New mutation in the *PTEN* gene in a Brazilian patient with Cowden's syndrome

Nova mutação no gene PTEN *em um paciente brasileiro com síndrome de Cowden*

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SUMMARY

Cowden syndrome is characterized by hamartomatous polyps, trichilemmomas, increased risk of developing neoplasms, and is associated with germline mutations in the *PTEN* gene. We searched for germline mutations in *PTEN* in a 49-year-old female patient who presented trichilemmoma with previous history of breast carcinoma, and thyroidectomy for a thyroid nodule. We also searched for somatic mutations in breast and thyroid tumoral tissues. DNA was extracted from peripheral leukocytes, paraffin samples of breast carcinoma, and cytological smears of thyroid nodule fine-needle aspiration biopsy, whose final histopathological diagnosis was adenomatous goiter. *PTEN* was amplified and sequenced. We identified a novel mutation, due to aT>A inversion at position 159 and A>T inversion at position 160, leading to valine-to-aspartic acid substitution at position 53. The p.Val53Asp was also found in homozygous state in samples obtained from adenocarcinoma breast and thyroid biopsy, denoting loss of heterozygosity. Here, we demonstrated a novel germline mutation in *PTEN,* as well as somatic loss of the wild-type *PTEN* allele in breast and thyroid tumors in a patient with Cowden syndrome. Arq Bras Endocrinol Metab. 2012;56(8):592-6

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SUMÁRIO

A síndrome de Cowden é caracterizada por pólipos de hamartoma, triquelomomas, risco aumentado em desenvolver neoplasias e está associada a mutações germinativas no gene *PTEN*. Procuramos por mutação germinativa no *PTEN* de uma paciente de 49 anos que apresentou triquilemomas com história pregressa de carcinoma de mama e realizou tireoidectomia devido a nódulo de tireoide. Investigamos também uma mutação somática em tecidos tumorais de mama e tireoide. O DNA foi extraído de leucócitos periféricos, de amostras de parafina de carcinoma de mama e exame citológico de nódulo de tireoide obtido de biópsia por agulha fina, cujo diagnóstico histopatológico foi de bócio adenomatoso. O *PTEN* foi amplificado e sequenciado. Identificou-se uma nova mutação em decorrência de uma inversão de T>A na posição 159 e A>T na posição 160, levando à substituição de valina para ácido aspártico na posição 53. A mutação p.Val53Asp também foi encontrada em estado homozigoto em amostras obtidas do adenocarcinoma de mama e da biópsia de nódulo tireoidiano, denotando perda de heterozigosidade. Portanto, demonstramos uma mutação germinativa no *PTEN* e também a perda somática do alelo selvagem PTEN no tumor de mama e da tireoide de uma paciente com síndrome de Cowden. Arq Bras Endocrinol Metab. 2012;56(8):592-6

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INTRODUCTION

Cowden syndrome (CS [MIM 158350]), first described by Lloyd and Dennis in 1963 (1), is characterized by hamartomatous polyps of the gastrointestinal tract, mucocutaneous lesions, and increased risk for developing neoplasms, particularly breast, endometrium, thyroid, kidney and colorectal cancers. CS has been estimated to affect around 1 in every 200,000 individuals, although this rate is probably underestimated due to the highly variable phenotype (2). Approximately 80% of CS patients have *PTEN* mutations. The *PTEN* gene (10q23.3) [MIM 601728] encodes a ubiquitously expressed tumor suppressor dual-specificity phosphatase that antagonizes the PI3K signalling

pathway by means of its lipid phosphatase activity, and negatively regulates the MAPK pathway by means of its protein phosphatase activity (3).

Major organs involved include breast, thyroid, uterus, brain, and mucocutaneous tissues. Breast cancer numbers among the tumor types in which germline *PTEN* mutation have been documented. Somatic *PTEN* mutation also occurs, but at a low frequency, and is not associated with CS (4). Thyroid tumors were also found to have somatic deletions of the *PTEN* gene, predominantly benign forms, suggesting that *PTEN* might also act as a tumor suppressor for these cancers (5).

Based on the Criteria of the International Cowden Consortium (6), we searched for *PTEN* mutation in a patient with trichilemmoma, breast carcinoma, and goiter.

CASE REPORT

We studied a 49-year-old female patient with pathognomonic criteria of CS: several mucocutaneous lesions measuring 0.2 to 0.4 cm, diagnosed by biopsy as trichilemmoma and nasal mucosal papillomatosis (Figure 1). At 46 years of age, she presented a unilateral breast cancer classified as ductal carcinoma *in situ* with micropapillary, solid, and cribriform pattern. She was submitted to radio and hormonal (tamoxifen) therapy after surgery. During follow-up, a solid hypoechoic and well-delimited nodule measuring 1.3 x 1.4 x 1.8 cm was noted in the left lobe on thyroid ultrasonography, and the patient was referred to our Endocrinology Department. Fine-needle aspiration biopsy determined the classification of the nodule as Class II of the Bethesda system, and total thyroidectomy was performed, as findings were indicative of CS (7). Histopathological diagnosis was adenomatous goiter. Gastrointestinal mucosa was normal on endoscopy and colonoscopy. The patient's parents and son were both asymptomatic. Nevertheless, DNA analysis was performed for genetic counseling.

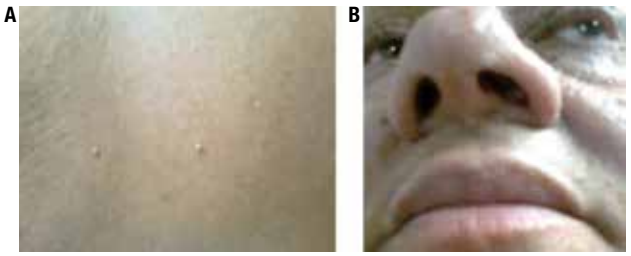

Figure 1. Pathognomonic mucocutaneous features of Cowden syndrome (CS). **(A)** Trichilemmomas in the patient's abdomen characterized as well-defined, smooth, asymptomatic papules. **(B)** Nostril polyposis.

DNA analysis

DNA was extracted from peripheral leukocytes as described elsewhere (8). DNA was also extracted from breast cancer tissue obtained from paraffin material and from thyroid cells from FNAB slides, as described elsewhere (9,10).

The *PTEN* gene was amplified by PCR, including the coding region and exon-intron boundaries, followed by automatic sequencing (ABI Prism 3130 *xl*, Applied Biosystems, Foster City, CA) and results were compared with the normal sequence. Primers and PCR conditions were amplified as described in table 1.

Table 1. Primers, annealing temperature, and respective fragment size obtained after amplification of coding region and exon-intron boundaries of the *PTEN* gene

Exon	Primers	T°C	Fragment size
Exon 1.A	5′CAG CTA CAC TGG GCA TGC T 3′ 5′AAA TGG TGA CAG GCG ACT 3′	57°C	709 bp
Exon 1.B	5′ CTT CCT CGG CTT CTC CTG A 3′ 5′ CAT CCG TCT ACT CCC ACG TT3′	51°C	768 bp
Exon 2	5′ACA TTG ACC ACC TTT TAT 3′ 5′CAA AGT ATC TTT TTC TGT 3′	45°C	321 bp
Exon 3	5′ATA TTC TCT GAA AAG CTC TGG3′ 5′TTA ATC GGT TTA GGA ATA CAA 3′	53°C	435 bp
Exon 4	5′GGG GGT GAT AAC AGT ATC TA 3′ 5′CTT TAT GCA ATA CTT TTT CCT A 3′	52°C	404 bp
Exon5	5′ACC TGT TAA GTT TGT ATG CAA C 3′ 5′TCC AGG AAG AGG AAA GGA AA 3′	57°C	382 bp
Exon 6	5′CAT AGC AAT TTA GTG AAA TAA CT3′ 5′GAT ATG GTT AAG AAA ACT GTT C 3′	50°C	274 bp
Exon7	5′TGA CAG TTT GAC AGT TAA AG 3′ 5′CTT ATT TTG GAT ATT TCT CC 3′	49°C	271 bp
Exon 8	5′CTC AGA TTG CCT TAT AAT AGT C 3′ 5′ TCA TGT TAC TGC TAC GTA AAC 3′	50°C	565 bp
Exon 9.A	5′AAG GCC TCT TAA AGA TCA TG3′ 5′ TTT TCA TGG TGT TTT ATC CCT C3′	55°C	378 bp
Exon 9.B	5′TCC AGA GGC TAG CAG TTC CAA 3′ 5′ TCT GAG CAT TCC CTC CAT TC3′	55°C	677 bp
Exon 9.C	5′TTC ACA TCC TAC CCC TTT GC 3′ 5′ AGC ACA TGA AGC ATC CAC AG 3′	55°C	645 bp
Exon 9.D	5′ CGA CTT CTC CAT CTC CTG TG 3′ 5′ GGG GGA GCA CTA TGA AGA AA 3′	55°C	601 bp
Exon 9.E	5′ CGT TCC ACC CTT TTG ACC T 3′ 5′ TGC CTA ATC TAT TTG CCA TCA A 3′	54°C	690 bp
Exon 9.F	5′TTG GTG CTG AAA TTG TTC ACT 3′ 5′ ATG CCA TTT TTC CAT TTC CA 3′	54°C	647 bp
Exon 9.G	5′ TGG AAA TGG AAA AAT GG3′ 5′ CCC CCA CTT TAG TGC ACA GT 3′	54°C	614 bp
Exon 9.H	5′GTT TAC CGG CAG CAT CAA AT 3′ 5′GCT TTG AAG GAC AGC AGG AA 3′	55°C	655 bp

bp: base pair.

RESULTS

The DNA sequence from peripheral leukocytes showed a T-to-A inversion at position 159 and an A-to-T inversion at position 160 in exon 2, leading to a valine-to-aspartic acid substitution at position 53 of the protein (p.V53D) (Figure 2A). This p.Val53Asp mutation was also found in the adenocarcinoma breast tissue and in the thyroid nodule, in a homozygous status (Figure 2B). We have not identified this mutation in 200 control alleles. The patient's father also presented this novel mutation in DNA extracted from peripheral leukocytes. Her son and mother showed normal sequences (Figure 2C).

This Valine-53 is a highly conserved amino acid among different species (Figure 3A). Predictive analysis of the protein secondary structure with the p.Val53Asp mutation showed minor structural changes in *PTEN*: (1) strand elongation at position 124; (2) reduced coil at position 129; (3) coil stretching at positions 123 and 227 of the PTEN protein; (4) coil structure for the strand exchange at position 269; (5) reduced helix elongation at position 290 (Figure 3B).

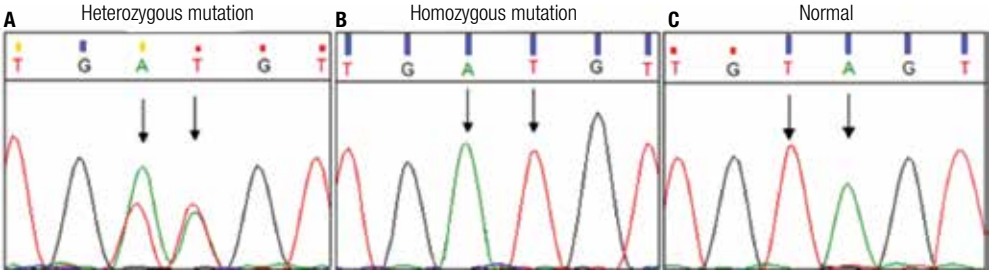

Figure 2. Sequence analysis of exon 2 of the *PTEN* gene in: **(A)** index case-DNA from peripheral leukocytes: T-to-A inversion – at position 159 and A-to-T inversion at position 160 (arrows), leading to a heterozygous missense mutation (GTA – GAT) at codon 53 (p.V53D). **(B)** breast tumor cells: homozygous mutation (GTA – GAT) at codon 53, denoting loss of heterozygosity. **(C)** son (normal).

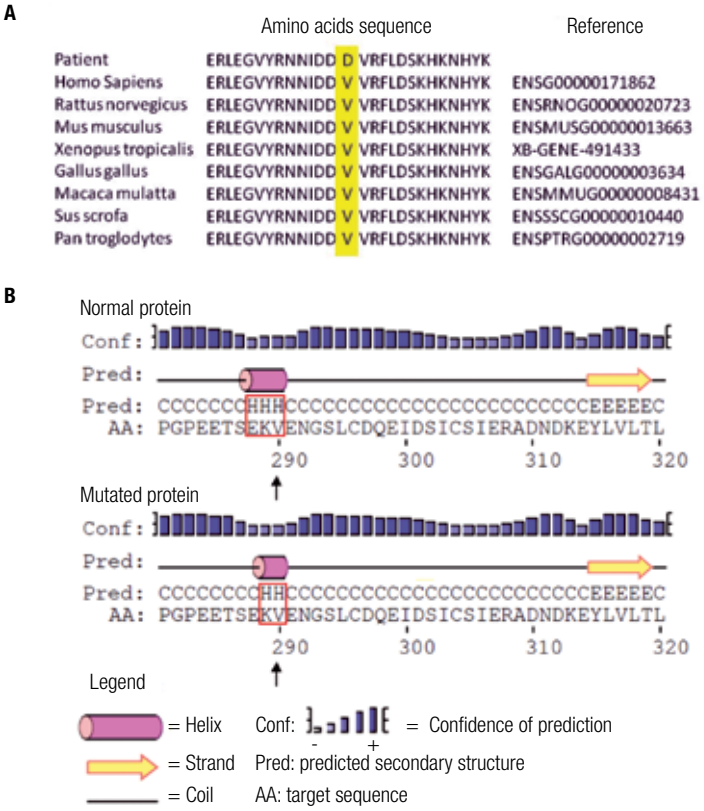

Figure 3. (A) Alignment of sequences among different species to evaluate the conservation of the amino acid valine (V53). **(B)** Analysis of secondary structure of normal and mutated PTEN protein. Arrows indicate predicted changes in protein structure caused by p.Val53Asp mutation. H: helix, C: coil, E: strand (β-sheet).

DISCUSSION

Germline mutations in *PTEN* have been described in a variety of rare syndromes, collectively known as PTEN hamartoma tumor syndromes (PHTS). The defining clinical feature of PHTS is the presence of hamartomatous tumors, which are the disorganized growth of native cells in native tissues (11). The phenotypic spectrum of PHTS is seen in Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and adult Lhermitte-Duclos disease. Lhermitte-Duclos disease is characterized by a dysplastic expansion of ganglion cells within the cerebellum, leading to replacement of the cerebellar internal granule cell layer and, consequently, various degrees of neurological signs, such as macrocephaly, mental retardation, seizures, and tremors (12). Bannayan-Riley-Ruvalcaba syndrome presents macrocephaly, benign hamartomas, pigmented macules of the glans penis, lipomas, hemangiomas, and developmental delay or mental retardation (11). Cowden syndrome is characterized by mucocutaneous features, including trichilemmomas and papillomatous papules. PTEN hamartoma tumor syndromes are inherited in an autosomal dominant manner across all subtypes.

PTEN is a tumor suppressor gene with dual specificity phosphatase activity, and the N-terminal domain contains the phosphatase domain active site, where most *PTEN* mutations occur (13). PTEN negatively regulates the phosphatidylinositol 3-kinase-AKT and mammalian target of rapamycin (mTOR) signalling pathways, which are critical for cell proliferation, cell cycle progression, and apoptosis. Loss of function in this gene is a risk factor for oncogenesis and *PTEN* is therefore considered a tumor suppressor gene (3). Loss of function in *PTEN* activates these pathways and leads to increased cellular growth, migration, proliferation, and survival.

Gastrointestinal polyps, also typically found in CS, are usually asymptomatic and can occur anywhere in the tract, but mostly at the colon (13,14). Cowden syndrome patients are at increased risk of developing breast, thyroid, and endometrium cancer. Lifetime risk of breast cancer in women with CS is estimated to be as high as 50% as compared to 11% within the general population (15,16). As with other hereditary breast cancer syndromes, breast cancer is diagnosed at younger ages compared with the general population. The most frequently observed breast cancer histology is invasive ductal adenocarcinoma. As our patient had no familial history of breast cancer, but presented breast cancer at a young age, the molecular study should clarify the prognosis (16).

Benign thyroid lesions occur in up to 75% of patients with CS and may include adenomas, hamartomas, multinodular goiter, and Hashimoto's thyroiditis (17). If a molecular study is not available, total thyroidectomy is recommended, even after benign cytological diagnosis, given the increased risk of developing subsequent thyroid cancer (6). If molecular study on FNAB material is performed, total thyroidectomy should be indicated, as clinicians are aware of the increased thyroid cancer risk when the *PTEN* mutation is found. We studied the LOH in thyroid cells obtained from FNAB to clarify whether *PTEN* had a role in the thyroid cell proliferation observed in our patient. The role of *PTEN* in her abnormal cellular proliferation was consequently reinforced, despite being a benign lesion (adenomatous goiter).

It is noteworthy that the patient's father also carried the p.V53D mutation and is apparently asymptomatic. To date, he has tested normal on prostate clinical evaluation and serum PSA, as well as on kidney ultrasound and colonoscopy. These exams will be performed annually to exclude prostate, colorectal and kidney cancer, respectively. His thyroid ultrasound was also normal. As phenotype and genotype may be poorly related in CS, he is being followed up closely. As her son does not bear the *PTEN* mutation, he is not considered at risk. As recommended by the International Cowden Consortium, our patient and her father must undergo cancer screening annually (endometrium, kidney, skin, prostate, colon, and thyroid) (6).

Most of the described mutations are frameshift or stop codon mutations throughout the whole gene, except exons 1 and 9 (17). A mutational hotspot was found in exon 5, which encodes the phosphatase catalytic core motif (18). The p.Val53Asp mutation described here is located at exon 2 of the *PTEN* gene, and is located in a highly conserved amino acid (Figure 3A). Twelve different germline mutations have been identified in exon 2 and are distributed as shown in Figure 4, all related to patients with CS (15). Given the absence of a described mutation in 200 normal alleles, p.V53 has shown to be a highly conserved amino acid among species, and changes in secondary mutated protein structure corroborate to link phenotype and genotype (Figure 3). Furthermore, deletion of one amino acid (D52) was described previously, and was associated with CS (19).

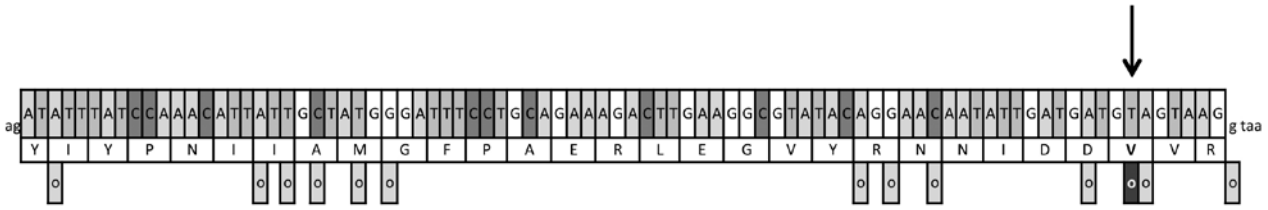

Figure 4. Representation of exon 2 of the *PTEN* gene with described mutations (circles). The arrow shows the mutation at position 159 (T to A) leading to p.Val53Asp (Mutation Database® modified).

In conclusions, we identified a novel germline p.Val53Asp mutation in the *PTEN* gene in a patient with Cowden syndrome, and demonstrated its involvement in breast cancer and thyroid lesions. Clinical management of patients with CS should include early and frequent screening for associated malignancies.

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