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Germline and somatic fumarate hydratase testing in atypical uterine leiomyomata

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Abstract

Women with germline pathogenic variants in the *fumarate hydratase* (*FH*) gene develop cutaneous and uterine leiomyomata and have an increased risk of developing aggressive renal cell carcinomas. Many of these women are unaware of their cancer predisposition until an atypical uterine leiomyoma is diagnosed during a myomectomy or hysterectomy, making a streamlined genetic counseling process after a pathology-based atypical uterine leiomyoma diagnosis critical. However, the prevalence of germline pathogenic/likely pathogenic variants (PVs) in *FH* among atypical uterine leiomyomata cases is unknown. To better understand *FH* germline PV prevalence and current patterns of genetic counseling and germline genetic testing, we undertook a retrospective review of atypical uterine leiomyomata cases at a single large center. We compared clinical characteristics between the *FH* PV, *FH* wild type (WT), and unknown genetic testing cohorts. Of the 144 cases with atypical uterine leiomyomata with evaluable clinical data, only 49 (34%) had documented genetic test results, and 12 (8.3%) had a germline *FH* PV. There were 48 immunohistochemistry-defined *FH*-deficient cases, of which 41 (85%) had *FH* testing and nine had a germline *FH* PV, representing 22% of the tested cohort and 18.8% of the FH-deficient cohort. Germline *FH* PVs were present in 8.3% of evaluable patients, representing 24.5% of the cohort that completed genetic testing. These data highlight the disconnect between pathology and genetic counseling, and help to refine risk estimates that can be used when counseling patients with atypical uterine leiomyomata.

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INTRODUCTION:

Germline pathogenic/likely pathogenic variants (PVs) in the *fumarate hydratase* (*FH*) gene cause *FH* tumor predisposition syndrome, which is also referred to as Reed's syndrome or previously hereditary leiomyomatosis and renal cell carcinoma (HLRCC) (1,2). Germline *FH* PVs confer an increased risk for cutaneous leiomyoma(ta), atypical uterine leiomyoma(ta), *FH*-deficient renal cell carcinoma (RCC), and potentially paraganglioma/pheochromocytoma (3). *FH*-deficient RCCs are associated with early age of onset and poor outcomes (3). Thus, it is critical to identify *FH* PV carriers to allow for lifelong intensive RCC surveillance as a means of early diagnosis and thus more effective treatment, since *FH*-deficient RCCs are currently the subject of clinical trials of promising targeted therapies (3,4).

Some women with germline *FH* PVs only become aware of their cancer predisposition when an atypical uterine leiomyoma is identified at the time of a myomectomy or hysterectomy, making a standardized referral pathway from pathology to genetic counseling in these cases critical (2). *FH*-deficient uterine leiomyomata can occur either due to germline *FH* PVs or tumor-specific somatic *FH* or *FH*-pathway alterations (5). *FH*-deficient uterine leiomyomata have a bizarre histologic appearance characterized by nuclei with prominent eosinophilic nucleoli amongst other features; and have historically been confused with uterine leiomyosarcoma, leading to the potential for misdiagnosis, mismanagement, and patient distress (3,5–8). Therefore, FH immunohistochemistry (IHC) is performed during histologic assessment of atypical appearing leiomyomata to help determine if the lesion is *FH* deficient; and if so, this raises concern for a potential germline *FH* PV, and language may be included in the pathology report to prompt the clinician to refer the patient for genetic counseling and testing (3,5,9). Adherence to these recommendations is not tracked. Additionally, the prevalence of germline *FH* PVs among individuals with atypical appearing and/or *FH*-deficient uterine leiomyomata is limited by few studies conducted in patient cohorts selected for young age at presentation (9,10).

Given this, the goal of this work was to enumerate the frequency of germline *FH* PVs in patients with atypical uterine leiomyomata in an unselected patient population to provide more precise risk estimates and help facilitate genetic counseling and testing for individuals with atypical uterine leiomyomata.

MATERIALS AND METHODS:

Human Subjects:

The human subjects work in this study was approved by the Mass General Brigham (MGB) Institutional Review Board (IRB) and conducted in accordance with the U.S. Common Rule. Limited chart review was conducted and limited discarded human formalin fixed paraffin embedded tissue samples were obtained and used for research after diagnosis under excess discarded tissue MGB IRB approved protocol 2017P001623, which waives the patient consent requirement.

Case selection and chart review:

Patients were identified by performing a search 1) for uterine leiomyomata cases classified as atypical (n=339) or with concern for Reed's syndrome (n=158) from 1988–2022 by the Brigham and Women's Hospital Pathology Department, or 2) for atypical uterine leiomyoma cases from Dana-Farber Cancer Institute's Cancer Genetics and Prevention Disease Center (n=26). In total, there were 144 unique cases identified with evaluable clinical data (Figure 1A). Electronic health records (EHR) were reviewed for clinical data and personal and family tumor histories (Table 1). Germline genetic testing (GGT) was documented for 49 of the 144 cases, and was completed through four different commercial CLIA-certified laboratories. Most (n=32, 65%) had multigene panel testing while the remaining were tested for *FH* alone. *FH* variants were classified as pathogenic/likely PVs, and thus clinically actionable, based on American College of Medical Genetics and Association for Molecular Pathology guidelines.

Statistical Analysis:

Descriptive statistics are reported and p-values were calculated using a Wilcoxon rank sum test for continuous outcomes or a Fisher's exact test for categorical outcomes. All tests were two-sided, and a p-value less than 0.05 was considered statistically significant.

Immunohistochemistry:

Of the 144 cases that underwent chart review, 20 cases with additional sufficient tissue sections available for research were selected to examine fumarate hydratase (FH) and S-(2-succino)-cysteine (2SC) expression by immunohistochemistry. Immunohistochemistry was performed on the Leica Bond III automated staining platform using the Leica Biosystems Refine Detection Kit in the Dana-Farber/Harvard Cancer Center Specialized Histopathology Core at Brigham and Women's Hospital. The Fumarate Hydratase antibody from Santa Cruz Biotechnology, catalog number sc-100743, clone J-13 was run at 1:800 dilution with EDTA antigen retrieval. The 2SC antibody from Discovery Antibodies, catalog number crb2005017D/6773, was run at 1:500 dilution with citrate antigen retrieval. A hematoxylin and eosin (H&E) stain was also performed on tissue sections from each leiomyoma for comparison to the immunohistochemistry and to allow assessment of bizarre nuclei.

Genomic DNA preparation, DNA library preparation, sequencing, and variant analysis:

Of the 144 cases that underwent chart review, 20 cases with additional sufficient tissue available for research were selected for the immunohistochemistry described above and somatic genomic analysis of the *FH* gene. Archived tissue was requested, H&E stained slides were examined to identify the atypical leiomyoma(ta), the lesions were circled, and tissue was scraped from unstained slides within the circled area. Genomic DNA was prepared with on-column RNASE digest using Qiagen's QIAamp DNA FFPE Tissue Kit (Cat. #56404). Genomic DNA was sent in a de-identified fashion to GENEWIZ (South Plainfield, NJ) where targeted sequencing of the *FH* genomic locus was performed. Please see Supplementary Materials and Methods for verbatim methods for library preparation, sequencing, and variant analysis provided by GENEWIZ.

Data Availability:

Due to patient privacy requirements and per the IRB approved protocol, none of the data generated are publicly available and the majority cannot be shared. The *FH* somatic sequencing data in Table 2 is the only data that can be shared and can be supplied by the corresponding authors upon reasonable request via MTA as per the IRB approved protocol.

RESULTS:

Assessment of genetic counseling outcomes after an atypical uterine leiomyoma histology result in a subset of the patient cohort:

We identified a cohort of 144 atypical leiomyomata cases with paired clinical data for analysis (Figure 1A and Table 1). Before exploring this larger cohort of cases, we sought to determine how often in current practice pathology report recommendations for referral to genetic counseling after diagnosis of an atypical uterine leiomyoma are followed. To test this, we obtained FFPE tissue sections from the 20 most recent atypical uterine leiomyoma cases from the study. We performed both somatic *FH* sequencing and IHC for FH and 2SC on the leiomyomata and assessed if patients who we found to have protein or somatic sequencing markers suggestive of *FH* tumor predisposition syndrome received genetic counseling and GGT (Figures 1B, 1C, 1D; Table 2). FH and 2SC IHC were both performed as reports now indicate that both stains together are more effective in detecting patients with *FH* deficiency (9,11).

For *FH*-deficient leiomyomata, we would expect negative FH and positive 2SC staining, which is what we observed in the majority of cases (Figures 1B, 1C, 1D; Table 2). However, sample 104 showed retained FH protein and positive 2SC by IHC. This case was from a patient with a germline missense *FH* PV that retained immunoreactivity to the FH IHC stain which has been observed previously (12,13).

We also examined the frequency of genetic counseling and GGT of the other 19 cases. Only 12 of the 20 cases (60%) had genetic counseling. Of those, nine had negative germline *FH* testing, and three had germline *FH* PVs. All 20 patients had intronic *FH* variants (ranging from one to eight) detected by somatic sequencing, and all three individuals with germline *FH* variants had their variant identified in their leiomyoma tissue (Table 2). Overall, these results indicated that the current atypical uterine leiomyoma diagnosis and referral system may be failing to both identify and connect patients with genetic counseling services.

Assessment of genetic counseling and/or clinical outcomes in a larger atypical uterine leiomyoma cohort:

Given the above results, we next examined a larger cohort of atypical uterine leiomyoma patients to better define the prevalence of *FH* PVs, the frequency of genetic counseling, and the clinical outcomes within this cohort. We identified an additional 124 cases beyond our original 20 cases for a total of 144 atypical uterine leiomyomata cases with evaluable clinical data, 12 (8.3%) of which had a germline *FH* PV (Figure 1A and Table 1). Retrospective chart review revealed 77 (53%) had FH IHC performed, of which 48 (62%) were FH deficient and 29 (38%) had retained or intact FH expression (Figure 1A). Among

the 48 FH-deficient cases, 41 (85%) had *FH* germline testing and 9 had a germline *FH* PV, representing 22% of the tested cohort and 18.8% of the FH-deficient IHC cohort (Figure 1A).

We also searched for differences between patients with atypical leiomyoma(ta) who had unknown GGT status compared to those with completed GGT to test for potential bias in who is referred to or completes genetic counseling. The median age at earliest resected leiomyoma was 13 years younger in the GGT cohort compared to the unknown GGT group (35 [Interquartile range (IQR) 31, 43] vs. 48 [IQR 40, 54.4] p<0.001). The median age at hysterectomy was 6 years younger in the GGT cohort compared to the unknown GGT group (44 [IQR 38, 49.5] vs. 50 [IQR 46, 57] p=0.002). Every examined clinical parameter including history of hysterectomy, history of myomectomy, absence of FH on IHC, presence of cutaneous leiomyomata, and history of cancer or tumor were all significantly different between the completed GGT and unknown GGT status cohorts, with the tested cohort globally having more myomectomies, fewer hysterectomies, and more syndromic features (e.g., cutaneous leiomyomata) (Table 1).

Overall, these results support that the current system fails to identify and connect patients with genetic counseling and that there is an ascertainment bias in patients who do complete counseling and GGT.

Comparisons between *FH* WT patients and those with a *FH* PV:

We next sought to determine if there were clinical differences between germline *FH* WT and *FH* PV patients. Of the 49 individuals with atypical uterine leiomyomata with *FH* GGT, 12 (24.5%) had a germline PV while the rest were *FH* WT (n=37) (Supplementary Table S1). The median ages at earliest resected leiomyoma(ta) (30.5 [IQR 26.3, 35] vs. 37 [IQR 32, 45] P=0.015) and at hysterectomy (38 [IQR 37, 41] vs. 46.5 [IQR 43.5, 51] P=0.01) were younger among the *FH* PV cohort than among the *FH* WT cohort. Of the 12 cases with an *FH* PV, 10 (83%) had FH IHC performed, nine (90%) of which had FH deficient IHC, and one (10%) of which had intact expression of FH on IHC. While 33% (4/12) of individuals with *FH* PVs had cutaneous leiomyomata, none in the *FH* WT cohort reported cutaneous leiomyomata (p=0.002). There were no significant differences in any of the other examined phenotypic variables (Table 1).

Family history of cutaneous leiomyomata was significantly different between the *FH* PV cohort where 25% endorsed a family history compared to 0% of the *FH* WT cohort (p=0.01) (Supplementary Table S2). Other family history comparisons including family histories of uterine leiomyomata or pheochromocytoma and paraganglioma were not significant (Supplementary Table S2). Interestingly there were more families with RCC reported in the WT cohort (14%) than in the PV cohort (8%), though this difference was not significant (Supplementary Table S2).

DISCUSSION:

In this study, we sought to enumerate the prevalence of germline *FH* PVs in an unselected cohort of patients with atypical uterine leiomyoma(ta). We demonstrate gaps in current

practice and significant ascertainment bias in who receives GGT and counseling. We found germline *FH* PVs in 8.3% (12/144) of evaluable atypical uterine leiomyomata patients. Germline *FH* PVs were identified in 24.5% (12/49) of the cohort that completed GGT (irrespective of FH IHC) and 18.8% (9/48) of the cohort with FH-deficient IHC. Thus, we found that most *FH*-deficient uterine leiomyomata do not occur in individuals with a germline *FH* PV. Our findings differ from a recent study among individuals with uterine leiomyomata under age 30 which showed a prevalence of somatic *FH* PVs in 6/7 (86%) of *FH*-deficient leiomyomata, of which 50% were found to be germline *FH* PVs (10). Our study expands on this prior finding by including individuals of all ages with atypical uterine leiomyomata. Our findings suggest multiple ways of refining genetic counseling, histologic analysis, and pathology-genetic counseling referral for patients with atypical uterine leiomyoma(ta).

First, our findings suggest the rate of germline *FH* PVs is far lower than previous estimates, which may be due to having no age criterion in our study eligibility (10,14). However, the 8.3% (12/144) prevalence of *FH* PVs among evaluable atypical uterine leiomyoma(ta) is above the 5% threshold often used to justify GGT and may underestimate the prevalence of *FH* PVs, as many patients did not have germline testing. Based on this finding, we support referral and evaluation for all individuals with atypical uterine leiomyomata; however, counseling about risk for a germline *FH* PV should align with the lower likelihood of detecting a germline PV rather than prior estimates suggesting a higher chance of a PV.

Second, while there were significant differences in age at atypical uterine leiomyoma(ta) diagnosis, we would not endorse use of age cutoffs in recommending GGT, although early age at onset can be informative when counseling and delivering risk assessment. Specifically, we found the age at earliest resected leiomyoma was younger in patients with germline *FH* PVs compared to the *FH* WT cohort; however both groups had wide ranges in age at presentation. Two prior studies estimated 2% and 2.6% of women under ages 30 and 40 respectively with uterine leiomyomata of all morphological types tested positive for germline *FH* PVs, and surmised the age cutoff likely missed germline *FH* PV carriers (9,10).

Third, only one third of the *FH* PV cohort had cutaneous leiomyomata suggesting the absence of this feature should not preclude GGT. Clinicians should be careful not to falsely reassure patients without cutaneous leiomyomata. Likewise, family history of RCC was not a sensitive indicator of germline *FH* PVs. This is unlike our prior work which found RCC and younger age at diagnosis of RCC was associated with *FH* PVs (15).

Also, among the 12 *FH* germline PV cases, one had intact FH IHC but also expressed 2SC (Figure 1A). The germline variant in this case was a missense alteration, c.1097G>A (p.Ser366Asn), which likely maintains immunoreactivity, but is functionally null resulting in the abnormal buildup of the metabolite 2SC and thus detection by IHC. Similar findings were reported previously (9,12). This result supports performing both FH and 2SC IHC staining on all atypical uterine leiomyomata to ensure detection of loss of FH function.

Finally, our somatic sequencing cohort of 20 patients indicates that there is loss to follow up and incomplete uptake of genetic counseling based on histology and/or IHC (Table 2). Only

60% (12/20) of analyzed cases had genetic counseling. Of the eight patients who had not undergone GGT, we detected possible FH dysfunction warranting further investigation in the atypical uterine leiomyoma tissue 1) by IHC in at least three patients, and 2) by somatic *FH* analysis in at least two patients (Table 2). There is a need to improve completion of genetic counseling among patients with atypical uterine leiomyoma(ta).

Limitations of this study included small numbers of patients that were evaluable; however, as heterozygous *FH PVs* causative of *FH* tumor predisposition syndrome are rare, these data represent the largest data set of tested individuals ascertained through serially evaluated atypical uterine leiomyomata. This study was limited to a single, albeit large-volume, academic center (Brigham and Women's Hospital and Dana-Farber Cancer Institute). There was missing data as most patients did not have documented GGT, including some of the cases that underwent somatic tumor sequencing. We were unable to report total leiomyomata numbers due to limitations in the counting of leiomyomata from myomectomy and hysterectomy specimens. Uterine smooth muscle tumor of uncertain malignant potential (STUMP) was not reported, as prior cases of the same histology may have been classified as uterine sarcoma in the past, but today would be classified as STUMP. Somatic sequencing was limited to the 20 most recent atypical uterine leiomyomata, and all possible mechanisms of loss of heterozygosity were not assessed.

Overall, these data support both FH and 2SC IHC staining of atypical appearing uterine leiomyomata as a standard practice and more uniform GGT in individuals with atypical uterine leiomyomata. This large data set of atypical uterine leiomyomata with germline *FH* testing provides more precise estimates of the frequency of germline *FH* PVs for genetic counseling. Additional research on underlying causes of *FH*-deficient uterine leiomyomata in the absence of germline *FH* PVs is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest Disclosure Statement:

S.J.H. currently receives sponsored research support from Merck, Sharp & Dohme Corporation and Eli Lilly and Company, and previously received support from AstraZeneca. None is related to this work. All other authors have nothing to report.

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PREVENTION RELEVANCE STATEMENT:

Women diagnosed with *fumarate hydratase* (*FH*) deficient uterine leiomyomata are at increased risk of renal cancer. This work suggests a more standardized pathology-genetic counseling referral pathway for these patients, and that research on underlying causes of *FH*-deficient uterine leiomyomata in the absence of germline *FH* pathogenic/likely pathogenic variants is needed.

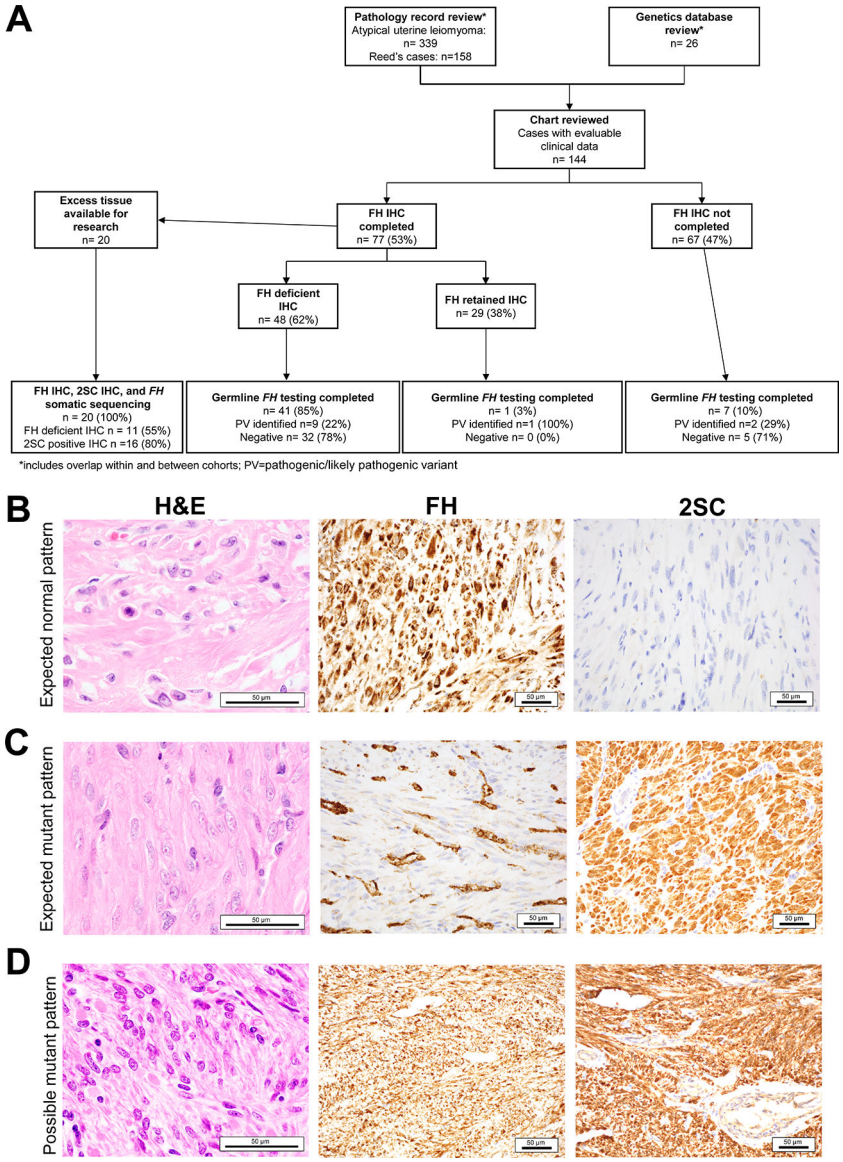

Figure 1.

Details of study cohort and differences in immunohistochemistry staining for FH and 2SC in different settings: A) Shown here is a diagram of how patients were identified and classified in the cohort of patients analyzed in the study. (PV=pathogenic/likely pathogenic variant; FH=fumarate hydratase; IHC=immunohistochemistry). **B, C, and D)** Lesions from a subset of patients were stained and scored by IHC for FH and S-(2-succino)-cysteine (2SC). Representative images are shown for B) the expected wild type *FH* staining pattern, C) the currently expected mutant *FH* staining pattern, and D) a possible mutant *FH* staining pattern missed by FH IHC alone. Hematoxylin and Eosin (H&E) stain photos are shown at 100x to demonstrate typical bizarre nuclei of an atypical uterine leiomyoma, and FH and 2SC IHC photos are shown at 40x with representative normal cells within where possible as controls. Scale bars=50μm.

Table 1.

Characteristics of subjects ascertained through review of pathology and disease center records for the terms atypical uterine leiomyoma(ta), or atypical uterine leiomyoma/leiomyomata concerning for Hereditary Leiomyomatosis and Renal Cell Carcinoma or Reed's Syndrome

Clinical Characteristics	Unknown germline genetic testing (GGT) (n=95)	GGT (n=49)	p-value (unknown vs GGT)	*FH* Wild type (WT) (n=37)	*FH* Pathogenic/ likely pathogenic variant (PV) (n=12)	p-value (*FH* WT vs PV)
Age at earliest resected leiomyoma						
Median [IQR]	48 [40, 54.4]	35 [31,43]	<0.001	37 [32, 45]	30.5 [26.3, 35]	0.015
Range	25–88	18–61		19–61	18–41	
Hysterectomy performed						
At initial presentation of leiomyoma	63	14		12	2	
After additional leiomyomas	12	9		3	6	
Total hysterectomies for any reason *	78 (82%)	24 (49%)	<0.001	16 (43%)	8 (67%)	0.2
Age at hysterectomy						
Median [IQR]	50 [46, 57]	44 [38, 49.5]	0.002	46.5 [43.5, 51]	38 [37,41]	0.01
Range	30–88	35–61		36–61	35–45	
Myomectomy performed						
At initial presentation of leiomyoma	32 (34%)	35 (71%)	<0.001	25 (68%)	10 (83%)	0.47
After additional leiomyomas	6	6		3	3	
Age at germline testing						
Median [IQR]	n/a	37 [33, 45]		37 [33, 47]	37 [32.8, 39]	0.21
Range	n/a	18–63		20–63	18–45	
FH IHC of leiomyoma						
Performed	35 (37%)	42 (86%)	<0.001	32 (86%)	10 (83%)	1
Loss of FH (absent staining) among cases tested	7 (20%)	41 (98%)	<0.001	32 (100%)	9 (90%)	0.24
Personal history of cutaneous leiomyomata						
	0 (0%)	4 (8%)	0.01	0 (0%)	4 (33%)	0.002
Personal history of cancer or tumor						
	46 (48%)	10 (20%)	0.001	9 (24%)	1 (8%)	0.41

p-values calculated using Wilcoxon rank sum test for continuous outcomes or Fisher's exact test for categorical outcomes

* Total number of hysterectomies for any reason, not the sum of those performed at initial presentation of leiomyoma and after additional leiomyomas.

FH=Fumarate hydratase, GGT=Germline genetic testing, IHC=Immunohistochemistry, IQR=Interquartile range, PV=pathogenic/likely pathogenic variant, WT=Wild type

Table 2:

FH and S-(2-succino)-cysteine (2SC) IHC staining with germline and somatic *FH* results for 20 patients with available atypical uterine leiomyoma tissue for research

Case ID	Age at specimen collection	Germline *FH* results	FH IHC Results	2SC IHC Results	Somatic *FH* sequencing
102	45	Pathogenic variant (c.1293del, p.Glu432Lysfs*17)	Negative	Positive	One inactivating frameshift variant (c.1293del, p.Glu432Lysfs*17), one intronic variant
103	35	Likely pathogenic variant (c.1020T>A, p.Asn340Lys)	Negative	Positive	One likely inactivating germline missense variant (c.1020T>A, p.Asn340Lys), four intronic variants
104	36	Likely pathogenic variant (c.1097G>A, p.Ser366Asn)	Positive	Positive	One likely inactivating germline missense variant (c.1097G>A, p.Ser366Asn), one likely inactivating frameshift variant (c.1188del, p.Gly397fs), three intronic variants
105	26	Negative	Negative	Positive	One inactivating missense variant (c.152G>A, p.Arg51Gln), one synonymous VUS (c.1267C>T, p.Leu423Leu), one intronic variant
106	37	Negative	Negative	Positive	Two intronic variants
107	50	Negative	Negative	Positive	Five intronic variants
108	44	Negative	Negative	Positive	Two intronic variants
109	29	Negative	Negative	Positive	One likely inactivating missense variant (c.1357C>A, p.Leu453Ile), four intronic variants
110	46	Negative	Negative	Positive	One likely inactivating nonsense variant (c.641T>G, p.Leu214*), eight intronic variants
111	37	Negative	Negative	Positive	One inactivating missense variant (c.583A>G, p.Met195Val), six intronic variants
112	32	Negative	Negative	Positive	One synonymous VUS (c.1264C>T, p.Leu422Leu), eight intronic variants
113	52	Negative	Positive	Negative	One inactivating missense variant (c.1256C>T, p.Ser419Leu), five intronic variants
114	47	Unknown (not tested)	Positive	Negative	Four intronic variants
115	24	Unknown (not tested)	Positive	Diffuse weak cytoplasmic positivity	Six intronic variants
116	41	Unknown (not tested)	Positive	Negative	One likely inactivating frameshift variant (c.422G>A, p.Trp141*), eight intronic variants
117	25	Unknown (not tested)	Positive	Positive	One synonymous VUS (c.1342C>T, p.Leu448Leu), four intronic variants
118	50	Unknown (not tested)	Positive	Diffuse weak cytoplasmic positivity	One intronic variant
119	43	Unknown (not tested)	Negative	Positive	One inactivating frameshift variant (c.1205del, p.His402fs), seven intronic variants
120	31	Unknown (not tested)	Positive	Positive	Five intronic variants

Case ID	Age at specimen collection	Germline *FH* results	FH IHC Results	2SC IHC Results	Somatic *FH* sequencing
121	38	Unknown (not tested)	Positive	Negative	Seven intronic variants

VUS=Variant of unknown significance, Coding=c., Protein=p.