Original Article

Clinical and Genetic Investigation of a Multi-generational Chinese Family Afflicted with Von Hippel-Lindau Disease

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Abstract

Background: Von Hippel-Lindau (*VHL*) disease is a hereditary tumor disorder caused by mutations or deletions of the *VHL* gene. Few studies have documented the clinical phenotype and genetic basis of the occurrence of *VHL* disease in China. This study armed to present clinical and genetic analyses of *VHL* within a five-generation *VHL* family from Northwestern China, and summarize the *VHL* mutations and clinical characteristics of Chinese families with *VHL* according to previous studies.

Methods: An epidemiological investigation of family members was done to collect the general information. A retrospective study of clinical *VHL* cases was launched to collect the relative clinical data. Genetic linkage and haplotype analysis were used to make sure the linkage of *VHL* to disease in this family. The *VHL* gene screening was performed by directly analyzing DNA sequence output. At last, we summarized the *VHL* gene mutation in China by the literature review.

Results: A five-generation North-western Chinese family afflicted with *VHL* disease was traced in this research. The family consisted of 38 living family members, of whom nine were affected. The individuals afflicted with *VHL* exhibited multi-organ tumors that included pheochromocytomas (8), central nervous system hemangioblastomas (3), pancreatic endocrine tumors (2), pancreatic cysts (3), renal cysts (4), and paragangliomas (2). A linkage analysis resulted in a high maximal LOD score of 8.26 (theta = 0.0) for the marker D3S1263, which is in the same chromosome region as *VHL*. Sequence analysis resulted in the identification of a functional C>T transition mutation (c. 499 C>T, p.R167W) located in exon 3 of the 167th codon of *VHL*. All affected individuals shared this mutation, whereas the unaffected family members and an additional 100 unrelated healthy individuals did not. To date, 49 mutations have been associated with this disease in Chinese populations. The most frequent *VHL* mutations in China are p.S65 W, p.N78 S, p.R161Q and p.R167 W.

Conclusions: The results supported the notion that the genomic sequence that corresponds to the 167th residue of *VHL* is a mutational hotspot. Further research is needed to clarify the molecular role of *VHL* in the development of organ-specific tumors.

Key words: Cancer; Linkage Analysis; Mutation; Von Hippel-Lindau Disease

Introduction

Von Hippel-Lindau (*VHL*) syndrome is a rare, autosomal inherited disorder with an estimated incidence of 1/52,000–1/36,000 and a penetrance of more than 97%.[1] The syndrome is caused by mutations or deletions of the *VHL* gene located on human chromosome 3p25,[2] and is characterized by multi-organ tumors involvement, which include hemangioblastomas (HBs) in the central nervous system, retinal angiomas, renal clear cell carcinomas (RCCs) and cysts, pheochromocytomas, pancreatic cysts, and pancreatic endocrine tumors (PETs).[3-5] *VHL* is an important tumor suppressor gene that encodes the *VHL* protein (p.*VHL*) that consists of 232 amino acids. The mutated p.*VHL* is thought to disrupt tumor-suppression through hypoxia-inducible factor (HIF)-1α-mediated effects that result in the degradation of HIF. Through transcriptional regulation, HIF can increase the glucose uptake and the expression of genes (i.e. vascular endothelial growth factor, platelet-derived growth factor, erythropoietin and transforming growth factor-β) that have been associated with the regulation of pathogenic angiogenesis.[6-9] Recently, Neal *et al.*[10] focused on roles of *VHL*-regulated microRNAs in tumorigenesis, and found that the expression of miR-210 is increased in renal cancers and might contribute to the *VHL*-associated RCC.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.4103/0366-6999.147802

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VHL is involved in a complex functional network that is important for tumor suppression.[11] Faulty genes within the network can act synergistically to induce tumors and the network can result in a cascade effect that affects tumor suppression by activating and inhibiting downstream genes. Burnichon *et al*[12] found that the succinate dehydrogenase x family and *VHL* act synergistically in the development of the pheochromocytoma. Similarly, Gimelli *et al*[13] found that haploinsufficiency of ring finger protein 139 (RNF139) might facilitate the development of clear-cell RCC in association with *VHL* mutations.

Clinically, *VHL* disease can be classified into four subtypes based on the presence of pheochromocytoma and/or RCC. Type I *VHL* is not associated with pheochromocytoma, and type II is associated with both hemangioblastoma and pheochromocytoma, with either a relatively low incidence (Type IIA) or a high incidence (Type IIB) of RCC and pancreatic tumors. In contrast, *VHL* IIC is characterized by a pheochromocytoma-only phenotype.[14,15]

Currently, based on the analysis of more than 300 families afflicted with *VHL* disease, more than 823 distinct mutations have been detected and registered in the Universal *VHL*-Mutation Database.[16] To date, a limited number of studies of *VHL* disease have been conducted in China. In the present study, we describe a five-generation *VHL* family from Northwestern China and present clinical and genetic analyses of *VHL* within this family. Moreover, we summarize the *VHL* mutations and clinical characteristics of Chinese families with *VHL* according to previous studies.

Methods

Patients

The Chinese family study included five-generations comprising of 38 living family members, of whom nine were affected [Figure 1]. All living members were examined clinically. The clinical and radiographic images were published with the consent of the patient. The study was approved by the research Ethics Committees of Xi'an Jiaotong University School of Medicine. Written informed consents were obtained from all participants.

Epidemiological investigation of the family afflicted with Von Hippel-Lindau

Family members were interviewed to collect socio-demographic information that included age, gender, locality, education, income, and occupation. Blood pressure and ultrasound data were collected during the initial screening, and additional examinations were performed on the affected subjects at Shaanxi Sengong Hospital. The medical charts of the *VHL*-patients were reviewed, and relevant social and clinical data (e.g. biochemical tests, images, pathologic reports, etc.) were collected.

Genetic linkage and haplotype analyses

Peripheral blood leukocytes were collected from 33 of the 38 living family members, and human genomic DNA was extracted using the DNA Isolation Kit for Mammalian Blood (Tiangen Biotech Co., Ltd., Beijing, China). We genotyped three polymorphic microsatellite markers that flanked *VHL* on chromosome 3p25 (D3S3691, D3S1597 and D3S1263). The microsatellite markers were amplified by PCR using fluorescently labeled primers. The products were analyzed with an Applied Biosystems 3730 Genetic

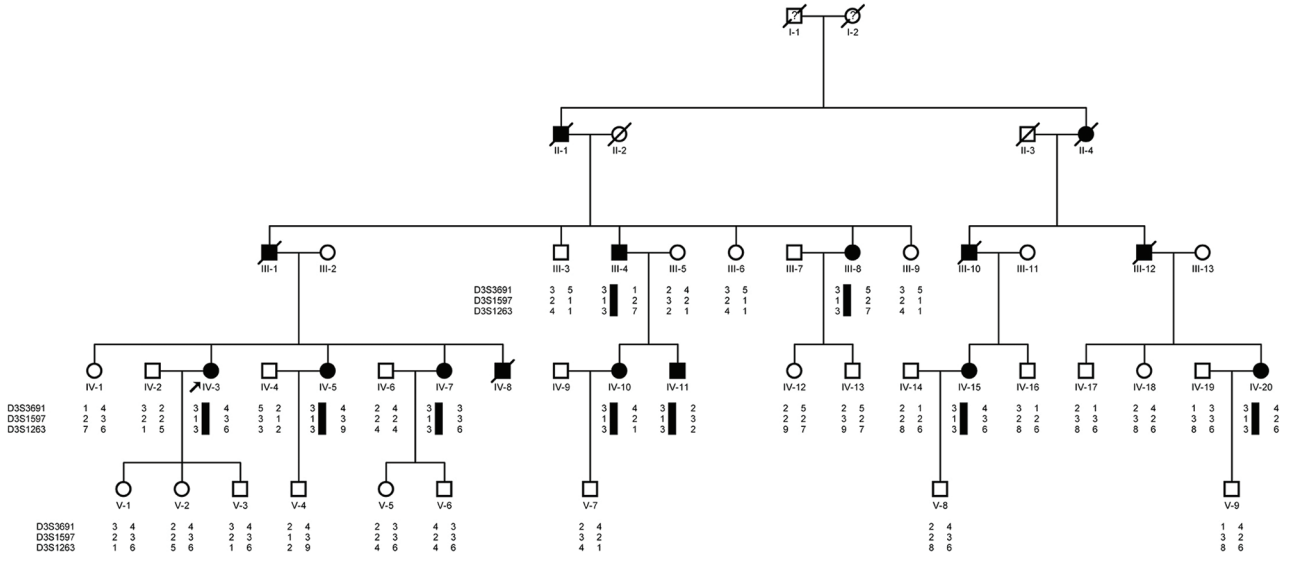

Figure 1: Haplotypes of the Chinese family affected by Von Hippel-Lindau (*VHL*). The open and filled blackened symbols indicated unaffected and affected individuals, respectively. The squares and circles symbolized the males and females, respectively. The proband, IV-3, was indicated by an arrow. The haplotyping was conducted using three polymorphic microsatellite markers. The marker order was determined from the Marshfield map and the UCSC Human Genome database (February 2009). The dark boxes symbolized haplotypes that cosegregated with the affected individuals and suggested linkage of *VHL* to disease in this family. The boxes with slash symbolized the dead family members.

Analyzer (Applied Biosystem, Foster City, CA, USA). LOD scores were calculated using the MLINK program of the LINKAGE package 5.1 (Perkin Elmer, Waltham, USA). The parameters used for linkage analysis were autosomal dominant inheritance, complete penetrance, a mutation rate of 0, equal male-female recombination rates, equal allele frequency, and the disease allele frequency of 1 in 10,000.[17]

Von Hippel-Lindau sequence analysis

Mutation screening was performed by directly analyzing the DNA sequence output. The exons of *VHL* were amplified with primers flanking the exon-intron boundaries [Table 1]. The PCR thermal cycling program was as follows: One cycle of 2 minutes for denaturation at 95°C, 35 cycles for 30 seconds at 95°C, 35 seconds at 57°C, 45 seconds at 72°C, and one 7 minutes extension step at 72°C. The PCR products were sequenced on an Applied Biosystems 3730 Genetic Analyzer.[17,18]

Summary of the mutations in the Chinese families with Von Hippel-Lindau disease

We searched for studies in PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed) using the following terms: "*VHL*," "Mutation" and "Chinese." All of the analyzed data have previously been published.[3]

Results

Clinical analysis

The proband in the family was a 42-year-old female [Figure 1: IV-3] who was first admitted to The First Affiliated Hospital of Xi'an Jiaotong University with a 2-week history of right upper abdomen pain in August 2011. An abdominal radiograph revealed neoplasms in the pancreas, bilateral adrenal gland, and spleen, bilateral renal cysts, cholecystolithiasis and pancreas atrophy with calcification [Figure 2]. Based on the radiographic findings and the endocrinological results, a surgical treatment was performed. Ultimately, the histopathologic examination confirmed the diffuse PETs and the right pheochromocytoma.

A total of 38 living members (19 males and 19 females) of the family were identified, nine of these members (two males and seven females) were affected. The ages of the living family members ranged from 16 to 61 years (mean age: 32.20 ± 4.75 years). The onset ages of the living patients ranged from 20 to 40 years (mean age: 29.11 ± 5.74 years). In the nine affected subjects, there were three cases of HBs, two cases of PETs, three cases of pancreatic cysts, four cases of renal cysts, eight cases of pheochromocytomas (including four hemi- and four bilateral cases), and two cases of paragangliomas. Patient III-4 had a combination of HBs and pheochromocytoma, IV-7 exhibited HBs, pancreatic cysts, renal cysts and pheochromocytoma; IV-10 exhibited HBs and renal cysts; IV-3 and IV-5 exhibited combined pancreatic lesions (PETs and cysts), renal cysts, pheochromocytomas and paragangliomas, III-8, and IV-11, IV-15 and IV-20 suffered only from pheochromocytomas. Blood pressures above 140/90 were observed in 13 members, of whom six were also diagnosed with pheochromocytomas. The relevant imaging, social, and clinical data of the proband and the affected relatives were shown in Figure 2, Tables 2 and 3.

Linkage and haplotype analyses

We performed linkage analysis using three markers that spanned *VHL*. A maximal LOD score of 8.26 was obtained for marker D3S1263 at $\theta = 0.00$, which suggested linkage [Table 4]. Haplotype analysis revealed that haplotype 3-1-3 cosegregated with the disease in this family,

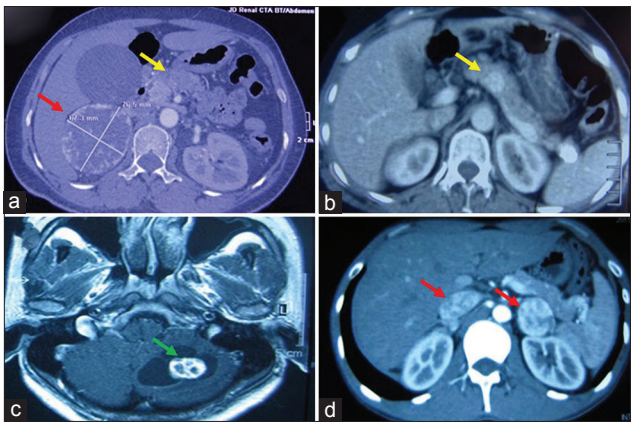

Figure 2: Representative clinical radiographic data from four patients with Von Hippel-Lindau. (a) Clinical radiograph from the proband (IV-3) who was 41-year-old woman. Contrast-enhanced computerized tomography (CT) scan showed masses in the right adrenal gland (red arrow) and pancreas (yellow arrow) that indicated a right pheochromocytoma and pancreatic endocrine tumors (PETs), respectively. (b) Clinical radiographs from a 37-year-old female patient (IV-5). Contrast-enhanced CT scan showed masses in the pancreas (yellow arrow), and post-operation pathology confirming the PETs. c Clinical radiographs from a 34-year-old female patient (IV-7). Magnetic resonance imaging (MRI) (T2W) showed lesions in the cerebellum that were indicative of hemangioblastoma (green arrow). (d) Clinical radiographs from a 24-year-old male patient (IV-11). Contrast-enhanced CT scan showed masses in the bilateral adrenal glands that were indicative of bilateral pheochromocytomas (red arrow).

Table 1: Primers used for amplification and sequencing of *VHL*

Exon	Physical Location*	Primers' sequence		Annealing Temperature (°C)
		Forward	Reverse	
1	10,183,426	5'-CGCGAAGACTACGGAGGT	5'-GGATGTGTCCTGCCTCAAG	57
2	10,188,045	5'-AGACGAGGTTTCACCACGTTAG	5'-CAAAGTGCTTTTGAGACACCAT	57
3	10,191,389	5'-GTAGTTGTTGGCAAAGCCTCTT	5'-GTTTGCCCCTAAACATCACAAT	57

*UCSC browser, February 2009; http://genome, ucsc.edu/cgi-bin/hgGateway.

which indicated that the disease locus was linked to the chromosome region harboring *VHL*, and hence, indicated *VHL* as a candidate gene [Figure 1].

Mutation analysis

A functional C>T transition mutation located within exon 3 of *VHL* (c. 499 C>T, p.R167W) was identified by mutation screening. This alteration was observed only in the affected members of the family and was not detected in any of the unaffected family members [Figure 3]. Moreover, this nucleotide substitution was not found in 100 healthy control individuals (200 alleles), which indicated that this alteration was not a polymorphic variant of *VHL*.

Summary of the studies of Von Hippel-Lindau mutations in afflicted Chinese families

Currently, nine studies including the present study have been conducted on 77 Chinese families and have described 49 mutations of *VHL*.[19-27] The mutations were summarized according to the position, nucleotide change, effect on coding sequence, and associated *VHL* phenotype (s) [Table 5]. Independent of phenotype, the most frequent mutations were p.S65W, p.N78S, p.R161Q and p.R167W. The majority of the mutations (40) were associated with phenotype I. Mutation p.R167W was associated with phenotypes IIA, IIB, and IIC. Mutation p.Y98C was associated with phenotype IIA. p.N78S, p.S80I, p.P86S, p.V130F, and intron 1 splice mutation c. 340 + 5G>C were associated with phenotype IIB, and p.H125P and p.R161Q were associated with phenotype IIC.

Table 2: Socio-demographic characteristics of the living members in the *VHL* family

Variables	Living members (n = 38), n (%)
Gender	
Male	19 (50)
Female	19 (50)
Age (years)	
16-20	3 (7.89)
21-30	4 (10.53)
31-40	7 (18.42)
41-50	18 (47.37)
≥50	6 (15.78)
VHL patients	
Patients	9 (23.68)
Healthy	29 (76.32)
Income/month (CNY)	
<3000	15 (39.47)
≥3000	23 (60.53)
Occupation	
Farmer	22 (57.89)
Worker	11 (28.95)
Student	2 (5.26)
Teacher	3 (7.90)
Education	
Illiterate	1 (2.63)
Primary	7 (18.42)
Secondary	17 (44.74)
High school certificate	9 (23.68)
Bachelor	4 (10.53)
Marital status	
Married	25 (65.79)
Unmarried	13 (34.21)
Blood pressure (highest)	
≥140/90 mmHg	13 (34.21)
<140/90 mmHg	25 (65.79)

VHL: Von Hippel-Lindau, CNY:ChinaYuan.

Discussion

VHL disease is an autosomal dominant familial neoplasm syndrome caused by mutations in *VHL* that can lead to the development of tumors and cysts in multiple organs. In our study, we identified a functional C>T transition mutation (c. 499 C>T, p.R167 W) in the *VHL* gene in a North-western Chinese family suffering from *VHL* disease by linkage and sequencing analyses. A high maximal-LOD-score of 8.26 (θ =0.0) for the marker D3S1263 was obtained through the linkage analysis. The p.R167W mutation resulted in the substitution of arginine at position 167 with a tryptophan residue that co-segregated only in affected individuals. This mutation was only present in the affected *VHL* patients and was completely absent from the other healthy family members and an additional 100 healthy controls. The results of this research firmly indicated that the p.R167W mutation of *VHL* caused the disease in this family. According to the diagnostic and classification criterion, one patient were classified as Type I, four as Type IIA, and four as Type IIC. To our knowledge, there are very few studies of PETs in the world, and this is the first such report in China.

Mutations in *VHL* that result in amino acid changes between codons 157–189 tend to be associated with increases in the

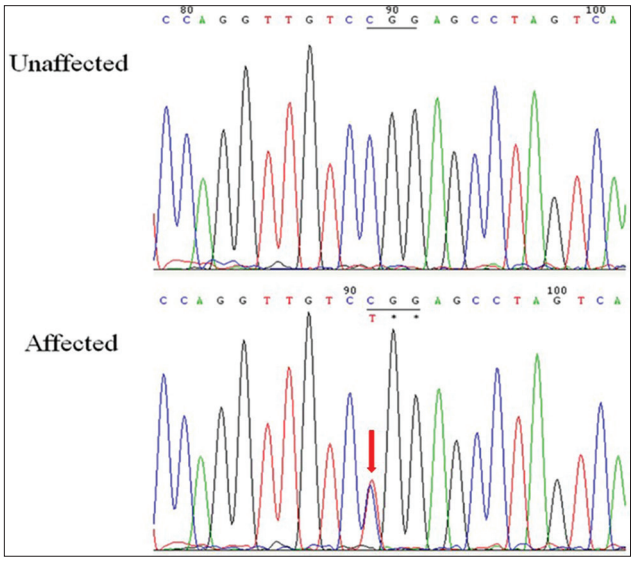

Figure 3: Identification of the c. 499C > T (p.R167W) mutation in Von Hippel-Lindau (*VHL*) in a Chinese family with *VHL* disease. The DNA sequences of a normal family member (above) and the proband IV-3 (below) were shown. The sequence of codon 167, in which the mutation occurred, was marked with red arrow. The C to T change in the proband resulted in the substitution of an arginine residue (CGG) with a tryptophan residue (TGG) in the *VHL* protein.

Table 3: The social and clinical characteristics of the nine living patients

Items	III - 4	III - 8	IV - 3	IV - 5	IV - 7	IV - 10	IV - 11	IV - 15	IV - 20
Age/OSA/OA (years)	56/38/38	45/26/30	41/31/41	37/20/24	34/34/34	28/23/28	24/24/24	42/35/35	32/29/29
Gender	Male	Female	Female	Female	Female	Female	Male	Female	Female
Education	Primary	Secondary	Secondary	Secondary	Secondary	Bachelor	Bachelor	Secondary	High school certificate
Occupation	Farmer	Worker	Farmer	Farmer	Farmer	Teacher	Worker	Worker	Worker
Marital status	Married	Married	Married	Married	Married	Married	Unmarried	Married	Married
Highest blood pressure (mmHg)	160/110	180/100	200/110	170/100	110/80	120/80	110/80	130/90	140/80
HBs									
Cerebellar	+	–	–	–	+	+	–	–	–
Pancreatic lesions									
PETs	–	–	+	+	–	–	–	–	–
Cysts	–	–	+	+	+	–	–	–	–
Renal lesions									
Cysts	–	–	+	+	+	+	–	–	–
Pheochromocytoma									
Hemi	–	–	+	–	+	–	–	+	+
Bilateral	+	+	–	+	–	–	+	–	–
Paraganglioma	–	–	+	+	–	–	–	–	–
VHL phenotype	IIA	IIC	IIA	IIA	IIA	I	IIC	IIC	IIC

OSA: Onset age; OA: Operation age; +: Affected; –: Not affected; VHL: Von Hippel-Lindau; PETs: Pancreatic endocrine tumors; HBs: Hemangioblastomas.

Table 4: Two-point LOD scores obtained from linkage analysis between *VHL* and chromosome 3p25.3 in the pedigree

Markers	Physical location*	LOD score at θ values							Z_{max}	θ_{max}
		0.0	0.01	0.05	0.1	0.2	0.3	0.4		
D3S3691	8,840,396	4.45	4.38	4.10	3.71	2.84	1.86	0.80	4.45	0.0
D3S1597	9,365,414	5.09	4.99	4.58	4.05	2.93	1.74	0.57	5.09	0.0
D3S1263	11,517,247	8.26	8.12	7.58	6.86	5.32	3.60	1.71	8.26	0.0

*UCSC browser, February 2009; http://genome.ucsc.edu/cgi-bin/hgGateway.

severity of tumors. The mutation presented in this study occurred at codon position 167, which is critical to the normal functioning of *VHL*.[28,29] Notably, the p.R167W mutation was found in four *VHL* phenotypes. Therefore, further examination of this codon might provide key insight into the function of *VHL*. Previous research conducted by Neumann *et al*.[30] reported that germline mutations of codon 167 convey a high risk for the development of pheochromocytoma. Moreover, the codon 167 mutation is correlated with the pheochromocytoma coalition with PETs. Zbar *et al*.[16] found the mutant *VHL* alleles that are associated with the subtypes that are characterized by pheochromocytoma with (p.R167Q and p.Rl67W) or without renal carcinoma (p.Y98H and p.Y112H). However, in the family we studied, the p.Rl67W mutation was connected to the pheochromocytoma without renal carcinoma phenotype. This discrepancy might be due to genetic heterogeneity between the two different ethnic populations. In China, there were reports of associations of p.Rl67W with each subtype of *VHL*.[23,24] To the best of our knowledge, the first reported the association of p.R167W with Type II *VHL* in a family of Chinese origin.

The p.Rl67W mutation resulted in a change from arginine to tryptophan at position 167. The isoelectric point of tryptophan is 10.76, while that of arginine is 5.89 isoelectric. The isoelectric point is an important factor in the formation of the three-dimensional structures of proteins. Moreover, the benzene ring structure of tryptophan can affect the secondary and three-dimensional structure of proteins, and indeed, replacement of residues by tryptophan in other polypeptides has been associated with various diseases.[31-33] Indeed the p.*VHL* is the component of the *VHL*-Elongin BC-CUL2 (VCB) complex that acts as an ubiquitin-ligase E3 and directs proteasome-dependent degradation of HIF-1α. Interesting, the regional residues (p. 157–166) are involved in the interaction with the Elongin BC complex. Thus, the p.Rl67W mutation might affect the VCB complex by impeding binding to the Elongin BC complex.[28,34]

Our summary of the numbers of *VHL* mutations that have been reported in various Chinese families revealed that these numbers are substantially lower than the numbers of mutations that have been reported in populations of European origin. The most frequent mutations in China are p.S65W, p.N78S, p.R161Q and p.R167W, and the most frequent mutations in Western countries are p.F76del, p.N78S, p.Rl61Stop, p.R167Q, p.R167W and p.L178P. The most common *VHL* phenotype was Type I (40 mutations), and 11 mutations were associated with Type II (40:11). Zbar *et al*[16] also showed that 208 mutations were associated with Type I, and 66 mutations were associated with Type II (208:66). The reason for these differences might be related to genetic

Table 5: Summary of the *VHL* mutations in Chinese families

Position	Nucleotide change	Effect on coding sequence	*VHL* phenotype	Reference
Exon 1	Exon 1 deletion		I	Huang *et al.* 2012[19]
				Zhang *et al.* 2008[20]
Exon 1	c. 194C > G	p.S65W	I	Zhang *et al.* 2008[20]
				Zhou *et al.* 2010[21]
				Wu *et al.* 2012[22]
Exon 1	c. 204G deletion	Frameshift	I	Huang *et al.* 2012[19]
Exon 1	c. 208G > T	p.E70X	I	Zhang *et al.* 2008[20]
Exon 1	c. 217C > T	p.Q73X	I	Zhang *et al.* 2008[20]
Exon 1	c. 221T > G	p.V74G	I	Zhang *et al.* 2008[20]
Exon 1	c. 226T deletion	p.F76del	I	Zhang *et al.* 2008[20]
Exon 1	c. 233A > G	p.N78S	IIB	Zhang *et al.* 2008[20]
				Siu *et al.* 2011[23]
				Huang *et al.* 2004[24]
Exon 1	c. 233A > C	p.N78T	I	Zhang *et al.* 2008[20]
Exon 1	c. 239G > T	p.S80I	IIB	Zhou *et al.* 2010[21]
				Mao *et al.* 2009[25]
Exon 1	c. 257C > T	p.P86S	IIB	Wu *et al.* 2012[22]
Exon 1	c. 257C > T	p.P86L	I	Zhang *et al.* 2008[20]
Exon 1	c. 263G > A	p.W88X	I	Wu *et al.* 2012[22]
Exon 1	c. 265C > T	p.L89S	I	Zhang *et al.* 2008[20]
Exon 1	c. 269A > T	p.N90I	I	Wu *et al.* 2012[22]
Exon 1	c. 280G > T	p.E94X	I	Wu *et al.* 2012[22]
Exon 1	c. 286C > T	p.Q96X	I	Zhang *et al.* 2008[20]
Exon 1	c. 293A > G	p.Y98C	IIA	Zhang *et al.* 2008[20]
Exon 1	c. 319C > G	p.R107G	I	Siu *et al.* 2011[23]
Exon 1	c. 332G > A	p.S111N	I	Zhang *et al.* 2008[20]
Exon 2	Exon 2 deletion		I	Siu *et al.* 2011[23]
Exon 2	c. 344G > C	p.H115R	I	Zhou *et al.* 2007[26]
Exon 2	c. 346G > C	p.V116L	I	Mao *et al.* 2009[25]
Exon 2	c. 351G > A	p.W117X	I	Zhang *et al.* 2008[20]
Exon 2	c. 374A > C	p.H125P	IIC	Wu *et al.* 2012[22]
Exon 2	c. 388G > T	p.V130F	IIB	Zhou *et al.* 2010[23]
Exon 2	c. 432G insertion	Frameshift	I	Huang *et al.* 2012[19]
Exon 2	c. 433C > T	p.E145X	I	Huang *et al.* 2012[19]
Exon 2	c. 451A > T	p.I151F	I	Wu *et al.* 2012[22]
Exon 3	c. 481C > T	p.R161X	I	Zhang *et al.* 2008[20]
				Siu *et al.* 2011[23]
Exon 3	c. 481C > A	p.R161R	I	Wu *et al.* 2012[22]
Exon 3	c. 482G > A	p.R161Q	IIC	Siu *et al.* 2011[23]
				Tong *et al.* 2009[27]
				Wu *et al.* 2012[22]
Exon 3	c. 484T > C	p.Cl62R	I	Mao *et al.* 2009[25]
Exon 3	c. 485G > A	p.C162Y	I	Zhang *et al.* 2008[20]
Exon 3	c. 486C > G	p.C162W	I	Zhang *et al.* 2008[20]
Exon 3	c. 487C > T	p.L163F	IIC	Zhang *et al.* 2008[20]
Exon 2	c. 488del A	Frameshift	I	Zhang *et al.* 2008[20]
Exon 3	c. 499C > T	p.R167W	I, IIA, IIB, IIC	Siu *et al.* 2011[23]
				Wu *et al.* 2012[22]
Exon 3	c. 500G > A	p.R167Q	I	Zhou *et al.* 2010[21]
Exon 3	c. 503G > C	p.S168T	I	Mao *et al.* 2009[25]
Exon 3	c. 529A > T	p.R177X	I	Zhou *et al.* 2010[21]
Exon 3	c. 533A > T	p.L178R	I	Zhou *et al.* 2010[21]
Exon 3	Exon 3 deletion		I	Zhang *et al.* 2008[20]
Exon 3	c. 642 + 70C > A (in 3-untranslated region causing nontypical disease)		I	Wu *et al.* 2012[22]
Intron 1	c. 340 + 1G > A	Splice mutation	I	Zhou *et al.* 2010[21]
Intron 1	c. 340 + 5G > C	Splice mutation	IIB	Huang *et al.* 2012[19]
				Zhou *et al.* 2010[21]
Intron 1	IVS1 - 38C > T	Unknown	I	Zhang *et al.* 2008[20]
Intron 2	c. 463 + 1G > T	splice mutation	I	Siu *et al.* 2011[23]
	Complete deletion		I	Zhang *et al.* 2008[20]

A: Alanine; R: Arginine; N: Asparagine; C: Cysteine; Q: Glutamine; E: Glutamic acid; G: Glycine; H: Histidine; I: Isoleucine; L: Leucine; F: Phenylalanine; P: Proline; S: Serine; T: Threonine; W: Tryptophan; Y: Tyrosine; V: Valine; *VHL*: Von Hippel-Lindau.

differences between the populations or might be due to differences in the efficiency of the dissemination of case reports. More precisely, the tracking and management of families with genetic diseases in China might be sub-par compare to European countries. For example, there were many case reports written in Chinese, but thorough studies of the entire family have not been subsequently performed. An effective registration and follow-up system are urgently needed to allow for the description and proactively treatment of Chinese families with *VHL*.

In summary, the present study identified a functional C>T transition mutation (c. 499 C>T, p.R167W) located within exon 3 of *VHL* that is likely the cause of *VHL* disease in this family. *VHL* mutations suggest that the function of *VHL* might be similar to other check-point proteins in that when mutations are present, uncontrolled cell proliferation (or more precisely, neoplasias) arise in various tissues. Further research is needed to clarify the molecular mechanisms of *VHL*'s role in organ-specific tumors.

Acknowledgments

We are indebted to all individuals who participated in or helped with this research project.

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Received: 20-04-2014 **Edited by:** Xin Chen
How to cite this article: Zhang J, Ma J, Du X, Wu D, Ai H, Bai J, *et al*. Clinical and Genetic Investigation of a Multi-generational Chinese Family Afflicted with Von Hippel-Lindau Disease. Chin Med J 2015;128:32-8.
Source of Support: This study was supported by funding from the National Science Foundation of China (No. 81072051 and No.81272644).
Conflict of Interest: None declared.