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A novel nonsense mutation in the fumarate hydratase gene in a Chinese patient with recurrent leiomyomas

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Objective: To describe a novel nonsense mutation in the fumarate hydratase (FH) gene in a Chinese patient with recurrent multiple leiomyomas.
Design: Case report.
Setting: Medical school-affiliated tertiary hospital.
Patient(s): A nulligravida patient aged 30 years with large uterine leiomyomas (ULMs) and severe anemia.
Intervention(s): Clinical evaluation, abdominal myomectomy, targeted next-generation sequencing.
Main outcome measure(s): Fumarate hydratase gene mutation in ULMs.
Result(s): A novel nonsense mutation (c.771T>G) in the *FH* gene was identified in this patient. This mutation is located in exon 6, which encodes the N-terminal fumarate lyase domain. It leads to a predicted truncated protein with loss of the majority of the lyase domain, resulting in FH deficiency.
Conclusion(s): Because of the recurrent multiple leiomyomas, this patient received 2 myomectomies within 5 years. On immunostaining the leiomyoma, FH deficiency was detected, and targeted next-generation sequencing revealed a novel mutation of the *FH* gene. This patient was at risk for early disease relapse and developing renal cancer, and close disease monitoring is recommended. Meanwhile, the expanded mutation database should benefit patients in diagnosing *FH* gene-associated ULMs. (Fertil Steril Rep® 2023;4:410–5. ©2023 by American Society for Reproductive Medicine.)
Key Words: Fumarate hydratase, uterine leiomyoma, targeted next-generation sequencing

Uterine leiomyomas (ULMs), or fibroids, are noncancerous tumors arising from the overgrowth of smooth muscle and connective tissue in the uterus and are the most common disease in women of reproductive age (1). Although the pathogenic mechanism underlying ULMs remains unclear, hormonal processes, genetic predisposition, somatic alterations, and epigenetic disruptions have been implicated (2). Cytological studies demonstrated that 40%–50% of leiomyoma cells had chromosomal rearrangements and 70% had systemic mutations (3), suggesting an essential role for genetic abnormalities in the pathogenesis of ULMs. As revealed by genome-wide profiling, several hundred genes with functional roles in cell proliferation, differentiation, and extracellular matrix production are altered in ULMs. In spite of the fact that many of these dysregulated genes may function as either effectors or promoters of ULM growth, they are also likely to be secondarily induced and indirectly responsible for tumor growth in morbid and symptomatic ULMs. As of yet, only a limited number of specific genes or cytogenetic aberrations have been identified as being associated with ULMs.

Germline mutations in the *FH* gene, encoding fumarate hydratase, have been associated with hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome (4, 5). Affected individuals develop multiple cutaneous piloleiomyomas, ULMs, uterine leiomyosarcoma, and renal cell carcinoma (RCC). As of now, more than 130 germline mutations on the *FH* gene, including missense, frameshift, nonsense, indel, splice site mutations, and complete deletions, have been identified (6). This case report describes a new mutation of the *FH* gene in a patient with early-onset and recurrent ULMs.

CASE REPORT

The patient, a 30-year-old Chinese nulliparous woman, presented to our hospital with menorrhagia for half a year, anemia, and a palpable mass in her abdomen. She was diagnosed with ULMs 7 years ago and underwent

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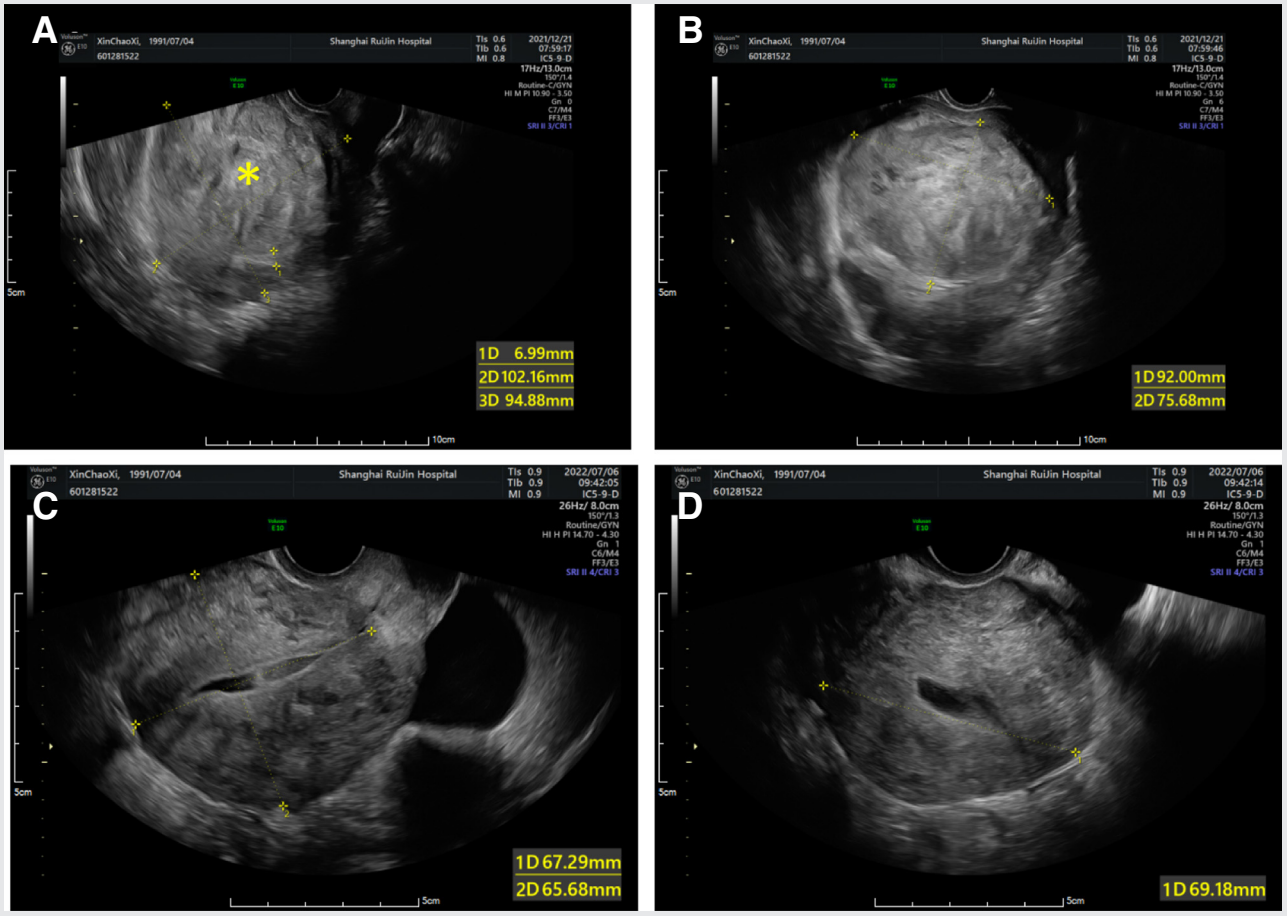
a myomectomy 5 years ago, during which 10 fibroids were removed, and the largest measured 7 × 8 × 6 cm. At that time, the postoperative pathology report showed no abnormalities. The patient continued to follow up with annual ultrasound scans after the first surgery, which showed no recurrence of ULMs until 2017. By 2017, an ultrasound scan indicated that ULMs had relapsed, with the biggest ULM having a diameter of 3 cm. In the fourth year, the ultrasound scan revealed a uterine volume of 102 × 94 × 98 cm and multiple uterine fibroids, the largest of which measured 92 × 76 × 83 mm in size (Fig. 1A). A myomectomy was conducted, and 16 fibroids were removed, with the largest fibroid measuring 10 ×10 × 10 cm. In contrast to regular fibroids, these fibroids were yellowish in color and soft. Intraoperative frozen section biopsies were collected and examined. Pathology showed that the tumors were composed predominantly of spindle-shaped cells with eosinophilic cytoplasm (Fig. 2A) and a few atypical cells. Staghorn vascularity (Fig. 2B) and an alveolar pattern of edema (Fig. 2C) were noted. Some cells had variably atypical nuclei. The mitotic activity was graded as (1/10) high power fields. The immunohistochemistry analysis (Fig. 3B–D) revealed that leiomyoma cells from this patient lacked fumarate hydratase (FH) expression, whereas endothelial cells of blood vessels were FH-positive, indicating the *FH* mutation was specific to the leiomyoma cells.

The patient experienced heavy vaginal bleeding 1 week after the surgery and received gonadotropin-releasing hormone antagonists and pitocine. Afterward, gonadotropin-releasing hormone antagonists were administered every 4 weeks for a total of 6 times. An ultrasound examination 3 months after surgery revealed several low-echo areas with diameters of 4–10 mm as remnant fibroids. After 6 months of surgery, ultrasound revealed heterogeneous echogenicity of the myometrium, possibly because of the postoperative changes or the presence of some small fibroids. Neither kidney abnormalities nor cutaneous lesions were detected at that time, and the patient then took oral contraceptives on a regular basis.

FIGURE 1



Before myomectomy: (A) Ultrasound of the enlarged uterus (102 × 94 × 98 cm) with multiple fibroids (asterisks) of the patient. (B) The largest fibroids (92 × 75 cm). Six months after the myomectomy: (C) ultrasound of the uterus (67 × 66 × 69 cm). (D) Myoma could not be detected using ultrasound; however, an irregular echo of myoma was observed, probably as a result of surgery.

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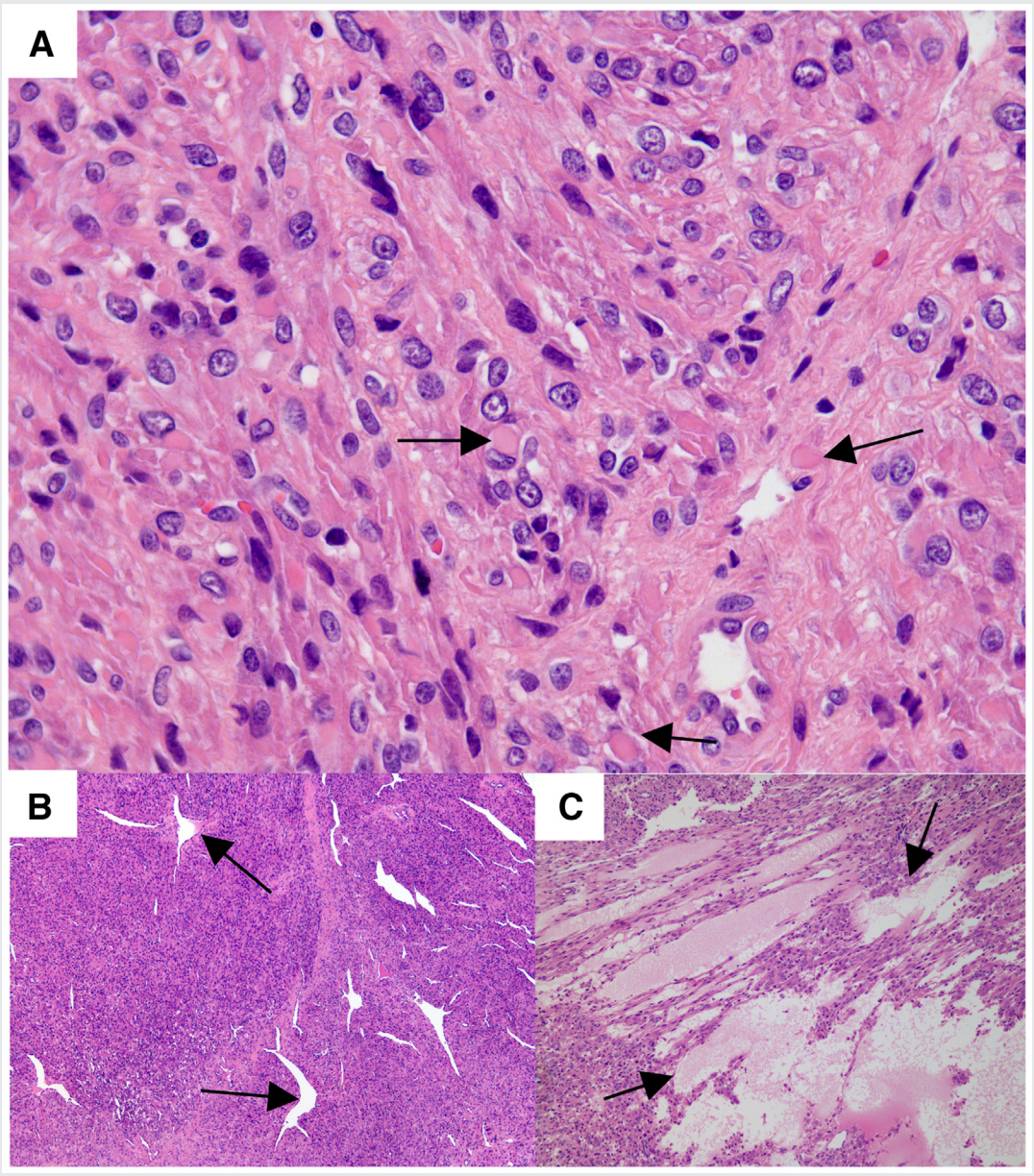
FIGURE 2



Morphologic features of uterine leiomyomas form the patient with the *FH* mutation. (A) Eosinophilic cytoplasmic inclusions, large prominent eosinophilic nucleoli with perinucleolar halos (arrow), (B) Staghorn vascularity (arrow), and (C) Alveolar pattern of edema (arrow) are noted.

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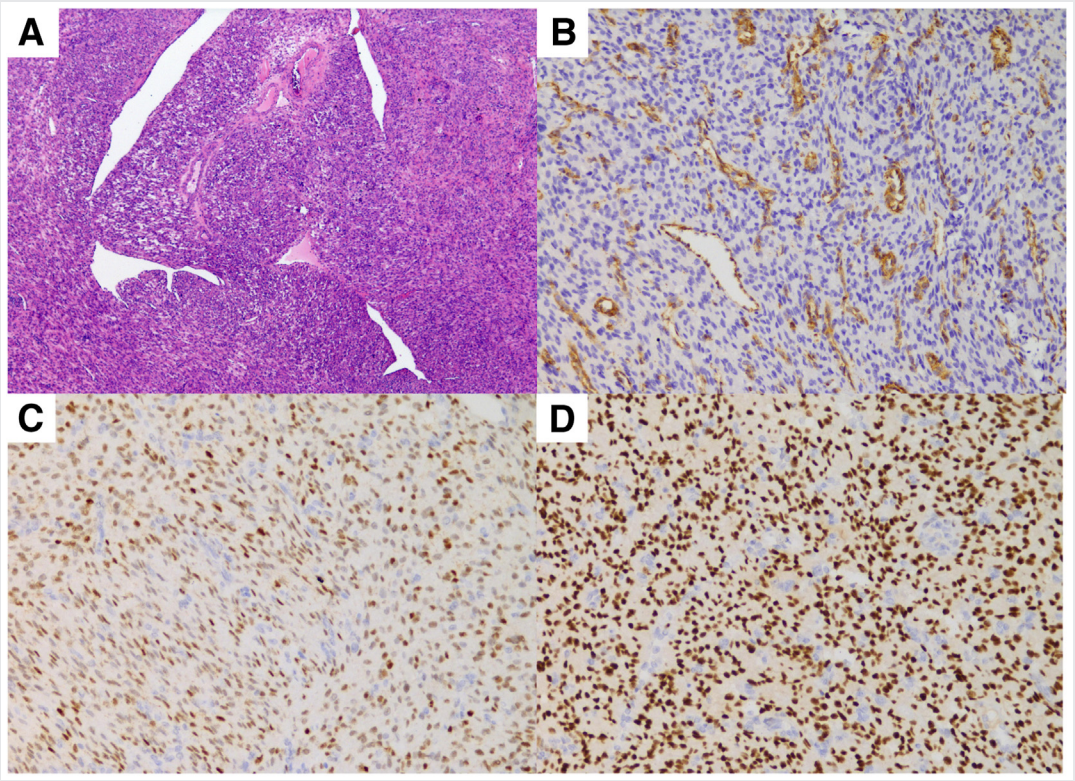
On January 7, 2022, the patient was referred for genetic testing because of the absence of FH protein expression in leiomyomas. Peripheral blood was collected with the patient's consent, and targeted next-generation sequencing was conducted (Human 61 gene mutation panel, ZSJY-3-748, AmoyDx Diagnostics [Xiamen, People's Republic of China]) on Illumina Miseq. The obtained DNA sequences were subjected to analysis with AmoyDx Diagnostics' data analytics. The region containing the detected mutation was amplified using polymerase chain reaction with primers (forward: ATG CAG TTA GAG TAA CTT GTA AGC; reverse: AAG TGC AGC CAC TTT TGC AG) and analyzed using Sanger sequencing.

A nonsense mutation in exon 6 of the *FH* gene (c.771T>G, p.Y257s*), which encodes the N-terminal fumarate lyase domain, was identified. (Fig. 4). This mutation leads to a predicted truncated protein with the loss of most of the lyase domain and results in FH deficiency. This mutation has not been documented previously according to the Leiden Open Variation Database.

FIGURE 3



Immunohistochemical features of leiomyomas from the patient with the *FH* mutation. (A) Topographical staining with hematoxylin and eosin. (B) The endothelial cells lining tumor vessels are FH-positive. The tumor cells lack FH expression and are both ER-positive (C) and PR-positive (D). ER = estrogen receptor; FH = fumarate hydratase; PR = progesterone receptor.

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DISCUSSION

Fumarate hydratase is located in mitochondria and cytoplasm and is an enzyme that catalyzes the conversion of fumarate to L-malate as part of the tricarboxylic acid cycle. In response to DNA damage, FH translocates into the nucleus and produces fumarate, which plays a crucial role in DNA damage repair (7). The FH encoding gene (*FH*) is located on the (q) arm of chromosome 1 (chr1q42.3-q43) and consists of 10 exons (8). Heterozygous germline *FH* mutations predispose to dominantly inherited cutaneous and ULMs as well as papillary renal cell cancer (HLRCC syndrome) (4), although somatic *FH* mutations occur at a low frequency and seem to be limited to counterpart tumor types segregating in HLRCC families. The biallelic inactivation of *FH*, which leads to fumarase deficiency, is associated with encephalopathy, developmental retardation, and nervous system abnormalities (9). Fumarate hydratase deficiency, as being associated with severe encephalopathy, was reported for the first time in 1986 by Zinn et al. (10), and Bourgeron et al. (11) outlined the molecular mechanism for *FH* mutations in 1994. In 2002, the Multiple Leiomyoma Alliance identified *FH* as a tumor suppressor gene in multiple cutaneous and uterine leiomyomatoses and HLRCC (4). Alam et al. (12) reported 45 types of *FH* mutations in 76 patients with cutaneous leiomyoma in 2004, including 58% (26/45) missense mutations, 27% (12/45) frameshift mutations, 9% (4/45) nonsense mutations, and 7% (3/45) different whole-gene deletions. So far, >130 germline mutations on the *FH* gene have been reported, and the number of reported mutations is ever-expanding (13). In this study, we reported a novel heterozygous nonsense mutation of the *FH* gene in a patient with recurrent ULMs.

Although fumarate hydratase-deficient uterine leiomyomas (FH-DUL) is a rare form of leiomyoma, accounting for 0.4%–1.6% of all ULMs (6, 14, 15), the underlying mechanism of somatic *FH* mutations remains unclear. Several mechanisms have been implicated in the association between *FH* mutations and ULMs, including pseudohypoxia, oxidative stress, decreased levels of adenosine monophosphate-activated protein kinase, and impaired homologous recombination of DNA repair mechanisms (16). Fumarate hydratase-deficient uterine leiomyomas has been reported in about 200 families worldwide and could be one of the clinical manifestations of HLRCC among female patients (17, 18) and those with somatic *FH* mutations. The clinical manifestations of females with HLRCC include early-onset multiple large uterine fibroids, anemia, irregular menstruation, dysmenorrhea, and infertility (20).

FIGURE 4

(A) Next-generation sequencing (NGS) sequencing read alignment using Integrated Genomics Viewer reports a variant at exon 6 of the *FH* gene [c.771 T > G, p.(Y257*)]. (B) Sanger sequencing validation of the identified mutation (K = T/G) revealed using the targeted NGS analysis.

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The pathological manifestations of FH-DUL are unique, including atypical nuclei, large eosinophilic nucleoli with perinuclear halos, intracytoplasmic hyaline bodies, interstitial "acinar" edema, and hemangiopericytoma-like (staghorn) vessels. With the development of next-generation sequencing, which has sharply reduced the cost and increased the efficiency, it now possible to detect affected individuals in their early stages.

The patient in this report was heterozygous for the *FH* mutation and had only uterine fibroids at present, without skin leiomyomas or renal tumors. In spite of the fact that not all individuals with HLRCC present with or develop RCC, close monitoring and routine examination are recommended. Considering that most RCCs are unilateral (19, 20), early intervention will improve the clinical outcome significantly (21). Meanwhile, it would be prudent for this

patient to adopt a careful fertility plan as a nulligravida, given the detrimental effects of ULMs on fertility and the potential risks associated with hysterectomy.

In summary, this report described a FH-DUL case caused by a novel germline heterozygous mutation of the *FH* gene, which has improved our understanding of this disease. For patients with early-onset and multiple or single large uterine fibroids, especially those with fibroids with unusual appearances, genetic testing for *FH* mutations is crucial to enabling an appropriate treatment plan for the women affected.

Declaration of interests: Y.R. has nothing to disclose. W.F. has nothing to disclose. C.Y. has nothing to disclose.

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