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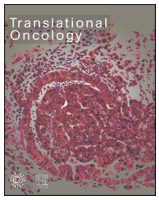

Original Research

# A new missense mutation c.1240A>G in fumarate hydratase gene leads to uterine leiomyoma associated hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome in Chinese

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ABSTRACT

*Objective:* This study presents a detailed analysis of the clinical and genetic characteristics of uterine leiomyoma associated with Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), combined with exploration of family history, pathology, and management procedures, supported by thorough evidence collection.
*Methods:* Blood samples were collected from the proband, and the pathogenic variant was verified using Sanger sequencing. A comprehensive review of family history, FH deficiency pathology, FH and 2SC immunohistochemistry staining was conducted. Functional evidence was derived from clinical and genetic information, supplemented by a literature collection and mutation was reclassified based on ACMG/AMP guidelines.
*Results:* The study successfully identifies a novel missense mutation (c.1240A>G; p.Lys414Glu) in exon 9 of FH, with no prior reports in existing databases. The patient's phenotype and family history, coupled with evidence collected from the literature, contribute to the preliminary determination of the variant as likely pathogenic. We also emphasize that the importance of combining FH-deficient morphology and immunohistochemical staining with 2SC for enhanced sensitivity.
*Conclusion:* This research adds a novel missense mutation to the repertoire of FH gene variants, emphasizing its likely pathogenic nature based on a multidimensional analysis of phenotype, family history, and literature evidence. The findings enhance our understanding of the genetic landscape associated with FH and underscore the importance of thorough characterization for accurate variant classification.

## Introduction

Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) syndrome stands as an exceedingly rare hereditary ailment distinguished by cutaneous leiomyomas (CLMs), uterine leiomyomas (ULMs), renal cysts (RCys), and renal cell carcinoma (RCC). The clinical presentation encompasses cutaneous or uterine leiomyomas, often with concurrent aggressive RCC [1,2], without a globally reported prevalence. The onset of uterine leiomyomas typically occurs in females aged 30–50 years. However, in the context of HLRCC, females may manifest multiple leiomyomas as early as 28–32 years, characterized by their considerable size and abundance [3,4]. Notably, uterine leiomyoma emerges as the most prevalent trait within HLRCC, with 80–90 % of affected women anticipated to receive a diagnosis during their lifetime. These leiomyomas exhibit early onset, multiplicity, substantial size, and symptomatic manifestations, culminating in the necessity for myomectomy or, in some instances, hysterectomy.

The Fumarate Hydratase (FH) gene, comprising ten exons spanning 22.15 kb of DNA, demonstrates a high degree of conservation across diverse species. Positioned at chromosome locus 1q42.3–43, FH serves as a tumor suppressor gene, encoding the fumarate hydratase enzyme pivotal in both the mitochondrial tricarboxylic acid (TCA) cycle and the repair of DNA double-strand breaks within the nucleus [5,6]. Mutation within the FH gene results in diminished or absent fumarate hydratase activity, thereby elevating intracellular fumarate levels. This escalation impedes the function of hypoxia-inducible factor (HIF) prolyl

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hydroxylases, fostering the accumulation of HIF-α. Subsequently, HIF-α activation initiates the HIF pathway, intricately linked to tumorigenesis and metastasis [7,8]. The pathogenic variant within the FH gene may induce HLRCC in monoallelic carriers in biallelic carriers[1].

Our investigation unveils a hitherto undocumented FH missense mutation, c.1240A>G:p(K414E), situated in exon 9. This mutation, previously unreported as a germline mutation associated with HLRCC, received a designation of likely pathogenicity through rigorous scrutiny involving segregation analysis, molecular profiling of blood and tumors, and a comprehensive multistep bioinformatic assessment.

## Case presentation

A 35-year-old female (III-4) presented at our esteemed medical institution, articulating her primary grievance of enduring uterine leiomyoma for a span exceeding six years, concomitant with hypermenorrhea persisting for more than a year. She possesses a robust familial history, with a healthy brother (III-2) and two sisters (III-3 and III-5). Both sisters received diagnoses of uterine leiomyoma at the age of 30, with the younger undergoing myomectomy and the elder opting for hysterectomy. Unfortunately, their father (II-3) succumbed to renal collecting duct carcinoma at the age of 50, while the matron (II-4) maintained a state of well-being. The patient's familial intricacies extend to her aunt (II-1) and the latter's daughter, both grappling with diagnoses of uterine leiomyoma. Furthermore, the ancestral matriarch (I-1), marked by a narrative of uterine leiomyoma, demised (Fig. 1).

Pelvic ultrasonography revealed the existence of an irregularly contoured uterine structure adorned with numerous hypoechoic masses, the most expansive of which measured 85 mm × 72 mm (Fig. 2).

The patient underwent transabdominal myomectomy, and laparotomy unveiled that the uterus mirrored a gestational age of six months, exhibiting an irregular configuration. Multiple fibroids protruded conspicuously from the muscular walls, with the larger ones positioned on the anterior wall of the uterus and the posterior aspect of the lower uterine segment, measuring 8–10 cm in diameter. Significantly, over 20 fibroids of diverse sizes were excised. The bilateral ovaries and fallopian tubes presented a normal external appearance, devoid of any conspicuous abnormalities.

In consideration of her family history encompassing uterine leiomyoma and renal cell cancer, the recommendation was proffered for the patient to undergo genetic testing to exclude the presence of germline mutations. The FH mutation c.1240A>G (p.Lys414Glu) in exon 9 of FH was identified in her, categorically classified as a Variant of Uncertain Significance (VUS) according to gene databases. However, her family members opted against genetic testing, citing personal reasons.

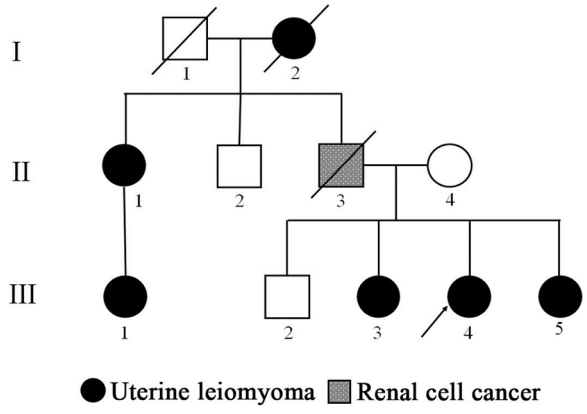


**Fig. 1.** Pedigree of the patient.
III-4, a 35-year-old female, served as the proband, presenting with uterine leiomyoma and harboring the FH missense mutation c.1240A>G(p.Lys414Glu). Her father (II-3) succumbed to renal collecting duct carcinoma at the age of 50, while the matron (II-4) maintained a state of well-being. Among the siblings, two sisters (III-3 and III-5) were diagnosed with uterine leiomyoma at the age of 30, while her brother (III-2) remains in good health. The patient's aunt (II-1) and the latter's daughter are both contending with diagnoses of uterine leiomyoma. Furthermore, the ancestral matriarch (I-1), marked by a narrative of uterine leiomyoma, demised.

## Materials and methods

The study was approved by the Ethics Committee of Liaocheng People's Hospital (No.2024027).

### *Case selection*

Patient demonstrating typical clinical phenotype of HLRCC were selected, albeit in the absence of established diagnostic criteria. Modified diagnostic criteria, as proposed by Smit et al. (2011)[9] and Schmidt et al. (2014)[10], delineated practical parameters for clinical diagnosis, comprising two major criteria and four minor criteria. Major criteria encompassed: (1) Presence of multiple cutaneous leiomyomas, particularly those associated with characteristic shooting pain; (2) Occurrence of one or more piloleiomyomas with characteristic shooting pain. Minor criteria included: (1) Solitary cutaneous leiomyoma coupled with a family history of hereditary leiomyomatosis and renal cell cancer syndrome; (2) Development of Type 2 papillary renal cell carcinoma before the age of 40; (3) Onset of severely symptomatic uterine leiomyomas in women before the age of 40; (4) Presence of a first-degree family member meeting any one of the aforementioned criteria. The manifestation of severely symptomatic uterine leiomyomas before the age of 40 in a second-degree paternal family member also held relevance. The diagnosis is considered likely when a patient satisfies the major criterion. Suspicions of HLRCC arise if the patient meets two or more minor criteria, necessitating additional tests such as immunohistochemistry (IMH) or gene sequencing to facilitate the conclusive diagnosis, as the definitive determination involves a positive germline fumarate hydratase mutation test[11].

### *Immunohistochemistry*

Immunohistochemical staining for FH and S-(2-succino)-cysteine (2SC) was conducted utilizing a Ventana Benchmark XT autostainer (Ventana Medical Systems, Tucson, AZ). The primary antibodies FH (clone No. J-13, acquired from MAIXIN BIO) and 2SC (polyclonal antibody, obtained from MAIXIN BIO) were subjected to staining under experimental conditions meticulously outlined in the antibody instructions and laboratory specifications. Standard practices for positive and negative controls were consistently implemented. Interpretation standards for FH and 2SC were established in accordance with pertinent references [12,13].

### *Detection of FH mutations*

DNA extracted from peripheral blood samples served as the substrate for FH genetic testing. The entire coding sequence and intron-exon boundaries underwent comprehensive analysis through polymerase chain reaction amplification and Sanger sequencing, focusing on single nucleotide variants and insertion/deletion variants. The description of identified variants adhered to the prevailing version of the human genomic variant search nomenclature[14], with NM_000143.4 designated as the transcript reference sequence.

### *Functional evidences analyze*

In light of the diverse functions attributed to Fumarate Hydratase (FH), an extensive literature review and database exploration were conducted to compile information concerning the properties of the FH missense mutation c.1240A>G(p.Lys414Glu) in exon 9. The aim was to

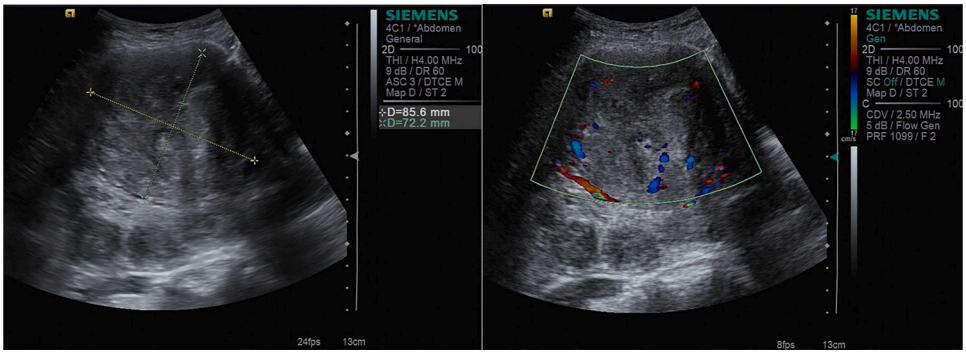


**Fig. 2.** Ultrasonic Images of Uterine leiomyoma in this case.
Irregularly contoured uterine structure adorned with hypoechoic masses measured 85 mm × 72 mm.

gather insights that could contribute to the interpretation of the pathogenicity of this genetic variant. The FH missense mutation c.1240A>G was scrutinized in databases such as 1000 Genomes, ExAC, gnomAD, and iJGVD to ascertain allele frequency, thereby furnishing evidence. Further inquiry into mutation sites encompassed ClinVar, OncoKB, VarSome, and other mutation databases, drawing evidence from existing literature or case reports. A meticulous search through publications addressing FH missense mutation c.1240A>G was conducted. The evidence level was subsequently assigned based on functional research, clinical trials, co-segregation, disease incidence, and other pertinent factors. Subsequently, FH c.1240A>G(p.Lys414Glu) underwent classification adhering to the criteria set forth by the American College of Genetics and Medical Genomics and the Association of Molecular Pathology (ACMG/AMP)[15].

## Results

### *Clinical features*

Six women within this familial lineage have been afflicted by uterine leiomyoma, each undergoing myomectomy before reaching the age of 30. Additionally, two individuals proceeded to undergo hysterectomy, one at the age of 32 and the other at 40. All female patients exhibited symptoms of heavy bleeding and/or bulk symptoms resistant to medical intervention, subsequently confirmed through preoperative ultrasonics to possess multiple, sizable fibroids.

### *Pathology findings*

The gross examination unveiled over 20 nodular formations, exhibiting diameters spanning from 0.4 cm to 8 cm. The incised surface presented a nuanced grayish-yellow hue, characterized by a resilient texture and a distinctive swirled or edema-like appearance (Fig. 3).

Microscopically, the majority of leiomyomas manifest elevated cellular density, with a minority displaying a moderate cellular composition. Distinctive characteristics encompass hemangiopericytomatous blood vessels distinguished by staghorn branching vessels, along with multifocal interstitial edema (Fig. 4A). Evident under scrutiny are prominently discernible eosinophilic macronucleoli with perinucleolar halos, accompanied by eosinophilic cytoplasmic inclusions consisting of round to ovoid pink globules within the cell cytoplasm. Focally, nuclei present peculiarities, marked by enlargement, irregularity, hyperchromasia, and multinucleation (Fig. 4B). Immunohistochemical analysis unveiled the negation of FH in leiomyoma cells, contrasting with the positive staining observed in adjacent vascular endothelial cells and vascular smooth muscle cells (Fig. 4C). Conversely, all cells within the uterine leiomyoma exhibited positive staining for 2SC, substantiating the absence of FH (Fig. 4D). Nevertheless, a selection of 8 leiomyoma samples from the proband underwent

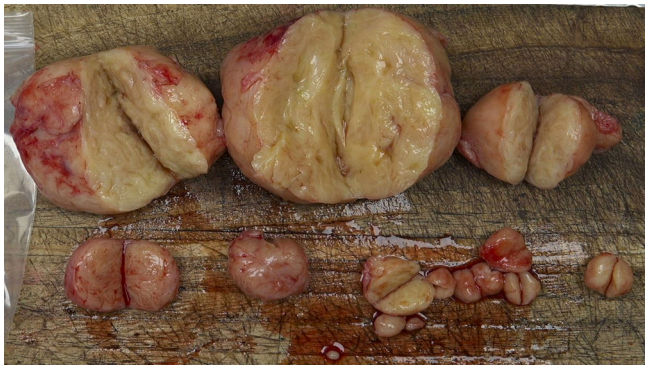

**Fig. 3.** Uterine Leiomyoma Samples.
The nodular formations exhibiting diameters spanning from 0.4 cm to 8 cm. The incised surface presented a nuanced grayish-yellow hue, characterized by a resilient texture and a distinctive swirled or edema-like appearance.

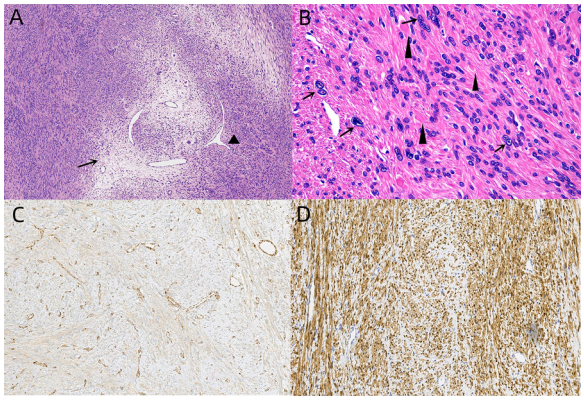


**Fig. 4.** Histopathology and Immunohistochemistry of Uterine Leiomyoma with FH deficiency.
A: At low magnification, staghorn blood vessels (arrowheads) and areas of patchy edema (arrows) are evident (100 ×).
B: High magnification reveals singular nuclei, eosinophilic nucleoli with perinucleolar halos (arrows), and cytoplasmic eosinophilic granules (arrowheads) (400 ×).
C: The expression of FH in tumor cells is completely lost, while the interstitial blood vessels serve as a positive internal control (200 ×).
D: The expression of 2SC in tumor cells is diffuse and strongly positive (200 ×).

immunohistochemical analysis for FH and 2SC. Among these, 5 samples exhibited negative FH staining, while 3 samples showed positive FH staining while notably all 8 samples displayed positive staining for 2SC. Confirmation of germline mutation was established through FH gene

sequencing (Fig. 5), revealing a nuanced pattern in staining across different leiomyomas. Retained staining was observed in some sections, while loss of staining was evident in others, indicating variable FH staining patterns. No discernible correlation was observed between the FH immunohistochemical staining results and the type of leiomyoma or the extent of FH-d morphology. Polymerase chain reaction (PCR)-based Sanger sequencing confirmed the presence of a germline mutation, specifically c.1240A>G(p.Lys414Glu) in exon 9, detected in both peripheral blood and tumor tissue.

*Evidences collection*

For this missense mutation, previously unreported as explicitly deleterious, we conducted a thorough search for evidence to assess its clinical significance. According to GenBank, the Homo sapiens fumarate hydratase (FH) transcript NM_000143.4 consists of 1791 bases encoding 510 amino acids, spanning 10 exons, with the FH c.1240A>G(p.Lys414Glu) variant located in exon 9. No records were found for the mutation site FH c.1240A>G(p.Lys414Glu) in the 1000 Genomes, ExAC, gnomAD, iJGVD databases, and there were no mentions in the databases of OncoKB. The reported evidence from the ClinVar database suggests uncertain significance of functional impact in a lower-level category. Liang Zheng et al. reported in their study that FH Lys414Glu mutants demonstrated stable characteristics but exhibited a loss of over 80 % of their normal activity in RCC[16].

Based on the online predictions from Mutation Taster and the integrated forecasting tool REVEL, both indicating the variation as "diseases-causing" and the splicing prediction software (NNSplice, NelGene2, FSPLICE, and SpliceAI) suggesting that the mutation does not affect splicing, there is consistent evidence for a potential deleterious impact.

VarSome database reported the uncertain significance, and the evidences was 2 points=2P - 0B (PM2 Supporting: Variant not found in gnomAD; PP2 Supporting: 156 out of 161 non-VUS missense variants in gene FH are pathogenic=96.9 % which is more than threshold of 80.8 %).

Considering a systematic diagnostic approach, which involves a comprehensive personal and family history analysis, and taking into account the patient's familial history of RCC, uterine leiomyoma, and the specific phenotype, the diagnosis of HLRCC is supported.

In accordance with the ACMG/AMP criteria, and considering the cumulative evidence, it can be concluded that the missense mutation site FH c.1240A>G(p.Lys414Glu) is likely pathogenic (Table 1).

## Discussion

This study marks the initial documentation of the FH c.1240A>G(p.Lys414Glu) mutation in the Chinese HLRCC population. Notably, a similar mutation was previously reported in a female from Belarus by Sánchez-Heras AB et al.[17]. Despite the reluctance of the proband's relatives to undergo FH gene sequencing, limiting the acquisition of additional co-segregation evidence, the identification of the FH c.1240A>G(p.Lys414Glu) variant in the Chinese population stands as a significant contribution and serves as an encouraging milestone for reporting. This novel finding underscores the importance of thorough personal and family history assessments for uterine leiomyomas (ULMs), cutaneous leiomyomas (CLMs), and renal cell carcinomas (RCCs) as essential components of a systematic diagnostic approach. It highlights the necessity for increased attention to the diagnosis, prevention, and management of HLRCC-related diseases associated with FH gene mutations, with particular emphasis on uterine leiomyomas as sentinel tumors.

A variant of uncertain significance (VUS) poses challenges in clinical decision-making[15] and may introduce confusion for healthcare providers and individuals receiving clinical reports, potentially causing anxiety. The associated uncertainty can contribute to an invisible burden on patients. Upon analyzing the variant through the ACMG/AMP criteria, FH c.1240A>G(p.Lys414Glu) variant detected in our patient, its evidence level was initially defined as PM2, PP2, and PP3, all supporting the classification as a VUS. However, detailed follow-up family information of the proband, coupled with a comprehensive review of the literature, revealed that the patient's phenotype and family history are highly gene-specific, providing supplementary evidence classified as PP4. Further strengthening the evidence, Liang Zheng et al. reported that FH Lys414Glu mutants, although stable, lost over 80 % of their normal activity in RCC[16], aligning with the PS3 criterion. Considering the accumulated evidence, FH c.1240A>G(p.Lys414Glu) can be reclassified from a VUS to "likely pathogenic" according to ACMG/AMP guidelines. This reclassification can contribute to more accurate genetic counseling, refined recommendations for the treatment of recurrent

**Table 1**
ACMG/AMP guidelines applied to FH c.1240A>G(p.Lys414Glu) variant reclassification.

| Criteria | Comments |
|---|---|
| PM2 | **Criterion: allele frequency of this variant in any population is <1 %; absent in population databases.** Description: There was no record about FH c.1240A>G(p.Lys414Glu) variant in 1000G,ExAC,gnomAD,iJGVD etc. population databases. |
| PS3 | **Criterion: Well-established functional studies show a deleterious effect.** Description: Liang Zheng et al. reported that FH Lys414Glu activity assay was the stable mutants but lost over 80 % of their normal activity in RCC (PMID: 37053010). |
| PP2 | **Criterion: Missense in gene with low rate of benign missense variants and path, missenses common.** Description: VarSome database indicates that among the 161 non-Variant of Uncertain Significance (VUS) missense variants in the FH gene, 156 are classified as pathogenic. This equates to a percentage of 96.9 %, surpassing the threshold of 80.8 %. |
| PP3 | **Criterion: Multiple lines of computational evidence support a deleterious effect on the gene/gene product.** Description- Online prediction tools, including Mutation Taster and the integrated REVEL, align in their designation of this mutation as disease-causing. |
| PP4 | **Criterion: Patient's phenotype or family history highly specific for gene.** Description: The proband received a diagnosis of FH-deficient leiomyoma following surgery for multiple uterine fibroids at the age of 35. Her father succumbed to "renal collecting duct cancer", and several female relatives, including her grandmother, aunt, cousin, and two sisters, all presented with severe symptoms and were diagnosed with uterine leiomyoma before the age of 40. This familial medical history aligns with the clinical diagnosis of HLRCC, which is associated with FH gene mutations. |

**Abbreviations:** ACMG/AMP=American College of Medical Genetics and Genomics / Association for Molecular Pathology; FH=Fumarate Hydratase; VUS =Variant of Uncertain Significance; HLRCC= Hereditary Leiomyomatosis and Renal Cell Cancer.

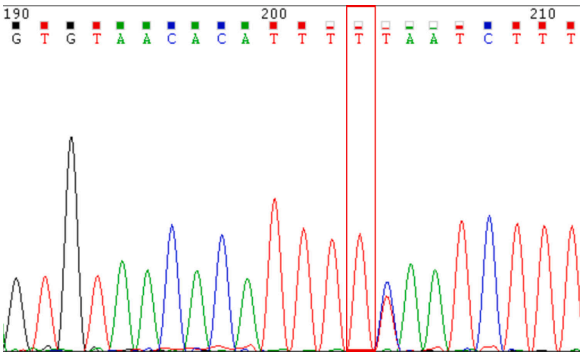


**Fig. 5.** Sanger sequencing test results of the patient.
Sanger sequencing data of the FH gene confirmed a point mutation c.1240A>G (p.Lys414Glu) in the blood of the patient (highlighted by a red box).

uterine fibroids, and early screening of kidney tumors. To enhance the reclassification of VUS and establish a clearer association between the variant and diseases, collaborative efforts involving patients, physicians, and laboratories are encouraged. This collaborative approach should focus on collecting comprehensive phenotypic data, detailed family histories, database exploration, and functional analysis to inform effective management strategies.

The predominant scenario for female individuals with HLRCC involves the development of symptomatic uterine leiomyomas, necessitating surgical intervention at a young age. This occurrence typically transpires prior to the onset of renal carcinoma [4,18,19]. According to a study, 57 % of female HLRCC patients underwent hysterectomy at or before the age of 30 years (mean, 30 y), significantly preceding the development of renal carcinoma, which typically occurs at a median age of 44 years[18]. Uterine leiomyoma, being the most prevalent benign neoplasm in the female reproductive system, poses a diagnostic challenge due to the rarity of the HLRCC syndrome[20]. Identifying patients with leiomyomas at a considerably younger age than renal carcinoma presents a distinct opportunity for early diagnosis and intervention if these individuals could be identified prospectively. In contrast to the rarity of the HLRCC syndrome, uterine leiomyomas are exceedingly common, affecting as many as 20 % to 50 % of women by the age of 30, and up to 80 % of females may have uterine leiomyomas by the age of 50 [21]. While the majority of those affected may not undergo surgery, uterine leiomyomas remain among the most prevalent visceral tumors encountered in diagnostic surgical pathology laboratories. Therefore, any screening test aiming to identify patients with HLRCC, who initially present with uterine leiomyomas, must exhibit high specificity[22]. In addition to their numerous and larger size, uterine leiomyomas associated with germline FH mutation exhibit distinctive morphological features. These include prominent nucleoli with symplastic-type nuclear atypia, heightened cellularity, perinucleolar clearing, staghorn/haemangiopericytoma (HPC) - like vessels, cytoplasmic globules, and stromal edema [12,23].

Our study reveals an absence of a discernible correlation between FH IHC staining results and the type of leiomyoma or the extent of FH-d morphology. In the case of eight leiomyomas from a patient with confirmed FH germline mutation, only 5 tumors (61.25 %) exhibited negative IHC staining for FH, while 3 samples displayed positive IHC staining for FH. However, all 8 tumors demonstrated positive staining for 2SC. Variable FH staining patterns were observed across different leiomyomas, with some sections retaining staining and others showing loss of staining. The inconsistency in FH IHC staining is not definitively explained, but speculation suggests a potential correlation with the type of second hit in the FH gene and/or the stability of the FH protein. Similar variations in FH staining have been observed in cutaneous leiomyomas from HLRCC patients[24]. Bennett et al.[25] identified a case with a somatic FH missense mutation that exhibited retained FH expression while also being 2SC-positive. In our study, we similarly incorporated leiomyomas with missense FH mutations that showed retained staining for FH and were 2SC-positive. This aligns with the observed phenomenon of retained FH IHC staining in RCCs and cutaneous leiomyomas with FH gene mutations, as reported in previous studies [24,26,27], 2SC IHC stain emerges as a more reliable and sensitive method for detecting FH deficiency.

The occurrence of retained FH staining has been previously documented in uterine leiomyomas and RCC with confirmed germline and somatic FH gene mutations. In a study by Emily Chan et al.[28], leiomyomas from two patients with missense FH germline mutations exhibited retained staining for FH in their tumors. This phenomenon was attributed to the production of a nonfunctional FH protein that remained detectable by immunohistochemical staining. Contrastingly, inconsistent FH staining was observed across different leiomyomas from patients with a nonsense mutation, an intronic mutation, and a missense mutation in the FH gene. The variability in staining patterns suggests the influence of mutation type on FH protein functionality and detectability through immunohistochemical analysis[28].

Our study emphasizes that the presence of retained FH staining should not serve as a sole basis for excluding the possibility of FH deficiency or HLRCC. In our clinical practice, when encountering a uterine leiomyoma with FH-deficient features, we conduct FH and 2SC staining. However, our diagnosis and comments remain consistent regardless of FH staining results. This prompts the consideration of the value of FH staining and raises the question of its continued necessity. In cases of young patients with uterine leiomyomas exhibiting FH-deficient morphology, even in the presence of retained FH IHC staining, referral for genetic counseling and testing should be contemplated[28].

In summary, we present a case involving a patient with a robust family history of maternal leiomyomas leading to early surgical interventions. The identification of a novel mutation, FH c.1240A>G (p.Lys414Glu), prompted comprehensive genetic counseling. Through an exhaustive evaluation of the entire family history and other pertinent information, we substantiated the diagnosis of Hereditary HLRCC, upgrading the evidence level from UVS to likely pathogenic.

Furthermore, we offered detailed guidance for the patient and her family's follow-up, recommending RCC screening, and advocating for early myomectomy and hysterectomy to mitigate the risk of uterine leiomyosarcoma in female HLRCC patients. The clinicopathological findings from myomectomy specimens in this novel mutation site were meticulously described, highlighting the variable topography of FH-deficient morphology within and between leiomyomas. In cases with equivocal findings, additional sections may prove beneficial.

It was observed that FH immunohistochemical staining, when combined with 2SC, offered enhanced sensitivity for diagnosing FH deficiency. Consequently, we question the additional value of FH IHC alone due to its limited sensitivity and variable staining patterns between leiomyomas from the same patient. As a result, we emphasize the consideration of referral for genetic counseling and testing in young patients with uterine leiomyomas displaying FH-deficient morphology, even when FH IHC staining is retained.

## CRediT authorship contribution statement

**Li Wang:** Conceptualization, Writing – original draft. **Ran Du:** Methodology, Writing – original draft. **Lin Han:** Data curation, Visualization. **Rui Yang:** Methodology, Software. **Yingxue Li:** Conceptualization, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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