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Linkage Analysis of BRCA1 in Japanese Breast Cancer Families

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We examined the involvement of BRCA1, which plays a major role in Western breast cancer families, in Japanese breast cancer families. Eleven families, in which at least three individuals within third degree relatives were affected by breast cancer, were collected. Five of them were early-onset breast cancer families, in which the average age at diagnosis was less than 45 years, and the other six were late-onset families. Ovarian cancer was observed in one patient in the early-onset families. Using seven polymorphic markers on chromosome 17q21, D17S250, ERBB2, THRA1, D17S579, D17S588, GIP and NME1, linkage to BRCA1 was analyzed. Linkage was not detected in any single family. Assuming homogeneity in an inherited component that confines the susceptibility to breast cancer in all families, we summed the LOD scores of all families. The cumulative LOD score obtained was -1.86 for D17S588 at $\theta = 0.001$, indicating no linkage with BRCA1. Since the proportion of families linked to BRCA1 is larger in Western early-onset breast cancer families than in late-onset ones, we also summed the LOD scores of five early-onset families. However, again a negative LOD score was obtained. These results suggest that BRCA1 is not a major breast cancer susceptibility gene in Japanese familial breast cancer.

Key words: Breast cancer — Family — BRCA1 — LOD score — Linkage

Breast cancer is one of the most common malignancies in Western countries. The cumulative incidence of breast cancer is about 10.3% among white female Americans of up to 75 years old.[1] Familial breast cancers, which include hereditary breast cancers and incidental breast cancers in a family, are considered to constitute 5–10% of all breast cancers.[2,3] At least three types of breast cancer families have been reported, (1) site-specific breast cancer families, (2) breast-ovarian cancer families (Lynch syndrome) and (3) Li-Fraumeni syndrome, although the third type is very rare in Western breast cancer families.[4–6]

It is now commonly accepted that breast-ovarian cancers and some fraction of site-specific breast cancers, especially an early-onset type, are associated with BRCA1 on chromosome 17q21.[7–9] A recent report showed that 45% of breast cancer families were BRCA1-linked families, indicating that BRCA1 is a major susceptibility gene in Western countries.[10] The proportion of BRCA1-linked families is more in early-onset than in late-onset families, being 67% in the former and 28% of the latter. Since loss of the wild-type allele in the region including BRCA1 was frequently observed in tumors in BRCA1-linked families, BRCA1 was suggested to be a tumor suppressor gene.[11]

The cumulative incidence of breast cancer in Japan is very low, being about one-fifth to one-third of those in Occidentals, although it is increasing remarkably, with westernization of life style.[12] A recent study suggested that about 5% of breast cancers are familial.[13] Thus, it is suspected that the population of those genetically predisposed to breast cancer is lower in Japan than in Western countries, although it is not known how much the incidence of familial breast cancer is affected by environmental factors including nutrition. There have been a few studies on the character of familial breast cancer in Japan, and no reports on linkage analysis.

In this study, we analyzed the involvement of BRCA1 in Japanese familial breast cancers.

MATERIALS AND METHODS

Families Eleven families were selected among those of the breast cancer patients who visited the National Cancer Center Hospital, on the criteria that at least three individuals among third degree relatives of the proband had breast cancer. A total of 69 individuals, including 24 affected individuals were analyzed.

Typing of DNA polymorphism Lymphocytes were collected from peripheral blood, and genomic DNA was extracted by the phenol/chloroform method.[14]

Families were typed by polymorphism at the D17S250, ERBB2, THRA1, D17S579, D17S588, GIP, and NME1 loci on chromosome 17q12-21, spanning a region of

approximately 25 cM, which included BRCA1,[10] as described[10, 15–19] with slight modifications. Briefly, DNAs were amplified by polymerase chain reaction (PCR) with primers based on the reported sequence, which were synthesized in a model 392 RNA/DNA synthesizer (Applied Biosystems Japan, Tokyo).

For typing of five CA-repeat microsatellites at the D17S250, THRA1, D17S579, D17S588 and NME1 loci, PCR amplification was carried out in a total of 5 μl of reaction mixture, containing 100 ng of genomic DNA, 0.4 pmol of each ^{32}P-labeled primer, 100 μM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin and 0.5 unit of AmpliTaq polymerase (Perkin-Elmer Cetus). The reaction mixture was heated at 94°C for 3 min, followed by 40 cycles of PCR with heating at 94°C for 30 s, primer annealing at 55°C for 1 min, and elongation at 72°C for 2 min. The final cycle was followed by a 7-min elongation period at 72°C. Samples of 5 μl of PCR products were diluted with 45 μl of SSCP buffer (0.1% SDS and 10 mM EDTA). Volumes of 2 μl of each were mixed with an equal volume of formamide dye (98% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue, 10 mM EDTA). After denaturation by heating at 95°C, 1 μl of the mixture was subjected to electrophoresis in 6% polyacrylamide gel under denaturing conditions. Then the gel was dried and exposed to XAR film (Kodak).

For typing of polymorphism at the GIP and ERBB2 loci, PCR was performed in a total of 25 μl of reaction mixture containing 100 ng of genomic DNA, 0.8 pmol of each primer, 200 μM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin and 0.75 unit of AmpliTaq polymerase. Samples of 10 μl of the PCR products were mixed with 2 μl of 6 × type III dye (0.25% bromophenol blue, 0.25% xylene cyanol FF and 30% glycerol in water), and subjected to electrophoresis in 0.9% agarose gel, followed by ethidium bromide staining. Polymorphism at the ERBB2 locus was detected in 4% agarose gel by subjecting the *Pvu*II and *Sau*3AI (Toyobo) digests of the PCR products to electrophoresis.

Linkage analysis Two-point linkage analysis was performed using a LINKAGE program (version 5.20).[20] As a model of inheritance, we used that proposed by Claus *et al.*,[21] in which breast cancer susceptibility is conferred by an autosomal dominant allele and the population frequency of the allele is 0.0033. We used the penetrance values determined in the age-specific manner by Easton *et al.*[10] The allele frequencies of the 7 loci used were determined by DNA typing of 74 normal Japanese volunteers, as shown in Table I.

Analysis of LOH in tumor DNA Loss of heterozygosity (LOH) was detected by the method described under "Typing of DNA polymorphism." Three regions for THRA1, D17S579 and D17S588 loci were examined. For PCR amplification of paraffin-embedded tissue materials, some primers were newly designed to minimize the size of PCR products. The sequences were 5′-ACCCTCAGCCTGCCACAGCC-3′ as sense and 5′-CATTGCCTTCCCATGTCCGT-3′ as antisense primers for amplification of the nucleotide sequences at the THRA1 locus,

Table I. Allele Frequencies of Seven Loci in Japanese

	Genotype	Frequency
D17S250	1	0.01
	2	0.02
	3	0.02
	4	0.02
	5	0.01
	6	0.10
	7	0.05
	8	0.16
	9	0.59
	10	0.02

	Genotype	(fragment size)	Frequency
ERBB2	1	(520 bp)	0.21
	2	(500 bp)	0.79
GIP	1	(1950 bp)	0.31
	2	(1800 bp)	0.68
	3	(1650 bp)	0.01

	Genotype	Frequency
THRA1	1	0.01
	2	0.01
	3	0.03
	4	0.07
	5	0.28
	6	0.05
	7	0.09
	8	0.27
	9	0.04
	10	0.14
	11	0.01
D17S588	1	0.01
	2	0.16
	3	0.14
	4	0.26
	5	0.18
	6	0.04
	7	0.16
	8	0.04
	9	0.01

	Genotype	Frequency
D17S579	1	0.01
	2	0.01
	3	0.01
	4	0.10
	5	0.21
	6	0.35
	7	0.02
	8	0.06
	9	0.01
	10	0.01
	11	0.10
	12	0.10
	13	0.01
NME1	1	0.06
	2	0.20
	3	0.50
	4	0.08
	5	0.14
	6	0.01
	7	0.01

5′-AGACATCATCCTGAAATCTA-3′ as the antisense primer for the D17S579 locus and 5′-ACCCCAGATG-GAGACACGTG-3′ as the sense primer for the D17S588 locus. The sense primer for amplification of the nucleotide sequence at the D17S579 locus and the antisense primer at the D17S588 locus were as reported previously (see above). Two tumors from the proband and her elder sister in family 266326, one of an early-onset breast cancer family, were examined.

SSCP analysis of p53 Exons 5–9 were examined by PCR-SSCP analysis. The primers were based on a previous report.[22] After end-labeling with T_4 polynucleotide kinase and [γ-^{32}P]ATP (ICN Radiochemicals), PCR and dilution of the products were performed as described under "Typing of DNA polymorphism." Samples of 1 μl of the mixture were denatured by heating at 95°C, separated by electrophoresis in 5% polyacrylamide gel with 0.1% bis acrylamide, and subjected to autoradiography.

RESULTS AND DISCUSSION

Families The families selected in this study included at least three of breast cancer patients within third degree relatives (Fig. 1). The characters of the families are summarized in Table II. Three patients in three families were affected bilaterally. The frequency of bilaterally affected patients in these families was higher than that in cases of sporadic breast cancer (7.5% versus 2.7%).[23] All breast cancer patients were females. In these 11 families, only one patient (family 306336) was affected by ovarian cancer, in contrast to 40 affected by breast cancer. The average age at diagnosis of all breast cancer patients in the 11 families was 46 years, which was lower than that of all Japanese breast cancer patients of 50 years, most of which were sporadic cases.[1] Five of 11 families were those of early-onset breast cancer, in which the average age at diagnosis was less than 45 years.

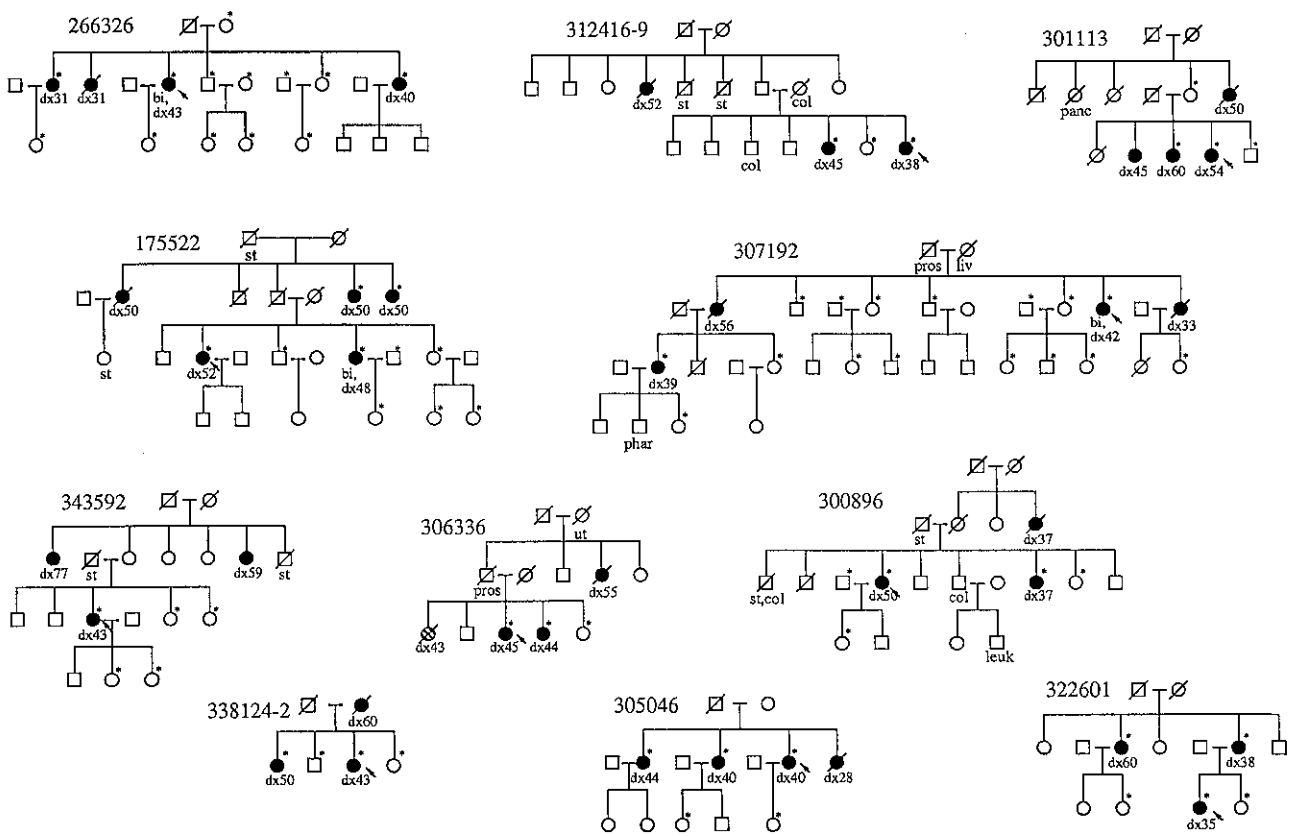

Fig. 1. Eleven Japanese breast cancer families. Circles denote females affected with breast cancer (closed) or ovarian cancer (hatched), or unaffected (open). Squares denote unaffected males. Arrows indicate proband in the family. Asterisks indicate individuals whose DNA was typed. dx: age at diagnosis, bi: bilateral case. Abbreviations of the organs of other malignancies are: st, stomach, col, colon; pros, prostate; ut, uterine cervix; leuk, leukemia; phar, pharynx; liv, liver and panc, pancreas.

Table II. Characters of the 11 Japanese Families

Family	No. of affected individuals (bilateral)	No. of ovarian cancer patients	Average age at diagnosis (years)	Other malignancies[a)]
266326	4 (1)	0	36	
305046	4	0	38	
300896	3	0	39	st (1), col (2), leuk (1)
307192	4 (1)	0	43	pros (1), liv (1), phar (1)
322601	3	0	44	
312416-9	3	0	45	st (1), col (1)
306336	4	1	47	st (1), ut (1), pros (1)
175522	5 (1)	0	50	st (2)
338124-2	3	0	51	
301113	4	0	52	panc (1)
343592	3	0	60	st (1)
Total	40 (3)	1	46	

a) st, stomach; col, colon; leuk, leukemia; ut, uterine cervix; pros, prostate; liv, liver; phar, pharynx; panc, pancreas. ( ), number of affected individuals.

Table III. LOD Scores for D17S579 and D17S588 in the 11 Families

Family	LOD score at recombination fraction of					
	0.001	0.01	0.1	0.2	0.3	0.4
D17S579						
Early-onset families						
266326	0.317	0.308	0.232	0.148	0.073	0.020
305046	−0.073	−0.070	−0.045	−0.025	−0.011	−0.003
300896	0.146	0.141	0.099	0.058	0.027	0.007
307192	−0.706	−0.620	−0.256	−0.107	−0.038	−0.008
322601	0.129	0.125	0.090	0.055	0.026	0.007
Total	−0.187	−0.116	0.120	0.129	0.077	0.023
Late-onset families						
312416-9	−0.098	−0.094	−0.060	−0.033	−0.014	−0.004
306336	−0.687	−0.625	−0.308	−0.146	−0.059	−0.014
175522	−1.040	−0.970	−0.528	−0.260	−0.106	−0.026
338124-2	0.086	0.083	0.057	0.033	0.015	0.004
301113	−0.001	0.000	0.000	0.000	0.000	0.000
343592	−0.003	−0.003	−0.002	−0.001	0.000	0.000
Total	−1.743	−1.609	−0.841	−0.407	−0.164	−0.040
All families	−1.930	−1.725	−0.721	−0.278	−0.087	−0.017
D17S588						
Early-onset families						
266326	0.120	0.117	0.085	0.052	0.025	0.007
305046	−0.697	−0.633	−0.311	−0.147	−0.059	−0.014
300896	0.082	0.080	0.055	0.032	0.014	0.004
307192	−0.729	−0.653	−0.301	−0.139	−0.055	−0.013
322601	0.145	0.140	0.097	0.057	0.026	0.007
Total	−1.079	−0.949	−0.375	−0.145	−0.049	−0.009
Late-onset families						
312416-9	0.091	0.088	0.061	0.035	0.016	0.004
306336	0.093	0.090	0.062	0.036	0.016	0.004
175522	−1.037	−0.967	−0.526	−0.257	−0.105	−0.025
338124-2	0.071	0.069	0.047	0.027	0.012	0.003
301113	−0.001	−0.001	0.000	0.000	0.000	0.000
343592	−0.004	−0.004	−0.003	−0.002	−0.001	0.000
Total	−0.787	−0.725	−0.359	−0.161	−0.062	−0.014
All families	−1.866	−1.674	−0.734	−0.306	−0.111	−0.023

An adequate model for the inheritance of Japanese familial breast cancer including the 11 families examined in this study is still unclear. Since the inheritance could be considered to be autosomally dominant, which is a characteristic of BRCA1, we used Claus' model[21] for linkage analysis.

Analysis of linkage to chromosome 17q21 Table III shows the LOD scores for D17S588 and D17S579, which were reported to be closely linked to BRCA1, in 11 families.[10] The maximum LOD scores were 0.317 at $\theta=0.001$ for D17S579 in family 266326 and 0.145 at $\theta=0.001$ for D17S588 in family 322601. Even on the estimated BRCA1 locus (1 cM telomeric to D17S579), the LOD scores were less than these values. In family 175522, LOD scores for D17S579 and D17S588 were less than -1, suggesting no linkage with BRCA1. However, the absolute values of LOD scores in each family were too small to allow a conclusion as to the presence or absence of linkage with BRCA1. These small LOD scores may be due to limitations of sample availability and information on family histories.

Assuming that the susceptible gene involved is homogeneous in all families, LOD scores of all families were summed (Table III). No linkage to BRCA1 was suggested (-1.866 at $\theta=0.001$). Since the ratio of BRCA1-linked families is higher in early-onset families in Western countries, the overall LOD score of early-onset families was also calculated. However, it was -1.079 at $\theta=0.001$ (Table III), again suggesting no linkage.

There is a report that in 27% of breast cancer families (57 of 214 families) in Western countries there is at least one case of ovarian cancer.[10] In Japan, 11% of breast cancer families are associated with ovarian cancer (2 of 15 families according to Nomizu *et al.*,[13] and 1 of 11 families in this study). Thus, the ratio of breast to ovarian cancer is much lower in Japan than in Western countries.[10] In this study, one individual in family 306336 was affected by ovarian cancer, but the LOD score of this family was 0.093 for D17S588 and -0.687 for D17S579 at $\theta=0.001$.

Analysis of LOH in tumors Since BRCA1 is suggested to be a tumor suppressor gene, we also examined LOH in two tumors obtained from family 266326, which was an early-onset family with a positive LOD score on D17S 579 (Zmax$=0.317$ at $\theta=0.001$). Using three markers for the THRA1, D17S579 and D17S588 loci, we demonstrated the absence of LOH in these tumors (Fig. 2). From all these results, we concluded that BRCA1 may not play a role in this family.

Analysis of germ line mutations in p53 As familial aggregation of breast cancer is observed in the Li-Fraumeni syndrome,[24] we examined germ-line mutations of the p53 gene. No mutations were detected in 22 affected individuals from 10 families (family 301113 was not tested). Thus involvement of the Li-Fraumeni syndrome was ruled out from results on these 10 families.

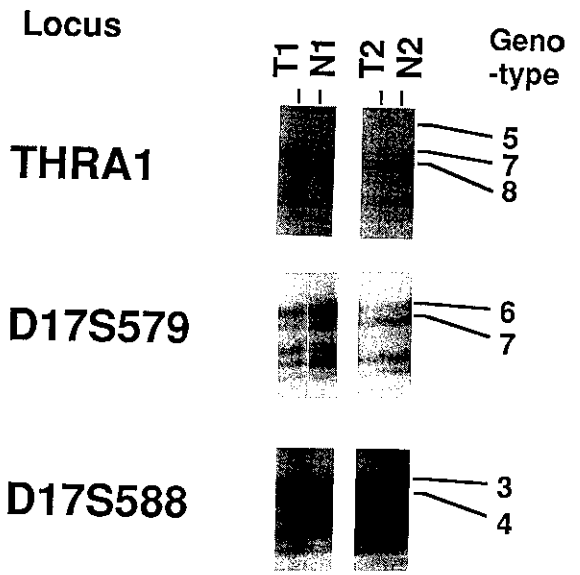

Fig. 2. Absence of allele losses of BRCA1 in two patients with breast cancer in family 266326. T1, tumor in the proband; T2, tumor in an elder sister; N1 and N2, respective normal counterparts of T1 and T2. In T1 and T2, both alleles at three loci, THRA1, D17S579 and D17S588, were retained.

Comparison of Japanese and Western breast cancers The clinicopathological characteristics of sporadic breast cancer are similar to those of familial breast cancer in Western countries.[25] Probably, this is also true in Japan, although no reports are available. Some differences between clinicopathological findings in sporadic breast cancer in Japanese and Caucasians have been reported. Histologically, a large proportion of Japanese breast cancer consists of well-differentiated carcinoma and a low proportion of lobular carcinoma.[26] A high degree of lymphocytic infiltration is also a characteristic of Japanese breast cancer.[27] Clinically, the prognosis of breast cancers in Japanese is better than that in Caucasians.[27] These differences in sporadic breast cancer in Japan and Western countries, such as the US or Western Europe, may be due to differences in environmental factors, such as dietary habits or levels of sex hormones. However, difference in genetic background cannot be excluded. The results of the present study suggest that the involvement of BRCA1 is not common in Japanese familial breast cancer.

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