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CLINICAL REPORT

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# Novel *FH* mutation associated with multiple uterine leiomyomas in Chinese siblings

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## Abstract

**Background:** Fumarate hydratase (FH) plays an important role in cell metabolism. Germline mutation of *FH* may cause hereditary leiomyomatosis and renal cell cancer syndrome. The correlation between various mutations of *FH* gene and the phenotype is controversial and needs further study. Therefore, this article described a novel mutation in siblings with multiple uterine leiomyomas.

**Methods:** Whole-exome sequencing was performed on the two patients and their family members using their peripheral blood. The function of the DNA variant was predicted in silico.

**Results:** Pathology results showed characteristics of leiomyoma. A novel missense mutation of *FH* gene (c.1214A > G, p.Leu405Ser) was identified in both patients and their father. This mutation was predicted to be probably pathogenic and deleterious.

**Conclusion:** This study indicated that the novel mutation may be responsible for the occurrence of multiple uterine leiomyomas. However, the risk of renal disease should not be ignored and regular screening was recommended.

**KEYWORDS**
fumarate hydratase, genotype-phenotype correlation, missense mutation, myomectomy, uterine leiomyoma

## 1 | INTRODUCTION

Uterine leiomyomas are the most common benign neoplasms in women's reproductive system. By the age of 50 years old, at least 1 uterine leiomyoma would have appeared in approximately 70% of white women and over 80% of black women (Bulun, 2013). The onset of age among most of the patients (over 95%) is after 30 years old (Wheeler, Warr, Warsetsky, & Barmat, 2016). Uterine leiomyomas may cause hemorrhage, anemia, recurrent miscarriage, premature labor, embryo implantation failure, and some other clinical symptoms, which will pose a threat to the fertility among women of childbearing age.

The occurrence of uterine leiomyomas often shows familial clustering between first-degree relatives and twins, attributed to several specific genetic defects inherited by germ cells (Commandeur, Styer, & Teixeira, 2015). It is reported that major genetic alterations associated with uterine fibroids may appear in *MED12*, *HMGA2,* and *FH* (OMIM *136850) (Mäkinen, Kämpjärvi, Frizzell, Bützow, & Vahteristo, 2017).

Fumarate hydratase (FH), encoded by the *FH* gene, is an enzyme that catalyzes the conversion of fumarate to malate in the Krebs cycle. Germline mutations in *FH* predispose women to multiple uterine leiomyomas (Bulun, 2013). Besides, *FH* mutations have been identified in most

families with hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome (Patel, Handler, Schwartz, & Lambert, 2017). The women with HLRCC syndrome may suffer from uterine leiomyomas characterized by early onset and multiple fibroids. Here we report a Chinese family with a novel *FH* mutation (c.1214A > G, p.Leu405Ser), where the female carriers suffer from multiple uterine leiomyomas while both the male carrier and the female noncarriers are healthy.

## 2 | CASE REPORT

### 2.1 | Patient 1

The index case was a 27-year-old nulliparous woman. She was diagnosed with multiple uterine leiomyoma at 20 years' old. At the age of 24 years, she underwent uterine myomectomy for the presence of menorrhagia and anemia caused by leiomyoma. However, ultrasound revealed a recurrence of uterine leiomyoma at postoperative 9 months, growing at a speed of approximately 2 cm per year followed by serial ultrasounds. She was referred to PUMCH (Peking Union Medical College Hospital) because of menorrhagia in the past 4 months and a bearing-down sensation in her lower abdomen. Magnetic resonance imaging (MRI) scan suggested multiple uterine leiomyomas with degeneration and the largest one was about 54 × 60 × 65 cubic millimeters in size (Figure 1a). Therefore, another myomectomy was performed and 42 uterine leiomyomas were resected (Figure 1b).

### 2.2 | Patient 2

This 23-year-old nulliparous woman was the proband's youngest sister. At the age of 20, she reported a palpable pelvic mass with heavy menstrual bleeding, and then was diagnosed with multiple uterine leiomyomas with degeneration. Subsequently, she underwent a myomectomy. At postoperative 2 years, menorrhagia occurred again. MRI scan showed a relapse of multiple uterine leiomyomas (Figure 1c).

### 2.3 | Histologic findings

Paraffin-embedded uterine leiomyoma sections of the patients were examined by immunohistochemistry with antibodies against caspase-3, α-smooth muscle actin (α-SMA) and FH. Hematoxylin-eosin staining showed spindle-shaped smooth muscle cells, without evident atypical nuclei and any other typical characteristics attributed to *FH* mutations (Miettinen et al., 2016; Reyes et al., 2014) (Figure 2a). Immunohistochemistry demonstrated that the tumor cells were positive for caspase-3 (Figure 2b) and α-SMA (Figure 2c), and there was no significant reduction in FH protein production (Figure 2d).

### 2.4 | Identification of a novel missense mutation in *FH* gene

The clinical manifestation of the two siblings shared some common characteristics including early onset, multiple uterine leiomyomas, and rapid relapses. A whole-exome sequencing was conducted among this family for more investigation of possible underlying genetic disorders. After obtaining written informed consent, we collected blood samples from the family. Genomic DNA obtained from peripheral blood was submitted to hybridization-based capture using the Illumina TruSeq panel and sequenced using an Illumina X10. A single rare missense variant in exon 8 of the *FH* gene (c.1214A > G, p.L405S) in both patients and their father (Figure 3b) was identified (RefSeq NM_000143.3,

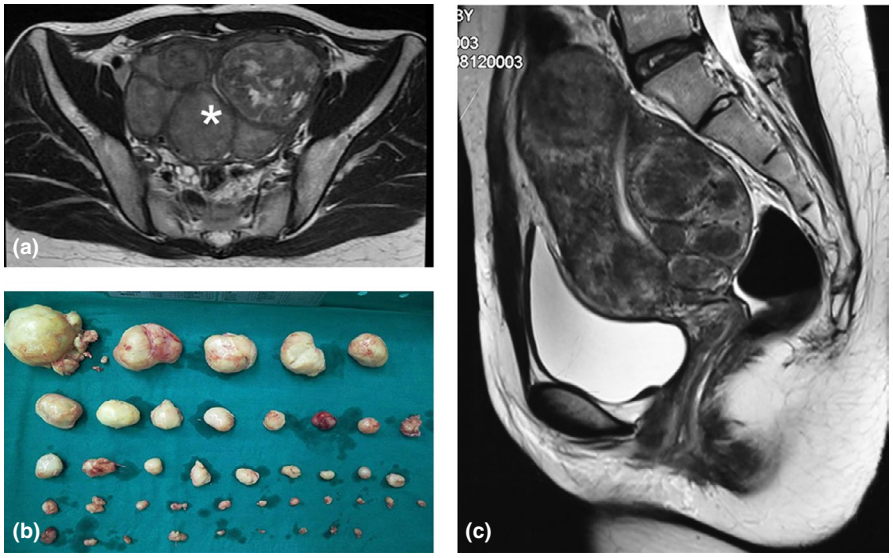


**FIGURE 1** Multiple uterine leiomyoma in situ and resected specimens. (a) Pelvic magnetic resonance T2-weighted imaging of horizontal segments of the uterus with multiple fibroids (*asterisk*) in patient 1. (b) The uterine leiomyomas removed from the same uterus through uterine myomectomy (patient 1). (c) Pelvic magnetic resonance T2-weighted imaging of sagittal segments of the uterus with multiple fibroids in patient 2

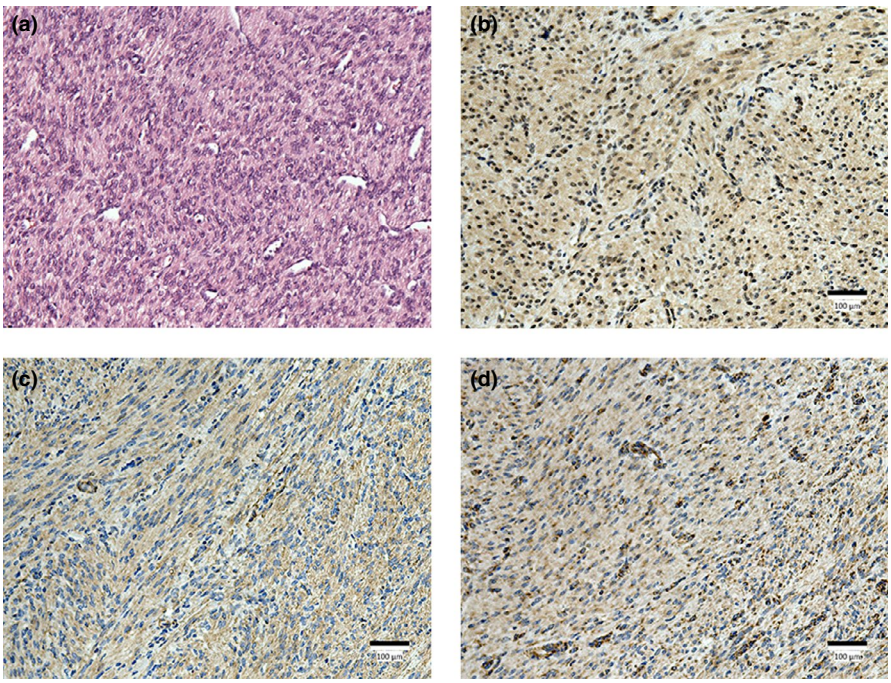


**FIGURE 2** Histologic sections showing characteristics of leiomyoma. (a) Hematoxylin-eosin staining of a uterine leiomyoma biopsy from patient 1. (b–d) Positive results of immunostain by caspase-3 (b), α-SMA (c) and fumarate hydratase (d). α-SMA, α-smooth muscle actin

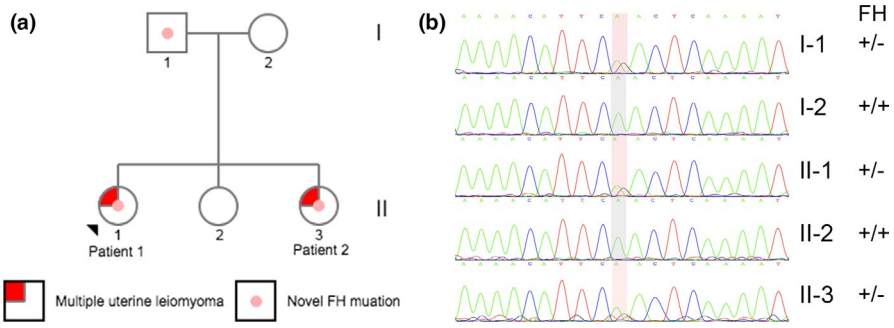


**FIGURE 3** Pedigree of the family and identification of a novel *FH* gene mutation (c.1214A > G, p.Leu405Ser). (a) Patient 1, patient 2, and their father carry the novel *FH* mutation. Patient 1 and patient 2 show the clinical symptom of multiple uterine leiomyoma. (b) Direct sequencing of the *FH* gene using genomic DNA from blood sample showed a heterozygous mutation c.1214A > G in exon 8 of the FH. The detected mutation is highlighted in pink and the normal allele is highlighted in gray. +/− means heterozygous *FH* mutation and +/+ means normal allele. (RefSeq NM_000143.3, GRCh37/hg19). FH, fumarate hydratase

GRCh37/hg19). Then sanger sequencing is performed to confirm the cosegregation of the mutated allele. This point mutation substitutes a serine for leucine at position 405 within the protein. The DNA variant has not appeared in any previous literature nor has its allele frequency been reported in any population database. Close to this position, some pathogenic mutations (c.1210G > T, c.1209delT, c.1205delA, c.1200delT, c.1189G > A, etc) have already been identified within exon 8, according to the database.

### 2.5 | Function prediction of *FH* gene with novel mutation

The mutation site (p.L405S) of *FH* gene possessed strong amino acid conservation among different species (Figure S1). It indicated that this novel mutation was probably located in a functional region of vital importance during the process of species evolution.

This mutation was predicted to be probably pathogenic by Polyphen and deleterious by PROVEAN (Table 1). The pedigree showed that the DNA variant in the two sisters was inherited from their unaffected father. It should be noted that another sister (aged 25) without the *FH* mutation has not developed uterine leiomyomas up till now. The finding led to a reasonable speculation that this novel mutation was pathogenic.

## 3 | DISCUSSION

In this article, we present a case series of two Chinese sisters who had multiple uterine leiomyomas in their 20s. A

**TABLE 1** Prediction of protein function

| | | | PolyPhen2 | | PROVEAN | |
|---|---|---|---|---|---|---|
| **Mutation gene** | **Mutation position** | **Amino acid change** | **Score[a]** | **Prediction** | **Score[b]** | **Prediction (cutoff = −2.5)** |
| *FH* | chr1:241665765 | p.L405S | 0.999 | Probably pathogenic | −5.962 | Deleterious |

[a]A larger score (close to 1) of PolyPhen2 revealed a probably damaging effect.

[b]A score smaller than −2.5 indicated a deleterious effect.

novel mutation (c.1214A > G, p.L405S) in exon 8 of the *FH* gene was identified in the siblings at the germ line level (Figure 3). As far as we know, it had not been reported previously and is likely a novel mutation. This mutation was inherited from their father who was free of any cutaneous or renal disease. With regard to the mother and another sibling without *FH* mutation, neither had multiple uterine leiomyomas. Coupled with results from in silico predictions of protein damage, the evidence indicated that the novel mutation in *FH* may be pathogenic and causative for the occurrence of multiple uterine leiomyomas with early onset. Although the pathologic results showed characteristics of common uterine leiomyoma with FH protein production, Joseph et al. (2015) illuminated the possibility of normal result for FH immunohistochemistry in uterine leiomyomas with missense *FH* germline mutation previously.

Pathogenic variants of *FH* gene may cause a rare disease called HLRCC syndrome. The syndrome puts affected individuals at risk for cutaneous piloleiomyomas, early-onset uterine leiomyomas, and type II papillary renal cell carcinoma (Adam, Yang, Soga, & Pollard, 2014). *FH* mutation was detected in 80%–90% of HLRCC family. In our case series, we tended to consider that the uterine leiomyomas of the siblings caused by the novel mutation (c.1214A > G, p.L405S) in *FH* gene were nonsyndromic. First, the siblings did not meet the diagnostic criteria of HLRCC syndrome according to Patel, where the major criteria include cutaneous symptoms (Patel et al., 2017). Besides, there was no reported personal or family history of cutaneous leiomyomas or renal cell cancer. And no typical pathological alteration caused by *FH* mutation was present in either patient.

However, possible appearance of renal cell carcinoma in the future should not be completely ignored because the correlations of genotype and phenotype are still ambiguous and controversial regarding different *FH* mutations. Wei et al. (2006) found different occurrence and types of renal tumors within families who carry the same germline mutation. Gardie also claimed that there was no convincing evidence of significant correlation between genotype and phenotype in HLRCC syndrome especially for renal cell carcinoma by studying 56 HLRCC families (Gardie et al., 2011). Some other studies, nevertheless, suggested the existence of correlation. Guinarda reported a big HLRCC family with no intrafamilial variability and all the mutation carriers in this family expressed cutaneous leiomyomas and uterine fibroids (the latter symptom only appropriate for women) (Guinard et al., 2016).

Although the family depicted here did not have renal disease history in the past, we strongly suggest the siblings as well as their father to take regular screening of renal disease in the future. Chan et al. (2017) reported an accidental identification of a renal tumor during kidney surveillance of a nonsymptomatic person with known FH deficiency, thus being able to perform a partial nephrectomy and save his life. For carriers of pathogenic *FH* germline mutations, it is generally recommended that kidney MRI should be performed each year after 8 years old in order to detect the possible development of renal cell carcinoma (Menko et al., 2014). But if some mutation sites like the one we found here would only cause uterine fibroids, it could be meaningful in genetic counseling because male fetus would be safe and female descendant should be recommended early reproduction when grown up. Therefore, it is necessary to further examine the existence of correlation between *FH* mutations and the phenotype, simultaneously taking into account of the effect of environment and epigenetics. Additionally, whether this novel mutation reported here is pathogenic should be further confirmed in future study.

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## CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

## AUTHORS CONTRIBUTION

ZZ designed the study and drafted the manuscript. WW collected patients' medical records and applied for the ethical approval. YY performed pathological analysis. LZ funded the study. FF recruited the patient in outpatient clinic, conceived and designed the study, and reviewed and edited the manuscript. All the authors read and approved the manuscript.

## ETHICAL COMPLIANCE

The project was approved by the Ethics Committees of Peking Union Medical College Hospital.

## INFORMED CONSENT

Written informed consent was obtained from the patients and their family.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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