Case Report

Familial and early recurrent pheochromocytoma in a child with a novel in-frame duplication variant of *VHL*

Yuri Suzuki[1], Ryosei Iemura[1], Akito Sutani[2], Yuki Mizuno[3], Eriko Adachi[1], Mineko Ushiama[4, 5], Teruhiko Yoshida[5], Makoto Hirata[5], Akihiro Hoshino[1], Kurara Yamomoto[6], Takumi Akashi[7], Yoshiko Nakano[5, 8], Takeshi Isoda[1], Kei Takasawa[1], Motohiro Kato[8], Masatoshi Takagi[1], Kentaro Okamoto[3], Tomohiro Morio[1], and Kenichi Kashimada[1]

[1]*Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Tokyo, Japan*
[2]*Department of Pediatrics, Kawaguchi Municipal Medical Center, Saitama, Japan*
[3]*Department of Pediatric Surgery, Tokyo Medical and Dental University Hospital, Tokyo, Japan*
[4]*Department of Clinical Genomics, National Cancer Center Research Institute, Tokyo, Japan*
[5]*Department of Genetic Medicine and Services, National Cancer Center Hospital, Tokyo, Japan*
[6]*Department of Human Pathology, Tokyo Medical and Dental University, Tokyo, Japan*
[7]*Department of Diagnostic Pathology, Tokyo Medical and Dental University, Tokyo, Japan*
[8]*Department of Pediatrics, The University of Tokyo Hospital, Tokyo, Japan*

Highlights

- We report a case of familial pheochromocytoma with a novel in-frame *VHL* variant.
- Genetic diagnosis is important to assess the risk of metastasis and recurrence.
- The in-frame duplication of *VHL* in specific regions may cause PPGL.

Abstract. Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors often linked to underlying genetic variants. Genetic analysis can promote gene-adjusted, specific follow-up, and surveillance protocols for both patients and their families at risk. We report the case of a 7-yr-old boy with bilateral pheochromocytoma, which recurred a year after partial adrenalectomy. The patient's father developed bilateral pheochromocytomas at 25 yr of age. Both individuals possessed a novel heterogeneous in-frame duplication germline variant of *VHL*, yet neither exhibited other clinical manifestations of von Hippel–Lindau disease (VHL). Traditionally, *VHL* missense mutations have been associated with a higher risk of PPGL development, whereas truncating mutations typically confer a lower risk. In-frame duplication variants are rarely observed in patients with VHL but may lead to changes in the three-dimensional structure of the translated protein, similar to truncating variants. Our analysis suggests that these in-frame duplications of amino acids in specific regions may cause pheochromocytomas in a manner similar to missense variants. Further accumulation of VHL cases with various genotypes and standardized open-access worldwide databases, including longitudinal and specific clinical data linked to genotypes, is required. It is crucial to consider genetic analyses for pediatricians who may diagnose childhood-onset PPGL.

Key words: pheochromocytoma, von Hippel–Lindau disease (VHL), in-frame variant

Introduction

Pheochromocytomas and paragangliomas (PPGLs) are rare tumors arising from catecholamine-producing chromaffin cells in the adrenal medulla and sympathetic nervous system. The incidence of this condition is approximately 0.04–0.95 cases per 100,000 per year, and more than one-third of patients with PPGLs possess a pathogenic germline variant (1, 2). All types of PPGLs are currently defined as potentially malignant tumors because they all have the potential for metastasis; however, there is currently no specific tool for predicting metastasis (3). Genetic analysis is becoming increasingly important for the early diagnosis of PPGLs and the targeted detection of associated diseases, not only for patients but also for their families at risk.

Genetic analysis can be useful for establishing follow-up protocols, as each germline variant can lead to different gene-related diseases. For example, a pathogenic variant of the *VHL* gene can cause von Hippel–Lindau disease (VHL), an autosomal dominant syndrome associated with variable manifestations in different tumors, including PPGLs, clear cell renal cell carcinomas, retinal/central nervous system hemangioblastomas, and pancreatic neuroendocrine tumors (4). These characteristics require whole-body surveillance throughout the patient's lifetime in addition to routine monitoring of catecholamine levels and blood pressure (5). In addition, the underlying germline variants define the long-term prognosis of pediatric patients with PPGLs in terms of prevalence, time of recurrence, malignant transformation, and survival.

Herein, we report a case of bilateral pheochromocytoma in a 7-yr-old boy with a family history of young-onset bilateral pheochromocytoma and a novel in-frame duplication *VHL* variant.

Case Presentation

A 7-yr-old Asian boy presented to a local clinic with vomiting, headache, and stomach pain. The patient had been experiencing thirst and hyperhidrosis for 1 mo. He was found to have proteinuria during a school checkup 2 wk prior to admission. Considering the possibility of nephrosis, the patient was admitted to a tertiary care hospital. On admission (day zero), his blood pressure was 160/80 mmHg (< 109/70 mmHg, within the normal range based on age, sex, and height percentiles (6)). Laboratory data at the first visit are shown in Supplementary Table 1. Brain magnetic resonance imaging (MRI) showed continuous T2 hyperintensities in the bilateral subcortices of the occipital lobe, basal nuclei, cerebellar tonsils, and medulla, which disappeared on follow-up MRI on day 16, suggesting posterior reversible encephalopathy syndrome. Abdominal ultrasonography and MRI revealed bilateral adrenal gland tumors (**Fig. 1A**). ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy revealed bilateral uptake in these tumors, with no evidence of metastasis (**Fig. 1B**). Following a clinical diagnosis of PPGL, administration of an alpha 1-blocker (doxazosin) was initiated and gradually increased. Proteinuria and hematuria improved immediately after correction of dehydration with fluid infusion and control of hypertension, indicating nephropathy associated with a hypertensive emergency. The patient's father had suffered from headaches and hypertension before the diagnosis of PPGL and had undergone resection for bilateral pheochromocytoma at 25 yr of age (Supplementary Figure 1 and Supplementary Table 1). The patient's father had no other tumors and did not undergo genetic analysis.

The patient was transferred to our hospital on day nine for further examination and treatment. His height and body weight were 122.1 cm (+0.11 SD) and 21.2 kg (–0.60 SD), respectively; there was no apparent growth retardation. The plasma and urine catecholamine levels were as follows: plasma noradrenaline: 14 ng/mL (< 0.57 ng/mL), plasma free normetanephrine: 6,410 pg/mL (< 506 pg/mL), urine metanephrine: 0.07 mg/d (650 ng/mgCr ($\leq$ 500 ng/mgCr)), urine normetanephrine: 6.9 mg/d (27,800 ng/mgCr ($\leq$ 500 ng/mgCr)). No elevations were detected for the plasma level of adrenaline (0.02 ng/mL; < 0.17 ng/mL) and free metanephrine level (50 pg/mL; < 130 pg/mL). Abdominal contrast-enhanced computed tomography (CT) revealed left hypervascular and right hypovascular adrenal tumors (**Fig. 1C**). ^{18}F-fluorodeoxyglucoose (^{18}F-FDG) positron emission tomography-CT showed increased uptake in both adrenal masses without signs of metastasis (**Fig. 1D**). No abnormalities were found in the cardiac function, thyroid glands, kidneys, or retinas. After blood pressure was well controlled by increasing the dose of doxazosin up to 16 mg/d, bilateral partial adrenalectomy with conservation of the normal adrenal gland was performed on day 21. Hydrocortisone was initiated preoperatively (50 mg/m^2/d), increased to 100 mg/m^2/d as a stress dose following resection, and gradually reduced to a physiological dose (10 mg/m^2/d). No hypotension or hypoglycemia was observed postoperatively. Plasma and urine catecholamine levels normalized following surgery (plasma-free normetanephrine, 202 pg/mL; urine metanephrine: 0.04 mg/d; urine normetanephrine: 0.32 mg/d). The patient was discharged on the 27th day after the administration. After 6 mo, we conducted an ACTH stimulation test, which confirmed that the patient's adrenal cortex functioned normally. Subsequently, we discontinued hormone replacement therapy. The excised masses measured 48 × 38 × 33 mm with central necrosis (right) and 33 × 30 × 28 mm (left). Immunohistochemical analysis revealed positive staining for chromogranin A, which was consistent with the diagnosis of pheochromocytoma (**Fig. 2**). In addition, the microscopic features, including relatively small and homogeneous nuclei, predominantly clear or eosinophilic cells, and the absence of hyaline globules, were consistent with VHL-associated pheochromocytomas (7). Ki-67 expression was positive in 9.5% and 2.3% of right and left lesions, respectively. The pheochromocytoma of the
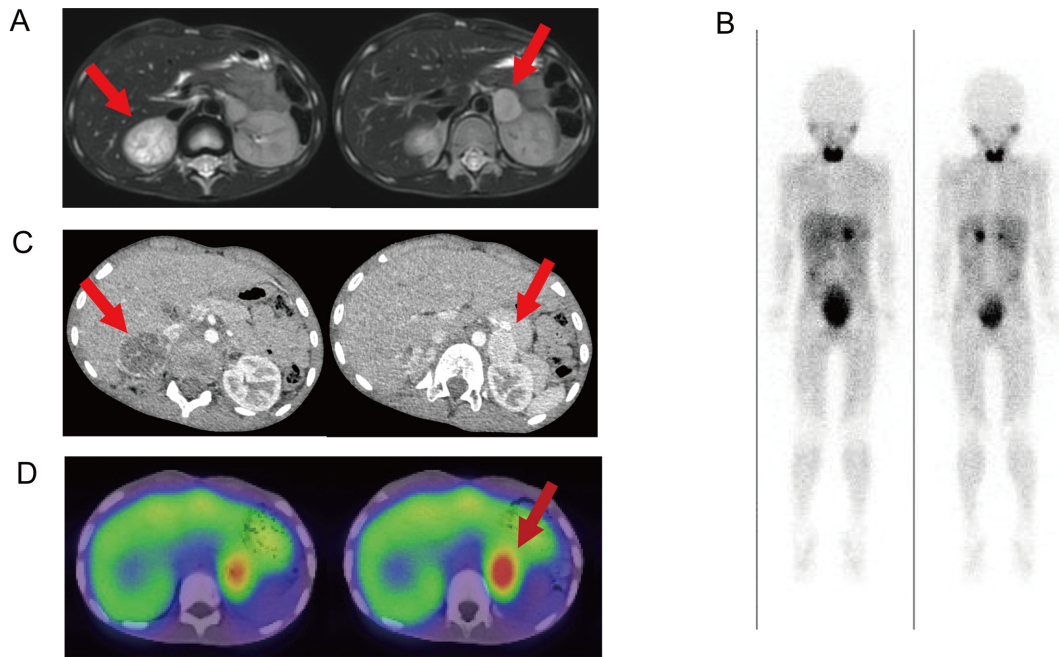
Fig. 1. Imaging and immunohistochemical analysis. Red arrows indicate lesions. A: Abdominal MRI shows a 4 cm lesion in the right adrenal gland and a 3 cm lesion in the left adrenal gland. B: ^{123}I-MIBG scintigraphy shows no evidence of metastasis. C: Abdominal CT revealing bilateral adrenal tumors. The right mass is 42 mm in size with predominant peripheral contrast and low internal contrast, whereas the left mass is 27 mm in size with homogeneous contrast. Enhanced CT with a nonionic contrast agent was conducted with written informed consent from the parent or guardian before surgery to better understand the positional relation between the masses and blood vessels (37). D: ^{18}F-FDG PET/CT shows no evidence of metastasis.
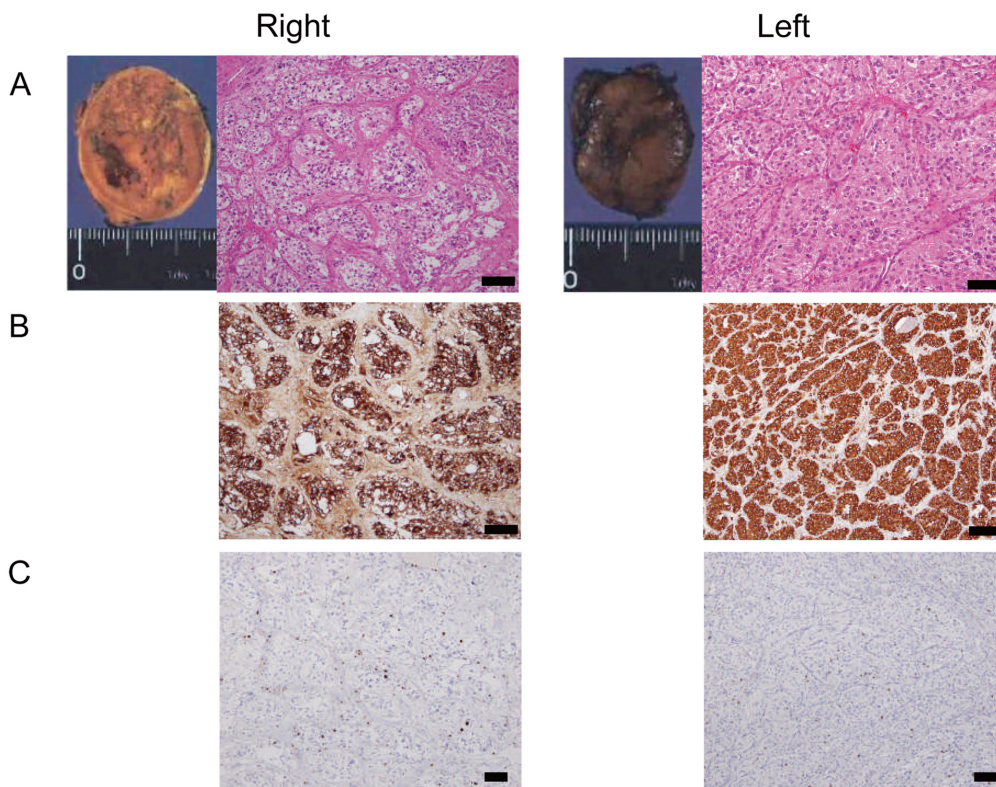

Fig. 2. Histopathological tissue analysis on bilateral adrenal tumors. Hematoxylin and eosin staining (A, × 400) and immunohistochemistry using chromogranin A (B, × 400) and Ki-67 (C, × 200). Bars indicate 100 μm. The left (33 × 30 × 28 mm) and right (48 × 38 × 33 mm) tumors scored one point (vascular invasion) and five points (central tumor necrosis, atypical mitotic figures, and vascular invasion) on the PASS. Both tumor tissues were positive for chromogranin A, and the positivity rates of Ki-67 were 2.3% and 9.5% for the left and right lesions, respectively.

adrenal gland scaled score (PASS)/grading system for adrenal pheochromocytoma and paraganglioma (GAPP) score (8) was 5/6 for the right mass and 1/3 for the left mass, indicating moderately and well-differentiated mass types, respectively.

One year after the first surgery, transabdominal ultrasonography revealed a recurrent tumor in the residual right adrenal gland with slightly elevated spot urine and plasma-free normetanephrine levels of 1,340 ng/gCr and 540 pg/mL, respectively. Although abdominal contrast-enhanced CT and ^{123}I-MIBG scintigraphy revealed a right hypervascular adrenal tumor with elevated uptake in the tumor (**Figs. 3A–C**), the patient had no symptoms. Following comprehensive discussions with the patient's caregivers, pediatric surgeons, pediatric endocrinologists, pediatric oncologists, and medical geneticists, right adrenalectomy was decided to be performed, and normal adrenal function was confirmed 6 mo after the second surgery. The excised masses measured 19 × 14 × 13 mm, and immunohistochemical analysis showed positive staining for chromogranin A and 3.6% positivity for Ki-67 (1/5 points of the PASS/GAPP score), suggesting the recurrence of a new pheochromocytoma (**Fig. 3D**).

Genetic analysis

Genetic and family analyses were performed after the initial surgical procedure. The patient and parents provided their informed assent and consent, respectively, to take part in the multi-institutional clinical genomics study, "Diagnosis and Research on Familial, Young-onset and Hereditary Cancers" (protocol no. 2013-303, approved by the research ethics committee at the National Cancer Center). A multigene panel, NOP_FC ver 3.0, was designed to sequence the exons and intronic regions of 147 genes (9), which were selected by the authors for known or possible relations with hereditary predispositions to cancers, including genes associated with PPGL, such as *SDHA*, *SDHB*, *SDHC*, *SDHD*, *VHL*, *RET*, *NF1*, *SDHAF2* and *TMEM127*. Multigene panel sequencing using Sanger sequencing of peripheral blood was performed on both the patient and his parents. Genetic analysis revealed a heterozygous in-frame germline variant of *VHL* (NM_000551.4, p.Glu189_Gln195dup) in both the patient and his father (**Fig. 4**). We were unable to identify this variant in the COSMIC variant databases (all databases were accessed on 1 April 2024), including the ClinVar database (http://
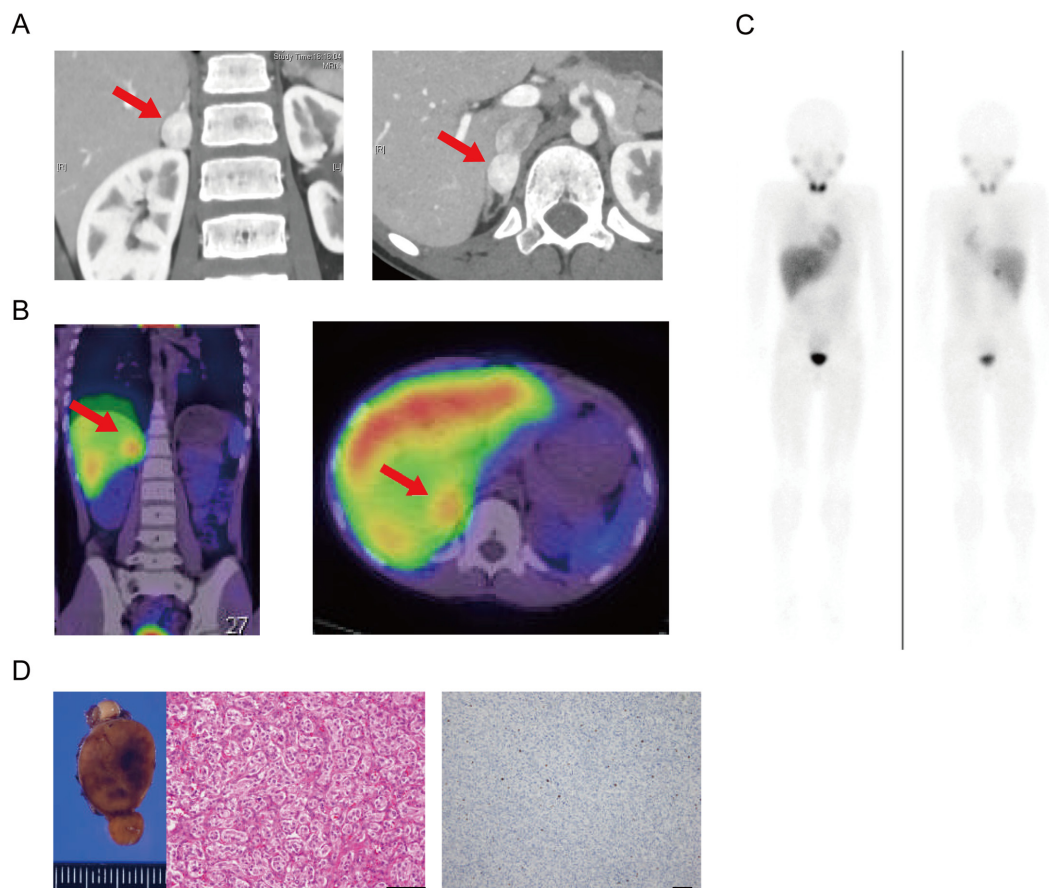

Fig. 3. Recurrent tumor on the right adrenal gland. Abdominal CT (A) and ^{123}I-MIBG scintigraphy (B and C) revealed a right adrenal tumor measuring 15 mm (red arrows) without metastasis. Histopathological tissue analysis (D) revealed a 9 × 14 × 13 mm tumor with hematoxylin-eosin (middle panel) and Ki-67 staining (× 400 and × 200, respectively). Bars indicate 100 μm. It scored one point (vascular invasion) on PASS. The positive rate of Ki-67 was 3.6%

www.clinvar.com/), the Human Gene Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/ac/index.php), the VHLdb database (http://vhldb.bio.unipd.it/), the UMD-VHL mutations database (http://www.umd.be/VHL/), the COSMIC database (https://cancer.sanger.ac.uk/cosmic), the Genome Aggregation database (gnomAD, https://gnomad.broadinstitute.org/), and the Japanese Multi Omics Reference Panel (jMorp database, https://jmorp.megabank.tohoku.ac.jp/). These findings indicated that the variant was novel, and the ACMG classification indicated that it was "likely pathogenic" (PM2, PM4, PP3, and PP4) (10). Collectively, these genetic, clinical, and pathological findings indicated that this novel variant was pathogenic. Based on the genetic diagnosis, active and periodic surveillance for other tumors (5), including renal cell carcinomas, retinal/central nervous system hemangioblastomas, pancreatic neuroendocrine tumors, and endolymphatic sac tumors, by ophthalmoscopy, abdominal and brain MRI, and audiography was initiated.
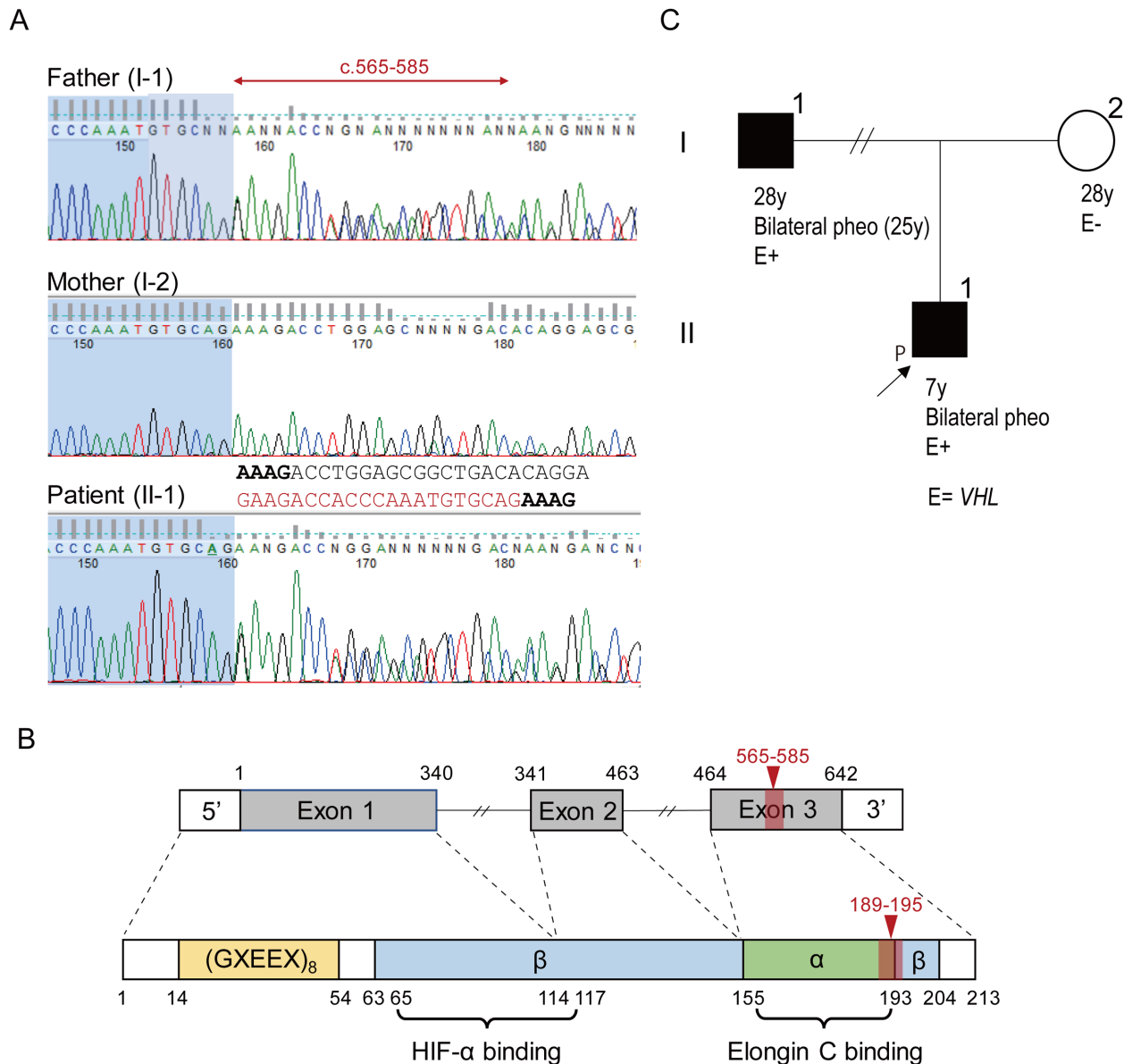

Fig. 4. Genetic analysis of *VHL* gene. A: Direct PCR sequencing analysis performed using DNA extracted from the peripheral blood of the patient and parents revealed a heterozygous in-frame germline variant in *VHL* (NM_000551.4, c.565_585dup, p.Glu189_Gln195dup) in the patient (II-1) and father (I-1). B: Schematic representation of the *VHL* gene and protein structure. The *VHL* gene comprises three exons (gray). Nucleotide numbers are indicated above the upper gene structures. The VHL protein structure with (GXEEX)$_8$ repeat motif (yellow), α-domain (green), and β-domain (blue). The hypoxia-inducible factor-alpha (HIF-α) binding site (residues 65–117) is within the β-domain, and the Elongin C binding site is indicated. Codon numbers are indicated below the lower protein structure. The variants are indicated in red. C: Family pedigree of the proband. The proband (II-1) and his father (I-1) harbored a *VHL* variant and developed bilateral pheochromocytomas (pheo).

Ethical consideration

All procedures performed in this study adhered to the ethical standards of the institutional and national research committees and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Genetic analysis was approved by the Research Ethics Committee of the University of Tokyo (protocol no. 2021226G) and performed in accordance with ethical standards. Written informed consent for publication of patient data, images, and genetic test results was obtained from the patient and his parents.

Discussion

Since the first disease-causing germline variant was reported in 1993, 19 responsible genes, including *SDHx*, *RET*, *NF1* and *VHL*, have been reported (8). According to international guidelines, genetic analysis is recommended for all patients with PPGLs because of the high frequency of germline variants (33.8%) and the possibility of metastasis (2). In the Japanese population, a recent study reported that 32.4% of patients with PPGL had pathogenic/likely pathogenic germline variants (11). This study also showed that the prevalence of *VHL* variants was 15.0% among affected patients and that patients with a family history of *VHL* variants (27.9%) had a higher prevalence than those without a family history (1.8%). Buffet *et al.* (2019) reported a positive outcome of genetic analysis, comparing patients with PPGL who had undergone genetic analysis within 1 yr after the first diagnosis of PPGL (genetic group) and those who were tested more than 1 yr later (historic group). They found that the metastatic tumors of the genetic group were significantly smaller, and the five-year survival rate was significantly higher than that of the historic group (12). These findings suggested that the early genetic diagnosis of hereditary PPGL may optimize personalized follow-up, thus leading to an improved prognosis. In our case, we considered that if genetic analysis of the father with bilateral young-onset pheochromocytoma had been performed, then the pheochromocytoma in the patient might have been detected before the symptoms became severe.

The incidence of PPGL in children and adolescents is low; however, when PPGL occurs, there is a high probability of an underlying germline variant. The occurrence of germline pathogenic variants in patients diagnosed with PPGL appears to be much higher earlier in life, with a prevalence of 80% in pediatric cohorts compared to that of 30% in adults (13, 14). Five other large retrospective cohort studies also identified hereditary diseases in 77–83% of the patients, with pathogenic variants primarily occurring in *VHL* (21–51%) and *SDHB* (15–50%) (14–18). Genetic analyses of patients with childhood-onset PPGL should be proactively considered for accurate prognostic predictions and treatment strategy decisions. The onset of PPGLs associated with *VHL* in childhood have a younger onset, a higher frequency of recurrence, and lower malignancy than those associated with *SDHB* (18). Seabrook *et al.* (2024) reported that almost half of patients with childhood- or adolescent-onset PPGL due to germline pathogenic variants experienced recurrence of primary PPGL within 5 yr (14). Indeed, early recurrence from the residual adrenal gland occurred 1 yr after partial adrenalectomy in this case. Our imaging follow-up revealed recurrence of PPGL before the development of symptoms and helped us conduct total resection of the right adrenal gland.

Since the identification of the *VHL* gene via positional cloning in 1993 (19), more than 600 *VHL* pathogenic variants have been documented worldwide. Clinically, VHL is classified as type 1 or type 2 disease based on the frequency of RCC and PPGL (20) (**Table 1**). In this case, the patient and father developed PPGL, but no other tumors, and were provisionally classified as type 2C VHL. The type of variant identified in the *VHL* gene has been shown to account for differences in PPGL risk, with a strong genotype–phenotype correlation (**Table 1**). A recently published database study (21) including 2,146 patients with 566 variants, revealed that 20% and 50% of patients with VHL presented with PPGL before the ages of 10 and 18 yr, respectively. In addition, missense variants were the most frequent variant type (62.4%), and there was a significant difference between the truncating/non-truncating distributions in the retention percentage of PPGL (11.1% vs. 50.1%), heavily favoring

Table 1. VHL subtypes

VHL subtype	Type 1	Type 1B	Type 2A	Type 2B	Type 2C
High risk	CNS/retinal HB RCC	CNS/retinal HB	Pheo CNS/retinal HB	Pheo CNS/retinal HB RCC	Pheo
Low risk	Pheo	Pheo RCC	RCC		CNS/retinal HB, RCC absent
Variant type	Deletions, insertions, truncations, missense	Contiguous gene deletions encompassing VHL	Missense (eg, p.Y98H, p.Y112H, p.V116F)	Missense (eg, p.R167Q, p.R167W)	Missense (eg, p.V84L, p.L188V)

Based on (21). Pheo, pheocromocytoma; HB, hemangioblastomas; RCC, renal cysts or carcinoma.

non-truncating variants.

By contrast, VHL patients with in-frame insertion/duplication variants are rare (0.6%), and only six variants in 20 patients have been reported (22–29). None of the variants have been functionally analyzed; however, most of them developed familial and type 1 VHL (21) (**Table 2**). Indeed, almost all in-frame insertion or deletion variants which may lead to change of the three-dimensional structure of the protein; therefore, these variants seem to act like truncating variants, such as nonsense or frameshift indels. Only two in-frame duplication variants (29), including the variant identified in our current case, are known to have developed, thus indicating type 2 VHL. However, the mechanism responsible for the development of pheochromocytoma in these in-frame duplication variants has yet to be elucidated. Although the molecular classification of PPGLs includes *VHL*-associated tumors in pseudohypoxia clusters, several studies have indicated that variations in the penetrance of different *VHL* variants in the development of pheochromocytomas within type 2C, and possibly within other VHL 2 types, may be associated with HIF-independent effects of VHL (30, 31). In contrast to truncating variants, which may cause a complete loss of VHL function, only partial insufficiency and partial residual VHL function may be required for PPGL development. The type 2C p.L188V variant, located in the Elongin-C-binding domain (residues 155–193), does not affect its ability to polyubiquitinate HIFs (32), and a high number of patients with PPGL due to *VHL* variants at residues within the Elongin-C-binding domain have been reported (33). The two in-frame duplication variants are located in a 3′ side junction of the α and β domains, which forms part of the α-β interface binding Elongin-C (34) and may predispose the patient to pheochromocytoma. These findings indicate that the in-frame duplication of amino acids in specific regions, especially those affecting Elongin-C binding, may cause pheochromocytomas in a manner similar to missense variants. Numerous studies have attempted to identify more specific genotype–phenotype correlations in VHL patients have been performed (21) but have provided isolated pieces of information. Thus, further accumulation of cases with various genotypes and a standardized open-access worldwide database, including longitudinal and specific clinical data, are required.

Genetic analyses may also provide personalized therapy, particularly for patients with multifocal tumors. Although surgical resection is the primary treatment for PPGL, radiolabeled MIBG or chemotherapy should be considered when surgery is not feasible (35). Molecular targeted therapy is an evolving approach tailored to specific mechanisms underlying PPGL development (36). Genetic analysis enables the categorization of each tumor into different clusters, which allows for precise therapy and consideration of follow-up frequency. Although molecular-targeted therapy has some limitations, it is expected to play an important role in future treatment strategies.

In conclusion, it is crucial to consider genetic analyses, particularly for pediatricians who may diagnose early-onset PPGL. Early diagnosis enables the provision of proper lifelong follow-up care, prompt detection of recurrent/metastatic tumors, and initiation of screening examinations for at-risk families before symptoms manifest.

Acknowledgments

Table 2. Genotype–phenotype correlations in in-frame insertion/duplication *VHL* variants

Variant (NM_000551.4)		Patients					References
		n	Pheo	RA	CHB	RCC	
c.230_231insTCT	p.Cys77_Asn78insLeu	4	0	0	3	1	(23–25)
c.322_324dup	p.Arg108dup	7	N	N	7	N	(24, 25)
c.342_343insGGT	p.Gly114dup	3	0	2	2	0	(26, 27)
c.386_387insAGA	p.Leu129_Val130insGlu	1	0	1	1	0	(28)
c.534_535insAGAGTAAAGCCTGAACTG	p.Leu178_Asp179insArgValLysProGluLeu	3	N	N	3	N	(29)
c.572_592dup	p.His191_Asp197dup	2	1	2	1	0	(30)
c.565_585dupGAAGACCACCCAAATGTGCAG	p.Glu189_Gln195dup	2	2	0	0	0	This study

Pheo, pheocromocytoma; RA, retinal angiomas; CHB, hemangioblastomas of the central nervous system; RCC, renal cysts or carcinoma; N, no data.

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