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Clinical Efficacy of S-1 in Pretreated Metastatic Breast Cancer Patients

Tadahiko Shien[1], Chikako Shimizu[2], Sadako Akashi-Tanaka[1], Kan Yonemori[2], Tsutomu Kohno[2], Takashi Hojo[1], Masashi Ando[2], Noriyuki Katsumata[2], Takayuki Kinoshita[1] and Yasuhiro Fujiwara[2]

[1]Division of Surgical Oncology and [2]Division of Medical Oncology, National Cancer Center Hospital, Chuo-ku, Tokyo, Japan

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Background: S-1, an oral fluoropyrimidine carbamate, is an active and well-tolerated agent against solid cancer. However, the clinical efficacy of S-1 in patients with metastatic breast cancer has not been determined.
Methods: We retrospectively evaluated the efficacy of S-1 and identified its adverse effects in patients with metastatic breast cancer who had failed to respond to prior chemotherapy regimens. All the patients were treated at the National Cancer Center Hospital and received S-1 twice daily at a dose of 80 mg/m^2 for 4 weeks, followed by a 2-week rest interval.
Results: Between 2003 and 2007, 37 women with metastatic breast cancer received S-1 as a third line or greater chemotherapy regimen. All the patients had been previously treated with both anthracyclines and taxanes prior to S-1 chemotherapy. The median order of S-1 administration was as a fifth-line treatment, and 23 patients (62%) received S-1 as their final anticancer drug. One (3%) partial response and two (5%) stable diseases were observed. The median time to progression (TTP) was 84 days. Grade 2 adverse events, such as diarrhea, stomatitis and neutropenia occurred in 5 (16%), 1 (3%) and 1 (3%) patients, respectively.
Conclusions: S-1 was safety administered to heavily treated metastatic breast cancer patients with limited efficacy. Further evaluation of S-1 is necessary to elucidate its clinical role in breast cancer treatment.

Key words: S-1 – metastatic breast – cancer – chemotherapy

INTRODUCTION

Treatment of patients with metastatic breast cancer (MBC) aims to prolong survival while relieving symptoms and maintaining a good quality of life (QOL).

Capecitabine is an orally administered fluoropyrimidine that has been reported to be effective in both monotherapy and combination therapy regimens. Capecitabine as a single agent produced an overall response rate (RR) of 29% and a median time to disease progression of 4.6 months in large phase II trials in taxane-pretreated MBC patients (1–3). Since capecitabine can sustain the QOL of MBC patients, it has been widely used as a third-line or subsequent chemotherapy regimen for heavily treated patients.

On the other hand, S-1 is another orally administered fluorinated pyrimidine that has been reported to be a well-tolerated and active agent against solid cancers. In a phase II study of S-1, the RR was 41.7% and the median survival time was 872 days among taxane-pretreated patients with MBC; S-1 has been approved in Japan as a salvage chemotherapy for patients who have received anthracycline and taxane (4,5). In addition, S-1 has been used mainly for the treatment of cancers of the digestive tract (6–8), and its efficacy is well known. However, the clinical usefulness of S-1 in patients with MBC is uncertain. Here, we describe the efficacy and tolerability of S-1 in a clinical setting.

PATIENTS AND METHODS

Patients

A retrospective analysis was performed on patients with MBC who received S-1 monotherapy between January 2003 and December 2006 at the National Cancer Center Hospital (NCCH). The patient population was identified from a

For reprints and all correspondence: Chikako Shimizu, Division of Surgical Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: cshimizu@ncc.go.jp

database at the NCCH. All the patients had received chemotherapy previously. They were followed up until death or, if they were still alive, to their last visit prior to March 2007.

The best response for each patient was assessed according to the WHO criteria (8). A complete response (CR) was defined as the disappearance of all clinical and radiographic evidence during two observations performed at least 4 weeks apart. A partial response (PR) was defined as a decrease of 30% or more in the sum of the products of the biperpendicular diameters of measurable lesions. Stable disease (SD) was defined as a $<30\%$ decrease and a $<25\%$ increase in the sum of the products of the biperpendicular diameters of measurable lesions and no appearance of new lesions; these conditions had to be maintained for at least 12 weeks. Progressive disease was defined as a greater than 25% increase in the sum of the products of the biperpendicular diameters of measurable lesions or the appearance of new lesions. The clinical benefit rate was defined as the proportion of patients who achieved either a CR, PR or SD. The National Cancer Institute common toxicity criteria (9) were adopted to determine toxicity.

Treatment

S-1 was administered orally twice daily (80 mg/m^2) for 28 days followed by 14 days of rest. Treatment was continued until disease progression, unacceptable adverse effects or withdrawal of the patient's consent. In the case of Grade 2 or worse toxicity, S-1 administration was interrupted and not resumed until the toxicity had resolved or improved to Grade 1.

The time to progression (TTP) was calculated from the day of commencement of S-1 administration until the day of documented progression. Overall survival (OS) was calculated from the start date of S-1 to the date of death from any cause. TTP and OS were analysed according to the Kaplan–Meier estimates.

RESULTS

Thirty-seven patients received S-1 as a greater than second-line chemotherapy for MBC between January 2003 and December 2006 at NCCH. Table 1 shows the patient's characteristics. The median age was 49 (28–70) years. The Eastern Cooperative Oncology Group (ECOG) performance statuses of the patients were all <2. The sites of metastatic disease were the bone and/or soft tissue in only six patients (16%) and involved visceral sites in 31 patients (84%). Table 2 shows the chemotherapy regimens that were administered prior to S-1. The median number of chemotherapy regimens used before the administration of S-1 including adjuvant and neoadjuvant treatments, was 4, and 23 patients (62%) received S-1 as their final chemotherapy regimen. All the patients had previously received both anthracyclines and taxanes, 13 patients (35%) had received vinorelbine and 11 patients (30%) had received oral 5FU-derivatives prior to the administration of S-1. All the patients who had responded to treatment had exhibited adequate progression-free intervals from the prior taxane administration until the subsequent taxane administration. Three patients received the same taxane regimen twice, once as adjuvant chemotherapy and the second time in combination with Trastuzumab after recurrence. Prior oral 5FU-derivatives included in other regimens were CMF (five patients), UFT (five patients), 5'DFUR (five patients) and CPT-11 (one patient). Sixteen patients (43%) with ER-positive diseases had received hormone therapy, and 13 patients (35%) with HER2-positive diseases had received Trastuzumab as a monotherapy or in combination with taxane or vinorelbine.

Table 1. Patient characteristics

	No. of patients ($n = 37$)	% of patients
Median age (years; range)	49 (28–70)	
Metastatic sites involved		
Bone/Soft tissue	6	16
Visceral	31	84
Oestrogen receptor		
Positive	16	43
Negative	21	57
Progesteron receptor		
Positive	17	46
Negative	20	54
HER2/neu status		
Positive	13	35
Negative	24	65

Table 2. Prior chemotherapy

Prior chemotherapy	No. of patients ($n = 37$)	% of patients
No. of regimens used		
2/3/4/5/6/7/8	4/10/10/4/2/0/3	
Median (range)	4 (2–8)	
Neoadjuvant chemotherapy	6	16
Adjuvant chemotherapy	17	46
S-1 was the last regimen	23	62
Prior chemotherapy		
Anthracycline	37	100
Taxane	37	100
Vinorelbine	13	35
Capecitabine	1	3

The median number of administration days was 70 (6–415 days). The RR was 3%, with no cCR and 3% (1/37) PR. The overall clinical benefit rate (CR, PR and SD for more than 6 months) was 8% (3/37). The median TTP was 84 days (range, 6–415) (Fig. 1; note that a colour version of this figure is available as supplementary data at http://www.jjco.oxfordjournals.org). The median OS from the start of S-1 treatment was 284 days (range, 14–1511), and six patients (16%) were still alive at the last follow-up. Nine patients (24%) received S-1 for more than 100 days. Six out of these nine patients had visceral involvement. Two out of seven patients had oestrogen receptor-positive diseases and four of them were HER2-positive.

Overall, S-1 was well tolerated. Table 3 shows the adverse events in response to S-1 chemotherapy. Toxicities of Grade 3 or more were not reported. The most common toxicities arising from S-1 administration were diarrhea (33%) and nausea (30%). Most of the adverse events were Grade 1, and none of the S-1-related adverse events were fatal. The most frequent reasons for treatment discontinuation were disease progression (30 patients, 81%) and adverse event (seven patients, 19%). The adverse events that were encountered were Grade 2 diarrhea (five cases), Grade 2 stomatitis (one case) and Grade 2 neutropenic fever (one case).

Table 3. Treatment-related adverse event of TS-1

	Gradre 1 (%)	Grade 2 (%)
Diarrhea	7 (19)	5 (14)
Stomatitis	5 (14)	1 (3)
Nausea/vomitting	11 (30)	0 (0)
Neutropenia	1 (3)	1 (3)
Disorder of liver function	2 (6)	0 (0)

DISCUSSION

The number of patients with MBC who have been pretreated with anthracyclines and/or taxanes are increasing. However, the optimal chemotherapy for patients with MBC who have been pretreated with both anthracyclines and taxanes has not been determined. These patients require palliative therapy that offers a chance of prolonging life with minimal toxicity according to the antitumor response and the alleviation of tumor-related symptoms.

In this study, S-1 chemotherapy produced a 3% RR and an 8% rate of clinical benefit in previously treated patients with MBC who were refractory to both anthracyclines and taxanes. The median TTP was 84 days, and 24% of the patients received S-1 for more than 100 days. These results were worse than those reported in clinical trials. This discrepancy is probably because 11 patients had received other 5FU-derivatives prior to S1, the median order of S-1 administration was fifth line (most of the patients received S-1 chemotherapy as their final treatment), and most of the patients had multiple metastatic sites (84% had visceral metastases). The toxicity of S-1, however, was mild in these heavily treated patients, and S-1 is considered to be a feasible palliative chemotherapy in heavily treated MBC patients.

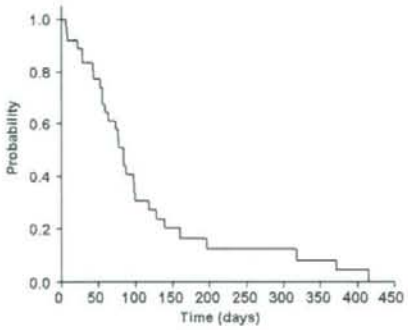

Figure 1. Kaplan–Meier curve for time to progression (TTP). Median TTP was 84 (range 6–415) days.

Several oral 5FU-derivatives have been used to treat MBC, but only S-1 and capecitabine have been tested in taxane-refractory MBC patients (10). The treatments were administered based upon physicians' decisions, but the reason why S-1, and not capecitabine, was selected in this study population is unclear. S-1 is a fluoropyrimidine that consists of 1-(2-tetrahydrofuryl)-5-fluorouracil (FTO), a pro-drug of 5-FU, and two other compounds, 5-chloro-2, 4-dihydroxypyrimidine (CDHP; gimestat) and potassium oxonate (OXO; otastat), in molar proportions of 1:0.4:1. CDHP is an inhibitor of dihydropirimidine dehydrogenase (DPD), which degenerates 80% of 5-FU in the liver and maintains the 5-FU level above a minimal effective concentration level. On the other hand, capecitabine is converted to 5'-DFUR either by human carboxyesterase (CE) or cytidine deaminase (CD), which is mainly localized in the human liver. 5'-DFUR is converted to the active form of 5-FU by thymidine phosphorylase (dThdPase) in human tumors. Low CE and CD activity levels are thought to protect the digestive wall and bone marrow from capecitabine toxicity.

Clinically, the reported RRs of capecitabine and S-1 in taxane-pretreated MBC patients are similar, but the toxicity profile seems to be different. Relatively severe diarrhea (14%, Grade 3) and hand-foot syndrome (10%, Grade 3) were observed in a phase II study for capecitabine (2,3), whereas the incidence of Grade 3 or severe diarrhea was relatively low (0.9%) and no hand-foot syndrome was observed in a phase II study of S-1 for MBC (4). A direct comparison of capecitabine and S-1 monotherapy is surely necessary, and since the antitumor activity of capecitabine might be relatively low in tumor cells with high DPD levels, an evaluation of the efficacy of S-1 after progression with

capecitabine or in tumors with high DPD expression levels is warranted.

Moreover, while the efficacy of capecitabine in combination therapy with other cytotoxics (11–16) or as first-line chemotherapy (17) has already been reported, few evidence of the efficacy of S-1 in combination therapy or first-line chemotherapy is available (18,19). The efficacy and safety of S-1 in combination with molecular-targeted drugs, such as antibodies and small molecule tyrosine kinase inhibitors, are also unknown. Further studies are thus required to elucidate the clinical role of S-1 in the management of breast cancer patients.

Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

Masahiro Uehara · Takayuki Kinoshita · Takashi Hojo
Sadako Akashi-Tanaka · Eriko Iwamoto
Takashi Fukutomi

Long-term prognostic study of carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) in breast cancer

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Abstract
Background. Tumor markers are frequently used for screening and monitoring in oncology. We investigated the use of preoperative tumor marker (carcinoembryonic antigen [CEA] and carbohydrate antigen [CA] 15-3) levels in estimating the prognosis of breast cancer patients.
Methods. We conducted a retrospective study in patients who underwent breast cancer surgery at National Cancer Center Hospital between 1975 and 1994 and whose serum CEA ($n = 1663$) and CA 15-3 ($n = 1500$) levels were measured prior to operation. When we excluded patients with stage IV disease from the study, the CEA level was within the normal range in 1470 patients, while 150 patients had an elevated CEA level. For CA 15-3, 1395 patients were within the normal range, while 70 patients exhibited an elevated level.
Results. The 5-year and 10-year survival rates for patients with normal CEA levels were 87% and 76%, respectively. However, the 5-year and 10-year survival rates for patients with elevated CEA levels were 76% and 65%, respectively. At both time points, patients with normal CEA levels had higher survival rates ($P < 0.05$). The 5-year and 10-year survival rates for the patients with normal CA 15-3 levels were 86% and 76%, respectively, while only 71% and 52% patients with elevated CA 15-3 levels survived at 5 and 10 years, respectively. These differences were also significant ($P < 0.05$). However, there were no significant differences in disease-free survival (DFS) according to CEA or CA 15-3 levels.
Conclusion. There was a positive correlation between CEA levels and CA 15-3 levels and patient prognosis. Thus, the levels of these tumor markers may help to determine prognosis in breast cancer patients.

Key words Breast cancer · Long-term survival · CEA · CA 15-3 · Retrospective study

M. Uehara (✉)
Department of Surgery, Kyoto Katsura Hospital, 17 Yamada-Hirao, Nishikyo, Kyoto 615-8256, Japan
Tel. +81-75-391-5811; Fax +81-75-381-4224
e-mail: muehara@katsura-hp.jp

T. Kinoshita · T. Hojo · S. Akashi-Tanaka · E. Iwamoto
Department of Breast Surgery, National Cancer Center Hospital, Tokyo, Japan

T. Fukutomi
Department of Breast and Endometrial Surgery, Aichi Medical College, Aichi, Japan

Introduction

Tumor markers, which are proteins or enzymes produced by tumor cells or generated by host cells in response to tumorigenesis, are frequently used for screening and monitoring in oncology. The expression of tumor-specific antigens varies, however, and, in general, tumor cells express several different unique antigens. Therefore, the most effective cancer screening protocols would combine multiple markers for increased specificity.

A number of tumor markers (e.g., carcinoembryonic antigen [CEA] and carbohydrate antigen 15-3 [CA 15-3]) are used clinically in the treatment of breast cancer, but the sensitivity of these markers is low, so that they are not useful as screening tools.[1] However, abnormally elevated levels of tumor markers prior to surgery in a patient with primary breast cancer suggest the presence of undetectable metastatic foci, and this is a negative prognostic factor. In addition, tumor marker levels tend to increase as tumor progression occurs; therefore, tumor markers, while of limited diagnostic use, are important for determining the prognosis of breast cancer.[1] In this study, we investigated the use of preoperative tumor marker (CEA and CA 15-3) levels in estimating the prognosis of breast cancer patients.

Patients and methods

We conducted a retrospective study in patients who underwent breast cancer surgery at National Cancer Center Hos-

Table 1. Characteristics of the patients

Stage	No. of patients							
	0	I	IIA	IIB	IIIA	IIIB	IV	Not known
CEA normal (n = 1495)	19	431	774	2	90	57	25	97
CEA high (n = 168)	1	29	69	1	24	22	18	4
CA15-3 normal (n = 1418)	18	413	744	3	80	49	23	88
CA15-3 high (n = 82)	0	4	32	0	13	20	12	1

pital between 1975 and 1994 and whose serum CEA and CA 15-3 levels were measured prior to operation. For serum CEA measurement, an enzyme immunoassay (EIA) was used until 1989 (n = 462), while a latex photometric immunoassay (LPIA) was used between 1990 and 1992 (n = 706). Until 1993, serum CA 15-3 was measured with a quantitative sandwich radioimmunoassay (RIA) utilizing two monoclonal antibodies (115D8, DF3; n = 1017). However, since 1993, a chemiluminescent enzyme immunoassay (CLEIA) has been used to measure both CEA and CA 15-3 (CEA, n = 495; CA 15-3, n = 483).

We set the criteria as follows. Normal values (thresholds) for CEA and CA 15-3 were set at less than 5.0 ng/ml and less than 28 U/ml, respectively. In this study, the CEA level was within the normal range in 1495 patients, while 168 patients had an elevated CEA level. As for CA 15-3, 1418 patients were within the normal range, while 82 patients exhibited an elevated level. When we excluded stage IV patients from the study, CEA level was within the normal range in 1470 patients, while 150 patients had an elevated CEA level. As for CA 15-3, 1395 patients were within the normal range, while 70 patients exhibited an elevated level. The clinical stages of the patients in each group are listed in Table 1. We used the Japanese Breast Cancer Society classification of breast cancer[2] for the stage classification.

Statistical analyses

The Kaplan-Meier method was used to calculate the cumulative survival rates for the different groups: CEA (normal and elevated levels), and CA 15-3 (normal and elevated levels). Statistical significance was tested using the log-rank test. P values of less than 0.05 were considered as significant.

Results

We found that the 5-year and 10-year survival rates for patients with normal CEA levels (n = 1470) were 87% and 76%, respectively. However, the 5-year and 10-year survival rates for patients with elevated CEA levels (n = 150) were 76% and 65%, respectively. At both time points, patients with normal CEA levels had higher survival rates (P < 0.05; Fig. 1). The 5-year and 10-year survival rates for the patients with normal CA 15-3 levels (n = 1395)

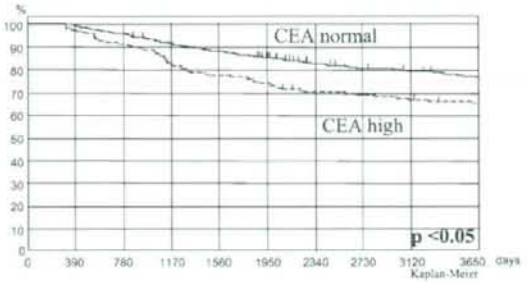

Fig. 1. The 5-year and 10-year survival rates for patients in relation to carcinoembryonic antigen (*CEA*) levels. *CEA normal* denotes normal CEA levels, and *CEA high* denotes elevated CEA levels

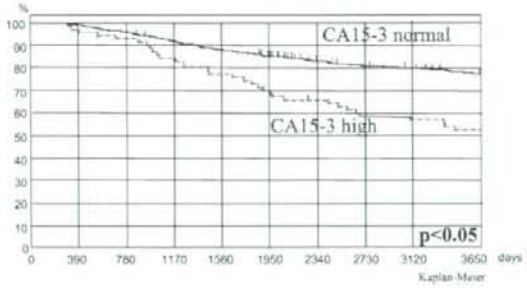

Fig. 2. The 5-year and 10-year survival rates for patients in relation to carbohydrate antigen 15-3 (*CA 15-3*) levels. *CA 15-3 normal* denotes normal CA 15-3 levels, and *CA 15-3 high* denotes elevated CA 15-3 levels

were 86% and 76%, respectively, and only 71% and 52% patients with elevated CA 15-3 levels (n = 70) survived at 5 and 10 years, respectively. These differences were also significant (P < 0.05; Fig. 2). However, there were no significant differences in disease-free survival (DFS) according to either CEA or CA15- levels. The 5-year DFS rate in patients with normal CEA levels was 82%, and the rate in patients with elevated CEA levels was 73% (Fig. 3). The 5-year DFS rate in patients with normal CA 15-3 levels was 83%, and the rate in those with elevated CA 15-3 levels was 67% (Fig. 4).

We also performed a prognostic analysis of the levels of these tumor markers in relation to disease stage. In patients with stage II disease, those with normal CA 15-3 levels had

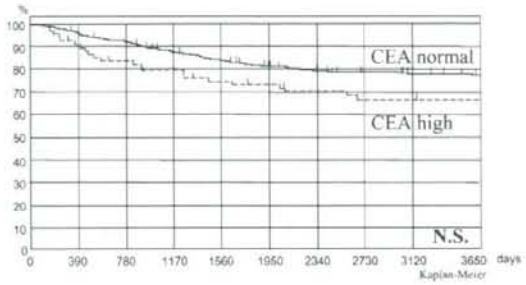

Fig. 3. Disease-free survival rates for patients in relation to CEA levels. *N.S.*, Not significant

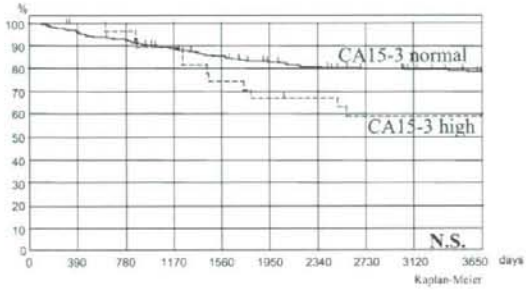

Fig. 4. Disease-free survival rates for patients in relation to CA 15-3 levels

a significantly better prognosis than those with elevated CA 15-3 levels. However, in patients with other disease stages, there was no significant difference in prognosis between those with normal levels and those with elevated levels of either tumor marker.

Discussion

During cellular transformation and progression to cancer, cancer cells release unique enzymes or proteins. Additionally, host cells can produce proteins in response to cancer. Such proteins are termed tumor markers and can be used to screen and monitor disease progression in oncology. Different tumor cells typically produce several unique tumor makers, and, while the specificity of any one marker may be low, the combination of several appropriate tumor markers is a powerful clinical tool.

Carcinoembryonic antigen (CEA) and carbohydrate antigen 15-3 (CA 15-3) are tumor markers commonly used in the screening for breast cancers. CEA is a glycoprotein that is overexpressed in various adenocarcinomas, while CA 15-3 is a mucin-like glycoprotein that is produced in stem cells in response to tumorigenesis.[1]

We conducted a retrospective study in breast cancer patients (excluding stage IV patients) treated at our hospital between 1975 and 1994, and investigated the relationship between CEA and CA 15-3 levels (measured at the time of first medical examination) and patient survival. Interestingly, the 5-year and 10-year survival rates of patients with normal CEA levels were 87% and 76%, while those of patients with elevated CEA levels were 76% and 65%, respectively. Thus, the prognosis of patients whose CEA level was within the normal range at the time of diagnosis was significantly better than the prognosis of those with elevated CEA levels (log-rank; $P < 0.05$). In addition, the 5-year and 10-year survival rates of patients with normal CA 15-3 levels were 86% and 76%, while these rates in the patients with elevated CA 15-3 levels were 71% and 52%, respectively. As with the CEA levels, the long-term survival of breast cancer patients with CA 15-3 levels within the normal range at the time of diagnosis was significantly better than the survival of patients with elevated CA 15-3 levels (log-rank; $P < 0.05$). Previous studies have identified an inverse relationship between tumor marker levels and prognosis when comparing patients with tumors of the same clinical stage.[3] Our results further demonstrate a relationship between preoperative tumor marker levels and long-term survival. Further work is needed to clarify which marker is superior for predicting prognosis, but both may be suitable. However, CA 15-3 may be more sensitive than CEA. The American Society of Clinical Oncology (ASCO) has reported that CA27-29, a MUC-1 marker, is better at tracking tumor recurrence than CA 15-3 (also a MUC-1 marker). Regardless of which marker is better, measuring the levels of MUC-1 markers is likely to be highly effective for monitoring tumor progression or recurrence.[1] Some studies have reported that tumor markers are effective for the early screening of recurrence,[4–7] but the sensitivity varies in these reports. Similar variation was observed when tumor markers were used in the diagnosis of primary breast cancer.[3,8,9] Of note, ASCO has reported that: (i) CEA is not recommended for screening, diagnosis, staging, or routine surveillance of breast cancer patients after primary therapy (1997, 2000, 2007 recommendation); (ii) routine use of CEA for monitoring the response of metastatic disease to treatment is not recommended; and (iii) in the absence of readily measureable disease, elevated levels of MUC-1 markers (CA 15-3 and/or CA27-29) or a rising CEA level may suggest treatment failure.[1]

Significant differences in CA 15-3 expression levels between those in benign tumors and those in stage III and IV disease have been reported.[10] However, Gion et al.[11] reported that no differences were seen in CA 15-3 expression between benign tumors and stage I and II disease. Tumor marker levels tend to rise as disease progresses; therefore, tumor markers may be important prognostic factors.[1] Detecting elevated levels of preoperative tumor markers in patients with primary breast cancer may suggest the presence of undetectable metastatic foci; therefore, increased tumor marker levels are one factor that may predict a poor prognosis. Molina et al.[3,8] conducted a study of locoregional breast cancer and reported that the preoperative sensitivities of CEA and CA 15-3 were 11.7%–13% and 12.7%–18.8%, respectively. In addition, the sensitivities of CEA and CA 15-3 after recurrence were 30%–70%

Table 2. Past reports

Author	Year	No. of patients	Tumor marker	Sensitivity (%)	Patients state
Present study	2007	1620/1465	CEA, CA 15-3	CEA=9.11; CA 15-3=5.36	Preoperative
Soletormos[15]	2004	406	CEA, CA 15-3, TPA	CEA=or CA 15-3 or TPA=44–69	Recurrence
Molina[5]	2003	503	CEA, CA 15-3	CEA=11.7; CA 15-3=12.7	Locoregional breast cancer
Molina[3]	2003	1057	CEA, CA 15-3	CEA=13; CA 15-3=18.8	Locoregional breast cancer
Guadagni[9]	2001	2191	CEA, CA 15-3	CEA=16.7, CA 15-3=33.0, CEA+CA 15-3=39	Stage I–IV or metastatic disease
				CEA=38.5, CA 15-3=60, CA 15-3 and/or CEA=60	Recurrence
Lumachi[16]	2000	62	CEA, CA 15-3	CEA=40.3, CA 15-3=41.9, CEA+CA 15-3=59.7	Recurrence
Lumachi[17]	1999	103	CEA, CA 15-3	CEA=38.1, CA 15-3=61.1	Recurrence
Sutterlin[10]	1999	664	CEA, CA 15-3	CEA=30.3, CA 15-3=48.7	Recurrence
Molina[18]	1999	250	CEA, CA 15-3	CEA=31.6,CA 15-3=46.3, CEA+CA 15-3=59	Recurrence
Sutterlin[19]	1999	76	CEA, CA 15-3	CEA=30, CA 15-3=49,	Recurrence
Lauro[20]	1999	70	CEA, CA 15-3	CEA=35, CA 15-3=79, CEA+CA 15-3=79	Recurrence
Pectasides[21]	1996	68	CEA, CA 15-3	CEA=34, CA 15-3=68, CEA+CA 15-3=68	Recurrence
Jezersek[22]	1996	56	CEA, CA 15-3	CEA=70, CA 15-3=75	Recurrence

and 41.9%–79%, respectively (Table 2). In addition to well-known prognostic factors such as T-factor, N-factor, and hormone receptors, several references have acknowledged the relevance of tumor markers and prognosis.

In the present study, there were no significant differences in DFS according to preoperative levels of the tumor markers CEA and CA 15-3. Many studies have examined the relationship between recurrence and rising tumor marker expression levels. However, there is a delay between increases in marker levels and the confirmation of clinical recurrence, and this time period differs for each patient. In current practice, although a rise in marker expression may be detected, a patient will not be treated unless clinical recurrence is confirmed. A recent study compared the 7-year survival rates of patients undergoing surveillance treatment upon the detection of a rise in marker expression (n = 36) and those who were treated after the recurrence was confirmed by imaging (n = 32). Interestingly, tumor marker-guided salvage treatment prolonged the survival of the breast cancer patients.[12] However, a large-scale study conducted in 1320 postoperative breast cancer patients by the GIVIO investigators[13] found no significant differences in time to detection of recurrence between an intensive surveillance group and a control group. Furthermore, another study of postoperative breast cancer patients (n = 1243) found no difference in 5-year overall mortality between an intensive surveillance group and a control group.[14] Despite differences in the accuracy of current test methods, most studies have not found any differences in survival between groups undergoing intensive surveillance treatment for recurrence at an early stage and control groups; therefore, we believe that tumor marker-guided salvage treatment may not improve patient prognosis.

While tumor marker monitoring may not be useful for the detection of disease recurrence, our data support a role for CEA and CA 15-3 levels at least in helping to determine preoperative prognosis. We found that patients with elevated preoperative tumor marker levels had a significantly worse long-term prognosis than those patients with levels in the normal range. In relation to disease stage, stage II patients with normal CA 15-3 levels had a significantly better prognosis than those with elevated CA 15-3 levels. However, there were no significant differences in prognoses according to either CEA or CA 15-3 levels in patients with any other disease stage. This result suggested that the tumor marker level at the time of diagnosis was an independent prognostic factor. The early detection of recurrent foci may be accomplished using highly sensitive tumor markers, together with modern imaging technologies. Although the results of randomized controlled trials have demonstrated that the timing to initiate treatment for recurrence does not affect the overall survival rate, advances in imaging technology and improved treatment regimens may allow the early detection of recurrent foci and lead to improved patient survival. As diagnostic and treatment techniques improve, tumor markers will likely become more important in cancer therapy.

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Cross-sectional analysis of germline *BRCA*1 and *BRCA*2 mutations in Japanese patients suspected to have hereditary breast/ovarian cancer

Kokichi Sugano,[1,4,13,17,24] Seigo Nakamura,[19] Jiro Ando,[2] Shin Takayama,[2,11] Hiroyuki Kamata,[3] Isao Sekiguchi,[3] Megumi Ubukata,[4] Tetsuro Kodama,[5,17] Masami Arai,[8] Fujio Kasumi,[9] Yasuo Hirai,[10] Tadashi Ikeda,[11] Hiromitsu Jinno,[11] Masaki Kitajima,[11] Daisuke Aoki,[12] Akira Hirasawa,[12] Yuko Takeda,[13,14] Kumiko Yazaki,[13] Takashi Fukutomi,[15,23] Takayuki Kinoshita,[15] Ryuichiro Tsunematsu,[16] Teruhiko Yoshida,[17,18] Masako Izumi,[17] Shino Umezawa,[17] Hiroshi Yagata,[19] Hiroko Komatsu,[20,21] Naoko Arimori,[20,21] Noriko Matoba,[20,21] Nobuhisa Gondo,[22] Shiro Yokoyama[22] and Yoshio Miki[6,7]

[1]Oncogene Research Unit/Cancer Prevention Unit, Tochigi Cancer Center Research Institute; [2]Division of Surgery, [3]Division of Gynecology, [4]Genetic Counseling and Cancer Prevention Clinic, [5]Director, Tochigi Cancer Center Hospital, 4-9-13 Yohnan, Utsunomiya, Tochigi Pref. 320-0834; [6]The Cancer Institute of Japanese Foundation for Cancer Research (JFCR), 3-10-6 Ariake, Koto-ku, Tokyo 135-8550; [7]Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-0034; [8]Clinical Genetic Oncology, [9]Department of Breast Surgery, [10]Department of Gynecology, The Cancer Institute Hospital of JFCR, 3-10-6 Ariake, Koto-ku, Tokyo 135-8550; [11]Department of Surgery, [12]Department of Gynecology, [13]Genetic Counseling Clinic, Keio University Hospital; [14]Faculty of Nursing and Medical Care, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582; [15]Division of Surgery, [16]Division of Gynecology, [17]Genetic Counseling Clinic, National Cancer Center Hospital; [18]Genetics Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045; [19]Breast Center, [20]Department of Clinical Genetics, St Luke's International Hospital; [21]St. Luke's College of Nursing, 9-1 Akashi-cho, Chuo-ku, Tokyo 104-8560; [22]FALCO biosystems, 346, Shimizu-cho Nijoagaru Kawaramachi-dori Nakagyo-ku Kyoto 604-0911; [23]Department of Surgery, Aichi Medical University, 21 Karimata, Yazako, Nagakute-cho, Aichi-gun, Aichi, 480-1195 Japan.

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The prevalence of *BRCA*1/2 germline mutations in Japanese patients suspected to have hereditary breast/ovarian cancer was examined by a multi-institutional study, aiming at the clinical application of total sequencing analysis and validation of assay sensitivity in Japanese people using a cross-sectional approach based on genetic factors estimated from personal and family histories. One hundred and thirty-five subjects were referred to the genetic counseling clinics and enrolled in the study. Full sequencing analysis of the *BRCA*1/2 gene showed 28 types of deleterious mutations in 36 subjects (26.7%), including 13 types of *BRCA*1 mutations in 17 subjects (12.6%) and 15 types of *BRCA*2 mutations in 19 subjects (14.1%). Subjects were classified into five groups and 22 subgroups according to their personal and family history of breast and/or ovarian cancer, and the prevalence of deleterious mutations was compared with previously reported data in non-Ashkenazi individuals. Statistical analysis using the Mantel-Haenszel test for groups I through IV revealed that the prevalence of Japanese subjects was significantly higher than that of non-Ashkenazi individuals ($P = 0.005$, odds ratio 1.87, 95% confidence interval 1.22–2.88). Family history of the probands suffering from breast cancer indicated risk factors for the presence of deleterious mutations of *BRCA*1/2 as follows: (1) families with breast cancer before age 40 within second degree relatives ($P = 0.0265$, odds ratio 2.833, 95% confidence interval 1.165–7.136) and (2) families with bilateral breast cancer and/or ovarian cancer within second degree relatives ($P = 0.0151$, odds ratio 2.88, 95% confidence interval 1.25–6.64). (*Cancer Sci* 2008; 99: 1967–1976)

In Japan, breast cancer is the most frequent malignancy in women and estimates of new cases and deaths in 2002 were 32 245 and 9178, respectively.[1] The standardized incidence ratio of breast cancer in Japan was approximately one-third that of the US (32.7 *vs* 101.7 per 100 000 women).[1] The incidence of breast cancer in Japanese women shows a steady increase; however, it is still much lower than in Western countries. In breast cancer, family history is the strongest risk factor for cancer predisposition. Epidemiological studies showed that 12% of women with breast cancer have one affected family member and 1% have two or more affected relatives.[2] Women with one, two, and three or more first-degree affected relatives have an increased breast cancer risk when compared with women who do not have an affected relative (risk ratios 1.8, 2.9, and 3.9, respectively).[2] Recent advances in molecular genetics elucidated *BRCA*1 and *BRCA*2 (*BRCA*1/2) as two major susceptibility genes for breast cancer predisposition.[3,4] Gene testing of *BRCA*1/2 is available as a routine clinical test for diagnosing hereditary breast/ovarian cancer (HBOC) in the US and other Western countries,[5,6] while only a few reports have been published concerning the prevalence of *BRCA*1/2 mutations among Japanese people.[7–12] The methods of genetic analysis employed in these studies varied, such as polymerase chain reaction (PCR)/ single strand conformational polymorphisms (SSCP), protein truncation test, and PCR/direct sequencing, but they were performed as preliminary in-house tests in the research setting. In the US, commercial *BRCA*1/2 gene testing was initiated by Myriad Genetic Laboratories in 1996. The test is based on the exon-by-exon PCR/direct sequencing approach comprising sequence analysis of over 17 500 base pairs of the protein-coding and adjacent non-coding regions of *BRCA*1 and *BRCA*2 genes of which test results performed in more than 10 000 individuals were reported.[5,6] Myriad Genetic Laboratories released some of the results to a public database, the Breast Cancer Information Core (BIC) and this test is now utilized as the de facto standard in the US.[13] Examinees with positive test results or pathogenic mutations may undergo close surveillance of breast and ovarian cancers or there might be other options such as risk-reducing surgeries including prophylactic salpingo-oophorectomy and/or mastectomy.[14,15] Chemoprevention using tamoxifen or oral contraceptives were also reported to reduce the risk for contralateral breast cancer or ovarian cancers.[16,17] Clinical application of *BRCA*1/2 gene testing brought a paradigm shift in cancer prevention strategies targeted to at-risk mutation carriers. The aim of the present study was to clarify the prevalence of germline *BRCA*1/2 mutations among Japanese patients suspected to have

[24]To whom correspondence should be addressed.
E-mail: ksugano@tcc.pref.tochigi.jp

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Family history within second-degree relatives						
Breast and/or ovarian cancer at any age		Yes				No
Breast cancer <50 years of age in ≥ one relative		No	Yes	No	Yes	
Ovarian cancer at any age		No	No	Yes	Yes	
Proband's personal history	Breast cancer ≥50 years of age	I-1	II-1	II-4	II-7	
	Breast cancer <50 years of age	I-2	II-2	II-5	IV-3	
	Ovarian cancer at any age, No breast cancer	I-3	II-3	II-6	IV-4	
	Breast cancer and ovarian cancer at any age	III	IV-1	IV-2	IV-5	V-5
	Male breast cancer at any age	V-1	V-2	V-3	V-4	V-6

Group I (64/828 7.7%)* Group II (364/1709 21.3%)
Group III (10/52 19.2%) Group IV (205/421 48.7%) Group V

Fig. 1. Classification and grouping of the enrolled subjects. *Numbers in parentheses indicate prevalence of *BRCA*1/2 mutations reported in non-Ashkenazi individuals in the US.[6]

HBOC using the standardized method available in the clinical setting and to establish the genetic basis required for the clinical application of *BRCA*1/2 gene testing for Japanese.

Materials and Methods

The study was designed to validate the sensitivity of *BRCA*1/2 gene testing among Japanese with relevance to personal and family histories of breast and/or ovarian cancer in comparison with the previously reported data of non-Ashkenazi individuals in the US.[6] The study was performed as a contract research by FALCO biosystems. The study protocol was approved by the institutional review board of each participating institution before study initiation. All patients gave written informed consent before registration.

Recruitment of subjects. Candidates were recruited from surgical or gynecologic clinics in five major hospitals in the Tokyo metropolitan area (The Cancer Institute Hospital of Japanese Foundation for Cancer Research, National Cancer Center Hospital, Keio University Hospital, St. Lukes' International Hospital, and Tochigi Cancer Center Hospital) or recruited through newspaper advertisements in the Tokyo metropolitan area. All participants were referred to genetic counseling clinics in these hospitals and checked for eligibility for enrollment in the study. As genetic counseling was mandatory in this study, they underwent genetic counseling before and after gene testing. All expenses for genetic counseling were covered by FALCO biosystems. From August 2003 through May 2006, 135 patients were enrolled in the study, among which 101 subjects were treated for breast and/or ovarian cancers in these hospitals, and 34 subjects were recruited through newspaper advertising. Patient records of their personal history of cancer, including diagnosis, treatment, and clinicopathological parameters, were obtained through a survey completed by their attending surgeons or physicians.

Patient selection. Patients were eligible for the study if they fulfilled the following conditions: (i) native Japanese over 20 years old; (ii) histological diagnoses of invasive breast cancer and/or ovarian cancer; and (iii) at least one first- or second-degree relative diagnosed with either or both cancers. Eligible patients were assigned to the matrix chart according to their personal and family history of cancer (Fig. 1). This matrix chart was formulated based on the reported prevalence of *BRCA*1/2 mutations in 3011 non-Ashkenazi individuals in the US.[6] Enrolled subjects were classified into five groups, i.e. Group I through Group V. All subjects assigned to Groups I through IV had some form of risk factor estimated from their personal and family history, while the risk of those in Groups I and III was personal rather than familial. The proportion of familial risk increased in Groups II and IV, and Group IV had a combination of the highest risk of both personal and familial history. Reportedly, the prevalence of *BRCA*1/2 mutations in Group I is relatively low, 7.7% (64/828 cases), while those in Groups II, III, and IV were 21.3% (364/1709), 19.2% (10/52), and 48.7% (205/421) respectively.[6] Group V included cases of male breast cancer at any age irrespective of their family history and sporadic cases of breast cancers with concomitant ovarian cancers. The prevalence of *BRCA*1/2 mutations in each matrix or group in the present study could be compared with those reported in the US, except for Group V that had no comparable counterpart.

Exclusion criteria for enrollment were as follows: (i) informed consent was provided not by a principal, but by a representative; (ii) a precise history of breast and/or ovarian cancers was not available; (iii) the patient was undergoing bilateral oophorectomy due to non-malignant disorders; (iv) correct diagnosis was not disclosed to the patient; (v) informed consent was not provided due to psychiatric problems; (vi) the patient had non-invasive breast cancer; (vii) borderline ovarian neoplasms; (viii) the patient was undergoing allogeneic bone marrow transplantation; (ix) another relative had undergone *BRCA*1/2 gene testing; and (x) when gene testing and disclosure of genetic information might cause serious sociopsychological problems.

***BRCA*1 and *BRCA*2 gene testing.** Analyses of *BRCA*1/2 were performed by direct sequencing, as described previously.[5,6] Briefly, 7 mL of anticoagulated blood was sent to FALCO biosystems for DNA extraction. Aliquots of patient DNA were sent to Myriad Genetic Laboratories (Salt Lake City, UT, USA) and subjected to PCR/direct sequencing analysis for *BRCA*1/2. This analysis also included the detection of the following five specific large genomic rearrangements of the *BRCA*1 gene (five-site rearrangement panel): 3.8-kb deletion of exon 13 and 510-bp deletion of exon 22 described in individuals of Dutch ancestry,[18] 6-kb duplication of exon 13 described in individuals of European (particularly British) ancestry,[19] 7.1-kb deletion of exons 8 and 9 described in individuals of European ancestry,[20] and 26-kb deletion of exons 14–20.[21] Nucleotide positions of mutations were expressed according to GenBank entries U14680 and U43746. All variants were interpreted according to the following criteria.[6]

Positive for deleterious mutation. Mutations were interpreted as positive deleterious mutations if they prematurely terminated (truncated) the protein product of *BRCA*1 at least 10 amino

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Table 1. Background of subjects enrolled in the study

Variables	All	Non-carrier	Deleterious mutations in		
			*BRCA*1/2	*BRCA*1	*BRCA*2
Number of patients	135	99	36	17	19
Age at enrollment	51.6 ± 12.4	52.5 ± 12.7*	49.1 ± 11.3*	48.9 ± 10.1	49.2 ± 12.5
Number of sessions for genetic counseling	3.36 ± 0.82 (1–8)[‡]	3.22 ± 0.56 (1–5)***	3.75 ± 1.20 (1–8)***	–	–
Sex					
Female	131	96	35	17	18
Male	4	3	1	0	1
Types of affected cancer					
Female					
Breast	113	83	30	12	18
Ovarian	9	8	1	1	0
Both	9	5	4	4	0
Male					
Breast	4	3	1	0	1
Genealogical information; number of relatives ascertained					
Relatives ≤ 1st degree	673 (4.99)[†]	487 (4.92)**	186 (5.16)**	92 (5.41)	94 (4.95)
Male	305 (2.26)	218 (2.20)	87 (2.42)	42 (2.47)	45 (2.37)
Female	368 (2.73)	269 (2.72)	99 (2.75)	50 (2.94)	49 (2.58)
1st < Relatives ≤ 2nd degree	1142 (8.46)	825 (8.33)**	317 (8.80)**	166 (9.76)	151 (7.95)
Male	526 (3.90)	383 (3.87)	143 (3.97)	72 (4.24)	71 (3.74)
Female	616 (4.56)	442 (4.46)	174 (4.83)	94 (5.53)	80 (4.21)
Relatives > 2nd degree	427 (3.16)	254 (2.57)**	173 (4.81)**	106 (6.24)	67 (3.53)
Male	192 (1.42)	112 (1.13)	80 (2.22)	55 (3.24)	25 (1.32)
Female	235 (1.74)	142 (1.43)	93 (2.58)	51 (3.00)	42 (2.21)

[†]Numbers in parentheses indicate average number of relatives in a family. [‡]Minimal and maximal. ***$P = 0.0007$. *$P = 0.1594$, unpaired t-test. **χ^2 value 26.90, d.f. = 2, $P < 0.0001$.

acids from the C-terminus or the protein product of *BRCA*2 at least 110 amino acids from the C-terminus, based on the documentation of deleterious mutations in *BRCA*1/2. In addition, specific missense mutations and non-coding intervening sequence mutations were interpreted as deleterious on the basis of data derived from linkage analysis of high-risk families, functional assays, biochemical evidence, or demonstration of abnormal mRNA transcript processing.

Genetic variant of uncertain significance. This group includes missense mutations and mutations that occur in analyzed intronic regions whose clinical significance has not yet been determined, chain-terminating mutations that truncate *BRCA*1 and *BRCA*2 distal to amino acid positions 1853 and 3308, respectively, and mutations that eliminate the normal stop codons of these proteins.

Mutational types were defined according to the international nomenclature system reported by Antonarakis *et al.*[22] All of the detected mutations were searched for in the BIC database.[13] The description of mutational types previously reported in the BIC database is indicated along with those defined by the international nomenclature system, in order to facilitate the comparison of the data with those reported in the database or other publications.

Multiplex ligation-dependent probe amplification (MLPA) analysis. To search for unknown genomic rearrangements, we performed MLPA analysis for all samples in which no deleterious mutation was detected, using Salsa MLPA Kits P002 and P087, which are commercially available from MRC-Holland (Amsterdam, The Netherlands). MLPA is a quantitative multiplex PCR approach to determine the relative copy number of each *BRCA*1/2 exon.[23,24] Assay procedures were performed according to the manufacturer's instructions.

Statistical analysis. Statistical significance was analyzed by Fisher's exact test and unpaired t-test using Prism 4 (GraphPad Software, San Diego, CA, USA). Comparisons of the prevalence of *BRCA*1/2 germline mutations divided by subgroups between Japanese and non-Ashkenazi individuals were analyzed by Fisher's exact test or the Mantel-Haenszel test using R package (version 1.1.2), available from the Comprehensive R Archive Network (CRAN) (http://strimmerlab.org/software/genets/). Cumulative incidence was analyzed by Kaplan–Meier plot (log-rank test) using SAS software (SAS Institute Japan, Tokyo, Japan).

Results

Characteristics of the enrolled subjects. A total of 135 subjects were examined for *BRCA*1/2 germline mutations, and deleterious mutations were found in 36 subjects (17 for *BRCA*1 and 19 for *BRCA*2). Backgrounds of all subjects and those divided by carrier status are shown in Table 1. In the analysis of all subjects, average age at enrollment was 51.6 ± 12.4 years and the average number of counseling sessions per client was 3.36 ± 0.82. There was no significant difference as to the age at enrollment between non-carriers and carriers with deleterious mutations. As for genetic counseling, significantly more sessions were performed for those carrying deleterious mutations of *BRCA*1/2 as compared to non-carriers (3.75 ± 1.20 *vs* 3.22 ± 0.56, $P = 0.0007$). Of the 135 subjects examined, 131 were women and 4 were men. All of the male subjects developed breast cancer, while 113 women developed breast cancers (9 women developed ovarian cancers and 9 women developed both breast and ovarian cancers). There was no statistical significance in these variables between *BRCA*1/2 mutation carriers and non-carriers. In the study, patient accrual was determined by personal and family histories within second-degree relatives. Family history was precisely assessed in genetic counseling clinics and familial information was obtained for 2242 family members, including probands. There were no statistical differences in the numbers of ascertained relatives within the first- or second-degree between non-carriers and carriers. As for relatives beyond the second-degree, significantly more relatives were ascertained in pedigrees with deleterious *BRCA*1/2 mutations as compared to

Table 2. Clinical characteristics of the subjects with breast or ovarian cancer

Variables		Breast cancer[†]	Ovarian cancer[‡]
Number of subjects		126	18
Sex	Female	122	18
	Male	4	–
Age at diagnosis	Female	46.2 ± 12.1	50.2 ± 11.8
	Male	64.5 ± 3.7	–
Tumor size (T)	Tis	2[¶]	–
	T1	66	11
	T2	49	1
	T3	4	4
	Missing data (No.)	5	2
Nodal status (N)	Negative	95	10
	Positive	24	5
	Missing data (No.)	7	3
Stage	0	2	–
	I	71	9 (IA 4, IC 5)
	II	38	1 (IIA 1)
	III	4	4 (IIIB 1, IIIC 3)
	IV	1	1
	Missing data (No.)	13	3
Histology	Non-invasive	2	Serous 3, mucinous 3,
	Invasive	111	endmetrioid 2, clear cell 2,
	Missing data (No.)	13	undifferentiated 2, mixed-cell type 2,
			others 2, sex-cord stromal tumor 1, germ cell tumor 1
Estrogen receptor status	Positive	72	
	Negative	37	
	Missing data (No.)	17	
Progesterone receptor status	Positive	64	
	Negative	44	
	Missing data (No.)	18	
Histological grade	Grade 1	24	
	Grade 2	37	
	Grade 3	24	
	Missing data (No.)	41	
Laterality	Unilateral	104	
	Bilateral	22	
Mutational status	No mutation	91	13
	*BRCA*1/2	35	5
	*BRCA*1	16	5
	*BRCA*2	19	0

[†]Includes subjects with ovarian cancer ($n = 9$). [‡]Includes subjects with breast cancer ($n = 9$). [¶]Subjects with multiple primary breast cancer, in which histology of the first primary cancer was pTis. They were enrolled in the study as the histology of the second primary breast cancer was ascertained to be invasive cancer.

those of non-carriers ($P < 0.0001$) (Table 1). The clinical characteristics of breast and ovarian cancers that developed in enrolled subjects are listed in Table 2. In this protocol, non-invasive cases, including pTis or DCIS, did not fulfill the eligibility, but two cases were enrolled in the study due to the subsequent occurrence of invasive breast cancers in the contralateral breast.

Results of *BRCA*1/2 gene testing. In the analysis of *BRCA*1, 13 types of deleterious mutations were detected in 17 subjects. One mutation (L63X, c.188T > A) was found in five subjects. Genetic variants of uncertain significance were detected in nine subjects, among which three subjects had the same mutational types (S1557P, c.4729T > C), substituting cytosine for thymine (Table 3).

In the analysis of *BRCA*2, 15 types of deleterious mutations were detected in 19 subjects. Each of four mutational types (c.1813delA, S1882X[c.5645C > A], c.5576_5579delTTAA, and R2318X[c.6952C > T]) was detected in two subjects. Eight types of genetic variants of uncertain significance were detected in 13 subjects, among which four types were detected in more than two subjects (Table 4). Of the deleterious mutations, five of 13 mutational types in *BRCA*1 and four of 15 mutational types in *BRCA*2 were not reported previously in the BIC database. As for genetic variants of uncertain significance, all of these variants were missense mutations, among which three in *BRCA*1 and four in *BRCA*2 were not reported in the BIC database. No genomic rearrangement of the *BRCA*1 gene was detected in analysis using a 5′-site rearrangement panel. In analysis using MLPA, genomic rearrangements were not detected in *BRCA*1/2 in all subjects.

Comparison of prevalence of *BRCA*1/2 germline mutations between Japanese and non-Ashkenazi individuals. Deleterious mutations of *BRCA*1/2 were detected in 26.7% (36/135) of the subjects enrolled in the study. The prevalence of deleterious mutations in non-Ashkenazi individuals in each matrix or group was calculated based on the data reported previously.[6] The prevalence of mutations in Groups I through IV in the Japanese cohort was 27.2% (34/125), while that in non-Ashkenazi individuals was 20.3% (590/2900), respectively (Table 5). The prevalence of *BRCA*1/2 mutations in each matrix or subgroup was compared with that in non-Ashkenazi individuals. In the analysis of each subgroup, statistical difference was observed only in the subgroup I-1 between Japanese and non-Ashkenazi individuals,

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Table 3. Deleterious mutations and genetic variants of uncertain significance detected in *BRCA1*

BRCA1: Deleterious mutations Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	(Breast Cancer Information Core [BIC] mutation database) BIC designation	Type	No. reported	Ethnicity
5	L63X(c.188T > A)	5	II-2, II-6, IV-3, V-5, III-1	NR	L63X	N	6	Asian: 5
5	c.190_193delTGTA	1	I-2	NR	(309del4)[a]	F	0	
7	Y130X(c.390C > A)	1	II-7	NR	Y130X	N	1	Asian: 1
11	c.1112delC	1	II-2	NR	(1231delC)	F	0	
11	K503X(c.1507 A > G)	1	IV-2	NR	(K503X)	N	0	
11	E908X(c.2722G > T)	1	II-5	NR	E908X	N	58	Asian: 0
11	Q934X(c.2800C > T)	1	IV-5	NR	Q934X	N	4	Asian: 2
11	c.3442delG	1	II-2	NR	3561delG	F	2	Asian: 2
11	c.3505_3509delGACAT	1	IV-3	NR	(3624del5)	F	0	
11	c.4041_4042delAG	1	I-2	NR	4160delAG	F	7	Asian: 0
13	IVS13 + 1G > T[a]	1	II-5	NR	IVS13 +1G > T	S	1	NR
13	R1443X(c.4327C > T)	1	IV-3	NR	R1443X	N	126	Asian: 1
24	c.5533_5534insT	1	II-2	NR	(5652insT)	F	0	

BRCA1: Genetic variant of uncertain significance Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	(BIC Mutation Database) BIC Designation	Type	No. reported	Ethnicity
5	L52F(c.154C > T)	1	II-1	NR	L52F	M	5	Asian: 2
10	P209L(c.626C > T)	1	II-1	NR	(P209L)	M	0	
11	S1217P(c.3649T > C)	1	II-1	NR	(S1217P)	M	0	
16	S1577P(c.4729T > C)	3	I-2, II-2, II-5	NR	S1577P	M	1	Asian: 1
20	R1753T(c.5258G > C)	1	II-3	NR	(R1753T)	M	0	
21	F1761S(c.5282T > C)	1	II-2	NR	F1761S	M	1	Asian: 0
24	Y1853C(c.5558 A > G)	1	II-5	NR	Y1853C	M	1	Asian: 0

[a]Mutation in the donor site of intron 13, suspected to be deleterious, resulting in a splicing error. [b]Mutations in parentheses indicate mutational types of unreported cases represented according to the style of BIC nomenclature. F, frameshift mutation; M, missense mutation; N, nonsense mutation; NR, not reported; P, genetic polymorphism; S, splice site mutation; Syn, synonymous mutation.

Table 4. Deleterious mutations and genetic variants of uncertain significance detected in *BRCA2*

BRCA2: deleterious mutation Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	(Breast Cancer Information Core [BIC] mutation database) BIC designation	Type	No. reported	Ethnicity
3	c.86_87delTT	1	I-1	NR	314delTT	F	1	NR
10	c.1813delA	2	II-2, II-2	NR	2041delA	F	16	Asian: 0
11	c.2612delCinsTTT	1	I-2	NR	(2840delC insTTT)[b]	F	0	
11	c.3847_3848delGT	1	V-6	NR	4075delGT	F	61	Asian: 0
11	c.4021delT	1	II-2	NR	(4249delT)	F	0	
11	S1882X(c.5645C > A)	2	I-2, II-1	NR	S1882X	N	28	Asian: 2
11	c.5207_5208delAA	1	II-5	NR	(5435delAA)	F	0	
11	c.5576_5579delTTAA	2	I-1, I-1	NR	5804del4	F	29	Asian: 0
11	c.6445_6446delAT	1	II-2	NR	6673delAT	F	3	Asian: 0
13	R2318X(c.6952C > T)	2	II-1, II-5	NR	R2318X	N	5	Asian: 2
18	c.8064_8065delCT	1	II-2	NR	(8292delCT)	F	0	
20	S2835X(c.8504C > A)	1	II-2	NR	S2835X	N	1	Asian: 0
23	Q3026X(c.9076C > T)	1	II-2	NR	Q3026X	N	3	Asian: 1
23	P3039P (c.9117G > A)[a]	1	II-2	rs28897756	P3039P	Syn	14	Asian: 0
25	R3128X(c.9382C > T)	1	II-2	NR	R3128X	N	50	Asian: 0

BRCA2: Genetic variant of uncertain significance Exon	Designation	No. detected	Subgroup assigned	dbSNP ID	(BIC Mutation Database) BIC designation	Type	No. reported	Ethnicity
10	K322Q(c.964 A > C)	2	I-1, II-1	rs11571640	K322Q	M	11	Asian: 7
10	M524I(c.1572G > C)	1	I-1	NR	(M524I)	M	0	
10	K610Q(c.1828 A > C)	1	II-2	NR	(K610Q)	M	0	
11	I770V(c.2308 A > G)	1	II-5	NR	(I770V)	M	0	
11	K1132R(c.3395 A > G)	1	I-2	rs1801406	K1132R	M	1	Asian: 1
11	G2044V(c.6131G > T)	3	II-2, II-2, V-5	NR	G2044V	M	10	Asian: 10
11	V2109I(c.6325G > A)	2	I-1, I-2	NR	V2109I	M	8	Asian: 5
17	S2616F(c.7847C > T)	2	I-1, II-1	NR	(S2616F)	M	0	

[a]Synonymous mutation in the exon–intron junction of exon 23, suspected to be deleterious, resulting in a splicing error. [b]Mutations in parentheses indicate mutational types of unreported cases represented according to the style of BIC nomenclature. F, frameshift mutation; M, missense mutation; N, nonsense mutation; NR, not reported; P, genetic polymorphism; S, splice site mutation; Syn, synonymous mutation.

Table 5. Prevalences of *BRCA*1/2 germline mutations between non-Ashkenazi individuals and Japanese

Group	Subgroup	Prevalence of mutations in *BRCA*1/2		*P*-values*	Odds ratio (95% CI)*
		Non-Ashkenazi individuals (Myriad)[6]	Japanese (FALCO)		
I	I-1	4/172 (2.3%)	3/14 (21.4%)	0.010	11.11 (1.450–75.21)
	I-2	55/579 (9.5%)	4/26 (15.4%)	0.307	1.730 (0.418–5.360)
	I-3	5/77 (6.5%)	0/2 (0%)	ND	ND
II	II-1	34/315 (10.8%)	2/16 (12.5%)	0.689	1.180 (0.125–5.491)
	II-2	206/806 (25.6%)	13/33 (39.4%)	0.103	1.892 (0.848–4.079)
	II-3	25/67 (37.3%)	0/1 (0%)	ND	ND
	II-4	4/87 (4.6%)	0/5 (0%)	ND	ND
	II-5	41/236 (17.4%)	4/11 (36.4%)	0.119	2.704 (0.554–11.22)
	II-6	35/111 (31.5%)	1/6 (16.7%)	0.665	0.437 (0.009–4.112)
	II-7	19/87 (21.8%)	1/3 (33.3%)	0.534	1.776 (0.029–35.87)
III	–	10/52 (19.2%)	1/1 (100%)	ND	ND
IV	IV-1†	16/39 (41.0%)	0/0	ND	ND
	IV-2	9/20 (45.0%)	1/1 (100%)	ND	ND
	IV-3	126/267 (47.2%)	3/5 (60%)	0.671	1.675 (0.189–20.35)
	IV-4†	38/71 (53.5%)	0/0	ND	ND
	IV-5	16/24 (66.7%)	1/1 (100%)	ND	ND
V	V-1	NR	0/0		
	V-2	NR	0/0		
	V-3	NR	0/0		
	V-4	NR	0/0		
	V-5	NR	1/4 (25.0%)		
	V-6	NR	1/6 (16.7%)		
Subtotal for Group I		64/828 (7.7%)	7/42 (16.7%)	0.0471†	2.613 (1.108–6.162)†
Subtotal for Group II		364/1709 (21.3%)	21/75 (28.0%)	0.134†	1.554 (0.915–2.640)†
Subtotal for Group IV		151/311 (48.6%)	5/7 (71.4%)	0.455†	2.589 (0.484–13.87)†
Subtotal for Group V		NR	2/10 (20.0%)	ND	ND
Total for Groups I–IV		589/2900 (20.3%)	34/125 (27.2%)	0.005†	1.873 (1.217–2.884)†

*Fisher's exact test. †Estimated by Mantel-Haenszel test. ‡Excluded from Mantel-Haenszel test due to the absence of enrolled subjects.
CI, confidence interval; ND, not done.

which was characterized as the presence of breast cancers in both the proband and her relatives at over 50 years of age. In Subgroup I-1, the prevalence of *BRCA*1/2 mutations was 21.4% (3/14) in Japanese *versus* 2.3% (4/172) in non-Ashkenazi individuals, showing significantly higher prevalence in Japanese (odds ratio [OR] 11.11, 95% confidence interval [CI] 1.450–75.21, $P = 0.01$). All three mutations detected in Group I-1 were in *BRCA*2, of which two subjects showed the same mutational type, i.e. c.5576_5579delTTAA in exon 11 of the *BRCA*2 gene, formerly designated as '5804del4,' '5804_5807delTTAA', or '5802delAATT' in the BIC database or references[10,11] (Table 4). One subject developed breast cancer at 57 years of age and her sister developed breast cancer at 58 years of age, and they were found to be identical twins. In this pedigree, no other relatives suffered from breast and/or ovarian cancer. Another subject developed lt-breast cancer at 52 years of age and her aunt developed lt-breast cancer at 63 years of age. The prevalence of *BRCA*1/2 mutations in each group or all subgroups between Japanese and non-Ashkenazi individuals was analyzed by the Mantel-Haenszel test, which is an extended method of analyzing multiple 2×2 contingency tables in retrospective studies and can exclude the effect of confounding factors between subgroups.[25] It was elucidated that the prevalence of *BRCA*1/2 mutations in Japanese was significantly higher than that in non-Ashkenazi individuals in the analysis of all subjects enrolled in Groups I through IV ($P = 0.005$, OR 1.873, 95% CI 1.217–2.884) or subtotal for Group I ($P = 0.0471$, OR 2.613, 95% CI 1.108–6.162). Subtotals for Groups II and IV were not significant, although the prevalence was higher in Japanese than non-Ashkenazi people in Group II (28.0% *vs* 21.3%) and in Group IV (71.4% *vs* 48.6%), respectively. In Group V, two deleterious mutations were detected in 10 subjects, comprising four subjects with male breast cancer and six subjects with concomitant breast and ovarian cancers. All subjects assigned to Group V were sporadic, without familial predisposition.

Clinical characteristics showing significant association with *BRCA*1/2 mutational status. Statistical analysis was carried out to search for clinical characteristics showing an association with *BRCA*1/2 mutational status (Table 6). In the analysis of female subjects with breast cancer, ages at diagnosis were significantly younger in *BRCA*1/2-positive subjects than *BRCA*1/2-negative ones (42.4 ± 11.0 years *vs* 47.3 ± 12.0 years, $P = 0.0272$), while in the analysis of ovarian cancer, there was no significant differences between *BRCA*1/2-positive and -negative groups (52.2 ± 8.6 years *vs* 49.4 ± 13.1 years, $P = 0.6647$). In the analysis of four subjects with male breast cancer, mutation of the *BRCA*2 gene was detected in one subject. Age at onset was high (more than 60 years) in male breast cancer regardless of *BRCA*1/2 mutational status (Table 6). Statistical analysis of other clinicopathological indices showed significant results in stage ($P = 0.026$), estrogen receptor (ER) status ($P = 0.0002$), progesterone receptor (PgR) status ($P = 0.0020$), and histological grade ($P = 0.0386$) between non-carriers and *BRCA*1/2 mutation carriers suffering from breast cancer (Table 7). There seemed to be more stage II or III tumors in *BRCA*1/2 positive cases ($P = 0.026$). In *BRCA*1 mutation carriers, ER or PgR status was negative in 84.6% (11/13) of subjects and all tumors were either Grade 2 (2/7) or Grade 3 (5/7). In *BRCA*2 mutation carriers, positivities for ER and PgR were 76.4% (13/17) and 56.2% (9/16), respectively, and higher than *BRCA*1. As for histological grades, frequencies of Grade 1 tumors were 0% (0/7) and 14.2% (2/14) in *BRCA*1-positve or *BRCA*2-positive subjects, significantly lower than in non-carriers. In the analysis of ovarian cancer, five subjects showed mutation of *BRCA*1, while no subjects showed mutation of *BRCA*2. No

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Table 6. Ages at onset of participants with breast and/or ovarian cancer

	All	Breast cancer (female)	Breast cancer (male)	Ovarian cancer
Number of subjects	135	122	4	18
Age at diagnosis				
Overall	46.5 ± 12.1	46.2 ± 12.1	64.5 ± 3.7	50.2 ± 11.8
No deleterious mutations	47.7 ± 12.1 (n = 99)	47.3 ± 12.0 (n = 86)	64.0 ± 4.4 (n = 3)	49.4 ± 13.1 (n = 13)
Deleterious mutations in *BRCA1* or *BRCA2*	43.1 ± 11.3 (n = 36)	42.4 ± 11.0 (n = 34)	66 (n = 1)	52.2 ± 8.6 (n = 5)
P-values	0.052	0.0272	0.7295	0.6647
Deleterious mutations in *BRCA1*	42.0 ± 11.9 (n = 17)	41.8 ± 9.6 (n = 16)	–	52.2 ± 8.6 (n = 5)
Deleterious mutations in *BRCA2*	44.2 ± 13.1 (n = 19)	42.9 ± 12.3 (n = 18)	66 (n = 1)	–
P-values	0.574	0.757	–	–

statistical significance was observed in clinicopathological indices such as tumor size, nodal status, and stage by *BRCA1* mutational status.

In 122 subjects with female breast cancer, cumulative incidences were analyzed by Kaplan–Meier plot and log-rank test (Fig. 2). In subjects with deleterious *BRCA1/2* mutations, median ages at onset were 42.5 years (*BRCA1/2*), 42 years (*BRCA1*), and 42.5 years (*BRCA2*), respectively, indicating that breast cancer developed at a significantly younger age than in non-carriers (P = 0.0144 between *BRCA1/2*-positive and -negative subjects, P = 0.0338 between *BRCA1*-positive, *BRCA2*-positive, and -negative subjects) (Fig. 2). Similar analysis was extended to relatives and it was shown that the age at onset of breast cancer could be a significant predictor of *BRCA1/2* mutational status (Table 8). Breast cancer developing before age 40 within second-degree relatives indicated a significantly higher prevalence of *BRCA1/2* mutations (P = 0.0265). Of the 122 pedigrees among which index patients suffered from female breast cancer, 26 pedigrees (21.3%) fulfilled this condition and the frequency of deleterious *BRCA1/2* mutations was significantly higher than in the controls (46.2% [12/26] *vs* 22.9% [22/96], OR 2.833, 95% CI 1.165–7.136). This feature was further emphasized when the disease history of cousins was included in the family history, in which 29 of 122 pedigrees (23.8%) fulfilled the criteria and the prevalence of *BRCA1/2* mutations showed a significant increase compared to the controls (48.3% [14/29] *vs* 21.5% [20/93], OR 3.407, 95% CI 1.412–8.217). When the index patient suffered from breast cancer, the presence of ovarian cancer and/or bilateral breast cancer within second-degree relatives was shown to be a strong indicator of *BRCA1/2* mutations. Half of the enrolled subjects (49.2% [60/122]) fulfilled this condition and the prevalence of *BRCA1/2* mutations in this group was considerably high (38.3% [23/60] *vs* 17.7% [11/62], OR 2.882, 95% CI 1.252–6.637).

Discussion

A total of 135 subjects were enrolled in the study, 131 women and four men, all of whom had a history of breast and/or ovarian cancers (Table 1). All patients were selected and enrolled in the study from genetic counseling clinics of the corresponding hospitals and the average number of sessions of genetic counseling was 3.36 ± 0.82. Patients usually underwent one or two sessions before and one session after gene testing when test results were negative. For patients with deleterious mutations, additional sessions were performed as follow-up because of the risk of developing psychosocial issues; hence, the number of sessions for mutation carriers was significantly more than for non-carriers (3.75 ± 1.20 *vs* 3.22 ± 0.56, P = 0.0007). In all subjects, the course was uneventful until the end of the study. Genealogical information was obtained in counseling sessions and the numbers of relatives greater than second-degree were significantly more in mutation carriers than non-carriers

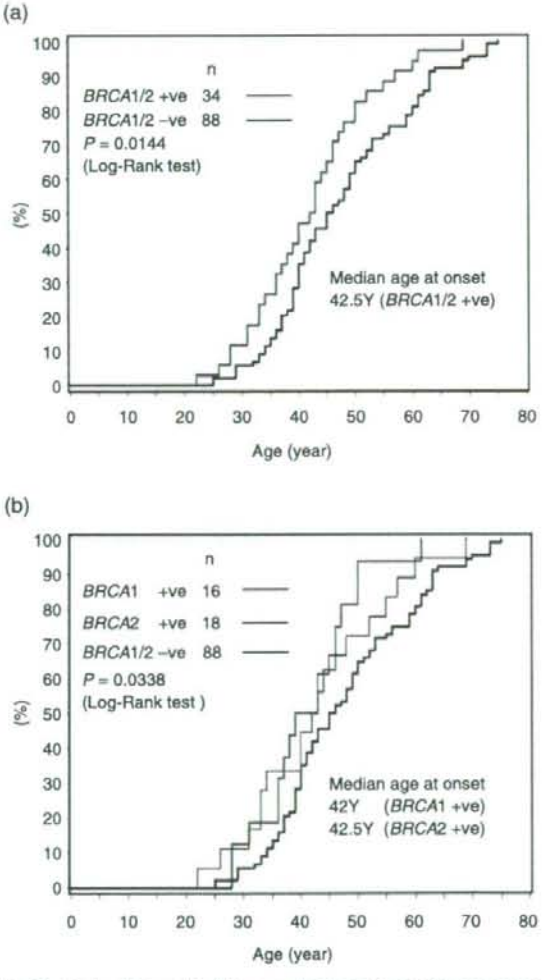

Fig. 2. Kaplan–Meier plot of the cumulative incidence of breast cancer in female index patients examined for germline *BRCA1/2* mutations. (a) Grouped in *BRCA1/2* positive (n = 34) or negative cases (n = 88), (b) grouped in *BRCA1* positive (n = 16), *BRCA2* positive (n = 18) and *BRCA1/2* negative cases (n = 88).

Table 7. Clinicopathological characteristics of the breast or ovarian cancers by *BRCA*1/2 mutational status

Variables		Breast cancer					Ovarian cancer		
		Non-carrier*	Deleterious mutations in			*P*-values*	Non-carrier	Deleterious mutations in	*P*-values
			*BRCA*1/2	*BRCA*1*	*BRCA*2*			*BRCA*1	
	No. of subjects	91	35	16	19		13	5	
Tumor size (T)	Tis	2	0	0	0	0.3884	–	–	0.8338
	T1	50	16	7	9		8	3	
	T2	35	14	7	7		1	0	
	T3	1	3	1	2		3	1	
	Missing (No.)	(3)	(2)	(1)	(1)		(1)	(1)	
Nodal status (N)	Negative	70	25	11	14	0.7859	7	3	1.000
	Positive	17	7	4	3		4	1	
	Missing (No.)	(4)	(3)	(1)	(2)		(2)	(1)	
Stage	0	2	0	0	0	0.026	–	–	0.9875
	I	56	15	6	9		6	3	
	II	25	13	6	7		1	0	
	III	1	3	3	0		3	1	
	IV	1	0	0	0		1	0	
	Missing (No.)	(6)	(4)	(1)	(3)		(2)	(1)	
ER status	Positive	57	15	2	13	0.0002			
	Negative	22	15	11	4				
	Missing (No.)	(12)	(5)	(3)	(2)				
PgR status	Positive	53	11	2	9	0.0020			
	Negative	26	18	11	7				
	Missing (No.)	(12)	(6)	(3)	(3)				
Histological grade	Grade 1	22	2	0	2	0.0386			
	Grade 2	28	9	2	7				
	Grade 3	14	10	5	5				
	Missing (No.)	(27)	(14)	(9)	(5)				
Laterality	Unilateral	75	29	13	16	0.9711			
	Bilateral	16	6	3	3				

*χ^2-test was performed between non-carriers, subjects with *BRCA*1 mutations, and *BRCA*2 mutations. ER, estrogen receptor; PgR, progesterone receptor.

Table 8. Univariate analysis of family-based clinical variables associated with germline *BRCA*1/2 mutations in 122 pedigrees with female probands affected with breast cancers

Variables		*BRCA*1/2 deleterious mutations		*P*-values (χ^2-test)	Odds ratio (95% confidence interval)
		Yes	No		
Breast cancer before age 40 Within second-degree relatives	Yes No	12 22	14 74	0.0265	2.833 (1.165–7.136)
Breast cancer before age 40 Within second-degree relatives and cousins	Yes No	14 20	15 73	0.0084	3.407 (1.412–8.219)
Ovarian cancer and/or bilateral breast cancer within second-degree relatives	Yes No	23 11	37 51	0.0151	2.882 (1.252–6.637)

($P < 0.0001$). Patient accrual was decided by family history within second-degree relatives, and in greater than second-degree relatives, family history might be investigated more precisely in subjects with deleterious *BRCA*1/2 mutations after disclosure of test results; therefore, genealogical information on relatives greater than second-degree may be biased in carriers.

Among mutations detected in *BRCA*1, L63X (c.188T > A), Q934X (c.288C > T), and K503X (c.1507 A > G) were previously reported in Japanese.[7,9–11] Sekine *et al.* reported L63X and Q934X as the two common founder mutations in Japanese.[11] L63X was detected in five subjects and Q934X in only one subject in this study. In a search of the BIC database, five mutational types were previously unreported, of which K503X (c.1507 A > G) was detected in one Japanese subject,[10] and the other four mutational types were considered to be previously unreported deleterious mutations. Genetic variants of unknown significance were detected in nine subjects; all were missense mutations and three types were thus far unreported in the BIC database (Table 3).

In the analysis of *BRCA*2, 5804del4 (c.5576_5579delTTAA), Q3026X (c.9076C > T), and R3128X (c.9382C > T) were previously reported in Japanese people (Table 4).[9–11] As for genetic variants of uncertain significance, two mutational types (G2044V [c.6131G > T] and V2109I [c.6325G > A]) were reported in Japanese, and G2044V was found in at least one of 28 Japanese healthy volunteers.[12] M524I (c.1572G > C), K610Q (c.1828 A > C), I770V (c.2308 A > G), and S2616F (c.7847C > T) were previously unreported mutational types.

The prevalence of *BRCA*1/2 mutations in Japanese subjects classified to each subgroup was compared with that of non-

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Ashkenazi individuals, and statistical difference was observed in subgroup I-1, in which two mutational types were detected in three subjects in Japanese. They were 314delTT in exon 3 of *BRCA*2 and two subjects with c.5576_5579delTTAA or 5804delTTAA in exon 11 of *BRCA*2. Two cases with 5804delTTAA have been reported so far in Japanese.[9,11] Ikeda *et al.* reported seven cases of 5802del AATT,[10] which is the same mutational type as 5804delTTAA as there is a repeated sequence in this region; therefore, this mutational type seems to be rather common in Japanese. In this study, c.5576_5579delTTAA were detected in two subjects. In the former, breast cancer developed in identical twins at 57 and 58 years of age, respectively. Reportedly, statistically significant effects of heritable factors were observed for breast cancers coincidentally developing in identical twins, some of which would be attributed to polygenic inheritance.[26] In the latter case, lt-breast cancer developed at 52 years of age in the proband and her aunt developed lt-breast cancer at 63 years. No other relatives suffered from breast and/or ovarian cancer in these pedigrees and we strongly suspected that c.5576_5579delTTAA might be a relatively common mutation with low penetrance in Japanese. If these two subjects were excluded, no statistical significance was observed between Japanese subjects and non-Ashkenazi individuals in the subgroup I-1. Further studies are required to elucidate the prevalence of this particular mutational type in a Japanese healthy cohort.

A significantly higher frequency of mutation was found in patients with breast cancer older than 50 years of age who had a family history of breast cancer at older than 50 years within second-degree relatives (Subgroup I-1) compared to the corresponding non-Ashkenazi individuals (Table 5). This may imply that Japanese carriers of *BRCA*1/2 mutation suffer from breast cancer with later onset than non-Ashkenazi carriers. As for non-Ashkenazi individuals, the clinical backgrounds of the enrolled subjects, such as age at onset, were not available except for the data so far reported;[6] therefore, it is hard to produce a Kaplan–Meier plot of cumulative incidences in non-Ashkenazi individuals. Likewise, few reports have shown the prevalence of *BRCA*1/2 mutations analyzed by full sequencing in non-Ashkenazi individuals as a population-based study or cross-sectional study. Recently, John *et al.* estimated the prevalence of *BRCA*1 mutations in white, non-Hispanic breast cancer patients without Ashkenazi ancestry younger than 65 years at diagnosis. They analyzed 508 breast cancer patients enrolled in the Breast Cancer Family Registry and pathogenic mutations of *BRCA*1 were found in 14 subjects, of which six subjects (42.8%) and 11 (78.6%) developed breast cancer before age 35 and age 50, respectively.[27] Not exclusive to non-Ashkenazi individuals, Metcalfe *et al.* analyzed 927 women with unilateral breast cancer and with positive *BRCA*1/2 mutations from eight countries (Austria, Canada, France, Israel, Italy, Norway, Poland, and the US) and their average age at diagnosis of the first breast cancer was 42.2 years.[28] In our study, average age at onset of breast cancer in *BRCA*1/2 carriers was 42.4 years and these data look similar to those reported in Western countries.

The numbers of subjects classified into each group were 42 in Group I, 75 in Group II, one in Group III, seven in Group IV, and 10 in Group V (Table 5). Originally, we assumed that more subjects would be enrolled into Group I than Group II, as the first recruitment of all eligible patients was made by the attending doctors, and a considerable number of the breast cancer patients fulfilling the eligibility criteria had a modest family history. It seemed likely that patients with a higher risk wished to be enrolled in the study and those with a modest risk did not visit the clinic for genetic counseling. This may be why fewer subjects were enrolled in Group I than in Group II. We designed the study as an unbiased hospital-based, cross-sectional study in which all subjects with a family history of breast and/or ovarian cancer were enrolled, but the results seemed closer to a family-based study rather than a hospital-based study. This trend was similar to the subjects enrolled in the study through press advertising.

The results of the Mantel-Haenszel test showed that the prevalence of *BRCA*1/2 mutations in Japanese was significantly higher than those reported in non-Ashkenazi individuals. As the sample size was too small to reach a conclusion, one reason may be that all gene tests were carried out in genetic counseling clinics, where clients were more likely to be prone to hereditary cancers. It should be noted that the prevalence of *BRCA*1/2 mutations in Japanese was as high or even higher than that of non-Ashkenazi individuals reported in the US.

Ikeda *et al.* examined 113 Japanese breast cancer patients showing a modest to minimal familial risk, i.e. those with at least one breast cancer or one ovarian cancer patient within their first-degree relatives.[10] They reported that families with early onset patients diagnosed at younger than 40 years of age showed a higher frequency (38%, 19/50 subjects) of *BRCA*1/2 mutations than those without early onset patients, and families with bilateral breast cancer patients showed a higher frequency (40%, 6/15 subjects) than those with only unilateral breast cancer patients, but all these differences were statistically insignificant.[10] In the present study, we found statistical significance in families with breast cancer before age 40 within second-degree relatives (46.2%, 12/26 subjects) and within second-degree relatives and cousins (48.3%, 14/29 subjects). Families with ovarian cancer and/or bilateral breast cancer within second-degree relatives exhibited statistically significant *BRCA*1/2 mutation frequency (38.3%, 23/60 subjects). Predisposition to breast cancer in cousins seems informative for assessing familial risk, particularly in cases where the responsible genes are likely to be transmitted from the paternal side. In genetic counseling, precise family history is a key point in assessing genetic risk and the inclusion of familial risk within second-degree relatives or cousins would be helpful for proper risk assessment.

In conclusion, this is the first cross-sectional study elucidating the prevalence of *BRCA*1/2 mutations among Japanese people with varying genetic susceptibility. Genetic counseling performed prior to gene testing in genetic counseling clinics is an effective approach to assess the risk for cancer predisposition and subsequent indication for gene testing. Full sequencing analysis of *BRCA*1/2 genes would be a useful modality for diagnosing HBOC and the results of the present study provide a basis for the clinical application of a cancer prevention strategy targeted to *BRCA*1/2 mutation carriers in Japanese.

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