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

Taeko Mizoo1, Naruto Taira1*, Keiko Nishiyama1, Tomohiro Nogami1, Takayuki Iwamoto1, Takayuki Motoki1, Tadahiko Shien1, Junji Matsuoka1, Hiroyoshi Doihara1, Setsuko Ishihara2, Hiroshi Kawai3, Kensuke Kawasaki4, Youichi Ishibe5, Yutaka Ogasawara6, Yoshifumi Komoike7 and Shinichiro Miyoshi1

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
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-rs1281615 [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], FGFRI/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 (SNP) 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.

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. In 2 models were also used in this analysis, with 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).
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 OR = 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, rs3803662 (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 decreased breast cancer risk 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 decreased 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 unmarried status. 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 underwent 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* (95% CIs) |
|------------------------------|---------------|-------------------|---------------|
| 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/m²)                  |               |                   |               |
| 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)

| Lifestyle Factor                  | Ref. 1 | 2-4 | ≥5  | Ref. 2   | Odds Ratio 95% CI |
|-----------------------------------|--------|-----|-----|----------|------------------|
| 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)| Ref.|
| 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)| Ref.|
| 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)| Ref.|
| ≥4                                | 48 (10)| 55 (12)| Ref.|
| Leisure-time exercise             |        |     |     |          |                  |
| None                              | 254 (54)| 214 (46)| Ref.|
| Current                           | 214 (46)| 248 (54)| 0.70 | (0.54-0.91) |
| Intensity of physical activity (METs/week) |        |     |     |          |                  |
| 0                                 | 254 (56)| 214 (47)| Ref.|
| >6.0                              | 51 (11)| 42 (9)| Ref.|
| 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 | (0.61-1.25) |
| 13                                | 109 (23)| 113 (25)| Ref.|
| ≤14                               | 217 (47)| 144 (31)| 1.25 | (0.88-1.78) |
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 (HapMap-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 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) |

**Notes:** *OR is adjusted for age. #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.*
| SNP | Gene/location | Genotypea | All women (n = 936) | Premenopausal (n = 385) | Postmenopausal (n = 551) |
|-----|---------------|-----------|---------------------|------------------------|-------------------------|
|     |               |           | No. of Adjusted ORb | Multivariate ORc | No. of Adjusted ORb | Multivariate ORc | No. of Adjusted ORb | Multivariate ORc |
|     |               |           | 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. |
| /8q24 | TC | 96/102 | 0.54 (0.14-1.85) | 0.62 (0.15-2.32) | 33/42 | 1.24 (0.19-8.95) | 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) |
| rs889132 | AA | 76/91 | Ref. | Ref. | 34/36 | 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) | 1.82 (1.08-2.44) |
| rs13283615 | AA | 75/75 | Ref. | Ref. | 29/31 | 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) | 1.05 (1.00-1.15) |
| rs981782 | TT | 166/149 | Ref. | Ref. | 67/64 | 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) | 1.07 (0.74-1.38) |
| rs3803662 | CC | 74/91 | Ref. | Ref. | 24/42 | 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) |
| rs381798 | TT | 339/347 | Ref. | Ref. | 138/140 | 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) |
| rs2046210 | GG | 213/244 | Ref. | Ref. | 83/107 | 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) |
| SNP             | Allele   | Ref. | Ref. | 71/64 | Ref. | Ref. | Ref. | Ref. | Ref. | 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. | Ref. | Ref. | Ref. | Ref. |
|                 | /5q      | 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. | 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. | 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. | 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) |

*Alleles on upper line are common alleles; bAdjusted for age; cMultivariate 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.
| Risk allele carriers (AA + AG) n = 474 | Non-risk allele carriers (GG) n = 457 |
|--------------------------------------|--------------------------------------|
|                                      | Case n = 255/Control n = 219        | Case n = 213/Control n = 244    |
| Age (years)                          | n/n OR (95% CI) p  OR (95% CI) p    | n/n OR (95% CI) p  OR (95% CI) p |
| 54.0/53.9                            | 55.8/53.2                          |
| Menopausal status                    |                                      |
| Pre                                  | 148/133                             |
| Post                                 | 107/86                              |
|                                      | 83/107                              |
| Height (cm)                          |                                      |
| ≤150                                 | 1.03 (0.58-1.83) 0.93 0.96 (0.53-1.74) 0.89 | 1.34 (0.78-2.9) 0.29 1.19 (0.66-2.14) 0.57 |
| 151-155                              | 1.38 (0.88-2.16) 0.16 1.44 (0.91-2.29) 0.12 | 0.76 (0.48-1.3) 0.27 0.89 (0.53-1.48) 0.64 |
| >160                                 | 1.41 (0.81-2.47) 0.23 1.62 (0.91-2.91) 0.10 | 0.59 (0.32-1.08) 0.09 0.51 (0.25-0.99) 0.05 |
| BMI (Kg/m²)                          |                                      |
| 20                                   | 1.27 (0.75-2.14) 0.37 1.13 (0.67-1.94) 0.64 | 1.62 (0.93-2.81) 0.09 1.54 (0.84-2.82) 0.16 |
| 20-21.9                              | 1.09 (0.66-1.80) 0.75 0.97 (0.58-1.63) 0.92 | 1.40 (0.82-2.40) 0.22 1.47 (0.83-2.63) 0.19 |
| ≥224                                 | 1.17 (0.71-1.94) 0.53 1.09 (0.65-1.82) 0.74 | 2.07 (1.26-3.43) <0.01 1.91 (1.11-3.29) 0.02 |
| Smoking status                       |                                      |
| Never                                | 1.78 (0.93-3.51) 0.08 1.61 (0.83-3.21) 0.16 | 3.82 (1.94-7.98) <0.01 3.86 (1.87-8.37) <0.01 |
| Current or former                    | 1.29 (0.67-1.40) 0.97 1.07 (0.73-1.57) 0.74 | 1.06 (0.62-1.33) 0.61 0.87 (0.56-1.33) 0.51 |
| Alcohol drinking                     |                                      |
| Never                                | 1.12 (0.72-1.74) 0.61 1.22 (0.78-1.92) 0.39 | 0.99 (0.64-1.54) 0.98 0.98 (0.60-1.61) 0.94 |
| Current or former                    | 0.75 (0.42-1.34) 0.34 0.88 (0.49-1.60) 0.68 | 0.94 (0.51-1.72) 0.85 0.92 (0.46-1.80) 0.80 |
| Alcohol intake (g/day)               |                                      |
| 0                                    | 1.12 (0.72-1.74) 0.61 1.22 (0.78-1.92) 0.39 | 0.99 (0.64-1.54) 0.98 0.98 (0.60-1.61) 0.94 |
| ≤5                                   | 0.75 (0.42-1.34) 0.34 0.88 (0.49-1.60) 0.68 | 0.94 (0.51-1.72) 0.85 0.92 (0.46-1.80) 0.80 |
| >5                                   | 0.88 (0.44-1.74) 0.71 0.94 (0.46-1.89) 0.85 | 0.70 (0.35-1.38) 0.31 0.55 (0.24-1.22) 0.14 |
| Leisure-time exercise                |                                      |
| No                                   | 1.62 (0.43-0.89) 0.01 0.60 (0.41-0.87) <0.01 | 1.01 (0.52-1.1) 0.17 0.74 (0.49-1.11) 0.14 |
| Yes                                  | 0.77 (0.52-1.12) 0.17 0.74 (0.49-1.11) 0.14 |
| Intensity of physical activity (met/week) |                                      |
| >0.6                                 | 25/13                               |
| 0.6-1.19                             | 0.49 (0.26-0.92) 0.03 0.46 (0.24-0.86) 0.02 |
| 12.0-23.9                            | 0.52 (0.29-0.91) 0.02 0.53 (0.30-0.94) 0.03 | 0.48 (0.26-0.85) 0.01 0.45 (0.24-0.83) 0.01 |
| ≥24.0                                | 30/12                               |
| Age at menarche (year)               |                                      |
| ≤12                                 | 0.73 (0.45-1.19) 0.73 0.72 (0.44-1.19) 0.20 | 0.72 (0.44-1.19) 0.20 0.72 (0.44-1.19) 0.20 |
| >14                                 | 116/68                              |

Mizoo et al. BMC Cancer 2013, 13:565  http://www.biomedcentral.com/1471-2407/13/565
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. | 31/40 Ref. | 123/122 0.63 (0.38-1.04) 0.07 | 0.66 (0.40-1.10) 0.11 | 0.95 (0.55-1.64) 0.85 | 1.12 (0.61-2.09) 0.71 |
|--------------|----------|------------|------------|-----------------------------|----------------------|----------------------|----------------------|
| ≥2 1-2       | 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 |
| ≥3 124/143 0.95 (0.55-1.64) 0.85 | 1.12 (0.61-2.09) 0.71 |
| Age at first childbirth | 78/68 1.21 (0.77-1.90) 0.40 | 1.08 (0.68-1.71) 0.74 | 0.72/74 1.22 (0.78-1.91) 0.38 | 1.17 (0.71-1.91) 0.54 |
| (year) 75/97 Ref. | Ref. |
| 25-29 | 87/89 Ref. | 75/97 | Ref. |
| ≥30 33/22 1.55 (0.84-2.90) 0.16 | 1.45 (0.77-2.76) 0.25 | 1.39 (0.77-2.54) 0.27 | 1.77 (0.92-3.45) 0.09 |
| Breastfeeding | 72/51 Ref. | 51/53 | Ref. |
| Yes 178/165 0.76 (0.50-1.16) 0.21 | 0.77 (0.50-1.17) 0.22 | 0.83 (0.53-1.30) 0.42 | 1.02 (0.62-1.69) 0.93 |
| No 209/180 Ref. | Ref. |
| Family history of Breast cancer | 31/24 1.11 (0.63-1.97) 0.55 | 1.12 (0.63-2.00) 0.71 | 0.72/28 0.82 (0.45-1.50) 0.75 | 1.07 (0.57-2.05) 0.83 |
| Yes 178/192 Ref. | Ref. |
| Education | High school or less 135/99 Ref. | 123/96 Ref. |
| Two-year college 81/63 0.93 (0.61-1.42) 0.74 | 0.95 (0.62-1.47) 0.83 | 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 | 0.35 (0.21-0.59) <0.01 | 0.38 (0.22-0.66) <0.01 |

*aOR is adjusted for age.
*bMultivariate adjusted for leisure-time exercise and education.
*cMultivariate adjusted for BMI, smoking state, intensity of physical activity and education.
*dIntensity of physical activity and education. Significant dates are showed in boldface.
OR, odds ratio; CI, confidence interval; BMI, body mass index.
| Risk allele carriers(AA + AG) n = 397 | Non-risk allele carriers(GG) n = 530 |
|----------------------------------------|--------------------------------------|
|                                        | **Case n = 216/Control n = 181**     | **Case n = 249/Control n = 281** |
|                                        | n/n OR (95% CI) p | OR (95% CI) p | n/n OR (95% CI) p | OR (95% CI) p |
| **Age (years)**                        | 54.23/53.30 | 55.28/53.76 |
| **Menopausal status**                  |              |              |
| Pre                                    | 124/101     | 154/170     |
| Post                                   | 92/80       | 95/111      |
| **Height (cm)**                        |              |              |
| ≤150                                   | 36/28       | 58/50       |
| 151-155                                | 62/63       | 84/80       |
| 156-160                                | 78/51       | 72/105      |
| >160                                   | 36/38       | 34/43       |
| **BMI(Kg/m²)**                         |              |              |
| <20                                    | 48/37       | 54/59       |
| 20-21.9                                | 59/60       | 54/90       |
| 22-23.9                                | 47/35       | 57/66       |
| ≥24                                    | 57/48       | 81/63       |
| **Smoking status**                     |              |              |
| Never                                  | 186/168     | 214/262     |
| Current or former                      | 25/11       | 34/17       |
| **Alcohol drinking**                   |              |              |
| Never                                  | 114/90      | 124/127     |
| Current or former                      | 101/89      | 125/153     |
| **Alcohol intake**                     |              |              |
| 0                                      | 114/90      | 124/127     |
| 5-10                                   | 27/27       | 25/35       |
| 10>                                    | 13/16       | 22/29       |
| **Leisure-time Exercise**              |              |              |
| No                                     | 122/80      | 127/133     |
| **Exercise**                           |              |              |
| Yes                                    | 93/101      | 119/146     |
| **Intensity of physical activity/met/week** |              |              |
| >6.0                                   | 23/17       | 28/25       |
| 6.0-11.9                               | 21/25       | 23/34       |
| 12.0-23.9                              | 19/32       | 29/48       |
| ≥24.0                                  | 23/26       | 27/35       |
| **Age at menarche**                    |              |              |
| ≤12                                    | 63/73       | 73/127      |
| >12                                    | 13          | 57/61       |
| ≥14                                    | 99/56       | 115/88      |
Table 4 Age-adjusted odds ratio and multivariate adjusted odds ratio with 95% confidence intervals for lifestyle factors in rs3757318 (Continued)

| Parity | 0  | 49/24 | Ref. | Ref. | 37/50 | Ref. | Ref. |
|--------|----|-------|------|------|-------|------|------|
|        |    |       |      |      |       |      |      |
| 1-2    | 110/105 | 0.48 (0.27-0.84) | <0.01 | 0.55 (0.19-1.54) | 0.25 | 132/160 | 0.98 (0.60-1.62) | 0.95 | 1.19 (0.70-2.05) | 0.52 |
| ≥3     | 36/48 | 0.34 (0.17-0.65) | <0.01 | 0.35 (0.12-1.04) | 0.06 | 65/58 | 1.36 (0.77-2.40) | 0.29 | 1.74 (0.95-3.21) | 0.07 |
| Age at first childbirth |       |       |      |      |       |      |      |
| <25    | 60/60 | 1.05 (0.64-1.71) | 0.86 | 0.97 (0.56-1.66) | 0.90 | 88/82 | 1.35 (0.89-2.05) | 0.15 | 1.19 (0.77-1.84) | 0.43 |
| ≥30    | 34/19 | 1.96 (1.03-3.80) | 0.04 | 1.82 (0.88-3.85) | 0.11 | 29/31 | 1.17 (0.66-2.10) | 0.59 | 1.27 (0.69-2.33) | 0.45 |
| Breastfeeding |       |       |      |      |       |      |      |
| No     | 65/38 | Ref. | Ref. | Ref. | Ref. | Ref. | Ref. |
| Yes    | 150/143 | 0.60 (0.38-0.95) | 0.03 | 0.93 (0.36-2.43) | 0.89 | 183/211 | 0.91 (0.61-1.38) | 0.67 | 1.07 (0.69-1.65) | 0.77 |
| Family history of Breast cancer |       |       |      |      |       |      |      |
| No     | 173/143 | Ref. | Ref. | Ref. | Ref. | Ref. | Ref. |
| Yes    | 24/19 | 1.04 (0.55-2.00) | 0.79 | 1.30 (0.56-3.07) | 0.54 | 28/33 | 0.91 (0.53-1.57) | 0.93 | 0.90 (0.51-1.58) | 0.72 |
| Education |       |       |      |      |       |      |      |
| High school or less | 113/80 | Ref. | Ref. | Ref. | Ref. | Ref. | Ref. |
| Two-year college | 74/54 | 0.99 (0.62-1.57) | 0.96 | 1.02 (0.58-1.79) | 0.94 | 66/90 | 0.60 (0.40-0.91) | 0.01 | 0.63 (0.42-0.96) | 0.03 |
| University | 27/45 | 0.43 (0.24-0.76) | <0.01 | 0.33 (0.16-0.67) | 0.00 | 36/74 | 0.40 (0.25-0.64) | <0.01 | 0.45 (0.28-0.73) | <0.01 |

*OR is adjusted for age.
*Multivariate adjusted for smoking state, leisure-time exercise, party, age of first children, breastfeeding and education. *Multivariate adjusted for BMI, smoking state, and education. *Intensity of physical activity and education. Significant dates are showed in boldface. OR, odds ratio; CI, confidence interval; BMI, body mass index.
located 200 kb upstream of ESR1. The risk allele frequency of rs3757318 is 6.6% in Europeans (HapMap-CEU), 33% in Chinese (HapMap-HCB) and 25% in Japanese (HapMap-JTP) [19]. We found a 22% risk allele frequency, consistent with HapMap-JTP. Thus, the risk allele frequency for rs3757318 varies between Europeans and Asians. In an analysis of the association between rs2046210 and rs12662670 as a surrogate for rs3757318 and breast cancer risk, Heins et al. found that that per allele OR for rs3757318 was higher in Asians (1.29, 95% CI 1.19–1.41) than in Europeans (1.12, 95% CI 1.08–1.17) [31]. These results suggest that screening for the rs3757318 genotype may be important in Asian women.

We also found that SNPs associated with breast cancer differed with regard to menses state, with rs2046210 and rs3803662 associated with breast cancer risk in premenopausal women. rs3803662 lies 8 kb upstream of TNRC9 and was found to have a significant association with breast cancer risk by Easton et al. [12]. TNRC9 is located on chromosome 16q12 and consists of seven exons. The protein encoded by this gene is a member of the high mobility group box (HMG-box) family. TNRC9 is expressed in brain and breast tissue, and has a higher expression level in breast cancer compared to that in normal tissue [37]. The risk allele frequency of rs3803662 is 24% in Europeans (HapMap-CEU), 72% in Chinese (HapMap-HCB) and 60% in Japanese (HapMap-JTP) [19]. Thus, Asian populations have a higher risk allele frequency than Europeans. However, Chen et al. found that rs3803662 was significantly associated with breast cancer in Europeans [17], but that this relationship was unclear in Asians [38]. Among the breast cancer-associated SNPs found in the current study, rs2046210 and rs3757318 are located near ESR1 and are related to breast cancer risk in Asians. To examine whether lifestyle factors associated with breast cancer risk vary in risk allele and non-risk allele carriers, we performed a subgroup analysis. Leisure-time exercise were associated with a decreased breast cancer risk in rs2046210 risk allele carriers. Although low-penetrance susceptibility SNPs may confer only a small effect on breast cancer risk alone, the risk for development of breast cancer in a risk allele carrier is about 1.2-1.3 fold higher than those in non-carriers. However, our results suggest that risk allele carriers can reduce their breast cancer risk through exercise, whereas obesity and smoking may increase breast cancer risk in non-risk-allele carriers. An understanding of the mechanisms underlying the different lifestyle factors associated with breast cancer in rs2046210 and rs3757318 risk allele and non-risk allele carriers may clarify the effects of these SNPs located near ESR1. Examination of interactions between SNPs and lifestyle factors in a larger Japanese population is needed to confirm the current findings for SNPs, lifestyle factors and breast cancer.

Conclusions

This case–control study showed that rs2046210 and rs3757318 located near the ESR1 gene and rs3808662 located on TNRC9 are associated with breast cancer risk in Japanese women. Our results suggest that leisure-time exercise can reduce the breast cancer risk in rs2046210 risk allele carriers, whereas smoking and obesity may increase the breast cancer risk in non-risk allele carriers. Further studies are required to confirm the validity of the association of these SNPs and lifestyle factors with breast cancer risk in the Japanese population.

Abbreviations

SNPs: Single nucleotide polymorphisms; WCRF/AICR: World Cancer Research Fund/American Institute for Cancer Research; NIC: National Cancer Institute; GWAS: Genome-wide association studies; LD: Linkage disequilibrium; BMI: Body mass index; MET: Metabolic equivalent; OR: Odds ratio; CI: Confidence interval; ERα: estrogen receptor α.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

NT designed the study, TM carried out genotyping, performed statistical analysis, and wrote the manuscript with NT. KN participated in genotyping and statistical analysis. TN, TI, TM, TS, JM, HD, SJ, RK, KK, YI and YO obtained informed consent from subjects, collected blood samples and data from subjects, and provided advice on the study. YK designed the study and served as an advisor. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Author details

1Department of General Thoracic Surgery and Endocrinological Surgery, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama-city, Okayama 700-8558, Japan. 2Department of Radiology, Okayama Saiseikai General Hospital, 1-17-18 Ifuku-cho, Okayama-city, Okayama 700-8511, Japan. 3Tokyo Metropolitan Komagome Hospital, 1-17-19 Chikkoumidorimachi, Okayama-city, Okayama 702-8055, Japan. 4Department of Breast Surgery, Kagawa Prefectural Cancer Detection Center, 587-1 Tougou-cho, Takamatsu-city, Kagawa 761-8031, Japan. 5Department of Breast Surgery, Mizushima Kyodo Hospital, 1-1 Mizushima, Minamikasuga-cho Kurashiki-city, Okayama 712-8567, Japan. 6Department of Breast and Endocrinological Surgery, 5-4-16, Ban-cho, Takamatsu-city, Kagawa 760-8557, Japan. 7Faculty of Medicine, Kinki University Hospital, 377-2 Ohnohigashi, Osaka 589-8511, Japan.

Received: 26 July 2013 Accepted: 18 November 2013

Published: 1 December 2013

References

1. Matsuda A, Matsuda T, Shibata A, Katanoda K, Sobue T, Nishimoto H, The Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2007: a study of 21 population-based cancer registries for the Monitoring of Cancer Incidence in Japan (MCIJ) project. Jpn J Clin Oncol 2013, 43:328–336.
2. Ministry of Health, Ministry of Health, Labour and Welfare: Vital Statistics; 2011. http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/geppo/men/a11/kekka03html#3_2.
3. World Cancer Research Fund/American Institute for Cancer Research: Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR; 2007.
4. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Shihadeh C, Mulvihill JJ: Projecting individualized probabilities of developing breast cancer for
white females who are being examined annually. J Natl Cancer Inst 1989, 81:1879–1886.
5. Breast Cancer Risk Assessment Tool. http://www.cancer.gov/bcrisktool/
Default.aspx.
6. The Japanese Breast Cancer Society: Clinical Practice Guidelines for Breast Cancer. Tokyo: KANEHARA & Co., Ltd. 2012.
7. Hindloft LA, Sethupathy P, Jenkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA: Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. Proc Natl Acad Sci USA 2009, 106:9362–9367.
8. Resource Council, Science and Technology Agency & the Government of Japan: Standard Tables of Food Composition in Japan. 5th edition. Tokyo: National Printing Bureau, 2005.
9. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Connor JK, Pettei DM,Craig SW, Bassett DR Jr, Schmitz KH, Emplaincourt PQ, Jacobs DR Jr, Leon AS: Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000, 32(Suppl):948–504.
10. Travis RC, Reeves GK, Green J, Bull D, Tipper SJ, Baker K, Beral V, Petro R, Bell J, Zelenika D, Lathrop M, Million Women Study Collaborators: Gene-environment interactions in 7610 women with breast cancer: prospective evidence from the Million Women Study. Lancet 2010, 375:2143–2151.
11. Tumblin C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, Seal S, Ghousaini M, Hines S, Healey CS, Hughes D, Warren-Perry M, Tapper W, Eccles D, Evans DG, Breast Cancer Susceptibility. Collaboration, Hooning M, Schatte M, van den Oudewael A, Houton R, Ross G, Langford C, Pharoah PD, Stratton MR, Dunning AM, Rahman N, Easton DF: Genome-wide association study identifies five new breast cancer susceptibility loci. Nat Genet 2010, 42:504–507.
12. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson DJ, Smuevung JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Boweman R, Meyer KB, Haiman CA, Khoe US, Iwasiw M, Santella RM, Zhang L, Fair AM, Wu PE, Wang HC, Sukup O, Fletcher O, Johnson N, dos Santos S, Petro R, etc: Genome-wide association study identifies new breast cancer susceptibility loci. Nature 2007, 47:1087–1093.
13. Long J, Shu XO, Cai G, Gao YQ, Zheng Y, Li G, Li C, Gu K, Wen W, Xiang YB, Lu W, Zheng W: Evaluation of breast cancer susceptibility loci in Chinese women. Cancer Epidemiol Biomarkers Prev 2010, 19:2357–2365.
14. Qian B, Zheng H, Yu H, Chen K: Genotypes and phenotypes of IGFI and IGFBP-3 in breast tumors among Chinese women. Breast Cancer Res Treat 2011, 130:217–226.
15. Zheng W, Long J, Gao YT, Li G, Zheng Y, Xiang YB, Wen W, Levy S, Deming SL, Haines JL, Gu K, Fair AM, Cai G, Lu W, Shu XO: Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet 2011, 43:324–328.
16. Stacey SN, Sulem P, Zanoni C, Gujindosoa SA, Thorleifsson G, Helgason A, Jonassdotir A, Besenbacher S, Kostic JP, Fackenthal JD, Huo D, Adebowale C, Ogundiran T, Olsson JE, Fredericksen ZS, Wang X, Look MP, Sveum WS, Martens JW, Pajaroa I, Garcia-Prats MD, Ramon-Cajal JM, de Juan A, Panadero AV, Marchand L, Brennan P, Sangrajrang S, Gabarev V, O'Decessary F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Petro R, et al: Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2010, 47:1087–1093.
17. Hein R, Maranian M, Hopper JL, Kapuscinski MK, Southey CM, Park DJ, Schmidt MK, Broeks A, Hogervorst FB, Bueno-de-Mesquit HB, Miur KR, Lophathanon A, Rattanamongkongul S, Pattuwal P, Faasching P, Heiman A, Eki AB, Beckmann MM, Fletcher O, Johnson N, dos Santos S, Petro R, etc: Comparison of 6q25 breast cancer hits from Asian and European Genome Wide Association Studies in the Breast Cancer Association Consortium (BCAC). PLoS One 2012, 7:e43380.
18. Cai Q, Wen W, Wu S, Li G, Egan KM, Chen K, Deming SL, Shen H, Shen CY, Gammom MO, Botl WJ, Matsuo K, Haiman CA, Khoe US, Iwasiw M, Santella RM, Zhang L, Fair AM, Hu Z, Wu PE, Signorello LB, Titus-Ernstoff L, Tajima K, Henderson BE, Chan KY, Kusaka Y, Newcomb PA, Zeng H, Cui Y, Wang F, et al: Replication and functional genomic analyses of the breast cancer susceptibility locus at 6q25.1 generalize its importance in women of Asian, Japanese, and European ancestry. Cancer Epidemiol Biomarkers Prev 2011, 20:1355–1363.
19. Guo H, Ming J, Liu C, Li Z, Zhang N, Cheng H, Wang W, Shi W, Shen N, Zhao Q, Li D, Yi P, Wang L, Wang R, Xin Y, Zhao X, Nie X, Huang T: A common polymorphism near the ESRI1 gene is associated with risk of breast cancer: evidence from a case–control study and a meta-analysis. PLoS One 2012, 7:e45250.
20. Jones JD, Chin SF, Wang-Taylor LA, Leaford D, Ponder BA, Caldas C, Maia AT: TOX3 Mutations in breast cancer. PLoS one 2013, 8:e74102.
21. Liang J, Chen P, Hu Z, Shen H, Wang F, Chen L, Li M, Tang J, Wang H, Shen H: Genetic variants in truncleotide repeat-containing 9 (TNRC9) are associated with risk of estrogen receptor positive breast cancer in a Chinese population. Breast Cancer Res Treat 2010, 124:237–241.

Cite this article as: Mizoo et al: Effects of lifestyle and single nucleotide polymorphisms on breast cancer risk: a case–control study in Japanese women. BMC Cancer 2013 13:565.