Original Article

A novel *PTEN* gene promoter mutation and untypical Cowden syndrome

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Abstract: Cowden syndrome (CS), an autosomal dominant disorder, is one of a spectrum of clinical disorders that have been linked to germline mutations in the phosphatase and tensin homolog (*PTEN*) gene. Although 70-80% of patients with CS have an identifiable germline *PTEN* mutation, the clinical diagnosis presents many challenges because of the phenotypic and genotypic variations. In the present study, we sequenced the exons and the promoter of *PTEN* gene, mutations and variations in the promoter and exons were identified, and a *PTEN* protein expression negative region was determined by immunohistochemistry (IHC). In conclusion, a novel promoter mutation we found in *PTEN* gene may turn off *PTEN* protein expression occasionally, leading to the disorder of *PTEN* and untypical CS manifestations.

Key Words: Cowden syndrome; PTEN; immunohistochemistry

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Introduction

Cowden syndrome (CS) is a multi-system disorder involving increased risks for a number of malignancies as well as benign hamatomatous overgrowth of various tissues (1,2). CS was first described in 1963 (3), and was named after the family in which it was reported. Approximately 40% to 60% of CS patients inherit the disease. The International Cowden Consortium developed the original diagnostic criteria. More than 80% of patients who strictly met these criteria were subsequently found to have a phosphatase and tensin homolog (*PTEN*) mutation.

The *PTEN* gene is located on chromosome subband 10q23.3 and codes for a major lipid phosphatase. It has a central role in regulating the phosphatidylinositol-3-kinase (PI3K) signal transduction cascade (4). It is now known to be critically important both during embryonic development and as a tumor suppressor in mature organisms. Inactivation of *PTEN* may be caused by germline mutations of exons or promoter (5). Germline mutations in *PTEN* gene occur in 85% of patients with CS (6). Teresi *et al*. had emphasized the importance of *PTEN* promoter nucleotide variations and their ability to lead to CS progression (7). In addition to screening for deletions involving PTEN, using sequence analysis of the *PTEN* promoter to identify *PTEN* mutations is currently one of the criteria of CS diagnosis.

In the present study, we sequenced the exons and the promoter of *PTEN* to determine the mutation of *PTEN* gene. We found mutations in the *PTEN* promoter, which might induce the dysfunction of *PTEN*.

Methods and materials

Patient characteristics

A 72-year-old woman was admitted to Peking Union

Table 1 Primers of *PTEN* exons and promoter

Primer	Product length
PTEN E1	85
Forward: GAGGATGGATTCGACTTAGACTTGA	
Reverse: CCCACGTTCTAAGAGAGTGACAGAA	
PTEN E2	163
Forward: GTTTGATTGCTGCATATTTCAG	
Reverse: TGAAATAGAAAATCAAAGCATTC	
PTEN E3	178
Forward: AAAATCTGTCTTTTGGTTTTTC	
Reverse: TTGCAAGCATACAAATAAGAA	
PTEN E4	205
Forward: CATTATAAAGATTCAGGCAAT	
Reverse: GACAGTAAGATACAGTCTATC	
PTEN E5	282
Forward: CTTTTTACCACAGTTGCACA	
Reverse: GGAAAGGAAAAACATCAAAA	
PTEN E6	120
Forward: CCTGTTAAAGAATCATCTGGA	
Reverse: AAGGATGAGAATTTCAAGCA	
PTEN E7	172
Forward: AGGCATTTCCTGTGAAATAA	
Reverse: TTGATATCACCACACACAGG	
PTEN E8	245
Forward: CTCAGATTGCCTTATAATAGTC	
Reverse: TCTGAGGTTTCCTCTGGTC	
PTEN E9	260
Forward: TCATATTTGTGGGTTTTCATT	
Reverse: TCATGGTGTTTTATCCCTCT	
Promoter	683
Forward: GCGTGGTCACCTGGTCCTTT	
Reverse: GCTGCTCACAGGCGCTGA	

Medical College Hospital (PUMCH) for an investigation of her personal history of multiple neoplasms. She suffered medullar carcinoma in the left breast without lymph node invasion at the age of 56. She underwent a modified radical mastectomy followed by taking Tamoxifen for 7 years without any signs of cancer relapse. She suffered colon tubulovillous carcinoma mosaicking with tubulovillous adenoma and severe atypical hyperplasia and papillary carcinoma in the right lobe of thyroid gland at the age of 69. In the following year, she was found to harbor a myoschwannoma at the right C3-4 intervertebral foramen. This benign tumor was again surgically removed concerning the possibility of spinal cord compression. A brief survey of diseases of her family members was performed.

Samples collection

Tissues of the breast cancer, colon cancer, thyroid cancer, and myoschwannoma were collected in operation when tumors were removed from the patient. A part of the tissues were paraffin-embedded for pathologic analysis. A part of tissues were snap-frozen with liquid nitrogen, and were stored in –80 °C refrigerator until further assay. Also the clinical characteristics of the patient were collected.

Immunohistochemical analysis

5-μm-thick paraffin-embedded tissue sections were deparaffinized with xylene and rehydrated with ethanol. Endogenous peroxidase was blocked with 0.3% H_2O_2. Antigen retrieval was performed in 0.1 mol/L sodium citrate buffer (pH 6.0) with a microwave. Samples were incubated with rabbit anti-human PTEN polyclonal antibody (ab31392, 1:1,000, Abcam, Cambridge, UK) at room temperature and detected with a horseradish peroxidase (HRP) conjugated compact polymer system, and 3,3'-diaminobenzidine (DAB) was used as the chromogen. Slides were counterstained with hematoxylin and mounted with depex. Photographs of immunohistochemically stained sections were taken by a camera mounted on a Keyence BZ-8000 digital microscope (Keyence, Osaka, Japan). Immunochemical staining was examined by two pathologists blinded to the origin of the sections independently.

DNA extraction and sequencing

Genomic DNA was isolated from 30 mg tissues using ChargeSwitch® gDNA Mini Tissue Kit following the producer's instruction. To amplify 9 exons and promoter between –1,389 bp and –707 bp upstream of the translation start codon of *PTEN*, poly chain reaction(PCR) was performed using the primers listed in *Table 1* with 5 μg genomic DNA as template. The PCR products were purified and sequenced with Sanger Sequencing which was performed by lifetechnology.

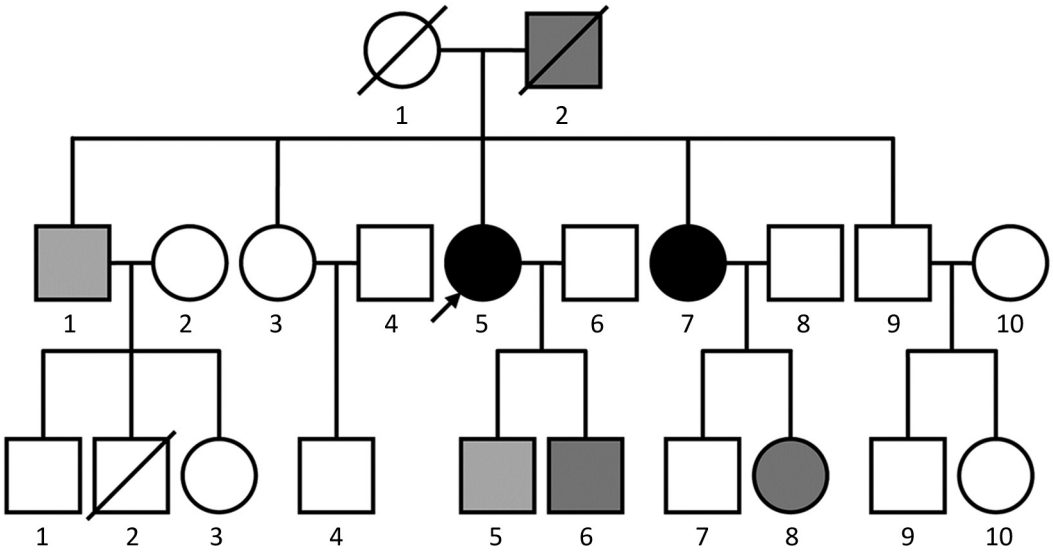

Figure 1 The pedigree of the family. If this patient could be diagnosed as CS according to the operational criteria proposed by the International Cowden Syndrome Consortium, some members of this family could be diagnosed likewise. The darker color, the more likely. I-2, urinary cancer; II-1, gall bladder polypus; II-3, endometrial cancer; II-7, breast cancer, multiple uterine fibroids; III-5, multiple skin lipomas; III-6, multiple hamartomas in the gall bladder, multiple skin lipomas; III-8, breast fibrocystic disease

Results

Outcomes of patient and pedigree of family

The patient still survived when the article was prepared. The pedigree of the family showed a relatively high incidence of malignancies in this family (*Figure 1*). The pedigree of the family showed that seven persons had suffered cancer. One person was the 1st generation, 3 persons were the 2nd generation and 3 persons were the 3rd generation. The types of cancers were showed in *Figure 1*.

Expression of PTEN in tumors

To investigate the expression of PTEN in the four surgically resected tumor tissues, immunohistochemical analysis was performed. In most slices, PTEN expressed normally (*Figure 2A*,*C*,*D*). A PTEN-negative region was found at the basal part of the colon tubulovillous carcinoma (*Figure 2B*).

Mutation of PTEN

To further explore the mechanisms of PTEN expression in the malignance, we performed a series of analyses on *PTEN* gene. We sequenced 9 exons of *PTEN*, and found no mutation of the exons (data not shown). Then the *PTEN* promoter was sequenced with primers designed to amplify the region between –1,389 bp and –707 bp upstream of the translation start codon. Which included the full–length *PTEN* promoter between –1,344 bp and –745 bp. We examined the downstream effect of *PTEN* promoter variants (such as –861G/T, –853C/G, –834C/T, –798G/C, and –764G/A), which have been reported to be associated with CS. But we found no mutations of above promoter variants. A G>T mutation was found at –1,312 (*Figure 3*).

Discussion

CS is a rare autosomal dominant inherited disorder characterized by multiple tumor-like growths called hamartomas with an increased risk of certain forms of cancer (1). CS follows an autosomal dominant inheritance pattern in which a mutation happens in only one copy of the gene. Thus the descendants of the patients may suffer CS. The pedigree of the family may implicit this, but the limitation of the current study is that we could not get the DNA species and sequence the DNA of all members of the family. At least four genes, *PTEN*, *SDHB*, *SDHD*, and *KLLN*, have been identified in people with CS or Cowden-like syndrome. Most cases of CS and a small percentage of cases of Cowden-like syndrome result from mutations in *PTEN* gene (8). According to the International Cowden Consortium Operational Diagnostic Criteria, about 80% of

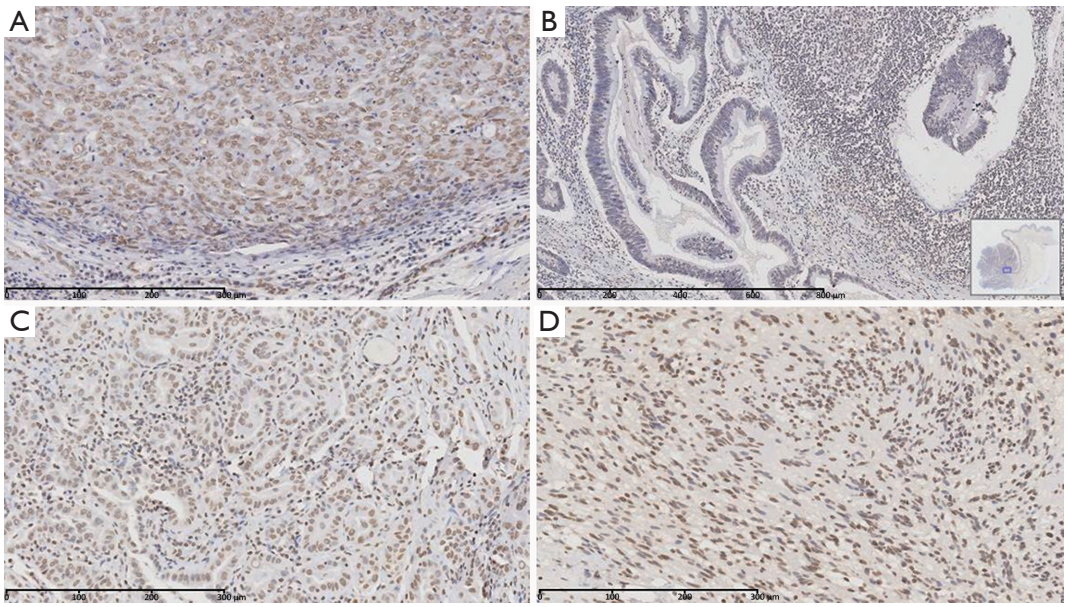

Figure 2 Immunohistochemistry with anti-PTEN staining. A. Breast medullar carcinoma (40×), normal expression; B. Colon tubulovillous carcinoma mosaicking with tubulovillous adenoma (20×), carcinoma, absent expression; C. Thyroid papillary carcinoma (40×), normal expression; D. Myoschwannoma (40×), normal expression

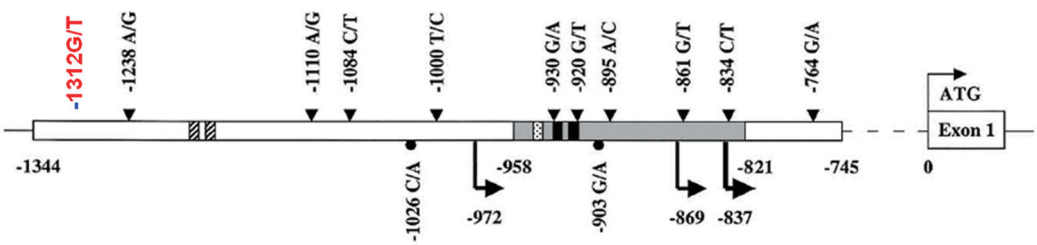

Figure 3 Mutation in the *PTEN* promoter. Mutations of *PTEN* promoter which were associated with CS were sequenced. G>T mutation was found at –1,312 of the *PTEN* promoter in the current patient

patients with CS demonstrate germline *PTEN* mutations (9). In the current study, we found the dysfunction of *PTEN*, and the sequencing results showed the mutation of *PTEN* which located in the promoter.

PTEN has dual protein and lipid phosphatase activity, and its tumor suppressor activity is depend on its lipid phosphatase activity, which negatively regulates the PI3K-AKT-mTOR pathway (10). Dysfunction of PTEN protein results in disorder of signal pathway of PTEN/AKT (4). This may caused by mutation of exons or promoter (11). The mutations of exons down-regulate the expression of PTEN protein and contribute to PTEN-related diseases such as cancers (12,13). Reports have shown the roles of PTEN in breast tumor, gastric cancer and lung cancer (14-16). Germline mutations in *PTEN* have been described in a variety of rare syndromes that are collectively known as the PTEN-hamartoma tumor syndromes (PHTS). And CS is the best-described syndrome within PHTS (6). Patients with CS have an increased incidence of cancers of the breast, thyroid and endometrium, which correspond to sporadic tumor types that commonly exhibit somatic PTEN inactivation. PTEN is a constitutively expressed in normal cells (17). Zhou *et al.* have revealed a reduction in protein with *PTEN* promoter mutation-positive CS patients (18). In the present study, we found the patient have tubulovillous carcinoma, breast cancer and myoschwannoma. In this case, we did find a PTEN-negative region in the immunohistologically stained slices, which was located

on the basal part of the colon tubulovillous adenoma. Subsequent sequencing revealed six mutations, five of which were located within the previously reported *PTEN* promoter region. These mutations may result in underexpression of PTEN, and increase cancer susceptibility. Teresi *et al.* have revealed that *PTEN* promoter was regulated by statins and SREBP (18). Sheng *et al.* have reported that a P53-binding sequence is located within the 599 bp fragment (–1,344/–745) in *PTEN* promoter (19). In the present study, we found the mutation of *PTEN* located in –1,312, which is the region of P53-binding sequence. We hypothesized that the mutation of this location may interfere the P53-induced PTEN expression, but this needs further investigation. And the successive incidence of tumors with germline mutations of *PTEN* needs further study.

Moreover, we should not ignore other possible explanation for the oncogenesis in this case, for instance, the germline *KILLIN* or *PTEN* methylation (20,21). As it is found recently that even subtle changes in expression of the *PTEN* tumor suppressor gene, such as hyper-methylation, can significantly increase cancer susceptibility in several organs, and may account for CS and Cowden-like syndrome (22). And the patients with germline variations in succinate dehydrogenase (*SDH*) genes also have higher thyroid and breast cancer prevalence in Cowden and Cowden-like syndrome. Studying *PTEN* in the continuum of rare syndromes provides insight into the role of *PTEN* in progression of rare disease and will inform targeted drug development.

In conclusion, the promoter of *PTEN* may turn off occasionally leading to the disorder of PTEN and the incidence of CS.

Acknowledgements

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