Novel FH mutation associated with multiple uterine leiomyomas in Chinese siblings

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FUNDING INFORMATION
This study was supported by CAMS Initiative for Innovative Medicine (CAMS-2017-I2M-1-002).

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

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, HMG2A, 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 uterus 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).
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
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 cutane-
ous 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 predic-
tions 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 character-
istics of common uterine leiomyoma with FH protein pro-
duction, Joseph et al. (2015) illuminated the possibility of
normal result for FH immunohistochemistry in uterine lei-
omyomas 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 carci-
noma (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 cor-
relations 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 signifi-
cant 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,
evertheless, suggested the existence of correlation. Guinarda
reported a big HLRCC family with no intrafamilial variabili-
and all the mutation carriers in this family expressed cutaneous
leiomyomas and uterine fibroids (the latter symptom only ap-
propriate 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 dis-
ease 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 fe-
male 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.

**ACKNOWLEDGMENTS**

We would like to thank the staffs from Thorgene CO., LTD
for their contribution in gene sequencing and immunohis-
tochemical staining. This study was supported by CAMS
Initiative for Innovative Medicine (CAMS-2017-I2M-1-002).

**CONFLICT OF INTEREST**

No potential conflicts of interest were disclosed.

**AUTHORS CONTRIBUTION**

ZZ designed the study and drafted the manuscript. WW col-
lected 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 manu-
script. 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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REFERENCES
Adam, J., Yang, M., Soga, T., & Pollard, P. J. (2014). Rare insights into cancer biology. Oncogene, 33(20), 2547–2556. https://doi.org/10.1038/onc.2013.222
Bulun, S. E. (2013). Uterine fibroids. New England Journal of Medicine, 369, 1344–1355. https://doi.org/10.1056/NEJMr a1209993
Chan, M. M. Y., Barnicoat, A., Mumtaz, F., Aitchison, M., Side, L., Brittain, H., … Gale, D. P. (2017). Cascade fumarate hydratase mutation screening allows early detection of kidney tumour: A case report. BMC Medical Genetics, 18, 79. https://doi.org/10.1186/s12881-017-0436-1
Commandeur, A. E., Styer, A. K., & Teixeira, J. M. (2015). Epidemiological and genetic clues for molecular mechanisms involved in uterine leiomyoma development and growth. Human Reproduction Update, 21(5), 593–615. https://doi.org/10.1093/humupd/dmv030
Gardie, B., Remenieras, A., Kattygnarath, D., Bombled, J., Lefèvre, S., Perrier-Trudova, V., … Richard, S.; French National Cancer Institute “Inherited predisposition to kidney cancer” Network. (2011). Novel FH mutations in families with hereditary leiomyomatosis and renal cell cancer (HLRCC) and patients with isolated type 2 papillary renal cell carcinoma. Journal of Medical Genetics, 48, 226–234. https://doi.org/10.1136/jmg.2010.085068
Guinard, E., Legendre, L., Kramkimel, N., Avril, M. F., Chassaing, N., Cabaret, O., … Mazereeuw Hautier, J. (2016). Complete penetrance and absence of intrafamilial variability in a large family with hereditary leiomyomatosis and renal cell carcinoma. Dermatology, 232, 293–297. https://doi.org/10.1159/000445430
Joseph, N. M., Solomon, D. A., Frizzell, N., Rabban, J. T., Zaloudek, C., & Garg, K. (2015). Morphology and immunohistochemistry for 2SC and FH aid in detection of fumarate hydratase gene aberrations in uterine leiomyomas from young patients. The American Journal of Surgical Pathology, 39, 1529–1539. https://doi.org/10.1097/PAS.0000000000000520
Mäkinen, N., Kämpjärvi, K., Frizzell, N., Bützow, R., & Valteristo, P. (2017). Characterization of MED12, HMGA2, and FH alterations reveals molecular variability in uterine smooth muscle tumors. Molecular Cancer, 16, 101. https://doi.org/10.1186/s12943-017-0672-1
Menko, F. H., Maher, E. R., Schmidt, L. S., Middleton, L. A., Aittomäki, K., Tomlinson, I., … Linehan, W. M. (2014). Hereditary leiomyomatosis and renal cell cancer (HLRCC): Renal cancer risk, surveillance and treatment. Familial Cancer, 13(4), 637–644. https://doi.org/10.1007/s10689-014-9735-2
Miettinen, M., Felisiak-Golabek, A., Wasag, B., Chmara, M., Wang, Z., Butzow, R., & Lasota, J. (2016). Fumarase-deficient uterine leiomyomas: An immunohistochemical, molecular genetic, and clinicopathologic study of 86 cases. The American Journal of Surgical Pathology, 40(12), 1661–1669. https://doi.org/10.1097/PAS.00000 0000000703
Patel, V. M., Handler, M. Z., Schwartz, R. A., & Lambert, W. C. (2017). Hereditary leiomyomatosis and renal cell cancer syndrome: An update and review. Journal of the American Academy of Dermatology, 77(1), 149–158. https://doi.org/10.1016/j.jaad.2017.01.023
Reyes, C., Karamurzin, Y., Frizzell, N., Garg, K., Nonaka, D., Chen, Y. B., & Soslow, R. A. (2014). Uterine smooth muscle tumors with features suggesting fumarate hydratase aberration: Detailed morphologic analysis and correlation with S-(2-succino)-cysteine immunohistochemistry. Modern Pathology, 27(7), 1020–1027. https://doi.org/10.1038/modpathol.2013.215
Wei, M. H., Toure, O., Glenn, G. M., Pithukpakorn, M., Neckers, L., Stolle, C., … Toro, J. R. (2006). Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. Journal of Medical Genetics, 43, 18–27. https://doi.org/10.1136/jmg.2005. 033506
Wheeler, K. C., Warr, D. J., Warsktsky, S. I., & Barmat, L. I. (2016). Novel fumarate hydratase mutation in a family with atypical uterine leiomyomas and hereditary leiomyomatosis and renal cell cancer. Fertility and Sterility, 105(1), 144–148.

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

How to cite this article: Zhao Z, Wang W, You Y, Zhu L, Feng F. Novel FH mutation associated with multiple uterine leiomyomas in Chinese siblings. Mol Genet Genomic Med. 2020;8:e1068. https://doi.org/10.1002/mgg3.1068