Relationships of physical and breast cancer phenotypes with three single nucleotide polymorphisms (rs2046210, rs3757318, rs3803662) associated with breast cancer risk in Japanese women

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Abstract

Background: Recent genome-wide association studies have shown that many single nucleotide polymorphisms (SNPs) are associated with breast cancer risk. However, it is often unclear how these SNPs are related to breast cancer. Analysis of associations between SNPs and phenotypes may be important for determining mechanisms of action, including carcinogenesis.

Methods: In previous case-control studies, we found three SNPs (rs2046210, rs3757318, rs3573318) associated with breast cancer risk in Japanese women. Among these SNPs, two (rs2046210 and rs3757318) are located at 6q25.1, in proximity to the *estrogen receptor 1 gene* (*ESR1*). Using data from these studies, we examined associations between factors related to breast cancer risk, such as height, weight, and breast density, and the three SNPs in cases and controls. We also investigated whether the SNPs correlated with breast cancer features, such as estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor type 2 (HER2) status, and clinical stage.

Results: There was a significant difference in mean height between risk and non-risk allele carriers for rs2046210 (156.0 ± 5.8 vs. 154.3 ± 5.5 cm, p=0.002), and rs3757318 (155.8 ± 5.7 vs. 154.7 ± 5.6 cm, p=0.035) in cases, but no significant associations between height and these SNPs in controls. There was also a significant difference in breast density between risk and non-risk allele carriers for rs2046210 (p=0.040) and rs3757318 (p=0.044) in cases. rs2046210 and rs3757318 risk allele carriers tended to have higher breast density in all subjects and in controls. In cases, rs3757318 risk allele carriers were also significantly more likely to be ER-negative compared to non-risk allele carriers (ER-positive rate: 77% vs. 84%, p=0.036).

Conclusions: SNPs rs2046210 and rs3757318, which are associated with breast cancer risk in Japanese women, were significantly associated with height and high breast density, and this association was particularly strong in those with breast cancer. These findings suggest that SNPs in the *ESR1* gene region affect phenotypes such as height and breast density.

Keywords: single nucleotide polymorphism, breast cancer, Japanese, obesity, breast density, height

Introduction

Since 1975, the number of breast cancer patients has consistently increased in Japan, and more than 107,000 new cases of breast cancer were reported in 2016, according to the National Cancer Registry [1]. Therefore, identifying high-risk populations is critical for efficient screening and preventive measures. Breast cancer risk estimation using the Gail model used in Europe and the United States has been attempted, but there are no breast cancer risk models strictly for the Japanese population, which is a challenging clinical concern.

Many studies have shown associations of breast cancer risk with lifestyle, reproductive factors, breast density, and specific genetic mutations in genes such as *BRCA1/2*. Single nucleotide polymorphisms (SNPs) occur at a specific frequency across the genome sequence of a population, and specific SNPs result in individual genetic and physical characteristics, and have relationships with various diseases, including cancer. In recent years, several genome-wide association studies (GWAS) have identified numerous SNPs associated with breast cancer risk. However, many of these studies have been in European, Chinese, and Afro-American populations, with only a few in Japanese people [2]. The frequency of alleles and degree of linkage disequilibrium associated with breast cancer risk are significant factors for heterogeneity across individual populations and races, leading to a need to verify whether the reported SNPs affected breast cancer risk in Japanese women.

In a case-control study in Japanese women, we identified rs2046210, rs3757318 and rs3803662 as SNPs associated with breast cancer risk [3]. rs2046210 and rs3757318 are located near the *estrogen receptor 1* (*ESR1*) gene, and their association with breast cancer risk in various populations has been shown, with rs2046210 suggested to be associated with a greater risk of ER-negative breast cancer [4]. This observation indicates that

SNPs may affect breast cancer phenotype. However, only a few studies have examined relationships between phenotypic changes in individuals with SNPs and cancer risk, and this remains as an important area of study. Physical characteristics of patients with breast cancer are also associated with the risk of breast cancer. For example, obesity markedly increases the risk of breast cancer in postmenopausal women, but almost certainly reduces the risk in premenopausal women [5], with these results supported by a subsequent large-scale systematic review and meta-analysis [6]. In a study in Japanese women, obesity increased breast cancer risk after menopause, although a large-pool analysis showed that the risk increased even before menopause [7]. Based on European and American research outcomes, taller women have a higher risk of breast cancer, and in the JPHC study in Japan, women of height ≥160 cm had a higher risk of breast cancer compared to those ≤148 cm, regardless of menopause status [8]. Breast density determined by mammography is also a risk factor, with a high breast density considered to increase breast cancer risk, regardless of race and ethnicity [9].

Numerous SNPs have been linked to breast cancer risk, but the associated mechanisms are poorly understood. Thus. analysis of the association between SNPs and phenotypes may be important for examining the mechanism of action, including in carcinogenesis. Here, we used a dataset from our case-control study to analyze the phenotypes of Japanese women with specific SNPs associated with breast cancer risk. Obesity, height, breast density, and breast cancer characteristics were investigated to understand the biological function of these SNPs and the mechanisms of increased breast cancer risk.

Materials and Methods

In a previous case-control study, we investigated relationships among SNPs in Japanese women, breast cancer risk, and environmental factors [3]. We registered patients who had undergone treatment for breast cancer at six facilities in Okayama and Kagawa prefectures from 1987 to 2011 as “cases”. Women who visited Kagawa Prefectural Cancer Detection Center and the screening department of Mizushima Kyodo Hospital in Okayama for screening, and had no history of breast cancer, were included as “controls”. Physical and environmental factors in all subjects were collected using a questionnaire. These included date of birth, height, weight (current, at the time of diagnosis, and at 18 years of age), smoking, drinking, 15 food types eaten, 4 drink types consumed, leisure exercise (current, at the time of diagnosis, and at 18 years of age), menstrual status, age of menarche, age of first birth, birth history, number of births, breastfeeding, hormone replacement therapy, history of mammary gland disease, history of breast cancer in relatives, profession, and educational history. We extracted four physical factors associated with Japanese breast cancer risk; height, weight, BMI, and breast density.

Breast density was determined using digital mammography at breast cancer diagnosis in cases, and at screening in controls [9]. Digital mammography images were exported and interpreted on a monitor display. Two specialists who were qualified in mammographic interpretation and certified by The Japan Central Organization on Quality Assurance of Breast Cancer Screening determined the breast density. Breast density was classified into four categories according to the Breast Imaging Reporting and Data System (BI-RADS): 1, fatty breast (<25% gland); 2, glandular breast (25-50%); 3, uneven high breast (51-75%); and 4, high breast (>75%) [10]. The two examiners shared their grading, reevaluated the mammograms if there was a conflict, and made a final decision after discussion. If the density of the two breasts differed, the higher value was used as the

final breast density.

Breast cancer characteristics, such as estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor type-2 (HER2) status, tumor size, regional lymph node metastasis, and distant metastasis were collected from medical records. Blood samples were collected from all subjects, and the relationship between 17 SNPs predicted to be associated with breast cancer from previous GWAS and each survey item was investigated [11]. The risk alleles associated with breast cancer were identified, with reference to the Japanese Single Nucleotide Polymorphism (JSNP) database [12].

Age-adjusted odds ratios (ORs) and multivariate ORs with 95% confidence intervals (CIs) for independent SNPs in all subjects and stratified by menopausal status were investigated. In all women, three SNPs were significantly associated with breast cancer risk after 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]) [3]. To investigate the possibility of the risk allele of each breast cancer risk-related SNP affecting a phenotype, relationships between the three SNPs and physical phenotypes (height, body weight, body mass index (BMI), and breast density), and breast cancer characteristics were investigated using the same dataset.

All procedures involving human participants met the ethical standards of institutional and/or national research committees and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical analysis

Height (cm) was analyzed as four categorical variables (≤150, 151-155, 156-160, and >160 cm) based on quartiles, weight was used as a continuous variable, and BMI was analyzed as six categorical variables (<18.5, 18.5-24.9, 25.0-29.9, 30.0-34.9, 35.0–39.9, and >40.0 kg/m^2) based on the WHO classification. Breast density was calculated for the four BI-RADS categories, as mentioned above. Expression of ER, PgR, and HER2 in cases was defined as positive or negative: ER and PgR were defined as positive based on immunohistochemical (IHC) expression of ≥1%, and HER2 as positive based on an IHC score of 3 or *HER2* gene amplification confirmed by FISH positivity.

To compare risk and non-risk allele carriers for each factor, categorical variables were evaluated by chi-square test, and continuous variables by t-test (ANOVA), with the 95% CI calculated. Associations of breast density with possible related factors (number of deliveries, history of breastfeeding, genotype of SNPs) were analyzed using age-adjusted multivariate ordinal logistic regression analysis. A p value < 0.05 was considered significant. JMP ver. 13.2.1 (SAS Institute) was used for all statistical analyses.

Results

A total of 515 patients (cases) and 527 controls provided written informed consent to participate in the study. Of the subjects, 476 cases (92.4%) and 464 controls (88.8%) returned self-administered questionnaires. In two cases, blood samples could not be obtained owing to brittle vessels, and in two other cases, SNP genotyping could not be performed due to poor DNA amplification. Thus, the final dataset for analysis included 472 cases and 464 controls with completed questionnaires and SNP genotyping information.

Physical characteristics of people with SNP rs2046210

rs2046210 was evaluated in 931 people (468 cases and 463 controls) in the dataset. The numbers of risk allele (AA + AG) and non-risk allele (GG) carriers were 474 (50.9%) and 457 (49.8%), respectively, including 255 (54.3%) and 213 (45.5%) cases, and 219 (47.3%) and 244 (52.7%) controls (Table 1). Risk allele carriers were significantly taller than non-risk allele carriers in cases (156.0 ± 5.8 vs. 154.3 ± 5.5 cm, p=0.002), but there was no significant difference in all subjects or in controls. Risk allele carriers also had significantly lower BMI at diagnosis in all subjects (22.3 ± 3.3 vs. 22.8 ± 3.6 kg/m^2, p=0.026) and in cases (22.3 ± 3.3 vs. 23.3 ± 3.9 kg/m^2, p= 0.003), but not in controls. In risk allele carriers, there was a significant difference in distribution of breast density in cases (p=0.040), and breast density showed a tendency to be higher in cases and controls (Figure 1).

Physical characteristics of people with SNP rs3757318

rs375318 was evaluated in 927 people (465 cases and 462 controls). The numbers of risk allele (AA + AG)

and non-risk allele (GG) carriers were 397 (42.8%) and 530 (57.2%), respectively, including 216 (46.5%) and 249 (53.5%) cases, and 181 (39.2%) and 281 (60.8%) controls (Table 2). Risk allele carriers were significantly taller than non-risk allele carriers in cases (155.8 ± 5.7 vs. 154.7 ± 5.6 cm, p= 0.035), but there was no significant difference in all subjects and in controls. In risk allele carriers, there was a significant difference in distribution of breast density in cases (p=0.044), and breast density showed a tendency to be higher in cases and controls (Figure 1).

Physical characteristics of people with SNP rs3803662

rs3803662 was evaluated in 927 people (464 cases and 462 controls). The numbers of risk allele (AA + AG) and non-risk allele (GG) carriers were 716 (77.2%) and 156 (16.8%), respectively, including 390 (84.1%) and 74 (15.9%) cases, and 369 (79.9%) and 91 (19.9%) controls (Table 3). Height, body weight, BMI, and breast density did not differ significantly between risk and non-risk carriers in all subjects, cases, and controls.

Factors influencing breast density

Age-adjusted multivariate ordinal logistic regression analysis in the all subject showed that the number of deliveries, and the rs2046210 genotype were independent significant factors that influenced breast density (Table 4). However, in the stratified analysis of the case group and control group, the number of deliveries was the only significant factor affecting the breast density.

Breast cancer characteristics and SNPs

Risk and non-risk allele carriers in cases were compared to evaluate the relationship between genotype and breast cancer phenotypes. rs3757318 risk allele carriers were significantly more likely to be ER-negative (ER positive rate: 77% vs. 84%, p= 0.036). There was no difference in breast cancer subtype, tumor size, lymph node metastasis, or distant metastasis among risk and non-risk allele carriers for any of the three SNPs (Table 5).

Discussion

Taller height has been shown to be associated with breast cancer risk [5], and rs2046210 and rs3757318 risk allele carriers were significantly taller in the patients in this study. However, this was not observed in controls, which makes interpretation of this result difficult. An analysis of the association between height and genes using whole-genome sequence data from 1,037 Japanese subjects did not include these SNPs [13], which suggests that rs2046210 and rs3757318 are not universal factors affecting the height of Japanese women. An association between these SNPs and height was found only in breast cancer patients, which suggests that other factors (genetic predispositions and environmental factors) affect the SNP effects. rs2046210 is present in chromosome 6q25.1, 29 kb upstream of the start of the *ESR1* gene, 180 kb upstream of the transcriptional exon, and 6 kb downstream of C6orf97, whereas rs3757318 is in the intron of C6of97, 200 kb upstream of *ESR1*, and 34,253 bp upstream of rs2046210. However, whether these SNPs are involved in *ESR1* expression or ERα function has not been determined [14, 15]. A potential association between *ESR1* and height has been described in a study of adult males from two Swedish population cohorts and in a Swedish case-control study of breast cancer [16, 17].

Risk allele carriers of rs2046210 had significantly lower BMI at diagnosis in all subjects and in patients only. Obesity is an established risk factor for breast cancer, but this risk allele does not appear to be related to obesity in Japanese women. The reason why BMI was significantly lower in risk allele carriers may be due to the taller height of rs2046210 risk allele carriers.

Interestingly, rs2046210 and rs3757318 are both associated with *ESR1* and were also both associated with breast density, with risk allele carriers having a higher breast density. There have been many reports on the

association of breast density with genetic polymorphisms associated with breast cancer. A systematic review showed strong evidence for a genetic component to breast density. Candidate gene approaches yielded replicated associations of *COMT* Val^{158}Met and *IGF-I* rs6220 A>G with premenopausal breast density and of *ESR1 (Xba I and Pvu II*) polymorphisms with postmenopausal breast density, which possibly translate to breast cancer risk [18]. However, many polymorphisms have only been examined in a few studies, which limits the conclusions. The current study does not allow for a definitive conclusion due to the small sample size, but our results suggest that rs2046210 and rs3757318 may be related to breast density in Japanese women. In particular, the rs2046210 genotype was a significant factor affecting breast density in multivariate analysis in all subjects, and its association with breast density was strong.

rs3803662 is associated with the *TNRC9* gene and is one of the four most influential breast cancer risk-related SNPs found in GWAS across 4,000 breast cancer samples [19]. A meta-analysis by Yan et al. suggested no association of rs3803662 with breast cancer risk in Asians [20], and in the current study we found no significant association between rs3803662 and physical phenotypes associated with breast cancer risk.

Following identification of breast cancer risk loci in early GWAS, subsequent studies have investigated associations between these loci and breast tumor histopathology [21]. To date, many SNPs associated with overall breast cancer are also associated with ER-positive breast cancer. A large scale case-control GWAS study conducted in Europe aimed at finding susceptibility variants associated with a risk of ER-negative breast cancer found 125 such variants that explained approximately 16% of the familial risk of this breast cancer subtype [22]. In our case-control study, the sample size was small and risk assessment by subtype-specific polymorphism was

not possible. However, rs3757318 risk allele carriers had a significantly higher rate of ER-negative breast cancer, which suggests the need for a further study of the association of rs3757318 with ER-negative breast cancer risk.

There were certain limitations in the study. The small sample size limited the conclusions that could be drawn. The multiple tests were not adjusted and the possibility of α error cannot be excluded. Also, additional SNPs associated with new breast cancer risk have been identified recently, and thus, only limited SNPs were considered. Within these limitations, we conclude that SNPs rs2046210 and rs3757318, which are associated with breast cancer risk in Japanese women, are significantly associated with height and high breast density, and that this association is particularly strong in patients with breast cancer. These findings suggest that SNPs in the *ESR1* region affect phenotypes such as height and breast density in breast cancer cases.

Compliance with Ethical Standards

Disclosure of potential conflicts of interest: The authors declare that they have no conflicts of interest.

Ethical approval: All procedures were performed with appropriate approval. No animal studies were performed.

Informed consent: All subjects and controls gave written informed consent.

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Table 1. Physical characteristics of people with SNP rs2046210

Variables	All subjects (n=931)			Case group (n=468)			Control group (n=463)		
	AA+AG (n=474)	GG (n=457)	*p* value	AA+AG (n=255)	GG (n=213)	*p* value	AA+AG (n=219)	GG (n=244)	*p* value
Age (year)	56.2±0.5	56.8±0.6	0.415	58.1±0.7	60.9±0.8	0.012	53.9±0.7	53.2±0.7	0.511
Height (cm)	155.7±5.7	155.4±5.7	0.473	156.0±5.8	154.3±5.5	0.002	155.4±5.7	156.4±5.6	0.065
≤150	86 (18)	97 (21)		44 (17)	57 (27)		42 (19)	40 (16)	
151-155	153 (32)	136 (30)	0.249	76 (30)	68 (32)	0.028	77 (35)	58 (28)	0.189
156-160	155 (33)	152 (33)		89 (35)	63 (30)		66 (30)	89 (36)	
>160	80 (17)	72 (16)		46 (18)	25 (12)		34 (16)	47 (19)	
Weight at diagnosis (kg)	54.2±8.7	55.1±8.6	0.094	54.3±8.6	55.6±9.2	0.143	54.0±8.6	55.6±9.2	0.321
Weight at 18 years old (kg)	50.0±6.8	49.9±6.4	0.982	50.1±7.0	49.4±6.5	0.325	49.8±6.6	50.4±6.2	0.360
BMI at diagnosis (kg/m^2)	22.3±3.3	22.8±3.6	0.026	22.3±3.3	23.3±3.9	0.003	22.3±3.3	22.4±3.2	0.800
<18.5	35 (8)	26 (6)		22 (9)	12 (6)		13 (6)	14 (6)	
18.5-24.9	347 (75)	324 (72)		180 (72)	140 (68)		167 (77)	184 (76)	
25.0-29.9	70 (15)	78 (17)	0.339	41 (16)	40 (19)	0.123	29 (13)	38 (16)	0.745
30.0-34.9	12 (3)	18 (4)		5 (2)	12 (6)		7 (3)	6 (2)	
>35.0	1 (0)	3 (1)		1 (1)	2 (1)		0	1 (0)	
BMI at 18 years old (kg/m^2)	20.5±2.5	20.7±2.5	0.403	20.5±2.6	20.8±2.6	0.280	20.6±2.4	20.6±2.4	0.910
<18.5	91 (20)	71 (16)		48 (21)	35 (18)		43 (30)	36 (15)	
18.5-24.9	332 (75)	339 (78)		174 (74)	148 (75)		158 (75)	191 (81)	
25.0-29.9	20 (4)	20 (5)	0.502	11 (5)	13 (7)	0.506	9 (4)	7 (3)	0.401
30.0-34.9	2 (0)	2 (0)		1 (0)	0		1 (1)	2 (1)	
>35.0	0	0		0	0		0	0	
Breast density (%)*									
≤25	72 (17)	98 (24)		33 (16)	45 (28)		39 (18)	53 (22)	
26-50	186 (44)	163 (40)	0.061	102 (50)	69 (43)	0.040	84 (39)	94 (39)	0.686
51-75	135 (32)	121 (30)		56 (27)	41 (25)		79 (36)	80 (33)	
>75	30 (7)	21 (5)		15 (7)	7 (4)		15 (7)	14 (6)	

Data are shown as the number of subjects (%) or as a mean ± SD

*Each mammogram was assessed for breast density according to the BI-RADS breast density categories

BMI: body mass index

Table 2. Physical characteristics of people with SNP rs3757318

Variables	All subjects (n=927)			Case group (n=465)			Control group (n=462)		
	AA+AG (n=397)	GG (n=530)	*p* value	AA+AG (n=216)	GG (n=249)	*p* value	AA+AG (n=181)	GG (n=281)	*p* value
Age (year)	56.1±0.6	56.8±0.5	0.388	58.4±0.8	60.1±0.8	0.124	53.3±0.8	53.8±0.7	0.663
Height (cm)	155.9±5.7	155.3±5.7	0.105	155.8±5.7	154.7±5.6	0.035	156.0±5.8	155.8±5.6	0.726
≤150	69 (17)	112 (21)		40 (19)	59 (24)		29 (16)	53 (19)	
151-155	125 (31)	164 (31)	0.255	62 (29)	84 (34)	0.176	63 (35)	80 (28)	0.081
156-160	129 (32)	177 (33)		78 (36)	72 (29)		51 (28)	105 (37)	
>160	74 (19)	77 (15)		36 (17)	34 (14)		38 (21)	43 (15)	
Weight at diagnosis (kg)	54.5±8.6	54.7±8.8	0.694	54.4±8.5	55.3±9.3	0.296	54.6±8.8	54.2±8.2	0.647
Weight at 18 years old (kg)	50.0±6.7	49.9±6.5	0.833	49.8±7.0	49.8±6.5	0.972	50.3±6.3	50.3±6.5	0.675
BMI at diagnosis (kg/m^2)	22.4±3.3	22.7±3.5	0.213	22.4±3.4	23.1±3.8	0.053	22.4±3.2	22.4±3.2	0.897
<18.5	26 (7)	35 (7)		16 (8)	18 (7)		10 (6)	17 (6)	
18.5-24.9	289 (74)	381 (73)		152 (73)	167 (68)		137 (76)	214 (77)	
25.0-29.9	64 (16)	82 (16)	0.605	35 (17)	45 (18)	0.613	29 (16)	39 (14)	0.724
30.0-34.9	9 (2)	21 (4)		5 (2)	12 (5)		4 (2)	9 (3)	
>35.0	1 (0)	3 (1)		1 (1)	2 (1)		0	1 (0)	
BMI at 18 years old (kg/m^2)	20.5±2.4	20.68±2.5	0.403	20.5±2.6	20.8±2.6	0.173	20.6±2.2	20.6±2.5	0.594
<18.5	73 (20)	88 (17)		40 (21)	43 (18)		33 (19)	45 (17)	
18.5-24.9	278 (75)	391 (77)		144 (74)	176 (76)		134 (77)	215 (79)	
25.0-29.9	18 (5)	22 (4)	0.153	11 (6)	13 (6)	0.685	7 (4)	9 (3)	0.318
30.0-34.9	0	4 (1)		0	1 (0.4)		0	3 (1)	
>35.0	0	0		0	0		0	0	
Breast density (%)*									
≤25	63 (18)	107 (23)		29 (17)	49 (25)		34 (18)	58 (21)	
26-50	155 (44)	193 (41)	0.315	86 (50)	85 (44)	0.044	69 (39)	108 (39)	0.843
51-75	108 (31)	146 (31)		42 (24)	53 (27)		66 (37)	93 (33)	
>75	25 (7)	26 (6)		15 (9)	7 (4)		10 (6)	19 (7)	

Data are shown as the number of subjects (%) or as a mean ± SD

*Each mammogram was assessed for breast density according to the BI-RADS breast density categories

BMI: body mass index

Table 3. Physical characteristics of people with SNP rs3803662

Variables	All subjects (n=924)			Case group (n=464)			Control group (n=460)		
	AA+AG (n=759)	GG (n=164)	*p* value	AA+AG (n=390)	GG (n=74)	*p* value	AA+AG (n=369)	GG (n=91)	*p* value
Age (year)	56.5±0.4	56.0±0.9	0.576	59.3±0.6	59.7±1.4	0.807	53.7±0.6	53.1±1.1	0.639
Height (cm)	155.5±5.7	155.8±5.6	0.594	155.3±5.8	154.8±5.2	0.526	155.8±5.6	156.5±5.8	0.236
≤150	153 (20)	28 (17)		80 (21)	17 (23)		70 (19)	11 (12)	
151-155	225 (30)	62 (38)	0.235	119 (31)	24 (32)	0.460	106 (29)	38 (42)	0.017
156-160	256 (34)	48 (29)		124 (32)	26 (35)		132 (36)	22 (24)	
>160	125 (16)	27 (16)		64 (16)	7 (9)		61 (17)	20 (22)	
Weight at diagnosis (kg)	54.6±8.7	54.9±8.7	0.700	55.0±85.9	54.3±8.9	0.573	54.2±8.4	55.3±8.6	0.254
Weight at 18 years old (kg)	49.9±6.6	50.5±6.4	0.308	49.8±6.7	49.9±7.2	0.905	49.9±6.6	50.9±5.6	0.210
BMI at diagnosis (kg/m^2)	22.6±3.5	22.6±3.3	0.922	22.8±3.7	22.5±3.4	0.534	22.3±3.3	22.6±3.3	0.535
<18.5	51 (7)	10 (6)		27 (7)	7 (10)		25 (7)	3 (3)	
18.5-24.9	543 (73)	123 (75)		269 (71)	49 (68)		274 (75)	74 (81)	
25.0-29.9	119 (16)	28 (17)	0.158	64 (17)	16 (22)	0.077	55 (15)	12 (13)	0.147
30.0-34.9	29 (4)	1 (1)		17 (4)	0		12 (3)	1 (1)	
>35.0-39.9	3 (0.4)	1 (1)		3 (1)	0		0	1 (1)	
BMI at 18 years old (kg/m^2)	20.6±2.5	20.7±2.4	0.581	20.6±2.6	20.7±2.6	0.858	20.6±2.5	20.7±2.3	0.541
<18.5	141 (20)	22 (14)		71 (20)	13 (19)		70 (20)	9 (10)	
18.5-24.9	537 (75)	127 (81)		24 (74)	54 (78)		273 (76)	73 (84)	
25.0-29.9	33 (5)	7 (4)	0.207	22 (6)	2 (3)	0.612	11 (3)	5 (6)	0.077
30.0-34.9	4 (1)	0		1 (0.3)	0		3 (1)	0	
>35.0-39.9	0	0		0	0		0	0	
Breast density (%)*									
≤25	151 (22)	20 (14)		72 (23)	7 (12)		79 (22)	13 (15)	
26-50	273 (40)	72 (50)	0.060	136 (44)	32 (56)	0.200	137 (37)	40 (45)	0.352
51-75	209 (31)	46 (32)		81 (26)	15 (26)		128 (35)	31 (35)	
>75	42 (6)	7 (5)		19 (6)	3 (5)		23 (6)	4 (5)	

Data are shown as the number of subjects (%) or as a mean ± SD

*Each mammogram was assessed for breast density according to the BI-RADS breast density categories

BMI: body mass index

Table 4. Results of analysis of factors with a potential influence on breast density

Factor	All subjects			Case group			Control group		
	Estimate	Standard error	*p* value*	Estimate	Standard error	*p* value*	Estimate	Standard error	*p* value*
Number of deliveries	0.35	0.09	<0.001	0.29	0.13	0.033	0.40	0.13	0.0015
No history of breast feeding	0.10	0.11	0.348	0.07	0.16	0.650	0.13	0.15	0.399
Risk allele carrier (rs2046210)	-0.17	0.08	0.033	-0.13	0.15	0.396	-0.19	0.10	0.053
Risk allele carrier (rs3757318)	0.04	0.08	0.614	0.03	0.15	0.827	0.02	0.10	0.849
Risk allele carrier (rs3803662)	0.11	0.09	0.215	0.20	0.15	0.183	0.07	0.12	0.559
Case	-0.08	0.07	0.267	-	-	-	-	-	-

*Multivariate ordinal logistic regression analysis, age adjusted

Table 5. Breast cancer characteristics and SNPs

Variables	rs2046210			rs3757318			rs3803662		
	AA+AG (n=255)	GG (n=213)	*p* value	AA+AG (n=216)	GG (n=249)	*p* value	AA+AG (n=390)	GG (n=74)	*p* value
ER									
positive	194 (79)	175 (84)	0.158	161 (77)	206 (84)	0.036	311 (82)	57 (79)	0.587
negative	53 (21)	34 (16)		49 (23)	38 (16)		69 (18)	15 (21)	
PgR									
positive	148 (65)	138 (70)	0.234	128 (66)	156 (68)	0.640	241 (69)	43 (61)	0.2436
negative	81 (53)	59 (30)		66 (34)	73 (32)		110 (31)	27 (39)	
HER2									
positive	42 (19)	24 (14)	0.135	36 (20)	30 (14)	0.161	60 (18)	6 (10)	0.0773
negative	175 (81)	151 (86)		147 (80)	179 (86)		267 (82)	56 (90)	
Triple negative									
yes	34 (13)	17 (8)	0.061	30 (14)	21 (8)	0.061	40 (10)	9 (12)	0.6307
no	221 (87)	196 (92)		185 (86)	228 (92)		350 (90)	65 (88)	
ER negative and HER2 positive									
yes	16 (6)	9 (4)	0.322	15 (7)	10 (4)	0.163	22 (6)	3 (4)	0.5659
no	239 (94)	204 (96)		201 (93)	239 (96)		368 (94)	71 (94)	
Size of primary tumor									
in situ	41 (17)	37 (18)		28 (13)	75 (17)		67 (18)	9 (13)	
<1 cm	127 (52)	100 (48)		111 (53)	227 (50)		187 (50)	38 (54)	
>1 to 2 cm	69 (28)	60 (29)	0.941	61 (29)	129 (29)	0.353	106 (28)	22 (31)	0.7652
>2 to 5 cm	4 (2)	4 (2)		5 (2)	8 (2)		7 (2)	1 (1)	
>5 cm	5 (2)	6 (3)		4 (2)	11 (2)		10 (3)	1 (1)	
Axillary lymph node metastasis									
negative	171 (69)	135 (65)	0.394	139 (66)	162 (67)	0.859	257 (68)	45 (62)	0.3246
positive	78 (31)	73 (35)		72 (34)	81 (33)		123 (32)	28 (38)	
Distant metastasis									
positive	248 (99)	204 (97)	0.089	210 (99)	239 (98)	0.208	376 (98)	72 (99)	0.778
negative	2 (1)	6 (3)		2 (1)	6 (2)		7 (2)	1 (1)	

Data are shown as the number of subjects (%)
ER-positive cases defined as ≥1% ER-positive tumor cells
PgR-positive cases defined as ≥1% PgR-positive tumor cells
HER2-positive cases defined as IHC 3+ or FISH-positive.

Figure 1

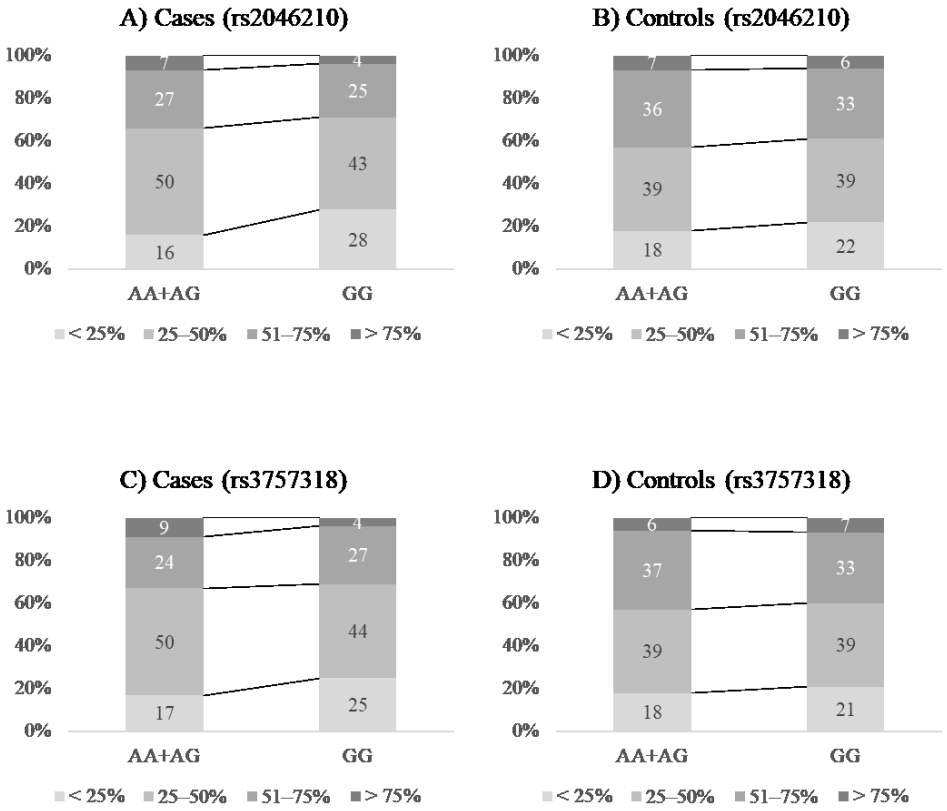
A) Cases (rs2046210)
100%
80%
60%
40%
20%
0%
7
27
50
16
4
25
43
28
AA+AG
GG
< 25%
25–50%
51–75%
> 75%
B) Controls (rs2046210)
7
36
39
18
6
33
39
22
C) Cases (rs3757318)
9
24
50
17
4
27
44
25
D) Controls (rs3757318)
6
37
39
18
7
33
39
21

Figure Legend

Figure 1. Relationship of breast density with genotypes of rs2046210 [A) cases, B) controls] and rs3757318 [C) cases, D) controls].