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Genetic variants in microRNA and microRNA biogenesis pathway genes and breast cancer risk among women of African ancestry

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Compliance with ethical standards

The authors declare no conflicts of interest. This study is compliant with the ethical standards of human research. Written informed consent was obtained from all participants.

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Abstract

Background—MicroRNAs (miRNA) regulate breast biology by binding to specific RNA sequences, leading to RNA degradation and inhibition of translation of their target genes. While germline genetic variations may disrupt some of these interactions between miRNAs and their targets, studies assessing the relationship between genetic variations in the miRNA network and breast cancer risk are still limited, particularly among women of African ancestry.

Methods—We systematically put together a list of 822 and 10,468 genetic variants among primary miRNA sequences and 38 genes in the miRNA biogenesis pathway, respectively; and examined their association with breast cancer risk in the ROOT consortium which includes women of African ancestry. Findings were replicated in an independent consortium. Logistic regression was used to estimate the odds ratio (OR) and 95% confidence intervals (CI).

Results—For overall breast cancer risk, three single nucleotide polymorphisms (SNPs) in miRNA biogenesis genes *DROSHA* rs78393591 (OR=0.69, 95% CI: 0.55–0.88, P=0.003), *ESR1* rs523736 (OR=0.88, 95% CI: 0.82–0.95, $P=3.99\times10^{-4}$), and *ZCCHC11* rs114101502 (OR=1.33, 95% CI: 1.11–1.59, P=0.002) and one SNP in primary miRNA sequence (rs116159732 in miR-6826, OR=0.74, 95% CI: 0.63–0.89, P=0.001) were found to have significant associations in both discovery and validation phases. In a subgroup analysis, two SNPs were associated with risk of estrogen receptor (ER)-negative breast cancer and three SNPs were associated with risk of ER-positive breast cancer.

Conclusion—Several variants in miRNA and miRNA biogenesis pathway genes were associated with breast cancer risk. Risk associations varied by ER status, suggesting potential new mechanisms in etiology.

Keywords

Breast Neoplasms; Genome Wide Association Studies; microRNA; African Americans; Biogenesis

Introduction

Breast cancer is the most common cancer among women world-wide and is a leading cause of cancer death (Coughlin and Ekwueme 2009). While common in high-income countries, the incidence of breast cancer is also increasing rapidly in many middle and low-income countries, including Sub-Saharan Africa (Parkin et al. 2008; Sitas et al. 2008). Numerous high-risk genes, such as *BRCA1* and *BRCA2*, and low-penetrance common genetic variants have been identified as breast cancer risk factors, but a large proportion of heritability remains unexplained, suggesting that there are still uncharacterized genetic variants that modify the risk of breast cancer. Furthermore, already identified variants that affect breast cancer risk may not do so in all population groups because of differences in population characteristics (Chen et al. 2011; Chen et al. 2014; Yao et al. 2013; Zheng et al. 2013).

MicroRNAs (miRNAs) are ~22 nucleotide long, single-stranded RNA molecules that function in post-transcriptional gene regulation by binding to messenger RNA (mRNA) and leading to mRNA degradation (Kim 2005; Kim et al. 2009; Krol et al. 2010). The role of miRNAs in the initiation and progression of cancer has been confirmed in numerous studies and is a notable source of interest for developing targeted cancer therapy (Blenkiron and Miska 2007; He et al. 2015; Lee and Dutta 2009; Lin and Gregory 2015; Nana-Sinkam and Croce 2013; Ryan et al. 2010; Serpico et al. 2014). Furthermore, the genes involved in the synthesis of miRNAs have also been of interest given some evidence of association with cancer risk (De Santa et al. 2013; Gregory and Shiekhattar 2005; Ha and Kim 2014). miRNAs have frequently been found to be located in fragile sites (FRA) or cancer-associated genomic regions (CAGRs), including those implicated in breast cancer (Calin et al. 2004). Several studies have examined the single nucleotide polymorphisms (SNPs) of miRNA biogenesis genes and their association with breast cancer risk among Asian populations, but the observations are rather inconclusive (Jiang et al. 2013; Sung et al. 2011; Sung et al. 2012). To our knowledge, there has been no large scale study that has taken a genome-wide approach of screening SNPs in the miRNA network for their association with breast cancer risk among women of African ancestry.

In this case-control study, we hypothesized that SNPs in miRNA and miRNA biogenesis genes are associated with risk of breast cancer and that this risk is further modified by factors such as hormone receptor status. Through a literature review, we identified 35 genes involved in miRNA biogenesis and three additional genes known to be involved in the hormonal regulation of miRNA biogenesis (Alanazi et al. 2013; Krol et al. 2010; Leaderer et al. 2011; Sung et al. 2011; Sung et al. 2012; Tchatchou et al. 2009). In addition, we examined genetic variants found in primary miRNA sequences using the miRBase version 20 (http://www.mirbase.org/) (Griffiths-Jones et al. 2006). We comprehensively evaluated these genetic variants in the Genome-Wide Association Study of Breast Cancer in the African Diaspora - the ROOT consortium, and validated the findings in independent samples from the African American Breast Cancer consortium (AABC).

Materials and Methods

Study participants

The study populations of the ROOT consortium have been described previously (Boersma et al. 2006; Huo et al. 2012; Nemesure et al. 2009; Qian et al. 2014; Zheng et al. 2013). Briefly, this study included 3,686 participants of African ancestry (1,657 breast cancer cases and 2,029 controls). Ascertainment of cases and controls occurred in Ibadan, Nigeria (711 cases and 624 controls), Barbados (92 cases and 229 controls), and four sites in the USA (854 cases and 1,176 controls). Samples in the replication stage of the study were from the AABC consortium, previously described in detail (Chen et al. 2013). In brief, the AABC consists of 5,984 African Americans (3,153 cases and 2,831 controls) from nine case-control studies (John et al. 2007a; John et al. 2007b; Kolonel et al. 2000; Marchbanks et al. 2002; Newman et al. 1995; Prorok et al. 2000; Signorello et al. 2005; Smith et al. 2008).

SNP genotyping and imputation

Genotyping in the ROOT consortium was conducted using the Illumina HumanOmni2.5–8v1 array, which included approximately 2.4 million genetic variants. Genotyping was attempted for a total of 3,909 study samples, of which 3,859 were successful. These samples were derived from 3,774 participants, with 85 of these with duplicate samples. After genotyping, quality control analysis was carried out by checking gender discordance, chromosomal anomalies, participant relativeness, population structure, missing call rates, batch effects, duplicate sample discordance, Mendelian errors, Hardy-Weinberg equilibrium, and duplicate SNP probes. First, duplicate samples from 85 pairs were excluded. Chromosomal anomalies > 5 Mb and anomalies on chromosomes where the sum of the anomaly lengths > 10 Mb were identified. No samples were excluded for this reason but all genotypes were set to missing for regions with chromosome anomalies. According to the identical by descent analysis, samples from 59 participants were excluded because they were possibly relatives of other participants. Additionally, 20 samples (18 African Americans and 2 African Barbadians) were excluded because principal components analysis showed that they were clustered with the CEU/TSI HapMap populations (i.e., high percentage of European ancestry). We further filtered out 7 samples with a missing call rate > 2% and 2 samples with possible tumor contamination. After these exclusions, a total of 3,686 participants (1,657 cases and 2,029 controls) were included for the final analysis. After SNP level quality control analysis, genotype imputation was conducted using IMPUTE2 software (Howie et al. 2009). With the 1000 Genomes Project phase I integrated variant set as the reference panel, 16,147,413 were imputed and passed the imputation quality filter (imputation score > 0.3).

Genotyping in the AABC consortium was conducted using the IlluminaHuman1M-Duo BeadChip (Chen et al. 2013). Samples based on the following exclusion criteria were removed: (1) unexpected replicates (≥ 98.9% genetically identical) that were confirmed through discussions with study investigators; (2) unknown replicates that could not be confirmed; (3) samples with call rates < 95% after a second genotyping attempt; (4) samples with < 5% African ancestry; and (5) samples with ≤ 15% mean heterozygosity of SNPs on the X chromosome and/or similar mean allele intensities of SNPs on the X and Y

chromosomes, as these are likely to be males. African ancestry was assessed using principal components analysis and the first eigenvector was used to distinguish between participants of African and those of other ancestries. This was confirmed by using input genotypes from the HapMap populations, CEU (CEPH Utah), YRI (Yoruba), and JPT (Japanese). Lastly, SNPs with <95 % call rate were removed.

A total of 17,291 candidate SNPs were extracted from the 38 miRNA biogenesis genes (Supplemental Table S1) using the dbSNP database (National Center for Biotechnology Information), including all SNPs found 5kb upstream and downstream of the known coding region. SNPs in this list were then matched to the ones present in stage 1 of our study. For miRNA variants, using the NCBI build 37 positions, 1,283 SNPs in our study that laid within the starting and ending positions of the primary miRNA sequence in miRBase version 20 were extracted. Variants that had MAF < 0.01 among controls or imputation score <0.7 were excluded from the stage 1 analysis. After these selections, 10,468 SNPs in biogenesis genes and 822 SNPs in the miRNA sequences were included in the association analysis.

Statistical analysis

Case–control differences in demographic and disease characteristics were compared using chi-squared tests for categorical variables and t-tests for continuous variables. Within each of the SNPs identified in miRNA or miRNA biogenesis genes in stage 1, we identified the most significant markers by testing the association of each SNP with breast cancer risk using logistic regression models, adjusting for age, study site (Nigeria, USA, or Barbados), and the first 10 eigenvectors from the principal component analysis (PCA). In both the discovery and replication stages, the first ten principal components were computed using the smartpca program in the EIGENSOFT package (Patterson et al. 2006). No eigenvectors from the principal component analysis were strongly associated with case status after controlling for the matching factors, DNA source, and study. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated in logistic regressions as estimates for allele dosage effect using SNPTEST v2.5 (Oxford University). As an exploratory analysis, we also examined whether SNPs were associated with risk of estrogen receptor (ER)-negative or ER-positive breast cancer. SNPs with $P < 0.05$ in stage 1 were examined in the stage 2 replication study in AABC. ORs and 95% CIs were estimated using unconditional logistic regression, adjusting for age, the first 10 eigenvectors from the PCA, and study site. An overall odds ratio or OR_{meta}, its 95% CI, and *P*-value were estimated by inverse-variance weighting of the stage 1 and 2 results.

The top SNP signal in each gene was determined by the smallest *P*-value from the genetic association test. After identifying the top signal, a conditional analysis was conducted by including this SNP in the genetic association test model as a covariate and examining whether there were additional signals that reached statistical significance. SNPs found to be statistically significant in the same miRNA biogenesis gene were examined for linkage disequilibrium (LD) using HaploReg v2 (Broad Institute, Massachusetts Institute of Technology) using $r^2 > 0.2$ as the threshold.

We performed corrections for multiple testing by estimating number of independent variants for each miRNA biogenesis gene. Using Haploview (Barrett et al. 2005), we identified the

number of SNPs that can tag all SNPs with MAF ≥ 0.05 in the genomic region 5kb upstream and downstream of each miRNA biogenesis gene for Yoruba in Ibadan, Nigeria (YRI) population from the International HapMap Project. The method of aggressive tagging was used in which up to two SNPs can be used to tag a SNP if they are in LD with $r^2 > 0.8$. The regional corrected alpha level was calculated by dividing 0.05 by the number of tagging SNPs in each region, whereas a genome-wide correction was calculated by dividing by the total number of SNPs from all regions. For miRNA variants, we applied a genome-wide correction based on the number of SNPs we tested that had MAF ≥ 0.05 in our stage 1 population.

Statistical analysis was conducted using the SAS 9.3 package (SAS Institute, Cary, NC) and Stata 14.0 (StataCorp, College Station, TX). All *P*-values are two-sided and a *P*-value < 0.05 was considered statistically significant for demographic and disease characteristics and SNP identification in stage 1 analysis.

Results

The initial analysis included 1,657 cases and 2,029 controls among women of African ancestry from Nigeria, USA, and Barbados (Table 1). The mean age of cases was 49.3 years whereas the mean age of controls was 48.4. The majority of cases and controls in the sample were African American. Compared to controls, higher proportions of cases were post-menopausal, had a family history of breast cancer, and had ever consumed alcohol. Controls had a higher mean BMI (29.7) than cases (28.4) and were more likely to have ever used oral contraceptives. In the replication analysis in AABC consortium, 3,153 cases and 2,831 controls were analyzed. The age of the cases and controls in the replication sample ranged from 22 to 87 years, with median ages of 55 and 58 years, respectively (Table 1). Roughly 11% of the participants in both our discovery study and the replication sample had a first-degree family history of breast cancer. Approximately 60% of the cases in the replication sample had ER positive disease, which is slightly higher than the percentage in the discovery study.

Across the miRNAs and 38 miRNA biogenesis genes, 1,743 SNPs (1,638 from biogenesis genes and 105 from miRNA) were significantly associated with breast cancer risk at significant level of 0.05 in stage 1. Out of these SNPs, 575 were associated with overall breast cancer risk (Supplementary Table S2). In addition, 736 were associated with ER-negative breast cancer risk (Supplementary Table S3) whereas 676 were associated with ER-positive breast cancer risk (Supplementary Table S4). These SNPs were further examined in stage 2 of our study.

Using Haploview, we found that 622 SNPs could be used to tag all SNPs with MAF>0.05 in the miRNA biogenesis genes and gene-level multiple testing corrected significance level were presented in Supplementary Table S1. The genome-wide corrected alpha level for the SNPs in the miRNA biogenesis genes was calculated to be 8.04×10^{-5}. For SNPs in primary miRNA sequences, we found that 439 of the SNPs we tested had MAF ≥ 0.05, which can be used to calculate a genome-wide corrected alpha level of 1.14×10^{-4}. These corrected alpha-values were applied to identify significant variants in the pooled analysis.

Table 2 displays the 14 individual SNPs from miRNA biogenesis pathway genes that were associated with overall breast cancer risk in pooled analysis. The most significant SNPs in each miRNA biogenesis pathway gene are presented. Three SNPs were consistently found to be associated with breast cancer risk in both stages 1 and 2, including *DROSHA* rs78393591 (G vs. A, OR_{meta} = 0.69, 95% CI: 0.55 – 0.88, P = 0.003), *ESR1* rs523736 (G vs. A, OR_{meta} = 0.88, 95% CI: 0.82 – 0.95, $P = 3.99 \times 10^{-4}$), and *ZCCHC11* rs114101502 (G vs. C, OR_{meta} = 1.33, 95% CI: 1.11 – 1.59, P = 0.002). Table 3 displays the 14 individual SNPs located in miRNAs that were associated with overall breast cancer risk. One SNP was consistently found to be associated with breast cancer risk in both stages 1 and 2: *mir-6826* rs116159732 (C vs. T, OR_{meta} = 0.74, 95% CI: 0.63 – 0.89, P = 0.001).

Table 4 shows the association between individual SNPs and breast cancer risk stratified by ER status. Two SNPs were found to be associated with risk of ER-negative breast cancer: *mir-4725* rs73991220 (A vs. G; OR_{meta} = 1.27, 95% CI: 1.09 – 1.48, P = 0.002), and *PAPD4* rs146287903 (A vs. T; OR_{meta} = 0.49, 95% CI: 0.33 – 0.72, $P = 3.27 \times 10^{-4}$) in both stages 1 and 2. Three SNPs were found to be associated with risk of ER-positive breast cancer in both stages, including *miR-339-3p* rs72631820 (T vs. C, OR_{meta} = 1.36, 95% CI: 1.10 – 1.69, P = 0.004), *ESR1* rs9479113 (C vs. T, OR_{meta} = 0.86, 95% CI: 0.78 – 0.95, P = 0.003), *SMAD3* rs79730172 (T vs. C; OR_{meta} = 1.30, 95% CI: 1.11 – 1.54, P = 0.001).

Discussion

In this comprehensive investigation of miRNA-related SNPs in 9,670 women of African ancestry (4,810 cases and 4,860 controls), we identified four SNPs in miRNA biogenesis genes or miRNA sequences were consistently significantly associated with breast cancer risk, and additional five SNPs in miRNA biogenesis genes or miRNA sequences were associated with breast cancer risk after stratification by ER status. None of these SNPs were in linkage disequilibrium with genetic variants previously identified in GWAS (Chen et al. 2013; Michailidou et al. 2015; Michailidou et al. 2013; Song et al. 2013).

We found a total of five SNPs that differed in their relative association with breast cancer risk according to tumor ER status, which is consistent with prior studies that showed that the patterns of miRNA expression vary according to the specific breast tumor subtype (Blenkiron et al. 2007; Iorio et al. 2005; Lowery et al. 2009). For ER-negative breast cancers, we found two novel SNPs (rs73991220 and rs146287903) which conferred breast cancer susceptibility. rs146287903 was in the *PAPD4* 3'UTR region, which may implicate this particular variant in the transcriptional regulation of *PAPD4* expression. For ER-positive breast cancers, rs72631820 in *miR-339-3p* was consistently found to be associated with increased risk in both consortia. In previous studies, altered expression of *miR-339-3p* was associated with risk of prostate cancer and also served as a prognostic factor in diffuse large B-cell lymphoma (Lim et al. 2015; Medina-Villaamil et al. 2014). Furthermore, there is some evidence that expression levels of the related *miR-339-5p* have direct effects on the pathogenesis of breast cancer, including initial invasion and subsequent metastasis (Wang et al. 2014; Wu et al. 2010). One other SNP located in *miR-202* (rs12355840) was found to be associated with breast cancer in stage 1 of our study (OR = 0.78, P = 0.005), but not in the stage 2 replication (OR = 0.94, P = 0.27), with the pooled result (OR_{meta} = 0.90, P = 0.02).

This particular SNP and miR-202 were found to be associated with breast cancer risk and prognosis in several other studies, indicating a potential functional role of this miRNA, and warranting further studying of this miRNA in breast cancer diagnosis, prevention, and treatment (Joosse et al. 2014; Rawlings-Goss et al. 2014; Schrauder et al. 2012).

Our finding of SNPs in the gene *ZCCHC11* being associated with breast cancer risk may be based on a unifying pathway in the regulatory circuit of the *let-7* miRNA family, which has been found to be associated with tumorigenesis (van Kouwenhove et al. 2011; Yu et al. 2007). *LIN28* has been found to recruit *ZCCHC11* to mediate *LIN28*'s blockage of *let-7* miRNA biogenesis (Hagan et al. 2009; Lin and Gregory 2015; Piskounova et al. 2011). The possible changes in let-7 expression through this pathway may be a driver for the development of many types of cancers. The complex interactions of these proteins in addition to genetic variants that may alter their functional capabilities warrant further in-depth investigations.

We found SNPs in the *SMAD3* gene that were associated with both with overall and ER-positive-specific risk of breast cancer. Several recent studies have examined the pathways through which *SMAD3* may influence breast cancer pathogenesis. First, through the mediation of transformation growth factor β (TGF-β), *SMAD3* can interact directly with *miR-21*, which has been shown previously to be upregulated during breast cancer (Davis et al. 2008; Li et al. 2014). A second pathway is through the interaction of the *BRCA1* gene with *DROSHA* along with the *SMAD3/p53/DHX9* pathway in promoting miRNA maturation (Dubrovska et al. 2005; Kawai and Amano 2012). This result may confer an additional pathway through which *BRCA1* modulates breast cancer pathogenesis, particularly among women of African ancestry, who have higher rates of *BRCA1/BRCA2* mutations and may be more susceptible to the deleterious effects of *BRCA1/BRCA2* on inducing triple-negative breast cancer (Dietze et al. 2015; Hall et al. 2009).

ER plays a prominent role in breast cancer signaling, progression, and metastasis, and is also a potent target for hormonal therapies such as tamoxifen (Clarke et al. 2003; Fuqua 2001). The differential associations of miRNA with ER-negative and ER-positive breast cancer suggests that miRNA may interact directly with or is modulated by the ER. In a prior study, variants in a miRNA binding site in *ESR1* were associated with familial breast cancer risk (Tchatchou et al. 2009). The specific *ESR1* variant, rs2747648, was noted to affect the binding capacity of *miR-453*. Another study demonstrated that miRNA processing could involve complex regulatory pathways with both ER and c-Myc, leading to differential levels of miRNA expression in ER-positive or ER-negative breast cancers (Castellano et al. 2009). The interaction between miRNA and ER is hence of particular interest as a better understanding of this pathway could unveil additional therapeutic targets for breast cancer. While we were not able to replicate this finding in the present study, we did find one SNP in *ESR1* (rs523736), the gene encoding ERα that was consistently associated with breast cancer risk regardless of ER status. This SNP was not found to be in strong LD with GWAS identified *ESR1* SNPs, such as rs2046210 or rs12662670, which were both found to be strongly associated with breast cancer risk in European and Asian populations (Garcia-Closas et al. 2013; Hein et al. 2012; Yang et al. 2013). This suggests a possible novel mechanism by which ERα influences breast cancer risk among African populations.

Our study has several strengths, including its large sample size and replication which reduces the likelihood of false positive findings, a comprehensive search of genes/miRNA of interest, and the high coverage of known SNPs using a genome-wide scan. We also assessed the LD between the SNPs in our study and known breast cancer susceptibility loci and did not find any notable linkages. The limitations of this study include the need to determine the functionality of the particular SNPs in altering the expression level or function of the biogenesis gene or miRNA and the resultant effects on breast cancer risk. Additional research is needed to confirm the associations found for these SNPs and determine how these miRNAs and biogenesis genes alter the disease pathway of breast cancer. The majority of the variants found in our study were intronic variants which may exert their effect through effects on alternative splicing, binding of regulatory miRNA or RNA binding proteins, and additional interactions with enhancers or repressors (Ward and Kellis 2012). In addition, we did not have complete hormone receptor status for all breast cancer cases the in stage 1 and 2 studies, thus decreasing the number of cases and controls tested in stratified analyses. Furthermore, while our study examined the association of individual SNPs with the risk of breast cancer, growing research shows that the joint variations of multiple miRNAs (Zhong et al. 2014) have profound effects on breast cancer risk; understanding these complex interplays will be key in determining novel preventive, diagnostic and therapeutic strategies. Lastly, we cannot rule out the possibility that variants found in this study could be false positives. Since there were statistically significant differences in several baseline characteristics in our discovery cohort, we performed a sensitivity analysis in which we additionally adjusted for these variables. Overall, the effect estimates are not materialistically different from our main analysis, though several variants that previously had borderline statistical significance were no longer significant (Supplementary Table 5). In addition, we applied a more liberal form of correction by using regional tagging SNPs for each gene and a combined genome-wide correction. Additional studies in populations of African ancestry as well as other populations will be needed to confirm our findings, since the SNPs examined in our study did not reach our corrected genome-wide significance. Nevertheless, our study provides further evidence that miRNA play a potentially important regulatory role in breast cancer and highlight the importance of using miRNA as novel biomarkers for clinical risk assessment as well as potential therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Demographic and disease characteristics for participants in the stage 1 and stage 2 analysis

	Stage 1: ROOT Consortium			-	Stage 2: AABC Consortium	
	Case	**Control**	***P*-value[a]**		**Case**	**Control**
	N = 1,657	**N = 2,029**			**N = 3,016**	**N = 2,745**
Age, mean ± SD	49.3 ± 12.4	48.4 ± 12.9	**0.04**	Age, median	55	58
Race, n (%)			**< 0.001**	Body mass index, (%)		
Nigerian	7 11 (42.9)	6 24 (30.8)		<25	22	22
African American	8 54 (51.5)	1 176 (58.0)		≥25	76	76
African Barbadian	9 2 (5.6)	2 29 (11.3)		First degree family history of breast cancer, (%)		
Body mass index (kg/m^2), mean ± SD	28.4 ± 7.0	29.7 ± 7.0	**< 0.001**	Yes	18	11
Height (cm), mean ± SD	162.4 ± 7.7	162.0 ± 7.2	0.11	No	79	86
Menopausal status, n (%)			**< 0.001**	Age at menarche (%)		
Pre-menopausal	781 (47.1)	1080 (53.2)		< 12	49	50
Post-menopausal	876 (52.9)	948 (46.7)		13–14	33	35
Family history of breast cancer, n (%)			**< 0.001**	15+	12	13
Yes	272 (17.9)	167 (10.0)		Age at first birth (%)[b]		
No	1248 (82.1)	1499 (90.0)		0	15	13
Alcohol consumption, n (%)			**0.03**	≤ 20	42	46
Ever	436 (27.9)	423 (24.6)		20+	37	38

	Stage 1: ROOT Consortium			-	Stage 2: AABC Consortium	
	Case	Control	*P*-value[a]		Case	Control
	N = 1,657	**N = 2,029**			**N = 3,016**	**N = 2,745**
Never	1 125 (72.1)	1 294 (75.4)		Parity (%)		
Hormonal contraceptive use, n (%)			**0 .001**	0	15	13
Yes	6 93 (48.4)	8 15 (54.5)		1–2	40	41
No	7 40 (51.6)	6 80 (45.5)		3+	39	45
Age at menarche, mean ± SD	1 3.7 ± 2.3	1 3.6 ± 2.3	0 .27	Estrogen receptor status, n (%)		
Parity, n (%)			**0 .001**	Positive 1	520 (50)	
Nulliparous	1 74 (11.1)	1 88 (11.1)		Negative 9	88 (33)	
1–2	5 20 (33.3)	5 86 (34.7)		Progesterone receptor status, n (%)		
3–4	4 38 (28.0)	5 49 (32.5)		Positive 1	239 (41)	
5+	4 31 (27.6)	3 66 (21.7)		Negative 1	144 (38)	
Age at first live birth, mean ± SD	2 2.3 ± 5.1	2 2.1 ± 5.2	0 .33	Tumor stage, n (%)[c]		
Estrogen receptor status, n (%)				DCIS 7	5 (2)	
Positive	4 03 (51.9)			Localized 1	477 (49)	
Negative	3 74 (48.1)			Advanced 9	65 (32)	
Progesterone receptor status, n (%)				Tumor grade, n (%)		
Positive	3 15 (41.8)			Well/moderate 1	072 (36)	
Negative	4 39 (58.2)			Poor/undifferentiated 1	109 (37)	
HER2 receptor status, n (%)						

	Stage 1: ROOT Consortium		-	Stage 2: AABC Consortium	
	Case	**Control**	***P*-value[a]**	**Case**	**Control**
	N = 1,657	**N = 2,029**		**N = 3,016**	**N = 2,745**
Positive	7 1 (19.9)				
Negative	2 86 (80.1)				
Tumor stage, n (%)[c]					
I	1 39 (19.9)				
IIA	1 24 (17.7)				
IIB	1 12 (16.0)				
IIIA	8 1 (11.6)				
IIIB	8 2 (11.7)				
IV	1 62 (23.1)				
Tumor grade, n (%)					
1	7 3 (9.9)				
2	2 74 (37.1)				
3	3 92 (53.0)				

Notes: DCIS: ductal carcinoma in-situ.

Some variables in the table have missing data. Percentages were calculated out of participants who had data. Bold values indicate statistical signficance at $P<0.05$

[a] *P*-values were calculated using χ^2 test for categorical variables and student's t-test for continuous variables. All *P*-values are two sided.

[b] Among parous women

[c] Regional/Metastatic based on SEER and AJCC coding.

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Table 2

SNPs in miRNA biogenesis pathway genes and overall breast cancer risk

SNPs (rs#)	Biogenesis Gene	Cytogenetic locus	Alleles[a]	Imputation Score[b]	Functional Annotation	MAF (%)		Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						Case	Control	OR (95% CI)[c]	*p*[d]	OR (95% CI)[c]	*p*[d]	OR_{meta} (95% CI)[c]	*p*[e]
rs3738030	*ADAR*	1q21.3	A/C	0.97	Intronic	5.96	4.28	1.41 (1.13, 1.75)	0.002	1.13 (0.96, 1.33)	0.136	1.22 (1.07, 1.40)	0.002
rs11423272	*AGO1*	1p34.3	T/A	0.90	Intronic	3.60	4.57	0.72 (0.56, 0.92)	0.009	0.87 (0.65, 1.16)	0.335	0.78 (0.64, 0.94)	0.009
rs2977458	*AGO2*	8q24.3	G/T	0.78	Intronic	24.93	27.60	0.84 (0.74, 0.94)	0.004	0.96 (0.87, 1.07)	0.484	0.91 (0.84, 0.98)	0.015
rs10144436	*DICER1*	14q32.13	C/A	Genotyped	3'-UTR	10.29	11.71	0.86 (0.74, 1.00)	0.047	0.93 (0.81, 1.06)	0.286	0.90 (0.81, 0.99)	0.034
rs7839359 1	*DROSHA*	5p13.3	G/A	Genotyped	Intronic	1.72	2.39	**0.69 (0.50, 0.96)**	**0.027**	**0.70 (0.49, 1.00)**	**0.047**	**0.69 (0.55, 0.88)**	**0.003**
rs18515731	*KHSRP*	19p13.3	G/A	0.94	Intronic	6.08	5.10	1.24 (1.01, 1.54)	0.043	1.11 (0.93, 1.33)	0.254	1.16 (1.02, 1.33)	0.030
rs523736	*ESR1*	6q25.1	G/A	0.94	Intronic	28.51	26.59	**0.88 (0.79, 0.99)**	**0.027**	**0.88 (0.81, 0.96)**	**0.006**	**0.88 (0.82, 0.95)**	**3.99E-04**
rs5994340 4	*RAD52*	12p13.33	T/G	0.97	Intronic	33.04	30.60	1.12 (1.01, 1.24)	0.030	1.05 (0.97, 1.14)	0.197	1.08 (1.01, 1.15)	0.019
rs11598748 5	*SMAD2*	18q21.1	C/T	Genotyped	Intronic	2.44	3.38	0.69 (0.53, 0.91)	0.009	0.83 (0.64, 1.07)	0.147	0.76 (0.63, 0.92)	0.005

SNPs (rs#)	Biogenesis Gene	Cytogenetic locus	Alleles[a]	Imputation Score[b]	Functional Annotation	MAF (%)		Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						Case	Control	OR (95% CI)[c]	P[d]	OR (95% CI)[c]	P[d]	OR_{meta} (95% CI)[c]	P[e]
rs5805668 0	*SMAD3*	15q22.33	G/C	0.95	Intronic	6.72	8.25	0.78 (0.65, 0.94)	0.009	0.85 (0.72, 1.00)	0.054	0.82 (0.73, 0.93)	0.002
rs1291253 4	*SMAD6*	15q22.31	G/A	0.95	Intronic	20.15	17.96	1.16 (1.03, 1.31)	0.018	1.09 (0.99, 1.21)	0.080	1.12 (1.04, 1.21)	0.004
rs1694257 3	*SRSF1*	17q22	T/C	Genotyped	3'-UTR	2.26	3.28	0.73 (0.55, 0.97)	0.031	0.83 (0.67, 1.03)	0.097	0.80 (0.67, 0.94)	0.009
rs1338127 0	*SMAD7*	18q21.1	A/G	0.92	Intronic	12.69	14.44	0.82 (0.72, 0.95)	0.007	0.90 (0.80, 1.02)	0.109	0.87 (0.79, 0.95)	0.003
rs11410150 2	*ZCCHC11*	1p32.3	G/C	1.00	Intronic	4.12	2.87	**1.35 (1.05, 1.74)**	**0.021**	**1.30 (1.00, 1.68)**	**0.046**	**1.33 (1.11, 1.59)**	**0.002**

Notes: MAF: minor allele frequency; OR: odds ratio; OR_{meta}: pooled odds ratio; CI: confidence interval

[a] Effect allele vs. comparison allele

[b] Indicates if the SNP is genotyped or the imputation score

[c] Adjusted for age, site of study, and principal components in unconditional logistic regression models

[d] *P* value for trend test from additive models of effect

[e] *P* value was calculated from additive models of effect after weighting the for the size of each study

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Table 3

SNPs in miRNA and overall breast cancer risk

SNPs (rs#)	miRNA	Chromosome	Alleles[a]	Imputation Score[b]	Functional Annotation	MAF (%)		Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						Case	Control	OR (95% CI)[b]	*p*[d]	R (95% CI)[b]	*p*[d]	OR_{meta} (95% CI)[b]	*p*[e]
rs13840818 7	miR-6742-3p	1	C/T	0.97	Intronic (*TRIM11*)	1.87	1.28	1.53 (1.03, 2.26)	0.034	1.21 (0.89, 1.64)	0.226	1.32 (1.04, 1.68)	0.024
rs11185152 3	mir-1285-2	2	C/T	0.99		3.17	2.11	1.59 (1.18, 2.13)	0.002	1.03 (0.79, 1.36)	0.814	1.26 (1.03, 1.53)	0.026
rs11358083 3	mir-1285-2	2	C/A	0.99		3.19	2.12	1.60 (1.19, 2.15)	0.002	1.03 (0.78, 1.35)	0.840	1.26 (1.03, 1.54)	0.026
rs11615973 2	mir-6826	3	C/T	0.99	Intronic (*COPG1*)	2.49	3.24	**0.74 (0.55, 0.98)**	**0.033**	**0.75 (0.60, 0.93)**	**0.010**	**0.74 (0.63, 0.89)**	**0.001**
rs6787734	mir-3135a	3	C/T	1.00	Intronic	42.60	46.81	1.18 (1.07, 1.29)	0.001	0.99 (0.92, 1.07)	0.863	1.06 (1.00, 1.13)	0.044
rs11262814 8	miR-4802-5p	4	T/C	0.94	Intronic	1.82	2.68	0.63 (0.46, 0.87)	0.005	0.85 (0.65, 1.12)	0.244	0.75 (0.61, 0.92)	0.007
rs11576916 9	mir-4457	5	C/T	0.99		4.29	3.19	1.32 (1.03, 1.69)	0.031	1.20 (0.98, 1.47)	0.083	1.24 (1.06, 1.46)	0.007
rs77966622	mir-5706	5	G/A	0.99	Intronic	14.72	12.64	1.18 (1.03, 1.36)	0.016	1.09 (0.96, 1.23)	0.171	1.13 (1.03, 1.24)	0.009

SNPs (rs#)	miRNA	Chromosome	Alleles[a]	Imputation Score[b]	Functional Annotation	MAF (%)		Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						Case	Control	OR (95% CI)[b]	P[d]	R (95% CI)[b]	P[d]	OR_{meta} (95% CI)[b]	P[e]
rs77674981	miR-4641	6	G/A	0.95	Intronic	5.65	6.54	0.81 (0.66, 0.99)	0.044	0.87 (0.72, 1.04)	0.133	0.84 (0.74, 0.97)	0.014
rs67042258	mir-6128	11	G/A	1.00		19.83	21.93	0.88 (0.78, 0.98)	0.026	0.95 (0.87, 1.04)	0.268	0.92 (0.86, 0.99)	0.024
rs75258105	mir-5700	12	G/T	0.92		1.97	1.44	1.80 (1.21, 2.67)	0.004	1.24 (0.96, 1.60)	0.104	1.38 (1.12, 1.72)	0.003
rs11415353 0	mir-4708	14	C/T	0.99		4.21	3.47	1.29 (1.01, 1.66)	0.042	1.15 (0.94, 1.42)	0.176	1.21 (1.03, 1.42)	0.019
rs73410309	mir-4739	17	G/C	0.90		45.73	42.33	1.11 (1.01, 1.23)	0.039	1.07 (0.97, 1.17)	0.163	1.09 (1.02, 1.16)	0.015
rs11404107 1	mir-8074	19	A/G	1.00		2.02	1.26	1.89 (1.29, 2.77)	0.001	0.96 (0.65, 1.42)	0.842	1.36 (1.03, 1.79)	0.029

Notes: MAF: minor allele frequency; OR: odds ratio; OR_{meta}: pooled odds ratio; CI: confidence interval

[a] Effect allele vs. comparison allele

[b] Indicates if the SNP is genotyped or the imputation score

[c] Adjusted for age, site of study, and principal components in unconditional logistic regression models

[d] *P* value for trend test from additive models of effect

[e] *P* value was calculated from additive models of effect after weighting the for the size of each study

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Table 4

Distributions of selected SNPs in miRNA and biogenesis genes and breast cancer risk by estrogen receptor status

SNPs (rs#)	miRNA/Biogenesis Gene	Chromosome/ Cytogenetic Locus	Alleles[a]	Functional Annotation	MAF among controls (%)	Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						OR (95% CI)	*p*[c]	OR (95% CI)[b]	*p*[c]	OR_{meta} (95% CI)[b]	*p*[d]
ER−											
rs18464736565	mir-3129		G/2A	Intronic	3.01	0.56 (0.32, 0.98)	0.044	0.77 (0.50, 1.19)	0.234	0.68 (0.49, 0.96)	0.029
rs11262814848	miR-4802-5p		T/4C	Intronic	2.79	0.51 (0.29, 0.88)	0.016	0.70 (0.46, 1.07)	0.096	0.62 (0.44, 0.87)	0.005
rs7381453 8	mir-4638		T/5G		3.92	0.60 (0.36, 0.99)	0.045	0.75 (0.49, 1.13)	0.170	0.68 (0.49, 0.94)	0.020
rs7767498 1	miR-4641		G/6A	Intronic	6.02	0.65 (0.46, 0.92)	0.014	0.84 (0.64, 1.09)	0.193	0.76 (0.62, 0.94)	0.011
rs7366530 3	mir-7150		T/9C	Intronic (*DENND1A*)	18.69	0.73 (0.59, 0.92)	0.006	0.96 (0.82, 1.12)	0.564	0.88 (0.77, 1.00)	0.042
rs7533047 4	mir-323b	14	C/T		9.30	1.53 (1.13, 2.08)	0.006	1.18 (0.95, 1.46)	0.135	1.29 (1.08, 1.54)	0.005
rs7399122 0	mir-4725	17	A/G		13.95	**1.33 (1.02, 1.74)**	**0.038**	**1.24 (1.03, 1.50)**	**0.023**	**1.27 (1.09, 1.48)**	**0.002**
rs595055	*AGO1*	1p34.3	T/C	Intronic	30.29	1.24 (1.04, 1.49)	0.019	1.09 (0.96, 1.22)	0.175	1.13 (1.02, 1.25)	0.015

SNPs (rs#)	miRNA/Biogenesis Gene	Chromosome/ Cytogenetic Locus	Alleles[a]	Functional Annotation	MAF among controls (%)	Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						OR (95% CI)	p[c]	OR (95% CI)[b]	p[c]	OR_{meta} (95% CI)[b]	p[d]
rs297458	*AGO2*	8 q24.3	G/T	Intronic	27.60	0.80 (0.65, 0.98)	0.036	0.93 (0.80, 1.08)	0.352	0.88 (0.78, 1.00)	0.046
rs8019464	*DDX20*	1 p13.2	C/T	Intronic	4.91	0.60 (0.40, 0.89)	0.011	0.91 (0.68, 1.23)	0.555	0.84 (0.73, 0.97)	0.015
rs6725432	*DICER1*	14q32.13	G/A	Intronic	3.81	0.74 (0.58, 0.94)	0.015	0.89 (0.75, 1.07)	0.208	0.20 (1.03, 1.41)	0.020
rs1927906	*DROSHA*	5 p13.3	C/T	Intronic	0.73	0.35 (1.03, 1.78)	0.033	1.14 (0.94, 1.38)	0.172	0.88 (0.80, 0.97)	0.010
rs7775047	*ESR1*	6 q25.1	G/C	Intronic	5.38	0.83 (0.70, 0.98)	0.028	0.91 (0.81, 1.02)	0.099	0.83 (0.72, 0.95)	0.006
rs1052186	*FMR1*	X q27.3	C/A	upstream variant 2KB	4.46	0.76 (0.60, 0.95)	0.017	0.87 (0.74, 1.02)	0.093	0.78 (0.62, 1.00)	0.046
rs1419721	*GEMIN4*	17p13.3	A/G	Intronic	3.44	0.77 (0.60, 0.98)	0.033	0.92 (0.78, 1.09)	0.327	0.87 (0.76, 1.00)	0.043
rs1134635	*ILF3*	19p13.2	C/T	Intronic	41.06	0.53 (1.10, 2.14)	0.013	0.09 (0.87, 1.38)	0.452	0.22 (1.01, 1.47)	0.042
rs1851573	*KHSRP*	19p13.3	G/A	Intronic	10.55	0.56 (1.09, 2.24)	0.015	0.23 (0.97, 1.56)	0.084	0.32 (1.09, 1.61)	0.005
rs1911364	*LIN28A*	1 p36.11	T/C	3′-UTR	75.41	0.47 (0.24, 0.92)	0.027	0.62 (0.38, 1.00)	0.052	0.56 (0.38, 0.83)	0.004

SNPs (rs#)	miRNA/Biogenesis Gene	Chromosome/ Cytogenetic Locus	Alleles[a]	Functional Annotation	MAF among controls (%)	Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						OR (95% CI)	*p*[c]	OR (95% CI)[b]	*p*[c]	OR_{meta} (95% CI)[b]	*p*[d]
rs11303126 3	*NANOG*	12p13.31	C/T	Intronic	10.37	0.73 (0.55, 0.96)	0.026	0.88 (0.72, 1.07)	0.189	0.83 (0.70, 0.97)	0.019
rs14628790 3	*PAPD4*	5q14.1	A/T	3′-UTR	1.69	**0.43 (0.22, 0.84)**	**0.014**	**0.53 (0.33, 0.85)**	**0.008**	**0.49 (0.33, 0.72)**	**3.27E-04**
rs11937875	*SMAD1*	4q31.21	C/T	Intronic	27.69	0.80 (0.66, 0.96)	0.019	0.95 (0.83, 1.08)	0.398	0.89 (0.80, 1.00)	0.042
rs2864729 0	*SMAD3*	15q22.33	G/A	Intronic	12.39	0.68 (0.52, 0.90)	0.007	0.93 (0.78, 1.11)	0.420	0.85 (0.73, 0.99)	0.034
rs18796194 8	*SRRT*	7q22.1	G/A	Intronic	1.29	2.91 (1.33, 6.37)	0.008	1.28 (0.76, 2.15)	0.357	1.64 (1.06, 2.54)	0.025
rs7440457 0	*TRIM71*	3p22.3	C/G	Intronic	4.50	1.50 (1.01, 2.21)	0.044	1.15 (0.87, 1.52)	0.326	1.26 (1.00, 1.58)	0.049
rs14506523 8	*XPO5*	6p21.1	G/A	Intronic	13.54	0.78 (0.61, 0.99)	0.037	0.86 (0.72, 1.03)	0.104	0.83 (0.72, 0.96)	0.011
ER+											
rs72631820	miR-339-3p	7	T/C	Intronic	2.96	**1.57 (1.01, 2.45)**	**0.046**	**1.36 (1.04, 1.76)**	**0.022**	**1.36 (1.10, 1.69)**	**0.004**
rs56195815	mir-548aw	9	C/T		25.06	1.32 (1.09, 1.59)	0.005	1.05 (0.94, 1.17)	0.384	0.81 (0.68, 0.96)	0.017

SNPs (rs#)	miRNA/Biogenesis Gene	Chromosome/ Cytogenetic Locus	Alleles[a]	Functional Annotation	MAF among controls (%)	Stage 1 (1,657 cases, 2,029 controls) OR (95% CI)	Stage 1 (1,657 cases, 2,029 controls) p[c]	Stage 2 (3,153 cases, 2,831 controls) OR (95% CI)[b]	Stage 2 (3,153 cases, 2,831 controls) p[c]	Stage 1 + Stage 2 (4,810 cases, 4,860 controls) OR_{meta} (95% CI)[b]	Stage 1 + Stage 2 (4,810 cases, 4,860 controls) p[d]
rs12355584	mir-202	10	C/T		2.37	0.78 (0.65, 0.93)	0.005	0.94 (0.85, 1.05)	0.274	0.90 (0.82, 0.98)	0.020
rs17091403	mir-2110	10	C/T	5′-UTR	4.20	0.60 (0.41, 0.87)	0.007	0.82 (0.67, 1.02)	0.073	0.76 (0.63, 0.92)	0.004
rs11527501	mir-6762	12	A/C	Intronic (*TPCN1*)	8.62	0.69 (0.49, 0.98)	0.038	0.85 (0.69, 1.05)	0.125	1.11 (1.01, 1.22)	0.031
rs7525810	mir-5700	12	G/T		2.43	2.25 (1.18, 4.29)	0.014	1.29 (0.95, 1.75)	0.103	1.41 (1.12, 1.76)	0.003
rs11415350	mir-4708	14	C/T		3.42	1.87 (1.24, 2.84)	0.003	1.22 (0.95, 1.56)	0.121	1.32 (1.03, 1.69)	0.027
rs7356047605	mir-1292	20	C/T	Intronic	3.03	1.62 (1.01, 2.59)	0.044	1.22 (0.91, 1.63)	0.178	1.43 (1.08, 1.88)	0.012
rs17864386	*ADAR*	1q21.3	T/C	Missense	3.60	2.07 (1.38, 3.10)	4.68E-04	1.07 (0.82, 1.39)	0.642	1.30 (1.04, 1.63)	0.021
rs2944773	*AGO2*	8q24.3	A/G	Intronic	4.68	1.23 (1.04, 1.46)	0.015	1.08 (0.98, 1.20)	0.113	1.12 (1.03, 1.22)	0.009
rs5604613	*DICER1*	4q32.13	A/G	Intronic	1.63	1.40 (1.09, 1.81)	0.010	1.08 (0.93, 1.25)	0.308	1.15 (1.01, 1.31)	0.029
rs8021428	*DROSHA*	5p13.3	A/G	Intronic	1.67	1.25 (1.00, 1.57)	0.050	1.09 (0.95, 1.24)	0.232	1.13 (1.00, 1.27)	0.041

SNPs (rs#)	miRNA/Biogenesis Gene	Chromosome/ Cytogenetic Locus	Alleles[a]	Functional Annotation	MAF among controls (%)	Stage 1 (1,657 cases, 2,029 controls)		Stage 2 (3,153 cases, 2,831 controls)		Stage 1 + Stage 2 (4,810 cases, 4,860 controls)	
						OR (95% CI)	*p*[c]	OR (95% CI)[b]	*p*[c]	OR_{meta} (95% CI)[b]	*P*[d]
rs9479113	*ESR1*	6q25.1	C/T	Intronic	37.46	**0.79 (0.65, 0.96)**	**0.017**	**0.89 (0.79, 0.99)**	**0.035**	**0.86 (0.78, 0.95)**	**0.003**
rs18632894	*HNRNPA1*	12q13.13	G/A	Intronic	1.18	0.35 (0.16, 0.76)	0.008	0.74 (0.46, 1.18)	0.205	0.60 (0.40, 0.90)	0.014
rs14120962	*ILF2*	1q21.3	G/T	upstream variant 2KB	3.50	1.74 (1.08, 2.81)	0.023	1.18 (0.89, 1.57)	0.238	1.31 (1.03, 1.67)	0.030
rs14137706	*RAD52*	12p13.33	T/C	3'-UTR	1.82	2.40 (1.21, 4.77)	0.012	1.40 (0.98, 2.00)	0.061	1.57 (1.15, 2.16)	0.005
rs7973017 2	*SMAD3*	15q22.33	T/C	Intronic	7.32	**1.44 (1.06, 1.95)**	**0.020**	**1.25 (1.03, 1.52)**	**0.023**	**1.30 (1.11, 1.54)**	**0.001**
rs5617079 9	*SMAD6*	15q22.31	T/A	Intronic	10.65	0.70 (0.53, 0.91)	0.008	0.89 (0.76, 1.05)	0.172	0.84 (0.73, 0.96)	0.012
rs13943458	*SMAD7*	18q21.1	G/A	Intronic	2.42	0.56 (0.33, 0.96)	0.036	0.84 (0.60, 1.17)	0.303	0.75 (0.56, 1.00)	0.047
rs2273013	*SNIP1*	1p34.3	C/T	Synonymous	6.88	0.72 (0.53, 0.99)	0.040	0.83 (0.69, 1.00)	0.051	0.80 (0.68, 0.94)	0.006
rs7838156 3	*TRIM71*	3p22.3	G/A	Intronic	8.11	1.46 (1.08, 1.98)	0.014	1.15 (0.95, 1.40)	0.151	1.24 (1.05, 1.46)	0.011
rs11503724 0	*XPO5*	6p21.1	G/A	Intronic	5.25	0.63 (0.43, 0.92)	0.016	0.84 (0.67, 1.06)	0.148	0.78 (0.64, 0.95)	0.013

Notes: MAF: minor allele frequency; OR: odds ratio; OR_{meta}: pooled odds ratio; CI: confidence interval

[a] Effect allele vs. comparison allele

[b] Adjusted for age, site of study, and principal components in unconditional logistic regression models

[c] *P* value for trend test from additive models of effect

[d] *P* value was calculated from additive models of effect after weighting the for the size of each study