able, only BMI and number of deliveries emerged as significant factors determining breast density. In multivariate analysis in premenopausal women, only BMI was a significant factor determining breast density (parameter estimate: −0.403, p value: 0.0005), and breast density decreased as BMI increased. In postmenopausal women, BMI (parameter estimate: −0.196, p value: 0.0143) and number of deliveries (parameter estimate: −0.388, p value: 0.0186) were significant factors determining breast density, and the density decreased as BMI and number of deliveries

Table 2 Concordance rate of breast density evaluation

Judgment category (breast density)		Expert 1 (S. I.)			
		1	2	3	4
Expert 2 (Y. I.)	1	218	3	0	0
	2	5	397	11	0
	3	0	8	345	1
	4	0	0	6	50

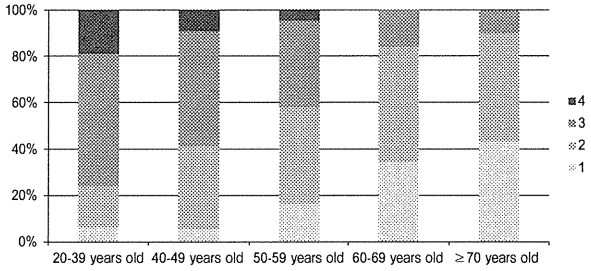

Fig. 1 Final breast density classifications by age group. Following the BI-RADS criteria, breast density was classified as 1, breast is almost entirely fat (<25% glandular); 2, scattered fibroglandular densities (25-50%); 3, heterogeneously dense breast tissue (51-75%); and 4, extremely dense (>75% glandular). Breast density tended to decrease with aging.

Table 3 Results of breast density evaluation

	Number of samples (%)		
	All N=522	Premenopausal N=219	Postmenopausal N=303
1 Breast is almost entirely fat (<25% glandular)	95 (18)	17 (8)	78 (26)
2 Scattered fibroglandular densities (25-50%)	202 (39)	64 (29)	138 (46)
3 Heterogeneously dense breast tissue (51-75%)	192 (37)	114 (52)	78 (26)
4 Extremely dense (>75% glandular)	33 (6)	24 (11)	9 (3)

Table 4 Results of analysis of factors influencing breast density

	Univariate analysis (All subjects, age-adjusted)			Multivariate analysis (All subjects)			Multivariate analysis (Premenopausal)			Multivariate analysis (Postmenopausal)		
Factor	Estimate	Standard error	p value	Estimate	Standard error	p value	Estimate	Standard error	p value	Estimate	Standard error	p value
Body weight	−0.084	0.011	<.0001	0.013	0.024	0.6006	0.043	0.039	0.2634	−0.011	0.032	0.731
BMI	−0.258	0.030	<.0001	−0.271	0.065	<.0001	−0.403	0.116	0.0005	−0.196	0.080	0.0143
Age at first menstruation	0.161	0.062	0.0092	0.118	0.068	0.0827	0.201	0.117	0.0858	0.116	0.087	0.1809
Number of deliveries	−0.365	0.090	<.0001	−0.321	0.129	0.0128	−0.340	0.223	0.1267	−0.388	0.165	0.0186
No history of breast feeding	0.205	0.103	0.0463	0.016	0.154	0.9188	0.152	0.268	0.5707	−0.068	0.197	0.7289
No familial history of breast cancer	0.289	0.135	0.0328	0.275	0.144	0.0571	0.461	0.251	0.0664	0.175	0.179	0.3281

increased.

The influences of BMI and the number of deliveries on breast density in each age group (20–30s, 40s, 50s, 60s, and ≥70 years old) were investigated by ordinal logistic regression analysis using BMI and number of deliveries as explanatory variables and breast density as the response variable (Table 5). BMI significantly influenced breast density in all women except those aged ≥70 years old. The number of deliveries mostly strongly influenced breast density in women in their 50s and 60s, but there was no significant correlation in other age groups.

Discussion

Age markedly influenced breast density in our subjects, as also previously reported [3]. Breast density showed a general decrease with aging; thus, all factors were analyzed after age adjustment. In univariate analysis, body weight, BMI, number of deliveries, history of breast feeding, age at first menstruation, and familial history of breast cancer showed a significant association with breast density. In multivariate analysis performed to account for confounding factors, only BMI and the number of deliveries remained as significant factors associated with breast density. In menopause-stratified analysis, BMI significantly influenced breast density in pre- and postmenopausal women, with the density decreasing as BMI rose. Menopause-stratified analysis has not been performed in many previous studies of the association between breast density and BMI [4–6], but one report showed no association between BMI and breast density in premenopausal women [7]. However, a significant association was found in all generations from the 20s to 60s in the age-stratified analyses in our study. This association was lost in elderly subjects aged ≥70 years old, which may have been due to an overall age-related decrease in breast density.

The number of deliveries also significantly influenced breast density in postmenopausal women, with the density decreasing as the number of deliveries increased. Age-stratified analysis showed that the number of deliveries had a particular influence on breast density in women in their 50s and 60s. There was no significant association in other age groups. An association between number of deliveries and breast density has been shown in previous studies [4, 6–8], but the menopause-stratified analysis in our study provides new details on this association.

Lifestyle factors such as dietary habits, physical activity, alcohol intake, and cigarette smoking showed no relationships with breast density. Alcohol intake is thought to increase the risk of breast cancer, and previous studies have associated alcohol intake with increased breast density [4, 7]. The type of alcoholic beverage and alcohol intake markedly vary among seasons, cultures, and regions, and these factors need to be taken into account in future investigations of the association between alcohol intake and breast density. We also found no association of breast density with any of the diet-related factors surveyed. Previous studies of various diet-related factors have suggested that olive oil and cheese ingestion reduced breast density [4] and that high-fat and high-sugar diets increased breast density [9]. We surveyed the frequency of ingestion of various types of food, but no significant association was found between the ingestion frequency of any food and breast density. However, we did not investigate the intake per single ingestion,

Table 5 Influences of BMI and number of deliveries on breast density in each age group

	BMI			Number of deliveries		
	Estimate	Standard error	*p* value	Estimate	Standard error	*p* value
20–30s	−0.281	0.087	0.0012	−0.465	0.244	0.0565
40s	−0.266	0.064	<.0001	−0.245	0.177	0.1675
50s	−0.277	0.055	<.0001	−0.336	0.160	0.0354
60s	−0.186	0.063	0.0031	−0.468	0.216	0.0302
≥70 years old	−0.194	0.109	0.0762	0.030	0.495	0.9523

which may have been a methodological limitation in the survey method that prevented identification of an association between the type of food and breast density.

High physical activity has also been suggested to reduce the breast cancer risk in postmenopausal women. However, in a previous study of the relationship between physical activity and mammographic density in postmenopausal women, breast density tended to be higher in the most physically active group, although without a significant difference. We also found no significant association between breast density and physical activity, suggesting that physical activity influences the risk of breast cancer independently of breast density. Regarding the history of breast feeding, this has been reported to be both associated [4] and not associated [6, 8] with breast density, and no consensus has been reached. Breast feeding is thought to reduce the risk of breast cancer, but it may have an association that differs from that of delivery.

In our study, breast density decreased as BMI rose regardless of age. However, the risk of breast cancer decreases as BMI increases in premenopausal women, but increases as BMI increases in postmenopausal women. This would appear to contraindicate the use of breast density as a predictor of the risk of breast cancer; it may also indicate that pre- and postmenopausal women should be separated in analyses of associations of BMI with breast density and risk of breast cancer, and that the significance of breast density as a predictor of the risk of breast cancer may be limited to premenopausal women. Alternatively, the cause of the contradiction concerning the associations of BMI with breast cancer risk and breast density may be due to the method of breast density measurement. In the BI-RADS approach used in this study, breast density was evaluated visually, whereas in other reports the percentage of high-density area was calculated by measuring the high-density and whole breast areas using computer-aided diagnosis [10]. Investigations using the whole breast may lead to different findings.

In conclusion, only BMI and number of deliveries were significant factors influencing mammographic breast density. BMI showed an inverse correlation with breast density at all ages below 70 years old, before and after menopause. The number of deliveries significantly influenced breast density in postmenopausal women in their 50 and 60s.

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Original Article

p53 Expression in Pretreatment Specimen Predicts Response to Neoadjuvant Chemotherapy Including Anthracycline and Taxane in Patients with Primary Breast Cancer

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While clinical and pathologic responses are important prognostic parameters, biological markers from core needle biopsy (CNB) are needed to predict neoadjuvant chemotherapy (NAC) response, to individualize treatment, and to achieve maximal efficacy. We retrospectively evaluated the cases of 183 patients with primary breast cancer who underwent surgery after NAC (anthracycline and taxane) at the National Cancer Center Hospital (NCCH). We analyzed EGFR, HER2, and p53 expression and common clinicopathological features from the CNB and surgical specimens of these patients. These biological markers were compared between sensitive patients (pathological complete response; pCR) and insensitive patients (clinical no change; cNC and clinical progressinve disease; cPD). In a comparison between the 9 (5%) sensitive patients and 30 (16%) insensitive patients, overexpression of p53 but not overexpression of either HER2 or EGFR was associated with a good response to NAC. p53 ($p = 0.045$) and histological grade 3 ($p = 0.011$) were important and significant predictors of the response to NAC. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR in insensitive patients between CNB and surgical specimens were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The pathologic response was significantly associated with p53 expression and histological grade 3. The correspondence rate of p53 expression between CNB and surgical specimens was higher than that of other factors. We conclude that the level of p53 expression in the CNB was an effective and reliable predictor of treatment response to NAC.

Key words: breast cancer, neoadjuvant chemotherapy, predictors

Neoadjuvant chemotherapy (NAC) is the standard therapy for patients with advanced local breast cancer and is used increasingly for operable disease. Clinical and pathologic responses are important prognostic parameters, but cannot be accurately predicted. Unfortunately, approximately 20% of breast cancer patients do not benefit from NAC (*i.e.*, they continue to show stable or progressive disease). One of the aims of NAC is to confirm the sensitivity of tumors to chemotherapy. Using NAC, we can directly determine the sensitivity to chemotherapy based on whether or not the primary tumor is diminished, whereas we

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cannot confirm the efficacy by adjuvant chemotherapy itself. However, non-sensitive patients have to endure relatively needless therapy for about 6 months, so it is very important to make the pre-diagnosis of sensitivity to chemotherapy if possible. Several biological markers that might predict response are under investigation [1–9]. Estrogen receptor, progesterone receptor, and HER2 are very useful markers for the selection of anticancer drugs and prediction of prognosis, but are not useful for predicting the response to chemotherapeutic agents such as anthracycline and taxane. Therefore, other biological markers from pretreatment core needle biopsy are needed to predict the response to NAC, to individualize treatment, and to achieve maximal efficacy.

In this study, we investigated biological markers from pre-treatment core needle biopsies of highly sensitive tumors and non-sensitive tumors and identified additional prognostic markers that might predict the response to NAC and aid in the selection of treatment strategy.

Materials and Methods

All patients with operable breast cancer who were treated between May 1998 and July 2006 at the National Cancer Center Hospital with anthracycline and/or taxane as NAC were included in this retrospective study. NAC was indicated for clinical stage II breast cancer patients with tumors larger than 3 cm and stage III breast cancer patients. Core needle biopsy was performed before NAC to allow pathological diagnosis. Doxorubicin (DOX, 50 mg/m^2) and docetaxel (DTX, 60 mg/m^2) were administered for four 3-week cycles before surgery. Additional adjuvant treatment with DOX/DTX was given if patients achieved complete or partial remission after NAC. Otherwise, patients were treated with four cycles of iv cyclophosphamide, methotrexate, and 5FU. Trastuzumab was not administered to the patients with HER2-overexpressing tumors. Tamoxifen (20 mg/day) or anastrozole (10 mg/day) was administered for 5 years after surgery if either the pretreatment biopsy specimen or the surgical specimen post-chemotherapy was positive for estrogen-receptor or progesterone receptor.

Pretreatment diagnosis was established by our pathologists using samples from core needle biopsy or surgical resection. Overexpression of hormone receptors, p53, HER2 and EGFR was examined by immunohistology. Surgical specimens were sectioned at about 7–10 mm and classified for pathological response. Pathological features were described and invasive ductal carcinomas were classified into 3 subtypes (papillotubular, solid tubular, and scirrhous) according to the General and Pathological Recording of Breast Cancer guidelines established by the Japanese Breast Cancer Society [10]. The criteria for histological grading of IDC were based on a modification of those recommended by the WHO [11, 12]. The response criteria used in this study include Fisher's system [13], complete pCR denotes no histological evidence of tumor cells, pCR with DCIS denotes no histological evidence of invasive tumor cells (specimens with only noninvasive cells included), and pINV denotes the presence of invasive tumor cells. Overexpression of ER (1D5, Dako Cytomation, Baltimore, MD, USA), PgR (1A6, Novocastra), HER2 (Herceptest, Dako), p53 (DO7, Dako), and EGFR (2-18C9, Dako) were examined by immunohistology using the noted antibodies. The criterion for ER, PgR, and p53 was staining of more than 10% of cancer cell nuclei, regardless of intensity. HER2 and EGFR grading is as follows: 0: negative, 1+: slightly positive in more than 10% of cancer cells, 2+: moderately positive in more than 10% of cancer cells, 3+: markedly positive in more than 10% of cancer cells. 2+ and 3+ were considered positive for HER2 and EGFR.

Clinical response to NAC was decided from the 2 greatest perpendicular diameters (before each chemotherapy treatment and before surgery) of tumors in the breast and axillary lymph nodes. Absence of clinical evidence of palpable tumors in the breast and axillary lymph nodes was defined as a clinical complete response (cCR). Reduction in total tumor size of 30% or greater was graded as clinical partial response (cPR). An increase in total tumor size of more than 20% or appearance of new suspicious ipsilateral axillary adenopathy was considered progressive disease (cPD). Tumors that did not meet the criteria for objective response or progression were classified as stable disease (cSD). In this study, we analyzed biological markers from core needle biopsies before NAC in complete pCR cases and non-sensitive tumors (clinical SD and PD), and demonstrated bio-

logical predictors of pathological response to PST.

Statistical analysis was carried out using JMP version 6.0 (SAS Institute Inc., Cary, NC, USA). Associations between ordinal variables were assessed using χ^2 analyses or the Fisher exact test for two-by-two variables. The statistical significance (P) was taken as a measure of the strength of evidence against the null hypothesis, and $p \leq .05$ was considered statistically significant.

Results

One hundred and eighty-three patients with operable breast cancer were treated with NAC at National Cancer Center Hospital between May 1998 and October 2001. Table 1 lists the patient and tumor characteristics. The median age was 50 years (range: 29–70). At diagnosis, 41 (22%) patients were in stage IIA, 63 (34%) were in stage IIB, 37 (20%) were in stage IIIA, and 42 (23%) were in stage IIIB. Breast conserving surgery was performed for 55 (30%) patients after NAC. The overall clinical response rate to NAC was 83% (cCR+cPR) and the pCR rate was 13%. 30 (17%) patients were insensitive to NAC (cSD or cPD). Among the responsive patients, 9 (5%) exhibited complete pCR (pathologically no tumor in the breast) and 14 (8%) exhibited pCR with DCIS.

Immunohistological characteristics from core needle biopsy before NAC are listed in Table 2. There were 62 (34%) cases of solid tubular primary tumor, 65 (36%) scirrhous, 34 (19%) papillotubular, 9 (5%) ILC, and 3 (2%) mucinous carcinomas. 88 (48%) cases were histological grade 3. 66 (36%) were ER positive and 72 (39%) were PgR positive. 73 (40%) were HER-2 positive (2+ and 3+ in immunohistological examination).

We evaluated age, histological type, histological grade, ER, PgR, HER2, EGFR, and p53 as predictive factors for response to NAC by comparing 9 (5%) sensitive (complete pCR) and 30 (17%) insensitive (cSD and cPD) tumors (Table 3). In univariate analysis, histological grade 3 ($p = 0.011$) and p53 ($p = 0.045$) were significant predictors of complete pCR. However, EGFR and HER2 were not predic-

Table 1 Patient and tumor characteristics

Parameter	No. of patients (%)
Total	183
Age (median)	50 (29–70)
Clinical stage	
Stage IIA	41 (22%)
Stage IIB	63 (34%)
Stage IIIA	37 (20%)
Stage IIIB	42 (23%)
Operation	
Bt + Ax	128 (70%)
Bp + Ax	55 (30%)
Clinical response	
cCR	32 (17%)
cPR	121 (66%)
cNC	29 (16%)
cPD	1 (1%)
Pathological response	
complete pCR	9 (5%)
pCR with DCIS	14 (8%)
pINV	160 (87%)

Bt, total mastectomy; Bp, partial mastectomy; Ax, axillary lymph node dissection.

Table 2 Immunohistological characteristics of CNB before PST

Parameter	No. of patients (%)
Histological type	
IDC	161 (88)
Solid tubular	62 (34)
Scirrhous	65 (36)
Papillotubular	34 (19)
ILC	9 (5)
mucinous	3 (2)
others	10 (5)
Histological grade	
3	88 (48)
2	88 (48)
1	7 (4)
ER	
positive	66 (36)
negative	117 (64)
PgR	
positive	72 (39)
negative	111 (61)
HER2	
positive (2 + and 3 +)	73 (40)

tors.

We analyzed the immunohistological features of CNB specimens. The correspondence rates of these features in insensitive patients between CNB and surgical specimens are shown in Table 4. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The correspondence rate of EGFR was not low; however, in almost all patients with a discrepancy between CNB and surgical specimens, EGFR overexpression changed from negative to positive.

Discussion

The identification of predictive factors for NAC is very important for order made cancer treatment. The development of new medicines has diversified chemotherapeutic regimens, and the selection of treatment strategy according to individual cancer characteristics has become more difficult. To aid in selection, translational research has begun to demonstrate important correlations between prognostic factors and sensitivity to chemotherapy.

Table 4 Correspondence rates of biological markers in insensitive patients between CNB and surgical specimens

Parameter	%
Histological type	70
Histological grade 3	73
ER	67
PgR	70
HER2	80
p53	93
EGFR	73

In this study, we retrospectively evaluated response to NAC including anthracycline and taxane and a number of biomarkers. We found that pathologic response significantly associated with p53 expression and histological grade 3.

In our analysis, p53 could predict response of NAC. p53 accumulation was reported to be associated with a poor response to anthracycline in node-negative breast cancer patients [14], and may compromise the efficacy of anthracycline but not of taxane [15]. All patients in this study received both anthracycline and taxane, and p53 was an independent predictive factor of response to NAC similar to these reports. We cannot analyze the response of anthracycline and taxane respectively. However commonly we use both drugs in NAC. If the tumor has p53 mutation before NAC, we should check the response of anthracycline tightly and change to taxane when the response is wrong.

Previous studies reported poor prognosis for patients with HER2-overexpression. Several studies indicate that HER2 expression can predict sensitivity to anthracycline chemotherapy [16]; however, in this study, HER2 was not a predictor of pCR to NAC. HER2 negative patients rate were 22% of good responders and 33% of poor responders. In this study trastuzumab was not administered to patients with HER2 overexpression tumors. However, in these days, trastuzumab significantly improved the prognosis and the response to chemotherapy in these patients [17]. It was reported that the rate of pCR patients administered trastuzumab was significantly high. HER2 expression was not predictor of response to anthracycline and taxane in this study. We need to examine the relationship between HER2-overexpression and response to chemotherapy with trastuzumab.

Table 3 Univariate analysis of clinicopathological features between sensitive (pCR) and insensitive cases (cNC + cPD)

Parameter	Sensitive (n = 9) (%)	Non-sensitive (n = 30) (%)	*p*-value
Age < 50	3 (33)	19 (63)	N.S.
Histological type (so.)	6 (67)	12 (40)	N.S.
Histological grade 3	8 (89)	13 (43)	0.011
ER negative	8 (89)	17 (57)	N.S.
PgR negative	6 (67)	17 (57)	N.S.
HER2 positive	2 (22)	10 (33)	N.S.
p53 positive	5 (56)	6 (20)	0.045
EGFR positive	3 (33)	7 (23)	N.S.

so, solid tubular carcinoma

A previous study observed EGFR expression in 37-80% of basal-like tumors, as identified by DNA microarray, and reported poorer prognosis for this phenotype [18-20]. We hypothesized that EGFR expression might distinguish the basal-like phenotype and predict poorer response to NAC. However, in this study, EGFR was not an independent predictive factor of response to NAC. It was reported that EGFR is expressed in 7-36% of breast carcinomas with high grade conventional invasive ductal carcinoma (IDC) [21-24] and EGFR expression was seen in 272 (20%) of 1388 cases. In a univariate analysis, Tsutsui *et al.* showed a significantly poorer clinical outcome for patients with EGFR-positive tumors compared with those who were EGFR-negative, both for overall survival and disease-free survival [21]. The correspondence rate of EGFR overexpression between core needle biopsy and surgical specimens was higher than the correspondence rates of common predictive factors (ER, PgR, and HER2) between the 2 types of specimens. However, the rates of EGFR expression were relatively low in both sensitive (33%) and insensitive patients (23%). In addition, in cases in which EGFR expression did not correspond between CNB and surgical specimens, EGFR was always negative in CNB, but positive in the surgical specimen. Therefore, it is possible that core needle biopsy specimens are inadequate to evaluate EGFR overexpression, or that EGFR expression was stimulated by chemotherapy. Following NAC, highly malignant EGFR-positive tumor cells increased in number, while EGFR-negative cells decreased in number. In these specimens, other common predictive factors did not change pre- and post-NAC; therefore it is not certain that all of the CNB specimens were inadequate. Indeed, it may be that NAC changed the characteristics of some tumors.

We evaluated EGFR, HER2, p53 and other common markers in specimens from pretreatment core needle biopsies as predictors of response to NAC. p53 was a more significant predictor than ER and histological grade, factors that have been previously reported. These results may have been influenced by the uncertainty of core needle biopsy results and the heterogeneity of cancer cells in the tumors. The correspondence rates of these common markers between CNB and surgical specimens were relatively low. However, the correspondence rate of p53 was significantly high. This result indicates that p53 is a stable parameter and suitable for predicting the response to neoadjuvant chemotherapy and for pretreatment diagnosis from CNB specimens.

Pretreatment diagnosis from CNB specimens is necessary to decide the strategy for primary breast cancer treatment. Therefore, identifying prognostic factors is very important, and we need a greater sample size to establish a classification system to predict patient outcome.

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Effects of lifestyle and single nucleotide polymorphisms on breast cancer risk: a case–control study in Japanese women

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Abstract

Background: Lifestyle factors, including food and nutrition, physical activity, body composition and reproductive factors, and single nucleotide polymorphisms (SNPs) are associated with breast cancer risk, but few studies of these factors have been performed in the Japanese population. Thus, the goals of this study were to validate the association between reported SNPs and breast cancer risk in the Japanese population and to evaluate the effects of SNP genotypes and lifestyle factors on breast cancer risk.

Methods: A case–control study in 472 patients and 464 controls was conducted from December 2010 to November 2011. Lifestyle was examined using a self-administered questionnaire. We analyzed 16 breast cancer-associated SNPs based on previous GWAS or candidate-gene association studies. Age or multivariate-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) were estimated from logistic regression analyses.

Results: High BMI and current or former smoking were significantly associated with an increased breast cancer risk, while intake of meat, mushrooms, yellow and green vegetables, coffee, and green tea, current leisure-time exercise, and education were significantly associated with a decreased risk. Three SNPs were significantly associated with a breast cancer risk in multivariate analysis: rs2046210 (per allele OR = 1.37 [95% CI: 1.11-1.70]), rs3757318 (OR = 1.33[1.05-1.69]), and rs3803662 (OR = 1.28 [1.07-1.55]). In 2046210 risk allele carriers, leisure-time exercise was associated with a significantly decreased risk for breast cancer, whereas current smoking and high BMI were associated with a significantly decreased risk in non-risk allele carriers.

Conclusion: In Japanese women, rs2046210 and 3757318 located near the ESR1 gene are associated with a risk of breast cancer, as in other Asian women. However, our findings suggest that exercise can decrease this risk in allele carriers.

Keywords: Japanese women, Asian, Breast cancer, Lifestyle, Leisure-time exercise, Parity, Single nucleotide polymorphisms, rs2046210, rs3757318, ESR1

Background

Data in the National Statistics of Cancer Registries by Region (1975–2004) indicate that the prevalence of breast cancer in Japan has increased steadily since 1975. More than 60,000 patients had breast cancer in 2008 and the mammary gland is the most common site of a malignant tumor in Japanese women [1]. Additionally, the Vital Statistics Japan database of the Ministry of Health, Labor and Welfare indicates that mortality due to breast cancer in Japan has increased since 1960, with more than 10,000 deaths from breast cancer in 2011 [2].

The relationship of lifestyle factors, including food and nutrition, physical activity, body composition, environmental factors, and reproductive factors, with breast cancer risk have been widely studied, mainly in Europe and the United States, and much evidence linking cancer to these factors has been accumulated. According to the

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2007 World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) Second Expert Report, the evidence that breastfeeding decreases the breast cancer risk and that alcohol increases this risk is described as "convincing" [3]. In postmenopausal women, evidence that body fat and adult attained height increase breast cancer risk is also stated to be "convincing". However, the evidence of a relationship of other foods with breast cancer risk remains at the level of "limited-no conclusion". Thus, it is important to identify risk factors for breast cancer with the goal of prevention through efficient screening and surveillance.

In the United States, a breast cancer risk assessment tool based on a statistical model known as the "Gail model" has been produced by the National Cancer Institute (NCI) [4,5]. However, this model has been developed from epidemiological data in Caucasians and it may be inappropriate to apply the Gail model in the Japanese population [6]. However, there are few epidemiological studies of breast cancer risk in Japanese women and a breast cancer risk model applicable to Japanese women has yet to be established.

Regarding genetic factors, genome-wide association studies (GWAS) have identified several breast cancer susceptibility single nucleotide polymorphisms (SNPs) [7]. However, most of these studies were also conducted in subjects with European ancestry, with some in populations with Chinese ancestry or in African Americans. There is only one such study in subjects with Japanese ancestry. However, allele frequencies related to breast cancer risk and the extent of linkage disequilibrium differ among races. Thus, the validity of the reported associations of SNPs with breast cancer needs to be tested in a Japanese population.

Current findings suggest that the interactions between breast cancer susceptibility SNPs and breast cancer risk are not as strong as those for BRCA1 or BRCA2 gene mutation. However, carriers of risk SNP alleles are more common compared with carriers of BRCA1 or BRCA2 mutation. Evaluation of the need to incorporate SNPs into a breast cancer risk model requires examination of the influence of these SNPs and established breast cancer risk factors to determine whether these are mutually confounding factors. Moreover, such findings might allow risk allele carriers to reduce their incidence of breast cancer through guidance on lifestyle habits.

The current study was performed to add to the relatively small number of studies that have examined genomic factors such as SNPs in combination with non-genomic factors such as those associated with lifestyle. We first aimed to validate whether reported breast cancer susceptibility SNPs are applicable in the Japanese population. We then examined the possible confounding effects on breast cancer risk of SNPs and lifestyle factors such as food, nutrition, physical activity, body composition, environment factors and reproductive factors.

Methods

Subjects

A multicenter population-based case–control study was conducted between December 2010 and November 2011 in Japan. The subjects were consecutive patients with non-invasive or invasive breast cancer aged over 20 years old who were treated at Okayama University Hospital, Okayama Rousai Hospital and Mizushima Kyodo Hospital in Okayama and at Kagawa Prefecture Central Hospital in Kagawa. The controls were women aged over 20 years old without a history of breast cancer who underwent breast cancer screening at Mizushima Kyodo Hospital and Okayama Saiseikai Hospital in Okayama and at Kagawa Prefectural Cancer Detection Center in Kagawa. All subjects gave written informed consent before enrollment in the study. A blood sample (5 ml) used for SNP analysis was collected from each subject. Subjects were also given questionnaires that they completed at home and mailed back to Okayama University Hospital. The study was approved by the institutional ethics committee on human research at Okayama University.

Survey of lifestyle

A survey of lifestyle was performed using an 11-page self-administered questionnaire that included questions on age, height and body weight (current and at 18 years old), cigarette smoking, alcohol drinking, intake of 15 foods items, intake of 4 beverages, leisure-time exercise (current and at 18 years old), menstruation status, age at first menstruation, age at first birth, parity, breastfeeding, age at menopause, hormone replacement therapy (HRT), history of benign breast disease, familial history of breast cancer, and education. Controls answered the survey based on their current status and patients referred to their prediagnostic lifestyle.

Body mass index (BMI) was calculated as body weight/square of height. Former or current alcohol drinkers were asked to give the frequency per week and type of drink usually consumed (beer, wine, sake, whisky, shochu, or others). The alcoholic content of each drink was taken to be 8.8 g per glass (200 ml) of beer, and 20 g per glass of sake (180 ml), wine (180 ml), shochu (110 ml) and whisky (60 ml) [8]. Alcohol intake per day (g/day) was calculated as follows: (total alcohol content per occasion × frequency of consumption per week)/7. Women who currently engaged in leisure-time exercise were asked to give the intensity of physical activity per occurrence and frequency per week. Metabolic equivalent (MET) values of 10, 7, 4, and 3 METs were assigned for strenuous-, moderate-, low-, and very low intensity activities per occurrence, respectively [9], to allow calculation of the intensity of

physical activity in leisure-time exercise per week (METs/week). A family history of breast cancer included mother, sisters and daughters (first-degree family history). History of benign breast disease included the non-cancerous breast. Clinical data on patients were obtained from hospital medical records.

Selection of SNPs

Sixteen breast cancer-associated SNPs were identified from previous GWAS [7] and candidate-gene association studies: ATM/11q22-rs1800054 [10], 8q24-rs1562430 [11], MAP3K1/Chr5-rs889132 [10,12], 2q-rs4666451 [10], 8q24-rs13281615 [10,12,13], TTNT3/11p15-rs909116 [11], 5q-rs30099 [10], IGF1/12q23.2-795399 [10,14], ESR1/6q25.1-rs2046210 [15,16], CAPSP8/2q33-34-rs1045485 [10], 2q35-rs13387042 [10], ESR1/6q25.1-rs3757318 [11], TNRC9/16q12-rs3803662 [12,17], FGFR2/10q26-rs2981282 [10,12], LSP1/11p15.5-rs381798 [12], and HCN1/5p12- rs98178 [10]. Risk alleles associated with breast cancer were identified with reference to the Japanese Single Nucleotide Polymorphism (JSNP) database [18].

SNP genotyping

Genomic DNA was isolated from whole blood with a TaqMan® Sample-to-SNP™ kit (Applied Biosystems, Foster City, CA, USA). Samples were analyzed by a TaqMan genotyping assay using the StepOne™ real-time polymerase chain reaction (PCR) system (Applied Biosystems) in a 96-well array plate that included four blank wells as negative controls. The PCR profile consisted of an initial denaturation step at 95°C for 10 min, 40 cycles of 92°C for 15 sec, and 60°C for 1 min. PCR products were analyzed by StepOne™ Software Ver2.01 (Applied Biosystems). To assess the quality of genotyping, we conducted re-genotyping of a randomly selected 5% of samples and obtained 100% agreement.

Statistical analysis

For all analyses, significance was defined as a p-value <0.05. Associations between lifestyle and breast cancer risk were estimated by computing age adjusted odds ratios (OR) and their 95% confidence intervals (CI) from logistic regression analyses. Height was categorized as ≤150, 151–155, 156–160 and >160 according to quartile. Weight was categorized as <50, 50–54.9, 55–59.9 and ≥60 according to quartile. BMI was categorized as ≤20, 20–21.9, 22–23 and ≥24 according to quartile. Alcohol intake per day (g/day) was categorized as 0, <5, 5–10 and ≥10 g/day according to quartile. Food intake, including meat, fish, egg, soy, milk, fruits, green and yellow vegetables and mushrooms, was categorized as ≤1, 2–4 and 5 times/week. Beverage intake including coffee and green tea was categorized as ≤1, 2–3 and ≥3 cups/day. Intensity of physical activity in leisure time was categorized as 0, <6, 6–11.9, 12–23.9 and ≥24 METs/week. Age at menarche was classified as ≤12, 13 and ≥14 years old, parity as 0, 1–2 and ≥3, and age at first childbirth as <25, 25–29 and ≥30 years old. Education level was categorized as high school or less, two-year college, and university or higher.

In analysis of SNPs, accordance with the Hardy-Weinberg equilibrium was checked in controls using a chi-squared test. The associations between genotype and the risk of breast cancer were estimated by computing OR and the 95% CI from logistic regression analyses. Per allele OR was calculated using 0, 1 or 2 copies of the risk allele (a) as a continuous variable. The reported OR and 95% CI denote the risk difference when increasing the number of risk alleles by one. Two models of analyses were performed, with the first model adjusted only for age and the second model adjusted for factors that were significantly associated with breast cancer risk in this study (multivariate adjustment).

For SNPs associated with breast cancer, we classified subjects as risk allele carriers or non-risk allele carriers and examined associations of lifestyle factors with breast cancer risk in these subgroups. Two models were also used in this analysis, with the second model adjusted for factors that were significantly associated with breast cancer risk in the first model.

All statistical analyses were performed with Statistical Analysis System software JMP version 9.0.3 (SAS Institute).

Results

A total of 515 patients and 527 controls agreed to participate in the study and gave written informed consent. Of these women, 476 patients (92.4%) and 464 controls (88.8%) returned self-administered questionnaires. In 2 cases, blood samples could not be obtained because of brittle vessels and in another 2 cases SNP genotyping could not be performed because of poor DNA amplification. Thus, the final data set for analysis included 472 patients and 464 controls with completed questionnaires and SNP genotyping.

Adjusted OR with 95% CIs for lifestyle factors are shown in Table 1. BMI ≥24 (vs. 20–21.9) and current or former smoker (vs. never) were associated with a significantly increased risk for breast cancer. Meat intake ≥2 times/week (vs. ≤once/week), mushroom intake (vs. ≤once/week), yellow and green vegetable intake (vs. ≤once/week), coffee intake 2–3 cups/day (vs. <1 cup/day), green tea intake 2–3 cups/day (vs. <1 cup/day), current leisure-time exercise (vs. none), intensity of physical activity in leisure-time exercise 6–23.9 METS/week (vs. 0 METS/week), and university education (vs. high school or less) were all associated with a significantly decreased risk for breast cancer. Height, alcohol intake, age at first menstruation, parity, age at first birth, and familial history of breast cancer have generally been considered to be associated with breast

cancer risk, but did not show a significant association in this study.

In analysis of SNPs, deviation from the Hardy-Weinberg equilibrium (P <0.05 by chi square test) was found for rs1800054 and rs1045485, and thus these SNPs were excluded from analysis. The minor allele frequencies were <0.05 for rs4666451 and rs104548, and these SNPs were also excluded, leaving 12 SNPs for analysis. Multivariate ORs were adjusted for factors that were found to be significantly associated with breast cancer: BMI, smoking status, meat intake, mushroom intake, yellow and green vegetable intake, coffee intake, green tea intake, leisure-time exercise and education level.

Age adjusted ORs and multivariate ORs with 95% CIs for independent SNPs in all subjects and in subjects stratified by menopausal status are shown in Table 2. In all women, three SNPs were significantly associated with breast cancer risk in multivariate adjustment: rs2046210 (per allele OR = 1.37 [95% CI:1.11-1.70]), rs3757318 (per allele OR = 1.33 [1.05-1.69] and rs3803662 (per allele = 1.28 [1.07-1.55]). rs2046210 and rs3757318, both of which are located on 6q25.1, are not in strong linkage disequilibrium (LD) (D = 0.68, r2 = 0.21) according to Hap-Map JTP [19]. Among pre-menopausal women, s3803662 (per allele OR = 1.58 [95% CI: 1.17-2.16]) and rs2046210 (per allele OR = 1.70 [95% CI: 1.24-2.35]) were significantly associated with breast cancer risk in multivariate adjustment. Among post-menopausal women, there were no SNPs significantly associated with breast cancer risk.

A subgroup analysis was performed for rs2046210 and rs3757318. For rs2046210, leisure time exercise was associated with a significantly decreased breast cancer risk in risk allele carriers (AA + AG), but not in non-risk allele carriers (GG). In contrast, BMI ≥ 24 and current smoking were associated with a significantly increased breast cancer in non-risk allele carriers (GG), but not in risk allele carriers (AA + AG). Intensity of physical activity in leisure exercise of 12.0-23.9 METS/week and university education were associated with breast cancer risk in risk allele and non-risk allele carriers (Table 3). For rs3757318, BMI ≥ 24 was associated with a significantly increased breast cancer risk in risk allele carriers (GG), but not in risk allele carriers (AA + AG). University education and current smoking were associated with breast cancer risk in risk allele and non-risk allele carriers (Table 4).

Discussion

Associations of breast cancer risk with lifestyle factors and SNPs alone and in combination were examined in a case–control study in 472 patients and 464 controls. Reproductive factors such as early age at first menstruation, late age at menopause, late age at first birth, nulliparity, and no breastfeeding have been associated with an increase in breast cancer risk [20], including in the Japanese population [21]. In our study, parity and breastfeeding showed a tendency for an association with decreased breast cancer risk, but this association was not significant; and age at first menstruation, age at first birth, and age at menopause were not significantly associated with breast cancer risk. In most previous studies, comparisons were made using categories for age at first menstruation of 12–13 and >15 years old [22] and age at first birth of ≤24 and >30 years old [23]. In the current study, the sample sizes for women who were >15 years old at first menstruation and >30 years old at first birth were too small to analyze correctly, which is a limitation in the study.

The associations of food and nutrition with breast cancer risk have been summarized by the WCRF/AICR [3]. The effects of some foods on breast cancer are unclear, but we found that intake of meat, mushrooms, yellow and green vegetables, coffee and green tea was associated with decreased breast cancer risk. The evidence that alcohol is associated with breast cancer was judged to be "convincing" by the WCRF/AICR, but we did not find this association, which is consistent with other Japanese studies. The frequency and amount of food consumption depends on cultures and customs in different countries, and this may cause the factors and threshold level for breast cancer risk to also vary in the respective countries.

Cigarette smoking [24,25] is also considered to be associated with increased breast cancer risk, while leisure-time exercise [26] is associated with decreased breast cancer risk, including in the Japanese population. The mean BMI of the Asian population, including the Japanese population, is lower than that in non-Asians [27]. However, we found that BMI ≥24 is associated with increased breast cancer risk, as found in other Japanese studies [28].

A high education level has been associated with increased breast cancer risk, but this may be explained by highly educated women having a high rate of nulliparity and being older at first birth. However, in Japan, social advances and college attendance have only become more common for women in recent years, and thus education level may not correlate well with social status and an unwed state. Instead, more highly educated women are more likely to be involved in preventive health behavior such as exercise, non-smoking, no alcohol intake and avoidance of obesity, compared to women with less education, and some studies have associated a higher education level with a decreased breast cancer risk [29,30].

The current study has several limitations. First, selection bias may have influenced the results because we enrolled women who underwent breast cancer screening as controls. In Japan, the rate of breast cancer screening was no more than about 25% in 2010 [31]. Thus, women who undergo screening may have more interest in trying to maintain their health and may have a family history of cancer, which may have eliminated the significant

Table 1 Adjusted odds ratios and 95% confidence intervals for lifestyle factors in 472 cases and 464 controls (recruitment period: December 2010 to November 2011)

Variables	Case (n = 472)		Control (n = 464)		OR[a] (95% CIs)	
	n (%)		n (%)			
Age (year) (mean ± SD)	54.72 ± 12.45		53.56 ± 11.00			
Menopausal status						
Pre	280	(59)	271	(58)		
Post	192	(41)	193	(42)		
Height (cm)						
≤150	95	(20)	78	(17)	1.16	(0.78-1.71)
151-155	147	(32)	145	(32)	Ref.	
156-160	152	(33)	156	(34)	0.99	(0.72-1.36)
>160	72	(15)	81	(18)	0.93	(0.63-1.38)
Weight (Kg)						
≤50	159	(34)	173	(37)	0.97	(0.69-1.36)
51-55	112	(24)	118	(26)	Ref.	
56 -60	92	(20)	78	(17)	1.24	(0.83-1.85)
>60	104	(22)	93	(20)	1.18	(0.80-1.73)
BMI (Kg/m^2)						
20	102	(22)	96	(21)	1.39	(0.96-2.01)
20-21.9	118	(25)	150	(33)	Ref.	
22-23.9	104	(22)	102	(22)	1.28	(0.89-1.84)
≥24	139	(30)	112	(24)	**1.54**	**(1.08-2.19)**
Smoking status						
Never	406	(87)	432	(94)	Ref.	
Current or former	60	(13)	28	(6)	**2.49**	**(1.56-4.06)**
Alcohol drinking						
Never	240	(51)	218	(47)	ref.	
Current or former	231	(49)	243	(53)	0.91	(0.70-1.18)
Alcohol intake (g/day)						
0	240	(51)	218	(48)	ref.	
<5	140	(30)	130	(29)	1.02	(0.75-1.39)
5-10	53	(11)	62	(14)	0.82	(0.54-1.24)
10>	36	(8)	45	(10)	0.75	(0.46-1.21)
Meat intake (times/week)						
≤1	101	(22)	66	(14)	Ref.	
2-4	297	(64)	307	(67)	**0.65**	**(0.45-0.92)**
≥5	67	(14)	88	(19)	**0.51**	**(0.32-0.80)**
Soy intake (times/week)						
≤1	45	(10)	49	(11)	Ref.	
2-4	236	(50)	227	(50)	1.12	(0.72-1.76)
≥5	188	(40)	182	(40)	1.09	(0.69-1.72)
Fish intake (times/week)						
≤1	103	(22)	94	(20)	Ref.	
2-4	297	(64)	314	(68)	0.85	(0.62-1.18)
≥5	67	(14)	53	(11)	1.09	(0.68-1.74)

Table 1 Adjusted odds ratios and 95% confidence intervals for lifestyle factors in 472 cases and 464 controls (recruitment period: December 2010 to November 2011) *(Continued)*

Eggs intake (times/week)						
≤1	108	(23)	95	(21)	Ref.	
2-4	238	(51)	247	(54)	0.86	(0.62-1.20)
≥5	120	(26)	112	(25)	0.96	(0.66-1.41)
Milk intake (times/week)						
≤1	84	(18)	82	(18)	Ref.	
2-4	157	(34)	135	(30)	1.14	(0.78-1.67)
≥5	226	(48)	238	(52)	0.92	(0.64-1.31)
Fruit intake (times/week)						
≤1	112	(24)	112	(24)	Ref.	
2-4	172	(37)	149	(32)	1.11	(0.79-1.57)
≥5	184	(39)	199	(43)	0.86	(0.61-1.21)
Mushrooms intake (times/week)						
≤1	156	(34)	120	(26)	Ref.	
2-4	247	(53)	261	(57)	**0.73**	**(0.54-0.98)**
≥5	61	(13)	77	(17)	**0.60**	**(0.40-0.91)**
Green and yellow vegetables intake (times/week)						
≤1	47	(10)	28	(6)	Ref.	
2-4	231	(50)	204	(46)	0.66	(0.39-1.09)
≥5	183	(40)	212	(48)	0.48	(0.29-0.80)
Coffee intake (times/week)						
<1	132	(28)	103	(22)	Ref.	
1	154	(33)	158	(34)	0.77	(0.55-1.09)
2-3	135	(29	160	(35)	**0.68**	**(0.48-0.96)**
≥4	45	(10)	40	(9)	0.91	(0.55-1.51)
Green tea intake (times/week)						
<1	200	(43)	182	(40)	Ref.	
1	151	(33)	133	(29)	0.97	(0.71-1.33)
2-3	63	(14)	87	(19)	**0.63**	**(0.43-0.93)**
≥4	48	(10)	55	(12)	0.72	(0.46-1.12)
Leisure-time exercise						
None	254	(54)	214	(46)	Ref.	
Current	214	(46)	248	(54)	**0.70**	**(0.54-0.91)**
Intensity of physical activity[b] (METs/week)						
0	254	(56)	214	(47)	Ref.	
>6.0	51	(11)	42	(9)	1.05	(0.67-1.65)
6.0-11.9	44	(10)	60	(13)	**0.61**	**(0.39-0.93)**
12.0-23.9	48	(11)	80	(17)	**0.51**	**(0.34-0.75)**
≥24.0	52	(12)	61	(13)	0.70	(0.46-1.07)
Age at menarche (year)						
≤12	140	(30)	201	(44)	0.88	(0.616-1.25)
13	109	(23)	113	(25)	Ref.	
≤14	217	(47)	144	(31)	1.25	(0.882-1.78)

Table 1 Adjusted odds ratios and 95% confidence intervals for lifestyle factors in 472 cases and 464 controls (recruitment period: December 2010 to November 2011) *(Continued)*

Parity						
0	86	(20)	75	(17)	Ref.	
1-2	247	(57)	265	(59)	0.74	(0.511-1.06)
≥3	102	(23)	107	(24)	0.76	(0.495-1.15)
Age at first childbirth (year)						
<25	151	(40)	142	(37)	1.22	(0.89-1.68)
25-29	162	(43)	187	(49)	Ref.	
≥30	63	(17)	50	(13)	1.46	(0.96-2.25)
Breastfeeding						
No	125	(27)	104	(23)	Ref.	
Yes	339	(73)	355	(77)	0.77	(0.57-1.04)
History of benign breast disease						
No	351	(79)	354	(79)	Ref.	
Yes	93	(21)	92	(21)	1.03	(0.74-1.42)
Family history of breast cancer						
No	391	(88)	373	(88)	Ref.	
Yes	53	(12)	52	(12)	0.98	(0.65-1.47)
History of HRT use						
No	424	(92)	412	(90)	Ref.	
Yes	35	(8)	45	(10)	0.76	(0.47-1.21)
Education						
High school or less	259	(55)	196	(43)	Ref.	
Two-year college	144	(31)	144	(31)	0.78	(0.57-1.05)
University	64	(14)	120	(26)	**0.41**	**(0.29-0.59)**

[a]OR is adjusted for age. [b]Intensity of physical activity in leisure-time exercise. Significant dates are showed in boldface. OR, odds ratio; CI, confidence interval; BMI, body mass index; HRT, hormone replacement therapy.

association of a family history of breast cancer with breast cancer risk in our study. Second, recall bias may have influenced the results because of the use of self-administered questionnaires. In particular, data from patients might lack accuracy because their answers reflected their behavior before diagnosis.

In all subjects, 3 of the 16 SNPs analyzed in the study were significantly associated with breast cancer risk. These included rs2046210 and rs3757318, which are located at 6q25.1, in proximity to the estrogen receptor 1 gene (ESR1). ESR1 encodes an estrogen receptor (ERα), a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription [32]. ERα is mainly expressed in the uterus, ovary, bone, and breast in females [33], ERα is also overexpressed in 60-70% of cases of breast cancer and is involved in the disease pathology. Although these SNPs are located in the same chromosome region, they are not in strong LD based on the HapMap Project. Potential involvement of both SNPs in regulation of ESR1 is unclear [14,34]. rs2046210 is located 29 kb upstream of the first untranslated exon. The risk allele frequency of rs2046210 is 33.3% in Europeans (HapMap-CEU), 37.8% in Chinese (Hap Map-HCB) and 30.0% in Japanese (HapMap-JTP) [19]. Our result indicated a 27% risk allele frequency, which was about the same as that in HapMap-JTP. Thus, the risk allele frequency of Asians differs little from that of Europeans. Several studies have associated rs2046210 with breast cancer risk [15,34-36]. Guo et al. found a significant association between rs2046210 and breast cancer risk in the overall population (per allele OR 1.14, 95% CI =1.10–1.18) and in Asians (per allele OR 1.27, 95% CI =1.23–1.31) and Europeans (per allele OR 1.09, 95% CI =1.07–1.12), indicating that rs2046210 has a larger effect in Asians [34]. Our results also suggest that rs2046210 is significantly associated with breast cancer risk in Japanese subjects.

Turnbull et al. first reported a significant association of rs3757318 with breast cancer risk [11]. rs3757318 is

Table 2 Odds ratio with 95% confidence intervals for individual SNPs in all subjects and in subjects stratified by menopausal status

		All women (n = 936)					Premenopausal (n = 385)					Postmenopausal (n = 551)				
SNP		No. of	Adjusted OR[b]		Multivariate OR[c]		No. of	Adjusted OR[b]		Multivariate OR[c]		No. of	Adjusted OR[b]		Multivariate OR[c]	
Gene/location	Genotype[a]	Case/Control	OR (95% CI)		OR (95% CI)		Case/Control	OR (95% CI)		OR (95% CI)		Case/Control	OR (95% CI)		OR (95% CI)	
rs1562430	CC	7/4	Ref.		Ref.		2/3	Ref.		Ref.			Ref.		Ref.	
/8q24	TC	96/102	0.54	(0.14-1.85)	0.62	(0.15-2.32)	33/42	1.24	(0.19-9.85)	1.10	(0.15-10.05)	5/1	0.24	(0.01-1.54)	0.35	(0.02-2.80)
	TT	369/351	0.61	(0.16-2.05)	0.67	(0.16-2.45)	155/146	1.64	(0.27-12.63)	1.72	(0.24-15.14)	63/60	0.24	(0.01-1.52)	0.29	(0.01-2.25)
	Per allele		1.05	(0.79-1.39)	1.02	(0.75-1.39)		1.08	(0.81-1.45)	1.62	(1.08-2.44)	214/205	1.07	(0.85-1.36)	0.80	(0.56-1.14)
rs889132	AA	76/91	Ref.		Ref.		34/36	Ref.		Ref.			Ref.		Ref.	
MAP3K1/5q	CA	227/211	1.27	(0.89-1.83)	1.27	(0.86-1.88)	91/95	0.96	(0.55-1.65)	0.82	(0.45-1.50)	42/55	1.59	(0.98-2.58)	1.57	(0.91-2.76)
	CC	164/160	1.21	(0.83-1.76)	1.21	(0.81-1.81)	64/61	1.07	(0.60-1.92)	0.98	(0.52-1.84)	136/116	1.35	(0.82-2.23)	1.30	(0.74-2.30)
	Per allele		1.07	(0.89-1.29)	1.07	(0.88-1.31)		1.08	(0.81-1.45)	1.11	(0.83-1.49)	100/99	1.07	(0.85-1.36)	1.05	(0.81-1.36)
rs13283615	AA	75/75	Ref.		Ref.		29/31	Ref.		Ref.			ref.		ref.	
/8q24	GA	211/206	1.04	(0.71-1.51)	1.09	(0.73-1.65)	73/80	0.97	(0.53-1.76)	1.13	(0.60-2.17)	46/44	1.10	(0.68-1.79)	1.17	(0.67-2.05)
	GG	180/177	1.03	(0.70-1.51)	1.02	(0.67-1.55)	86/78	1.14	(0.63-2.05)	1.18	(0.62-2.24)	138/126	0.97	(0.58-1.61)	1.09	(0.61-1.97)
	Per allele		1.01	(0.84-1.21)	1.00	(0.81-1.22)		1.11	(0.84-1.47)	1.03	(1.00-1.05)	94/99	0.95	(0.74-1.21)	0.99	(0.76-1.28)
rs981782	TT	166/149	Ref.		Ref.		67/64	Ref.		Ref.			Ref.		Ref.	
HCN1/5p12	TG	220/234	0.85	(0.64-1.14)	0.82	(0.60-1.13)	88/98	0.85	(0.54-1.33)	0.78	(0.48-1.26)	99/85	0.87	(0.59-1.27)	0.83	(0.54-1.29)
	GG	82/76	0.96	(0.66-1.41)	0.88	(0.58-1.34)	31/28	1.03	(0.56-1.91)	0.97	(0.50-1.90)	132/136	0.93	(0.57-1.52)	0.76	(0.43-1.34)
	Per allele		0.95	(0.79-1.14)	0.97	(0.80-1.17)		1.00	(0.75-1.35)	1.01	(0.74-1.38)	51/48	0.93	(0.73-1.18)	0.86	(0.66-1.13)
rs3803662	CC	74/91	Ref.		Ref.		24/42	Ref.		Ref.			Ref.		Ref.	
TNRC9/16q12	TC	230/227	1.25	(0.88-1.79)	1.32	(0.89-1.96)	89/96	1.59	(0.90-2.85)	1.50	(0.81-2.80)	50/49	1.08	(0.68-1.72)	1.25	(0.73-2.16)
	TT	160/142	1.41	(0.97-2.08)	**1.61**	**(1.06-2.45)**	72/53	**2.29**	**(1.25-4.26)**	**2.29**	**(1.20-4.46)**	141/131	1.04	(0.63-1.71)	1.27	(0.72-2.24)
	Per allele		1.18	(0.98-1.42)	**1.28**	**(1.07-1.55)**		**1.54**	**(1.15-2.09)**	**1.58**	**(1.17-2.16)**	88/89	1.00	(0.78-1.28)	1.07	(0.83-1.39)
rs381798	TT	339/347	Ref.		Ref.		138/140	Ref.		Ref.			Ref.		Ref.	
LSP1/11p15.5	CT	120/107	1.14	(0.85-1.55)	1.07	(0.77-1.49)	46/49	0.92	(0.58-1.48)	1.00	(0.60-1.68)	201/207	1.30	(0.87-1.94)	1.18	(0.75-1.86)
	CC	10/5	2.04	(0.72-6.60)	1.63	(0.52-5.66)	4/1	3.98	(0.58-78.39)	3.29	(0.42-68.89)	74/58	1.65	(0.46-6.55)	1.39	(0.32-6.31)
	Per allele		1.19	(0.91-1.56)	1.11	(0.83-1.49)		1.07	(0.70-1.64)	1.21	(0.77-1.90)	6/4	1.27	(0.90-1.81)	1.14	(0.78-1.66)
rs2046210	GG	213/244	Ref.		Ref.		83/107	Ref.		Ref.			Ref.		Ref.	
ESR1/6q25.1	AG	194/185	1.21	(0.92-1.59)	1.22	(0.90-1.64)	78/72	1.41	(0.92-2.17)	1.63	(1.03-2.61)	130/137	1.11	(0.78-1.59)	0.99	(0.67-1.48)
	AA	61/34	**2.03**	**(1.29-3.25)**	**2.16**	**(1.32-3.59)**	27/14	**2.46**	**(1.23-5.10)**	**2.93**	**(1.40-6.40)**	116/113	1.69	(0.93-3.14)	1.69	(0.84-3.50)
	Per allele		**1.34**	**(1.10-1.63)**	**1.37**	**(1.11-1.70)**		**1.49**	**(1.10-2.03)**	**1.70**	**(1.24-2.35)**	34/20	1.23	(0.95-1.59)	1.14	(0.86-1.51)

Table 2 Odds ratio with 95% confidence intervals for individual SNPs in all subjects and in subjects stratified by menopausal status *(Continued)*

rs909116	CC	166/178	Ref.		Ref.		71/64	Ref.		Ref.			Ref.		Ref.	
LSP/11p15.5	CT	225/228	1.08	(0.81-1.43)	1.04	(0.77-1.42)	88/106	0.76	(0.49-1.18)	0.90	(0.55-1.47)	95/114	1.36	(0.94-1.97)	1.20	(0.79-1.83)
	TT	79/57	1.49	(0.99-2.24)	1.40	(0.90-2.19)	30/23	1.21	(0.64-2.30)	1.23	(0.62-2.48)	137/122	**1.72**	**(1.02-2.90)**	1.69	(0.94-3.09)
	Per allele		1.18	(0.97-1.42)	1.15	(0.93-1.41)		0.98	(0.72-1.32)	1.11	(0.81-1.52)	49/34	**1.32**	**(1.03-1.69)**	1.24	(0.95-1.63)
rs30099	CC	225/216	Ref.		Ref.		93/84	Ref.		Ref.			Ref.		Ref.	
/5q	TC	205/198	0.82	(0.52-1.29)	1.08	(0.80-1.45)	82/84	0.87	(0.57-1.33)	0.96	(0.61-1.53)	132/132	1.08	(0.76-1.54)	1.21	(0.80-1.83)
	TT	42/50	0.99	(0.76-1.30)	0.86	(0.52-1.41)	15/25	0.53	(0.26-1.06)	0.51	(0.24-1.08)	123/114	1.12	(0.61-2.06)	1.19	(0.58-2.45)
	Per allele		0.93	(0.76-1.13)	0.98	(0.79-1.22)		0.78	(0.57-1.06)	0.85	(0.92-1.16)	27/25	1.04	(0.81-1.36)	1.12	(0.83-1.50)
rs2981282	CC	220/226	Ref.		Ref.		86/94	Ref.		Ref.			Ref.		Ref.	
FGFR2 /10q26	TC	210/190	1.15	(0.87-1.50)	1.19	(0.89-1.60)	91/81	1.23	(0.81-1.87)	1.48	(0.94-2.35)	134/132	1.10	(0.77-1.58)	1.08	(0.72-1.62)
	TT	41/45	0.92	(0.58-1.47)	0.84	(0.50-1.40)	13/17	0.89	(0.41-1.92)	1.07	(0.46-2.50)	119/109	0.95	(0.53-1.71)	0.76	(0.38-1.48)
	Per allele		1.03	(0.84-1.25)	1.02	(0.82-1.27)		1.04	(0.75-1.43)	1.27	(0.91-1.78)	28/28	1.04	(0.80-1.34)	0.94	(0.71-1.24)
rs795399	TT	255/249	Ref.		Ref.		90/107	Ref.		Ref.			Ref.		Ref.	
IGF1/12q23.2	CT	180/173	0.84	(0.51-1.36)	1.05	(0.78-1.41)	82/65	1.49	(0.97-2.30)	1.56	(0.98-2.48)	165/142	0.80	(0.56-1.15)	0.78	(0.52-1.18)
	CC	34/41	1.03	(0.78-1.35)	0.85	(0.49-1.45)	15/20	0.86	(0.41-1.77)	1.04	(0.46-2.27)	98/108	0.87	(0.44-1.70)	0.93	(0.43-1.99)
	Per allele		0.96	(0.79-1.18)	0.97	(0.78-1.21)		1.13	(0.83-1.55)	1.25	(0.91-1.72)	19/21	0.87	(0.66-1.14)	0.88	(0.66-1.17)
rs3757318	GG	249/281	Ref.		Ref.		95/111	Ref.		Ref.			Ref.		Ref.	
ESR1/6q25.1	AG	182/162	1.27	(0.97-1.67)	**1.25**	**(0.93-1.69)**	76/72	1.25	(0.82-1.91)	1.22	(0.77-1.92)	154/170	1.27	(0.88-1.81)	1.20	(0.79-1.80)
	AA	34/19	**2.01**	**(1.13-3.68)**	**2.05**	**(1.09-3.97)**	14/8	2.02	(0.83-5.25)	1.90	**(0.73-5.25)**	106/90	1.96	(0.92-4.37)	2.14	**(0.88-5.49)**
	Per allele		**1.34**	**(1.08-1.66)**	**1.33**	**(1.05-1.69)**		1.30	(0.93-1.83)	1.34	**(0.95-1.91)**	20/11	1.32	(1.00-1.76)	1.27	**(0.93-1.75)**

[a]Alleles on upper line are common alleles; [b]Adjusted for age; [c]Multivariate adjusted for age, BMI, smoking, meat intake, mushroom intake, green and yellow vegetable intake, coffee intake, green tea intake, leisure-time exercise and education. Significant dates are showed in boldface. OR, odds ratio; CI, confidence interval.

Table 3 Age-adjusted odds ratio and multivariate adjusted odds ratio with 95% confidence intervals for lifestyle factors in rs2046210

		Risk allele carriers (AA + AG) n = 474 Case n = 255/Control n = 219							Non-risk allele carriers (GG) n = 457 Case n = 213/Control n = 244						
		n/n	OR[a] (95% CI)		p	OR[b] (95% CI)		p	n/n	OR[a] (95% CI)		p	OR[c] (95% CI)		p
Age (years)		54.0/53.9							55.8/53.2						
Menopausal status	Pre	148/133							130/137						
	Post	107/86							83/107						
Height (cm)	≤150	40/39	1.03	(0.58-1.83)	0.93	0.96	(0.53-1.74)	0.89	55/39	1.34	(0.78-2.9)	0.29	1.19	(0.66-2.14)	0.57
	151-155	76/77	Ref.			Ref.			68/68	Ref.			Ref.		
	156-160	89/66	1.38	(0.88-2.16)	0.16	1.44	(0.91-2.29)	0.12	63/89	0.76	(0.48-1.3)	0.27	0.89	(0.53-1.48)	0.64
	>160	46/34	1.41	(0.81-2.47)	0.23	1.62	(0.91-2.91)	0.10	25/47	0.59	(0.32-1.08)	0.09	0.51	(0.25-0.99)	0.05
BMI (Kg/m^2)	20	59/46	1.27	(0.75-2.14)	0.37	1.13	(0.67-1.94)	0.64	43/50	1.62	(0.93-2.81)	0.09	1.54	(0.84-2.82)	0.16
	20-21.9	69/67	Ref.			Ref.			48/82	Ref.			Ref.		
	22-23.9	58/50	1.09	(0.66-1.80)	0.75	0.97	(0.58-1.63)	0.92	43/52	1.40	(0.82-2.40)	0.22	1.47	(0.83-2.63)	0.19
	≥24	65/53	1.17	(0.71-1.94)	0.53	1.09	(0.65-1.82)	0.74	74/59	**2.07**	**(1.26-3.43)**	**<0.01**	**1.91**	**(1.11-3.29)**	**0.02**
Smoking status	Never	222/201	Ref.			Ref.			180/230	Ref.			Ref.		
	Current or former	29/15	1.78	(0.93-3.51)	0.08	1.61	(0.83-3.21)	0.16	31/13	**3.82**	**(1.94-7.98)**	**<0.01**	**3.86**	**(1.87-8.37)**	**<0.01**
Alcohol drinking	Never	129/107	Ref.			Ref.			108/111	Ref.			Ref.		
	Current or former	125/109	0.97	(0.67-1.40)	0.97	1.07	(0.73-1.57)	0.74	105/133	0.91	(0.62-1.33)	0.61	0.87	(0.56-1.33)	0.51
Alcohol intake (g/day)	0	129/107	Ref.			Ref.			108/111	Ref.			Ref.		
	<5	75/56	1.12	(0.72-1.74)	0.61	1.22	(0.78-1.92)	0.39	64/73	0.99	(0.64-1.54)	0.98	0.98	(0.60-1.61)	0.94
	5-10	28/32	0.75	(0.42-1.34)	0.34	0.88	(0.49-1.60)	0.68	25/30	0.94	(0.51-1.72)	0.85	0.92	(0.46-1.80)	0.80
	10>	20/19	0.88	(0.44-1.74)	0.71	0.94	(0.46-1.89)	0.85	16/26	0.70	(0.35-1.38)	0.31	0.55	(0.24-1.22)	0.14
Leisure-time exercise	No	143/97	Ref.			Ref.			110/116	Ref.			Ref.		
	Yes	110/121	**0.62**	**(0.43-0.89)**	**0.01**	**0.60**	**(0.41-0.87)**	**<0.01**	101/127	0.77	(0.52-1.12)	0.17	0.74	(0.49-1.11)	0.14
Intensity of physical activity[d] (met/week)	0	143/99	Ref.			Ref.			109/119	Ref.			Ref.		
	>6.0	25/23	0.79	(0.42-1.48)	0.45	0.72	(0.38-1.37)	0.32	25/19	1.35	(0.70-2.63)	0.37	1.20	(0.59-2.48)	0.61
	6.0-11.9	20/28	**0.49**	**(0.26-0.92)**	**0.03**	**0.46**	**(0.24-0.86)**	**0.02**	22/32	0.63	(0.34-1.17)	0.15	0.66	(0.34-1.28)	0.22
	12.0-23.9	27/36	**0.52**	**(0.29-0.91)**	**0.02**	**0.53**	**(0.30-0.94)**	**0.03**	21/44	**0.48**	**(0.26-0.85)**	**0.01**	**0.45**	**(0.24-0.83)**	**0.01**
	≥24.0	30/32	0.65	(0.37-1.14)	0.13	0.68	(0.38-1.20)	0.18	22/29	0.74	(0.40-1.38)	0.35	0.70	(0.36-1.36)	0.30
Age at menarche	≤12	70/92	0.73	(0.45-1.19)	0.73	0.72	(0.44-1.19)	0.20	68/109	1.07	(0.63-1.81)	0.80	0.98	(0.56-1.70)	0.93
(year)	13	66/55	Ref.			Ref.			43/58	Ref.			Ref.		
	≤14	116/68	1.20	(0.74-1.93)	1.20	1.15	(0.71-1.89)	0.57	99/75	1.32	(0.78-2.25)	0.29	1.62	(0.93-2.84)	0.09

Table 3 Age-adjusted odds ratio and multivariate adjusted odds ratio with 95% confidence intervals for lifestyle factors in rs2046210 *(Continued)*

Parity	0	54/35	Ref.			Ref.			31/40	Ref.			Ref.		
	1-2	123/122	0.63	(0.38-1.04)	0.07	0.66	(0.40-1.10)	0.11	124/143	0.95	(0.55-1.64)	0.85	1.12	(0.61-2.09)	0.71
	≥3	54/53	0.65	(0.36-1.15)	0.14	0.65	(0.36-1.17)	0.15	46/53	0.94	(0.50-1.76)	0.84	1.29	(0.64-2.62)	0.48
Age at first childbirth	<25	78/68	1.21	(0.77-1.90)	0.40	1.08	(0.68-1.71)	0.74	72/74	1.22	(0.78-1.91)	0.38	1.17	(0.71-1.91)	0.54
(year)	25-29	87/89	Ref.			Ref.			75/97	Ref.			Ref.		
	≥30	33/22	1.55	(0.84-2.90)	0.16	1.45	(0.77-2.76)	0.25	30/28	1.39	(0.77-2.54)	0.27	1.77	(0.92-3.45)	0.09
Breastfeeding	No	72/51	Ref.			Ref.			51/53	Ref.			Ref.		
	Yes	178/165	0.76	(0.50-1.16)	0.21	0.77	(0.50-1.17)	0.22	159/189	0.83	(0.53-1.30)	0.42	1.02	(0.62-1.69)	0.93
Family history of	No	209/180	Ref.			Ref.			178/192	Ref.			Ref.		
Breast cancer	Yes	31/24	1.11	(0.63-1.97)	0.55	1.12	(0.63-2.00)	0.71	22/28	0.82	(0.45-1.50)	0.75	1.07	(0.57-2.05)	0.83
Education	High school or less	135/99	Ref.			Ref.			123/96	Ref.			Ref.		
	Two-year college	81/63	0.93	(0.61-1.42)	0.74	0.95	(0.62-1.47)	0.83	60/81	**0.62**	**(0.40-0.95)**	**0.03**	**0.59**	**(0.37-0.94)**	**0.03**
	University	36/55	**0.48**	**(0.29-0.79)**	**<0.01**	**0.48**	**(0.29-0.79)**	**<0.01**	28/65	**0.35**	**(0.21-0.59)**	**<0.01**	**0.38**	**(0.22-0.66)**	**<0.01**

[a]OR is adjusted for age.
[b]Multivariate adjusted for leisure-time exercise and education.
[c]Multivariate adjusted for BMI, smoking state, intensity of physical activity and education.
[d]Intensity of physical activity and education. Significant dates are showed in boldface.
OR, odds ratio; CI, confidence interval; BMI, body mass index.