

Germline Mutations of the *PTEN/MMAC1* Gene in Japanese Patients with Cowden Disease

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Cowden disease (CD) is an autosomal dominant disorder which confers a high susceptibility to diverse benign and malignant tumors. The *PTEN/MMAC1* gene was identified as being responsible for CD, since its germline mutations have been identified in affected individuals in the United States and Europe. We identified three novel germline *PTEN* mutations, a 2-bp deletion, a 1-bp insertion and a missense mutation, in three of five Japanese patients with CD. The missense mutation resided outside of the region encoding a putative phosphatase domain of the predicted *PTEN* protein, where previously reported missense mutations in CD patients have been clustered. The present result suggests that a wide range of germline *PTEN* mutations may play a role in the pathogenesis of CD.

Key words: Cowden disease — *PTEN* — Germline mutation — Breast cancer

Cowden disease (CD) is an autosomal dominant cancer predisposition syndrome associated with an elevated risk for tumors of the breast, thyroid and skin.¹⁾ The common feature of this disease is a predisposition to hamartomas, which are benign tumors containing differentiated but disorganized cells indigenous to the tissue of origin. Linkage analysis has indicated that a single locus within chromosome 10q23 is likely to be responsible for the disease.²⁾ The *PTEN/MMAC1* gene, which encodes a protein tyrosine/serine/threonine phosphatase, was identified as a tumor suppressor gene at 10q23.3, that is inactivated in various types of human tumors, including glioblastoma, prostate cancer, malignant melanoma, endometrial carcinoma, and lung cancer.^{3–6)} Recently, this gene was also identified as being responsible for CD, since germline mutations have been identified in affected individuals of CD families in the United States and Europe.^{7–10)} Furthermore, it was revealed that the wild-type allele of the *PTEN* gene was lost in tumors of CD patients with germline *PTEN* mutations.¹¹⁾ Therefore, inactivation of both alleles of the *PTEN* gene, conforming to Knudson's "2-hit" model,¹²⁾ should be responsible for tumorigenesis in CD patients. Up to the present, 56 CD families/patients have been examined for *PTEN* alterations, and 23 were found to carry germline *PTEN* mutations. Seventeen of them had nonsense or frameshift mutations leading to premature termination of the *PTEN* protein, while 5 had missense mutations. The remaining one had a splice site

mutation. Information on germline *PTEN* mutations in patients with CD should be accumulated so as to define the spectrum of *PTEN* mutations associated with CD and thereby to understand the pathogenic significance and etiologic role of *PTEN* mutations in CD.

More than forty cases of CD have been reported in Japan.¹³⁾ However, *PTEN* mutations have not yet been investigated in Japanese patients with CD. In the present study, we examined *PTEN* germline mutations in five Japanese patients with CD. After having obtained informed consent, we collected blood samples from one familial (patient #1) and 4 sporadic (patients #2–5) CD patients, who were diagnosed based on the criteria formulated by the international CD consortium (Table I). An abridged pedigree of the CD family including patient #1 is shown in Fig. 1. All the patients showed multiple polyps in the gastrointestinal tract, as well as multiple facial trichilemmomas, which are one of the major criteria for the diagnosis of CD. Four patients had multiple adenomas of the thyroid, and three of them were affected by breast cancers.

Genomic DNA was extracted from blood samples by proteinase K digestion and phenol-chloroform extraction, and polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis was performed using 9 sets of primers which amplified exons 1–9.⁶⁾ Three cases, patients #1–3, showed bands with distinct electrophoretic mobilities in exons 6, 4 and 5, respectively (Fig. 2A). Direct sequencing analysis revealed that these three patients were heterozygous for the alleles of mutations leading to changes in the predicted *PTEN/MMAC1* gene products (Fig. 2B, Table I). Patients #1 and #2 had frame-

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Table I. Clinical Features and *PTEN* Mutations in Japanese Patients with Cowden Disease

Patient	Sex	Age	Skin	Thyroid	Breast ^{a)}	Gastrointestinal tract	Others	Mutation	Predicted effect
1	F	56	trichilemmomas fibroma	multiple adenomas	cancer (53)	multiple polyps		del AT in Y177 (exon 6)	frameshift
2	M	49	trichilemmomas			multiple polyps		ins T in N82 (exon 4)	frameshift
3	M	46	trichilemmomas angioma	multiple adenomas		multiple polyps	macrocephaly	C to T in H93 (exon 5)	H to Y
4	F	47	trichilemmomas lipoma	multiple adenomas	cancer (36)	multiple polyps		ND	
5	F	62	trichilemmomas angioma	multiple adenomas	cancer (62)	multiple polyps	endometrial cancer	ND	

M, male; F, female; ND, not detected.
a) Ages at diagnosis are shown in parentheses.

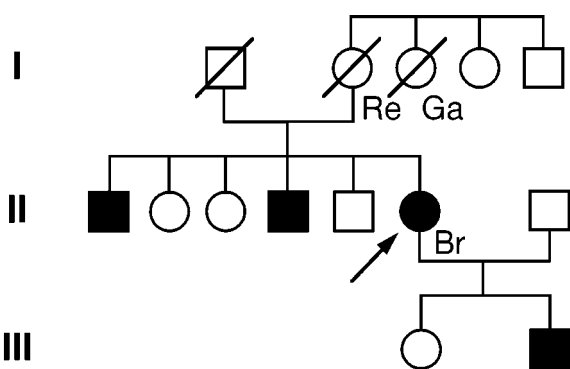


Fig. 1. Abridged pedigree of a Japanese CD family. Squares and circles indicate male and female, respectively. Filled symbols indicate members evaluated as having features of CD. Roman numerals indicate generation numbers, and an arrow indicates the proband. The following abbreviations are used for the cancer types: Br, breast cancer, Ga, gastric cancer; and Re, rectal cancer.

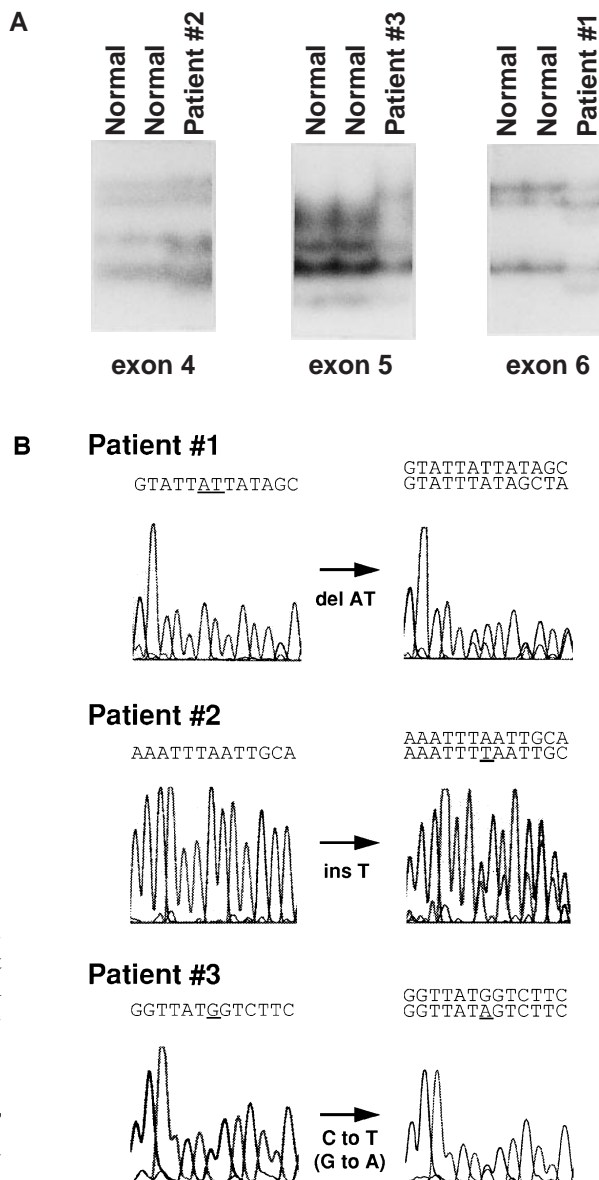


Fig. 2. Germline *PTEN* mutations in Japanese CD patients. A, PCR-SSCP analysis. Results for exons 4, 5, and 6 are shown. B, Direct sequencing. PCR products were purified using a QIA quick-spin PCR purification kit (QIAGEN Inc.), and directly sequenced using the 373S DNA sequencer with a DNA sequencing kit (Applied Biosystems). All PCR products were sequenced in both directions. A 2-bp deletion in codon 177 was detected in patient #1, while a 1-bp insertion in codon 82 was detected in patient #2. In patient #3, a C-to-T transition in codon 93 was detected (reverse sequence). The mutated regions are underlined.

shift mutations in codons 177 and 82, which caused premature termination of the protein. Patient #3 showed a sequence variation in codon 93, which caused a change of an amino acid residue flanking the putative phosphatase domain. To determine whether the sequence variant in codon 93 represents a germline mutation or a genetic polymorphism, we performed PCR-SSCP analysis of DNA from 50 unrelated Japanese individuals and found no evidence of this variation in 100 alleles. Furthermore, we directly sequenced all exons of the *PTEN* gene in patient #3, but no other sequence variation than that in codon 93 was detected. Thus, this variant is likely to be a germline mutation. Patients #4 and #5 did not show bands with distinct electrophoretic mobilities in the PCR-SSCP analysis. Therefore, we again sequenced all exons of the *PTEN* gene, but no sequence variation was detected in these cases.

Three of the 5 Japanese patients with CD carried germline *PTEN* mutations, indicating that this gene is involved in the pathogenesis of CD in Japan. Up to the present, 22 different types of *PTEN* mutations have been identified in 23 cases of germline *PTEN* mutations.⁷⁻¹⁰ In this study, we have further identified three novel types of *PTEN* mutations in patients with CD. A missense mutation detected in patient #3 resided outside the region encoding a putative phosphatase domain of the *PTEN* gene, where previously reported missense mutations in CD patients have been clustered.^{7,9} This result indicates that a wide

range of germline *PTEN* mutations may play a role in the pathogenesis of CD.

PTEN mutations were not detected in two of the five patients with CD. This result is consistent with those of previous studies showing that *PTEN* mutations are undetectable in a significant proportion of CD patients, including those from families linked to 10q22-q23.⁷⁻¹⁰ In those cases, mutations might be present in untranslated regions, introns or the promoter region of the *PTEN* gene. Other mechanisms, including large deletions and methylational silencing, may also be involved in the inactivation of the *PTEN* gene. Alternatively, it is also possible that CD is a genetically heterogeneous disease, as indicated by a recent linkage study.¹⁰ CD is a complex disease associated with a high risk of developing breast cancer and other lesions, including benign and malignant diseases of the thyroid, intestinal polyps and genitourinary abnormalities. Analysis of *PTEN* alterations in a large number of CD patients, as well as a search for other genes relevant to CD, will be needed to elucidate the molecular mechanisms of the development of CD.

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