Clinical diagnosis, treatment and screening of the VHL gene in three von Hippel-Lindau disease pedigrees

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Abstract. The present study aimed to investigate the clinical characteristics of von Hippel-Lindau (VHL) disease and the clinical significance of VHL gene detection. The clinical materials of patients with VHL disease were collected from 3 different families between May 1985 and October 2017. A systematic pedigree study and VHL gene detection at the germline level were performed together with a literature review. Of the 22 patients from 3 VHL pedigrees, 10 exhibited VHL gene mutations (3 genotypes) at the germline level. The genotypes of pedigree were VHL- p.R161Q (c.482G>A), VHL-p.N78S (c.233A>G), and VHL- p.R167Q (c.500G>A). During the follow-up period, the symptoms were stable in 10 patients, including 2 cases of central nervous system hemangioblastomas (CNS-HB), 3 cases of bilateral multiple renal cell carcinoma (RCC) and 5 cases of adrenal pheochromocytoma without local recurrence or distant metastasis. Patients with p.R161Q and p.N78S were not associated with CNS-HB, which was different from the clinical phenotype of previously reported families. RCC were Fuhrman II grade, which was consistent with the previous study. The results of the present study indicated that the standardization of early diagnosis and the improvement of long-term efficacy may be achieved by combining clinical screening and VHL gene detection.

Introduction

Von Hippel-Lindau (VHL) disease (1) is a rare autosomal dominant inherited disease that predisposes the affected individual to various benign or malignant tumors with an incidence rate of 1 in every 36,000-50,000 worldwide in 2018 (2-4). Currently, >40 lesions for VHL disease have been primarily observed in the central nervous system (CNS) and in 14 different organs, including hemangioblastomas (HB; 44-72% of cases), renal cell carcinoma (RCC; 25-45% of cases), pheochromocytoma (PHEO)/paragangliomas (PGL; 10-30% of cases), endolymphatic sac tumors (ELSTs; 6-15% of cases), renal cysts (60% of cases), pancreatic neuroendocrine tumors (PNETs; 5-10% of cases), pancreatic serous cystadenoma/cysts (72-90% of cases), and papillary cystadenoma (PC) of the epididymis and the broad ligament of the uterus (2-15). VHL disease is primarily caused by inactivation of the VHL tumor-suppressive protein. The VHL gene (OMIM, 608537) is located on human chromosome 3p25.3 and encodes the VHL protein (pVHL), which forms a complex with elongation factor and is critical for pVHL to function as an E3 ligase (16). VHL germline mutations result in the dysfunction of E3 ligase and the accumulation of hypoxia-inducible factor-α (HIF-α; VHL-Elongin-HIF-α complex), leading to decreased ubiquitination and proteasomal degradation of HIFs, namely HIF-1α and HIF-2α. Elevated levels of HIFs subsequently regulate overactivation of the downstream pathways in which vascular endothelial growth factor (VEGF), platelet-derived growth factor-β (PDGF-β) and transforming growth factor-α (TGF-α) are involved, which accelerates tumorigenesis (14). The present study analyzed the clinical data of 9 patients from 3 families with VHL disease. The mutations in the VHL gene of patients and their family members were determined.

Materials and methods

Patients. Between May 1985 and October 2017, three Han pedigrees with VHL disease were recruited separately and followed up by Lishui People's Hospital (Lishui, China), the Second Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou, China) and the First People's Hospital of Wenling City (Wenling, China), respectively. The patients underwent collection of their medical history, family survey (pedigree analysis) and a detailed physical examination and associated auxiliary examination by a specialist (GL, ZZ and HZ, respectively) from each of the three hospitals aforementioned in a blinded manner. There were 21 members in the pedigrees, including 15 males and 6 females, aged 9-66 years (Fig. 1 and Table I). The present study was
approved by the Ethics Committee of Lishui People's Hospital and written informed consent was obtained from all patients.

**Detection of VHL gene mutation.** A total of 5 ml peripheral blood (EDTA anticoagulant) was collected from each of the 21 family members from the 3 families, including the 3 probands (F1-II6, F2-II1 and F3-III2). Extraction of genomic DNA from peripheral blood leukocytes was performed using a QIAamp Blood kit (Qiagen GmbH) according to the manufacturer's protocol. The primer sequences were designed based on the VHL gene sequence found in GeneBank using the Primer-Blast online tool (https://omictools.com/primer-blast-tool) and synthesized by Sangon Biotech Co., Ltd. The primer sequences were forward, 5’-ACCGGTGTGGCTCTTTAACA-3’ and reverse, 5’-TCCTGTACTTACCACAACCTT-3’. The 20 µl solution used for the PCR amplification consisted of 17 µl KAPA2G Robust HotStart ReadyMix (cat. no. KK5701; Beijing Huaruikang Technology Co., Ltd.), 1 µl each of the aforementioned upstream and downstream primers (10 µM) and 1 µl of genomic DNA (200 ng) from both the patients and family members. PCR was performed as follows: Pre-denaturation at 94˚C for 5 min, followed by 30 cycles of denaturation at 94˚C for 30 sec, annealing at 65˚C for 30 sec, 72˚C for 1 min; and extension at 72˚C for 5 min. The 2 µl PCR amplified product was used for subsequent electrophoresis with 1% agarose gel for identification and purification. The gel was stained with ethidium bromide (cat. no. E7637; Sigma-Aldrich; Merck KGaA) for visualization. The purified product was sent to Shanghai Xiang Yin Biotechnology Co., Ltd. for sequencing with an ABI 3730XL sequencer. All mutations were confirmed by bi-directional sequencing.

**Imaging examination and detection of biochemical indexes.** The brain, adrenal glands, kidney, and pancreas were imaged using ultrasound, CT or MRI scans and fundus screening was performed in patients with VHL mutations. Serological tests, including the measurement of catecholamine and/or urine vanillicmandelic acid for 24 h, were performed using an AU400 Automated Chemistry Analyzer (Olympus Corporation).

**Follow-up.** All patients were followed up by telephone or hospital visits for 5 years. The follow-up interval was every 2 months in the first year, then every 6 months in the second year and once every year thereafter. During follow-up, general conditions, including blood pressure, blood biochemical indices, recurrence or metastasis of tumor were assessed for every patient.

**Results**

**Family 1 (F1) investigation and general clinical material.**

Proband (F1-II6) was female and aged 50 years. The patient was admitted to Lishui People's Hospital (Lishui, China) in May 1985 due to paroxysmal headache, palpitation and hyperhidrosis that had occurred for 3 months. The results of physical examinations revealed that patient heart rate was 110 bpm and blood pressure was 210/120 mmHg. Ultrasound and CT results revealed a mass sized 5.5x4.5x2.8 cm on the right side of the adrenal gland and it was considered as right PHEO. Retinal hemangioblastoma was excluded by an ophthalmologist. After blood pressure and volume expansion were adjusted with phenylbenzylamine hydrochloride tablets, right adrenal tumor resection was performed under general anesthesia. The postoperative pathology results confirmed that the patient suffered from right PHEO.

The proband's son (F1-III6) was 25 years old and was admitted to Lishui People's Hospital (Lishui, China) in March 2002 due to paroxysmal headache and hypertension (140-160/100-110 mmHg). Ultrasound and CT results revealed a mass sized 5.5x4.5x2.8 cm on the right side of the adrenal gland. After blood pressure and volume expansion were adjusted with phenylbenzylamine hydrochloride tablets, right adrenal tumor resection was performed under general anesthesia. The postoperative pathology results confirmed that the patient suffered from right PHEO.

Figure 1. Pedigree tree of three families with VHL disease. F1, family 1; F2, family 2; F3, family 3; Squares, male; circles, female. VHL, von Hippel-Lindau; HB, hemangioblastoma; RCC, renal cell carcinoma; PHEO, pheochromocytoma.
Table I. Clinical phenotype and genotype characteristics of patients with VHL syndrome in 3 families.

| Patients | Sex   | Age at diagnosis (years) | VHL gene mutation | CNS-HB tumor size (cm) | RCC size (cm) | PHEO size (cm) | Operative type | Pathological diagnosis | Final diagnosis | VHL type |
|----------|-------|--------------------------|-------------------|------------------------|--------------|----------------|-----------------|-----------------------|----------------|---------|
| F1-II6   | Female | 18                       | p.R161Q           | -                      | -            | Right, 5.5x4.5x2.8 | Right PHEO resection | Right PHEO         | Right PHEO         | VHL-IIB      |
| F1-III2  | Male   | 9 (right)/17 (left)      | p.R161Q           | -                      | -            | Left, 7.0x5.5x3.4; right, 5.6x3.8x3.0 | Left and right PHEO resection | Bilateral PHEO (metachronous) | Bilateral PHEO (metachronous) | VHL-IIB      |
| F1-III6  | Female | 28                       | p.R161Q           | -                      | -            | Left, 6.0x4.7x3.5; right, 3.5x2.8x2.5 | Bilateral PHEO resection | Bilateral PHEO (synchronous) | VHL-IIB      |
| F2-II1   | Male   | 24                       | p.N78S            | Left, 1.0x1.0x0.8; right, 1.0x1.0x1.0 | Right, 4.1x3.4x3.9 | Right PHEO resection + right RCC resection + resection of bilateral epididymal cyst | Right PHEO + right RCC + bilateral epididymal cyst | Right PHEO | Right PHEO + right PHEO + bilateral epididymal cyst + multiple pancreatic cysts | VHL-IIB      |
| F2-II2   | Male   | 27                       | p.N78S            | -                      | Right, 4.0x3.0x2.5 | Right radical nephrectomy | Right RCC | Right RCC | Right PHEO + multiple cysts of kidney and pancreas + bilateral epididymal nodules | VHL-IIB      |
| F2-III1  | Female | 5                        | p.N78S            | -                      | -            | - | - | - | VHL gene mutation carrier | VHL-IIB      |
| F3-II1   | Male   | 66                       | p.R167Q           | Left, 1.0x1.0x0.8; right, 1.0x1.0x0.9 | Right, 4.5x3.5x2.6 | Right PHEO resection + right RCC resection | Right PHEO + right RCC | Right PHEO + bilateral RCC + multiple cysts of kidney and pancreas + PNETs | VHL-IIB      |
| F3-III1  | Female | 45                       | p.R167Q           | 3.2x2.1x1.0            | -            | - | Cerebellar HB resection | HB | Cerebellar HB | VHL-IIB      |
| F3-III2  | Male   | 34                       | p.R167Q           | Right 2.1x1.0x0.9; right, 0.6x0.5x0.5 | - | - | Gamma knife radiosurgery for CNS-HB | - | Multiple CNS-HB | VHL-IIB      |
| F3-IV1   | Male   | 21                       | p.R167Q           | -                      | -            | - | - | - | VHL variant carrier | VHL-IIB      |

*Bilateral multiple RCC; †Clinical diagnosis. VHL, von Hippel-Lindau; CNS, central nervous system; HB, hemangioblastomas; RCC, renal cell carcinoma; PHEO, pheochromocytoma; PNETs, pancreatic neuroendocrine tumors.
patient suffered from right PHEO. In 2010, this patient suffered from paroxysmal hypertension and headache for a second time and his levels of serum catecholamine were also increased, including dopamine (600.36 ng/l), adrenaline (297.94 ng/l) and norepinephrine (1,093.84 ng/l). The ultrasound and CT results revealed a mass sized 7.0x5.5x3.4 cm on the left side of the adrenal gland. After blood pressure and volume expansion were adjusted with phenylbenzylamine hydrochloride tablets, left adrenal tumor resection was performed under general anesthesia. The postoperative pathology results confirmed that the patient suffered from left PHEO. The final diagnosis of this patient was bilateral PHEO.

The proband's niece (F1-III2) was 38 years old and was admitted to Lishui People's Hospital in October 2002 due to persistent headache and dizziness that had lasted for 3 days. The results of physical examination demonstrated that the patient's blood pressure was 160/100 mmHg and their serum catecholamine levels were increased. Ultrasound and CT results revealed a mass sized 7.0x5.5x3.4 cm on the left side of the adrenal gland. After blood pressure and volume expansion were adjusted with phenylbenzylamine hydrochloride tablets, left adrenal tumor resection was performed under general anesthesia. The postoperative pathology results confirmed that the patient suffered from left PHEO. The father of this patient (F1-II2, the proband's brother) died from hypertensive intracerebral hemorrhage 26 years ago, aged 38 years.

Family 2 (F2) investigation and general clinical material. The proband (F2-II1) of F2 was male and aged 33 years. The patient was admitted to the Second Affiliated Hospital of Zhejiang University School of Medicine (Hangzhou, China) in September 2009 due to paroxysmal headache, dizziness and palpitation with hyperhidrosis. Physical examination results revealed that the patients' heart rate was 100 bpm and blood pressure was 105/78 mmHg. MRI results revealed a mass sized 4.1x3.4x3.9 cm at the right adrenal gland and multiple renal tumors (the largest diameter of the tumors, 1.0x1.0x1.0 cm on the left; 1.0x1.0x0.8 cm on the right) and multiple cysts in the pancreas (Fig. 2A and B). An ophthalmologist (from the Second Affiliated Hospital of Zhejiang University School of Medicine) excluded the existence of retinal hemangioblastoma preoperatively. The concentration of urine vanillicmandelic acid at 24 h was 138.3 µmol/24 h (normal reference value, <33 µmol/d). Combined with clinical
manifestations, the patient was considered as right PHEO, with
double renal masses and multiple pancreatic cysts. After the
patient's blood pressure and volume expansion was adjusted
with phenylbenzylamine hydrochloride tablets, resection of the
right adrenal tumor and the upper pole of the right kidney
were performed under general anesthesia. The postoperative
pathology results confirmed that the patient had right PHEO
and right RCC (Fuhrman II grade) (17). In April 2012, the
patient underwent bilateral epididymal nodule excision due to
the presence of multiple nodules in the bilateral epididymal
head. Postoperative pathology results confirmed that the
patient suffered from papillary cystadenoma of the bilateral
epididymal head.

The proband's younger brother (F2-II2) was aged 32 years.
In 2012, the patient presented with multiple masses in
both kidneys (left, 1.0x1.0x1.0 cm; right, 4.0x3.5x2.6 cm),
multiple cysts in both kidneys, and pancreas and epididymal
nodules (Fig. 2C and D). The patient underwent laparoscopic
right nephrectomy under general anesthesia. The post-
operative pathology results confirmed that the patient suffered
from multiple RCC in the right kidney (Fuhrman II grade).
IFN and IL-2 were used for postoperative treatment.

Family 3 (F3) investigation and general clinical material.
Proband (F3-II2) was male and aged 39 years. In April 2013,
the patient was admitted to the First People's Hospital of
Wenling City (Wenling, China) having suffered with dizziness
and headaches for 1 month. A cranial MRI examination
revealed that there was a mass sized 2.1x1.0x0.9 cm at the right
margin of the sellar region and a mass sized 0.6x0.5x0.5 cm at
the cerebellar vermis. The masses were markedly enhanced
with clear margins. The patient was considered to suffer from
CNS-HB. An ophthalmologist (The First People's Hospital
of Wenling City) excluded retinal hemangioblastoma. As
the patient refused surgical treatment, cranial gamma knife
radiotherapy (peripheral dose 5 Gy, central dose 10 Gy) was
performed 6 times with a 5 Gy dose at the peripheral and
10 Gy dose at the central positions. CNS-HB of the F3-II2
was stable after 5 years of follow-up.

The proband's elder sister was aged 45 years (F3-III1). In
May 2015, the patient was admitted to the First People's Hospital of
Wenling City due to dizziness lasting 3 months. A brain MRI revealed that there was a mass sized 3.2x2.1x1.0 cm at
the vermis of the cerebellum, with clear margins. The mass
was excised via surgery. The postoperative pathology results
confirmed that the patient suffered from cerebellar hemangioblastoma.

The proband's father (F3-II1) was aged 66 years. The
patient was hospitalized in the First People's Hospital of
Wenling City presenting with cough and expectoration with
low fever for 2 months in February 2014. Physical examination
results revealed that the patient's temperature was 37.8°C and
his blood pressure fluctuated between 104-123/66-96 mmHg.
CT examination results revealed that the patient suffered from
chronic obstructive pulmonary disease with pulmonary
infection. Ultrasound and CT results revealed a mass
sized 4.5x3.5x2.6 cm on the right side of the adrenal gland,
multiple solid lesions of both kidneys (left, 1.0x1.0x0.8 cm; right, 1.0x1.0x0.9 cm) with multiple small cysts and pancreatic
head lesions (2.6x2.0x2.0 cm). Following treatment of a lung
infection and the adjustment of blood pressure and volume
expansion with phenylbenzylamine hydrochloride tablets,
resection of the right adrenal tumor and right kidney tumor
were performed under general anesthesia. The postoperative
pathology results revealed that the patient suffered from right
PHEO and right RCC (Fuhrman II grade).

VHL gene detection results. Of the 22 patients from 3 VHL
families, 10 had three VHL germline missense mutations
within coding regions, respectively (Fig. 3). In the F1 family,
3 cases harbored the P.R161Q (c.482G>A) mutation at exon
3 (Fig. 3A); 3 cases in the F2 family harbored the P.N78S
(c.233A>G) mutation at exon 1 (Fig. 3B); and 4 cases in the
F4 family harbored the P.R167Q (c.500G>A) mutation at exon
3 (Fig. 3C). Apart from 8 patients with the VHL clinical
phenotype who had been surgically and pathologically diag-
nosed, 2 new cases (F2-III1 and F3-IV2) were revealed to be
asymptomatic carriers of VHL gene mutations. The remaining
12 family members had no VHL gene mutations and no
clinical manifestations or abnormalities following imaging
and serological tests associated with VHL disease. The
clinical phenotype and genotype characteristics are presented
in Table I.

Discussion

VHL disease, also known as VHL syndrome, is characterized
by a variety of benign, malignant tumors and multiple organ
cysts (18). VHL patients usually develop clinical symptoms
after reaching 20 years of age, with 90-100% penetrance of
clinical symptoms between 65 and 70 years old (19). Retinal
and cerebellar HB are typically the most common and earliest
presenting forms of VHL disease (4,7,9). VHL syndrome can
be divided into two types: Type I and type II. Type I syndrome has
CNS-HB and/or RCC among other tumors (except PHEO) and
can also be divided into type IA (high-risk RCC) and type IB
(without RCC). The patients with type II must have PHEO and
can be divided into type IIA (includes PHEO but not RCC),
type IIB (RCC + PHEO) and type IIC (only PHEO) (2,3,14).
The clinical diagnosis of this disease should be based on VHL
symptoms, heredity and family factors. If a patient possesses
one of the following three conditions, they may be diagnosed
with VHL disease: i) At least two positions where CNS-HB
exists; ii) CNS-HB at one position and one other organ tumor
(RCC, PHEO/PGL, PNETs or ELSTs); iii) at least one visceral
tumor (RCC, PHEO/PGL, PNETs or ELSTs) associated with
the VHL gene mutation at the pathogenic germline level, or
a parent has been diagnosed with VHL (3). Despite the high
heterogeneity of clinical phenotypes, VHL gene mutations
could be detected at the germline level in almost all patients
with VHL disease (2). The patients in the three families
included in the present study were diagnosed as VHL type II.
The patients in F2 and F3 were VHL type IIB and the patients
in F1 were VHL type IIC (Table I).

The VHL gene contains three exons and encodes a poly-
saccharide anchored membrane protein containing 213 amino
acids (20). The N-terminal region of the protein contains α
and β domains (20). The α domain binds to the elongation
factor C/B to form a complex and the β domain binds to the
HIF-α gene (21). pVHL downregulates the expression of
HIF-α, activates the ubiquitination of epidermal growth factor receptor (EGFR), regulates glucose metabolism-associated genes [glucose transporter 1 (GLUT1) and pspohonfructokinase 1], growth factors (TGF, PDGF and VEGF) and the cascade reaction of neuro growth factor/JunB/Egl nine homolog 3-associated apoptotic pathway, ultimately leading to the formation of benign and malignant multiple organ diseases (21). Existing evidence has indicated that HIF-1α serves an important role during clear cell (cc) RCC and HB development (21). Previously, almost 400 VHL gene mutants have been revealed to cause VHL disease (22). The majority of mutations are missense (27-38%), nonsense (13-27%), large fragment deletions (9-20%), microdeletions (10%), truncation and rearrangement (25%) mutations (22). Splicing site mutations are rare. Of all the mutations, ~20% of the patients exhibited new mutations (de novo) or their parents are chimeras (22). VHL type I disease is caused primarily by a large fragment deletion of the VHL gene, including C3orf10 gene inactivation, truncation and missense mutations, which lead to the loss of pVHL function or structural changes in the protein (3). VHL type II disease associated with PHEO is mostly caused by missense mutations (78-96%), which often lead to partial functional defects of the pVHL. The most frequent mutations of VHL type II disease are p.R167W and p.R167Q mutations at position 167 of exon 3 in the VHL gene (2,3). Notably, although the stabilization of HIF-α is closely associated with the occurrence of RCC, both IIA and IIB antimutagenesis can inactivate VHL function, which in turn inhibits the regulation of HIF-α activation. However, only patients in stage IIB exhibit ccRCC (23). Currently, certain hypotheses suggest that the occurrence of ccRCC is associated with the expression of HIF-2α (20,21). However, IIA mutations do not form enough HIF-2α to form HB, despite producing the appropriate quantity of HIF-α. This explains why HB is the most common clinical manifestation of VHL (21). Liu et al (15) demonstrated that patients with non-HIF-α binding sites demonstrated an improved survival rate when compared with those that exhibited HIF-α binding sites and truncated mutations (15). All patients in the three families of the present study were VHL type II with missense mutations in the VHL gene and the patients of the F3 family harbored p.R167Q (α) mutations accompanied with CNS-HB. No CNS-HB was observed in the F1 (p.R161Q, α) and F2 (p.N78S, β) families, which differed from the clinical phenotype of previously reported pedigrees (7,8). The presence of different clinical phenotypes or disease progression in different or identical pedigrees or members indicated that there may be other pathogenic or modifier factors, and/or secondary mutation attacks at the somatic cell level in addition to genetic factors (3). This also indicated that individualized treatment should be adopted for heterogeneous patients of different or the same pedigrees.

The typical genotype-phenotype correlation dictates that VHL missense mutations are responsible for type II disease with a high risk of PHEO and that truncating mutations are responsible for type I disease with a low risk of PHEO (24). Deletion of all or part of the VHL gene as well as the nearby gene BRK1 (also known as HSPC300 and C3orf10) leads to retinal HB and CHB with a low risk of RCC, which is sometimes called type IB VHL disease (25). The results of the present study supported the notion that missense mutations tend result in PHEO. In addition, mutations at different sites are associated with diverse risks of VHL-associated lesions (22). Patients with VHL that present with missense mutations in the HIF-α binding site (HM) are associated with a lower risk of PHEO and higher risks of CHB and pancreatic tumors or cysts, while missense mutations located at sites other than that of the HIF-α binding site (nHM) are associated with a higher risk of PHEO, which results in better survival time (24). Furthermore, patients with truncating mutations are more likely to develop RCC than those with HM (26).

In contrast to the present study, it has been indicated that p.N78S (HM)-associated PHEO alone, p.R161Q (nHM)-associated RCC/PHEO and p.R167Q (nHM)-associated CNS/RCC/PHEO exhibit diverse phenotypes (16). Of note, the pathogenesis of CHB and RCC, upregulated HIF-α expression and consequent overexpression of VEGF and other HIF-associated genes are the primary causative factors of tumor progression (14). In PHEO, HIF-α (particularly HIF-2α) dysregulation results in the overexpression of various HIF-inducible genes, including GLUT1 and VEGF, which themselves serve important roles in tumor development (27). However, the development of PHEO appears to be HIF-independent. In patients with the typical IIC phenotype, as exhibited by the F1 family of the current study, mutant pVHL is able to degrade HIFs, and it has been hypothesized that mutations associated with PHEO may induce gain of function through an intact but altered pVHL (28). In addition, Gossage et al (14) reported that mutations in the elongin C binding domain of pVHL are associated with PHEO. These binding sites are implicated in the p53-mediated apoptosis of sympathetic neuronal precursor cells, which then go on to form PHEO (14). It is speculated that such large phenotypic variation may result from numerous other factors that are potentially environmental and may affect the phenotypes induced by specific mutations (7).

The current clinical treatment strategy of VHL disease focuses primarily on the treatment of VHL disease-associated CNS-HB, RCC, PHEO and PNETs (19). The average age of CNS-HB at diagnosis is 33 years (range, 7-78 years) (8,19). For CNS-HB, 90% presented in multiple forms and the majority occurred in the cerebellum (45-50%) and spinal cord (40-45%) (8,20). Complete excision of CNS-HB based on microsurgical treatment was rare (<1%) with recurrence of the tumor (8,20). Mild nerve defects usually recovered within 2 weeks to 6 months after excision (9). Stereotactic radiotherapy is a potential treatment option for patients who cannot tolerate surgery, or where resection of CNS-HB was difficult. The 2- and 15-year remission rates were 91 and 51%, respectively (10,11). A previous study also demonstrated that a gamma ray single dose of 20 Gy at the peripheral position and a 40 Gy dose at the central position could effectively treat CNS-HB (12). Consistently, the F3-II12 in this study was only treated by cranial gamma knife radiotherapy and was stable after 5 years of follow-up. These results indicate that radiotherapy may be an alternative treatment method for patients with CNS-HB who refuse to undergo surgical excision, but the long-term effectiveness requires continued observation. Retinal HB can occur in ~50% of patients with VHL (10). The mean age at diagnosis is 25 years (range, 1-68 years) and the disease is characterized by multiple and bilateral tumors, with sizes varying from <1 to several optic discs in diameter (29). In general, small lesions can be treated with greater success and fewer complications compared with those that are larger (30).
The majority of peripheral retinal HB cases can be treated with laser photocoagulation (small peripheral HB), cryotherapy (large HB) or photodynamic therapy (31). However, these treatments cannot be used when the tumor is near the optic nerve, in which case the therapeutic approach is only surveillance, resulting in a higher risk of damage to the optic nerve (32). The average age at diagnosis of VHL-RCC is 39 years (range, 13-70 years) (13,20). VHL-RCC is characterized by simultaneous or heterogeneous bilateral tumors that are multifocal and low grade with a slow progression (13,20). The metastasis rate of VHL-RCC is very low when tumors are <3 cm and physical therapy, such as waiting for observation or radiofrequency ablation, may be used in clinical treatment (13,22). Nephron-sparing surgery should be performed if the tumor is >3 cm, which should effectively decrease renal insufficiency despite the high recurrence rate of localized neoplasms (13).

In the present study, the diagnostic ages of 3 patients with VHL-RCC (F2-II1, F2-II2 and F3-II1) were 33, 32 and 66 years, respectively. F2-II2 underwent radical resection due to multiple and large tumors of the right kidney. The other two cases underwent unilateral single tumor enucleation (similar to biopsy for definite pathology), all of which were Fuhrman grade II and RCC was relatively stable after follow-up. The average age at diagnosis of VHL-PHEO was 27 years (range, 2.75-58.00 years) (33,34). Of the VHL-PHEO cases, 90% of tumors were located in the adrenal gland, and 20-50% of VHL-PHEO were bilateral (33). Malignant VHL-PHEO was rare (1-5%) (33). VHL-PHEO can be asymptomatic at an early stage and often secretes large quantities of norepinephrine, which differs from MEN2-PHEO, where large quantities of adrenaline are secreted (2,33-35). PHEO excision or partial adrenalectomy decreases the risk of or avoids adrenal cortical insufficiency or crisis (36). It is worth noting that, 50% of patients developed a second PHEO within 30 years after initial diagnosis (2,36,37). In the present study, the average diagnostic age of 5 patients with VHL-PHEO was 34.6 years. There were 3 patients (F1-II6, F2-II1 and F3-II1) with unilateral PHEO and two patients (F1-II12 and F1-II16) with bilateral PHEO, including one patient (F1-II12) with first-time diagnosis of unilateral PHEO and contralateral PHEO occurring 8 years after surgery. Patients received PHEO resection, after which adrenal function was normal. A previous study determined that the average diagnostic age of PNETs was 35 years (range, 10-75 years). PNETs were asymptomatic and grew slowly. Of the PNETs, 80-93% were <3.0 cm. The phenomena of tumor >3.0 cm, doubling time <500 days and codon 161 and 167 mutations at exon 3 were potentially malignant signs (3,38,39). In the present study, one patient (F3-III1, 66 years old) with PNETs required close monitoring for the possibility of malignancy, although no definite signs or symptoms were identified via clinical imaging. VHL-associated renal and pancreatic cysts and epididymis/broad ligament cystadenoma of the uterus is usually asymptomatic and often not treated clinically. The average diagnostic age of ELSTs is 22 years (range, 12-50 years). Of the ELSTs recorded in a previous study, 30% were bilateral and the majority were benign. Clinical symptoms of ELSTs include hearing loss (84-100%), tinnitus (73-77%), dizziness (62-68%) and facial paralysis (8%). Surgical resection of the tumor is the primary treatment method for ELSTs (2,3,9). In the present study, 10 patients with VHL disease were not associated with ELSTs and it was speculated that the phenomenon may be associated with the small sample size of the family and patients. In addition, two asymptomatic VHL gene carriers (F2-III1, 5 years of age, p.N78S; F3-IV2, 21 years of age, p.R167Q) were identified via gene testing. Early diagnosis and genetic counseling, regular cancer screening and surveillance are therefore helpful for disease management and for the improvement of prognosis (40).

In conclusion, VHL is a complex disease that can be easily misdiagnosed. Future studies should aim to improve the understanding and attention to molecular diagnosis and management based on VHL gene detection, which may be helpful for early diagnosis and standardization of treatment, improvement of patient prognosis and quality of life, and for the decrease in clinical risk of VHL disease.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
HZ designed the study. GL collected the clinical data, performed the experiments and wrote the manuscript. YZ and ZZ collected clinical data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Lishui People's Hospital, The Second Affiliated Hospital of Zhejiang University School of Medicine and the First People's Hospital of Wenling City. Written informed consent was obtained from all participants.

Patient consent for publication
Patients provided their written consent for the publication of these images.

Competing interests
The authors declare that they have no competing interests.

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