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VHL mutation as a cause of three generations familial pheochromocytoma

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Abstract

Background Pheochromocytoma is a rare disease, and its familial occurrence is quite uncommon. The aim of this paper is to report a three-generation phenotypical expression of a case familial occurrence of pheochromocytoma.

Case presentation A 25-year-old female, with a history of adrenalectomy for pheochromocytoma, arrived at the shock room during her third pregnancy with an adrenergic crisis and hypoglycemia. To prevent perinatal complications, the patient was stabilized and the newborn was delivered through a Kerr-type cesarean section. A detailed history revealed that the paternal grandfather of the patient had an unilateral pheochromocytoma, whereas her paternal uncle had a bilateral pheochromocytoma. Additionally, a brother of the patient presented a unilateral pheochromocytoma. Amplicons for PCR assays were designed to span the protein-coding segments of the three Von Hippel–Lindau (*VHL)* exons, and the PCR products were sequenced using the Sanger method. In the trace of exon 3, we detected in the sample of the proband a heterozygous guanine to adenine transition (NM_000551.4 c. 552G > A) within the protein-coding segment of exon 3 of the *VHL* gene, which leads to a substitution of the arginine residue at position 161 by a glutamine residue in the encoded peptide (NP_000542.1p.R161Q). This mutation was absent in two unaffected daughters.

Conclusion A *VHL* mutation was suspected and confirmed in this family that was not transmitted to a fourth generation. This case illustrates the importance of molecular genetics methodologies to assist genetic counseling in cases of pheochromocytoma where familial aggregation is presumed.

Keywords Familial pheochromocytoma, Molecular diagnosis, Sequencing, *VHL*

Background

Pheochromocytomas are rare tumors of chromaffin cells derived from the neural crest and mainly found in the adrenal medulla, although they can appear in other sites (paragangliomas). These are difficult to diagnose catecholamine-producing neuroendocrine tumors, and the familial aggregation of cases is quite uncommon. Most pheochromocytomas are isolated and apparently sporadic, while approximately one in ten cases occurs in the context of a syndrome (neurofibromatosis type I, Multiple Endocrine Neoplasia type II, Von Hippel–Lindau (VHL) syndrome), sometimes displaying Mendelian patterns of inheritance [1, 2].

The annual incidence of pheochromocytomas is estimated around 2–8 cases per million [3, 4], being responsible for less than 0.1–0.2% of hypertension cases without sex preference, and most are diagnosed

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between the fourth and fifth decade of life, although they can appear at any age [4, 5].

About 80–85% of pheochromocytomas are found in the adrenal medulla, and the other 15–20% in extra-adrenal areas, mainly the Zuckerkandl organ, although it can occur in other areas of the thorax and abdomen; extra-adrenal pheochromocytomas are more common in children [6, 7].

Pheochromocytomas are classified as benign or malignant, the difference being that the malignant type is capable of metastasizing [1, 3, 5]. The formation of pheochromocytomas has been linked to germline mutations in more than 20 loci, causing activation of three signaling pathways: (1) kinase signaling-related genes, (2) pseudohypoxic Krebs cycle-related genes and (3) Wnt signaling-related genes [1, 6].

The most commonly occurring symptoms in pheochromocytoma cases are blurred vision, chest and abdominal pain, constipation, diaphoresis, fatigue, fever, flushing, headache, heat intolerance, hyperglycemia, hypertension, nausea, palpitations, pallor, panic attacks and anxiety disorders, papilledema, polyuria and polydipsia, sweating, vomiting, tremors and weight loss [2, 3, 5, 6].

Diagnosis is based on confirmation of clinical suspicion by biochemical tests and image studies [2, 8]. Biochemical tests are indicated in asymptomatic patients with family history of pheochromocytoma or germline mutations associated with increased risk for pheochromocytoma as well as patients with either (1) signs or symptoms suggesting an excess of catecholamines, with or without arterial hypertension, (2) arterial hypertension that requires three or more antihypertensive drugs for its management, (3) unexplained blood pressure variability, (4) paradoxical response in surgical interventions after the use of anesthesia and beta adrenergic receptor blockers or (5) in the event of the incidental finding of an adrenal tumor [8, 9].

The preferred diagnosis test is the determination of free metanephrines (normetanephrine and metanephrine) in urine and plasma [2, 5]. Plasma determination is considered superior to the urinary one, with a sensitivity of 97.9% and a specificity of 94.2% [8, 10, 11]. The treatment of pheochromocytoma is based on a multidisciplinary intervention, including the management of symptoms such as hypertension, headache, kidney damage. However, the definitive treatment is surgery to remove the neuroendocrine tumor [5, 8].

Pheochromocytoma is a rare disease, and its familial presentation is quite uncommon. The aim of this paper is to report a three-generation phenotypical expression of familial pheochromocytoma.

Case report

The proband, a 25-year-old female, was diagnosed with a pheochromocytoma located at the right adrenal gland and later submitted to right adrenalectomy, developing right renal atrophy after the procedure. She had a grandfather with unilateral pheochromocytoma, an uncle with bilateral pheochromocytoma and a brother with unilateral pheochromocytoma (Fig. 1), the last two treated surgically.

During her third pregnancy, the patient was received at the shock room with hypoglycemia, neuroglycopenic symptoms and fluctuating sleepiness. To avoid perinatal complications an emergency Kerr-type cesarean section was performed, obtaining a healthy female newborn. The patient had two previous pregnancies, one spontaneous and complete abortion at 3 weeks of gestation. During her second pregnancy, she had altered fasting glucose and severe urinary infections that led to sepsis, making necessary the obstetrical interruption with a satisfactory evolution for her and the product. Table 1 shows the main paraclinical studies.

Genetic analysis

Samples (1 mL of peripheral venous blood) were collected from the patient, her affected brother and her unaffected two daughters, in tubes containing ethylenediaminetetraacetic acid. Genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following directions from the manufacturer. Briefly, 400 µL of blood were mixed with 20 µL of proteinase K and 250 µL of lysis buffer and mixed. After an incubation at

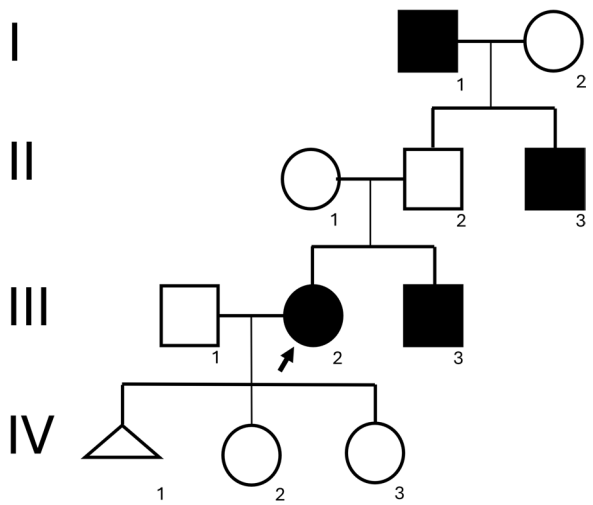

Fig. 1 Genealogy of the family affected by pheochromocytoma described in the present report. Individuals (I,1), (III,3) and the proband (III,2) had unilateral tumors, whereas individual (II,3) had a bilateral tumor

Table 1 Main laboratorial and paraclinical studies

Date	Procedure
17/05/2011	She went to the emergency room due to intermittent headache of one year's of duration with diaphoresis and high blood pressure of 3 months' of duration. Diagnosis of pheochromocytoma is made
20/05/2011	Normal echocardiogram
12/06/2011	Right adrenalectomy
08/07/2011	Discharged
23/09/2011	Thoraco/abdomino/pelvic tomography with surgical absence of the right adrenal gland and morphological changes of the ipsilateral kidney Hospitalization due to fall, dizziness of 3-day duration was reported
25/02/2012	Hospitalization due to fall, dizziness of 3-day duration was reported
26/02/2012	A diagnostic impression of canalolithiasis of the left superior semicircular canal is made
02/03/2012	Normal single ear resonance
27/07/2012	Diagnosis of benign paroxysmal vertigo is established
11/09/2016	Spontaneous abortion
30/01/2018	Positive pregnancy immunology test
11/02/2018	Platelets of 486,000/µL
11/02/2018	Triglycerides 234.3 mg/dL
28/02/2018	ICU admission due to hypoglycemia
28/02/2018	C-peptide: 24.4 ng/mL, insulin: 63.3 IU/mL, serum cortisol: 30.8 µg/dL
28/02/2018	Liver and bile duct ultrasound showing gallbladder polyp
28/02/2018	Glucose 134 mg/dL, sodium 130 mg/dL, lactate dehydrogenase 205, amylase 158 U/L
28/02/2018	General urine test with positive proteins
28/02/2018	Leukocytes 12.1 X 10^3/µL, lymphocytes 4.7%, granulocytes 93.8%
01/03/2018	Albumin 2.4 mg/dL, urinary sodium 15 mg/dL, urinary chlorine 34 mg/dL
01/03/2018	Leukocytes 19.4 X 10^3/µL, hematocrit 35.5, lymphocytes 4.1%, granulocytes 18%
01/03/2018	Urine test with positive proteins
02/03/2018	Triglycerides 201 mg/dL, total proteins 5.3 mg/dL, albumin 2.2 mg/dL, indirect bilirubin 0.04 mg/dL, urinary potassium 9.8 mEq/L and urinary BUN 378 mg/dL
02/03/2018	Hemoglobin 10.7 g/dL, hematocrit 32.5%
03/03/2018	Glucose 167 mg/dL, triglycerides 257 mg/dL, calcium 7 mg/dL, total proteins 5.2 mg/dL, albumin 2.2 mg/dL, BUN 743 mg/dL
03/03/2018	Leukocytes 12.3 X 10^3/µL, hematocrit 30.8, lymphocyte percentage 6.5%
04/03/2018	ICU discharge
06/03/2018	Simple magnetic resonance imaging of the upper abdomen without evidence of abdominal tumor lesion and with moderate right renal atrophy
28/03/2018	Performing an ECG that reports atypical block of the abnormal QRS bundle branch
30/05/2018	Admission due to pregnancy and hypoglycemia
31/05/2018	Performing a Kerr-type cesarean section
31/05/2018	ICU admission
31/05/2018	Glucose of 46 mg/dL
31/05/2018	Urine test with glucose > 1000 mg/dL
01/06/2018	Cholesterol 210 mg/dL, alkaline phosphatase 157 IU/L, urinary creatinine 9.1, urinary sodium 15 mg/dL, urinary potassium 7.7 mg/dL, urinary chloride 21 mg/dL, lipase 111 U/L, urinary BUN 107
02/06/2018	Glucose 137 mg/dL, BUN 6 mg/dL, calculated urea 12.8, triglycerides 240 mg/dL, calcium 7 mg/dL, total protein 4.9 g/dL, alkaline phosphatase 144 IU/L, urinary creatinine 185.8, urinary potassium 102.3, urinary BUN 706
02/06/2018	EGO with hemoglobin+++, proteins+, countless erythrocytes
02/06/2018	Leukocytes 15.6 X 10^3/µL, lymphocytes 5.1%, granulocytes 90%
03/06/2018	Urine culture for *E. coli* 120,000 sensitive to amikacin, imipenem, meropenem
03/06/2018	Leukocytes 12.6 X 10^3/µL, hemoglobin 11.4 g/dL, hematocrit 34.6,% lymphocytes 7.2%, granulocytes 88.1%
03/06/2018	Glucose 119 mg/dL, triglycerides 216 mg/dL, calcium 7.1 mg/dL, total proteins 4.9 g/dL, indirect bilirubin 0.11 mg/dL, amylase 124 U/L, urinary creatinine 23, urinary chloride 53, lipase 367, urinary BUN 492
04/06/2018	Leukocytes 11.6 X 10^3/µL, hemoglobin 10.7 g/dL, hematocrit 32.1%, lymphocytes 18.1%
04/06/2018	Triglycerides 209 mg/dL, indirect bilirubin 0.11 mg/dL, urinary potassium 5.2, urinary creatinine 14.5, calcium 6.5 mg/dL

Table 1 (continued)

Date	Procedure
05/06/2018	Cholesterol 208 mg/dL and triglycerides 217 mg/dL
06/06/2018	Glucose 109 mg/dL, cholesterol 212 mg/dL, triglycerides 348 mg/dL, total proteins 5.3 g/dL, alkaline phosphatase 141 IU/L, urinary creatinine 23.4, urinary sodium 132, urinary chloride 129, urinary BUN 434
06/06/2018	Urine test with hemoglobin++ 200 and leukocytes++ 70
06/06/2018	Negative 48/hour urine culture
06/06/2018	ICU discharge
13/02/2021	Hospital admission
13/02/2021	Performing Kerr-type cesarean section and bilateral tubal occlusion
13/02/2021	Glucose 48 mg/dL, potassium 3.08 mmol/L, cholesterol 245 mg/dL, triglycerides 310 mg/dL, calcium 8 mg/dL, alkaline phosphatase 205 IU/L, total proteins of 8 mg/dL
14/02/2021	Glucose 115 mg/dL, lactate dehydrogenase of 225 U/L
14/02/2021	Renal US with diagnostic impression of right renal hypoplasia
15/02/2021	Urine test with negative glucose
15/02/2021	Hospital discharge

56ºC for 10 min, 300 µL of ethanol was added, transferring the mix to a DNA isolation column, which was spun to 6,000 g for 1 min twice, discarding the flow-through. The column was first washed with 500 µL of AW1 buffer and spun at 6,000 g for 1 min and washed again with 500 µL of buffer AW2, centrifuging it at 17,000 g for 3 min a first time and for 1 min a second time, discarding the flow-through. The DNA was eluted from the column to a 1.5-mL microfuge using 50 µL of elution buffer and spun at 6000 g for 1 min. Purity and concentration were determined by UV absorbance at 260 nm and 280 nm of wavelength and integrity by agarose gel electrophoresis.

Sanger sequencing

Amplicons for PCR assays were designed to span the protein-coding segments of the three *VHL* exons using primers at a final concentration of 3.2 µM. Exon 1 was amplified using primers VHL-1F (5'-acagtaacgagttggcctagc-3') and VHL-1R (5'-ttcagaccgtgctatcgtcc-3'), exon 2 was amplified using primers VHL-2F (5'-gtgtaggtcaggggaaatgg-3') and VHL-2R (5'-ggataacgtgcctgacatc-3'), and exon 3 was amplified using primers VHL-3F (5'-actacagaggcatgaacacc-3') and VHL-3R (5'-ACTTCT CTAATGGGCAGGC-3', capital letters denote exonic sequence). 600 ng of genomic DNA was PCR-amplified using the GoTaq Green Master Mix (Promega, Madison, WI, USA), following directions from the manufacturer. An initial denaturation step was carried out at 96 °C for 3 min, followed by 30 cycles, each one with a denaturation step at 96 °C for 30 s, a annealing step at 60 °C for 30 s and an extension step at 72 °C for one minute. After the 30 cycles, a final extension step was performed at 72 °C for five minute. The PCR products were purified from excised 2.5% agarose gel bands using the QIAquick Gel Extraction Kit (Qiagen) and quantified with a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). 50 ng of purified PCR product was used to generate 20 µL Sanger sequencing reactions with the BigDye Terminator kit v3.1 (Thermo Fisher Scientific). Sequencing reactions were in turn purified employing Centri-Sep columns (Thermo Fisher Scientific). Capillary electrophoresis was run in a Genetic Analyzer 310 (Thermo Fisher Scientific) following manufacturer's instructions.

Results

The PCR assays performed resulted in efficient amplification of the intended targets as revealed by the Sanger sequencing electropherograms. In all three samples screened (the proband, her affected brother and her two unaffected daughters), no variants were detected in the traces corresponding to exons 1 and 2 of the *VHL* gene. In the trace of exon 3, we detected in samples from the proband and her affected brother a heterozygous guanine to adenine transition (NM_000551.4 c.552G > A) within the protein-coding segment of exon 3 of the *VHL* gene, which leads to a substitution of the arginine residue at position 161 by a glutamine residue in the encoded peptide (NP_000542.1p.R161Q). Upon sequencing of the relevant VHL gene segment, we observed that this mutation was not present in two unaffected daughters of the proband (Fig. 2). All sequences were performed in both directions, being internally consistent.

Discussion

According to various studies, 10% of pheochromocytoma occurrences correspond to familial cases and reports of this tumor affecting several members of a family are

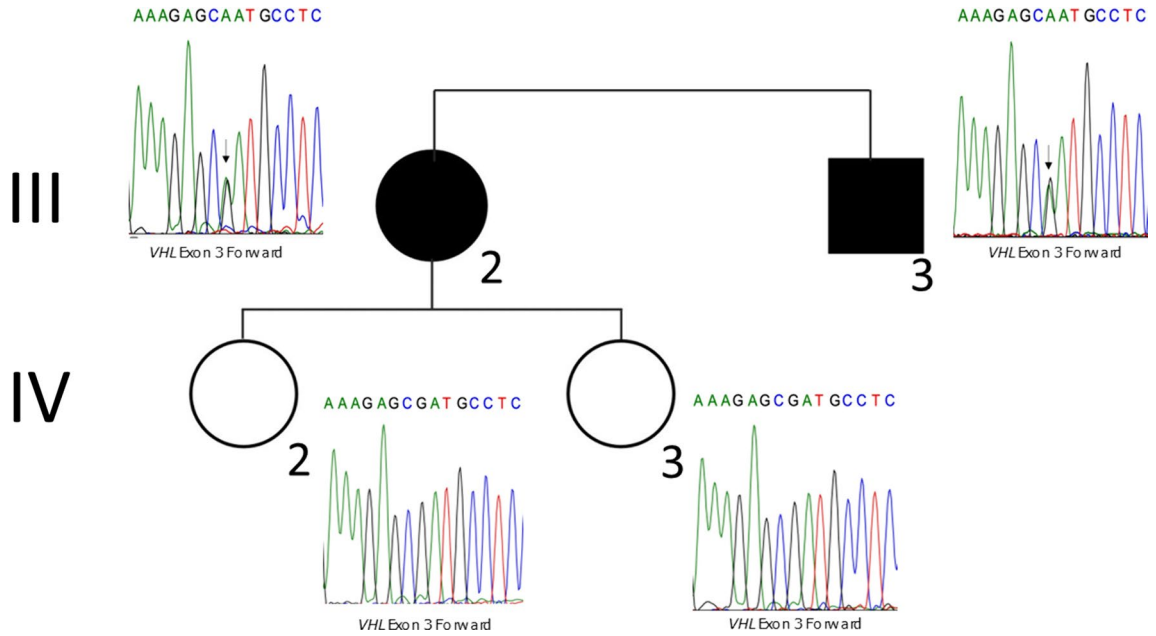

Fig. 2 Sanger sequencing traces obtained from samples from affected (III,2 and III,3) and unaffected (IV,2 and IV,3) subjects from the genealogy depicted in Fig. 1

uncommon. For example, a familial case consisting of a male patient and three affected daughters was registered among a series of 24 pheochromocytoma cases treated within a 15-year period in a referral center. Within this family, the most characteristic finding in these patients was the malignant hypertension. After the two daughters underwent subtotal adrenalectomy, they remained normotensive and with normal cortisol values [12].

The familial aggregation of pheochromocytoma cases has been described in other articles, including one of them reporting a case of a mother and daughter affected by this tumor and neurofibromatosis 1. Both underwent successful surgical resection and the daughter had healthy offspring [13].

Another group reported two further familial cases. Regarding the first of them, the proband had an extra-adrenal tumor causing severe renal artery stenosis, with pheochromocytomas present in three successive generations of the family. The second familial occurrence included multiple pheochromocytomas cases associated with von Hippel–Lindau syndrome and a family member with multiple endocrine neoplasia type 2. These genetic entities and syndromes share peculiar interrelationships, pathologically related to an aberration in the migration, growth and differentiation of the neural crest cells, with a common neuroectodermal origin [14].

The tumor-suppressor protein pVHL, the product of the *VHL* gene, is a E3 ubiquitin ligase that forms a complex with Elongin B and C to mediate the degradation of HIF1α y HIF2α and among other functions; it also stabilizes the tumor-suppressor p53, enhancing its transcriptional activity [15, 16]. The mutation identified in the family studied here, Arg161Gln, is located within the p53 and Elongin C binding site (residues 157–172). Previously, it has been shown that the Arg161Gln mutation impairs the induction of apoptosis caused by p53 activation in comparison with the wild-type pVHL [16].

Conclusion

The majority of pheochromocytomas are related to abnormal gene expression of neuroectoderm-derived tissues, usually because of germline or somatic mutations affecting specific genes. This kind of tumor can occur as an isolated entity of as part of a monogenic syndrome. Isolated tumors can also display Mendelian patterns of inheritance when familial aggregation of cases occurs. Given the severity and often life-threatening nature of complications of pheochromocytomas, effective and accurate genetic counseling to affected families should be provided. Here, we describe an assay that aided us to provide genetic counseling in a three-generation pheochromocytoma family.

Abbreviation

VHL Von Hippel–Lindau

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Author contributions
JTGR and HMZ provided the overall design of the manuscript. MCME, HDG and RSU performed the genetic analysis. MDRP, ALAG, MARL and KAMC contributed to the acquisition of the data and clinical assessment. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was granted by the Maternal-Perinatal Hospital "Mónica Pretelini Sáenz" Research Ethics Committee (2024-01-01).

Consent for publication
Non applicable.

Competing interests
The authors declare that there are no competing interests.

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