

## Nonsense Mutation at Codon 63 of the *BRCA1* Gene in Japanese Breast Cancer Patients

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The involvement of abnormalities of the *BRCA1* gene in breast cancers in Japanese patients without any family history of this cancer was investigated by polymerase chain reaction-based single-strand conformation polymorphism analysis of the DNA sequences corresponding to the zinc finger domain (exons 2, 3 and 5) and the binding domain with Rad51 (exon 11) of the *BRCA1* protein. An identical nonsense mutation at codon 63 (TTA to TAA) was found in 2 of 56 (3.5%) breast cancers from independent patients. The nucleotide change was also detected in the DNAs from non-cancerous tissues of both patients and therefore was a germline mutation. One of the patients was a member of a pedigree involving 3 ovarian cancer and 1 gastric cancer patients, while the other patient had no family history of malignancy. The same germline mutation at codon 63 was reported in four other independent Japanese pedigrees with frequent breast cancer, but not in such families in other countries. These observations suggest that the mutation commonly originated from a single Japanese ancestor. No other mutation of the *BRCA1* gene was observed in the samples analyzed in this study. A low incidence of germline mutation and the absence of somatic mutation suggest that the aberration of the *BRCA1* gene is involved only in a subset of Japanese breast cancers.

Key words: *BRCA1* — Germline mutation — Breast-ovarian cancer — Japanese breast cancer

Aberration of the *BRCA1* gene is responsible for an increased susceptibility to familial breast and ovarian cancers.<sup>1,2</sup> Inactivating mutations in the inherited allele and loss of the wild-type allele in tumors that have developed in familial cases demonstrate that the *BRCA1* gene is one of the tumor suppressor genes.<sup>3,4</sup> The gene, mapped to chromosomal region 17q21,<sup>5,6</sup> was isolated by positional cloning in 1994.<sup>7</sup> It is composed of 22 coding exons distributed over approximately 100 kb of the genomic DNA and the size of the transcript is 7.8 kb, encoding 1,863 amino acids.<sup>7</sup> A zinc finger motif near the N-terminus, encoded by exons 2, 3 and 5, could be involved in the transcriptional activation of other genes,<sup>7</sup> while the central portion of the protein corresponding to exon 11 was demonstrated to interact with Rad51, a human homolog of the bacterial RecA protein.<sup>8</sup>

In Western countries, germline mutations in the *BRCA1* gene are estimated to account for approximately 50% of early onset familial breast cancers in females and most of the early onset breast and ovarian cancers.<sup>1,2</sup> So far, over 130 distinct germline mutations have been identified among pedigrees of hereditary breast and ovarian cancer syndrome.<sup>9</sup> In Japan, germline mutations have also been reported in 17 pedigrees with breast or ovarian cancers<sup>10-13</sup> and in unselected primary breast cancers.<sup>14</sup>

However, patients with a strong genetic predisposition are restricted to at most 10% of all breast cancers. Thus, an understanding of breast cancers in patients without any family history of this cancer at the molecular level is required to control these types of cancers. Previous studies have demonstrated that loss of heterozygosity at chromosome 17q, including the *BRCA1* locus, is observed in 30 to 70% of breast cancers in patients without family history, suggesting that *BRCA1* could also act as a tumor suppressor gene for non-familial breast cancers.<sup>15-17</sup>

The present study was initiated to clarify the possible involvement of *BRCA1* gene mutations in non-familial breast cancers. DNAs from 56 Japanese breast cancer patients without any family history of breast cancers were analyzed for mutations in exons 2, 3, 5 and 11 of the *BRCA1* gene using polymerase chain reaction-based single-strand conformation polymorphism analysis (PCR-SSCP).

### MATERIALS AND METHODS

**Human tissue samples** All 56 breast cancer specimens were surgically resected and histologically diagnosed at the Second Department of Surgery, Tohoku University School of Medicine, Sendai, Japan from 1986 to 1994. The tumors included 14 scirrhous carcinomas, 13 solid-tubular carcinomas, 5 papillotubular carcinomas, one each

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of lobular carcinoma, squamous cell carcinoma and mucinous carcinoma and 18 unknown. The patients without any family history of breast cancers were selected for the analysis. The family history of other cancers including ovarian cancers was not taken into consideration in the selection of patients.

Corresponding constitutional DNAs were obtained from the peripheral blood leukocytes or non-cancerous tissues of each patient. Four patients with onset at ages less than 35 were included. The mean age of the patients analyzed was 51.8 years.

**PCR-SSCP analysis** DNA was extracted from the tissues by proteinase K-phenol-chloroform extraction.<sup>18)</sup> Exons 2, 3 and 5 of the *BRCA1* gene, encoding a zinc finger domain, and exon 11, which is reported to be a mutation cluster region and to encode a binding domain with Rad51, were examined.<sup>9)</sup> The nucleotide sequences of the primers for exons 2, 3 and 5 were provided by Myriad Diagnostic Services, Inc. (Salt Lake City, UT), while 19 primers for exon 11 were synthesized according to the report by Castilla *et al.*<sup>19)</sup>

The target DNA sequences were amplified by PCR in a mixture (5  $\mu$ l) containing 50 ng of genomic DNA, 2.5 pmol of each primer, 0.6 nmol of each dNTP and 0.12 units of *Taq* polymerase. The 5'-ends of the primers were labeled using [ $\gamma$ -<sup>32</sup>P]ATP and polynucleotide kinase (Boehringer Mannheim, Mannheim, Germany). PCR products were denatured and subjected to electrophoresis at 40 W for 4 to 5 h in 5% non-denaturing polyacrylamide gels containing 10% glycerol and those without glycerol, followed by autoradiography.<sup>20)</sup>

**DNA sequencing** DNA fragments showing a mobility shift by SSCP analysis were eluted from the polyacrylamide gel as described previously,<sup>21)</sup> and were amplified by 30 cycles of PCR using the same primers under the same conditions as described earlier.<sup>21)</sup> The reaction mixture was diluted and purified in a Microcon 100 micro-concentrator (Millipore Corp., "Amicon," Bedford, MA). The amplified DNAs were annealed with the 5'-labeled primers, and nucleotide sequences were determined using a double-strand DNA Cycle Sequencing Kit, Cyclist (Stratagene, La Jolla, CA).

## RESULTS

The regions of exons 2, 3, 5 and 11 of the *BRCA1* gene and their flanking sequences were analyzed by PCR-SSCP for detecting mutations. The same mobility shift of the DNA fragment of exon 5 was detected in tumors from 2 out of 56 patients (3.5%) (patients 51 and 76) (Fig. 1). Nucleotide sequence analysis revealed a T-to-A transversion at the second letter in codon 63 (TTA) in both tumors, which resulted in a premature termination of synthesis of the *BRCA1* protein (Fig. 2). The identical mobil-

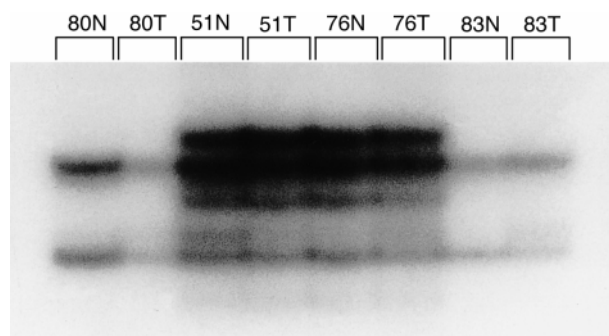


Fig. 1. PCR-SSCP analysis for exon 5 of the *BRCA1* gene. Note the aberrant shifted bands in both the normal and tumor tissue samples of patient 51 (51N and 51T) and patient 76 (76N and 76T). No mutations were detected in patients 80 and 83.

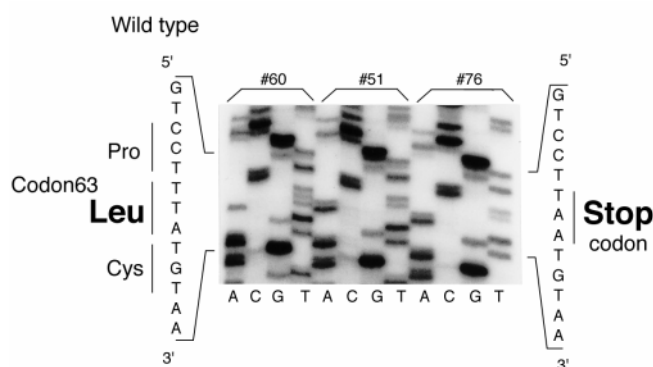


Fig. 2. A T-to-A transversion at the second nucleotide of codon 63 in the *BRCA1* gene. This nucleotide change alters the leucine (Leu) codon to a termination (Stop) codon at this site. The nonsense mutation was detected in both patients 51 and 76 by direct sequencing analysis. This mutation also existed in normal tissue samples from each patient.

ity shift and mutation were also observed in the DNAs from non-cancerous tissues of the same patients (Fig. 1). The results indicated that the T-to-A transversion was due to a germline mutation. If the nonsense mutation was involved in the genesis of the tumors, the normal allele of the *BRCA1* gene should be inactivated in any of several ways, including allelic loss. However, the signal intensity of the DNA fragment from the normal allele did not differ between normal and tumor DNA samples (Fig. 1), indicating either no loss of the normal allele in the tumors or the presence of significant amounts of normal DNA in the tumor DNA samples. Therefore, the involvement of this nonsense mutation in the genesis of the tumors remained to be clarified.

Patient 76 was 66 years old at onset with no metastasis ( $T_1N_0M_0$ ) and is alive, free from the disease, 8 years after

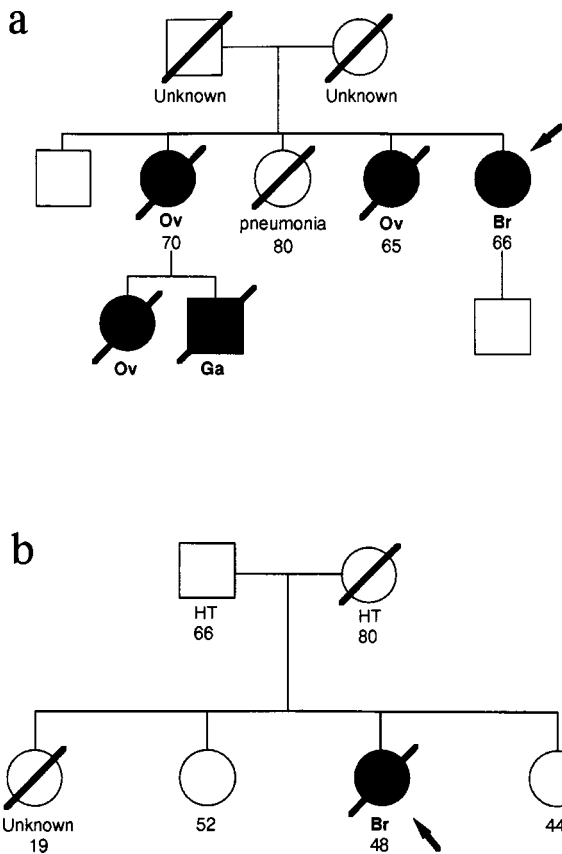


Fig. 3. Pedigrees of two patients with the germline mutation of the *BRCA1* gene. In the breast-ovarian cancer family of patient 76, three members were affected with ovarian cancer and one with gastric cancer (a). In the pedigree of patient 51, no family member was affected with malignancy (b). The arrows indicate patients with breast cancer; Br, breast cancer; Ga, gastric cancer; Ov, ovarian cancer; and HT, hypertension.

the surgical resection. Histopathological diagnosis revealed that the tumor of this patient was an invasive ductal carcinoma of a solid-tubular type. This patient had a strong family history of cancers as shown in Fig. 3a; three ovarian cancers and a gastric cancer, but not breast cancer, within relatives to the second degree. Assessment of the mutation in these family members, however, could not be performed because they had already died at other hospitals.

Patient 51 was 48 years old at onset with peritoneal metastasis ( $T_4N_2M_1$ , stage IV) and died of cancer within a year of diagnosis. The tumor expressed estrogen receptors and showed invasive ductal carcinoma of scirrhous type in histopathological examination. This patient did not have any family history of cancers (Fig. 3b).

No other abnormal mobility shifts of any DNA fragments were detected in the other 54 tumors except for

those due to nucleotide sequence polymorphisms in exons 3 and 11 that were reported previously.<sup>9,22)</sup>

## DISCUSSION

An identical germline mutation in codon 63 was detected in breast cancers from 2 independent individuals out of 56 Japanese patients. This mutation resulted in the production of a short truncated polypeptide lacking most of the BRCA1 protein, including its functional domains. The same mutation was reported in four other Japanese pedigrees including two families with breast cancers, one family with breast-ovarian cancers and one family with ovarian cancers.<sup>10,13)</sup> In contrast, the mutation in codon 63 has not been found among 132 aberrations reported in families with breast or ovarian cancer in Western countries.<sup>9)</sup> These findings suggest that the mutation in codon 63 originated from an ancestral allele in Japan, although the affected families lived in different geographical areas in Japan. A similar feature has been reported previously<sup>23-26)</sup>; deletion of 2 bp in codon 185 of the *BRCA1* gene was detected in approximately 1% of Ashkenazi Jewish women.<sup>27)</sup>

Together with the two cases reported in the present study, 5 of 6 Japanese pedigrees with the codon 63 mutation showed a family history of breast and/or ovarian cancers. The relatively high penetrance of ovarian cancers (3 of 6 pedigrees) might be due to the specific features of the truncated protein of BRCA1 at its N-terminus. This does not correspond with the previous report that ovarian cancers occur predominantly in cases carrying truncated BRCA1 proteins at their C-terminal regions.<sup>28)</sup> Ovarian cancers might develop, alternatively, based on the different genetic background and/or environmental factors in these Japanese families. The presence of a gastric cancer patient in the patient's pedigree is also interesting, because the incidence of gastric cancer is much higher in Japan than in Western countries. Likewise, gastric cancer patients were also reported in Li-Fraumeni syndrome in Japan, although we could not determine whether the mutated allele was segregated in this patient.<sup>29)</sup> It is noteworthy that no family history of malignancy was observed in the pedigree of patient 51.

A few somatic mutations of the *BRCA1* gene have been reported in ovarian cancers, but none in breast cancers so far. We confirmed the absence of a somatic mutation in Japanese breast cancers in this study. However, Chen and colleagues recently demonstrated the aberrant subcellular localization of the BRCA1 protein in non-familial breast cancers,<sup>30)</sup> suggesting that the protein might be altered at the post translational level. Analysis using a specific antibody against the BRCA1 protein is required to elucidate the involvement of the altered BRCA1 protein in non-familial breast cancers.

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