BACKGROUND

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

The causal relationship between heterozygous \textit{FH} pathogenic variants and HLRCC was first reported by Tomlinson et al. in 2002 \textsuperscript{8} and has since been well described in the literature. \textit{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.\textsuperscript{9,10} This role in dsDNA break repair is thought to be related to the tumor suppressor role of \textit{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.\textsuperscript{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.\textsuperscript{2}

The \textit{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 \textit{FH} gene, with one study reporting a 93\% pathogenic variant detection rate in individuals with clinical HLRCC\textsuperscript{5} and another reporting an 89\% pathogenic variant detection rate in HLRCC families.\textsuperscript{12} As of January 2022, the ClinVar database includes 217 pathogenic and 102 likely pathogenic single gene variants within the \textit{FH} gene.\textsuperscript{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 \textit{FH} gene. Further evidence presented includes variant interpretation and bioinformatic assessment of the rare variant in \textit{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, multi-cancer panel testing was pursued at a commercial laboratory which revealed a variant of uncertain significance (VUS) in the \textit{FH} gene NM_000143.4(FH): c.977G>A (p.Gly326Glu). In addition, a low penetrance pathogenic variant was discovered in the \textit{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 \textit{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\textsuperscript{14} pathologically confirmed cutaneous leiomyomas.\textsuperscript{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.\textsuperscript{2} Given the identified \textit{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 \textit{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 \textit{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.
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. 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.

### 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. \( FH \) Gly326 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 \( \alpha \)-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”. Additionally, heterozygous LOF \( FH \) mutations have been found to be highly
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. 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. 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.

ACKNOWLEDGMENTS
We thank the research subjects for participating in this research study. We would also like to thank the University of Utah and Huntsman Cancer Institute for their contributions to this study.

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

FRANKE ET AL.
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

**REFERENCES**

1. Muller M, Ferlicot S, Guillaud-Bataille M, et al. Reassessing the clinical spectrum associated with hereditary leiomyomatosis and renal cell carcinoma syndrome in French FH mutation carriers. *Clin Genet*. 2017;92(6):606-615. doi:10.1111/cge.13014

2. Smit DL, Mensenkamp AR, Badele S, et al. Hereditary leiomyomatosis and renal cell cancer in families referred for fumarate hydratase germline mutation analysis. *Clin Genet*. 2011;79(1):49-59. doi:10.1111/j.1399-0004.2010.01486.x

3. Bholta PT, Gilpin C, Smith A, Graham GE. A retrospective review of 48 individuals, including 12 families, molecularly diagnosed with hereditary leiomyomatosis and renal cell cancer (HLRCC). *Fam Cancer*. 2018;17(4):615-620. doi:10.1007/s10689-018-0076-4

4. Menko FH, Maher ER, Schmidt LS, et al. Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. *Fam Cancer*. 2014;13(4):637-644. doi:10.1007/s10689-014-035506

5. Wei MH, Toure O, Glenn GM, et al. Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. *J Med Genet*. 2006;43(1):18-27. doi:10.1136/jmg.2005.033506

6. Castro-Vega LJ, Buffet A, De Cubas AA, et al. Germline mutations in FH confer predisposition to malignant pheochromocytomas and paragangliomas. *Hum Mol Genet*. 2014;23(9):2440-2446. doi:10.1093/hmg/ddt639

7. Clark GR, Sciacovelli M, Gaude E, et al. Germline FH mutations presenting with pheochromocytoma. *J Clin Endocrinol Metab*. 2014;99(10):E2046-E2050. doi:10.1210/jc.2014-1659

8. Tomlinson IP, Alam NA, Rowan AJ, et al.; Multiple Leiomyoma Consortium. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet*. 2002;30(4):406-410. doi:10.1038/ng849

9. Jiang Y, Qian X, Shen J, et al. Local generation of fumarate promotes DNA repair through inhibition of histone H3 demethylation. *Nat Cell Biol*. 2015;17(9):1158-1168. doi:10.1038/ncb3209

10. Yogev O, Yogev O, Singer E, et al. Fumarase: a mitochondrial metabolic enzyme and a cytosolic/nuclear component of the DNA damage response. *PLoS Biol*. 2010;8(3):e1000328. doi:10.1371/journal.pbio.1000328

11. Schmidt LS, Linehan WM. Hereditary leiomyomatosis and renal cell carcinoma. *Int J Nephrol Renovasc Dis*. 2014;7:253-260. doi:10.2147/IJNRD.S42097

12. Toro JR, Nickerson ML, Wei MH, et al. Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am J Hum Genet*. 2003;73(1):95-106. doi:10.1086/376435

13. NCBI, ed. FH[Gene] - ClinVar - NCBI. n.d. Accessed January 7, 2022. https://www.ncbi.nlm.nih.gov/clinvar/?term=fh%5Bgene%5D

14. National Comprehensive Cancer Network. *Kidney Cancer (Version 1.2022)*. 2021. https://www.nccn.org/professionals/physician_gls/pdf/kidney.pdf. Accessed on April 2021.

15. Feng BJ. PERCH: a unified framework for disease gene prioritization. *Hum Mutat*. 2017;38(3):243-251. doi:10.1002/humu.23158

16. Shorthouse D, Hall M, Hall BA. Computational saturation screen reveals the landscape of mutations in human fumarate hydratase. *J Chem Inf Model*. 2021;61(4):1970-1980. doi:10.1021/acs.jcim.0c00063

17. Bulku A, Weaver TM, Berkmen MB. Biochemical characterization of two clinically-relevant human fumarase variants defective for oligomerization. *Open Biochem J*. 2018;12:1-15. doi:10.2174/1874091X01812010001

18. Lorenzato A, Olivero M, Perro M, Brière JJ, Rustin P, Di Renzo MF. A cancer-predisposing “hot spot” mutation of the fumarase gene creates a dominant negative protein. *Int J Cancer*. 2008;122(4):947-951. doi:10.1002/ijc.23209

19. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

20. Sexton A, Rawlings L, McKavanagh G, Simons K, Winship I. A novel von Hippel Lindau gene intronic variant and its reclassification. *Fam Cancer*. 2014;13(4):637-644. doi:10.1007/s10689-014-9735-2

21. Slavin TP, Manjarrez S, Pritchard CC, Gray S, Weitzel JN. How to cite this article: Franke K, Vagher J, Boyle J, Hall A, Smith-Simmer K. Rare variant in the fumarate hydratase gene found in patients with clinical features of hereditary leiomyomatosis and renal cell cancer (HLRCC): A case series. *Clin Case Rep*. 2022;10:e05513. doi:10.1002/ccr3.5513