

Molecular Analysis of the *Von Hippel-Lindau (VHL)* Gene in a Family with Non-Syndromic Pheochromocytoma: The Importance of Genetic Testing

ABSTRACT

The two index patients of the family analyzed in this study had undergone bilateral adrenalectomy for pheochromocytomas. This prompted genetic analyses of the probands and seven first-degree relatives. The two pheochromocytoma patients and two additional asymptomatic family members were found to harbor a mutation c496G>T in exon 3 of the *VHL* gene. The family was then lost to systematic follow-up. Three years after performing the initial genetic evaluation, the sister of the probands, who was known to carry the same *VHL* germline mutation, was referred to our service after a pregnancy that was complicated by preeclampsia. She reported paroxysms suggestive for pheochromocytoma, but her urinary metanephrines were negative. However, computerized tomography of the abdomen showed an adrenal mass that was also positive on metaiodobenzylguanidine (MIBG) scintigraphy. This study illustrates that molecular analysis of the index patient(s) can lead to the identification of presymptomatic relatives carrying the mutation. Moreover, despite negative urinary metanephrines, the identification of a specific mutation has led to an increased suspicion and detection of a pheochromocytoma in the sister of the probands. (*Arq Bras Endocrinol Metab* 2007;51/9:1463-1467)

Keywords: Von Hippel-Lindau disease; Pheochromocytoma; Genetic testing; Mutation

RESUMO

Análise Molecular do Gene *Von Hippel-Lindau (VHL)* em uma Família com Feocromocitoma Não-Sindrômico: A Importância do Teste Genético.

Dois pacientes índices da família analisada neste estudo foram submetidos a adrenalectomia bilateral devido a feocromocitoma. Foi, então, realizado o estudo genético dos pacientes e de sete parentes de primeiro grau. Os dois pacientes com feocromocitoma e dois outros membros assintomáticos da família apresentaram a mutação c496G>T no exon 3 do gene *VHL*. A família perdeu seguimento médico. Três anos após a realização da avaliação genética, a irmã dos pacientes, portadora da mutação, foi encaminhada para o nosso serviço após uma gestação complicada por pré-eclampsia. Ela referia paroxismos sugestivos de feocromocitoma, mas as metanefrinas urinárias eram negativas. Entretanto, a tomografia computadorizada de abdômen evidenciou uma massa adrenal que também se contrastou na cintilografia com metaiodobenzilguanidina (MIBG). Esse estudo mostra que a análise molecular do paciente índice pode levar à identificação de parentes assintomáticos portadores da mutação. Além disso, mesmo com as metanefrinas urinárias negativas, a identificação de uma mutação específica levou a um aumento da suspeita e detecção de feocromocitoma na irmã dos afetados pela doença. (*Arq Bras Endocrinol Metab* 2007;51/9:1463-1467)

Descritores: Doença de Von Hippel-Lindau; Feocromocitoma; Teste genético; Mutação

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PHEOCHROMOCYTOMAS ARE CATECHOLAMINE-secreting tumors that develop from neural crest chromaffin cells. About 24% of them are associated with complex hereditary diseases, namely Von Hippel-Lindau (VHL) disease, multiple endocrine neoplasia type 2 (MEN 2), neurofibromatosis type 1 (NF1), and the syndrome consisting of familial pheochromocytoma/paraganglioma associated with glomus tumors caused by mutations in succinate dehydrogenase subunit D or B (SDHD, SDAB) (1-3).

Von Hippel-Lindau disease (VHL; OMIM 608537) is an autosomal dominant disease that is caused by mutations in the *VHL* tumor suppressor gene located on chromosome 3p26-p25 (HUGO L15409) (4). The clinical spectrum is highly variable and can include, among others, hemangioblastomas of the central nervous system (CNS) and the retina, clear cell renal carcinomas, and pheochromocytomas (5). Type and location of the mutations permit dividing the VHL syndrome into several subtypes (4). VHL type 1 is associated with a low risk for developing pheochromocytomas. These patients present with renal carcinomas, CNS hemangioblastomas, and renal, pancreatic, or epididymal cysts. In contrast, VHL type 2 is associated with a high risk for pheochromocytomas. Type 2A has a low risk for renal cell carcinomas and pancreatic lesions, type 2B is associated with a high risk for renal cell carcinomas, and type 2C is usually limited to the development of pheochromocytomas (6,7).

VHL type 1 is caused by mutations leading to a truncated or misfolded VHL protein (pVHL) (8). This deprives target cells of the ability to orchestrate the expression of a diverse set of genes involved in several signal transduction pathways, cellular responses to hypoxia, and the regulation of the metabolic microenvironment in tumors. One of the best-understood pVHL functions is the controlled degradation of the α -1 subunits of the heterodimeric transcription factor HIF (hypoxia inducible factor) (9). In contrast, VHL type 2 is predominantly caused by missense mutations leading to conformational alterations in the pVHL (4). Deletion of the whole *VHL* gene may be associated with a lower risk for developing renal cell carcinomas and a preponderance of central nervous system hemangioblastomas (10).

The Human Gene Mutation Database lists currently more than 300 mutations in the *VHL* gene (<http://archive.uwcm.ac.uk/uwcm/mg/search/120488.html>). They include, among others, 164 missense or nonsense mutations. Most of these mutations have deleterious functional consequences on the VHL protein (pVHL) and result in a loss of inhibition of HIF1

(hypoxia inducible factor 1). This, in turn, results in cell proliferation in selected tissues and to the development of tumors associated with VHL type 1. Other mutations, predominantly missense substitutions, result in partial retention of VHL protein function that conserve inhibition of HIF1, but are associated with proliferation of sympathoadrenal cells and ultimately result in the development of pheochromocytomas (VHL type 2) (4).

Here we present a kindred with isolated familial pheochromocytomas and illustrate the importance of genetic testing for carrier identification in first-degree relatives in the absence of positive biochemical tests, and its impact on clinical management and therapy.

SUBJECTS AND METHODS

Patients

At the age of fourteen years, the index patient II-1 reported the onset of paroxysms (headache, hypertension, and diaphoresis). On physical examination he was found to be tachycardic, he had an elevated blood pressure of 190/140 mmHg, and he had an increased precordial impulse in the fifth intercostals space. He had increased perspiration. His thyroid was not enlarged, and he did not have any palpable abdominal mass. The funduscopy revealed an abnormal arterio-venous crossing in the left eye and arteriolar spasms. There was no papilledema, nor any evidence for the presence of a hemangioblastoma.

Based on history and clinical exam, the possibility of a pheochromocytoma was considered and biochemical testing was therefore initiated. His urinary metanephrines were clearly elevated with a value of 14.6 mg/g creatinine (< 1 mg/g creatinine). A computerized tomography (CT) of the abdomen then revealed bilateral adrenal masses with large central calcifications. There were no pancreatic or renal lesions. The bilateral adrenal lesions were also positive on metaiodobenzylguanidine (MIBG) scintigraphy. Of note, there was no evidence on CT and MIBG imaging that the paraaortic ganglia would also be involved. Magnetic Resonance Imaging (MRI) of the brain did not show any alterations. His echocardiogram was normal.

He was then treated with an alpha-blocker (prazosin) in preparation for an open adrenalectomy. Both adrenals were surgically removed. Histology and immunohistochemistry confirmed the presence of bilateral pheochromocytomas.

One year later, a 10-year-old sister of the proband (II-2) was referred to our service because of palpitations, weight loss, diaphoresis, and shooting pain attacks that had started about two weeks earlier. Based on this clinical picture and because of her family history, a pheochromocytoma was suspected immediately. Her urinary metanephrines were elevated to 12.2 mg/g creatinine (< 1 mg/g). A CT of the

abdomen revealed a mass in the left adrenal; there were no lesions in the pancreas or the kidney. This mass was also positive on MIBG scintigraphy. As in her brother, there was no suggestion that the paraganglia would also be involved. Her MRI of the brain, and her echocardiogram were both normal. She was then treated with an alpha-blocker (prazosin) in preparation for an open adrenalectomy. Histology and immunohistochemistry confirmed diagnosis of a pheochromocytoma in the left adrenal gland.

Because of the familial occurrence, and the absence of signs suggesting multiple endocrine neoplasia type 2 (MEN2) or neurofibromatosis type 1 (NF1), we considered the possibility that the patients may have VHL type 2C. Therefore, genetic testing was initiated in the probands and seven first-degree relatives (figure 1A). The father was not available for genetic testing because he had died, apparently secondary to a hypertensive crisis. The molecular analysis led to the detection of a mutation in the *VHL* gene in the two affected siblings, as well as in an asymptomatic sister (II-4) and one of her daughters (III-1). The mother of the carriers was negative for the mutation, and she did not report any signs or symptoms suggestive for a pheochromocytoma. Unfortunately, the family was then lost to medical follow-up. Three years later, the sister of the probands (II-4), identified as a carrier of the same *VHL* gene mutation, was referred to our service after a pregnancy complicated by preeclampsia. Moreover, she reported the onset of paroxysms after delivery.

Ancillary studies

Urinary metanephrines were determined by standard chromatography. Imaging studies included computerized tomography of the abdomen, metaiodobenzylguanidine (MIBG) scintigraphy, MRI of the brain, echocardiography, and fundoscopy.

Molecular analysis

After obtaining informed consent of all patients and their relatives, blood was collected for genetic testing. Analysis of the *VHL* gene was performed using genomic DNA isolated from peripheral blood leukocytes. Exons 1, 2, and 3 were amplified by PCR with the following primers: 1F - 5' CCAT CCTTACCGAGCGCGC 3'; 1R - 5' GGGCTTCAGACCGTGCTATCG 3'; 2F - 5' TGCCAGCCACCGGTGTG 3'; 2R - 5' GTCTATCCTGTA CTTACCACAACA; 3F - 5' CACACTGCCACATACATGCACTC 3'; 3R - 5' ACTCATCAGTACCATCAAAAAGCTG 3'. Amplification was performed with 100 ng of genomic DNA in a 25 μ L reaction containing 20 pM of each primer, 10 X buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4), 1.0 mM MgCl₂, 0.2 mM each of dNTP, and 1.25 U Taq DNA Polymerase (Gibco-BRL). The following PCR conditions were used: initial denaturation for 1 min at 94°C, 35 cycles with 30 s at 60°C, 30 s at 72°C, and a terminal extension at 72°C for 5 min. PCR products were analyzed on 1% agarose gels, stained with ethidium bromide and visualized with UV light. After PCR amplification, both strands were sequenced using

a Genetic Analyzer 3100 Sequencer (Applied Biosystem, Foster City, CA, USA). Sequencing was performed in the Molecular Biology Laboratory, Department of Clinical Medicine, Botucatu School of Medicine — UNESP.

RESULTS

Sequence analysis

The two index patients, a brother (II-1) and a sister (II-2), were found to harbor a monoallelic germline mutation in the *VHL* gene (Figure 1B). This transversion c496G>T in exon 3 leads to substitution of valine by phenylalanine at position 166 (V166F). Subsequently, analysis of the first-degree relatives led to the detection of the same germline missense mutation in

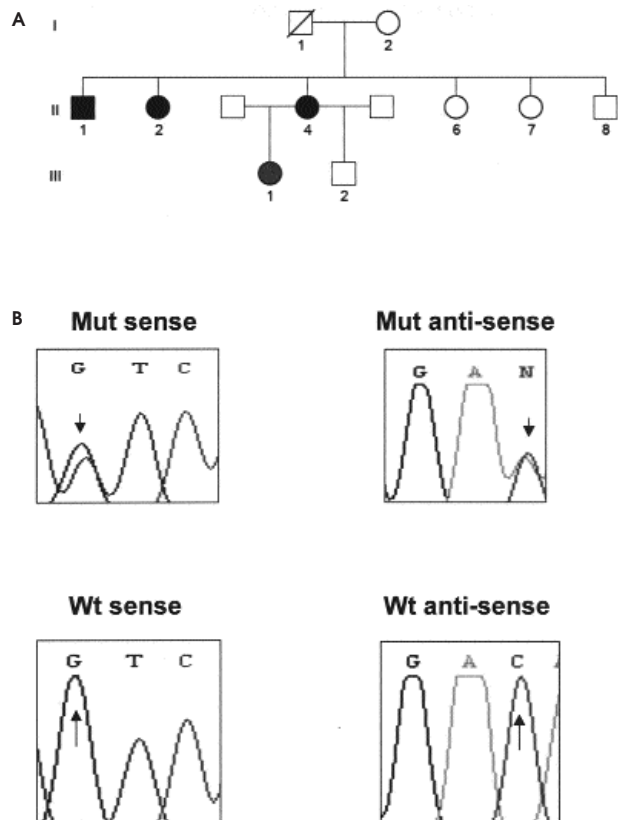


Figure 1. A) Pedigree of the kindred with familial pheochromocytomas caused by mutations in the *VHL* gene. The index patients II-1 and II-2 presented with bilateral pheochromocytomas. After genetic testing and identification of a germline mutation (c496G>T; V166F), II-4 and III-1 were identified as asymptomatic carriers. Initially lost to follow-up, II-4 came to medical attention because of post-partum paroxysms and a pregnancy complicated by preeclampsia; although her metanephrines were negative, she was known to carry the mutation and imaging studies led to the detection of a unilateral pheochromocytoma. **B)** Segment of the chromatograms illustrating the monoallelic germline mutation c496G>T; V166F and wild type.

an asymptomatic sister (II-4) and her daughter (III-1). The five other family members undergoing genetic testing did not have a sequence alteration in the analyzed regions of the *VHL* gene.

Clinical evaluation of the patient with preeclampsia

After initial evaluation, the family was lost to medical follow-up. However, three years later, patient II-4 was again referred to our service after a pregnancy complicated by preeclampsia. Her urinary metanephrines were repeatedly negative on two collections (0.86 µg/mg creatinine and 0.8 µg/mg creatinine; normal value: < 1.0 µg/mg creatinine). However, computerized tomography of the abdomen showed a right-sided adrenal mass; non-contrast images revealed a density of 36 Hounsfield units, findings that are highly suggestive for a pheochromocytoma (11). Moreover, this mass was also positive on MIBG scintigraphy. Echocardiography showed concentric myocardial remodeling of the left ventricle; funduscopy and MRI of the brain were normal. She was then treated with an alpha-blocker (prazosin) in preparation for an open adrenalectomy. The histology confirmed the diagnosis of a pheochromocytoma and immunohistochemistry was only weakly positive for COX-2 (data not shown), a finding that is suggestive of a benign tumor (12).

DISCUSSION

In this study, we detected a germline mutation (c496G>T, pV166F) in the two index patients (II-1 and II-2) and subsequently in two asymptomatic relatives (II-4 and III-1). This mutation has been described previously by Gross and colleagues (13), and these authors reported a strong association between the V166F mutation and the development of both benign and malignant neoplasias of the adrenal and extradrenal chromaffin tissues. Thus, this mutation appears to fall into the VHL type 2C category characterized by the predominant development of pheochromocytomas without other features of the VHL syndrome (1).

This study highlights several important aspects in the management of patients with pheochromocytomas. The molecular analysis of the two index patients led to the detection of a mutation in the *VHL* gene, which prompted screening of first-degree relatives. This led to the identification of two asymptomatic carriers of the mutation. Although these individuals were then lost to follow-up, the work-up of II-4, who was referred

because of a pregnancy complicated by preeclampsia, included imaging studies despite normal urinary metanephrines and this led to the detection of a unilateral pheochromocytoma amenable to elective surgery. In agreement with recently published guidelines (14), we conclude that genetic testing should be performed in the index case(s) with pheochromocytomas in the following instances: 1) young onset (particularly before age 20), 2) bilateral and multicentric pheochromocytomas, and 3) clinical features suggestive of a tumor syndrome such as VHL, neurofibromatosis type 1, MEN2 (and rarely MEN1), or the familial paraganglioma/pheochromocytoma syndrome. As illustrated by this study, this may lead to the detection of additional carriers that may be oligo- or asymptomatic, and to early treatment before the development of the potentially deleterious consequences of these tumors.

REFERENCES

1. Neumann HP, Berger DP, Sigmund G, Blum U, Schmidt D, Parmer RJ, et al. Pheochromocytomas, multiple endocrine neoplasia type 2, and von Hippel-Lindau disease. **N Engl J Med** 1993;329(21):1531-8.
2. Neumann HP, Bausch B, McWhinney SR, Bender BU, Gimm O, Franke G, et al. Germ-line mutations in nonsyndromic pheochromocytoma. **N Engl J Med** 2002;346(19):1459-66.
3. Benn DE, Gimenez-Roqueplo AP, Reilly JR, Bertherat J, Burgess J, Byth K, et al. Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes. **J Clin Endocrinol Metab** 2006;91(3):827-36.
4. Zbar B, Kishida T, Chen F, Schmidt L, Maher ER, Richards FM, et al. Germline mutations in the Von Hippel-Lindau disease (VHL) genes in families from North America, Europe, and Japan. **Hum Mutat** 1996;8(4):348-57.
5. Maher ER, Webster AR, Richards FM, Green JS, Crossey PA, Payne SJ, et al. Phenotypic expression in von Hippel-Lindau disease: correlations with germline VHL gene mutations. **J Med Genet** 1996;33(4):328-32.
6. Dahia PLM. Patogênese molecular do feocromocitoma. **Arq Bras Endocrinol Metab** 2001;45:507-17.
7. Weirich G, Klein B, Wöhl T, Engelhardt D, Brauch H. VHL2C phenotype in a German von Hippel-Lindau family with concurrent VHL germline mutations P81S and L188V. **J Clin Endocrinol Metab** 2002;87(11):5241-6.
8. Gimm O. Pheochromocytoma-associated syndromes: genes, proteins and functions of RET, VHL and SDHx. **Fam Cancer** 2005;4(1):17-23.
9. Ivan M, Kaelin WG Jr. The von Hippel-Lindau tumor suppressor protein. **Curr Opin Genet Dev** 2001;11(1):27-34.
10. Hes F, Zewald R, Peeters T, Sijmons R, Links T, Verheij J, et al. Genotype-phenotype correlations in families with deletions in the von Hippel-Lindau (VHL) gene. **Hum Genet** 2000;106(4):425-31.
11. Hamrahian AH, Ioachimescu AG, Remer EM, Motta-Ramirez G, Bogabathina H, Levin HS, et al. Clinical utility of noncontrast computed tomography attenuation value (Hounsfield units) to differentiate adrenal adenomas/hyperplasias from nonadenomas: Cleveland Clinic experience. **J Clin Endocrinol Metab** 2005;90(2):871-7.
12. Salmenkivi K, Haglund C, Ristimäki A, Arola J, Heikkilä P. Increased expression of Cyclooxygenase-2 in Malignant Pheochromocytomas. **J Clin Endocrinol Metab** 2001;86:5615-9.

13. Gross DJ, Avishai N, Meiner V, Filon D, Zbar B, Abeliovich D. Familial pheochromocytoma associated with a novel mutation in the von Hippel-Lindau gene. **J Clin Endocrinol Metab** 1996;81(1):147-9.
14. Jiménez C, Cote G, Arnold A, Gagel RF. Review: Should patients with apparently sporadic pheochromocytomas or paragangliomas be screened for hereditary syndromes? **J Clin Endocrinol Metab** 2006;91(8):2851-8.

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