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CASE REPORT

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Rare variant in the fumarate hydratase gene found in patients with clinical features of hereditary leiomyomatosis and renal cell cancer (HLRCC): A case series

Keith Franke[1] | **Jennie Vagher**[2] | **Julie Boyle**[2] | **April Hall**[1,3] | **Kelcy Smith-Simmer**[1,4]

[1]Master of Genetic Counselor Studies Program, Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[2]Huntsman Cancer Institute at the University of Utah, Salt Lake City, Utah, USA

[3]Center for Human Genomics and Precision Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[4]Oncology Genetics, University of Wisconsin Carbone Cancer Center, UW Health, Madison, Wisconsin, USA

Correspondence
Kelcy Smith-Simmer, Oncology Genetics, University of Wisconsin Carbone Cancer Center, UW Health, Madison, WI, USA.
Email: ksmith-simmer@uwhealth.org

Present address
Keith Franke, Department of Molecular and Medical Genetics, Oregon Health & Science University, Portland, Oregon, USA

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Abstract

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an inherited cancer predisposition syndrome caused by autosomal dominant heterozygous pathogenic variants in the fumarate hydratase (*FH*) gene. *FH* pathogenic variant carriers are at an increased risk for cutaneous leiomyomas, renal cell cancer, and uterine fibroids. We present a case series of patients identified at two different medical institutions with clinically diagnostic features of HLRCC and a shared rare variant in the *FH* gene.

KEYWORDS

FH, HLRCC, leiomyomatosis, renal cancer

1 | BACKGROUND

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is a hereditary cancer predisposition condition characterized by cutaneous leiomyomas, an increased risk for renal cell cancer, and uterine leiomyomas (uterine fibroids). Heterozygous germline pathogenic variants of the fumarate hydratase (*FH*) gene, located on chromosome 1q43, are responsible for the clinical features of HLRCC. The most distinguishing feature of HLRCC is multiple cutaneous leiomyomas, which had previously been reported as occurring in nearly all patients with heterozygous *FH* pathogenic variants.[1,2] However, with increased population screening and the ability to detect milder presentations, the incidence of cutaneous leiomyomas among individuals with HLRCC is estimated to be around 50%.[3] HLRCC is also associated with an approximate 15% lifetime risk of renal cell cancer (RCC), with a tendency for aggressive type 2 papillary RCC.[4] Uterine leiomyomas are the last of the three hallmark features of HLRCC, and while common in the general population, they occur in a vast majority (86%–100%) of females with *FH* pathogenic variants.[2,5] The associated uterine leiomyomas also tend to be more numerous and of an earlier onset than in the general population,

often resulting in early total abdominal hysterectomies.[2,5] Additional studies have proposed that individuals with *FH* pathogenic variants may also be at increased risk of pheochromocytoma(s) and paraganglioma(s).[6,7]

The causal relationship between heterozygous *FH* pathogenic variants and HLRCC was first reported by Tomlinson et al. in 2002 [8]and has since been well described in the literature. *FH* encodes for the enzyme fumarate hydratase (FH), which converts fumarate to malate in the Krebs cycle, and research suggests that this enzyme plays a role in repairing double strand DNA (dsDNA) breaks.[9,10] This role in dsDNA break repair is thought to be related to the tumor suppressor role of *FH*.

While there is not current consensus on the diagnostic criteria for HLRCC, experts suggest that the major criteria, which signifies a high likelihood of HLRCC, is multiple cutaneous leiomyomas with at least one biopsy-proven/pathologically confirmed lesion.[2,11] Proposed minor criteria that indicates a suspicion for HLRCC includes (a) surgical treatment for severely symptomatic uterine leiomyomas before age 40, (b) type 2 papillary RCC before age 40, and/or (c) a first-degree relative that meets one of the previous criteria.[2]

The *FH* gene is currently the only reported gene associated with HLRCC. While true for most patients, not all individuals with a clinical diagnosis of HLRCC will have a detectable pathogenic variant in the *FH* gene, with one study reporting a 93% pathogenic variant detection rate in individuals with clinical HLRCC[5] and another reporting an 89% pathogenic variant detection rate in HLRCC families.[12] As of January 2022, the ClinVar database includes 217 pathogenic and 102 likely pathogenic single gene variants within the *FH* gene.[13]

We present a case series of patients identified at two different medical institutions with clinically diagnostic features of HLRCC and a shared rare variant c.977G>A (p.Gly326Glu) in the *FH* gene. Further evidence presented includes variant interpretation and bioinformatic assessment of the rare variant in *FH*.

2 | CASE REVIEW

The three patients identified from two families underwent germline genetic testing via blood samples at the same commercial CAP/CLIA certified clinical testing laboratory. The testing laboratory reports that analysis was performed using Illumina next-generation sequencing with reported >99% sensitivity and specificity for single nucleotide variants and insertions and deletions <15 bp.

2.1 | Family A, Patient #1

Patient #1, a 24-year-old woman, was referred for genetic counseling to discuss genetic testing for HLRCC following an evaluation by her dermatologist for two groupings of 11 total pathologically confirmed leiomyomas (Figure 1, Images A & B). Prior to the genetic counseling consult, the patient underwent a transvaginal combo with limited pelvis and renal ultrasounds to evaluate for uterine leiomyomas and renal cancer with unremarkable results.

The patient's initial genetic counseling consult revealed a family history striking for features associated with HLRCC (Figure 2), including a paternal uncle with a history of papillary RCC (unknown type) in his 40s, a paternal grandmother with a history of papillary RCC (unknown type) in her 50s and a hysterectomy at a young age for unknown reasons, a paternal great aunt (grandmother's sister) with a history of renal cancer (unknown type) in her 60s and a hysterectomy due to uterine fibroids, and a paternal great grandmother (grandmother's mother) with a history of renal cancer (unknown type) at an unknown age.

Given the patient's family and personal history, multicancer panel testing was pursued at a commercial laboratory which revealed a variant of uncertain significance (VUS) in the *FH* gene NM_000143.4(*FH*): c.977G>A (p.Gly326Glu). In addition, a low penetrance pathogenic variant was discovered in the *CHEK2* gene c.470T>C (p.Ille157Thr), which is expected to be non-contributory for the personal and family history that is concerning for HLRCC.

At the time of testing, the clinical laboratory reported the *FH* VUS had not been seen in their laboratory previously, did not appear in population databases, and was not published in the general literature. Despite this variant being classified as a VUS, the patient met proposed clinical criteria for HLRCC given her personal history of multiple[14] pathologically confirmed cutaneous leiomyomas.[2,11] The patient was advised to follow surveillance recommendations for HLRCC including annual gynecological ultrasound examination for uterine fibroids, annual renal cancer screening, and continued dermatologic monitoring of cutaneous leiomyomas.[2] Given the identified *FH* VUS and concerning paternal family history, the patient's father and sister were encouraged to undergo genetic counseling with consideration of testing for the familial *FH* VUS.

2.2 | Family A, Patient #2

Patient #2, a 55-year-old man, presented for evaluation and discussion of genetic testing for the familial VUS in *FH* c.977G>A (p.Gly326Glu) identified in his daughter, patient #1. This patient reported he received renal ultrasounds every 18 months due to his family history of renal cancer (Figure 2), he was not followed by a dermatologist, and he did not have any known cutaneous leiomyomas.

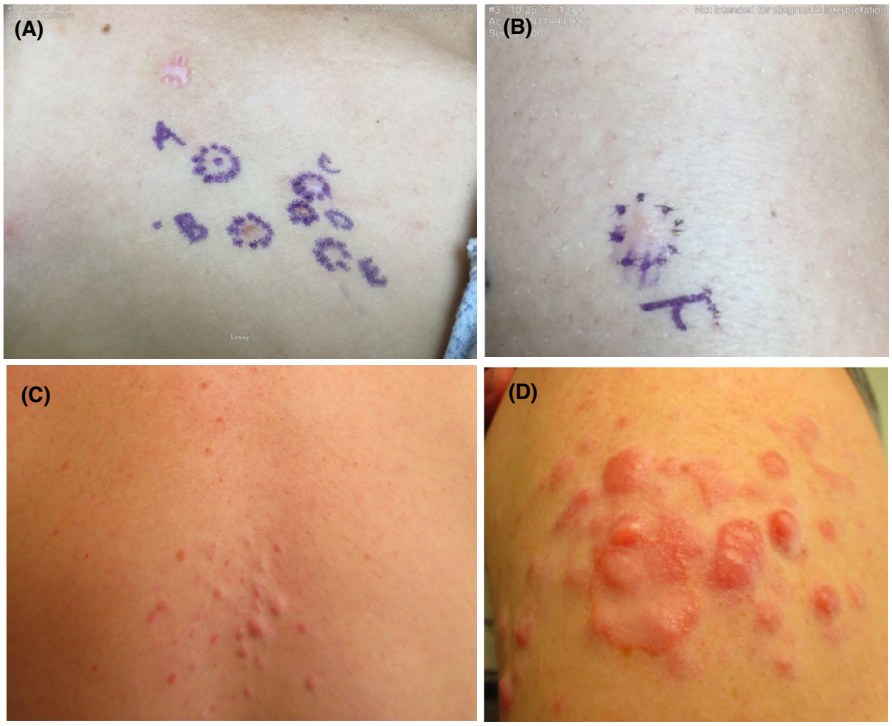

FIGURE 1 Dermatology images of pathologically confirmed cutaneous leiomyomas. (A) Patient #1, six leiomyomas of the chest. (B) Patient #1, one leiomyoma of the calf. (C): Patient #3, leiomyomas of the back. (D) Patient #3, leiomyomas of the arm

Patient #2 decided to pursue genetic testing for the familial *FH* VUS in order to aid segregation analysis, and also elected to pursue a multi-gene panel due to the family history of multiple cancers.

The results of patient #2's genetic testing revealed the same *FH* variant c.977G>A (p.Gly326Glu) identified in his daughter. Given these testing results and his daughter's clinical diagnosis of HLRCC, patient #2 was encouraged to follow surveillance guidelines for *FH* pathogenic variants.[2] In addition, full gene analysis of the *CHEK2* gene revealed a pathogenic deletion of exons 9–10, a different pathogenic variant than the one discovered in his daughter. This *CHEK2* variant is expected to be non-contributory for the concerning family history of HLRCC. Since his initial genetic counseling consult, patient #2 has passed away.

2.3 | Family B, Patient #3

Patient #3, a 31-year-old man, presented to a separate medical institution for genetic testing due to multiple biopsy-proven cutaneous leiomyomas (Figure 1C,D). The patient first noticed the cutaneous lesions at age 16, and the lesions were biopsied on two separate occasions, confirming the diagnosis of leiomyoma. Genetic testing revealed a VUS in *FH* c.977G>A (p.Gly326Glu) (the same *FH* VUS identified in Family A). The patient's maternal family history was unremarkable for features of HLRCC, and the paternal family history was unavailable. Although the testing laboratory classified this variant as a VUS when the result was reported, a variant interpretation specialist at the ordering hospital reviewed the *FH* VUS and was suspicious that the variant was pathogenic. The variant specialist, in conjunction with the genetic counseling team at the ordering hospital, recommended increased follow-up for the patient based on proposed HLRCC management guidelines because of the personal history of multiple biopsy-proven cutaneous leiomyomas and unknown family history.[2,14]

3 | VARIANT INTERPRETATION

Analysis of the *FH* c.977G>A (p.Gly326Glu) variant using in-silico tools designed to predict impact on protein function (BayesDel, Sift, Polyphen2, GERP++, Mutation Taster, SiPhy29way, CADD, rhapsodyscore, Foldx energy, Rosetta energy, RSA), predicts deleterious or destabilizing impact on FH function.[15,16] *FH* Gly326Glu is absent from the gnomAD population database. *FH* Gly326 is a highly conserved amino acid in the highly conserved D2 subunit (amino acid residues 189–439) within α-helical structure three of six, contributing to the 20-helix bundle core that interacts to form the FH homotetramer. Two D2 mutants underwent in vitro enzymatic and oligomerization assays to determine impact of mutations in this region: "A308T and H318Y render human fumarase enzymatically inactive via defective oligomerization. Therefore, some forms of FH deficiency and HLRCC can be linked to improperly folded quaternary structure".[17] Additionally, heterozygous LOF *FH* mutations have been found to be highly

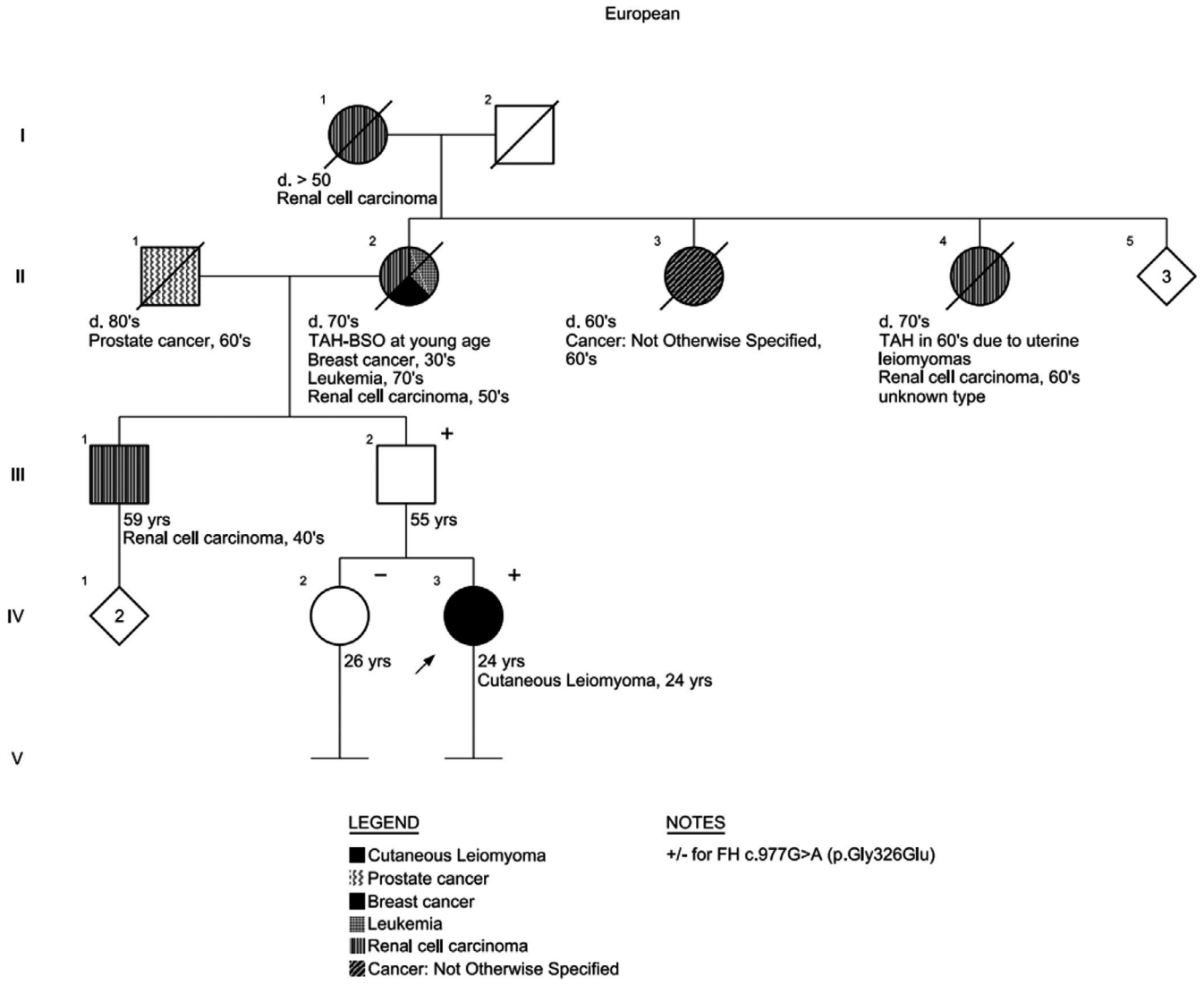

FIGURE 2 Pedigree of Family A. Family pedigree, taken at the time of the initial appointment, detailing the cancer history and HLRCC related features. Assigned females at birth are represented as circles. Assigned males at birth are represented as squares. Lines through a symbol indicate the individual is deceased. Patient #1 is IV-3, and indicated as the proband with an arrow. Patient #2 is III-2. BSO, bilateral salpingo-oophorectomy; d., deceased at age; TAH, total abdominal hysterectomy

penetrant, with FH loss of activity reported in D2 domain R190H-FH and E319Q-FH cells by Lorenzato et al: "expression of equal amount of wild-type and R190H-FH in the same cell... mutated FH protein directly inhibited enzymatic activity by nearly abrogating the FH homotetramer formation. These data demonstrate the dominant negative effect of the R190H missense mutation in the *FH* gene and suggest that the FH tumor-suppressing activity might be impaired in cells carrying a heterozygous mutation".[18] A loss of enzymatic activity due to loss of function (LOF) *FH* mutations in the D2 subunit is also supported by metabolomics data.[16] Overall, available data suggest that heterozygous *FH* c.977G>A (p.Gly326Glu) may have a deleterious impact on FH activity; however, additional supportive data are needed for a robust likely pathogenic or pathogenic classification. These data would include functional studies demonstrating disruption of FH homotetramer formation, or otherwise inhibited enzymatic activity, in heterozygous Gly326Glu cells and additional segregation data within proband relatives.

4 | DISCUSSION

We report a case series of individuals from two families with clinical diagnoses of HLRCC who carry a shared rare variant in the gene *FH* c.977G>A (p.Gly326Glu). The presence of pathologically confirmed cutaneous leiomyomas in patient #1 indicates a clinical diagnosis of HLRCC, and the segregation analysis of this variant through patient #2 links this variant to the paternal family history of renal cancer and uterine fibroids. Additionally, the presence

of pathologically confirmed cutaneous leiomyomas, and thus, clinical diagnosis of HLRCC in patient #3 adds evidence toward the pathogenicity of this variant. Finally, literature review of similar variants and bioinformatic tools adds evidence that this rare variant in *FH* may be deleterious to the protein function of fumarate hydratase.

Classification of genetic variants is an important tenant of clinical genetics, and steps have been taken within the field to standardize the variant classification system used to provide evidence toward pathogenic or benign status for any given variant. In 2015, the American College of Medical Genetics (ACMG) released an updated guideline with which to classify variants in clinical laboratories.[19] Using this ACMG framework and their own internal processes, clinical genetic testing laboratories classify variants between the categories of benign, likely benign, variant of uncertain significance, likely pathogenic, and pathogenic. When new information about a variant becomes available, such as evidence of disease in carriers, segregation of the variant in families with multiple affected individuals, functional studies of protein expression, RNA analysis, and/or variant predictions from functional models, laboratories may consider reclassifying the variant.

At the time these patients were seen, this variant was classified as a VUS by the clinical genetic testing laboratory which performed the analysis. This variant was also detected once by two other commercial clinical genetic testing laboratories (representing two patients). Each of the laboratories had varying degrees of evidence toward its pathogenicity, though all classified it as a VUS.

Since the commencement of this project, the clinical testing laboratory where the patients described in this study were tested has internally reclassified this variant from a VUS to a likely pathogenic variant. Personal communication with the commercial clinical testing laboratory revealed that evidence toward this likely pathogenic classification includes the absence of the variant from healthy population databases, clinical phenotypes of the patients identified with the variant, and internal modeling of the predicted protein sequence and biophysical properties of the protein product of this variant.

Research has shown that reclassification of variants from VUS to pathogenic is beneficial to patient care.[20,21] Reclassification is especially important in cancer genetics, since patients identified to have pathogenic variants in cancer predisposition genes may choose to undergo screening for earlier detection of cancer. For the *FH* c.977G>A (p.Gly326Glu) variant, broad reclassification from a VUS to a pathogenic variant could increase access to renal cancer screening, improve insurance coverage for recommended HLRCC management, and reduce psychological burden from uncertain results. These potential outcomes are important for the families identified in this study and for other families who carry this specific variant.

In summary, this case series presents information regarding the characteristics and family history of individuals identified to have a rare c.977G>A (p.Gly326Glu) variant in the *FH* gene who meet proposed clinical diagnostic criteria for HLRCC. To our knowledge, there have been no other detailed publications regarding this variant, and in light of the presented information, a broader reclassification of this variant from VUS to likely pathogenic or pathogenic is indicated.

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CONFLICTS OF INTEREST

Jennie Vagher serves on the advisory board for medical oncology at Invitae Genetics. The authors do not have any additional relevant conflicts of interest to report.

AUTHOR CONTRIBUTIONS

Keith Franke (Primary author for the publication) involved in project initiation, IRB application, data collection, writing, editing, and manuscript submission. Jennie Vagher (Co-author for the publication) involved in IRB coordination, data collection, participant recruitment, writing, and editing. Julie Boyle (Co-author for the publication) involved in IRB coordination, variant interpretation, and writing. April Hall (Co-author for the publication) involved in project oversight, IRB application, IRB coordination, and editing. Kelcy Smith-Simmer (Corresponding author for the publication) involved in project oversight, IRB application, data collection, participant recruitment, participant consenting, and editing.

ETHICAL APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. This study underwent scientific review and was approved by the UW Carbone Cancer Center Protocol Review and Monitoring System and was approved by the UW-Madison Health Sciences Institutional Review Board (IRB #2020-1489).

CONSENT

Written consent was obtained from all patients or their legal representatives for inclusion in this study and this research study to be published.

CODE AVAILABILITY

Research reported in this publication utilized the High-Throughput Genomics and Bioinformatic Analysis Shared

Resource at Huntsman Cancer Institute at the University of Utah, and this tool was supported by the National Cancer Institute of the National Institutes of Health under Award Number P30CA042014. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Keith Franke https://orcid.org/0000-0001-7826-9999

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