GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 20, Number 12, 2016

Pp. 771–776
DOI: 10.1089/gtmb.2016.0204

VHL Germline Mutations in Argentinian Patients with Clinical Diagnoses or Single Typical Manifestations of Type 1 von Hippel–Lindau Disease

Cecilia Mathó,[1] Gabriela Sansó,[1] Blanca Diez,[2] Marta Barontini,[1] and Patricia A. Pennisi[1]

Aims: von Hippel–Lindau (VHL) disease is caused by mutations in the *VHL* tumor suppressor gene. As tumors that develop in the context of VHL also occur in a sporadic context, the frequency of this syndrome may be underestimated. Our aim was to identify *VHL* gene mutations in Argentinian patients who fulfilled the clinical criteria for type 1 VHL disease and in patients with VHL-associated manifestations that did not meet these criteria. *Methods:* We performed a retrospective cohort study, including patients who met current diagnostic criteria for type 1 VHL (Group 1, $n = 19$) and patients with VHL-associated manifestations that did not meet these criteria (Group 2, $n = 21$). Genomic DNA was extracted from peripheral blood leukocytes. Mutation analysis involved DNA sequencing, while large deletions were determined by universal primer quantitative fluorescent multiplex polymerase chain reaction (UPQFM-PCR) and multiplex ligation-dependent probe amplification (MLPA) analysis. *Results: VHL* mutations were detected in 16/19 (84.2%) patients in Group 1 and included: gross deletions (4/16); nonsense mutations (6/16); frameshift mutations (4/16); missense mutations (1/16); and splicing mutations (1/16). Three of these mutations were novel. No alterations were found in 3 of 19 VHL patients. In Group 2, one nonsense *VHL* mutation was detected in a young patient with a solitary central nervous system hemangioblastoma without familial history. A study of 30 first-degree relatives revealed four carriers with *VHL* mutations. *Conclusions:* We found three novel mutations in the VHL gene in our population. Our results emphasize the importance of a complete genetic study of *VHL* to confirm type 1 VHL disease, not only in patients with clinical diagnostic criteria but also in those presenting a single typical manifestation.

Keywords: von Hippel-Lindau disease, VHL gene, hemangioblastoma, retinal angioma, ccRCC

Introduction

Von Hippel–Lindau (VHL) disease (OMIM No. 193300) is an autosomal dominant syndrome (Friedrich, 1999) characterized by the development of central nervous system (CNS) hemangioblastomas and retinal angiomas, clear cell renal carcinomas (ccRCC) and cysts, pheochromocytoma, pancreatic cysts and tumors, endolymphatic sac tumors, and cystadenomas of the epididymis and broad ligament (Latif *et al.*, 1993). VHL is caused by mutations in the *VHL* tumor suppressor gene (Latif *et al.*, 1993); its incidence ranges from 1/36000 to 1/45000 live births (Latif *et al.*, 1993; Cho *et al.*, 2009) and achieves over 90% of penetrance by the age of 65 years (Chou *et al.*, 2013).

Identified in 1993, *VHL* was found to map to the short arm of chromosome 3 (3p25-26) (Latif *et al.*, 1993). Its coding sequence spans three exons and encodes a 213-amino-acid protein (pVHL) (Kim and Kaelin, 2004). pVHL is widely expressed in human tissues, and its expression is not restricted to organs at risk for the disease (Los *et al.*, 1996). Clinical diagnosis of VHL is confirmed by molecular genetic analysis of the *VHL* gene in patients, and analysis is subsequently extended to relatives to identify carriers of the disease before the onset of clinical manifestations. Up to 23% of VHL patients carry *de novo* mutations (Sgambati *et al.*, 2000; Cybulski *et al.*, 2002; Cho *et al.*, 2009).

Clinically, VHL can be divided into two subtypes based on the absence (type 1) or presence (type 2) of pheochromocytoma (Kim and Kaelin, 2004). In an individual with a familial history of type 1 VHL disease, the finding of a single CNS hemangioblastoma, retinal angioma, or ccRCC is sufficient to make a diagnosis. In the absence of a family history of VHL, two or more CNS hemangioblastomas or retinal angiomas or one hemangioblastoma plus another typical manifestation should be present to make a clinical diagnosis (Friedrich, 2001).

[1]Centro de Investigaciones Endocrinológicas ''Dr. César Bergadá'' (CEDIE) CONICET–FEI–División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, CABA, Argentina.
[2]Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia, CABA, Argentina.

Hemangioblastomas (Hb) are highly vascular tumors of the CNS that are generally benign, and their symptomatology occurs following the local compression of neural structures. Hemangioblastomas can be either sporadic or a component of VHL disease (10–40%; Hes *et al.*, 2000; Bamps *et al.*, 2013). They are sporadic in ~75% of all cases and arise in the fourth and fifth decade of life. In general, sporadic Hb are solitary, and complete surgical resection can offer definitive therapy.

Hemangioblastomas associated with VHL disease appear earlier (second to third decade) and are usually present in multiples. They are the most frequent lesions associated with this syndrome, affecting from 60% to 85% of VHL patients.

ccRCC are the most common sporadic renal cell carcinoma histologic type (75%) and are associated with *VHL* loss of function. It is the major cause of death in patients with VHL disease. Patients with ccRCC who are 46 years old or younger should consider genetic counseling and germline mutation testing, even in the absence of secondary clinical manifestations (Ho and Jonasch, 2014).

Previous studies have shown that 60–80% of VHL families carry point mutations, which are detected by DNA sequencing, while 20–40% of VHL patients have large deletions that cannot be detected by this methodology. Type 1 VHL patients typically have large *VHL* deletions or protein-truncating mutations, while type 2 patients primarily carry missense mutations (Friedrich, 2001; Chou *et al.*, 2013). In recent years, universal primer quantitative fluorescent multiplex polymerase chain reaction (UPQFM-PCR) and multiplex ligation-dependent probe amplification (MLPA) have been used to detect large deletions (Hes *et al.*, 2007; Cho *et al.*, 2009). We aimed to identify *VHL* mutations in patients with clinical diagnosis of type 1 VHL disease and patients with type 1 typical tumors who do not fulfill the diagnostic criteria.

Materials and Methods

Subjects

We performed a retrospective cohort study at a public children's hospital (Buenos Aires, Argentina). Eligible patients were all index cases with clinical diagnosis or clinical suspicion of type 1 VHL referred to the Centro de Investigaciones Endocrinológicas ''Dr. César Bergadá,'' Hospital de Niños ''Dr. Ricardo Gutiérrez,'' for genetic testing from 2003 to 2015. Follow-up was considered to begin starting from the first clinical manifestation of the disease.

Probands were categorized into two groups: Group 1 included patients who met the current clinical criteria for the diagnosis of type 1 VHL disease according to Melmon and Rosen (1964) and Group 2 included patients with VHL-associated manifestations that did not fulfill the clinical criteria, but presented with a single CNS Hb, retinal angioma, or ccRCC.

Patients with type 2 VHL were excluded from this study. The presence of pheochromocytoma was ruled out in all patients with 24-h urinary epinephrine, norepinephrine, and VMA measurements.

When a mutation was confirmed, we extended the genetic testing to all at-risk individuals who agreed to participate.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or National Research Committee and with the 1964 Declaration of Helsinki and its latter amendments or comparable ethical standards. An informed consent was obtained from all individual participants included in this study.

Mutation analysis

DNA sequencing. Genomic DNA was extracted from peripheral blood leukocytes using the cetyltrimethyl ammonium bromide (CTAB) method as previously described by Del Sal *et al.* (1989). The three exons and flanking regions of the *VHL* gene were amplified by PCR. Primer sequences were previously described by Sansó *et al.* (2004).

Amplification was performed in 50-μL reaction mixtures containing 1.5 μL of template DNA, 20 pmol of each primer, 0.2 mM of each deoxyribonucleotide triphosphate (dATP, dGTP, dCTP, and dTTP), 1.5 mM $MgCl_2$, 1.25 U of Taq DNA polymerase, and 10 μL of 5 × buffer, with annealing temperatures of 64°C, 60°C, and 57°C for exons 1, 2, and 3, respectively. PCR products were purified using an AccuPrep® PCR Purification Kit (Bioneer Corporation) and sequenced in an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA).

Universal primer quantitative fluorescent multiplex PCR. Two-step PCR was used to amplify the three *VHL* exons and a fragment of the β-globin gene (internal control) as reported previously by Cybulski *et al.* (2002). Briefly, DNA content was quantified, and 100 ng of genomic DNA was used as a template for the first round of PCR. The second round of PCR was performed in a final volume of 50 μL, using 4 μL of P1 (first PCR) product as the template. PCR primers and cycling conditions for both PCR steps were previously described (Cybulski *et al.*, 2002). PCR products were separated according to their length by capillary electrophoresis using an ABI 3730xl DNA Analyzer (Applied Biosystems), and GeneScan® Analysis Software (Applied Biosystems) was used to analyze the results. The detection of large deletions was based on comparisons of the peak heights for *VHL* exons and the β-globin gene using Peak Scanner™ Software v1.0 (Applied Biosystems). First, a ratio was calculated for the height of the peak obtained for each exon compared to the β-globin gene using 26 control individuals, and a normal interval was calculated to be the average ratio ±2 standard deviation. Ten samples with confirmed large deletions in the *VHL* gene were processed as positive controls. These were generously provided by Prof. Dr. Hartmut Neumann (University of Freiburg).

Patients who had a ratio below the normal interval (approximately half the ratio value) were considered to carry deletions. Positive and negative controls were included in every run.

Multiplex ligation-dependent probe amplification. MLPA analysis was carried out using the SALSA MLPA KIT P016-C2 VHL (MRC Holland, Amsterdam, Netherlands) following the manufacturer's instructions, starting with 1 μL of a 100 ng/μL genomic DNA template. Probes were labeled with 6-FAM, and products were separated according to their length by capillary electrophoresis using an ABI 3730xl DNA Analyzer (Applied Biosystems) using the GeneScan™-500 ROX™ STANDARD (Applied Biosystems) molecular size marker. Peak Scanner Software v1.0 (Applied Biosystems) was used to visualize and obtain peak heights, and data

analysis was performed using Coffalyser.Net Software (MRC Holland). Dose quotients in the range from 0.7 to 1.3 were considered normal, and values below 0.7 were considered deletions. In each MLPA test, three healthy control samples were included to determine the *VHL* gene dosage.

Results

Demographic data for the 40 patients studied are shown in Table 1. Group 1 included 19 patients (7 females, 12 males), while Group 2 included 21 patients (11 females, 10 males). The entire cohort of patients is still alive, with the exception of one individual. Follow-up ranged from 1 to 33 years after the first manifestation of the disease (Group 1: median age = 8, range 1–33 years; Group 2: median age = 3, range 1–15 years). Seven of the 19 patients in Group 1 had a familial history of VHL disease. We detected germline *VHL* mutations in 16 of 19 (84.2%) of these patients. DNA sequencing of the three exons of the *VHL* gene detected mutations in 12 of 16. The mutations identified were nonsense (6/12), frameshift (4/12),

Table 1. Demographic Data of Studied Patients

				Clinical manifestations				
ID	*Age at diagnosis (genetic testing)*	*Fulfills type 1 VHL clinical diagnosis*	*Family history*	*CNS Hb or RA*	*Renal cancer or cysts*	*Pancreatic cysts*	*Other*	*VHL nucleotide and protein change*
Group 1								
1	13	Yes	No	x			x	c.481C>T, p.R161*
2	19	Yes	No	x	x	x	x	del EXONS 2–3
3	20	Yes	No	x	x	x	x	c.481C>T, p.R161*
4	21	Yes	No	x		x	x	c.481C>T, p.R161*
5	21	Yes	Yes	x	x	x		c.430G>T, p.G144*
6	22	Yes	Yes	x	x	x		c.486C>G,.p.C162 W
7	22	Yes	No	x	x			c.343_395dup, p.Q132Hfs*45
8	23	Yes	No	x	x		x	Normal
9	25	Yes	No	x	x	x		c.481C>T, p.R161*
10	29	Yes	No	x	x			c.217dupC, p.Q73Pfs*59
11	30	Yes	No	x	x	x		c.263G>A, p.W88*
12	32	Yes	No	x	x		x	c.219_232delGGTCATCTTCTGCA, p.V74Sfs*53
13	34	Yes	No	x	x			c.463 + 1G>A
14	34	Yes	No	x				Normal
15	34	Yes	Yes		x	x	x	del EXON 3
16	41	Yes	Yes	x	x	x		del EXONS 2–3
17	44	Yes	Yes	x	x			Normal
18	57	Yes	Yes	x	x			c.361delG, p.D121Mfs*38
19	60	Yes	Yes	x	x			del EXONS 2–3
Group 2								
20	3	No	No	x				Normal
21	12	No	No	x				Normal
22	13	No	No	x				c.264G>A, p.W88*
23	14	No	No	x				Normal
24	14	No	No	x				Normal
25	20	No	No	x				Normal
26	23	No	No	x				Normal
27	24	No	No	x				Normal
28	26	No	No		x			Normal
29	28	No	No	x				Normal
30	33	No	No	x				Normal
31	34	No	No		x	x		Normal
32	37	No	No		x			Normal
33	40	No	No	x				Normal
34	46	No	No	x				Normal
35	48	No	No	x				Normal
36	50	No	No	x				Normal
37	54	No	No		x			Normal
38	54	No	No	x				Normal
39	55	No	No	x				Normal
40	61	No	No	x				Normal

This table shows patients sorted by increasing age of diagnosis within two groups: those who fulfill the clinical diagnosis (Group 1, patient Nos. 1–19) and those who do not fulfill the clinical diagnosis (Group 2, patient Nos. 20–40). ID refers to patient ID number. The NCBI accession number is NM_000551.3.

CNS, central nervous system; Hb, hemangioblastoma; RA, retinal angioma; VHL, von Hippel–Lindau.

missense (1/12), and splicing (1/12) mutations. Among these, three novel mutations were detected: one was a single base pair duplication (c.217dupC, p.Q73Pfs*59, patient No. 10), another was a 53-bp duplication (c.343_395dup, p.Q132Hfs*45, patient No. 7), and the final was a small deletion of 14 bp (c.219_232delGGTCATCTTCTGCA, p.V74Sfs*53, patient No. 12). These novel findings were confirmed by UPQFM-PCR. Patient No. 12 was confirmed to have a *de novo* mutation. Thus far, it has not been possible to study the parents or close relatives of patients Nos. 7 and 10.

In the remaining 4 of 16 patients, gross deletions were detected by both UPQFM-PCR and MLPA. We were unable to find any *VHL* alterations in 3 of the 19 patients with clinical diagnosis of VHL disease, one of which had a familial history.

In Group 2, 1 of 21 patients demonstrated a nonsense *VHL* mutation detected by DNA sequencing. This patient (No. 22) harbored a solitary CNS Hb at 13 years of age, with no familial history.

Table 2 shows the phenotypes of all patients presenting a VHL gene mutation ($n = 17$). Sixteen (94%) presented CNS Hb and/or retinal angioma. In terms of the first manifestation of the disease (highlighted cells), CNS Hb were present in 12 patients (70%, median age = 23). In one of these patients, CNS Hb were associated with retinal angioma. In the remaining four, the first manifestations were bilateral retinal angioma (one), epididymal cysts (two: at birth, and at 23), and an endolymphatic sac tumor at age 13 (one). Three patients had multiple manifestations at ages 21, 30, and 34.

Sixteen patients developed other manifestations of the disease within 1–9 years of the first (Table 2) between ages 16 and 46. Five patients had a relapse of CNS Hb, the majority after 5 years following the first event. One of these patients died after the third CNS hemangioblastoma surgery. Patient No. 22, who presented hemangioblastoma as the sole manifestation of the disease at age 13, is the only patient who did not develop other manifestations of the disease during a 5-year follow-up.

The index cases enabled the genetic study of 30 first-degree relatives. Four individuals carried *VHL* mutations and were asymptomatic. In three of these individuals, CNS Hb were found in one subject and pancreatic cysts in the other two subjects during screening after obtaining mutation-positive results. There were also 10 relatives with clinical history of VHL manifestations who died as a result of the disease (ages from 28 to 66) before a genetic study could be performed.

Discussion

The present study allowed us to detect germline *VHL* mutations in 84.2% of patients who fulfilled the clinical diagnostic criteria for type 1 VHL (Group 1) and in one young patient with a solitary Hb without a familial history of the disease, representing 7.7% of the patients in Group 2 bearing only CNS Hb (1/13).

More than 500 *VHL* mutations have been reported according to the Human Gene Mutation Database (HGMD® Professional 2015.2). Interestingly, in our group of patients, three novel germline mutations were detected in patients with no familial history of VHL disease. These patients harbored CNS Hb and/or retinal angioma or renal cysts; one patient had epididymal cysts and another had ccRCC. The mutations they carried produced frameshifts in the open reading frame, resulting in premature stop codons. Patient No. 12 had a *de novo* mutation and no descendants. Thus far, we have not been able to study the parents or close relatives of patient No. 7 or patient No. 10. Several mutations have previously been described in proximity to these three regions (Latif *et al.*, 1993; Cybulski *et al.*, 2002; Dollfus *et al.*, 2002; Hes *et al.*, 2007; Zhang *et al.*, 2008; Cho *et al.*, 2009; Gomy *et al.*, 2010; Limaverde-Sousa *et al.*, 2013), suggesting they may be unstable regions in the gene and prone to mutations. According to the ACMG Standards and Guidelines (Richards *et al.*, 2015), these three novel frameshift mutations are considered very strong evidence of pathogenicity (PVS1). This category

Table 2. Phenotype of Patients Presenting a VHL Gene Mutation

ID	Fulfills type 1 VHL clinical diagnosis	Family history	Clinical manifestations							Status
			CNS Hb	RA	ccRCC	pCy	rCy	eCy	ELST	
1	Yes	No		16					13	A
2	Yes	No	19, 25, 26		29	25	25	31		A
3	Yes	No	20, 25, 26		27	25	25	25		A
4	Yes	No	22	21		23		Birth		A
5	Yes	Yes	21, 26, 31	21		21	21			D
6	Yes	Yes	22	22		29	29			A
7	Yes	No		22 (2)			23			A
9	Yes	No	22		22	22	22			A
10	Yes	No	29		30		30			A
11	Yes	No	30	30		30	30			A
12	Yes	No	32	32			32	23		A
13	Yes	No	34				34			A
15	Yes	Yes			34	34	34		34	A
16	Yes	Yes	41	46	46	46				A
18	Yes	Yes	24, 34, 35, 43, 50, 56	35	41, 52					A
19	Yes	Yes	32, 57			32				A
22	No	No	13							A

ID refers to patient ID number. Numbers indicate the age in years at which the patient presented the manifestation. Highlighted cells indicate the first manifestation of the disease. Status: A (alive), D (deceased). (2) Refers to bilateral.

ccRCC, clear cell renal cancer; Cy, cysts; eCy, epididymal; ELST, endolymphatic sac tumors; pCy, pancreatic; rCy, renal.

includes null variants (nonsense, frameshift, canonical ± one or two splice sites, initiation codon, and single- or multiexon deletions) of a gene whose loss of function is a known disease mechanism, as is seen with the *VHL* gene.

Our results are in agreement with the reported genotype–phenotype correlation described by other authors, given that most of our patients carried gross deletions, nonsense, and frameshift mutations. The only exception was patient No. 6, who presented a missense mutation (p.C162 W) that was described previously in type 2 VHL patients (Zbar *et al.*, 1996; Dollfus *et al.*, 2002; Zhang *et al.*, 2008). Although association between a missense mutation and type 1 VHL is infrequent, other authors have also found this particular mutation in patients with type 1 VHL (Zhang *et al.*, 2008). The type 1 VHL classification of patient No. 6 is based on the absence of pheochromocytoma/paraganglioma (pheo/pgl) after 14 years of follow-up. Furthermore, three first-degree relatives of this patient died between the ages of 39 and 51 as a consequence of tumors related to the disease, without a record of pheo/pgl, supporting the VHL type 1 classification in this family. However, it is also important to consider that since subtype classification of VHL disease has limited clinical utility as additional tumors are discovered, all subjects with diagnosis of VHL, regardless of the subtype, must be followed according to the same surveillance protocol.

The splicing mutation found in patient No. 13 and the frameshift mutation found in patient No. 18 have been previously described in association with type 1 VHL only (Maher *et al.*, 1996; Yoshida *et al.*, 2000). The recurrent mutation p.R161*, which was found in 4 of 17 patients with positive genetic findings, and the mutations p.G144* (1/17) and p.W88* (2/17) have been reported to be associated with type 1 VHL (Zbar *et al.*, 1996; Cybulski *et al.*, 2002; Dollfus *et al.*, 2002; Ruiz-Llorente *et al.*, 2004; Hes *et al.*, 2007; Siu *et al.*, 2011; Wu *et al.*, 2012). However, other studies have reported families with type 2 VHL carrying the p.R161* (Zbar *et al.*, 1996) or p.W88* mutation (Zhang *et al.*, 2015). The observed differences in the outcomes of patients with the same *VHL* mutations in European, Chinese, and Japanese families (Zhang *et al.*, 2008) suggest that ethnic or environmental factors may play a role in the expression of VHL phenotypes.

We were unable to detect any *VHL* alterations in 3 of the 19 patients with clinical diagnosis of VHL disease. Other authors also observed a small proportion of patients with clinical diagnosis of VHL but negative genetic tests (Hes *et al.*, 2007; Ebenazer *et al.*, 2013). Mosaicism has been described in ∼5% of VHL patients (Sgambati *et al.*, 2000; Wu *et al.*, 2013; Coppin *et al.*, 2014). Recently, Coppin *et al.* (2014) found *VHL* mutations using next-generation sequencing in patients for whom conventional techniques (PCR and DNA sequencing, UPQFM-PCR, MLPA) gave negative results. In this report, the authors presented two patients with mosaicisms, in which only 5.7% and 1.7% of peripheral blood mononuclear cells carry the mutations. Such low frequencies are undetectable by conventional methods (Altimari *et al.*, 2013). Therefore, our patients may contain mosaicisms with very low frequencies, similar to the cases described by these authors (Coppin *et al.*, 2014); alternatively, undiscovered genes may be responsible for this phenotype.

Given that tumors that develop in the context of VHL disease also occur in a sporadic context, some authors believe that the disease is underestimated (Richard *et al.*, 2013). Hb are associated with VHL disease in 10–40% of cases (Hes *et al.*, 2000; Bamps *et al.*, 2013) and are commonly seen as the first presentation of the disease. VHL disease prevalence in patients with retinal Hb is approximately 30–58% (Niemelä *et al.*, 2000), and currently it is recommended that patients with this pathology should undergo genetic testing for VHL disease. The relatively high proportion of *de novo VHL* mutations also confirms the need for mutation analysis in patients with apparently sporadic VHL-related tumors (Hes *et al.*, 2007). In our group of patients without clinical diagnosis of VHL disease, 13 had only CNS Hb. In this group, we detected one patient with a *VHL* mutation (7.7%) that developed Hb at age 13. Our results are in agreement with an international multicenter study performed by Hes *et al.* (2000), in which the authors investigated the frequency of *VHL* mutations in a large group of 84 patients with solitary Hb and found that ∼4% had *VHL* germline mutations in patients younger than 50 years. Although our cohort includes fewer patients, it illustrates the relevance of genetic testing for all patients with CNS Hb to identify mutations in the *VHL* gene.

In summary, we found three novel mutations in the *VHL* gene in our population. Our results underline the importance of a complete genetic study of the VHL gene to confirm type 1 VHL disease, not only in patients with clinical diagnostic criteria but also in patients presenting a single typical manifestation, enabling their correct diagnosis and follow-up. The early identification of asymptomatic carriers allows for the implementation of a screening protocol to decrease morbidity and mortality rates in these patients, avoiding invasive procedures for noncarriers.

Acknowledgments

P.A.P. is an associated researcher from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). C.M. is a recipient of a doctoral fellowship from CONICET. The authors thank Prof. Dr. Hartmut Neumann for generously providing them with positive controls with *VHL* gene deletions, Dr. Ericka B. Trarbach for training C.M. to perform MLPA assays, and Gabriela Gutierrez Moyano for technical assistance. This study was funded by Grant No. 0100214, 2013–2015 CONICET, awarded to P.A.P.; Grant 2013–2015 from the Instituto Nacional del Cáncer, Ministerio de Salud awarded to P.A.P.; and funds obtained for technology services under STAN Res 3546/11 CONICET(GS).

Author Disclosure Statement

No competing financial interests exist.

References

Altimari A, De Biase D, De Maglio G, *et al.* (2013) 454 Next generation-sequencing outperforms allele-specific PCR, sanger sequencing, and pyrosequencing for routine KRAS mutation analysis of formalin-fixed, paraffin-embedded samples. Onco Targets Ther 6:1057–1064.

Bamps S, Calenbergh FV, Vleeschouwer SD, *et al.* (2013) What the neurosurgeon should know about hemangioblastoma, both sporadic and in Von Hippel-Lindau disease: a literature review. Surg Neurol Int 4:145.

Cho HJ, Ki CS, Kim JW (2009) Improved detection of germline mutations in Korean VHL patients by multiple ligation-dependent probe amplification analysis. J Korean Med Sci 24:77–83.

Chou A, Toon C, Pickett J, *et al.* (2013) von Hippel-Lindau syndrome. Front Horm Res 41:30–49.

Coppin L, Grutzmacher C, Crépin M, *et al.* (2014) VHL mosaicism can be detected by clinical next-generation sequencing and is not restricted to patients with a mild phenotype. Eur J Hum Genet 22:1149–1152.

Cybulski C, Krzystolik K, Murgia A, *et al.* (2002) Germline mutations in the von Hippel-Lindau (VHL) gene in patients from Poland: disease presentation in patients with deletions of the entire VHL gene. J Med Genet 39:E38.

Del Sal G, Manfioletti G, Schneider C (1989) The CTAB-DNA precipitation method: a common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. Biotechniques 7:514–520.

Dollfus H, Massin P, Taupin P, *et al.* (2002) Retinal hemangioblastoma in von Hippel-Lindau disease: a clinical and molecular study. Invest Ophthalmol Vis Sci 43:3067–3074.

Ebenazer A, Rajaratnam S, Pai R (2013) Detection of large deletions in the VHL gene using a real-time PCR with SYBR Green. Fam Cancer 12:519–524.

Friedrich CA (1999) Von Hippel-Lindau syndrome. A pleomorphic condition. Cancer 86:2478–2482.

Friedrich CA (2001) Genotype-phenotype correlation in von Hippel-Lindau syndrome. Hum Mol Genet 10:763–767.

Gomy I, Molfetta GA, de Andrade Barreto E, *et al.* (2010) Clinical and molecular characterization of Brazilian families with von Hippel-Lindau disease: a need for delineating genotype-phenotype correlation. Fam Cancer 9:635–642.

Hes FJ, McKee S, Taphoorn MJ, *et al.* (2000) Cryptic von Hippel-Lindau disease: germline mutations in patients with haemangioblastoma only. J Med Genet 37:939–943.

Hes FJ, van der Luijt RB, Janssen AL, *et al.* (2007) Frequency of von Hippel-Lindau germline mutations in classic and non-classic von Hippel-Lindau disease identified by DNA sequencing, Southern blot analysis and multiplex ligation-dependent probe amplification. Clin Genet 72:122–129.

Ho TH, Jonasch E (2014) Genetic kidney cancer syndromes. J Natl Compr Canc Netw 12:1347–1355.

Kim WY, Kaelin WG (2004) Role of VHL gene mutation in human cancer. J Clin Oncol 22:4991–5004.

Latif F, Tory K, Gnarra J, *et al.* (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260: 1317–1320.

Limaverde-Sousa G, de Andrade Barreto E, Ferreira CG, *et al.* (2013) Simulation of the mutation F76del on the von Hippel-Lindau tumor suppressor protein: mechanism of the disease and implications for drug development. Proteins 2:349–363.

Los M, Jansen GH, Kaelin WG, *et al.* (1996) Expression pattern of the von Hippel-Lindau protein in human tissues. Lab Invest 75:231–238.

Maher ER, Webster AR, Richards FM, *et al.* (1996) Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. J Med Genet 33:328–332.

Melmon KL, Rosen SW (1964) Lindau's disease. Review of the literature and study of a large kindred. Am J Med 36:595–617.

Niemelä M, Lemeta S, Sainio M, *et al.* (2000) Hemangioblastomas of the retina: impact of von Hippel-Lindau disease. Invest Ophthalmol Vis Sci 41:1909–1915.

Richard S, Gardie B, Couvé S, *et al.* (2013) Von Hippel-Lindau: how a rare disease illuminates cancer biology. Semin Cancer Biol 23:26–37.

Richards S, Aziz N, Bale S, *et al.* (2015) ACMG Standards and Guidelines Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424.

Ruiz-Llorente S, Bravo J, Cebrian A, *et al.* (2004) Genetic characterization and structural analysis of VHL Spanish families to define genotype-phenotype correlations. Hum Mutat 23: 160–169.

Sansó G, Rudaz MCG, Levin G, *et al.* (2004) Familial isolated pheochromocytoma presenting a new mutation in the von Hippel-Lindau gene. Am J Hypertens 17:1107–1111.

Sgambati MT, Stolle C, Choyke PL, *et al.* (2000) Mosaicism in von Hippel-Lindau disease: lessons from kindreds with germline mutations identified in offspring with mosaic parents. Am J Hum Genet 66:84–91.

Siu WK, Ma RC, Lam CW, *et al.* (2011) Molecular basis of von Hippel-Lindau syndrome in Chinese patients. Chin Med J (Engl) 124:237–241.

Wu P, Zhang N, Wang X, *et al.* (2012) Family history of von Hippel-Lindau disease was uncommon in Chinese patients: suggesting the higher frequency of de novo mutations in VHL gene in these patients. J Hum Genet 57:238–243.

Wu P, Zhang N, Wang X, *et al.* (2013) Mosaicism in von Hippel-Lindau disease with severe renal manifestations. Clin Genet 84:581–584.

Yoshida M, Ashida S, Kondo K, *et al.* (2000) Germ-line mutation analysis in patients with von Hippel-Lindau disease in Japan: an extended study of 77 families. Jpn J Cancer Res 91:204–212.

Zbar B, Kishida T, Chen F, *et al.* (1996) Germline mutations in the Von Hippel-Lindau disease (VHL) gene in families from North America, Europe, and Japan. Hum Mutat 8:348–357.

Zhang J, Huang Y, Pan J, *et al.* (2008) Germline mutations in the von Hippel-Lindau disease (VHL) gene in mainland Chinese families. J Cancer Res Clin Oncol 134:1211–1218.

Zhang M, Wang J, Jiang J, *et al.* (2015) Von Hippel-Lindau disease type 2 in a Chinese family with a VHL p.W88X truncation. Endocrine 48:83–88.

Address correspondence to:
Patricia A. Pennisi, PhD
Centro de Investigaciones Endocrinológicas
''Dr. César Bergadá''
(CEDIE) CONICET–FEI–División de Endocrinología
Hospital de Niños Ricardo Gutiérrez
Gallo 1330–C1425EFD, CABA
Argentina

E-mail: ppennisi@cedie.org.ar