

## A Novel *PTEN* Mutation in a Korean Patient with Cowden Syndrome and Vascular Anomalies

Byung Gi Bae<sup>1</sup>, Hee Jung Kim<sup>2</sup>, Sang-Guk Lee<sup>3</sup>, Jong Rak Choi<sup>3</sup>, Chul Hwang<sup>4</sup>, Jeung Hoon Lee<sup>4</sup>, Kyung-A Lee<sup>3</sup> and Min-Geol Lee<sup>1\*</sup>

<sup>1</sup>Department of Dermatology and Cutaneous Biology Research Institute, Brain Korea 21 project for Medical Science, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul, 120-752, <sup>2</sup>Department of Dermatology, Kangbuk Samsung Hospital, Sunkyunkwan University School of Medicine, Seoul, <sup>3</sup>Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, <sup>4</sup>Department of Dermatology, College of Medicine & Medical Research Institute, Chungnam National University, Daejeon, Korea. \*E-mail: mglee@yuhs.ac  
Accepted August 5, 2010.

Cowden syndrome (CS; OMIM #158350) is a rare autosomal dominant disorder characterized by multiple hamartomas of ectodermal, mesodermal and endodermal origins. CS patients have a high risk of developing breast, thyroid and endometrial cancers (1). Typical mucocutaneous symptoms include facial trichilemmomas (hamartomas of the infundibulum of the hair follicle), acral keratoses and mucocutaneous papules. Other mucocutaneous lesions include dermal fibromas, scrotal tongue and skin tags (2). Additional disease features may include adult-onset Lhermitte-Dulcros disease (LDD) (a dysplastic gangliocytoma of the cerebellum), macrocephaly, mental retardation and structural malformations in the genitourinary system (3). This list has recently been revised (4).

*PTEN* is a tumor suppressor gene located on chromosome 10q23.3. It encodes a phosphatase that influences the cell cycle, causing G1 arrest and apoptosis (5). *PTEN* hamartoma tumor syndromes (PHTS) are a group of disorders characterized by *PTEN* mutations and hamartomas of multiple organ systems. They include CS, Bannayan-Riley-Ruvalcaba syndrome (BRRS; OMIM #153480), Proteus syndrome, and Proteus-like syndrome (6). Some 85% of CS patients carry germline loss-of-function mutations in the *PTEN* gene. Among PHTS, it has been reported that BRRS and CS share clinical characteristics and represent a single entity. However, BRRS can be clinically differentiated from CS by hamartous polyposis, macrocephaly, lipomatosis, hemangioma and a speckled penis.

### CASE REPORT

A 28-year-old woman presented with multiple flesh-colored papules on her face and multiple palmar keratoses (Fig. 1). Additionally, she had a history of papillary thyroid cancer, a cervical arteriovenous malformation (AVM) and a hemangioma on the right forearm. All of these conditions had been treated prior to her visiting our department. A subtotal thyroidectomy, total excision of the cutaneous hemangioma and incomplete embolization therapy for the cervical paraspinous AVM were conducted. A recent thyroid ultrasound had shown that four thyroid masses remained. A fine-needle aspiration biopsy revealed benign adenomatous hyperplasia. She had no abnormalities of the oral mucosa, such as oral mucosal papillomatosis. She was not mentally disabled and had normocephaly (height 164 cm, 70<sup>th</sup> percentile; head circumference 55.4 cm, 55<sup>th</sup> percentile). The patient denied any family history of mucocutaneous lesions, hemangiomas, thyroid disease or mental retardation. Routine physical examinations and breast self-examinations

had revealed nothing abnormal. The results of laboratory tests, including a full blood cell count, biochemical analyses of the blood that assessed glucose levels and liver and renal function, and urinalysis, were either within normal limits or negative. Multiple biopsies from facial skin affected by papules revealed follicular plugging and hyperkeratosis (Fig. 2a). Histopathological analysis of biopsy specimens from skin affected by palmar keratoses showed epidermal hyperplasia with hypergranulosis and marked orthokeratotic hyperkeratosis (Fig. 2b).

Genomic DNA was prepared from peripheral blood leukocytes for use in germline mutation analyses. We performed a polymerase chain reaction amplification of exons 1-9 of the *PTEN* gene and sequenced all of the exon and intron junctions bilaterally. We identified a novel germline heterozygous mutation, c.209+4\_7delAGTA, in exon 3 (Fig. 3). Furthermore, her 2-year-old daughter, who did not have any mucocutaneous lesions and displayed normocephaly (head circumference 48.3 cm, 51.6%), was also found to be carrying this mutation. Through PCR amplification of *PTEN* mRNA, using following primers: *PTEN* F2, 5'-TCAAGAGGATGGATTCCGACTT-3'; *PTEN* R2, 5'-CGCCACTGAACATTGGAATA, we finally sequenced the patient's mRNA.

### DISCUSSION

According to the results of mRNA sequencing, the novel germline deletion mutation causes transcriptional skipping of exon 3. The novel germline mutation identified in this study is predicted to cause single amino acid substitution



Fig. 1. Multiple rice grain-sized papules were present on the face.

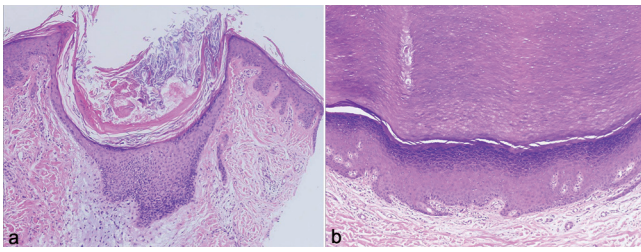


Fig. 2. Histopathological features. (a) Follicular keratotic plugging of a facial papule. (b) Acanthosis with hypergranulosis and marked orthokeratosis in a palmar keratosis. (a, b) H&E, original magnification  $\times 100$ .

(R55S) and deletion of amino acids 56-70 in PTEN's N-terminal phosphatase domain. We were unable to confirm whether or not the modified PTEN molecule was synthesized *in vivo*.

PTEN contains two key domains: the N-terminal domain and the C-terminal domain. Most *PTEN* mutations occur within the N-terminal phosphatase domain (amino acids 1-185). Analysis of the structure of PTEN revealed that its phosphatase domain, although similar to those of other phosphatases, has a slightly larger active site. This larger active site is accessible to phospholipid substrates, a unique feature of PTEN (7). It is also a mutational hot spot. This is especially true of exon 5, which accounts for 20% of the entire coding sequence and encodes the catalytic core. Exon 5 carries about 40% of the germline mutations in this gene. Approximately two-thirds of all mutations are found in exons 5, 7 and 8. Mutations in exon 3 have rarely been reported (8).

It has been demonstrated that genetic background and modifier genes more strongly influence the onset and spectrum of tumor formation than the specific *PTEN* mutation present (9).

Facial trichilemmomas, a hallmark of CS, were not detected, despite multiple skin biopsies of the facial papules being analyzed. Brownstein et al. (10) reported that biopsies of multiple facial skin lesions were necessary in order to detect evidence of trichilemmomas. It has been reported that punctate palmoplantar keratoses in childhood without other mucocutaneous lesions may be a clue for the early diagnosis of PHTS (11). Our patient's symptoms – multiple facial papules, palmar keratoses, papillary thyroid cancer, thyroid adenomatous hyperplasias and a germline *PTEN* mutation – strongly suggest that the *PTEN* hamartoma tumor syndrome she is suffering from is CS.

Our patient exhibited two vascular anomalies. One was a cutaneous hemangioma, and the other a cervical paraspinous AVM. Recently, Tan et al. (12) described 26 patients with vascular anomalies and *PTEN* mutations. They identified AVMs in 54% of the patients with *PTEN* mutations. This high frequency may be explained by the make-up of the study population, which included individuals who had been treated at a vascular anomalies center. None suffered from hemangiomas or other pure

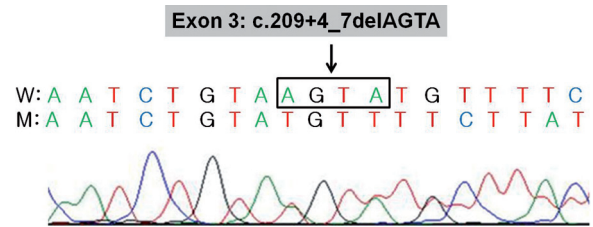


Fig. 3. A four-nucleotide deletion (c.209+4\_7delAGTA) in exon 3 of the *PTEN* gene. Arrow, deleted nucleotides; W, wild-type allele; M, mutated allele.

vascular tumors, and paraspinous AVM was found in only two cases.

There is growing evidence that PTEN has an anti-angiogenic effect (13) and it inhibits endothelial tube formation induced by vascular endothelial growth factor (VEGF) (14). Furthermore, loss of the *PTEN* gene results in subsequent VEGF expression (15).

Our patient wanted her daughter to undergo genetic testing, although she did not have any features of CS, including mucocutaneous lesions and macrocephaly. Examination of the daughter revealed her to be carrying the same germline *PTEN* mutation, and she was recommended for surveillance according to NCCN criteria (2).

The authors declare no conflict of interest.

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