A Nonsense Mutation in VHL Gene Causing Von Hippel-lindau Syndrome in a Large Chinese Family

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Research

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Abstract

Background

Von Hippel-Lindau (VHL) syndrome is a multi-organ neoplastic disease characterized by highly vascular and cystic tumors in central nervous system (CNS), retina and visceral lesions, which is mainly caused by germline mutations in the VHL gene.

Methods

Here, a large consanguineous four-generation family with variant phenotypes of VHL disease was recruited and molecular genetics tested via Sanger sequencing.

Result

Genetic investigation detected a c.351G>A nonsense mutation in the VHL gene, that altered the reading frame downstream and created a premature TGA stop signal, resulting in severely truncated pVHL (p.Trp117Ter). This mutation is absent from public databases, and the functional prediction bioinformatic tools demonstrated that the residue is conserved and the variant is highly likely to be deleterious.

Conclusion

The c.315G>A nonsense mutation in VHL gene is the causal mutation of this kindred that may lead to clear familial aggregation of VHL disease because of the dysfunction of truncated pVHL.

Background

VHL syndrome is a hereditary, autosomal dominant (AD), multi-organ, neoplastic disease, which is caused by genetic aberrations of the tumor suppressor gene VHL. It is characterized by highly vascular and cystic tumors, including hemangioblastomas (HGBs) of CNS and retina, and visceral lesions such as clear-cell renal cell carcinomas (ccRCCs) and renal cysts (RC), phaeochromocytomas (PCCs), pancreatic cysts and tumors (PCTs), and papillary cystadenomas in epididymis and broad ligament [1]. CNS hemangioblastomas are the most emblematic lesion of VHL disease, occurring in up to 80% of VHL patients, mainly in the cerebellum [2]. Along with RCCs, the two manifestations present as the major causes of mortality [3]. Retinal angioma is also the common presenting feature of VHL disease affecting multiple and bilateral fundus retinal vessel in about one half of cases [4]. VHL germline mutations affect 1 in 36,000 live births with an AD fashion, and its penetrance is estimated to be more than 90% by the age of 65 years old [5]. The onset of VHL disease occurs at a mean age of 26 years [6]. Approximately 80% of VHL patients have a multigenerational family history of the disease, and the remaining cases may due to de novo or somatic mutations of the VHL gene [5].

The VHL (OMIM: 608537) tumor suppressor gene, which is located on chromosome 3p25.3, consists of three exons, encoding two isoforms of VHL proteins: pVHL30 (30 kDa in length, 213 amino acids) and pVHL19 (19 kDa in length, 160 amino acids) which lacks the first 53 residues, due to alternative translation initiation site in the open reading frame of exon 54. Both the p30 and p19 isoforms have equivalent tumor suppressor effects, and both can regulate hypoxia-inducible factor-α (HIFα) [7, 8]. The pVHL consists of two tightly coupled domains, α and β, together with elongation factors C and B (Elongin C and Elongin B), Cullin 2 (CUL2) and the RING finger protein RBX1, forming the VCB–CR complex which is crucial for pVHL function. Then prolyl-hydroxylated HIFα is recognized and binded by the VCB–CR E3 ubiquitin ligase complex and targeted for ubiquitylation (Ub) and proteolytic degradation [8, 9]. By contrast, pVHL harbouring mutations that disrupt the complex construction is unstable and rapidly degraded by the proteasome, resulting in HIFα accumulation and then the upregulation of HIF target genes (e.g., VEGF, PDGF β, TGF α, CyclinD1, EPO etc.) involved in various processes, such as angiogenesis, proliferation, metabolism and apoptosis. The aberrant overexpression of downstream target genes thereby contribute directly to tumorigenesis. Many molecular genomic analysis studies on VHL disease have been reported, and about 585 different germline mutations within the whole coding sequence of VHL have been documented (http://www.umd.be/VHL/W_VHL). In non-mosaic patients with classical VHL disease germline mutation detection is almost up to 100% [10]. Approximately 11% are partial or complete deletions (ranging from 0.5 to 250 kb) that remove one or more VHL exons, while the remaining mutations fall into two groups, missense (52%) or small in-frame indel (6%) substitutions and mutations predicted to cause truncated protein (nonsense 11%, frameshift 13%, splice site 7%) [7]. And some researches have investigated that VHL mutation types, mutation regions and specific mutation codons can manifest different phenotypes of VHL disease [11].

In this study, we examined a large Chinese VHL family with 17 immediate members across four generations that includes 9 VHL patients and 1 mutation carrier. Correlated genetic findings, pathogenic mechanism, clinical characteristics and the genotype-phenotype connections are discussed.

Materials And Methods

Family Recruitment

We enrolled a family of Chinese Han ethnicity (Figure 1) with 9 members diagnosed with VHL syndrome. Participants were under detailed clinical manifestations questionnaire, physical examination, necessary imaging examination (MRI of the brain and spine, abdominal ultrasonography/CT and funduscopy) and pedigree investigation by clinicians. Two mL of peripheral blood samples were obtained from each available family members after the
informed written consents for experimentation with human subjects in this genetic study were obtained from all of the participants. This research was approved by the ethics committee of Beijing Ditan Hospital.

**Genomic DNA Preparation and Sanger sequencing**

Genomic DNA for each individual was extracted from peripheral blood lymphocytes via standard phenol-chloroform method. After the genomic DNA was isolated, the VHL gene sequence was amplified by polymerase chain reaction (PCR) with the primers designed with Primer3 online software (http://primer3.ut.ee/) summarized in Table 1. Then the products, purified with Axygene-AP-GX-50 Toolkit, were sequenced from both the DNA strands of the entire coding region and the junction regions on ABI Prism 3730 Avant DNA sequencer (Applied Biosystems). After that, the reads of the sequencing was compared to a reference sequence of GRCh38 human genome.

**Variant-detection and Pathogenicity prediction**

Sequence analysis was performed in software SnapGene viewer (version 2.8.3) for mutation exploring. UGENE (version 35.0) was used to investigate whether the identified amino acid substitution of pVHL was conserved between different species. Computational prediction tools (PROVEAN, Gerp++, PhyloP, PhastCons, Polyphen-2, MutationTaster, CADD, ClinGen Haploinsufficiency and ExAC pLI) were used to predict the conservation and pathogenicity of the detected variant and investigate the effect of the mutated amino acid on the protein's structure and function.

And the variant was compared against publicly available databases such as the 1000 Genomes Project (http://internationalgenome.org/), the Exome variant server, NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS/), the Exome Aggregation Consortium database (ExAC, http://exac.broadinstitute.org/), Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/) and the Single Nucleotide Polymorphism Database (dbSNP, https://ncbi.nlm.nih.gov/SNP/) to investigate the frequency of the variant in healthy populations.

**Modeling the three dimensional structure of pVHL**

IBS (version 1.0.3) were used to present the location of the variant in both the genome and protein level. The coordinates of the HIF-1c-pVHL-ElonginB-ElonginC complex were obtained from the RCSB protein data bank (PDB ID:1LM8). PyMol Viewer (version 1.7.4) were used to visualize the three dimensional protein model and map the mutated amino acid point onto the three dimensional model.

**Results**

**Clinical Information**

The proband (II-3) was a 23-years-old boy presented with one week history of mild diplopia, but with no obvious positive signs of the nervous system physical examination. Magnetic resonance imaging (MRI) of the brain revealed an enhancing lesion in the left cerebellum (Figure 2A-C). Axial T1 postcontrast images demonstrate an enhancing mural nodule (red arrowhead) with a large peritumoral cyst (red arrow) in the left cerebellar hemisphere exerting a mass effect upon the midline and fourth ventricle. Abdominal computed tomography (CT) scan revealed a cyst (red arrow) in the pancreatic head (Figure 2D). On fundus evaluation (Figure 2E), there is an atypical angiomata (white arrowhead) on his left eye retina and the optic disc presents edematous (black arrow) probably because of the intracranial hypertension leading to the clinical manifestation of diplopia. A diagnosis of HGB was confirmed by postoperative pathology of the cerebellum lesion. Because the patient had a family history of HGB, his father (III-3, Figure 2F-J) uncle (Ill-8) aunt (Ill-1) grandpa (II-1) and grandma (II-5), the entire family members were examined for VHL disease. Unfortunately, multiple enhancing nodules were revealed by brain MRI (Figure 2K-M) in the proband’s sister's cerebellum (Ill-4), and demonstrated to be HGBs after surgical excision of the biggest one near the brainstem (Figure 2N black arrowhead) and the other three surface lesions followed by histopathological analysis of the biopsy tissues. She also has bilateral involved retinal hemangioblastomas on fundoscopic view (Figure 2O-Q), which have easily recognizable globular reddish appearance with dilated feeding arteries. The proband’s cousin (II-2) was also diagnosed with HGB located in his cerebellum near the brainstem (Figure 2R-T). A further two affected members (II-1 and II-5) of the family had previously died from this syndrome before we got their blood. The proband’s great grandfather (I-1) suffered a sudden death in his 60s presenting with an episodic headache for 10 years suggesting the CNS HGB diagnosis. All findings consistent with Mendelian expectation for AD VHL disease trait in this family. The pedigree of the family is presented in Figure 1 and the clinical characteristics of the proband and his family members are indicated in Table 2.

**Mutation detection**

All the family members were further sequenced for VHL gene. A nonsense heterozygous variant NM_000551 c.351G>A (p.Trp117Ter) in exon 2 of the VHL gene was revealed to be present in the proband (Ill-3) (Figure 3A). And the target sequencing of this variant in the other family members revealed 6 another individuals, including 5 diagnosed patients (III-1, III-3, III-8, II-2, II-3 and II-4) and 1 asymptomatic mutation carrier (Ill-1), as revealed by Sanger sequencing (Figure S1).

**Pathogenicity prediction and bioinformatics analysis**

Alignment of VHL amino acid sequences in numerous species revealed that the tryptophane at the 117th amino acid site and the downstream residues is evolutionarily conserved (Figure 3B), indicating that its evolution may have preserved function. In addition, this nonsense mutation is absent from public databases (1000genome, ExAC, gnomAD, ESP5400 and dbSNP), and the functional prediction bioinformatics tools (PROVEAN, Gerp++, PhyloP,
PhastCons, Polyphen-2, MutationTaster, CADD, ClinGen Haploinsufficiency Score and ExAC pLI score) demonstrated that the residue is robustly
conserved and the variant is highly likely to be deleterious (Table 3). MutationTaster predicted that c.351G>A of *VHL* gene was a disease-causing
mutation through three possible pathogenic mechanisms: Nonsense-Mediated mRNA Decay (NMD), splicing abnormality, and known disease mutation
at this position. According to the prediction from the bioinformatics tools, it was indicated that the integrity and expression level of *VHL* protein might be
affected by c.351G>A.

The three dimensional protein model of HIF-1α-pVHL-ElonginB-ElonginC complex (Figure 4) was built via molecular modeling software. Ribbon diagram
shows that HIF-1α binds directly to pVHL β domain, made by the N segment, in particular by Hyp^564 of the HIF-1α. Meanwhile, the mutated amino acid
point of W117 is spatially close to Hyp^564 (at least 3.5 Å).

**Discussion**

Clinical diagnostic criteria introduced in 1964 [12] enabled the diagnosis of VHL disease in sporadic patients who had two manifestations (such as two
HGBs or a HGB and a visceral tumor), and in patients who had only a single simple manifestation (a CNS HGB or a visceral lesion) but with family
history of VHL disease. Molecular genetic testing for early identification of the patients improves diagnostic certainty and erases the psychological
burden of at-risk family members who have not inherited the pathogenic variant. In the present study, using Sanger sequencing, we successfully
identified a novel nonsense variant, c.351G>A (p.Trp117Ter), in the second exon of *VHL*, which was heterozygous in 6 VHL-diagnosed members (III-1, III-
3, III-8, III-2, III-3 and III-4) and 1 currently phenotype-normal mutation carrier (II-1) in this pedigree.

From the bioinformatics analysis, we found that the c.351G>A variant is absent from public databases, and predicted to be deleterious by bioinformatics
tools. The residue p.Trp117 in pVHL which is located within the β-domain (Figure 3C) and maps to hydrophobic core residue important for the structural
integrity of the β sandwich [13], is evolutionarily conserved, suggesting that this amino acid is important for maintaining the protein's structure and function.

pVHL contains two tightly coupled functional domains, the α-domain and the β-domain, held together by two short polypeptide linkers (residues 154 to
156 and 189 to 194) and a polar interface that is stabilized by hydrogen-bond networks from the H1 helix, the β sandwich, and Elongin C [13]. The α-
domain is responsible for directly binding to Elongin C, which consists of three α-helices (H1, H2 and H3) located at amino acid residues 155-192,
whereas the β-domain is the substrate recognition region of pVHL, which contains seven-stranded β sandwiches (residues 63 to 154) and an α-helix (H4;
residues 193 to 204) that packs against one of the β-sheets through hydrophobic interactions [13]. The pVHL−Elongin C complex nucleates a complex
containing Elongin B, CUL2 and RBX1, forming the VCB−CR complex (Figure 4), which is thus resistant to proteasomal degradation through their
interactions with each other [8]. The α-domain has an important role in the maintenance of the spatial conformation stability of pVHL [14]; the β-domain
binds directly to HIF-α (HIF-1α or HIF-2α) and participates in the degradation of the HIF subunit under aerobic conditions. Previous data shows that the
HIFα peptide binds exclusively to the β-domain of pVHL [15]. A six-residue NH2-terminal segment (residues 561 to 566) that is centered on Hyp^564 (Figure
4 in blue), a three-letter code for hydroxyproline, is central to the binding of HIF-1α to pVHL β-domain [9]. The pVHL residues that interact with Hyp^564,
including W117 which is spatially close to Hyp^564 (3.5 Å , Figure 4), are highly conserved. And W117R missense mutation of pVHL has been shown to
abolish HIF-1α binding [16].

Considering that the mutation c.351G>A introduced a premature stop codon which results in the replacement of tryptophane (TGG) with a stop codon
(TGA) at codon 117 (p.Trp117Ter), either it can lead to the production of a truncated protein missing 45% of its residues including the predicted
downstream α-domain (residues 155–192) and the α-helix (H4; residues 193 to 204) part of β-domain, failling to bind to Elongin C, Elongin B, CUL2 and
RBX1 to form the VCB−CR complex, or the protein may be entirely absent due to the Nonsense-Mediated mRNA Decay (NMD), a process that typically
degrades transcripts containing premature termination codons (PTCs) in order to prevent translation of unnecessary or aberrant transcripts. According
to the ClinGen Haploinsufficiency Score and the prediction of the aforementioned bioinformatics tools, it is likely that aberrant VHL transcripts with the
nonsense mutation p.Trp117Ter undergo NMD, thus no protein will be synthesized from the mutant allele. The haploinsufficiency of *VHL* expression will
lead to the loss of function (LoF) of the pVHL, then the accumulation of HiFa and subsequent overexpression of HIF target genes, including VEGF, PDGF
β, TGF α, CyclinD1 and EPO, which play a key role in the process of tumorigenesis, and consequently, results in VHL-associated tumors [17].

Two different nonsense mutations of residue 117 have previously been reported and enrolled in Human Gene Mutation Database (HGMD) (Table 3): a
somatic c.350G>A (p.Trp117Ter) mutation was detected in a 64 years female sporadic RCC patient [18], while a somatic c.351G>A (p.Trp117Ter)
mutation was found in cell lines UOK163 derived from tumor tissue from patients with renal cell carcinomas [19], and a germline c.351G>A
(p.Trp117Ter) mutation was discovered in a kindred with VHL disease without phaeochromocytoma phenotype [20].

Taken together, this variant is classified as pathogenic and the supporting evidence for the pathogenicity of *VHL* c.351G>A according to the American
College of Medical Genetics and Genomics (ACMG)(2015) was as follows: (1) PVS1 (very strong pathogenicity 1): variation may lead to loss of gene
function; (2) PS1 (strong pathogenicity 1): known disease mutation at this position enrolled in HGMD [18-20]; (3) PM2 (moderate pathogenicity 2):
absent from public databases; (4) PP1 (supporting pathogenicity 1): cosegregation with disease in all affected family members in the *VHL* gene that
was definitely known to cause the VHL disease; (5) PP3 (supporting pathogenicity 3): PROVEAN, Gerp++, PhyloP, PhastCons, Polyphen-2, MutationTaster,
CADD and ClinGen Haploinsufficiency Score predicted that the variant affects the gene products and is harmful in the conservativeness and structure of
the protein. (6) PP4 (supporting pathogenicity 4): patient's phenotype and the family history is highly specific for the VHL disease with a single genetic
etiology.
All of the diagnosed patients examined in this study were classified as type 1 VHL, in accordance with the fact that missense mutations are associated with the development of type 2 VHL disease, whereas deletions or mutations that lead to truncation of the VHL protein (pVHL) are primarily associated with the development of type 1 VHL disease [21]. In addition, previous studies have indicated that VHL deletions and protein truncating mutations appear to confer a higher risk of CNS HGBs than missense mutations [20, 22, 23]. In line with this observation, individuals in the family examined in our study all presented with CNS HGBs, but no manifestation of phaeochromocytoma. At least 4 out of 8 (50%) of our patients developed retinal angiomas (RA) diagnosed at an early age (2:3:9, 4:4:14, 8:8:16, 4:4:23), which is younger than the mean age (25 years old) of RA diagnosis compared to VHL patients in general [21]. This frequency is much higher than those of retinal lesions in VHL patients with intragenic mutations and partial deletions, suggesting that nonsense c.351G->A mutation may confer to a high risk of early onset of RA, which is in contrast to Maher's observation that the risk of RA is slightly higher (45% vs 37%) in the missense mutation group than in the deletion/protein truncation group [20]. With regard to the other manifestations, a single pancreatic cyst was detected in the proband, while RC or RCC was diagnosed in the proband’s father, aunt and grandfather, suggesting a relatively lower incidence of visceral organs lesion.

This is the first elaborately studied VHL family caused by p.Trp117Ter mutation. Further functional evidence research remains to be conducted to reveal the pathogenesis of p.Trp117Ter.

**Conclusions**

We conclude that the p.Trp117Ter nonsense mutation is the causal mutation of this kindred that may lead to clear familial aggregation of VHL disease because of the dysfunction of truncated pVHL via NMD mechanism, and this nonsense mutation is likely to be associated with a higher risk of CNS and retinal HGBs, but a lower risk of visceral organs lesion. Every members of a VHL family with the p.Trp117Ter nonsense mutation should be systematically and comprehensively examined considering the high penetrance (90%) among mutation carriers, especially in the CNS and retina, and regular follow-up should be strictly conducted to ensure that VHL complications are recognized at a curable stage.

**Abbreviations**

VHL, Von Hippel-Lindau; CNS, central nervous system; AD, autosomal dominant; HGB, hemangioblastoma; RA, retinal angiomas; ccRCC, clear-cell renal cell carcinoma; RC, renal cyst; PCC, phaeochromocytoma; PCT, pancreatic cyst and tumor; HIFα, hypoxia-inducible factor-α; PCR, polymerase chain reaction; NMD, Nonsense-Mediated mRNA Decay; HGMD, Human Gene Mutation Database; ACMG, American College of Medical Genetics and Genomics; LoF, loss of function.

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Institutional Review Board of Beijing Ditan Hospital.

**Consent for publication**

All consent for publication from persons in this study is available on request.

**Availability of data and materials**

All sequencing data analysed during the current study is available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

DX, LT, FE and WT conceived and designed the research and DX was a major contributor in writing the manuscript. LT and FE handled funding and supervision.

LB, WF, WS, CY, SJ, ZX and WX collected individuals’ clinical and imaging data.

LB, LP, CS and LJ analyzed and interpreted the patient clinical and imaging data. All authors read and approved the final manuscript.

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Tables
Table 1. Primers used for sequencing the VHL gene

| Gene name | Sequence of primers | Product Size (bp) |
|-----------|---------------------|------------------|
| VHL exon 1 | 5'- GCGTTCCATCCTCTACCGA -3' 5'- TTCAAGCGTGCTATCGTCC -3' | 523 |
| VHL exon 2 | 5'- GTGGCTCTTTAACAACCTTTG -3' 5'- CCTGTACTTACCACAAACCTTAC -3' | 208 |
| VHL exon 3 | 5'- GCAAAACCTCTTGGTTCCTTCC -3' 5'- CAAAATGCCACCACCTTCT -3' | 504 |

Table 2. Clinical characteristics of family members with VHL c.351G>A mutation.

| Age at diagnosis or last evaluation | Sex | Hypertension | VHL gene mutation status | Clinical type | Phenotype |
|-------------------------------------|-----|--------------|--------------------------|--------------|----------|
| I-1 b                              | M   | Y            | NA                       | Type 5       | CNS, HGB, RA, RCC/RC, PCT, PCC |
| II-1                               | M   | Y            | NA                       | Type 5       | +, -, +, -, - |
| II-5                               | F   | Y            | NA                       | Type 5       | +, -, -, -, - |
| III-1                              | F   | Y            | Y                        | Type 5       | +, +, +, -, - |
| III-3                              | M   | N            | Y                        | Type 5       | +, +, -, -, - |
| III-8                              | F   | N            | Y                        | ND           | - - - - - |
| II-2                               | M   | N            | Y                        | Type 5       | +, -, -, -, - |
| II-3                               | M   | N            | Y                        | Type 5       | +, +, -, -, - |
| II-4                               | F   | N            | Y                        | Type 5       | +, +, -, -, - |

Abbreviations: VHL, von Hippel-Lindau; M, man; F, female; Y, yes; N, no; NA, not available; ND, not diagnosed; CNS, central nervous system; HGB, hemangioblastoma; RA, retinal angioma; RCC, renal cell carcinoma; RC, renal cysts; PCT, pancreatic cysts or tumors; PCC, phaeochromocytoma; EC, epididymal/ovarian cystadenoma.

a Death before diagnosis.
b Diagnosis not confirmed.

c Disease-causing

Table 3. Prevalence and Pathogenicity prediction of VHL c.351G>A (p.Trp117Ter)

| Public database | 1000G | ESP6500 | ExAC | gnomAD | dbSNP |
|-----------------|-------|---------|------|--------|-------|
| Allele frequency| 0     | 0       | 0    | 0      | 0     |

| Bioinformatics tool | PROVEAN | Gerp++ | PhyloP | PhastCons | Polyphen-2 | MutationTaster | CADD | Haploinsufficiency | pLI |
|---------------------|---------|--------|--------|-----------|-------------|----------------|------|-------------------|-----|
| Prediction          | -16.57; D | 5.07   | 4.071  | 1         | 1; D       | 1; D          | 6.051 | 3                | 0.034 |

a Deleterious; b Probably damaging (>=0.909); c Disease-causing
Table 4. Published reports of VHL patients with nonsense mutation \textit{VHL} p.Trp117Ter described in the Human Gene Mutation Database (HGMD)

| Reference  | Nucleotide change (c.DNA) | Mutation type | Phenotype | Gender/Age |
|------------|---------------------------|---------------|-----------|------------|
| Bailly M [12] | c.350G>A            | Somatic       | RCC       | F/64       |
| Gnarra JR [13] | c.351G>A            | Somatic       | RCC       | Cell lines UOK163 |
| Maher ER [14] | c.351G>A            | Germline      | VHL       | NA         |

Figures

Figure 1

Pedigree and genotype segregation in the family with VHL disease. Squares indicate males; circles represent females; arrow indicates the proband. WT/WT represents both wild type alleles, i.e. bi-allelic, while M/WT designates VHL c.351G>A pathogenic variant as heterozygous allele.
Figure 2

Imaging manifestations of VHL patients in this family. (A-C) Axial, sagittal and coronal T1-weighted contrast-enhanced MRI reveals an enhancing mural nodule (red arrowhead) with a large peritumoral cyst (red arrow) in the left cerebellar hemisphere exerting a mass effect upon the midline and fourth ventricle. (D) Abdominal computed tomography scan reveals a cyst (red arrow) in the pancreatic head. (E) Fundoscopic view of an atypical angiomomas (white arrowhead) on the left eye upper retina and the optic disc presents edematous (black arrow). (F-J) Axial, coronal and sagittal T1-weighted contrast-enhanced MRI reveals an enhancing mural nodule (big red arrowhead) with two large accompanying cysts (red arrow) in the right cerebellar hemisphere and several isolated enhancing hemangioblastomas (little red arrowhead). (G) Axial T1-weighted contrast-enhanced MRI detects the left eye retinal hemangioblastoma (white arrowhead). (K-M) Axial and sagittal T1-weighted contrast-enhanced MRI shows multiple enhancing lesions (red arrowhead) widely distributed in the cerebellum with the biggest one (big red arrowhead) near the brainstem with an accompanying cyst (red arrow). (N) Intraoperative image of the brainstem-nearby hemangioblastoma nidus (black arrowhead) and its feeding artery (black arrows), posterior inferior cerebellar artery (PICA). (O-Q) Fundoscopic view of a angiomomas (white arrowhead) on the right eye upper retina, and of retinal hemangioblastomas on both eyes at the upper and lower poles of retina respectively, which have easily recognizable globular reddish appearance (white arrowhead) with dilated feeding arteries (white arrows). (R-T) Axial, sagittal and coronal T1-weighted contrast-enhanced MRI reveal a large enhancing hemangioblastoma (red arrowhead) located deep in the cerebellum near the brainstem.
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Sequencing detection, evolutionary conservation and location of the VHL c.351G>A variant. (A) Sanger sequencing results of the mutated fragments in normal members (upper panel) and heterozygous mutant patients (lower panel) in the family. (B) Multiple species sequence alignment of pVHL amino acids flanking the nonsense mutated residue highlighted by a red box revealed that the tryptophane at the 117th amino acid site and the downstream residues is evolutionarily conserved. (C) VHL c.351G>A (p.Trp117Ter) variant mapping, located in exon 2 of VHL gene, within the β-domain of pVHL.
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Figure 4

Schematic representation of HIF-1α bound to the β domain of pVHL in the pVHL-ElonginB-ElonginC complex in ribbon diagram. pVHL (yellow) contains two domains, the α-domain and the β-domain. The α-domain consists of three α-helices (H1, H2 and H3), whereas the β-domain contains seven-stranded β sandwich and an α-helix (H4). pVHL together with elongin C (cyan), elongin B (green), cullin 2 (not shown) and the RING finger protein RBX1 (not shown), forms the VCB–CR complex. HIF-1α (pink) binds directly to β-domain, made by the N segment, in particular by Hyp564 (blue) of the HIF-1α. The mutated amino acid point of W117 (red) is spatially close to Hyp564 (at least 3.5Å).
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