A patient with very early onset FH-deficient renal cell carcinoma diagnosed at age seven

Rieko Taniguchi[1], Hideki Muramatsu[1], Yusuke Okuno[2], Taro Yoshida[1], Manabu Wakamatsu[1], Motoharu Hamada[1], Chiyoe Shirota[1], Wataru Sumida[1], Akinari Hinoki[1], Takahisa Tainaka[1], Yoshimitsu Gotoh[3], Toyonori Tsuzuki [4], Yukichi Tanaka[5], Seiji Kojima[1], Hiroo Uchida[1], and Yoshiyuki Takahashi[1]

[1]Nagoya University Graduate School of Medicine Faculty of Medicine
[2]Nagoya University Hospital
[3]Nagoya Daini Red Cross Hospital
[4]Aichi Medical University Hospital
[5]Kanagawa Childrens Medical Center

March 30, 2022

Abstract

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is caused by heterozygous germline mutations in the fumarate hydratase (FH) gene and is associated with increased susceptibility to cutaneous leiomyomas, uterine leiomyomas, and renal cell carcinoma (RCC). This report describes a seven-year-old male who developed a large right kidney tumor with multiple cystic lesions that contained enhanced solid components. Whole-exome sequencing identified his germline mutation in the FH gene and its loss of heterozygosity in the tumor. This was the youngest-onset case of HLRCC-associated RCC to date. This report may affect the starting age for future RCC-surveillance programs for patients with HLRCC.

A patient with very early onset FH-deficient renal cell carcinoma diagnosed at age seven

Rieko Taniguchi [1], Hideki Muramatsu[1], Yusuke Okuno [2], Taro Yoshida[1], Manabu Wakamatsu [1], Motoharu Hamada [1], Chiyoe Shirota [3], Wataru Sumida [3], Akinari Hinoki [4], Takahisa Tainaka[3], Yoshimitsu Gotoh [5], Toyonori Tsuzuki [6], Yukichi Tanaka[7], Seiji Kojima [1], Hiroo Uchida[3], and Yoshiyuki Takahashi [1]

[1] Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan

[2] Medical Genomics Center, Nagoya University Hospital, Nagoya, Japan

[3] Department of Pediatric Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

[4] Department of Rare/ Intractable Cancer Analysis Research, Nagoya University Graduate School of Medicine, Nagoya, Japan

[5] Department of Pediatric Nephrology, Japanese Red Cross Nagoya Daini Hospital, Nagoya, Japan

[6] Department of Surgical Pathology, Aichi Medical University Hospital, Nagakute, Japan

[7] Department of Pathology, Kanagawa Children's Medical Center, Kanagawa, Japan

Corresponding authors:

Yoshiyuki Takahashi, M.D., Ph.D.

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Phone: +81-52-744-2294

Fax: +81-52-744-2974

E-mail: ytakaha@med.nagoya-u.ac.jp

Hideki Muramatsu, M.D., Ph.D.

65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Phone: +81-52-744-2294

Fax: +81-52-744-2974

E-mail: hideki.muramatsu@gmail.com

Word count for text: 1173 words

Word count for abstract: 100 words

Total number of figures, tables, and supplemental files: 1 figure, 0 tables, and 1 supplemental file

Running title: Very Early Onset FH-deficient RCC

Keywords: Fumarate hydratase-deficient renal cell carcinoma, Hereditary leiomyomatosis and renal cell cancer, Cystic renal disease

Abbreviations

Abbreviation	Full-term
HLRCC	hereditary leiomyomatosis and renal cell cancer
FH	fumarate hydratase
RCC	renal cell carcinoma
MRI	magnetic resonance imaging
WES	whole-exome sequencing
CT	computed tomography
MCDK	multicystic dysplastic kidney
FDG-PET	fluorodeoxyglucose-positron emission tomography
CD	cluster of differentiation
LOH	loss of heterozygosity

Abstract

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is caused by heterozygous germline mutations in the *fumarate hydratase*(*FH*) gene and is associated with increased susceptibility to cutaneous leiomyomas, uterine leiomyomas, and renal cell carcinoma (RCC). This report describes a seven-year-old male who developed a large right kidney tumor with multiple cystic lesions that contained enhanced solid components. Whole-exome sequencing identified his germline mutation in the *FH* gene and its loss of heterozygosity in the tumor. This was the youngest-onset case of HLRCC-associated RCC to date. This report may affect the starting age for future RCC-surveillance programs for patients with HLRCC.

INTRODUCTION

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant disease characterized by susceptibility to the development of cutaneous leiomyomas, uterine leiomyomas, and renal cell carcinoma (RCC). HLRCC is caused by heterozygous germline mutations in the*fumarate hydratase* (*FH*) gene, and the incidence of RCC in*FH* mutation carriers is estimated to be around 15%.[1–3] RCC in HLRCC usually occurs in

adolescents to middle-age adults, and the median age of RCC diagnosis is 40–44 years.[2, 3] FH-deficient RCC is often diagnosed at an advanced stage with metastases even if the primary tumor size is small. There is no standard treatment for FH-deficient RCC with metastasis. The mean survival of individuals diagnosed with advanced-stage FH-deficient RCC was significantly shorter than that of individuals diagnosed with early-stage RCC (15.8 vs. 80.8 months).[3] Since only diagnosis and early-stage intervention can improve disease-related mortality, regular abdominal magnetic resonance imaging (MRI) is recommended for carriers of *FH* mutations.[1]Based on the prior report of the then-youngest patient diagnosed to date, [4] it is recommended that screening start around age 8–10.[1] Here, we report a male with HLRCC and very early onset RCC at seven years old. This report may affect the starting age for future RCC-surveillance programs for patients with HLRCC.

METHODS

We performed whole-exome sequencing (WES) and Sanger sequencing as previously reported.[5] For WES, the sequencing library was prepared using SureSelect Human All Exon v5 bait (Agilent, Santa Clara, CA, USA), followed by paired-end sequencing using a HiSeq 2500 NGS system (Illumina, San Diego, CA, USA). Sanger sequencing, based on genomic DNA, was performed using an ABI 3130xL Genetic Analyzer and BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA, USA). The details of the analyses are presented in the supplementary methods.

Informed assent and written informed consent were obtained from the proband and proband's parents, respectively. This research was approved by the institutional review board of the Nagoya University Graduate School of Medicine.

RESULTS

Case presentation

A 7-year-old Japanese male presented with a right upper abdominal mass. He had no remarkable past medical history. His parents were non-consanguineous. His father underwent prostatectomy for prostate cancer at age 51. His mother and his elder brother had no remarkable medical history (**Fig. 1A**). His general condition was good. Physical examination revealed a large mass in the right upper abdomen. Brown spots were found on the left shoulder, lower back, and lower abdomen. His laboratory tests were normal, except for mildly elevated C-reactive protein (1.30 mg/dL). An enhanced computed tomography (CT) scan showed a large right kidney tumor with multiple cystic lesions that contained enhanced solid components (**Fig. 1B**) and a compensatory hypertrophic left kidney. Although he was initially misdiagnosed with multicystic dysplastic kidney (MCDK),[18]F-fluorodeoxyglucose-positron emission tomography (FDG-PET) revealed uptake on the solid components of the right kidney (**Fig. 1C**). Renal scintigraphy showed decreased right kidney function. There was no evidence of distant metastasis by enhanced CT and FDG-PET.

We performed a laparoscopic right nephrectomy. The tumor was inside the kidney with multiple cysts and focal nodules. Pathological examination revealed that cysts were lined by mild eosinophilic simple cuboidal epithelium with mild nuclear atypia and hardly mitotic activity. Tumor cells with relatively prominent nucleoli, surrounded by halos, were located in the limited area (**Fig. 1D**). Mild eosinophilic inorganic materials accumulated in a portion of the cysts. Continuous with the cysts, eosinophilic cells showed a papillary growth pattern with a thin vascular core. A nodular area and around the cysts showed a tubular structure and solid thick growth pattern. Multiple lesions were detected inside the kidney, suggesting a tumor of multifocal origin. The resected edge had no tumor cells. All of the hilar lymph node samples were negative for metastases. The *Transcription factor E3* split signal was negative in fluorescent *in situ* hybridization, and the Ki-67 expression was 2%–3%. Immunohistochemical staining was diffusely positive for succinate dehydrogenase B, a cluster of differentiation (CD) 10, epithelial membrane antigen, and paired box 8 and partially positive for pan-cytokeratin (AE1/AE3) and alpha-methylacyl coenzyme A racemase. The staining was negative for cytokeratin 7, carbonic anhydrase 9, CD117, transcription factor EB, and FH (**Fig. 1E**). On the basis of these findings, the tumor was pathologically diagnosed with HLRCC-associated RCC. Two years later, abdominal MRI and chest CT showed no postoperative recurrence or metastasis of

the tumor, and no appearance of left renal or skin lesions.

Identification of germline mutation

We performed whole-exome sequencing of the proband's tumor and peripheral blood. Splice site mutation in *FH*(c.378+1G>A; NM_000143) was detected on both tumor [variant allele frequency (VAF): 73.2%] and peripheral blood (VAF: 46.5%), and validated by Sanger sequencing (**Fig.1F**). The higher VAF in tumor cells suggests that somatic loss of heterozygosity (LOH) occurs in tumor cells in addition to germline *FH* gene mutation. Sanger sequencing was performed on his parents' peripheral blood, and the same heterozygous mutation was identified in his father, but not his mother (**Fig.1F**). His father had a medical history of prostate cancer, but no renal disease or cutaneous leiomyomas.

DISCUSSION

There is no standard treatment for advanced-stage HLRCC-related RCC. However, most cases are diagnosed in advanced stages with dismal prognosis.[3, 6] It is strongly recommended that the HLRCC patients' relatives receive genetic counseling and *FH* gene mutation screening. For confirmed HLRCC patients with *FH* gene mutation, the RCC surveillance program should start at 8–10 years old based on the age of the youngest case to date.[4] Here, we report a case of a boy who developed HLRCC-related RCC at 7 years old, which may affect the starting age of the future RCC-surveillance program for patients with HLRCC.

A clear association between *FH* gene mutation and renal cyst formation has been confirmed in mouse model.[7]Consistent with the mouse model phenotype, the prevalence of renal cysts in patients with HLRCC has been reported to be significantly higher than that in the normal population,[8] reaching 45%–50% in adult cases.[3, 9] Although it is controversial that HLRCC carrier with renal cyst developed kidney cancer more frequently than without cyst,[9] pathological examination of resected renal cysts of HLRCC patients revealed positive 2-succinyl-cysteine immunostaining,[10] suggesting a complete loss of function of the *FH* gene in renal cysts of HLRCC patients because of somatic genetic events. In the present case, the small solid lesions of RCC were located in the right renal cystic region. Renal cysts in these HLRCC patients may be precancerous lesions and should be carefully observed.

The present case was initially misdiagnosed with MCDK because of multiple renal cysts noted on an enhanced CT scan. Although it is rare, renal cyst formation can be seen in children with HLRCC.[11] HLRCC should be considered an important differential diagnosis for children with multiple renal cystic lesions and many other genetic disorders, including MCDK, Von Hippel–Lindau syndrome, or tuberous sclerosis.

In conclusion, we present a 7-year-old patient with HLRCC-related RCC with multiple renal cystic lesions. Since this is the youngest reported case to date, this report may affect the starting age for future RCC-surveillance programs for patients with HLRCC.

Conflict of Interest Statement

The authors report no potential conflicts of interest.

Acknowledgments

The authors would like to thank all of the clinicians and families who made this study possible by providing samples. The authors would also like to thank Mr. Akitaka Sugishita, Ms. Yoshie Miura, Ms. Hiroko Ono, and Ms. Chie Amahori for their valuable assistance. The authors acknowledge the help of the Division for Medical Research Engineering, Nagoya University Graduate School of Medicine, regarding technical support with next-generation sequencing. The authors also acknowledge the help of the Human Genome Center, University of Tokyo, for providing super-computing resources (http://sc.hgc.jp/shirokane.html).

REFERENCES

1. 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.

2. 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.

3. Forde C, Lim DHK, Alwan Y, et al. Hereditary leiomyomatosis and renal cell cancer: clinical, molecular, and screening features in a cohort of 185 affected individuals. Eur Urol Oncol 2019.

4. Nix J, Shuch B, Chen V, et al. 2008 Clinical features and management of hereditary leiomyomatosis and renal cell cancer (HLRCC). J Urol 2012;187(4S):e810-e810.

5. Taniguchi R, Muramatsu H, Okuno Y, et al. Comprehensive genetic analysis of donor cell derived leukemia with KMT2A rearrangement. Pediatr Blood Cancer 2018;65(2).

6. Park I, Shim YS, Go H, Hong BS, Lee JL. Long-term response of metastatic hereditary leiomyomatosis and renal cell carcinoma syndrome associated renal cell carcinoma to bevacizumab plus erlotinib after temsirolimus and axitinib treatment failures. BMC Urol 2019;19(1):51.

7. Pollard PJ, Spencer-Dene B, Shukla D, et al. Targeted inactivation of FH1 causes proliferative renal cyst development and activation of the hypoxia pathway. Cancer Cell 2007;11(4):311-319.

8. Terada N, Ichioka K, Matsuta Y, Okubo K, Yoshimura K, Arai Y. The natural history of simple renal cysts. J Urol 2002;167(1):21-23.

9. Lehtonen HJ, Kiuru M, Ylisaukko-Oja SK, et al. Increased risk of cancer in patients with fumarate hydratase germline mutation. J Med Genet 2006;43(6):523-526.

10. Ristau BT, Kamat SN, Tarin TV. Abnormal cystic tumor in a patient with hereditary leiomyomatosis and renal cell cancer syndrome: evidence of a precursor lesion? Case Rep Urol 2015;2015:303872.

11. Hol JA, Jongmans MCJ, Littooij AS, et al. Renal cell carcinoma in young FH mutation carriers: case series and review of the literature. Fam Cancer 2020;19(1):55-63.

Figure Legends

FIGURE 1 Morphological and molecular analysis of*FH*-deficient renal cell carcinoma

(A) Pedigree. (B) Enhanced computed tomography. (C) Positron emission tomography with 18F fluorodeoxyglucose. (D) Left kidney tumor sections were stained with hematoxylin and eosin staining. (E) Immunohistochemical staining is negative for FH. (F) Sanger sequencing: proband tumor, proband peripheral blood, peripheral blood of the patient's father, and peripheral blood of the patient's mother.