Hints from a Female Patient with Breast Cancer Who Later Presented with Cowden Syndrome

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INTRODUCTION

Cowden syndrome, or multiple hamartoma, is a rare autosomal dominant inherited disorder characterized by multiple-organ benign and malignant tumors [1]. Patients with Cowden syndrome are at increased risk of developing certain cancers, including breast, thyroid, uterine and colorectal cancers [2]. In 1997, germline mutations of the PTEN gene were found to be associated with Cowden syndrome [3], and most cases of Cowden syndrome resulted from mutations in the PTEN gene [4]. To date, 4 genetic loci, PTEN, SDHB, SDHD, and KLLN, have been shown to be associated with Cowden syndrome [5]. Several recent studies using whole-exome sequencing (WES) have demonstrated unexpected cancer-predisposition gene variants in patients with Cowden syndrome without underlying germline PTEN mutations [6]. Genotype-phenotype correlations require further investigation due to complex phenotypes and various clinical diagnoses of Cowden syndrome. The aim of this study is to elucidate the molecular pathogenesis and genotype-phenotype correlations in a woman with...
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Conceptualization: Wang WC; Formal analysis: Lai YC; Funding acquisition: Wang WC; Investigation: Hou TX, Kuo CY, Lai YC; Methodology: Hou TX, Kuo CY, Lai YC; Resources: Wang WC; Writing - original draft: Wang WC, Lai YC; Writing - review & editing: Wang WC, Hou TX, Kuo CY, Lai YC.

Cowden syndrome, based on the analysis of genetic variants via WES of DNA samples from peripheral blood lymphocytes and paraffin-embedded tumor specimens.

CASE REPORT

A 51-year-old Taiwanese female presenting with endometrial cancer came to our attention. The patient was diagnosed with endometrial carcinoma and surgery was recommended. She had been diagnosed with bilateral breast carcinomas 5 years earlier, and then developed thyroid carcinoma within the same year as the endometrial cancer diagnosis. Additional signs included an enlarged head (macrocephaly), with a circumference of 58 cm. Symptoms of multiple mucocutaneous lesions included acral keratosis: > 3 palmarplantar keratotic pits. The Institutional Review Board of Jen-Ai Hospital approved all procedures (reference 106–06) and informed consent was obtained prior to collection of genetic material for the study.

Immunohistochemistry was performed after antigen retrieval (0.01 M citrate buffer; pH 6.0) using a rabbit anti-PTEN antibody (1:100 dilution; clone 6H2.1; Zeta Corporation, Arcadia, USA) on 4-micrometer-thick formalin-fixed paraffin-embedded tissue sections. Immunostaining was conducted with an antibody against the C-terminal 100 amino acids of PTEN. The results show a loss of PTEN expression in the thyroid, endometrial, and bilateral breast carcinomas (Figure 1).

Genomic DNA was extracted from buffy coat leukocytes and paraffin-embedded sections using a QIAamp DNA blood mini kit (Qiagen, Taipei, Taiwan) and a QIAamp FFPE tissue kit (Qiagen), respectively, according to the manufacturer’s instructions. WES was carried out at 2 biotechnology companies (Welgene Biotech, Taipei, Taiwan; Genomics BioSci & Tech, Taipei, Taiwan). For further variant analyses, variant calling and annotations were created using a Genome Analysis Toolkit. Gene sequences were aligned to reference sequences based on human genome build GRCh37/UCSC hg19.

On analysis of the WES data, 10,246 single nucleotide variants (SNVs) were identified within exons. Among them, 8,971 were common variants that were detected after sequencing DNA from blood lymphocytes and paraffin-embedded tumor specimens. All were of moderate impact. The pattern of substitutions for each signature is shown in Figure 2. In addition to SNVs, the germline common variants detected after sequencing DNA from blood lymphocytes and paraffin-embedded tumor specimens included 331 deletions, 311 insertions, 4 sequence alterations, and 1 substitution within exons (Figure 3A). There were 205 variants within the 3′- and 5′-untranslated region without a change in proteins. Of the 647 constitutional genetic variants (Supplementary Table 1), 339 were heterozygous. Among them, 333 remained heterozygous in all 4 tumor specimens, 6 showed loss of heterozygosity in at least one tumor sample, and one showed loss of heterozygosity in all 4 tumor specimens along with a change in protein coding. As expected, the frameshift (c.1008delC, p.Thr336PhefsTer8) in codon 336 was a consequence of cDNA position 2,365 from AC to A deletion in exon 8 of the PTEN gene on chromosome 10 (depth 314, high impact, Supplementary Table 2). In addition, an inframe insertion from A to AAAP at codon 60 (c.181_189dupGCAGCGCC, p.Ala61_Pro63dup) was a consequence of cDNA position 258-259 from G to GCCGCAGCGC insertion in exon 1 of MutS homolog 3 (MSH3) on chromosome 5 (depth 1367, moderate impact, Supplementary Table 2). There were 161 variants of high impact in total (Supplementary Table 3). Among them, 11 were of a depth of more than 1,000 (Supplementary Table 2).
The somatic common variants detected after sequencing DNA from paraffin-embedded tumor specimens included 15 deletions, 5 insertions, and 1 sequence alteration within exons (Figure 3B). Among them were 9 variants with a change in protein coding due to frameshifts, inframe deletions, or inframe insertions (Supplementary Table 2). The specific variants detected after sequencing DNA from paraffin-embedded thyroid tumor specimens included 10 deletions and 9 insertions within exons (Figure 3C). Among them, 11 showed changes at the protein level (Supplementary Table 2). The specific variants detected after sequencing DNA from paraffin-embedded endometrial tumor specimens were 29 deletions and 32 insertions within exons (Figure 3C). Among them, 46 showed a change at the protein level or a gain of a stop codon and 12 were of high or moderate impact at a depth of more than 50 (Figure 3C and Supplementary Table 2). The specific variants detected after sequencing DNA...
from paraffin-embedded bilateral breast tumor specimens included only 2 deletions and 2 insertions within exons (Figure 3C). None of the samples analyzed demonstrated changes at the protein level or a gain of a stop codon.
DISCUSSION

More than 10,000 common germline variants with substitutions were detected after WES of blood lymphocytes or paraffin-embedded tumor specimens. The mutational signature of this patient was unique (Figure 2), with a bimodal distribution not reported by Alexandrov et al. [7]. Furthermore, 11 and 46 specific somatic mutations were detected in thyroid and endometrial cancers, respectively. No specific variant with change at the protein level or gain of stop codon was detected in bilateral breast cancers. This patient had a history of bilateral breast carcinomas 5 years prior to the other 2 cancers. The results of this study suggest that induction of the initial bilateral breast carcinomas requires fewer mutations, while induction of the later thyroid and endometrial cancers requires more mutations (Figure 4).

Similar mismatch repair deficiency, by MLH1 or MSH2 inactivation, with a PTEN frameshift mutation has been reported to be associated with endometrial carcinomas [8,9]. PTEN plays a critical role in DNA damage repair and response through its interaction with the ATM-p53 pathway in an AKT-independent manner [10]. The PTEN variant identified in this patient causes a frameshift that is not listed in the Genome Aggregation Database (gnomAD). No common germline variants in SDHB, SDHD, or KLLN have been found to be associated with Cowden syndrome [5]. Given this patient’s history, this variant should be classified as pathogenic. The MSH3 in-frame alanine/proline insertion (p.Ala61_Pro63dup) is a polymorphism with 8.86% frequency in the gnomAD East Asian population. This variant is multiallelic, and one allele (6 repeats, rs2001675) has been reported to be associated with many insertion-deletion polymorphisms [11].

Hundreds of common germline variants were detected after WES of blood lymphocytes and paraffin-embedded tumor specimens. Cowden syndrome tumors do not typically show this.
number of insertions/deletions, and most are due to replication errors resulting from slippage during DNA replication without mismatch repair. This implies that although a PTEN frameshift mutation determines the development of Cowden syndrome, the initial driver of the mutations could actually be a DNA repair dysfunction due to a MSH3 mutation. Moreover, in tumor suppression, a PTEN frameshift mutation prevents the establishment of a cooperative role with p53 to maintain genomic stability [12]. A similar synergistic effect has been proposed in colon tumor progression, where variants of the MSH3 gene behave as low-risk alleles [13].

This patient fulfilled the National Comprehensive Cancer Network criteria for Cowden syndrome [14]. She had germline mutations of both PTEN and MSH3, and her tumors were all found to be PTEN negative due to a frameshift mutation in the PTEN gene that altered protein function. There are several possible explanations for the development of Cowden syndrome-related malignancies in this case. First, the PTEN mutation alone induced all features of Cowden syndrome including the characteristic appearance and malignancies. Second, the PTEN mutation caused the characteristic appearance, while a combination of MSH3 and PTEN mutations caused the malignancies. Third, the PTEN mutation induced the characteristic appearance, while the MSH3 mutation induced the malignancies. We were unable to differentiate between these conditions.

The thyroid and breast are not typically involved in Lynch syndrome [15]. In terms of clinical implications, breast cancer may be induced by a PTEN mutation alone, and therefore, bilateral breast cancers were first to develop in this patient. Before other malignancies occur, a PTEN test for universal screening of breast cancer patients or those with only external phenomena fitting the criteria of Cowden syndrome is suggested. In the clinical setting, we met this patient when she presented with a leading tumor; if we had missed the opportunity for carrier status detection, we would also have missed the opportunity for early detection of other primary tumors associated with these mutations. As thyroid cancer does not usually occur in other syndromes, such as BRCA or Lynch syndrome, screening should be included in the follow up of breast cancer patients with suspected Cowden syndrome. Moreover, if genetic screening is unavailable, histochemical staining for PTEN should be carried out on breast cancer specimens. In addition, breast cancer patients with a clinical manifestation of Cowden syndrome should be carefully assessed for secondary malignancies, including thyroid and endometrial carcinomas.

In summary, this case study, conducted in Taiwan, is of a 51-year-old woman presenting with metachronous tumor development in bilateral breasts, thyroid and endometrium, in that order, and showing additional signs of Cowden syndrome. Immunohistochemical data showed loss of PTEN expression in all 4 tumors. On analysis of WES data, in addition to a germline PTEN frameshift, hundreds of common germline variants, including in the MSH3 gene, and 21 somatic mutations within exons, were detected in all tumors. This implies that although a PTEN frameshift mutation can determine the development of Cowden syndrome, a DNA repair dysfunction due to a MSH3 mutation could be the initial driver of the mutations. In addition, a PTEN frameshift mutation rules out a cooperative role with p53 to maintain genomic stability in tumor suppression. According to the specific somatic mutations detected in thyroid, endometrial, and bilateral breast cancers, induction of the initial bilateral breast carcinomas requires fewer mutations, while induction of the later thyroid and endometrial cancers requires more mutations. The findings of this case study provide important clues regarding the molecular pathogenesis of the multiple benign and malignant tumors that are observed in Cowden syndrome.
SUPPLEMENTARY MATERIALS

Supplementary Table 1
Constitutional genetic variants detected within exons after whole-exome sequencing

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Supplementary Table 2
Important genetic variants detected after whole-exome sequencing

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Supplementary Table 3
Germline common variants of high impact

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REFERENCES


