Retroperitoneal paraganglioma with loss of heterozygosity of the von Hippel–Lindau gene: a case report and review of the literature

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Abstract. Von Hippel–Lindau (VHL) disease is an autosomal dominant disease related to germline mutations in VHL. In VHL disease, pheochromocytoma develops in 10%–20% of patients because of germline mutations and loss of heterozygosity of VHL. However, the rate of paraganglioma associated with VHL is low compared with that of pheochromocytoma, and the reason is unknown. In this study, we performed germline and somatic mutation analyses of retroperitoneal paraganglioma that developed in a patient with clinically diagnosed VHL disease and investigated the tumorigenic mechanism of paraganglioma. The patient was a 25-year-old woman who was considered to have VHL disease on the basis of her family history. She was referred to our clinic to investigate a tumor at the bifurcation of the common iliac artery. The tumor was diagnosed as retroperitoneal paraganglioma by clinical evaluations. A left renal cell carcinoma was also suspected. Polymerase chain reaction direct sequencing analysis and polymorphic microsatellite analysis within the VHL locus suggested that loss of heterozygosity of VHL was associated with paraganglioma and renal cell carcinoma. Multiplex ligation-dependent probe amplification analysis showed a loss of the copy number of VHL exons in paraganglioma. These results suggest that VHL disease contributes to the development of paraganglioma. A literature review showed no reported common missense variants involved in the progression of paraganglioma. The loss of heterozygosity of VHL can be a tumorigenic mechanism of retroperitoneal paraganglioma in VHL disease. However, the low rate of paraganglioma compared with pheochromocytoma is not explained by their genetic background alone.

Key words: Von Hippel–Lindau disease, Paraganglioma, Polymorphic microsatellite, Multiplex ligation-dependent probe amplification, Loss of heterozygosity
to promote tumorigenesis through multiple pathways [2].

Pheochromocytoma occurs in approximately 10%–20% of patients with VHL disease [2]. However, the incidence of extra-adrenal pheochromocytoma, known as paraganglioma, associated with VHL is low [3, 4], and its tumorigenic mechanism is not understood.

Pheochromocytoma is generally associated with germ-line mutations of rearranged during transfection (RET), VHL, and neurofibromatosis 1 (NF-1). However, paraganglioma originates from thoracic and abdominal sympathetic paraganglia and is associated with other causative genes. Retroperitoneal paraganglioma is rare in patients with germ-line mutations of VHL, NF-1, Myc-associated protein X, and transmembrane protein 127. However, mutation of succinate dehydrogenase complex (SDHx) is common in paraganglioma [4]. These findings suggest that paraganglioma occurring in patients with VHL disease may not be associated with VHL. Determining the etiology of paraganglioma is important because the incidence of malignant paraganglioma, especially that associated with SDHx, is higher than that of pheochromocytoma [5]. According to Knudson’s two-hit hypothesis, tumorigenesis is initiated when both alleles of VHL are inactivated. In addition to missense mutations, large deletions in the contralateral allele and aberrant methylation modification of the promoter region can cause the neoplasm [6].

We report a case of VHL disease and retroperitoneal paraganglioma. In our case, the pathogenesis of paraganglioma was derived from VHL disease as shown by genetic analysis of VHL and succinate dehydrogenase subunit × (SDHB, SDHC, and SDHD). Multiplex ligation-dependent probe amplification (MLPA) analysis and polymorphic microsatellite analysis (PMA) showed that LOH of VHL was associated with tumorigenesis of paraganglioma.

Case Presentation

A 25-year-old woman was admitted to our clinic because of lower back pain. She had a history of surgery for cerebellar hemangioblastoma in her childhood. Her grandfather and father were clinically diagnosed with VHL disease. Her grandfather underwent surgery for RCC, and her father had two surgeries for cerebellar angioblastoma. She was diagnosed as having VHL disease by her past history and family history.

A computed tomography scan showed a 20-mm hypervascular retroperitoneal tumor at the bifurcation of the common iliac artery, a pancreatic head tumor, multiple pancreatic cysts, and left renal tumors (Fig. 1A, B). Endoscopic ultrasound-guided fine needle aspiration was
performed for the pancreatic head tumor and histologically showed a neuroendocrine tumor. There were no typical symptoms related to catecholamine excess, including hypertension and hyperglycemia. A summary of laboratory tests is shown in Table 1. An endocrinological evaluation showed that the 24-hour urinary normetanephrine concentration was slightly elevated to 0.53 mg/day. $^{123}$I-metaiodobenzylguanidine was greatly accumulated in the retroperitoneal tumor (Fig. 1C). These findings suggested that the retroperitoneal tumor was paraganglioma. The left renal tumors showed early phase staining on enhanced computed tomography and magnetic resonance imaging (Fig. 2A–E). These findings were consistent with multiple RCCs.

We scheduled two operations on the basis of radiological findings and endocrinological evaluation. The first operation was for resecting paraganglioma by laparoscopic procedures after medication of doxazocin and saline infusion to prevent a catecholamine crisis. The second operation was for resecting left RCCs by laparoscopic radical nephrectomy after the diagnosis of paraganglioma.

**Materials and Methods**

**Sample collection**

This study was conducted in accordance with the Declaration of Helsinki, in consideration of the ethics, human rights, and protection of personal information of the subjects/patients. The study protocol was reviewed and approved by the Ethics Review Committee of Shizuoka Prefectural Hospital and Tottori University Faculty of Medicine (19D011). Written informed consent was obtained from the proband who participated in the study. Peripheral blood was obtained during routine medical care. Samples were collected and placed in tubes with EDTA-2K as an anticoagulant. Tissues of retroperitoneal paraganglioma, RCC, and the normal kidney adjacent to RCC were removed at the patient’s surgery. Retroperitoneal paraganglioma was removed laparoscopically. A 5-mm$^2$ section in the center of the tumor was cut out and stored in RNA-stabilized solution. RCC in the left kidney was obtained by laparoscopic radical nephrectomy. The tumor was split from the removed left kidney, and half of the tumor was cut out and placed in RNA-stabilized solution. To compare tumor tissue with the normal kidney, a 10-mm$^2$ section from the normal kidney on gross observation was placed in RNA-stabilized solution. All samples were stored at 4°C, and genomic DNA was extracted within 1 week of collecting samples.

**Genetic analysis**

Genomic DNA from the patient was extracted from peripheral blood using the Genomic DNA Purification kit (Qiagen, Hilden, Germany or DNA genoTek, Ottawa,

| Table 1 | Laboratory test results on admission |
|---------|-------------------------------------|
| **Complete blood count** | **Biochemical tests** | **Endocrinological tests** |
| White blood cell 4,000/μL | Na 141 mEq/L | Thyroid stimulating hormone 1.39 μU/mL |
| Neutrophil 51% | K 4.4 mEq/L | Free T4 1.34 ng/dL |
| Lymphocyte 40% | Cl 107 mEq/L | Adrenocorticotropic hormone 33.0 pg/mL |
| Monocyte 4% | Ca 9.5 mg/dL | Cortisol 8.68 μg/dL |
| Eosinophil 5% | Urea nitrogen 13.0 mg/dL | Plasma renin activity 1.9 ng/mL/hr |
| Basophil 1% | Creatinine 0.51 mg/dL | Plasma aldosterone 165 pg/mL |
| Red blood cell 4.29 × 10$^6$/μL | eGFR 118.89 mL/min/1.73 m$^2$ | Dehydroepiandrosterone sulfate ≤0.01 ng/mL |
| Hemoglobin 12.2 g/dL | Total Protein 7.3 g/dL | adrenaline ≤0.01 ng/mL |
| Platelet 25.6 × 10$^4$/μL | Albumin 4.3 g/dL | noradrenaline 0.33 ng/mL |
| Total-bilirubin 0.4 mg/dL | AST 13 U/L | dopamine ≤0.02 ng/mL |
| ALP 116 U/L | U-metanephrine 0.11 mg/day |
| γ-GTP 8 U/L | Protein — |
| LDH 120 U/L | Glucose — |
| CK 61 U/L | Blood — |
| CRP 0.03 mg/dL | |

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The proband’s genomic DNA was screened for variants in VHL, SDHB, SDHC, and SDHD. All coding regions were analyzed by Sanger sequencing. The following oligonucleotides were used for VHL: exon 1, forward 5’-AAGACTACGGAGGTCGACTCGGGAGCGC-3’ and reverse 5’-CGGTAGAGGGCCTTCAGACCGTCT-3’; exon 2, forward 5’-CAGGTGTGGGCCACCGTCCACGC-3’ and reverse 5’-TCAAGTGGTCTATCCTGTACTTACC-3’; and exon 3, forward 5’-CCTCTTGTTCCTTGTACTGAG-3’ and reverse 5’-TCCTGTATCTAGATCAAGACTCATC-3’. The oligonucleotides used for SDHB, SDHC, and SDHD are listed in Supplementary Table 1. After purifying the PCR products using the QIAquick PCR purification kit (Qiagen), direct sequencing was performed using the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genomic DNA was also obtained from the surgically removed paraganglioma, left RCC, and normal kidney of the patient. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen or DNA genoTek) according to the manufacturer’s instructions. The extracted DNA was evaluated for VHL variants by Sanger sequencing. The peak amplitude obtained by PCR direct sequencing analysis was compared between both alleles. The ratio of c.194C>G peak signal intensity was calculated by the peak amplitude of the variant allele (C) divided by that of the normal allele (G).

**MLPA assay**

The MLPA assay was performed to investigate large deletions and abnormal methylation modification of the promoter region in the normal allele. Changes in copy

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**Fig. 2** Radiological and pathological presentation of left renal cell carcinoma.

(A) Plain computed tomography shows a 26- × 23- × 23-mm isodense tumor in the left kidney. (B) Contrast-enhanced computed tomography shows early enhancement with washout in the equilibrium phase. (C) Plain abdominal magnetic resonance imaging shows a high signal in T2-weighted images in the same area of early enhancement as that on computed tomography and contrast-enhanced magnetic resonance imaging by T1-weighted images (D). (E) T1-weighted images in magnetic resonance imaging also show a high signal that appears to be internal bleeding. (F) Histopathological tissue shows solid alveoli of tumor cells with round nuclei and an abundant clear cytoplasm, and a vascular stroma by hematoxylin–eosin staining (×400).
number were examined by MLPA using the SALSA MLPA kit (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. The two probe mixes P016-VHL (29 probes for each VHL exon, genes located close to VHL, and references for detecting sequences on other chromosomes) and P226 SDH (37 probes for each exon of SDHB, SDHC, SDHD, SDHAF1, and SDHAF2, and references for detecting sequences on other chromosomes) were used for the assays. Automated fragment and data analysis for MLPA was performed using Coffalyser Software (MRC-Holland). Dosage quotient areas outside the range of 0.70–1.3 were considered abnormal.

PMA

To determine the LOH, we also performed PMA for three microsatellite loci near VHL [7]. The following fluorescence-labeled primers (Thermo Fisher Scientific, Tokyo, Japan) were used for PCRs: D3S1597 (expected product: 170 bp), forward 5'-AGTACAAATACACACAAA TGCTC-3' and reverse 5'-GCAATCGTTCAATGGCT-3'; D3S1435 (expected product: 161 bp), forward 5'-CAAG GCAGTGGAGATGAG-3' and reverse 5'-TAAAGACGG AAGCAAGGAAG-3'; and D3S1263 (expected product: 231 bp), forward 5'-CTGTTGACCCATTGATACCC-3' and reverse 5'-AAAAATCACAGCAGAGGGTTTC-3'. PCR was performed for 40 cycles at annealing temperatures of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 68°C, was performed for 40 cycles at annealing temperatures of 30°C, 30°C, and 30°C, respectively. The amplified products were analyzed using electropherograms on GeneMapper of the ABI 3130-XL genetic analyzer (Applied Biosystems).

Results

Histopathological diagnosis

On gross observation, the retroperitoneal tumor was 20 × 15 mm, it was a solid mass, and the cut surface was homogeneously brown. On microscopic evaluation, hematoxylin–eosin staining, and the tumor cells were calculated by evaluation of the histological pattern as follows: Zellballen (0); cellularity: 190/U (1); comedo necrosis: absent (0); vascular/capsular invasion: absent (0); Ki67 labeling index: 3.6% (2); and catecholamine type: noradrenaline (1). The histopathological diagnosis was consistent with retroperitoneal paraganglioma.

Microscopically, the left renal tumor consisted of solid alveoli of tumor cells with round nuclei and an abundant clear cytoplasm, and a vascular stroma (Fig. 2F). A unicocular cyst was formed adjacent to the kidney parenchyma. The wall of the cyst was lined with clear cells that were identical to the tumor cells. All of these findings were consistent with clear cell renal cell carcinoma (ccRCC).

Genetic analysis

Genomic DNA was analyzed for VHL, SDHB, SDHC, and SDHD by PCR direct sequencing. A previously reported heterozygous mutation of VHL (NM_000551.4), p.Ser65Trp by single nucleotide substitution of c.194C>G in exon 1, was found in cells that originated from peripheral blood and the normal kidney (Fig. 3A). No previously reported mutations were observed in SDHB, SDHC, or SDHD (data not shown). The ratios of c.194C>G peak signal intensity were 0.574, 0.366, 0.325, and 0.618 for peripheral blood, paraganglioma, ccRCC, and the normal kidney, respectively. These data suggested that LOH was associated with paraganglioma and ccRCC (Fig. 3B).

MLPA analysis

Changes in the copy number of VHL exons 1–3 were examined by MLPA of cells that originated from peripheral blood, paraganglioma, ccRCC, and the normal kidney. All samples showed a lower copy number of VHL exons 1–3 (Fig. 4A–D) compared with that in the control without the VHL gene mutation (Fig. 4E). In particular, a considerably lower copy number was observed in paraganglioma compared with that in the other samples (Fig. 4B). The copy number of VHL exons 1–3 that originated from ccRCC was also low. However, no difference in the copy number of ccRCC was observed compared with that in the normal kidney (Fig. 4C, D). The copy number of exons in SDHX (SDHB, SDHC, SDHD, SDHAF1, and SDHAF2) was not different among cells that originated from paraganglioma (Supplementary Fig. 1).
VHL disease is an autosomal dominant disorder that occurs in 1/36,000 people. VHL is a tumor suppressor gene, and more than 500 different pathogenic mutations have been identified. We identified c.194C>G ([VHL:Ch3(GRCh37):g.10183725G>A, NM_000551.4:c.194C>G, p.Ser65Trp]) in exon 1, which

Fig. 3  PCR direct sequencing analysis of the von Hippel–Lindau gene.
PCR direct sequencing analysis (A) was performed using DNA extracted from peripheral blood, paraganglioma (PGL), clear cell renal cell carcinoma (ccRCC), and the normal kidney. A previously reported c.194C>G mutation in exon 1 associated with p. Ser65Trp was observed in all samples. The ratio of c.194C>G peak signal intensity (B) was lower in PGL and ccRCC compared with that in peripheral blood and the normal kidney.

Fig. 4  Multiplex ligation-dependent probe amplification analysis of the von Hippel–Lindau gene.
Multiplex ligation-dependent probe amplification analysis was performed using DNA extracted from peripheral blood cells (A), paraganglioma (B), clear cell renal cell carcinoma (C), the normal kidney (D), and a normal control (E). The probes were arranged in order from upstream of chromosome 3. All samples showed a reduction in the copy number of VHL exons 1–3. The copy number of paraganglioma was lower compared with that in the other samples.

Discussion
VHL disease is an autosomal dominant disorder that occurs in 1/36,000 people. VHL is a tumor suppressor gene, and more than 500 different pathogenic mutations have been identified. We identified c.194C>G ([VHL:Ch3(GRCh37):g.10183725G>A, NM_000551.4:c.194C>G, p.Ser65Trp]) in exon 1, which
has already been reported [8-10] in peripheral blood cells. Although pheochromocytoma is frequent in probands with VHL mutations, as well as RET and NF-1 mutations, paraganglioma is less frequent in probands with VHL mutations [4]. The underlying mechanism of how paraganglioma occurs in VHL disease is not well understood.

The two-hit hypothesis of the VHL gene has been proven to be the etiology of pheochromocytoma and ccRCC [11, 12]. In our analysis of tissue that originated from paraganglioma and ccRCC, PCR direct sequencing showed the same mutation in VHL as that extracted from the tumors. Additionally, the ratio of c.194C>G peak signal intensity was low in paraganglioma and ccRCC. Furthermore, MLPA analysis and PMA showed that the copy number of VHL exons and amplified products of microsatellite markers in the VHL locus were lower in paraganglioma and ccRCC compared with those in peripheral blood. These results suggested that LOH of VHL was associated with the progression of paraganglioma and ccRCC.

Although VHL disease was already clinically diagnosed in our case, more than 20 genes besides VHL have been reported in the development of paraganglioma [13]. We decided to conduct a genetic analysis involving development of paraganglioma. Germline mutations of SDHx are the most common in peritoneal paraganglioma, accounting for 25% of familial cases [13]. Although renal tumors and pheochromocytoma/paraganglioma tumor association syndrome are generally associated with VHL disease, SDHx mutations are the most frequent cause of non-VHL cases [14-16]. On the basis of these findings, SDHB, SDHC, and SDHD were also analyzed together with VHL, but no previously reported mutations were identified in our case.

We conducted a literature search to identify publications regarding paraganglioma associated with VHL disease. Original articles and case reports published from January 2000 to October 2021 were examined on 27 October 2021 using the following keywords: “VHL”, “paraganglioma”, and “genetic”. The search was performed in PubMed. The titles and abstracts were reviewed by two authors (M.A. and S.I.). Publications in languages other than English, as well as articles/reports involving head and neck paraganglioma, without genetic testing, and insufficient information on the patients’ background were excluded from this review.

We found 26 cases with paraganglioma related to VHL disease (Table 2) [17-25]. Their mean age at the diagnosis of VHL disease was 22.2 years, and the male to female ratio was 7:10. The complication of pheochromocytoma was observed in 12 of 26 (46.2%) cases. Multiple paragangliomas were reported only in two cases. All but one case, No. 23, presented with a missense mutation. The clinical phenotype type 2B of VHL disease is characterized by an increased risk of pheochromocytoma and RCC [26]. Previous findings have suggested that mutations in codon 161 and codon 167 are associated with the development of pheochromocytoma [18, 23, 27]. These missense mutations were reported in 30%-53% of the cases with VHL disease and pheochromocytoma. However, the cases with paraganglioma alone by missense mutations in codon 161 and codon 167 have not been reported previously.

Our literature search suggested the difference of the associated missense mutations between pheochromocytoma and paraganglioma in VHL disease. However, the previously reported highly prevalent missense mutations in pheochromocytoma [18, 23, 27] were accounted for...
tein dysfunction and its tissue specificity [28]. Faubert B et al. [17] association between inactivation of Arg161Gln). They demonstrated that the phenotype of ccRCC and pheochromocytoma by an atypical family bearing two mutations. X Ma et al. [18] et al. [25] J Favier et al. [19] 10 R Pandit et al. [23] 12 Male – + E1 13 Male – + E1 13 Male – + E2 12 U Srirangalingam et al. [21] X Ma et al. [18] 39 Female – – E2 14 J Favier et al. [19] 15 X Ma et al. [18] 19 Male – + E3 16 X Ma et al. [18] 10 Female – + E3 16 X Ma et al. [18] 10 Female – + E3 16 X Ma et al. [18] 10 Female – + E3 18 X Ma et al. [18] 9 Male – + E3 19 R Pandit et al. [23] 18 Female – + E3 19 R Pandit et al. [23] 18 Female – + E3 20 R Pandit et al. [23] 26 Female – + E3 21 J Favier et al. [19] 21 J Favier et al. [19] 24 J Welandt et al. [24] 25 R Pandit et al. [23] 22 Female – + E3 26 J Favier et al. [19] 26 J Favier et al. [19] 26 J Favier et al. [19] 26 J Favier et al. [19] 26 J Favier et al. [19] 26 J Favier et al. [19] 26 J Favier et al. [19] 26 J Favier et al. [19] Table 2 Reported cases of paraganglioma associated with VHL disease

| No. | Reference | Age at diagnosis (y) | Sex | Multiple PGLs | PCC | Exon number | DNA mutation | Protein change |
|-----|-----------|----------------------|-----|---------------|-----|-------------|--------------|---------------|
| 1   | J Favier et al. [19] | NR | NR | + | – | E1 | c.191G>C | p.Arg64Pro |
| 2   | J Favier et al. [19] | NR | NR | – | – | E1 | c.193T>G | p.Ser65Ala |
| 3   | Present case | 25 | Female | – | – | E1 | c.194C>G | p.Ser65Trp |
| 4   | S Yalcintepe et al. [17] | 33 | Male | NR | – | E1 | c.202T>C | p.Ser68Pro |
| 5   | M Castellano et al. [22] | 35 | NR | – | – | E1 | c.242C>T | p.Pro81Leu |
| 6   | J Favier et al. [19] | NR | NR | – | – | E1 | c.244C>G | p.Arg82Gly |
| 7   | X Ma et al. [18] | 30 | Female | – | – | E1 | c.250G>A | p.Val84Met |
| 8   | M Lacобone et al. [25] | 26 | Female | NR | + | E1 | c.277G>A | p.Gly93Ser |
| 9   | J Favier et al. [19] | NR | NR | – | – | E1 | c.293A>G | p.Tyr98Cys |
| 10  | R Pandit et al. [23] | 12 | Male | – | + | E1 | c.293A>C | p.Tyr98Ser |
| 11  | X Ma et al. [18] | 13 | Male | – | + | E1 | c.314C>T | p.Thr105Met |
| 12  | U Srirangalingam et al. [21] | 13 | Male | + | + | E2 | c.355T>G | p.Leu118Arg |
| 13  | X Ma et al. [18] | 39 | Female | – | – | E2 | c.460C>T | p.Pro154Ser |
| 14  | J Favier et al. [19] | NR | NR | – | – | E3 | c.475A>G | p.Lys159Glu |
| 15  | X Ma et al. [18] | 19 | Male | – | + | E3 | c.482G>A | p.Arg161Gln* |
| 16  | X Ma et al. [18] | 10 | Female | – | + | E3 | c.482G>A | p.Arg161Gln* |
| 17  | R Pandit et al. [23] | 11 | Female | – | + | E3 | c.500G>A | p.Arg167Gln* |
| 18  | X Ma et al. [18] | 9 | Male | – | + | E3 | c.499C>T | p.Arg167Trp* |
| 19  | R Pandit et al. [23] | 18 | Female | – | + | E3 | c.499C>T | p.Arg167Trp* |
| 20  | R Pandit et al. [23] | 26 | Female | – | + | E3 | c.499C>T | p.Arg167Trp* |
| 21  | J Favier et al. [19] | NR | NR | – | – | E3 | c.533T>C | p.Leu178Pro |
| 22  | J Crona et al. [20] | 28 | Male | NR | – | E3 | c.548C>T | p.Ser183Leu |
| 23  | U Srirangalingam et al. [21] | 30 | Female | – | – | E3 | c.555C>G | p.Tyr185Ter |
| 24  | J Welandt et al. [24] | NR | NR | – | + | E3 | c.593T>G | p.Leu198Arg |
| 25  | R Pandit et al. [23] | 22 | Female | – | + | E3 | c.593T>C | p.Leu198Pro |
| 26  | J Favier et al. [19] | NR | NR | – | – | E3 | c.642A>T | p.Xaa214Cys |

* Missense mutation of codon 161 and codon 167 in exon 3.

Abbreviations: E; exon, NR; not reported, PCC; pheochromocytoma, PGL; paraganglioma.

30%–53%, and insufficient for explaining the tumorigenic mechanism. Couvé S et al. previously validated the association between inactivation of VHL and occurrence of ccRCC and pheochromocytoma by an atypical family bearing two VHL mutations in cis (Arg200Trp and Arg161Gln). They demonstrated that the phenotype of VHL disease was correlated with a gradient of VHL protein dysfunction and its tissue specificity [28]. Faubert B et al. also reviewed that tumor progression was differently promoted by the characteristics of parental tissues, tumor cell intrinsic effects, tumor microenvironment, and metabolism of the patients [29]. VHL protein sensitivity to adrenal medulla and paraganglia may be differently associated with the tumorigenesis of pheochromocytoma and paraganglioma.

A limitation of our study is that MLPA analysis of ccRCC did not show a lower VHL exon copy number than that in the normal kidney. Amplified allelic fragments of D3S1435 in PMA showed the difference between samples from peripheral blood and normal kidney. In previous reports, a second hit to VHL was found to be earlier than progression of ccRCC [30, 31]. LOH itself did not lead to development of ccRCC. Additionally, VHL mutations in mouse models and cultured cells, mutations in Trp53 and Rhl1, and endoplasmic reticulum stress after a second hit to VHL may be involved in the development of ccRCC [32, 33]. We speculate that LOH of VHL in the normal kidney may already have occurred.
before the development of ccRCC. Other mutations in \textit{Trp53} and \textit{Rb1} and endoplasmic reticulum stress may be essential for the development of ccRCC. D3S1597 and D3S1263 in PMA showed similar results in the normal kidney as those in peripheral blood, which suggested that the findings in the normal kidney in MLPA analysis may have been a false positive result. MLPA analysis is based on the hybridization of a set of probes to genome DNA fixed on a solid support, followed by washing of the probes that did not bind. The presence of genome DNA on the solid support may pose a risk of contamination [34]. A possibility of contamination cannot be denied even though there was sufficient preparation in the sample collection.

The findings in our case show that peritoneal paraganglioma is associated with VHL disease similar to pheochromocytoma. However, the frequently reported missense mutations of pheochromocytoma associated with VHL disease were not reported in paraganglioma alone case. Further studies including a large number of patients with genomic DNA and associated products from tumors are necessary for validating the difference in progression of pheochromocytoma and paraganglioma related to VHL disease.

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**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.

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**Supplementary Table 1** Primers for genetic analysis of succinate dehydrogenase subunit \texttimes{} (\textit{SDHB, SDHC, and SDHD}).

| Gene | Exon number | Forward primer | Reverse primer |
|------|-------------|----------------|----------------|
| **SDHB** | 1 | 5'-GATGTCAGGCGGACCCGGCCGGAG-3' | 5'-CTCCGAGCCCCATCGCTCGGAC-3' |
| | 2 | 5'-AATCCAGGCTAACTGCTGTGGC-3' | 5'-AAGCTATGGCTAAATCAATAC-3' |
| | 3 | 5'-CTCCGATTATATATTGAGAAAGTGT-3' | 5'-CCAGCCCAAGGCCCTTTGAAAGCC-3' |
| | 4 | 5'-GAAGAAATATTTGGGGGAGGACTG-3' | 5'-AAACTAATAGCTAAATCAATAC-3' |
| | 5 | 5'-AAAGCTGAGTGGTGAATGGAATCTG-3' | 5'-CCACACTCTCTGGCAATCTCCTGTC-3' |
| | 6 | 5'-AAAGGTGACATTATATGAGTGAAGG-3' | 5'-TTCAAGCAATCTATTGTCCTGTGG-3' |
| | 7 | 5'-CTGCACTCCCAGAGCTTTGAGTG-3' | 5'-CTGCAATCCACTCTTTGAGGAC-3' |
| | 8 | 5'-CCAAGATGTTGGTTTCCTCCTTT-3' | 5'-CATCCCTGCGCAATGAAAGAC-3' |
| **SDHC** | 1 | 5'-AGGAGACATAAACCAGCAAAACACCAAGC-3' | 5'-CACAGGATCAAACGAGCCACAGCG-3' |
| | 2 | 5'-ATCCCTCACCCTAAATAGAGAATGG-3' | 5'-AGGCTGTCAGGAGCAGAGCCATCGGTC-3' |
| | 3 | 5'-CTTGAGCAAACCAGCTCTGGCCTTTGATGGC-3' | 5'-AAAGCTGAGGGAATGATGACATGCTAAG-3' |
| | 4 | 5'-TTGCGCAGAGTGAAGATCTTACCTGCTGGTTC-3' | 5'-CTGTTCCCTCTACGATGATCGTGAGCTGG-3' |
| | 5 | 5'-GAGGTGCCCAGGGGCTCCAGTTTATGATG-3' | 5'-AAACCAGTAATAGGAAAGAATCTTCCTCCC-3' |
| | 6 | 5'-GGGGTGTCGGGGTGTCGGAAGGAGGAG-3' | 5'-TTCGCCAGCTGCGAGATAAGATGACAAG-3' |
| **SDHD** | 1 | 5'-GTGTATTTTCTTCTCAGTCGGTG GG-3' | 5'-TCTGACACCCGCCAGATGACTCCCTC-3' |
| | 2 | 5'-CTCTGAGCTTTTCTGAGATGAGTC-3' | 5'-AGGAGCTAGCTCTAAGAGGTGAC-3' |
| | 3 | 5'-CTGTCACGGAAGTGGGTTCAAGAG-3' | 5'-GGGCGTTTCAATCCACTCTGTCCTC-3' |
| | 4 | 5'-GTTATGTGTCATGCTTTCTAATTTT-3' | 5'-TGAAGTCATCCAGCATGCAAAGC-3' |
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