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Clinical characteristics and risk factors for survival in affected offspring of von Hippel-Lindau disease patients

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ABSTRACT

Background Von Hippel-Lindau (VHL) disease is an autosomal dominant genetic tumour syndrome with poor prognosis. The clinical manifestation was found to be more serious in affected offspring of patients with VHL disease, but the risk factors and survival for them have never been reported before. We aimed to explore how these patients were influenced by genetic and clinical factors.

Methods In this retrospective study, we collected 372 affected offspring of VHL patients from 118 unrelated VHL families. Patients were stratified into different groups based on sets of variables. The age-related risk, overall survival and central nervous system haemangioblastoma (CHB)-specific survival were analysed between different groups using Kaplan-Meier survival analysis and Cox regression analysis.

Results The estimated median life expectancy and median age of onset for affected offspring of VHL patients were 66 years and 28 years, respectively. The later generation and patients with mutations in exon 3 had an earlier onset age. The first presenting symptom was the only independent risk factor influencing overall survival and CHB-specific survival. Patients that the first presenting symptom is central nervous system (CNS) significantly had a lower life expectancy both in overall survival and CHB-specific survival analysis than abdominal lesions group.

Conclusion This study indicated that affected offspring of VHL patients with CNS as the first presenting symptom was an independent risk factor for overall survival and CHB-specific survival. Generation and mutation region only had an effect on the onset age, which is helpful to clinical decision-making and generate a more precise surveillance protocol.

BACKGROUND

Von Hippel-Lindau (VHL) disease is an autosomal dominant disease caused by germline mutation of tumour suppressor gene *VHL*.^{1,2} Patients with VHL disease often develop multiple organ diseases, such as central nervous system (CNS) haemangioblastoma (CHB), retinal haemangioblastoma (RA), renal cell carcinoma (RCC), pheochromocytoma (PHEO), pancreatic neuroendocrine tumour (PNET), endolymphatic sac tumours (ELSTs) and genital system disorder (GS) that included

epididymal cystadenomas or broad ligament cystadenomas. In recent years, clinical manifestation in other rare parts such as liver adenomas or lung haemangioblastomas have also been reported.^{3–5} The VHL patients had an earlier onset age than other sporadic tumours. In addition, patients with VHL disease would develop multiple and recurrent tumors across the life course and this would improve the risk of death. Thus, the prognosis of VHL patients remains poor despite the continuous development of medical technology, which brings a substantial psychological and economic burden to the patients.^{2,6}

The survival of patients with VHL disease is significantly worse than that of the general population. In a retrospective study in Danish population, the hazard of death in VHL patients was four times than their non-VHL sibling.⁷ VHL patients with a positive family history (PFH) had a lower life expectancy when compared with other VHL patients. In our previous study, among the VHL patient population, 80% of them had a PFH and others carried *de novo* mutation.⁸ Patients with PFH should have longer life expectancies because of more active surveillance plan for the affected offspring, but the study showed that patients with PFH had a mortality hazard that were twice as patients with a negative family history.⁸ Genetic anticipation, which means affected offspring are affected at an earlier age or manifest more serious presentations than their parents, may partially account for this phenomenon.⁹ In addition to this, some Chinese parents' attention to the clinical outcomes of their affected offspring even outweighed themselves because of limited economic resources of the entire family. Unfortunately, though risk factors for patients with VHL disease had been reported before, the survival and risk factors for their affected offspring have never been studied all over the world.

The loss of the VHL protein function plays a crucial role in tumour development. VHL protein is an important E3 ligase, among which the most important substrate is HIF protein. The mutation or deletion of VHL gene will increase the accumulation of HIF and then activate the downstream hypoxia-induced related genes, leading to the occurrence and development of tumours.¹⁰ Also, VHL protein could regulate other non-HIF- α independent proteins to promote the development of



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tumours such as TBK1, SFMBT1 and ZHX2.^{11–13} Genotype of VHL disease was related to patients' phenotype and survival in some studies. The different mutation types or mutation sites could affect the interaction between the VHL and other molecules to cause disease progression.^{14,15}

At present, though there were a few cancer surveillance protocols for VHL patients and their affected offspring have been developed, most of which are based on expert experiences.¹⁶ In the present study, we screened clinical data and summarised clinical features of affected offspring in VHL diseases. Furthermore, age-related risk, overall survival and CHB-specific survival analysis were conducted to find independent influence factors of these patients. This study will improve clinical counselling, management and follow-up for affected offspring of patients with VHL disease, which will provide support for elaborating more precise surveillance.

METHODS

Patients and clinical data

A retrospective study was performed with all VHL patients enrolled in Peking University First Hospital during September 2010 to September 2020 (the only international VHLA Clinical Care Center in China). All VHL patients met one of the two criteria for diagnosis: (1) VHL germline mutation was identified by gene analysis. (2) The patients' symptoms met the clinical diagnosis of VHL disease and at least one person in this family met the genetic diagnosis.¹⁷ There were 605 patients with VHL disease enrolled in this study initially, 9 patients were excluded because of incomplete clinical data. Then, 224 first generation VHL patients in families were excluded. Finally, the 372 affected offspring of patients with VHL disease from 118 unrelated families were included in this study (online supplemental figure 1).

Among the 372 affected offspring of patients with VHL disease, 318 (85.5%) patients had VHL-related clinical symptoms and the remaining patients were asymptomatic during follow-up. According to the clinical criteria, the VHL patients were divided into two different cohorts: patients with VHL type 1 predominantly without PHEO and VHL type 2 predominantly with PHEO.¹⁸ Among the 372 patients, 322 patients were divided into VHL type 1 and the others 50 patients were divided into VHL type 2.

Genetic testing

The genomic DNA was extracted from peripheral blood leucocytes of all suspicious VHL patients using DNA extraction kit (Tiangen, China). VHL germline mutational status was determined by PCR amplification using previously described VHL primers and the PCR production was analysed by Sanger sequencing.¹⁹ The VHL mutational status mainly included missense mutation, small insert or deletion, frame shift and splice site mutation. For large fragment deletion, multiplex ligation-dependent probe amplification (MLPA) analysis was performed to identify mutational status using MRC Holland (Amsterdam, The Netherlands) SALSA MLPA probe according to the instruction. All results were reviewed by two different people to avoid wrong judgements. Mutation spectrum of the 372 affected offspring of patients with VHL disease is shown in online supplemental table 1.

All affected offspring of VHL patients were divided into two groups: missense mutation (all point mutation except stop mutation) and truncating mutation (stop mutation, nonsense mutation, small deletion and insert, splice site mutation, large fragment deletion). Afterwards, according to the location of

Table 1 Clinical characteristics of the affected offspring

	Patients (n)		
Overall	372	Onset	
Unrelated families	118	Affected	317 (85.5%)
Sex		Unaffected	55 (14.5%)
Male	214 (57.5%)	Onset age	
Female	158 (42.5%)	≤28 years	172 (54.4%)
Mutation type		>28 years	145 (45.6%)
Missense	187 (50.3%)	First lesion	
Truncating	185 (49.7%)	CHB	129 (40.7%)
VHL classification type		RA	40 (12.7%)
Type 1	322 (86.6%)	RCC	28 (8.8%)
Type 2	50 (13.4%)	PHEO	16 (5.0%)
Mutation region		PCL	26 (8.2%)
Exon 1	162 (43.6%)	GS	14 (4.4%)
Exon 2	48 (12.9%)	SO	64 (20.2%)
Exon 3	86 (23.1%)	CNS or abdomen	29
Other	76 (20.4%)	CNS and abdomen	35
Generation		Death and cause	
2	249 (66.9%)	CHB	47 (75.8%)
3	109 (29.3%)	RCC	11 (17.7%)
4	14 (3.8%)	Others	4 (6.5%)

CHB, central nervous system haemangioblastoma; CNS, central nervous system; GS, genital system (epididymis or broad ligament); PCL, pancreatic cystic lesion; PHEO, pheochromocytoma; RA, retinal haemangioblastoma; RCC, renal cell carcinoma; SO, simultaneous onset; VHL, von Hippel-Lindau.

mutation, patients were further categorised into exon 1 mutation, exon 2 mutation, exon 3 mutation and the other group (large fragment deletion or splice site mutation).

Statistical analysis

We included a total of 372 VHL patients in overall survival analysis and CHB-specific survival analysis. Kaplan-Meier (KM) curve, univariate and multivariate Cox regression model analysis were used in different groups of sex, mutation type, the location of mutation, VHL classification, the number of generation, onset age. According to the median age of onset of patients with symptoms, we divided the patients into two different cohorts: early-onset cohort and late-onset cohort. In the first presenting symptom analysis, because RA and CHB were of the same origin in the embryonic stage and retina is an important section of CNS, CHB and RA were combined as CNS group. Equally, RCC, PHEO, PCL and PNET were combined as abdominal group because most of them were noted on imaging examinations at the same time. All of the statistical results were conducted using the statistical software programmes (SPSS and GraphPad Prism 8) and p values <0.05 were considered statistically significant.

RESULTS

Clinical characteristics of affected offspring

One hundred twenty-nine (40.9%) patients developed CHBs as the first of manifestation followed by RAs, RCC, pancreatic cystic lesion, PHEO, genital system. Of the 64 patients who had manifestations in different systems when they were first diagnosed, 29 patients were observed only in CNS or abdomen, 35 patients were detected simultaneously in both the CNS and the abdomen (table 1). The distribution of the age frequency at last follow-up for patients and the distribution of the onset age in the two different first presenting symptom cohorts are shown in online supplemental figure 2. The median years of age at the

Table 2 Age at the onset of VHL-related manifestations

Manifestations	Mean±SD	Median (range)
CHB (59.1%)	29.5±11.0	28.0 (10–66)
RA (19.9%)	24.8±11.0	23.0 (2–55)
RCC (43.3%)	36.4±10.9	34.0 (14–74)
PCL (40.6%)	33.4±11.0	32.0 (10–66)
PHEO (13.4%)	31.7±11.9	31.5 (8–66)
GS (6.5%)	21.4±11.2	19.0 (4–56)

CHB, central nervous system haemangioblastoma; GS, genital system (epididymis or broad ligament); PCL, pancreatic cystic lesion; PHEO, pheochromocytoma; RA, retinal haemangioblastoma; RCC, renal cell carcinoma; VHL, von Hippel-Lindau.

onset of tumours in CNS groups and abdominal groups were 25 and 28 years. The age at the onset of VHL-related manifestations is shown in [table 2](#). The first manifestation presenting in CNS patients tend to have a much earlier disease onset age. Although the first manifestation presenting in abdominal patients had a later onset age, most of them concentrated on age 20–30 years. The distribution of each mutated residue of VHL protein is shown in [figure 1A](#), except that mutation is large fragment deletion or splice site mutation. High-frequency mutation site is amino acid 167 and followed by amino acid 65.

The mean age of onset was 28.44 ± 12.08 years and the median age of onset was 28 years in those affected offspring. The penetrance percentage of affected offspring was 98.17% before the age of 60 years. The average survival age was 36.81 ± 15.11 years and the median survival age was 66 years ([figure 1B](#)). In the present study, there were 62 deaths. Of these, 47 patients died because of CHB. There were 11 deaths because of RCC and 4 deaths from other reasons (1 death from PNETs and 3 deaths from other diseases).

Influence factors for age-related risk

For different generations, the results showed that there was earlier onset age for younger generation (online supplemental figure 3). However, there was no difference between the third generation and the fourth generation ($p=0.418$). Because the

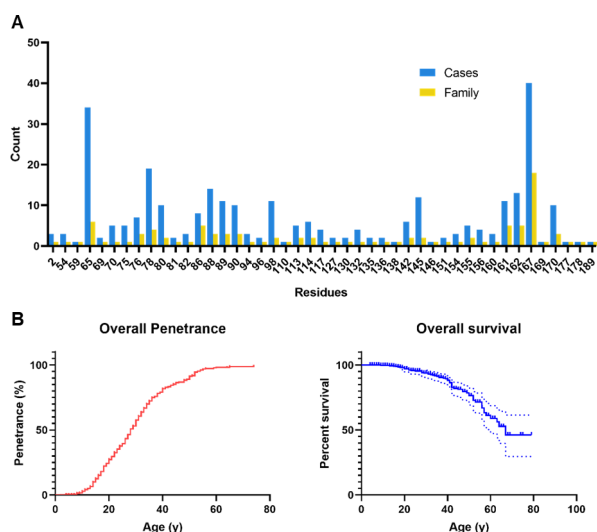


Figure 1 Genotype, age-related penetrance and overall survival of affected offspring. (A) The number of cases and families with VHL mutations on each residue of pVHL. (B) The penetrance of patients and the overall survival of patients. pVHL, von Hippel-Lindau protein; VHL, von Hippel-Lindau.

number of the fourth generation patients was too small, the third and fourth generation patients were combined as one group for further analysis. The results showed that the third and fourth generation had earlier onset age than the second generation ($p<0.001$). Furthermore, patients with truncating mutation had earlier onset age than patients with missense mutation ($p=0.022$). Meanwhile, the location of mutation site also influenced the status of onset age. The results showed that patients with exon 1 mutation had the latest onset age compared with other groups ([table 3](#)). The sex and type of VHL disease does not affect onset age in our analysis.

Multivariate Cox regression analysis suggested that the number of generation and the location of mutation site were the independent risk factors of onset age. The onset time of younger group was about three times later than that of the previous generation (HR 2.901, 95% CI 2.213 to 3.804, $p<0.001$). When compared with the different generation groups, the mean onset age was earlier in the later generation and the distribution of onset age was more concentrated (online supplemental table 2). Significantly, the mean onset age of RA was the youngest in all different generation groups. The minimum age of first discovered RA could reach 2 years. Also, the proportion of GS was increasing in next generation. For different mutation region groups, exon 1 group had the latest onset age and there were significant differences between exon 1 and exon 3 group (HR 1.555, 95% CI 1.169 to 2.069, $p<0.05$).

Risk factors for overall survival

KM curve analysis showed that different mutation types, VHL classifications, onset age groups and the sites of first presenting symptoms could influence the overall survival ($p<0.05$, online supplemental figure 4). The risk of death in truncating mutation group was almost 1.829 times when compared with missense mutation group ($p=0.021$) ([table 4](#)). Also, VHL type 2 group had better prognosis than VHL type 1 group ($p=0.011$). The analysis demonstrated that the earlier onset age group had a higher hazard of death (HR 1.821, 95% CI 1.051 to 3.154, $p=0.030$). The rate of death in CNS group was higher than that in abdominal group ($p=0.001$). Subsequently, multivariate Cox regression model was performed, only the sites of different first presenting symptom groups could independently influence overall survival. Patients in CNS group demonstrated a poorer survival than those in abdominal group. The risk of death in CNS group was about 2.2 times than that in abdominal group (HR 2.273, 95% CI 1.142 to 4.524, $p=0.019$).

Features and risk factors for CHB-specific survival

CHB is the primary cause of death in the VHL patients, so we further analysed the features and the risk factors for CHB-specific death patients. The median time for CHB-specific survival and RCC-specific survival was not reached (online supplemental figure 5A,B). However, the results showed that the median age for CHB-specific death was 36 years and for RCC-specific death was 49 years. Among 220 patients with CHB, 46 of them died of CHB and 5 patients died of RCC. Among 167 patients with RCC, 10 patients died of RCC and 11 patients died of CHB. CHB was the most common cause of death than RCC in affected offspring of VHL patients.

In the KM curve analysis, the VHL type and the site of first presenting symptom were associated with CHB-specific survival (online supplemental figure 5C–F). The type 2 VHL patients seems to have a more favourable survival than type 1 VHL patients ($p=0.029$). Meanwhile, patients in CNS group had a

Genotype-phenotype correlations

Table 3 Univariate and multivariate analysis for age-related risk

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Generation (4 and 3 vs 2)	2.590 (1.990 to 3.372)	<0.001	2.901 (2.213 to 3.804)	<0.001
Mutation (truncating mutation vs missense mutation)	1.280 (1.026 to 1.596)	0.029	1.204 (0.899 to 1.613)	0.212
Classification (VHL type 2 vs VHL type 1)	1.159 (0.855 to 1.572)	0.341		
Sex (female vs male)	0.984 (0.787 to 1.232)	0.891		
Mutation region		0.042		0.014
Exon 2 vs exon 1	1.368 (0.975 to 1.919)		1.406 (0.951 to 2.077)	
Exon 3 vs exon 1	1.318 (0.995 to 1.745)		1.555 (1.169 to 2.069)	
Other vs exon 1	1.469 (1.081 to 1.996)		1.442 (0.994 to 2.092)	

Significant p values ($p < 0.05$) are bolded.
VHL, von Hippel-Lindau.

poor survival than other groups ($p < 0.001$). In the multivariate analysis, the site of first presenting symptom was the only independent risk factor that influenced CHB-specific survival in affected offspring of VHL patients (table 5). According to our findings, the risk of CHB-specific death in CNS group would increase to 5.394 times than others affected by abdominal tumours (HR 5.394, 95% CI 1.910 to 15.232, $p = 0.001$).

DISCUSSION

The main causes of death for VHL disease were CHB (41%–60%) and RCC (27%–47%).^{6 7 20 21} In our previous study, CHB accounted for 66.2% of death patients in all patients with VHL disease.²² However, for affected offspring of VHL patients, the cause of death for CHB is 75.8% and for RCC is 15.7%. Our result further demonstrated that CHB has become the main cause of death especially for those patients diagnosed in recent decades. This may be caused by increasing awareness of clinical surveillance, evolving clinical detection technique and continuous improvement management strategy for these patients.²³ According to the results, there were significant differences in survival between VHL parents and affected offspring. We further explored the clinical features and risk factors for survival in affected offspring of VHL patients.

We found that VHL penetrance was 98.17% at the age of 60 for the affected offspring. This is much similar with previous study and penetrance has been reported to be 87%–100%.^{20 24} In our study, 14.5% (55 of 372) of the patients were asymptomatic mutation carriers, while other studies were 0.5%–14%.⁷ This may be because there was a higher proportion of young people in our cohort. The onset age of CHB was also earlier than

RCC, which is consistent with recent research.²⁵ In the previous study, the mean onset age was 31.2 ± 12.8 years. We could conclude that the mean onset age was slightly earlier in affected offspring when compared with the whole VHL patients.⁹ With the progress of techniques, the imaging examination and genetic testing have become the main diagnostic approach for VHL disease, which may explain the result. Furthermore, in multivariate analysis of onset age, later generation and exon 3 group showed an earlier onset age. Genetic anticipation is one of the most common phenotypes for hereditary disease, which means the next generation having an earlier onset age and more serious symptoms than their parents.^{26 27} The mechanism for genetic anticipation in VHL disease may be related to the telomere length. VHL children showed significantly shorter telomere length than their parents,^{9 28} though this conclusion needed further verification. Interestingly, we first described that exon 3 group showed an earlier onset age than other affected offspring. The region of exon 1 mainly corresponds to the binding site of HIF- α and the region of exon 3 mainly corresponds to the binding site of Elongin C which could make up VCB complexes. Previous study showed that the binding site of VCB complex could influence more molecules about tumour formation, which may relate to earlier onset age.¹⁴

Patients with CNS symptoms as the first manifestation had a lower life expectancy both in overall survival and in CHB-specific survival analysis. This result can be explained by development of advanced diagnosis technologies and treatment strategies such as targeted therapy, immune therapy and some novel drugs for RCC.²⁹ Also, affected offspring of VHL patients performed more active clinical surveillance for their symptoms

Table 4 Univariate and multivariate analysis for risk factors of overall survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Generation (4 and 3 vs 2)	1.377 (0.668 to 2.836)	0.386		
Mutation (truncating mutation vs missense mutation)	1.829 (1.096 to 3.052)	0.021	1.435 (0.815 to 2.526)	0.211
Classification (VHL type 2 vs VHL type 1)	0.253 (0.079 to 0.807)	0.020	0.420 (0.126 to 1.402)	0.159
Sex (female vs male)	0.881 (0.526 to 1.477)	0.632		
Mutation region		0.550		
Exon 2 vs exon 1	1.052 (0.499 to 2.217)			
Exon 3 vs exon 1	0.654 (0.319 to 1.341)			
Other vs exon 1	1.185 (0.603 to 2.327)			
Onset age (≤ 28 vs > 28)	1.821 (1.051 to 3.154)	0.033	1.490 (0.831 to 2.671)	0.181
First symptom (haemangioblastoma vs abdomen)	2.908 (1.495 to 5.653)	0.002	2.273 (1.142 to 4.524)	0.019

Significant p values ($p < 0.05$) are bolded.
VHL, von Hippel-Lindau.

Table 5 Univariate and multivariate analysis for risk factors of CHB-specific survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Generation (4 and 3 vs 2)	1.413 (0.655 to 3.047)	0.378		
Mutation (truncating mutation vs missense mutation)	1.760 (0.979 to 3.165)	0.056		
Classification (VHL type 2 vs VHL type 1)	0.110 (0.015 to 0.800)	0.029	0.116 (0.027 to 1.485)	0.116
Sex (female vs male)	0.920 (0.511 to 1.657)	0.780		
Mutation region		0.339		
Exon 2 vs exon 1	1.030 (0.443 to 2.397)			
Exon 3 vs exon 1	0.480 (0.196 to 1.175)			
Other vs exon 1	1.176 (0.559 to 2.475)			
Onset age (≤ 28 vs >28)	1.672 (0.904 to 3.095)	0.104		
First symptom (haemangioblastoma vs abdomen)	6.742 (2.408 to 18.877)	<0.001	5.394 (1.910 to 15.232)	0.001

Significant p values ($p < 0.05$) are bolded.

CHB, central nervous system haemangioblastoma; VHL, von Hippel-Lindau.

and selected surgical intervention when the RCC diameter is >3 cm or before metastases. However, although CHB is a benign tumour, its cystic characteristics and associated peritumoural oedema often leads to high mortality.^{30 31} For overall survival and CHB-specific survival, neither generation nor onset age was an independent factor in analysis. Meanwhile, we analysed the influence of generation and onset age for RCC-specific survival, which is another significant cause of death. The results showed that there were no risk factors for RCC-specific survival (online supplemental table 3). The later generation was associated with the earlier onset age in this study. According to the 'second-hit' model of VHL disease, both generation and onset age should have an effect on overall survival and CHB-specific survival because of longer pathogenesis process and higher risk for loss of function of the *VHL* allele. Indeed, our previous study revealed that onset age was an independent risk factor in two different survival analysis. However, our results did not show statistical significance between different onset age groups in affected offspring of VHL patients. We could attribute this discrepancy to that the VHL patients paid more attention to their children because of their own experiences and earlier onset symptom so that influence was reduced for both factors.

Type 2 patients only showed difference in univariate CHB-specific survival analysis. It seems that type 2 patients had a more favourable CHB-specific survival, which may attribute to low risk of developing CHB in type 2 patients than in type 1 patients (46.0% vs 61.2%) in this study. The previous study also revealed that type 1 VHL patients were associated with a higher risk of retinal and CNS.³² In multivariate Cox analysis, there was no difference between two groups. Patients with VHL disease could move from type 1 to type 2 during the follow-up period, which may relate to outcomes of patients. When compared with different mutation types, there seems to be a significantly higher death rate for truncating mutation carriers in univariate analysis. However, there was no difference in multivariate Cox analysis. Although this can be partly explained by heavier tumour burden and higher risks of CHB in truncating mutation carriers, the influence of first symptoms may play a decisive role especially in those affected offspring of VHL patients. Some study reported that the sex of patients may relate to survival, but we did not draw this result in our study.⁷

In summary, we could conclude that the site of the first presenting symptom was the biggest risk factor in affected offspring of VHL disease. Our previous study suggested that haemangioblastoma instead of RCC plays a major role in the overall survival of patients with VHL disease. The similar results

was found in their affected offspring. When we performed active surveillance for affected offspring, maybe we should put more effort on haemangioblastoma. Pharmacological and surgical improvement are more necessary for VHL-related haemangioblastoma when compared with RCC. There were some limitations that may cause unintentional biases in our study such as the small number of patients and the nature of retrospective study.

To our knowledge, this is the first survival analysis for affected offspring of VHL disease patients in the world. We found that the median life expectancy of these patients is 66 years, which is longer than total VHL disease cohorts. Generation and mutation site are independent risk factors for onset age. Patients with CNS as the first presenting symptom not only had effect on overall survival but also on CHB-specific survival. For VHL patients, affected offspring of VHL patients account for a large proportion in Chinese and there was no refined management strategy for them. Our findings may help guide a more elaborate clinical counselling and decision-making for different VHL patients.

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Contributors KG and LC were responsible for the study design, data acquisition and quality control. KZ and JQ deal with the clinical data. WY, KM, LL and YX collected the detailed information of VHL patients. KZ and JQ drafted the manuscript. YG, JZ and HX dealt with the final typesetting. KG is responsible for the overall content of the manuscript acting as guarantor. All authors have contributed significantly to the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

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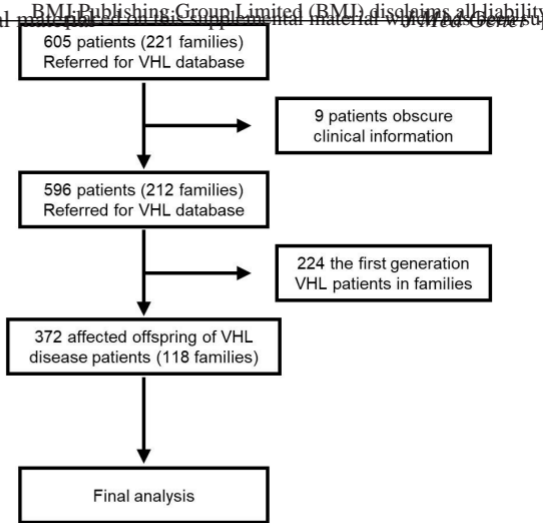
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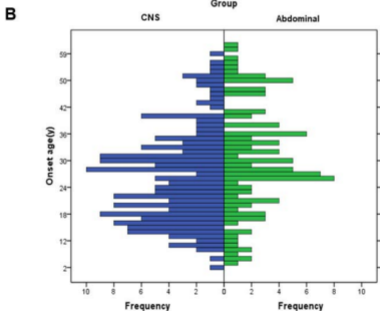
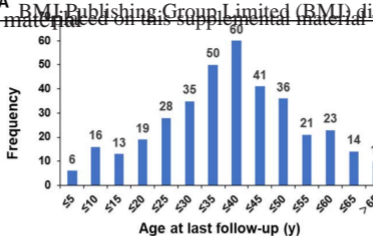
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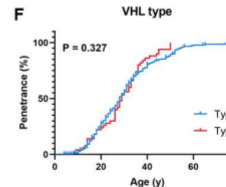
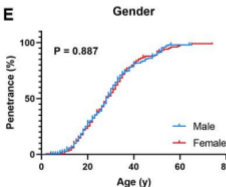
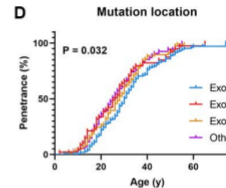
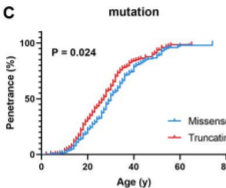
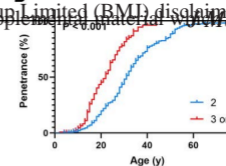
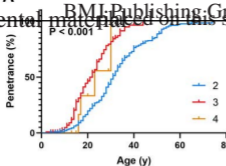
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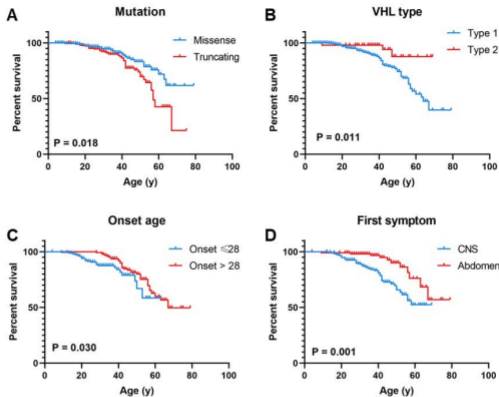
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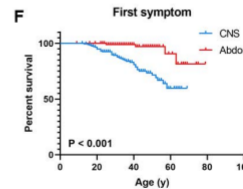
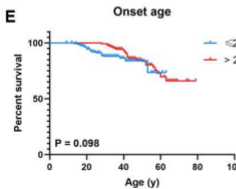
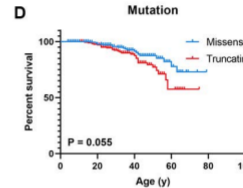
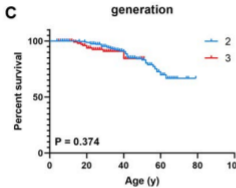
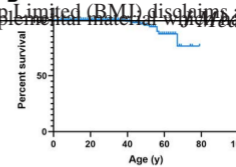
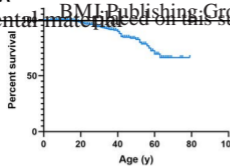
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Supplementary Table

Supplementary Table 1			
Mutation spectrum of the 372 affected offspring of von Hippel-Lindau disease patients			
Mutation site	Alteration of Protein	Family NO.	Patient NO.
c.4C>G	p.Pro2Ala	1	3
c.160delA		1	3
c.176insAGCA delTCCTGCGCT		1	1
c.193T>C	p.Ser65Pro	1	8
c.194C>T	p.Ser65Leu	4	23
c.194C>G	p.Ser65Trp	1	3
c.205insG		1	2
c.208G>T	p.Glu70stop	1	5
c.224delTCT		1	5
c.226delTTC		3	7
c.233A>G	p.Asn78Ser	4	19
c.239delGTCCGCG		1	7
c.240T>G	p.Ser80Arg	1	3
c.241delC		1	2
c.245G>C	p.Arg82Leu	1	3
c.256C>T	p.Pro86Ser	4	7
c.257C>T	p.Pro86Leu	1	1
c.262T>C	p.Ser88Arg	1	3
c.263G>A	p.Trp88stop	1	2
c.263G>C	p.Trp88Ser	1	9
c.266T>C	p.Leu89Pro	2	10
c.265delC		1	1
c.269A>T	p.Asn90Ile	3	10
c.280G>T	p.Glu94stop	1	3
c.288insA		1	2
c.292T>A	p.Tyr98Asn	1	4
c.293A>C	p.Tyr98Ser	1	7
c.329delA		1	1
c.337C>T		2	5
c.340delG		1	3
c.341ins CCCGATAGGTCACCTTTGGCTCTTCAGAGATGC		1	3
c.349T>G	p.Trp117Gly	1	4
c.381delGCTTinsC		1	2
c.388G>C	p.Val130Leu	1	2
c.395delA		1	4
c.404T>A	p.Leu135stop	1	2

c.406delT		1	2
c.414A > G	p.Pro138Pro	1	1
c.425delT		1	3
c.426insG		1	3
c.433delCAGCC		1	3
c.433C > T	p.Gln145stop	1	9
c.436insGG		1	1
c.452T>G	p.Ile151Ser	1	2
c.462delA		1	3
c.464delTGTAT		1	1
c.464delT		1	4
c.464-1G>C		1	8
c.464-1G>A		1	1
c.468insACTC		1	4
c.480delG		1	3
c.481C>T	p.Arg161stop	3	6
c.482G>A	p.Arg161Gln	2	5
c.485G>A	p.Cys162Tyr	1	2
c.486C>G	p.Cys162Trp	3	6
c.486C>A	p.Cys162stop	1	5
c.499C>T	p.Arg167Trp	10	20
c.499C > G	p.Arg167Gly	1	1
c.500G>A	p.Arg167Gln	7	19
c.506T > C	p.Leu169Pro	1	1
c.509T>A	p.Val170Asp	3	10
c.530_536delGACTGGA		1	1
c.533T>G	p.Leu178Arg	1	1
c.565-570delGAAGAC		1	1
Deletion		17	67

Supplementary Table 2 Age at the onset of VHL-related lesions in different generation

Lesion	Mean \pm SD	Median (range)
Generation 2	249	
CHB (66.7%)	32.0 \pm 10.7	31.0 (11-66)
RA (19.7%)	28.3 \pm 11.3	27.0 (8-55)
RCC (49.0%)	38.9 \pm 10.9	37.5 (18-74)
PCL (42.2%)	36.2 \pm 11.5	35.0 (10-66)
PHEO (15.7%)	33.1 \pm 12.3	34.0 (8-66)
GS (4.4%)	24.1 \pm 15.5	20.0 (4-56)
Generation 3	109	
CHB (49.5%)	21.8 \pm 8.2	21.5 (10-50)
RA (21.1%)	18.0 \pm 6.0	17.0 (2-27)
RCC (35.8%)	28.5 \pm 6.6	27.0 (14-44)

PCL (39.4%)	27.4 ± 6.73	28.0 (14-44)
PHEO (10.1%)	27.0 ± 9.5	26.0 (9-44)
GS (11.9%)	19.15 ± 5.2	19.0 (8-26)
Generation 4	14	
RA (14.3%)	16.5 ± 0.7	16.5 (16-17)
PCL (21.4%)	23.3 ± 6.5	23.0 (17-30)

Supplementary Table 3	
Univariate and multivariate analysis for risk factors of RCC-specific survival	
Variables	univariate analysis
	P value
Generation (3 and 4 vs 2)	0.321
Mutation (truncating mutation vs missense mutation)	0.054
Classification (VHL type 2 vs VHL type 1)	0.449
Gender (female vs male)	0.842
Mutation region	0.684
Onset age (≤ 28 vs >28)	0.351
First symptom (haemangioblastoma vs abdomen)	0.581

Supplementary table legends

Supplementary Table 1 Mutation spectrum of the 372 affected offspring of von Hippel-Lindau disease patients.

Supplementary Table 2 Age at the onset of VHL-related lesions in different generation.

Supplementary Table 3 Univariate and multivariate analysis for risk factors of RCC-specific survival.