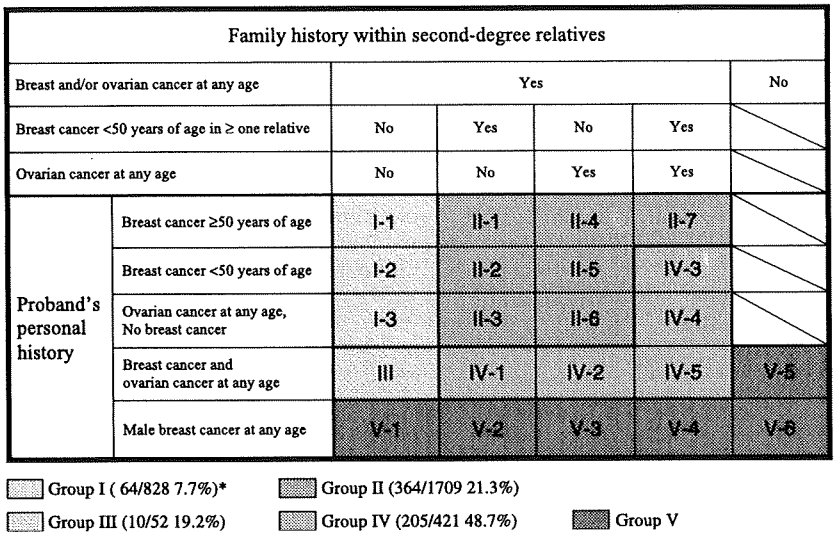

Fig. 1. Classification and grouping of the enrolled subjects. *Numbers in parentheses indicate prevalence of *BRCA*1/2 mutations reported in non-Ashkenazi individuals in the US.[6]

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)‡	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†	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

†Mutation in the donor site of intron 13, suspected to be deleterious, resulting in a splicing error. ‡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)‡	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)†	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	

†Synonymous mutation in the exon–intron junction of exon 23, suspected to be deleterious, resulting in a splicing error. ‡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

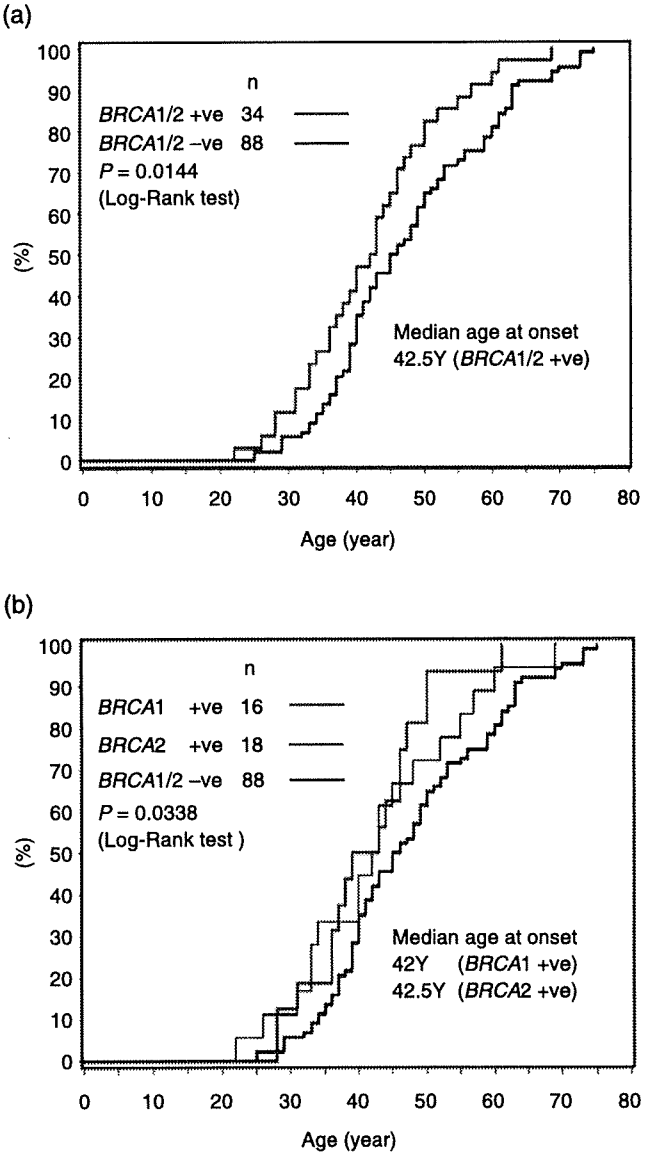

Fig. 2. Kaplan–Meier plot of the cumulative incidence of breast cancer in female index patients examined for germline *BRCA1/2* mutations. (a) Grouped in *BRCA1/2* positive (n = 34) or negative cases (n = 88), (b) grouped in *BRCA1* positive (n = 16), *BRCA2* positive (n = 18) and *BRCA1/2* negative 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	Yes	12	14	0.0265	2.833 (1.165–7.136)
Within second-degree relatives	No	22	74		
Breast cancer before age 40	Yes	14	15	0.0084	3.407 (1.412–8.219)
Within second-degree relatives and cousins	No	20	73		
Ovarian cancer and/or bilateral	Yes	23	37	0.0151	2.882 (1.252–6.637)
breast cancer within second-degree relatives	No	11	51		

($P < 0.0001$). 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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症　例

破骨細胞様巨細胞の出現を伴う乳癌の9例

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吉田亮介　木下貴之　北條　隆

1999年から2007年の9年間における破骨細胞様巨細胞（OCGC）の出現を伴う乳癌（OCGC乳癌）の自験例につき臨床病理学的，免疫組織化学的特徴を検討した．OCGC乳癌は9症例であり，全乳癌における頻度は0.3%であった．臨床病期はStage Ⅰ：4例，Stage ⅡA：3例，Stage ⅡB：2例であった．最大腫瘍径は1.5cm—6.0cm（平均3.1cm），リンパ節転移を4例（44%）に認めた．腫瘍組織型は全例浸潤性乳管癌，組織学的異型度は全例grade2であった．estrogen receptorは5例（56%），progesteron receptorは6例（67%）で陽性，HER2は7例で検討し，1例（14%）で陽性であった．予後は原病死1例を認めた以外は無再発生存中（平均観察期間4年10カ月）である．OCGC乳癌は臨床病期，組織学的異型度，リンパ節転移，ホルモン受容体発現状況等から悪性度は中等度，あるいは比較的良好の可能性があることが示唆された．

索引用語：乳癌，破骨細胞様巨細胞

緒　言

破骨細胞様巨細胞（Osteoclast-like giant cell，以下OCGC）の出現をみる乳癌（以下OCGC乳癌）は0.5～1.2%と極めて稀であり，OCGC出現機序は生物学的，臨床病理学的側面からも興味深い．今回われわれは1999年から2007年の間に当院で経験したOCGC乳癌9症例についてその臨床病理学，免疫組織化学的特徴について検討したので若干の文献的考察を加えて報告する．

対象および方法

1999～2007年の9年間に当院で手術した乳癌3546症例のうち，摘出標本にてOCGC乳癌と診断された9症例（0.3%）を対象とした．OCGC乳癌は組織学的に腫瘍胞巣内あるいは腫瘍間質内にOCGCの出現を認めるもの，と定義した（図1，2）．臨床病理学的検討は「乳癌取扱い規約」[1]に準拠した．さらにestrogen receptor（以下ER），progesteron receptor（以下PgR），HER2，p53の免疫組織化学的検討を行った．これらの結果を1999年～2006年に当院で手術された全浸潤性乳管癌症例（対照群）と比較検討した．

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成　績

年齢は38～72歳（平均50歳）で女性8例，男性1例であった（表1）．発生部位は右側7例，左側2例と右側に多く，C領域が6例で最も多かった．臨床病期はStage Ⅰ：4例（44%），Stage ⅡA：3例（33%），Stage ⅡB：2例（22%）であった．対照群ではStage Ⅰ：33.4%，Stage Ⅱ：57.6%，Stage Ⅲ：8.9%で両群間に差異を認めなかった．施行術式は乳房温存部分切除術＋腋窩リンパ節郭清術：5例，両胸筋温存乳房切除術＋腋窩リンパ節郭清術：3例，乳房温存部分切除術＋センチネルリンパ節生検術：1例であった．リンパ節転移を4例（44%）に認め，1例に5個以上の転移を認めた．リンパ節転移の頻度は対照群（44%）と同等であった．手術摘出標本の肉眼所見では腫瘍割面は赤褐色調を含むものが多く，通常経験される乳腺浸潤癌の肉眼像と異なっていた．病理学的最大腫瘍径は1.5cm～6.0cm（平均3.1cm），組織学的には全例浸潤性乳管癌であり，優位な組織像は乳頭腺管癌が8例，硬癌が1例であった（表2）．腫瘍胞巣内および腫瘍間質内にOCGCを瀰慢性に認めた．OCGC乳癌は組織学的に①腫瘍成分が未分化な肉腫様パターンを示し，高率に骨や軟骨化生を伴うmetaplastic carcinoma with OCGC，②比較的分化型の腺癌に伴うもので肉腫様成分や骨，軟骨化生のないcarcinoma with reactive

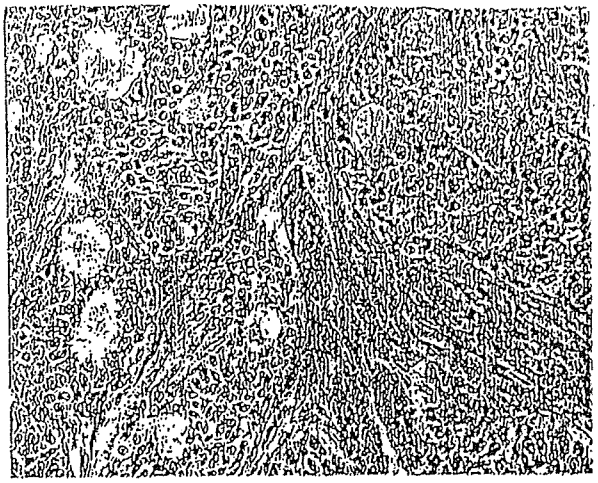

図1　病理組織所見：腫瘍は浸潤性乳管癌（乳頭腺管癌）であり，腫瘍間質にはOCGCの浸潤を散在性に認める（症例1，HE染色，対物20倍）．

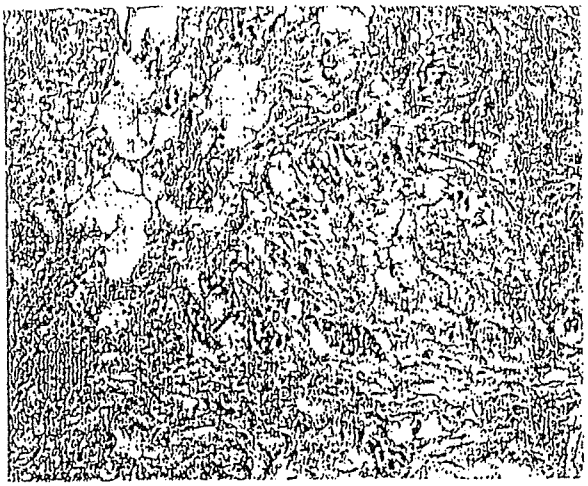

図2　病理組織所見：腫瘍周囲間質に出現した多数のOCGC（症例3，HE染色，対物20倍）．

stromal giant cells，③上皮性成分がなく，骨の巨細胞腫に類似性が求められるextraskeletal osteoclastomaに分類可能とされる[2]が，自験例は全て②に相当するものであった．OCGCの出現数には各症例間でばらつきがみられたが，その形態には違いがみられなかった．組織学的異型度（modified Bloom-Richardson分類）は全例grade2，リンパ管侵襲を3例に認めたものの，静脈侵襲は全例陰性であった．7例で脂肪浸潤を，1例で真皮への浸潤をきたしていた．高度乳管内進展を3例に認めた．いずれの症例においても扁平上皮化生や間質の骨・軟骨化生は認められなかった．対照群では組織学的異型度はgrade1：10%，grade2：48%，grade3：42%であり，対照群のほうが異型度が高い傾向にあった．免疫組織化学的検査ではERが5例（56%），PgRが6例（67%）で陽性であり，検討した7例中1例（14%）でHER2蛋白の過剰発現を認めた．またER，PgRがいずれも陽性であったのは3例（33%）であった．なおER，PgRは免疫染色で10%以上の腫瘍細胞が染まるものを陽性とした．p53は2例で陽性，5例で弱陽性であった．対照群ではERが72%，PgRが67%の症例で陽性，HER2の過剰発現を20%の症例で認めた．ER，PgRがいずれも陽性であったのは60%であった．OCGC乳癌症例では対照群と比較するとER陽性率が低い傾向にあった．術前穿刺吸引細胞診を施行された6例のうち当院で検討されたのは4例で，異型上皮細胞と多核巨細胞をともに認めたものは2例であった．予後は1例が術後5年10カ月で多発骨転移，多臓器転移にて死亡したものの，他症例は無再発生存中（平均観察期間4年10カ月）である．

考　察

OCGCの出現を伴う癌の報告はさまざまな臓器でみられるが極めて稀であり，乳癌においても同様でその頻度は0.5～1.2%[3)~5)]といわれている．1931年にLeroux[6)]が初めて報告して以来約100例の報告があるのみである．臨床的特徴として，通常型乳癌と比較して若年齢，閉経前の症例が多いとの報告が散見される[4)7)]が，自験例では平均50歳，閉経前が6例，自然閉経後と子宮卵巣摘出術後がそれぞれ1例ずつであり，通常型乳癌との差異を認めなかった．

男性に発症したOCGC乳癌の報告はなく，自験例が初めての症例であった．当症例は組織像においても，嚢胞内にポリープ状に発育し乳頭状～一部胞巣状に増殖した乳頭腺管癌で，本邦で従来報告されたOCGC乳癌はすべて充実性の発育を示している点からも極めて稀な症例と考えられる．本症例は術後2年で胸骨転移を，5年10カ月で上腕骨転移，多臓器転移をきたし死亡した．

腫瘍の組織型として1995年のViacavaらは多彩な組織型を報告している[8)]が，とりわけ本邦においては大多数が浸潤性乳管癌である[9)~12)]．自験例でも全症例が浸潤性乳管癌であった．

免疫組織化学的検討について，OCGC乳癌がER陽性，HER2陰性の傾向があることを示した報告[10)]や，それとは異なりER陰性，PgR陽性症例が多いとする報告[4)13)]もある．予後についても通常型乳癌と比較し良好とするもの[11)14)]や，差異がないとするもの[15)]，一方で比較的進行した症例が多いとするもの[4)]まで様々で一

表1　OCGC乳癌症例の臨床所見

症例	年齢（歳）	性別	TNM	術式	術後経過（術後観察期間）
1	41	女	T1cN0M0	Bp+SLN	no rec.（3M）
2	45	女	T2N0M0	Bt+Ax	no rec.（37M）
3	45	女	T1cN0M0	Bp+Ax	no rec.（42M）
4	57	女	T1cN0M0	Bp+Ax	no rec.（71M）
5	38	女	T2N0M0	Bp+Ax	no rec.（69M）
6	41	女	T2N0M0	Bp+Ax	no rec.（99M）
7	72	男	T2N1M0	Bt+Ax	胸骨転移（70M）
8	44	女	T1bN0M0	Bp+Ax	no rec.（51M）
9	64	女	T2N1M0	Bt+Ax	no rec.（81M）

Bp+SLN：乳房温存部分切除術＋センチネルリンパ節生検術，Bt+Ax：両胸筋温存乳房切除術＋腋窩リンパ節郭清術，Bp+Ax：乳房温存部分切除術＋腋窩リンパ節郭清術，no rec.：無再発生存中

表2　OCGC乳癌症例の病理組織所見と免疫組織化学所見

症例	腫瘍径（cm）	リンパ節転移	組織型（優位組織像）	G/NG	ly/v	ER/PgR	HER2	p53
1	5.1	n0(0/2)	IDC(pap)	2/3	(−)/(−)	(+)/(+)	(−)	…
2	3.5	n1(4/24)	IDC(pap)	2/1	(+)/(−)	(+)/(+)	(−)	(±)
3	1.5	n1(1/12)	IDC(pap)	2/2	(−)/(−)	(−)/(+)	(−)	(±)
4	2.0	n0(0/23)	IDC(pap)	2/3	(−)/(−)	(+)/(−)	(−)	(−)
5	2.1	n0(0/14)	IDC(pap)	2/2	(−)/(−)	(−)/(+)	(−)	(±)
6	2.3	n0(0/31)	IDC(pap)	2/2	(−)/(−)	(+)/(+)	…	(+)
7	5.0	n1(7/24)	IDC(pap)	2/2	(+)/(−)	(−)/(−)	…	(+)
8	0.8	n0(0/15)	IDC(sci)	2/2	(−)/(−)	(−)/(+)	(−)	(±)
9	6.0	n1(3/21)	IDC(pap)	2/2	(+)/(−)	(+)/(−)	(+)	(±)

IDC：浸潤性乳管癌，pap：乳頭腺管癌，sci：硬癌，G：組織学的異型度（modified Bloom-Richardson分類），NG：組織学的核異型度，ly：リンパ管侵襲，v：静脈侵襲，ER：estrogen receptor，PgR：progesteron receptor

定の見解が得られていない．自験例ではER陽性率が対照群より低い傾向にあった．津田[16]はリンパ節転移の程度と組織学的異型度（modified Bloom-Richardson分類）を最も重要な独立した予後因子としているが，今回の臨床病期，リンパ節転移の程度，ホルモン受容体発現状況の検討からはその悪性度は通常型乳癌とほぼ同等であるものと考えられる一方，臨床病期Stage IIBであった1例が術後5年10カ月後に原病死した以外全例無再発生存中であること，組織学的異型度がOCGC乳癌症例のほうが低い傾向にあったことは，OCGC乳癌の予後が比較的良好であることを示唆している．

術前画像診断についてOCGC乳癌は境界明瞭な腫瘤を形成する頻度が高いため，マンモグラフィにて良悪性の鑑別が困難であることが多く，そのことが予後を悪くしている可能性があるとの報告がある[4]．自験例では1症例がマンモグラフィ，超音波検査にて良悪性鑑別困難な嚢胞性腫瘤と診断され1年間経過観察となっている．他の1症例はマンモグラフィにて異常所見を認めず，超音波検査でも質的診断困難な低エコー腫瘤との診断であったが，造影MRIでの腫瘤の形状，造影パターンにて浸潤性乳管癌を疑われ，穿刺針生検にて浸潤性乳管癌と診断された．他の7症例ではマンモグラフィにて辺縁不整な，spiculaあるいは石灰化を伴う腫瘤等の所見により，乳癌あるいはその疑いと指摘された．自験例からは良悪性の鑑別が困難な傾向は認められなかった．

術前穿刺吸引細胞診についてOCGC乳癌の診断に有用であるとの報告があるが[17]，今回細胞診を検討した4例中，異型上皮細胞とOCGCをともに認め，OCGC乳癌が疑われたのは2例であった．OCGCの出現数に各症例間でばらつきがあったことがその原因と

考えられる.

OCGCの発生起源として過去の報告では間質系の組織球由来とする見解でほぼ一致している[9)~12)17)]. 1症例のみの検討であるが, 自験例でも免疫染色で単球・マクロファージ系に特異的なCD68が陽性となっておりその見解と矛盾しない.

OCGCの出現機序としては不明な点が多い. OCGCは腫瘍部位にのみ認められ, 電子顕微鏡による検討では組織球の融合にて形成されるとの報告もみられる[11)]. またマウスを用いた実験でinterleukin-1(IL-1)が破骨細胞の多核細胞化と骨吸収活性の誘導を促したという報告があり[18)], 癌細胞と関連してサイトカインが放出されOCGCが誘導されるとも考えられる. in-vitroでOCGCがparathormoneからの刺激にて溶骨能を持つことから, 骨転移との関連を指摘する報告もある[19)]. 因果関係は不明であるが, 自験例での死亡症例も骨転移をきたした. 今後更なる研究によりその出現機序が解明されればOCGC乳癌の生物学的特性, 予後等が明らかになっていくものと考えられる.

結　語

OCGCの出現を伴う乳癌は稀な特殊型である. 今回の検討ではその悪性度は中等度, あるいは比較的低い可能性があることが示唆された. OCGCの出現機序はまだ不明な点が多いが, その解明は腫瘍の生物学的特性を考える上で役立つものと考えられる.

稿を終えるにあたり, 病理学のご指導をいただきました当院病理部長谷部孝裕先生に深く感謝いたします.

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NINE CASES OF MAMMARY CARCINOMA CHARACTERIZED BY THE PRESENCE OF OSTEOCLAST-LIKE GIANT CELLS

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Mammary carcinoma containing osteoclast-like giant cells (OCGC) is an extremely rare tumor. We have encountered nine additional cases of this tumor, so we investigated is clinico-pathological and immunohistochemical characteristics. Four cases were in Stage I, three were in Stage II A, and two were in Stage II B. The tumor size varied from 1.5cm to 6.0cm. Lymph node metastasis was observed in four cases (44%). In all cases, histopathological examination revealed invasive ductal carcinoma containing OCGC among the tumor cell nests. The tumors were grade 2 in all cases, according to the modified Bloom-Richardson classification. Immunohistochemically, estrogen receptor, progesterone receptor and HER2 receptor were positive in five cases, six cases, and one case, respectively. One patient died of the disease five years and ten months after surgery while no signs of recurrence have been seen in the other cases. According to these results, it is possible that the prognosis of mammary carcinoma charaeterized by the piaeuce of OCGC is better than that of ordinary mammary carcinoma.

原著

2007.11.11受付

原発性乳がんに対するPrimary systemic therapy (PST)の適応—PST抵抗性乳がんを治療前に判定可能か？

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Clinicopathologic Features of Primary Breast Cancer Resisting Primary Systemic Therapy：Shien T[*1], Yoshida M[*1], Hojo T[*1], Shimizu C[*2], Kouno T[*2], Ando M[*2], Akashi-Tanaka S[*1], Seki K[*3], Katsumata N[*2], Fujiwara Y[*2] and Kinoshita T[*1] ([*1]Department of Surgery, [*2]Breast and Medical Oncology Division, [*3]Department of Pathology, National Cancer Center Hospital)

We evaluated the clinicopathologic and radiological features of patients with primary breast cancer resistant to PST to demonstrate the predictive factors of PST. Between 1998 and 2007, 443 PBC underwent curative surgical treatment after PST (anthracycline and/or taxane) at National Cancer Center Hospital (NCCH). We could evaluate 8 (2%) primary breast cancer patients who clearly judged clinical progressive disease (PD) with radiological examinations. Histological classifications were metaplastic carcinomas in 5 (63%) patients, but 2 of these patients were not correctly diagnosed before PST by core needle biopsy. All patients were triple negative (ER, PgR and HER2) by immunopathological examinations. On radiological examinations, the tumors were visualized as localized, round and non homogeneus masses. The clinicopathological and radiological features of PBC resistant PST were clear in this study. However, these features were similar to PBC with high sensitivity to PST. Another predictor is needed to accurately judge sensitivity to PST.

Key words：Breast cancer, Primary systemic therapy, Predictive features
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はじめに

現在乳がんに有効な抗がん剤の進歩に伴い，局所進行乳がんのみならず比較的早期の乳がんに対しても広くPrimary systemic therapy (PST) が行われるようになった．その，目的は[1]PSTにより原発腫瘍の完全消失（pCR）を得て予後を改善する．[2]原発腫瘍をできるだけ縮小させて切除範囲のできるだけ小さな乳房温存療法を可能にする．[3]原発腫瘍に対する抗がん剤の感受性を確認する．とされている[1]．しかし，治療前にPSTの効果を予測し効果にあわせたPSTの適応はいまだ議論されている最中である．

今回われわれは，PSTに抵抗性であった症例の臨床病理学的および画像的特徴を分類し，これらの症例を治療前に判定可能か検討した．

1．対象と方法

1998年5月から2007年9月までに国立がんセンター中央病院（NCCH）においてanthracyclineまたはtaxaneを含むPrimary systemic therapy (PST)を行った後治癒切除を行った原発性乳がん

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表1　患者背景および予後

症例	年齢	病期	PST	手術	再発・転移	DFS	死亡	OS
1	62	IIIB	AT	Bt+Ax	骨・肝	24	D	25
2	26	IIA	FECT	Bt+Ax	肺	14	D	21
3	42	IIIA	FECT	Bt+Ax	胸水	11	D	12
4	56	IIIB	ACT	Bt+Ax	局所	14	A	38
5	57	IIA	ACT	Bt+Ax	骨・肺	13	A	17
6	37	IIB	ACT	Bt+Ax	—	12	A	12
7	37	IIIB	ACT	Bt+Ax	肺	5	A	7
8	48	IIB	FECT	Bt+Ax	肺	6	D	9

AT：doxorubicin+Docetaxel. FECT：fluorouracil+epirubicin+cyclophosphamide followed by paclitaxel. ACT：doxorubicin+cyclophosphamide followed by paclitaxel. Bt：total mastectomy. Ax：axillary disection (level II). D：dead. A：alive

表3　画像所見

症例	組織型	画像所見
1	sq	限局性，境界明瞭，内部不均一
2	MPC	限局性，境界明瞭，内部不均一
3	sc	spiculaを伴う腫瘤
4	so	乳頭部に限局，境界明瞭
5	sq+sp	限局性，境界明瞭，内部不均一
6	so	限局性，境界明瞭，内部不均一
7	sp	限局性，境界明瞭，内部不均一
8	mix	限局性，境界明瞭，内部不均一

sq：squamous cell carcinoma. MPC：matrix producing carcinoma. so：solid tubular carcinoma
sc：scirrhous carcinoma. sp：adenocarcinoma with spindle cell metaplasia. mix：mixed epitherial metaplastic carcinoma.

患者は443名であった．そのうち，PSTに抵抗性で触診および画像上明らかに腫瘍の増大をみとめた症例8例（2％）について，病理学的特徴として治療前core needle biopsy (CNB) 検体および術後組織標本における，組織型，悪性度，リンパ節転移および免疫染色にてER, PgR, HER2, p53を比較検討した．また，治療前および術前画像結果から画像的特徴を検討した．

2. 結　果

表1に8例の臨床的特徴を示す．年齢中央値は45歳（26～62歳）．臨床病期はStage IIA 2名，Stage IIB 2名，Stage IIIA 1名およびStage IIIB 3名であった．PSTのレジメンはいずれもanthracyclineおよびtaxaneをともに含むもで，AT (doxorubicin；50mg/m²/docetaxel；60mg/m²) 1名，FEC followed by paclitaxel (fluorouracil；500mg/m²，epirubicin；100mg/m²，cyclophosphamide；500mg/m²，paclitaxel；80mg/m²) 3名およびAC followed by paclitaxel (doxorubicin；60mg/m²，cyclophosphamide；600mg/m², paclitaxel；80mg/m²) 4名であった．Trastuzumabを投与された症例はなかった．手術は全例レベル2郭清を伴う乳房切除術が行われていた．予後についても同様に**表1**に示す．8例中7例で2年以内に再発を認めそのうち6例は遠隔臓器転移で1例は局所再発であった．また，再発した7例中3例は再発後1年以内に死亡していた．

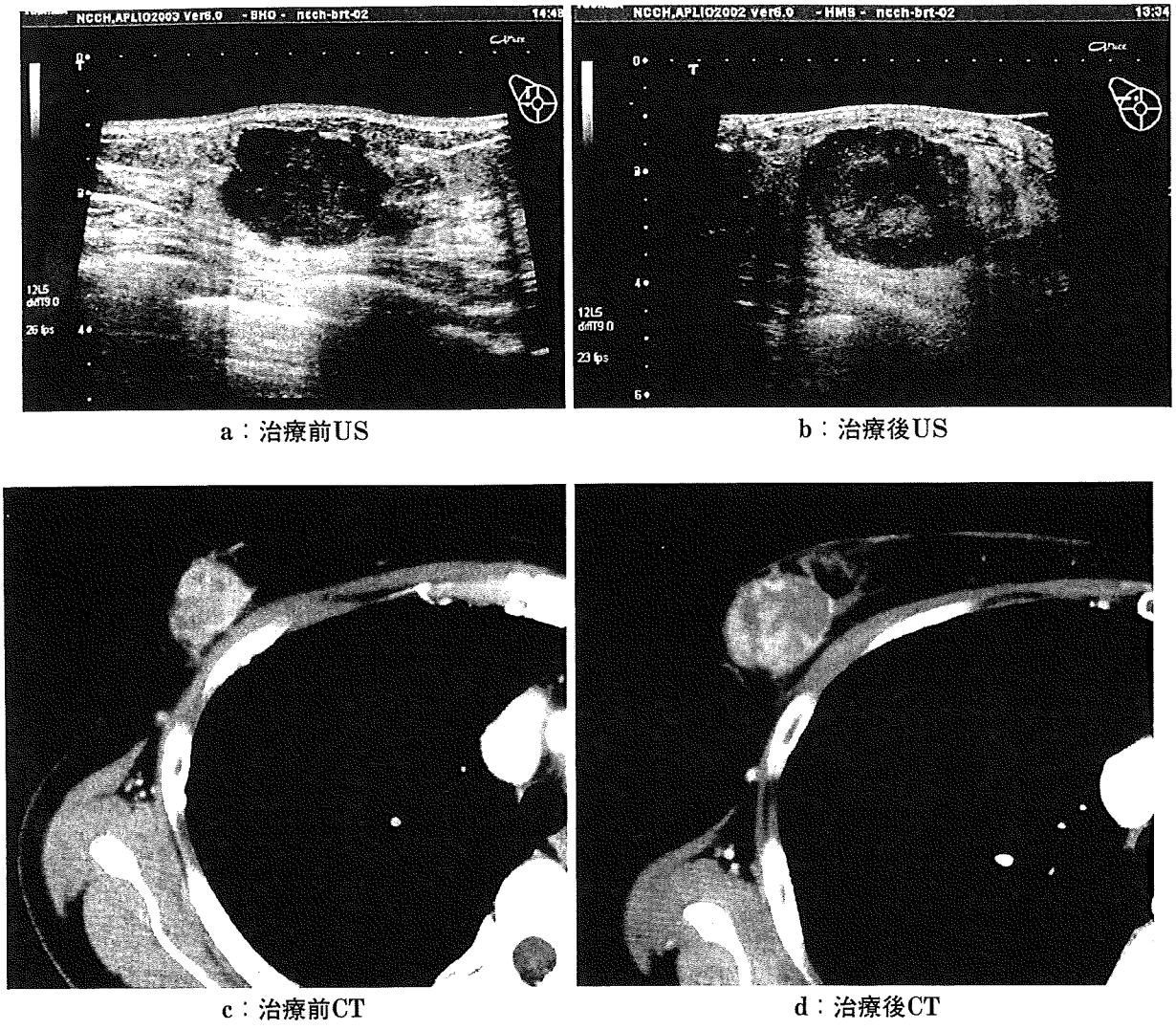
表2　免疫病理学的検査結果

症例	組織型(治療前CNB)	ER	PgR	HER2	G	p53	n
1	sq (sq)	0	0	0	3	2	21
2	MPC (so)	0	0	0	3	3	0
3	sc (sc)	0	0	0	3	3	19
4	so (so)	0	0	2+(0)	3	3	7
5	sq+sp (sq)	0	0	0	3	3	0
6	so (so)	0	0	0	3	3	2
7	sp (IDC)	0	0	0	3	3	1
8	mix (so)	0	0	0	3	3	3

sq：squamous cell carcinoma. MPC：matrix producing carcinoma. so：solid tubular carcinoma
sc：scirrhous carcinoma. sp：adenocarcinoma with spindle cell metaplasia. IDC：invasive ductal carcinoma. mix：mixed epitherial metaplastic carcinoma.
G：grade.
n：pathologically lymph node metastases

つづいて免疫病理学的検査結果を**表2**に示す．腫瘍の組織型は術後病理結果にて8例中5例（63％）でmetaplastic carcinomaの診断だった．しかし，そのうち2例は治療前CNBによる診断では浸潤性乳管癌であった．免疫組織学的検査結果ではホルモンレセプターはいずれも陰性でありHER2の発現は1例で2＋の結果であったもののFISH法にて陰性と判定されており，こちらも全例陰性の結果であった．つまり，8例ともtriple negativeであった．加えてp53は免疫染色にていずれも強陽性であり，組織学的悪性度はいずれもgrade 3（高悪性度）であった．リンパ節転移は8例中3例において7個以上（7～21個）の多数個認めたのに対してその他5例では3個以下（0～3個）と比較的転移個数は少なかった．

乳腺超音波またはCTによる画像検査結果を**表3**に示す．比較的リンパ節転移が多く組織型が硬癌の診断であった1例を除いていずれも比較的限局



a：治療前US　b：治療後US

c：治療前CT　d：治療後CT

図1　画像所見

症例5　57歳女性　AC 4コースでclinical CR後，paclitaxel 3週にて再増大組織型はSquamous cell and spindle cell metaplasia. USおよびCTにて限局性，境界明瞭および内部不均一な腫瘤を認める．

性，境界明瞭で内部は不均一なものであった（図1）．

3．考　察

近年抗がん剤治療の進歩および乳がん手術の縮小化に伴い，Primary systemic therapy (PST) は広く行われるようになった．当院においても，現在PST適応症例として治療前腫瘍径3 cm以上または治療前に明らかに腋窩リンパ節転移が疑われる症例としている．この理由は．腫瘍径3 cmにおいては乳房温存療法のガイドラインにおける温存療法の適応が腫瘍径3 cm以内である[2]ため，PSTを行って温存療法を可能にするためである．また，明らかに腋窩リンパ節転移陽性の症例については現在術後補助療法の指標とされているSt. Gallenのリスク分類[3]に従えば，術後必ず抗がん剤治療が行われることとなるために，PSTとして先に抗がん剤投与を行っても良いと考えられるためである．予後に関しては，基本的に同じ抗がん剤を手術前に行っても手術後に行っても腫瘍が完全消失するpCR症例以外予後は変わらないことが報告されている[4,5]．しかし，リンパ節転移に関しては近年PST後のリンパ節転移の個数がその後の予後を左右することが報告されており[6]，こういった面からも治療前にリンパ節転移陽性の症例がPSTにより転移個数が減少または消失した場合は良好な予後を得られる可能性があり，PSTの適応とされている．しかし，こういった適応を決める際に，腫瘍に対してPSTが予想しているような効果を挙げるかどうかは判定しておらず，PSTに

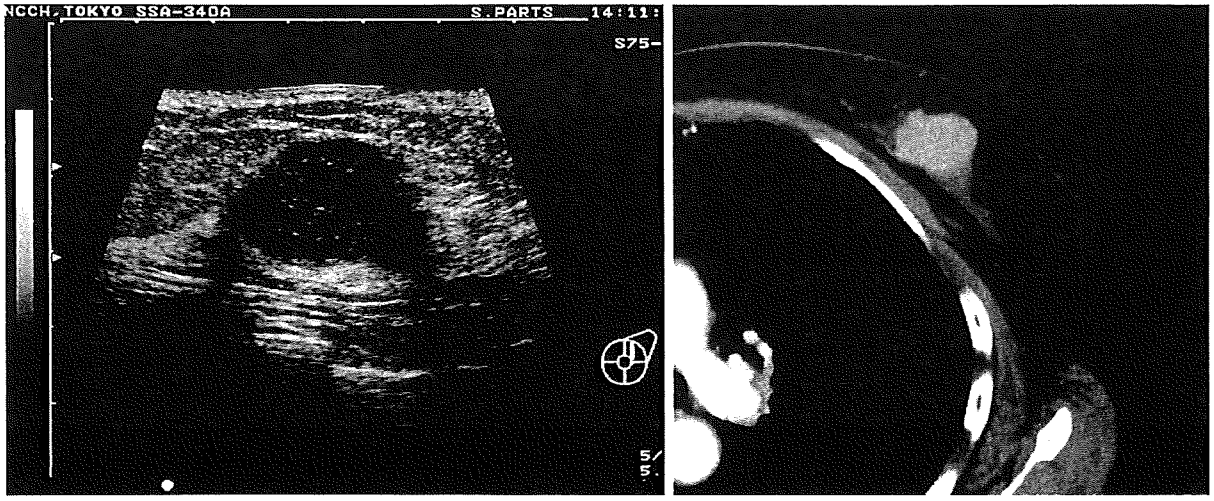


図2　pCR症例の画像所見

症例57歳女性．組織型は充実腺管癌．治療前CNBによる免疫組織学的検査結果はTriple negativeおよび組織学的悪性度grage 3

ACTによるPST効果はpCR（腫瘍の完全消失）．治療前画像所見では限局性，境界明瞭な腫瘤を認めた．

対する感受性は投与後に判定するのが現状である．

PSTの効果予測についてはさまざまな報告がある[7~10]が，いずれも決定的なものではなく，臨床に応用されているものは少ない．なかでもとくに有用とされ，コンセンサスを得られ始めているのは，PSTに非常に感受性があるとされるpCR症例の予測因子としてのホルモン陰性および組織学的高悪性度である．画像所見では，比較的限局性のものがびまん性に広がるものと比べて縮小効果が高いとされている[11,12]．ただし，これらの特徴のみでは適応を決めるまでにはいたっていない．

今回われわれは，当院において経験したPST症例のうち明らかにPSTに対して抵抗性で腫瘍の増大を認めた8例に絞って検討を行った．組織学的にはmetaplastic carcinoma症例が多かった．これまでにも，metaplastic carcinomaが治療抵抗性であり予後が悪いことは当院からも報告している[13,14]．しかし，今回の症例においても示されているとおり，CNB検体にて組織診断が必ず可能かどうかは不明である．これにはCNBの精度も関係するが，腫瘍内部の細胞が均一ではないことが考えられ，PSTによりPSTの効果のある腫瘍細胞は死滅するが，抵抗性の腫瘍は増大し最終的に残存する可能性も考えられるためである．とくに，これらの症例においてはこれまでの報告によりEGFRの発現や筋上皮への分化が示されているものもあり[13]，こういった症例を治療前に判定する上で新たな因子を検討する必要があると考える．

免疫学検査結果では全例ホルモンレセプター陰性およびHER2陰性であるいわゆるTriple negativeの症例であった．さらに組織学的悪性度も全例grade 3であった．さらに画像検査所見では，限局性，境界明瞭なものがほとんどであった．これらの因子は，上記のこれまでの報告や実臨床においてにおいても比較的PSTが良く効くと考えられている特徴とほぼ同一である（**図2**）．

Triple negativeに関しては抗がん剤に対する感受性が特殊であることから近年注目を集めている．それは，今回提示した症例のように抗がん剤に対して非常に抵抗性であるものと，逆に抗がん剤に対して著効を示すものがあるためである．当院における術前化学療法403例の検討においても，Triple negative症例のpCR達成率は15%であり，それ以外の症例の7%に比べて非常に高かった．Triple negative症例の中で今回提示したような症例が，Basal-like typeといわれる治療抵抗性の症例分類と同一かどうかは今回の検討では明らかではないが，現在日常的に行われている組織学的検査およびホルモンレセプターとHER2といった免疫学的検査だけではPSTの適応を決定することは非常に困難であり，これらとは別の因子の検討が必要と考えた．

今回PSTの適応を検討するため，とくにPSTに抵抗性である症例を除外することが可能かどうか検討した．結果いくつかの因子の中で，とくに有用な因子としてMetaplastic carcinomaの組織型が明らかとなった．しかし，治療前に確実にこの組織型を判定することは困難と考えられ，今後更なる症例の集積と新たな因子の検討が必要と考えた．

結　語

PSTに対してPDであった症例は，metaplastic carcinomaの組織型を持つものが多く，免疫染色でtriple negativeおよび画像所見では限局性，境界明瞭で内部不均一といった特徴があった．こういった症例はPSTの適応外と考えられたが，これらの特徴はPSTに非常に感受性のある症例と類似しており，治療前に全て診断するためには更なる検討および新しい予測因子が必要と考えた．

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原著

2008.2.5受付

80歳以上の超高齢者乳癌の治療

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Treatment for The Elderly Breast Cancer Patients Older Than 80 Years：Shien T*1, Kinoshita T*1, Yoshida M*1, Hojo T*1, Shimizu C*2, Kouno T*2, Ando M*2, Akashi-Tanaka S*1, Katsumata N*2 and Fujiwara Y*2 (*1Department of Surgery, National Cancer Center Hospital,*2Breast and Medical Oncology Division, National Cancer Center Hospital)

We examined the clinicopathological features and prognosis of elderly breast cancer patients over 80 years old to d define the optimad treatment strategy for these patients. Between 1966 and 2006, 117 primary breast cancer patients over 80 years of age at diagnosis underwent surgery in the National Cancer Center Hospital (NCCH). The median age was 82 (range 80-94). Operations were partial resection in 30 (27%), total resection 73 (62%), axillary lymph node dissection (ALND) in 64 (52%) and sentinel lymph node biopsy in 11 (9%) patients. On pathological diagnosis, the median tumor diameter was 2cm (range 0.2-5). Histological types were invasive ductal carcinoma in 73 (62%) and mucinous carcinoma in 16 (14%). Estrogen receptor (ER) and Progesteron receptor (PgR) were positive in 66 (57%) and 44 (38%) patients, respectively. Twenty-nine (44%) of 66 patients with ER-positive tumors received adjuvant hormone therapy and 10 (33%) of 30 patients who underwent partial resection received radiation therapy. The median overall survival time was 70 months. Clinical staging, distant and/or local metastasis and the number of axillary lymph node metastases were important predictors of overall survival (OS). ALND and/or SLNB were not important predictors of OS. In N0 patients with ER-positive tumors, the adjuvant hormone therapy was not an important predictor of OS. In elderly breast cancer patients over 80 years of age, the predictor of OS was not ALND but the number of ALN metastases, similar to that in young age. Adjuvant hormone therapy did not affect OS in N0 patients.

Key words：The elder breast cancer, Hormonal therapy, Prognosis
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はじめに

わが国の女性における臓器別のがん年齢調整罹患率は現在胃がんを抜いて乳癌が第1位でありその割合は今後もさらに上昇傾向にある．また日本人の平均寿命も年々延長しており高齢者乳癌症例は増加しており，日本乳癌学会による2004年次全国乳癌患者登録調査においても80歳以上の乳癌患者は全体の約5％と報告されている．現在乳癌が他の固形癌とくらべて比較的予後が良いことや，日常生活に不利益となる機能的な障害や症状をあまりきたさないことから，高齢者の乳癌治療は手術において低侵襲化し，放射線，薬物療法においても消極的になってきている．そういった流れにおいて，エビデンスが示されている部分はいまだ少なく議論の余地がある点が多い．さらに，近年アロマターゼ阻害剤などのホルモン療法薬剤の進

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