identified in ~2% of uterine

FIGURE 6
Somatic *YEATS4* mutations identified in leiomyomas from repeat operations



(**A**) Somatic mutations in *YEATS4* were identified in altogether 6 tumors from 3 patients. Number of years between the operations is presented. Tumors from the index operations are marked with an *arrow*. (**B**) Lollipop plot of the *YEATS4* mutations show that the mutations are distributed throughout the gene. Visualization through MutationMapper.[29]

WT, wild type; *YEATS4*, YEATS domain-containing protein 4.

leiomyomas.[14] We identified somatic *YEATS4* mutations in 6 leiomyomas from 3 patients with repeat operations (3/33; 9%). All mutations were unique, and the tumors were not clonally related. Similarly, Berta et al (2021) reported 6 patients who had had at least 2 concurrent SRCAP tumors, and 5 of those patients had tumors with *YEATS4* mutations. Recently, also germline mutations in SRCAP complex genes were reported, contributing to the moderate penetrance of uterine leiomyoma predisposition.[38] Further research is needed to elucidate the role of *YEATS4* in repeat leiomyoma occurrence.

Clinical and research implications

Repeat tumor occurrence remains a challenge for leiomyoma patients with uterine preserving treatment options. Compared to uterine artery embolization and high-intensity focused ultrasound, myomectomy is associated with the lowest reintervention risk.[5,39] Still, according to our results, 1 in 5 women undergo at least 1 reintervention within 11 years after myomectomy. The risk of reintervention is increased in women who have had multiple leiomyomas removed or who have experienced myomectomy at a young age, suggesting that hereditary factors may play a role in repeat tumor occurrence. Indeed, genome-wide association studies have identified several low-risk germline variants that associate with uterine leiomyomas,[40–43] and further research is warranted to understand how these variants contribute to leiomyoma development. Genetic predisposition due to germline *FH* mutations increases the risk for reintervention and should be kept in mind when treating women with multiple leiomyoma-related operations.[44] The role of *YEATS4* mutations in repeat leiomyomas warrants further studies, especially regarding the role of germline mutations and environmental factors affecting the uterus that may predispose to the development of *YEATS4* positive leiomyomas. A novel important finding of this study is that repeat leiomyomas only rarely are of clonal origin. Especially leiomyomas with *MED12* mutations—the most common molecular subtype—did not appear as clonally related tumors. Further, the rarity of clonally related tumors implies that the myomectomy procedure itself does not pose a significant risk to leiomyoma recurrence, even if power morcellation has been performed in the initial procedure.

Strength and limitations

Here, for the first time, we have systematically analyzed genetic driver alterations and clonal relationship of leiomyomas from repeat operations. The study presents a unique combination of detailed clinical information and molecular data from the tumors. Also, the follow-up time is significantly longer than in previous studies analyzing clinical risk factors for tumor recurrence or reintervention following myomectomy.[3,4,6,7,30,31]

Our study has some limitations. As the follow-up of patients was performed from medical records, we cannot exclude the possibility of missing information on repeat procedures or postoperative deliveries due to patients relocating to another area. The clonality analysis was not possible in all tumor pairs with an identical driver alteration due to lack of normal tissue sample and no SCNAs. The use of FFPE samples is known to affect high-throughput sequencing data quality. We thus applied strict parameters to remove false positives, but this may have resulted in the exclusion of true alterations. Expanding this research to larger cohorts and a multicenter setting is needed to validate and generalize the results.

Conclusions

Reinterventions are common after surgical myomectomy. The number of leiomyomas removed is a risk factor for later reintervention, while increasing age and postoperative parity reduce the risk. The reintervention risk is similar after laparoscopic and abdominal myomectomy. Molecular analyses revealed that while uterine leiomyomas usually form independently, a subset is clonally related. In some patients, leiomyoma-related reintervention can be attributed to genetic predisposition, especially to germline *FH* mutations. Further studies are needed to elucidate the role of *YEATS4* in repeat leiomyoma occurrence. ■

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Appendix

Supplemental Methods

DNA extraction

DNA was extracted from 7 to 14 whole tissue sections each with a thickness of 10 μm, or from 8 to 20 cores of 0.8 mm diameter each. Conventional phenol-chloroform DNA extraction method was used.

Sanger sequencing

Sanger sequencing of *MED12* (exons 1 and 2) and *YEATS4* (exons 2, 3, 4, 5, and 7) was performed at the Institute for Molecular Medicine Finland (FIMM), Helsinki, Finland, using Applied Biosystems ABI3730 Automatic DNA Sequencer. Electropherograms were analyzed by Mutation Surveyor (SoftGenetics, State College, PA) and FinchTV (Geospiza, Inc, Seattle, WA) and further confirmed through visual inspection.

Immunohistochemistry

HMGA2 expression level and FH status were analyzed by immunohistochemistry. We utilized an anti-2-succinylcysteine (2SC) antibody to detect FH status.[1,2] Immunohistochemistry was performed on 5 μm whole tissue sections. Following deparaffination, heat-induced antigen retrieval was carried out using citrate buffer (pH 6.0). Endogenous peroxidase blocking was followed by overnight incubation with the primary antibody anti-HMGA2 (1:2000, 59170AP, Biocheck, South San Francisco, CA) or anti-2SC (1:500, crb2005017d, Cambridge Research Biochemicals, Billingham, UK). Following post-antibody blocking (BrightVision plus, Immunologic BV, Duiven, The Netherlands), the samples were incubated with a secondary poly-HRP antibody (Poly-HRP-GAM/R/R IgG, Immunologic). Antibody detection was achieved by DAB Quanto (Thermo Fisher Scientific, Waltham, MA) system. Pathologists specialized in gynecological tumors (AP and RB) conducted the scoring for both stainings using 4 grades: ++ = strong staining, + = weak staining, (+) = single-cell positivity, and − = no staining. Samples with strong staining were classified as positive.

Whole-exome sequencing

Whole-exome sequencing was conducted on 52 tumor and 12 normal tissue samples. DNA libraries for most samples were produced with the Twist Library Preparation EF 2.0 kit (Twist Bioscience, San Francisco, CA) and enriched using Twist Comprehensive Exome probes (Twist Bioscience). DNA libraries for 3 samples (2 tumors and 1 normal) were created using the KAPA Hyper Prep kit (Roche NimbleGen, Madison, WI) and enriched with KAPA HyperExome probes (Roche). Sequencing was performed using the Illumina NovaSeq 6000 System (Illumina, San Diego, CA) at FIMM or the Illumina NextSeq500 System (Illumina) at the Biomedicum Functional Genomics Unit (FuGU), Helsinki, Finland. For detailed information on the samples in whole-exome sequencing, refer to Supplemental Table 1. Data preprocessing was performed with the Genome Analysis ToolKit 4 best practices.[3] The reads were trimmed by Trimmomatic and aligned against the Genome Reference Consortium Human Build 38 genome using Burrows—Wheeler Aligner BWA-MEM.[4,5] Duplicate reads were removed using Mark Duplicates and base quality scores were recalibrated using BaseRecalibrator.[3]

Paired and non-paired joint somatic variant calling was performed using Mutect2 with default parameters.[3] FFPE artifacts were identified using LearnReadOrientationModel.[3] Single-nucleotide variants and indels with an allelic fraction (AF) of at least 0.25, an allelic count of at least 6, and a sequencing depth of at least 12 were analyzed using BasePlayer.[6] We also considered lower allelic fraction mutations ($\geq$0.1 AF) if they had a minimum coverage of 60 reads. Detected variants were compared to an in-house panel of normals drawn from 59 exomes and 27 genomes and a panel of normals generated from the 1000 genomes project.[7] Variants present in the Genome Aggregation Database (exomes and genomes, v2.0.1 and v3, respectively) with an AF$\geq$0.0001 were filtered out.[8]

Somatic copy number alterations (SCNA) were identified using CNVkit with default parameters.[9] The SCNAs were compared against a pooled normal generated using 12 normal tissue samples from patients of this study. For visualization, heatmaps of the SCNAs were generated using -d option to de-emphasize low-amplitude segments. For the clonality assessment through shared SCNAs, we considered segments of $>$2 Mb in size and a threshold of <-0.4 for deletions and >0.75 for amplifications.

Statistical analyses

Statistical analyses were performed in SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Macintosh, version 28.0, Armonk, NY). Comparison of the clinical characteristics between patients with reintervention and patients without reintervention was performed with the Pearson chi-square test and the Fisher's exact test for categorical variables, and with the Mann-Whitney *U* test for continuous variables. The Kaplan-Meier estimator with log-rank test was used to describe and compare the risk of a leiomyoma-related reintervention across surgical approaches and molecular driver classes. The Cox proportional hazards regression model was used to further estimate the effect of clinical factors on the risk of a recurrent procedure. The proportional hazards assumption was assessed by visual inspection of Kaplan-Meier curves for categorical variables and by computing time-dependent covariates of the continuous variables. Two-sided *P* values $<$.05 were considered statistically significant.

Supplemental References

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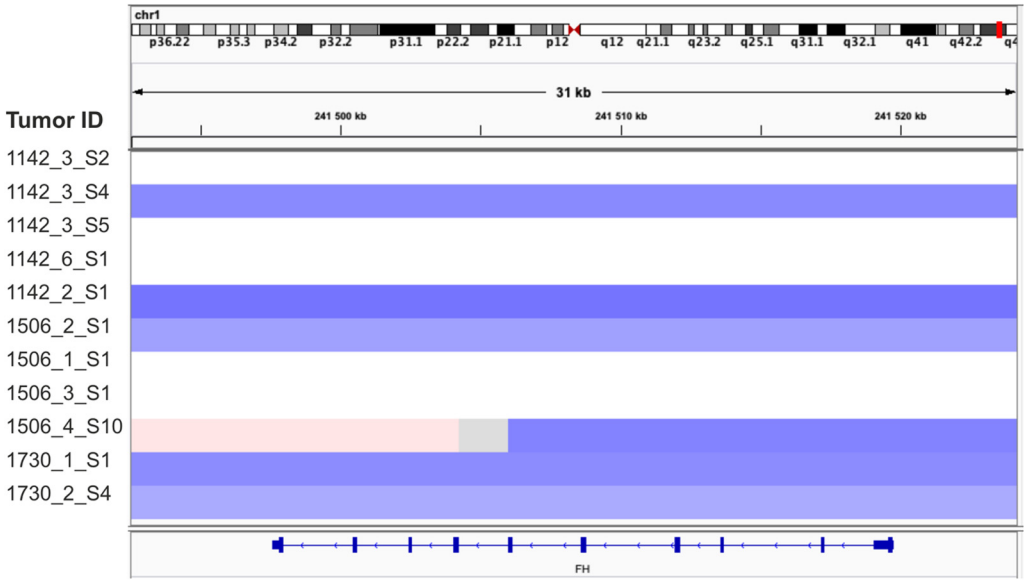
SUPPLEMENTAL FIGURE 1

Three patients had undergone multiple leiomyoma-related procedures due to FH-deficient tumors



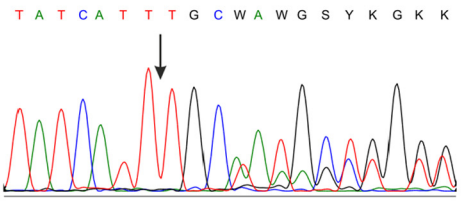
Altogether 11 tumors had been removed in these operations. Somatic copy number analysis showed deletions encompassing *FH* in 6 tumors. Visualization through IGV 2.5.0.[10]

SUPPLEMENTAL FIGURE 2
Sanger sequencing chromatograms of the identified *YEATS4* mutations

Patient 1138

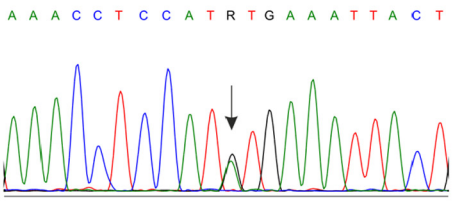
Tumor 1138_1_S1

c.353_380del
(p.L118Qfs*16)



Tumor 1138_2_S7

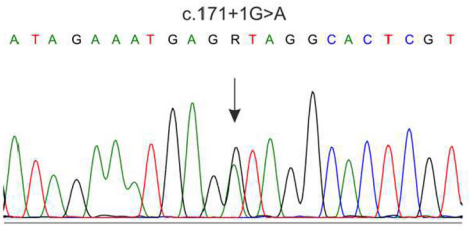
c.257A>G
(p.Y86C)



Patient 1597

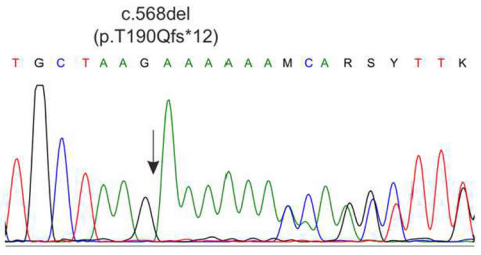
Tumor 1597_1_S1

c.171+1G>A



Tumor 1597_2_S1

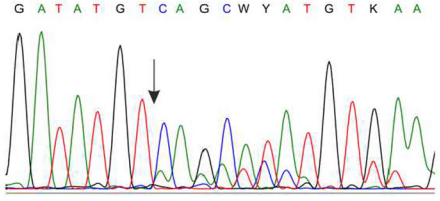
c.568del
(p.T190Qfs*12)



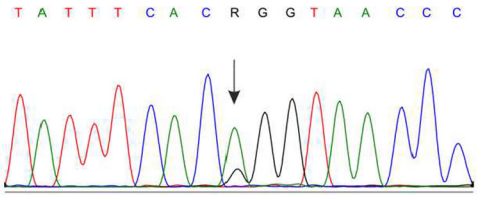
Patient 1613

Tumor 1613_3_S1

c.179_192del
(p.S60_A61delins*)

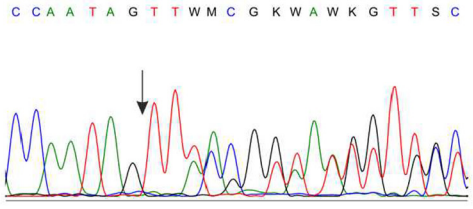


c.334-2A>G



Tumor 1613_2_S2

c.79del
(p.Y27Tfs*25)



SUPPLEMENTAL TABLE 1
Uterine leiomyoma and normal tissue samples in whole-exome sequencing

Patient ID	Sample ID	Tissue type	Cohort	Leiomyoma subtype	Whole-exome sequencing[a]	Average coverage
1038	1038_4_S6	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	88
1038	1038_3_S1	Leiomyoma	Index	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	59
1038	1038_4_S1	Leiomyoma	Nonindex	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	113
1047	1047_3_S2	Leiomyoma	Index	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	70
1047	1047_5_S2	Leiomyoma	Nonindex	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	80
1047	1047_6_S1	Leiomyoma	Nonindex	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	120
1138	1138_2_S12[b]	Ovarian	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome/ 2×75 bp, NextSeq500, KAPA HyperPlus, KAPA HyperExome*	102/95
1138	1138_1_S2	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	101
1138	1138_1_S1	Leiomyoma	Index	WT	*2×75 bp, NextSeq500, KAPA HyperPlus, KAPA HyperExome*	96
1138	1138_2_S6	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	91
1138	1138_2_S7	Leiomyoma	Nonindex	WT	*2×75 bp, NextSeq500, KAPA HyperPlus, KAPA HyperExome*	99
1142	1142_1_S1	Endometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	68
1142	1142_3_S2	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	78
1142	1142_3_S4	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	97
1142	1142_3_S5	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	84
1142	1142_6_S1	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	72
1142	1142_2_S1	Leiomyoma	Index	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	109
1224	1224_9_S1	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	155
1224	1224_6_S1	Leiomyoma	Index	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	78
1224	1224_7_S1	Leiomyoma	Nonindex	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	88
1506	1506_4_S4	Cervical	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	109
1506	1506_2_S1	Leiomyoma	Index	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	84

(continued)

SUPPLEMENTAL TABLE 1
Uterine leiomyoma and normal tissue samples in whole-exome sequencing *(continued)*

Patient ID	Sample ID	Tissue type	Cohort	Leiomyoma subtype	Whole-exome sequencing[a]	Average coverage
1506	1506_1_S1	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	86
1506	1506_3_S1	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	115
1506	1506_4_S10	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	127
1596	1596_1_S1	Leiomyoma	Index	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	80
1596	1596_2_S1	Leiomyoma	Nonindex	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	64
1597	1597_2_S2	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	107
1597	1597_1_S1	Leiomyoma	Index	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	107
1597	1597_2_S1	Leiomyoma	Nonindex	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	76
1613	1613_1_S1	Leiomyoma	Index	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	83
1613	1613_3_S1	Leiomyoma	Nonindex	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	108
1613	1613_2_S2	Leiomyoma	Nonindex	WT	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	87
1645	1645_1_S1	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	121
1645	1645_2_S1	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	81
1648	1648_2_S8	Adipose	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	131
1648	1648_1_S1	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	94
1648	1648_2_S2	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	117
1672	1672_2_S7	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	102
1672	1672_1_S1	Leiomyoma	Index	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	84
1672	1672_2_S2	Leiomyoma	Nonindex	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	122
1687	1687_1_S1	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	165
1687	1687_3_S1	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	98
1730	1730_1_S1	Leiomyoma	Index	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	49
1730	1730_2_S4	Leiomyoma	Nonindex	FH	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	145

(continued)

SUPPLEMENTAL TABLE 1
Uterine leiomyoma and normal tissue samples in whole-exome sequencing *(continued)*

Patient ID	Sample ID	Tissue type	Cohort	Leiomyoma subtype	Whole-exome sequencing[a]	Average coverage
1737	1737_2_S10	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	121
1737	1737_1_S3	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	69
1737	1737_2_S1	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	93
1752	1752_1_S1	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	45
1752	1752_2_S1	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	113
1756	1756_2_S1	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	122
1756	1756_1_S2	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	37
1756	1756_2_S5	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	85
1762	1762_3_S7	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	125
1762	1762_1_S2	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	114
1762	1762_3_S4	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	101
1774	1774_2_S8	Leiomyoma	Nonindex	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	67
1774	1774_1_S2	Leiomyoma	Index	HMGA2	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	135
1785	1785_1_S3	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	82
1785	1785_2_S1	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	123
1793	1793_2_S7	Myometrium	-	-	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	119
1793	1793_1_S1	Leiomyoma	Index	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	97
1793	1793_2_S3	Leiomyoma	Nonindex	*MED12*	*2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome*	86
1793	1793_2_S5	Leiomyoma	Nonindex	*MED12*	2×100 bp, NovaSeq 6000 System, Twist Library Preparation EF 2.0, Twist Comprehensive Exome	95

[a] Whole-exome sequencing method details are presented as follows: read length, sequencing machine, library kit, and capture probes; [b] Normal sample (1138_2_S12) was sequenced twice in 2 separate sequencing batches. Somatic copy number alterations from 1138_1_S1 and 1138_2_S7 tumors were compared against 1138_2_S12 normal sample that was sequenced with KAPA library kit and capture probes.

SUPPLEMENTAL TABLE 2
Status of leiomyoma driver alterations in 133 leiomyomas from 33 patients

Patient ID	Sample ID	Cohort	*MED12*	HMGA2	2SC
1036	1036_4_S1	Index	c.133_147del (p.F45_P49del)	NEG	NEG
1036	1036_4_S2	Index	c.120_140delinsAAC (p.N40_N47delinsKT)	NEG	NEG
1036	1036_4_S3	Index	c.130G>A (p.G44S)	NEG	NEG
1036	1036_5_S1	Nonindex	c.130G>T (p.G44C)	NEG	NEG
1036	1036_5_S2	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1038	1038_3_S1	Index	WT	NEG	NEG
1038	1038_4_S1	Nonindex	WT	NEG	NEG
1047	1047_3_S2	Index	WT	POS	NEG
1047	1047_5_S2	Nonindex	WT	POS	NEG
1047	1047_6_S1	Nonindex	WT	POS	NEG
1130	1130_1_S1	Index	c.131G>T (p.G44V)	NEG	NEG
1130	1130_2_S1	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1132	1132_1_S1	Index	c.131G>A (p.G44D)	NEG	NEG
1132	1132_1_S2	Index	c.130G>A (p.G44S)	NEG	NEG
1132	1132_1_S3	Index	WT	NEG	POS
1132	1132_2_S1	Nonindex	WT	POS	NEG
1138	1138_1_S1	Index	WT	NEG	NEG
1138	1138_1_S2	Index	c.131G>T (p.G44V)	NEG	NEG
1138	1138_2_S6	Nonindex	c.131G>T (p.G44V)	NEG	NEG
1138	1138_2_S7	Nonindex	WT	NEG	NEG
1142	1142_2_S1	Index	WT	NEG	POS
1142	1142_3_S2	Nonindex	WT	NEG	POS
1142	1142_3_S4	Nonindex	WT	NEG	POS
1142	1142_3_S5	Nonindex	WT	NEG	POS
1142	1142_6_S1	Nonindex	WT	NEG	POS
1224	1224_6_S1	Index	WT	POS	NEG
1224	1224_7_S1	Nonindex	WT	POS	NEG
1377	1377_1_S2	Index	c.146_166del (p.P49_E55del)	NEG	NEG
1377	1377_2_S2	Nonindex	c.130G>A (p.G44S)	NEG	NEG
1506	1506_1_S1	Nonindex	WT	NEG	POS
1506	1506_2_S1	Index	WT	NEG	POS
1506	1506_3_S1	Nonindex	WT	POS	POS
1506	1506_4_S10	Nonindex	WT	NEG	POS
1596	1596_1_S1	Index	WT	NEG	NEG
1596	1596_2_S1	Nonindex	WT	NEG	NEG
1597	1597_1_S1	Index	WT	NEG	NEG
1597	1597_2_S1	Nonindex	WT	NEG	NEG
1597	1597_2_S13	Nonindex	c.130G>C (p.G44R)	NEG	NEG
1613	1613_1_S1	Index	WT	NEG	NEG

(continued)

SUPPLEMENTAL TABLE 2
Status of leiomyoma driver alterations in 133 leiomyomas from 33 patients *(continued)*

Patient ID	Sample ID	Cohort	*MED12*	HMGA2	2SC
1613	1613_2_S2	Nonindex	WT	NEG	NEG
1613	1613_3_S1	Nonindex	WT	NEG	NEG
1642	1642_1_S1	Index	c.131G>A (p.G44D)	NEG	NEG
1642	1642_2_S1	Nonindex	c.130G>A (p.G44S)	NEG	NEG
1645	1645_1_S1	Index	c.131G>T (p.G44V)	NEG	NEG
1645	1645_2_S1	Nonindex	c.131G>T (p.G44V)	NEG	NEG
1648	1648_1_S1	Index	c.130G>A (p.G44S)	NEG	NEG
1648	1648_2_S1	Nonindex	c.130G>C (p.G44R)	NEG	NEG
1648	1648_2_S2	Nonindex	c.130G>A (p.G44S)	NEG	NEG
1648	1648_2_S3	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1672	1672_1_S1	Index	WT	POS	NEG
1672	1672_2_S2	Nonindex	WT	POS	NEG
1672	1672_2_S5	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1672	1672_2_S6	Nonindex	c.107_141del (p.L36Pfs*8)	NEG	NEG
1687	1687_1_S1	Index	c.130G>A (p.G44S)	NEG	NEG
1687	1687_3_S1	Nonindex	c.130G>A (p.G44S)	NEG	NEG
1703	1703_1_S1	Index	c.130G>A (p.G44S)	NEG	NEG
1703	1703_3_S1[a]	Nonindex	c.139_156del (p.N47_S52del)	NEG	NEG
1703	1703_3_S1[a]	Nonindex	c.107T>G (p.L36R)	NEG	NEG
1703	1703_3_S2	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1708	1708_1_S1	Index	c.100-8T>A	NEG	NEG
1708	1708_2_S2	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1730	1730_1_S1	Index	WT	NEG	POS
1730	1730_2_S4	Nonindex	WT	NEG	POS
1737	1737_1_S1	Index	c.100-6_129del	NEG	NEG
1737	1737_1_S2	Index	c.131G>T (p.G44V)	NEG	NEG
1737	1737_1_S3	Index	c.131G>A (p.G44D)	NEG	NEG
1737	1737_2_S1	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1737	1737_2_S2	Nonindex	c.131G>C (p.G44A)	NEG	NEG
1737	1737_2_S3	Nonindex	c.130G>T (p.G44C)	NEG	NEG
1737	1737_2_S4	Nonindex	c.107T>G (p.L36R)	NEG	NEG
1737	1737_2_S6	Nonindex	c.127_147del (p.Q43_P49del)	NEG	NEG
1744	1744_1_S1	Index	c.127_138del (p.Q43_N46del)	NEG	NEG
1744	1744_1_S2	Index	c.131G>C (p.G44A)	NEG	NEG
1744	1744_1_S3	Index	c.107T>G (p.L36R)	NEG	NEG
1744	1744_2_S2	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1744	1744_2_S3	Nonindex	c.131G>T (p.G44V)	NEG	NEG
1745	1745_1_S1	Index	c.131G>C (p.G44A)	NEG	NEG
1745	1745_1_S2	Index	c.122T>A (p.V41E)	NEG	NEG

(continued)

SUPPLEMENTAL TABLE 2
Status of leiomyoma driver alterations in 133 leiomyomas from 33 patients *(continued)*

Patient ID	Sample ID	Cohort	*MED12*	HMGA2	2SC
1745	1745_2_S1	Nonindex	WT	POS	NEG
1745	1745_4_S1	Nonindex	c.131G>T (p.G44V)	NEG	NEG
1745	1745_4_S2	Nonindex	c.139_150del (p.N47_A50del)	NEG	NEG
1745	1745_4_S3	Nonindex	c.131G>T (p.G44V)	NEG	NEG
1752	1752_1_S1	Index	c.131G>T (p.G44V)	NEG	NEG
1752	1752_1_S2	Index	c.131G>A (p.G44D)	NEG	NEG
1752	1752_2_S1	Nonindex	c.131G>T (p.G44V)	NEG	NEG
1756	1756_1_S1	Index	c.84_98del (p.D28_K32del)	NEG	NEG
1756	1756_1_S2	Index	c.130G>A (p.G44S)	NEG	NEG
1756	1756_2_S5	Nonindex	c.130G>A (p.G44S)	NEG	NEG
1762	1762_1_S2	Index	c.131G>C (p.G44A)	NEG	NEG
1762	1762_2_S1	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1762	1762_2_S2	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1762	1762_2_S3	Nonindex	c.130G>A (p.G44S)	NEG	NEG
1762	1762_3_S2	Nonindex	c.107T>G (p.L36R)	NEG	NEG
1762	1762_3_S3	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1762	1762_3_S4	Nonindex	c.131G>C (p.G44A)	NEG	NEG
1762	1762_3_S5	Nonindex	WT	NEG	NEG
1773	1773_1_S1	Index	c.130G>A (p.G44S)	NEG	NEG
1773	1773_1_S2	Index	c.130G>A, 131G>T (G44I)	NEG	NEG
1773	1773_1_S3	Index	c.130G>A (p.G44S)	NEG	NEG
1773	1773_1_S4	Index	c.130G>A (p.G44S)	NEG	NEG
1773	1773_2_S1	Nonindex	c.100-8T>A	NEG	NEG
1774	1774_1_S1	Index	c.117_128del (p.N40_Q43del)	NEG	NEG
1774	1774_1_S2	Index	WT	POS	NEG
1774	1774_2_S1	Nonindex	c.130G>T (p.G44C)	NEG	NEG
1774	1774_2_S2	Nonindex	c.102_110del (p.D34_T37delinsE)	NEG	NEG
1774	1774_2_S8	Nonindex	WT	POS	NEG
1775	1775_1_S1[b]	Index	c.131G>A (p.G44D)	NEG	NEG
1775	1775_1_S2	Index	c.128A>C (p.Q43P)	NEG	NEG
1775	1775_1_S3	Index	c.130G>T (p.G44C)	NEG	NEG
1775	1775_1_S4	Index	c.100-10_135del	NEG	NEG
1775	1775_2_S1	Nonindex	c.146_166del (p.P49_E55del)	NEG	NEG
1775	1775_2_S2	Nonindex	c.130G>C (p.G44R)	NEG	NEG
1775	1775_2_S3	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1785	1785_1_S1	Index	c.130G>A (p.G44S)	NEG	NEG
1785	1785_1_S2	Index	c.100-8T>A	NEG	NEG
1785	1785_1_S3	Index	c.107T>G (p.L36R)	NEG	NEG
1785	1785_1_S4	Index	c.131G>A (p.G44D)	NEG	NEG

(continued)

SUPPLEMENTAL TABLE 2
Status of leiomyoma driver alterations in 133 leiomyomas from 33 patients *(continued)*

Patient ID	Sample ID	Cohort	*MED12*	HMGA2	2SC
1785	1785_1_S5	Index	c.131G>C (p.G44A)	NEG	NEG
1785	1785_1_S6	Index	c.131G>T (p.G44V)	NEG	NEG
1785	1785_2_S1	Nonindex	c.107T>G (p.L36R)	NEG	NEG
1790	1790_1_S1	Index	c.130G>A (p.G44S)	NEG	NEG
1790	1790_1_S2	Index	c.131G>A (p.G44D)	NEG	NEG
1790	1790_2_S1	Nonindex	c.131G>C (p.G44A)	NEG	NEG
1793	1793_1_S1	Index	c.131G>A (p.G44D)	NEG	NEG
1793	1793_1_S2	Index	c.100-8T>A	NEG	NEG
1793	1793_1_S3[b]	Index	c.131G>A (p.G44D)	NEG	NEG
1793	1793_1_S4	Index	c.130G>A (p.G44S)	NEG	NEG
1793	1793_1_S5	Index	c.130G>C (p.G44R)	NEG	NEG
1793	1793_2_S1	Nonindex	WT	NEG	NEG
1793	1793_2_S2	Nonindex	c.130G>T (p.G44C)	NEG	NEG
1793	1793_2_S3	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1793	1793_2_S5	Nonindex	c.131G>A (p.G44D)	NEG	NEG
1793	1793_2_S6	Nonindex	c.131G>T (p.G44V)	NEG	NEG

[a] Sample 1703_3_S1 contained sections from 2 separate leiomyomas; [b] Tissue material was not sufficient for further analysis with whole exome sequencing.

SUPPLEMENTAL TABLE 3
Identical somatic point mutations in the clonally related tumors from patients 1038 and 1224

Patient ID	Sample ID	Hugo symbol	HGVSc	HGVSp	Variant allele fraction
1038	1038_3_S1	*ARHGAP32*	c.5045G>A	p.(C1682Y)	0.46
1038	1038_4_S1	*ARHGAP32*	c.5045G>A	p.(C1682Y)	0.4
1038	1038_3_S1	*CREBBP*	c.7087C>T	p.(P2363S)	0.45
1038	1038_4_S1	*CREBBP*	c.7087C>T	p.(P2363S)	0.31
1038	1038_3_S1	*IFNAR2*	c.136C>T	p.(R46*)	0.26
1038	1038_4_S1	*IFNAR2*	c.136C>T	p.(R46*)	0.38
1038	1038_3_S1	*KCNS2*	c.1320T>C	p.(P440=)	0.71
1038	1038_4_S1	*KCNS2*	c.1320T>C	p.(P440=)	0.37
1038	1038_3_S1	*STMN3*	c.442G>A	p.(E148K)	0.26
1038	1038_4_S1	*STMN3*	c.442G>A	p.(E148K)	0.31
1038	1038_3_S1	*UBR5*	c.6155A>G	p.(D2052G)	0.62
1038	1038_4_S1	*UBR5*	c.6155A>G	p.(D2052G)	0.35
1224	1224_6_S1	*CHCHD6*	c.366G>A	p.(T122=)	0.37
1224	1224_7_S1	*CHCHD6*	c.366G>A	p.(T122=)	0.27
1224	1224_6_S1	*CPEB2*	c.1903T>C	p.(F635L)	0.42
1224	1224_7_S1	*CPEB2*	c.1903T>C	p.(F635L)	0.4
1224	1224_6_S1	*GBP2*	c.286C>T	p.(L96F)	0.44
1224	1224_7_S1	*GBP2*	c.286C>T	p.(L96F)	0.33
1224	1224_6_S1	*NCKAP5*	c.2360G>A	p.(R787K)	0.41
1224	1224_7_S1	*NCKAP5*	c.2360G>A	p.(R787K)	0.37
1224	1224_6_S1	*NF1*	c.8204T>G	p.(L2735R)	0.78
1224	1224_7_S1	*NF1*	c.8204T>G	p.(L2735R)	0.42
1224	1224_6_S1	*RASGRP3*	c.1504T>G	p.(Y502D)	0.33
1224	1224_7_S1	*RASGRP3*	c.1504T>G	p.(Y502D)	0.42
1224	1224_6_S1	*ZNF260*	c.364A>G	p.(T122A)	0.42
1224	1224_7_S1	*ZNF260*	c.364A>G	p.(T122A)	0.34