

Novel Germline *PTEN* Mutation Associated with Cowden Syndrome and Osteosarcoma

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Abstract. *Background:* Cowden syndrome (CS) is a rare autosomal-dominant inherited disorder characterized by multiple hamartomas. While the hamartomas are benign, patients with CS have increased risk of osteosarcoma and of breast, thyroid, endometrial, soft-tissue and colonic neoplasms. Germline mutations of phosphatase and tensin homolog (*PTEN*) are implicated in CS and in the development of osteosarcoma. We report a patient with CS who presented with osteosarcoma, ganglioneuromatosis and a benign breast mass. Osteosarcoma, as presentation of CS, is rare (only one report in the English literature). Genomic DNA from the patient's peripheral blood was quantified by spectrophotometry, then underwent sequence enrichment, polymerase chain reaction and next-generation sequencing. Molecular analysis revealed a non-synonymous c.17_18delAA frameshift mutation in exon 1 of *PTEN* and a c.116G>T (p.R39L) missense mutation of serine/threonine kinase 11 (*STK11*) of unknown significance. *Conclusion:* We report a patient with CS presenting with ganglioneuromatosis, benign breast mass and osteosarcoma, harboring a novel molecular alteration in *PTEN* which to our knowledge has not been previously reported.

Cowden syndrome (CS) is an autosomal dominant disorder characterized by neoplasms originating from all three embryonic layers. Thirty-five to 85% of patients with CS will have gastrointestinal polyps (1). In a study of patients with CS, 25% (16/62) of the patients were found to have had ganglioneuromatous polyps. Ganglioneuromas are

pathologically benign outgrowths. These hamartomatous lesions are composed of ganglion cells, supporting cells and nerve fibers, and arise within the enteric nervous system. They can be further divided into three subcategories: polypoid ganglioneuroma, ganglioneuromatous polyposis, and diffuse ganglioneuromatosis. Out of these, only diffuse ganglioneuromatosis is a systemic/neurogenic disease (2). It has been shown that mutations in the tumor-suppressor genes "rearranged during transfection" (*RET*), neurofibromatosis type 1 (*NFI*) and phosphatase and tensin homolog (*PTEN*) influence the development of a ganglioneuroma and alterations of *PTEN* have been implicated in CS (3, 4). Here we present an interesting case of a 32-year-old female with CS manifesting with colonic ganglioneuromatous polyps, benign breast disease and osteosarcoma. Review of the literature revealed only one case of a patient with CS presenting with multiple neoplasms that included osteosarcoma (5).

Our intention here was to provide insight on a novel molecular alteration that may explain a unique clinical manifestation of Cowden syndrome.

Case Report

A 32-year-old female, presented in 2009 with an osteosarcoma of her left femur (Figures 1 and 2), which was treated with surgery and chemotherapy. The patient returned in 2014 with a right benign breast mass (fibroadenoma). Because of previous family history and previous presentation with osteosarcoma, the patient underwent genetic testing which revealed *PTEN* mutation. In view of this finding, the patient elected to undergo prophylactic bilateral mastectomy in 2015. A screening colonoscopy, performed in July 2015, revealed 75-100 polyps. During this procedure, biopsies from different areas of the colon were taken. Pathological examination revealed five ganglioneuromas in the splenic flexure, 16 ganglioneuromas in the descending colon, and four ganglioneuromas in the sigmoidal colon. The remaining

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polyps were hyperplastic polyps or large reactive lymphoid aggregates. Relevant patient family history included bladder cancer in her maternal grandfather; ovarian cancer in her maternal grandmother; brain, breast, colon, thyroid cancer in her mother; and a soft-tissue mass in her brother. The family members did not undergo genetic testing.

Biopsy analysis. The biopsies were processed using the routine method for hematoxylin & eosin stain and evaluated by pathologists with interest in gastrointestinal pathology (DC and MAH). Three-micron sections were immunohistochemically stained for Soluble 100 (S100) and neuron-specific enolase (NSE) using a Ventana automated immunostainer (Ventana BenchMark Ultra, Tucson, AZ, USA) employing a rabbit polyclonal antibody to S100 (IR504, dilution 1:250 and no antigen retrieval; DAKO, Carpinteria, CA USA) and mouse monoclonal antibody NSE (E27, no dilution and using Cell Conditioning 1 for 36 minutes; Cell Marque, Rocklin, CA USA).

Given the presentation of this patient with ganglioneuromatosis, benign breast mass and osteosarcoma, we analyzed the genetic profile of this patient's blood to try to detect molecular abnormalities that may be related to this unusual disease presentation. Genetic testing of the patient's blood was performed at Ambry Genetics (Aliso Viejo, CA, USA) using next-generation sequencing (NGS).

The CancerNext Assay used by Ambry Genetics is a comprehensive screen of 28 genes associated with hereditary cancer predisposition. Genomic deoxyribonucleic acid (gDNA) is isolated from the patient's specimen using standardized methodology and quantified using a spectrophotometer. Sequence enrichment of the targeted coding exons and adjacent intronic nucleotides is carried out by incorporating the gDNA into microdroplets along with primer pairs or by a bait-capture methodology using long biotinylated oligonucleotide probes followed by polymerase chain reaction (PCR) and NGS. Additional Sanger sequencing is performed for any regions missing or with insufficient read depth coverage for reliable heterozygous variant detection. Suspect variant cells other than those classified as "likely benign" or benign" are verified by Sanger sequencing in sense and antisense directions. Gross deletion/duplication of variant detection. Gross deletion/duplication analysis for all 28 genes is also performed utilizing a targeted chromosomal microarray. The analytical range of the CancerNext assay targets detection of DNA sequence mutations in 28 genes, including adenomatous polyposis coli (*APC*), ataxia-telangiectasia mutated (*ATM*), BRCA1-associated ring domain 1 (*BARD1*), bone morphogenetic protein receptor type 1A (*BMPRIA*), breast cancer susceptibility type 1 (*BRCA1*), *BRCA2*, BRCA1-interacting protein 1 (*BRIP1*), cadherin 1 (*CDH1*), cyclin-dependent kinase 4 (*CDK4*), CDKN2A, checkpoint kinase 2 (*CHEK2*), epithelial cell adhesion molecule (*EPCAM*), mutL homolog 1



Figure 1. Lateral radiograph showing the osteosarcoma of distal left femur as a radiodense lesion. A Codman's triangle of reactive bone can be seen in the lateral cortex.

(*MLH1*), meiotic recombination 11 homolog A (*MRE11A*), mutS homolog 2 (*MSH2*), MSH6, mutY homolog (*MUTYH*), nibrin (*NBN*), neurofibromatosis type 1 (*NF1*), partner and localizer of BRCA2 (*PALB2*), PMS1 homolog 2 (*PMS2*), phosphatase and tensin homolog (*PTEN*), DNA repair protein RAD50 (*RAD50*). *RAD51C*, *RAD51D*, mothers against decapentaplegic homolog 4 (*SMAD4*), serine/threonine kinase 11 (*STK11*), and tumor protein 53 (*TP53*). These mutations were detected by either NGS or Sanger sequencing of all coding domains and well into the flanking 5' and 3' ends of all the introns and untranslated regions. In addition, sequencing of the promoter region is performed for the following genes: *PTEN*, *MLH1* and *MSH2*. Gross deletion/duplication analysis determines gene copy number for all the covered exons and untranslated regions of all 28 sequenced genes and *EPCAM*.

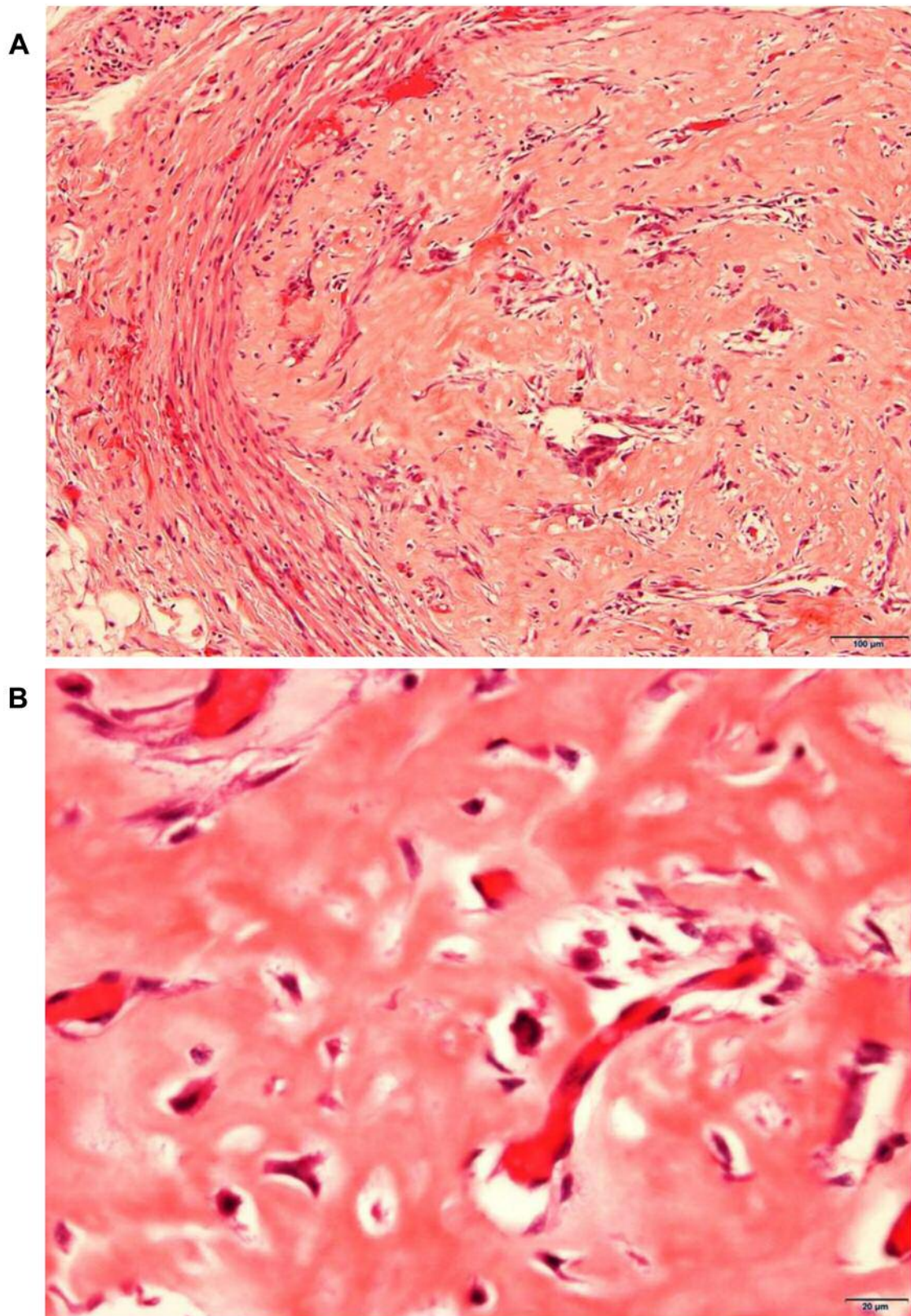


Figure 2. A: Low-power view of the osteosarcoma and its interphase with the adjacent soft tissue. B: At high power, neoplastic cells with nuclear atypia can be seen, surrounded by lace-like deposition of osteoid.

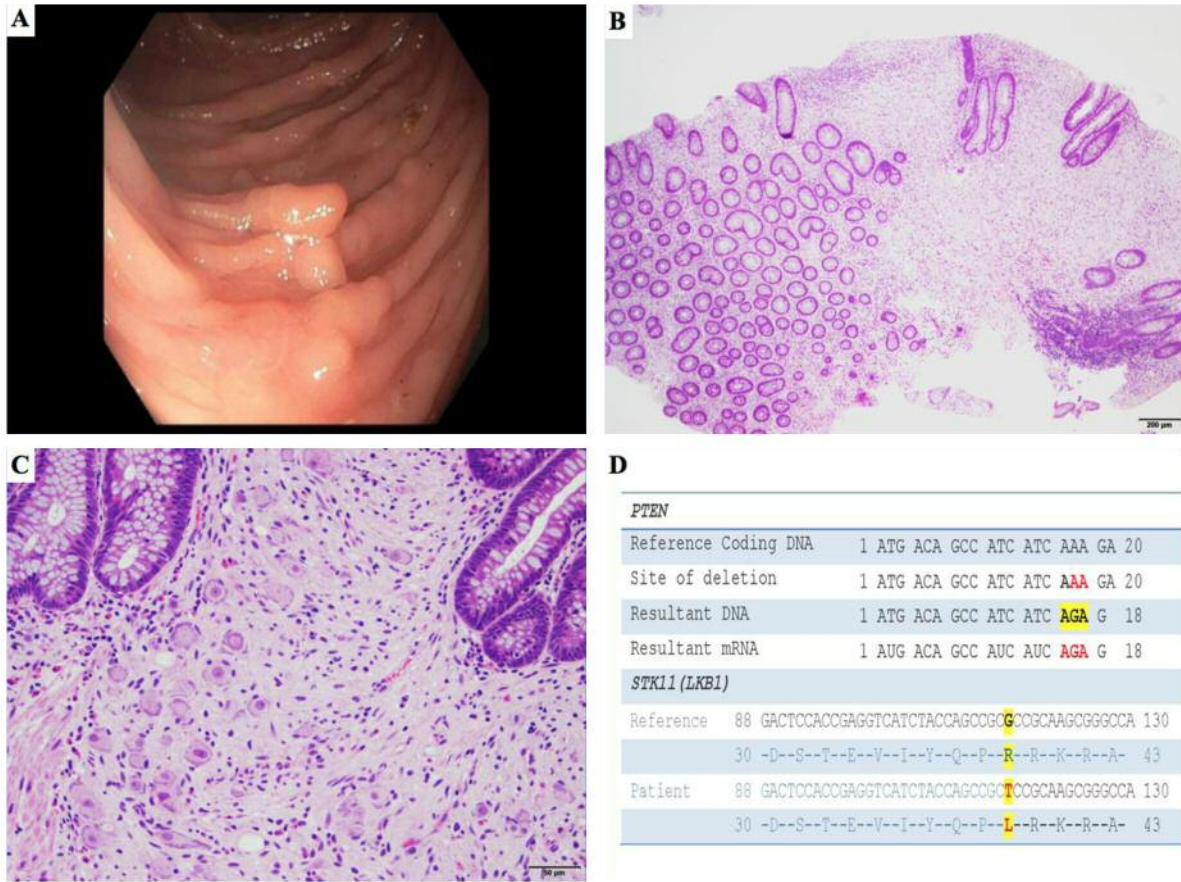


Figure 3. A: Endoscopy showing multiple mucosal polypoid lesions with broad base. B and C: Microscopically, each polyp was composed of a colonic mucosa expanded by a proliferation of spindle cells and ganglion cells, isolated or in clusters, displacing and distorting the crypts. D: Mutations found in the phosphatase and tensin homolog (*PTEN*) and serine/threonine kinase 11 (*STK11*) genes.

The following references were used by Ambry Genetics to identify variant analysis: the 1000 Genomes Project Consortium (<http://www.internationalgenome.org/about/>), Berkeley Drosophila Genome project (<http://www.fruitfly.org/>), Database of Single Nucleotide Polymorphisms (<https://www.ncbi.nlm.nih.gov/snp/>), ESE finder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/ese_finder.cgi?process=home), Exome Variant Server (<http://evs.gs.washington.edu/EVS/>), amino acid difference formula to help explain protein evolution (<http://science.sciencemag.org/content/185/4154/862.long>), HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>), Online Mendelian Inheritance in Man (<https://www.ncbi.nlm.nih.gov/omim>), PolyPhen (<http://genetics.bwh.harvard.edu/pph/data/>), American College of Medical Genetics and Genomics recommendations for standards for interpretation and reporting of sequence variations (<https://www.nature.com/articles/gim200844>), and Sorting Intolerant from Tolerant (<http://sift.bii.a-star.edu.sg/>) as per Ambry Genetics.

Results of biopsy analyses. Gross pathology: Colonoscopy revealed hundreds of polyps throughout the colon (Figure 3A). Biopsies of these polyps were performed from the transverse, splenic flexure, descending and sigmoidal colon. Each biopsy consisted of multiple fragments of soft tan-pink tissue ranging in size between 0.3 cm and 0.6 cm.

Microscopic pathology: Microscopically, each polyp was composed of a colonic mucosa expanded by a proliferation of spindle cells and ganglion cells isolated or in clusters, displacing and distorting the crypts (Figure 3B and C). The spindle cells were S100-positive and the ganglion cells were positive for NSE by immunohistochemistry.

Next-generation sequencing: Genetic testing results from Ambry Genetics revealed alterations in the genes *PTEN* and *STK11*. The patient was found to be heterozygous for a c.17_18delAA pathogenic mutation at exon 1 of the *PTEN* gene (Figure 3D). This mutation results in a translational frameshift with a predicted stop codon. A c.116G>T

missense mutation of unknown significance was also reported in the *STK11* gene (Figure 3D). Sequence analysis was performed based upon the following National Center for Biotechnology Information reference sequences: *PTEN*-NM_000314.4 and *STK11*-NM_000455.

Discussion

Molecular alterations of *PTEN* are associated with a class of syndromes called the *PTEN* Tumor Hamartoma Syndrome, of which, CS is the most common.

CS is a rare autosomal dominant disorder with variable expressivity that increases the probability of malignancies and is characteristic of hamartomatous growth in cells of all three embryonic layers. Patients with CS have a 25-50% increased risk of breast cancer and a 3-10% increased risk of non-medullary thyroid cancer. They also have increased prevalence of benign tumors arising in several organs including the thyroid (50-67%), breast (fibroadenoma/fibrocystic; 76% of affected females) and gastrointestinal system (hamartomatous polyps; 40%). Patients with CS are also at increased risk for brain lesions such as Lhermitte-Duclos (3). Eighty percent of individuals with CS have germline molecular alterations in the *PTEN* gene (1), the majority occurring in exons 5-8 (6). However, in this patient the *PTEN* mutation was found in exon 1 and as far as we are aware has not been previously reported.

Osteosarcoma is the most common primary malignant bone tumor. Down-regulation of *PTEN* has been implicated in many osteosarcomas and it has been related to either decreased *PTEN* copy number variations or to an increase in miRNAs specifically targeting *PTEN* transcripts (7-11). Thus, a germline *PTEN* alteration could have played a role in the tumorigenesis of both osteosarcoma and diffuse ganglioneuromatosis in this patient. Heterogeneity across tumors has made it difficult to identify the genetic aberrations which lead to the development of osteosarcoma. The only genes implicated with certainty are *TP53*, retinoblastoma transcriptional corepressor 1 (*RBI*), *CDKN2A* and myc protooncogene (*MYC*) (12). Moriarity *et al.* conducted a study seeking to identify new driver mutations during osteosarcomagenesis and found that *PTEN* was the most frequently mutated gene. Their results implied that a cooperative loss of *PTEN* and *TP53* could result in osteosarcomagenesis (13). In addition, copy number loss of *PTEN* was found in greater than 60% of osteosarcomas (13). Therefore, alteration of *PTEN* gene in this patient is in agreement with prior reports.

The patient reported here was found to have a rare heterozygous frameshift mutation with probable stop codon formation (c. 17_18delAA) in exon 1 of *PTEN*. Eighty percent of patients with CS have germline mutations in the *PTEN* tumor-suppressor gene. To date the majority of these

germline mutations reportedly occurred in exons 5 and 7 and none in exon 1 (14). Frameshift mutations are deleterious in nature and thus this type of molecular alteration results in a nonfunctional protein product.

Review of the English literature revealed only one report of a case of *PTEN* c.17_18delAA deletion. Zhou *et al.* described this deletion in a 53-year-old female diagnosed with Lhermitte-Duclos disease (15). Whether Zhou *et al.*'s patient had clinical characteristics of CS is unknown.

Our patient presented with a distinct manifestation of CS associated with a heterozygous frameshift mutation in exon 1 of the *PTEN* gene. To our knowledge, this is the first report of a deleterious germline *PTEN* mutation associated with CS and osteosarcoma.

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