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Rare coding variants and breast cancer risk: Evaluation of susceptibility loci identified in genome-wide association studies

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Abstract

Background—To date, common genetic variants in ~70 loci have been identified for breast cancer via genome-wide association studies (GWAS). It is unknown whether rare variants in these loci are also associated with breast cancer risk.

Methods—We investigated rare missense/nonsense variants with minor allele frequency (MAF) ≤ 5% located in flanking 500 kb of each of the index SNPs in 67 GWAS loci. Included in the study were 3,472 cases and 3,595 controls from the Shanghai Breast Cancer Study. Both single marker and gene-based analyses were conducted to investigate the associations.

Results—Single marker analyses identified 38 missense variants being association with breast cancer risk at $P < 0.05$ after adjusting for the index SNP. SNP rs146217902 in the *EDEM1* gene and rs200340088 in the *EFEMP2* gene were only observed in 8 cases ($P = 0.004$ for both). SNP rs200995432 in the *EFEMP2* gene was associated with increased risk with an odds ratio (OR) of 6.2 (95% CI: 1.4–27.6, $P = 6.2\times10^{-3}$). SNP rs80358978 in the *BRCA2* gene was associated with 16.5-fold elevated risk (95% CI: 2.2–124.5, $P = 2.2\times10^{-4}$). Gene-based analyses suggested eight genes associated with breast cancer risk at $P < 0.05$, including the *EFEMP2* gene ($P = 0.002$) and the *FBXO18* gene ($P = 0.008$).

Conclusion—Our results identified association of several rare coding variants neighboring common GWAS loci with breast cancer risk. Further investigation of these rare variants and genes would help to understand the biological mechanisms underlying the associations.

Impact—Independent studies with larger sample size are warranted to clarify the relationship between these rare variants and breast cancer risk.

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Competing Financial Interests
The authors declare no competing financial interests.

Keywords

susceptibility loci; breast cancer; rare variants; GWAS

Introduction

Breast cancer is one of the most commonly-diagnosed malignancies of women in the world (1). It is well established that genetic factors play an important role in breast cancer risk (2). Over the past several years, common variants, usually with minor allele frequency (MAF) > 5%, in approximately 70 loci have been identified as breast cancer risk factors via genome-wide association studies (GWAS) (3). However, these common variants together explained only a small portion of the heritability for breast cancer.

It has been increasingly recognized that the missing heritability for breast cancer and other complex diseases may be partially explained by low-frequency variants. There are a large number of low-frequency variants in the human genome, and these rare coding variants are enriched for functional importance (4). Rare coding variants have been associated with multiple diseases, such as the *MTNR1B* gene for type 2 diabetes (5), *IFIH1* gene for type 1 diabetes (6), *APOA5, GCKR, LPL* and *APOB* genes for hypertriglyceridemia (7) and *CHEK2, ATM, BRIP1, PALB2, RAD51C, RAD51D*, and *PPM1D* genes for breast cancer (8–13). Herein, we investigated low MAF coding variants in GWAS identified loci regions for their association with breast cancer risk. Focusing on the flanking 500kb regions of 67 GWAS identified loci, we investigated low MAF nonsense/missense variants and their corresponding genes in a total of 3,472 cases and 3,595 controls from the Shanghai Breast Cancer Genetics Study.

Materials and Methods

Study populations

Study participants in the present study were drawn from four population-based studies conducted in Shanghai, the Shanghai Breast Cancer Study (SBCS), Shanghai Women's Health Study (SWHS), Shanghai Breast Cancer Survival Study (SBCSS), and the Shanghai Endometrial Cancer Study (SECS, contributed control data only). Detailed descriptions of participating studies have been published elsewhere (14–16). In brief, the SBCS is a 2-stage (SBCS-I and SBCS-II), population-based, case–control study. SBCS-I recruitment occurred between August 1996 and March 1998; SBCS-II recruitment occurred between April 2002 and February 2005. Both studies identified patients with incident primary breast cancer through the population-based Shanghai Cancer Registry and randomly selected community controls from the general population in Shanghai. The SBCSS included newly diagnosed breast cancer cases ascertained via the Shanghai Cancer Registry between April 2002 and December 2006. The SECS is a population-based, case–control study of endometrial cancer conducted between January 1997 and December 2003 using a protocol similar to the SBCS; only community controls from the SECS were included in the present study. The SWHS is a population-based prospective cohort study of women from urban communities in Shanghai who were recruited between 1996 and 2000. The cohort has been followed by a combination of record linkage and active follow-ups to identify cause-specific mortality and cancer incidence by sites. All these studies are conducted among Chinese women in Shanghai, a genetically homogenous population, using very similar protocols in data and sample collection. Genomic DNA for all included participants was extracted using commercial DNA purification kits. Study protocols were approved by the institutional review boards of all institutions involved in the study, and informed consents were obtained from all study participants.

Genotyping array

Genotype assays were done by the Asian Exomechip, an expanded Illumina HumanExome-12v1_A Beadchip. The original Exome array includes 247,870 markers focused on protein-coding regions selected from >12,000 samples with exome and genome sequencing data. The vast majority of these samples were from European ancestry populations, and ~600 Asian samples were included. Details about SNP contents and characteristics are described at Exomechip design (17). In brief, nonsynonymous variants observed three or more times in at least two studies, and splicing and stop-altering variants observed two or more times in at least two studies were selected. Additional array content includes variants associated with complex traits in previous GWAS, HLA tags, ancestry-informative markers, markers for identity-by-descent estimation and random synonymous SNPs.

To improve the coverage for the low frequency variants in Asian population, we designed the Asian Exomechip by adding additional ~60K customer content variants onto the Illumina HumanExome-12v1_A Beadchip based on additional sequencing data. Included on the chip are also top SNPs selected from GWAS for follow-up. Three sequencing datasets were used to add additional nonsense/missense variants: exome sequencing in 581 Chinese women from SBCS, exome sequencing in 496 Singapore Chinese, and Asian data in the 1000 Genomes Project. Nonsynonymous, splicing and stop-altering variants observed two or more times in any of these datasets or once in any two of the three datasets, were added (N=33,342). Additional common variants (N=28,637) were added to the chip for various GWAS follow-up and GWAS loci fine-mapping projects.

Genotyping and quality control

All samples were genotyped at the Genome Quebec Innovation Centre (Montreal, Quebec, Canada) following Illumina's protocol. On each 96-well plate, blind duplicate samples and two HapMap samples were included as quality control (QC). Genotype calling was carried out using Illumina's GenTrain version 2.0 clustering algorithm in GenomeStudio version 2011.1. Cluster boundaries were determined using study samples. After clustering, ~80,000 variants were manually reviewed and clusters were edited for 27,506 variants.

Further QC procedures were conducted using plink (18). We evaluated concordance rates for HapMap samples genotyped in our study and sequenced by the 1,000 Genomes Project (4). Principal components analyses (PCA) were conducted based on 3,200 ancestry informative markers (AIMs) on the exomechip using EIGENSTRAT (19) to identify population outliers with the 1,000 Genomes Project data as reference. We also estimated pair-wise proportion of identify-by-descent (IBD) to identify potentially genetically identical, unexpected duplicated samples or close relatives. The samples were excluded if: (i) call rate<98%, or (ii) consistence rates between the HapMap samples with 1000 Genomes data <99%, or (iii) heterozygosity outlier, or (iv) ethnic outliers, or (v) samples with close relationship, or (vi) consistence rates among duplicated samples<99%, or (vii) samples with wrong sex. The SNPs were excluded if: (i) MAF=0, or (ii) call rate < 98%, or (iii) genotyping concordance rate < 98% in QC samples, or (iv) HWE test $P<10^{-5}$, or (v) redundant SNPs, or (vi) cautions SNPs discovered by the exomechip design group (17). A total of 8,200 samples plus 192 QC samples were genotyped. The final analysis dataset included 127,267 SNPs genotyped on 3,472 breast cancer cases and 3,595 controls.

Statistical analyses

We used ANNOVAR program (20) to annotate all SNPs. We included all missense/nonsense variants located flanking 500kb of the indexed SNP of 67 GWAS loci. If a protein-coding gene was partially covered within the flanking 500kb region, all missense/nonsense

variants in the whole gene were included for analyses. For single-variant analysis, we used logistic score test adjusted for age implemented in EPACTS package (21). Further conditional analyses were conducted by adjusting the corresponding index SNP in each locus.

For gene-based analysis, we used the SKAT-O test with default parameters implemented in the EPACTS package. SKAT-O (22) encompasses burden tests and SKAT (23). Low-frequency variants of MAF ≤ 5% or MAF ≤ 1% within each gene were aggregated.

Results

Characteristics of the study population are shown in Table 1. All the known risk factors were associated with breast cancer risk in this study setting. Cases had higher educational attainment and were more likely to have a first-degree relative with breast cancer, a history of benign breast disease, be postmenopausal, and report early menarche than controls.

Single marker analyses

In the flanking regions of those 67 GWAS loci, a total of 1,272 missense/nonsense variants were included on the chip; 1,080 were rare variants with 0 < MAF ≤ 5% (Table S1). A total of 38 rare variants (0 < MAF ≤ 5%) showed an association with breast cancer risk at $P < 0.05$ after adjusted for the corresponding index SNP (Table 2). Notably, five rare variants were associated with breast cancer risk at $P < 0.01$. SNP rs146217902 in the *EDEM1* at 3p26.1 and rs200340088 in the *EFEMP2* at 11q13.1 were observed in 8 cases but not in any controls (P =0.004 for both). Another SNP rs200995432 in the *EFEMP2* gene was associated with increased breast cancer risk with an odds ratio (OR) of 6.2 (95% CI: 1.4–27.6, $P= 6.2\times10^{-3}$). SNP rs80358978 in the *BRCA2* gene, 42 kb upstream from the GWAS SNP rs11571833, was associated with 16.5-fold elevated risk (95% CI: 2.2–124.5, $P = 2.2\times10^{-4}$). A rare variant rs143563006 in the *FBXO18* gene was associated with decreased risk of breast cancer with an OR being 0.60 (95% CI: 0.41–0.88) and a *P* value of 8.2×10^{-3}.

Gene-based analyses

Collapsing variants with MAF ≤ 5% within each gene suggested eight genes associated with breast cancer at $P < 0.05$ (Table 3 and Table S2). As the majority of rare variants whose MAF was ≤ 1%, similar results were found when MAF was set to ≤ 1%. These associations did not change materially after adjusting for corresponding GWAS index SNPs. At the locus 11q13.1, two genes, *EFEMP2* and *RNASEH2C*, showed an association with breast cancer risk with $P = 0.002$ and $P = 0.04$, respectively. The *EFEMP2* gene was approximately 61.3 kb downstream from the GWAS SNP rs12575663, and the *RNASEH2C* gene was 87 kb upstream from the index SNP. At the 10p15.1, the *FBXO18* (consisting of 5 variants with MAF < 0.05) was strongly associated with breast cancer risk ($P = 8.0\times10^{-3}$). The other five genes showing associations were *KLHL26, OR2A12, TGFBR2, TRIP13*, and *VTI1A*.

Discussion

In the present study, we investigated associations of 1,080 missense/nonsense variants with a MAF ≤ 5% in 337 genes at 67 GWAS loci among 3,472 Chinese breast cancer cases and 3,595 controls. Single marker analyses showed an association for 38 variants at $P < 0.05$. In particular, five variants were associated with breast cancer risk at $P < 0.01$, including rs200340088 and rs200995432 in *EFEMP2*, rs146217902 in *EDEM1*, rs143563006 in *FBXO18*, and rs80358978 in *BRCA2*. Gene-based analyses showed an association at $P < 0.01$ for *EFEMP2* and *FBXO18* genes and at $P < 0.05$ for six genes, including *RNASEH2C, KLHL26, OR2A12, TGFBR2, TRIP13*, and *VTI1A*.

The most significant association was observed for a missense variant, rs80358978 (Gly2508Ser), in the *BRCA2* gene. It was 42 kb upstream from the GWAS SNP rs11571833. This variant was observed in 16 heterozygous breast cancer cases and only one control participant. This variant was not present among the 1,092 individuals included in the 1,000 Genomes Project or the 6,400 individuals of European or African ancestry included in the NHLBI Exome Sequencing Project (24). This variant was found in four Asian breast cancer women in the Breast Cancer Information Core (25). Though the clinical importance of this variant was unknown, it may be potentially functional and it is predicted to be "probably damaging" based on its Polyphen-2 score (0.999) and "deleterious" based on its SIFT score (0).

In addition, of the 38 rare variants causing missense mutations, the predominantly single amino acid change is from a basic or acidic amino acid to a neutral amino acid, such is the case for *CCDC88C*, *MAP3K1*, and *SRRM5* genes are predicted to be deleterious based on the SIFT score (Table 2). It further suggests, to the some extent, that these rare missense mutations would affect the protein's topological structure and physicochemical properties.

For gene-based analysis, our results indicated that the significant association with breast cancer risk is driven by one single variant in each gene. The reason is greatly related to our focuses on the missense/nonsense variants with low-frequency. Generally speaking, the consequence of missense mutations has direct impacts on protein structure and function. Thus, it is more likely to undergo purifying selection (26, 27), making the probability of two or more rare missense mutations happening in the same gene quite low.

The most significant result from gene-based analyses is for the association observed with the *EFEMP2* gene, encoding a protein containing four EGF2 domains and six calcium-binding EGF2 domains. This gene is necessary for elastic fiber formation and connective tissue development (28). Several studies indicated that the expression level of the *EFEMP2* gene, even at an early cancer stage, was increased in cancer tissues of the colorectal and endometrial cancer patients (29–31). *RNASEH2C*, another gene located at the 11q13.1 locus, also showed a significant association in this study. This gene encodes one of Ribonuclease H2 (RNase H2) subunits, a major nuclear enzyme involved in the degradation of RNA/DNA hybrids and removal of ribonucleotides misincorporated in genomic DNA to maintain genomic integrity. Mutations in each of the three RNase H2 genes have been implicated in a human auto-inflammatory disorder, Aicardi-Goutières Syndrome (AGS) (32, 33). Crystal structure of RNase H2 complex indicated residues in the C-terminal kinked helix (RNASEH2C:143–160) contact both RNASEH2A and RNASEH2B (34), suggesting the detected variant (R145L) in the *RNASEH2C* gene may influence the complex formation of RNase H2.

FBXO18 (also called FBH1 or FBX18) is a member of the UvrD family of DNA helicases (35, 36). Its helicase activity induces DNA double-strand breakage and activation of ATM and DNA-PK and phosphorylation of RPA2 and p53 (37). The *ATM* and *p53* genes are two of the most well-established breast cancer susceptibility genes. A previous study has revealed a connection between rare missense variants in the *ATM* gene and breast cancer risk (11). Here we provide evidence that rare variants in the FBXO18 gene may also contribute to the risk of breast cancer.

It has been well established that TGF-β pathway plays a critical role in the development and progression of a large number of human cancers, including breast cancer (38–40). TGF-β1 is the most abundant form of TGF-β and regulates cellular processes by binding to TGFBR2. Therefore, defective expression of TGFBR2 may play a significant role in carcinogenesis. Our previous evaluation of the associations of genetic variants in the TGF-β signaling

pathway with breast cancer risk found that one common SNP (rs1078985) in the *TGFBR2* was associated with breast cancer risk (41). The gene-based results in this study provide further evidence that the *TGFBR2* gene is significantly associated with breast cancer risk.

In the present study, we identified multiple rare coding variants associated with breast cancer in GWA-identified loci. However, after adjusting multiple comparisons, some of them became insignificant. The statistical power in the present study is limited for rare variants, even though over 6,000 cases and controls were included. Independent studies with larger sample size are warranted to clarify the relationship between this rare variants and breast cancer risk.

In conclusion, we identified associations of additional genes/variants flanking the known susceptibility loci with breast cancer risk. These findings may provide new insights into the etiology of breast cancer as well as future potential therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Distribution of demographic characteristics and known breast cancer risk factors for cases and controls included in the study

Category	Cases (N=3,472)	Controls (N=3,595)	P value
Demographic Factors[a]			
Age (yr ± SD)	53.2 ± 10.0	53.0 ± 9.3	0.38
Education ≥ high school (%)	55.9	40	< 0.01
Reproductive Risk Factors			
Age at menarche (yr)	14.4± 1.7	14.9 ± 1.8	< 0.01
Postmenopausal (%)[b]	49.8	53.1	< 0.01
Age at menopause[b]	49.0 ± 4.2	48.8 ± 3.9	0.29
Age at first live birth (yr)[c]	26.9 ± 3.8	25.6 ± 4.1	< 0.01
Other Risk Factors			
First-degree relative with breast cancer (%)	5.3	2.2	< 0.01
Body mass index (BMI)	24.1 ± 3.5	23.9 ± 3.4	0.05
Body mass index (BMI)[b]	24.7 ± 3.7	24.4 ± 3.5	0.01

[a] Unless otherwise specified, mean ± sd are presented;

[b] Among postmenopausal women;

[c] Among parous women.

Table 2

SNPs associated with breast cancer risk at $P < 0.05$ in single marker analyses

Chromosome	Position	rs#	Alleles	Polyphen-2 score	SIFT score	Amino acid change	Gene	AF.case	AF.contrl	P-value	$P_{conditional}$	OR	Low 95% CI	Up 95% CI
3	5257572	rs146217902	A/G	0.096	0.12	Arg->Gln	EDEM1	0.0012	0.0000	4.09E-03	4.1E-03	1.67E+09	0.00	inf
5	917213	rs200263887	G/A	0	0	Ile->Val	TRIP13	0.0053	0.0028	0.02	1.7E-02	1.92	1.11	3.32
5	56155651	rs201579608	A/G	0.997	0	Arg->Gln	MAP3K1	0.0001	0.0013	0.01	1.3E-02	0.11	0.01	0.91
5	57750547	NA	G/A	0.088	0.1	Tyr->His	PLK2	0.0013	0.0029	0.04	3.4E-02	0.44	0.20	0.97
6	151914357	rs34563373	A/G	0.073	0.1	Arg->His	C6orf97	0.0006	0.0000	0.04	4.1E-02	1.67E+09	0.00	inf
6	152560708	rs190673256	T/C	0.001	0.78	Arg->Gln	SYNE1	0.0023	0.0008	0.03	3.7E-02	2.76	1.08	7.07
6	152603091	NA	T/C	0.006	0.11	Glu->Lys	SYNE1	0.0000	0.0006	0.05	4.9E-02	0.00	0.00	inf
10	5937039	rs143563006	C/G	0.262714	0	Val->Leu	FBXO18	0.0061	0.0100	0.01	8.1E-03	0.60	0.41	0.88
10	64005772	rs3765004	G/T	0.133	0.08	Lys->Thr	RTKN2	0.0207	0.0163	0.05	4.6E-02	1.28	1.00	1.64
10	114286892	rs184549091	C/T	0.973	0.01	Leu->Pro	VTI1A	0.0049	0.0076	0.04	3.6E-02	0.63	0.41	0.98
10	123843210	rs141547215	A/G	0.995	0	Gly->Arg	TACC2	0.0260	0.0324	0.02	1.9E-02	0.80	0.65	0.97
11	1491556	NA	T/C	na	0.2	Arg->Gln	AC091196	0.0010	0.0026	0.02	2.4E-02	0.38	0.16	0.90
11	65408708	rs3741379	T/G	0.001	0.88	Ala->Ser	SIPA1	0.0266	0.0214	0.05	4.2E-02	1.25	1.00	1.54
11	65487550	NA	A/C	0.991	0.14	Arg->Leu	RNASEH2C	0.0001	0.0010	0.04	4.0E-02	0.15	0.02	1.21
11	65629960	rs2298447	T/C	0.156	0.03	Leu->Phe	MUS81	0.0013	0.0029	0.04	3.8E-02	0.45	0.20	0.97
11	65630970	rs143145862	A/G	0.092	0.04	Arg->Gln	MUS81	0.0004	0.0018	0.02	1.5E-02	0.24	0.07	0.84
11	65636047	rs200340088	T/C	0.993	0.02	Glu->Lys	EFEMP2	0.0012	0.0000	4.07E-03	4.0E-03	1.67E+09	0.00	inf
11	65639801	rs200995432	T/G	0.966	0	Pro->Thr	EFEMP2	0.0017	0.0003	0.01	6.2E-03	6.18	1.38	27.62
12	95603070	NA	A/T	0	0.19	Ser->Cys	FGD6	0.0000	0.0006	0.05	4.9E-02	0.00	0.00	inf
12	96288809	rs140348782	G/A	0.924	0.15	Ser->Pro	CCDC38	0.0016	0.0032	0.05	4.8E-02	0.49	0.24	1.01
12	96379914	rs181887143	T/C	0.346	0.08	Arg->His	HAL	0.0009	0.0024	0.03	2.5E-02	0.36	0.14	0.92
13	32907359	rs80358457	C/A	0.561	0.06	Thr->Pro	BRCA2	0.0006	0.0019	0.02	2.3E-02	0.30	0.10	0.90
13	32930651	rs80358978	A/G	0.999	0	Gly->Ser	BRCA2	0.0023	0.0001	2.18E-04	2.2E-04	16.51	2.19	124.50
14	37132495	NA	G/A	0.985	0.28	Asn->Ser	PAX9	0.0000	0.0006	0.05	4.6E-02	0.00	0.00	inf
14	91739003	rs182423495	A/G	na	0.06	Pro->Leu	CCDC88C	0.0006	0.0018	0.03	3.5E-02	0.32	0.10	0.97
14	91763720	rs142539336	A/G	na	0	Arg->Cys	CCDC88C	0.0062	0.0036	0.03	3.4E-02	1.70	1.05	2.76
14	91780306	NA	G/C	na	0	Arg->Ser	CCDC88C	0.0035	0.0014	0.02	1.4E-02	2.38	1.15	4.92

Chromosome	Position	rs#	Alleles	Polyphen-2 score	SIFT score	Amino acid change	Gene	AF.case	AF.contrl	P-value	$P_{conditional}$	OR	Low 95% CI	Up 95% CI
16	53358202	NA	A/G	na	0	Gly->Ser	CHD9	0.0003	0.0013	0.04	4.4E-02	0.23	0.05	1.07
16	53682940	rs142349647	T/C	0.242	0.03	Arg->Gln	RPGRIP1L	0.0001	0.0013	0.01	1.4E-02	0.12	0.01	0.91
16	81075049	rs192089732	T/C	0.992	0	Pro->Leu	ATMIN	0.0006	0.0000	0.04	4.1E-02	1.68E+09	0.00	inf
19	17317529	rs186514880	A/G	na	0.13	Val->Ile	MYO9B	0.0014	0.0031	0.04	4.3E-02	0.47	0.22	0.99
19	17373389	NA	T/C	0.006	1	Arg->Gln	USHBP1	0.0007	0.0000	0.02	2.2E-02	1.69E+09	0.00	inf
19	17831801	NA	A/G	0.003	0.59	Val->Ile	MAP1S	0.0017	0.0004	0.02	1.6E-02	4.17	1.17	14.77
19	18546203	rs145899718	G/C	0.003	0.07	Glu->Gln	ISYNA1	0.0012	0.0026	0.04	3.7E-02	0.43	0.19	0.99
19	18779799	rs117020142	T/A	0.988	0.57	Tyr->Phe	KLHL26	0.0006	0.0018	0.03	2.7E-02	0.31	0.10	0.96
19	44118014	NA	T/C	na	0.07	Arg->Cys	SRRM5	0.0017	0.0006	0.04	3.8E-02	3.12	1.01	9.69
19	44470165	rs141927408	T/C	0.416	0.01	Arg->Cys	ZNF221	0.0000	0.0007	0.03	2.8E-02	0.00	0.00	inf
22	40417776	rs141861984	A/G	0.058	0.37	Arg->Gln	FAM83F	0.0000	0.0006	0.05	4.8E-02	0.00	0.00	inf

Table 3

Genes associated with breast cancer risk at $P < 0.05$ in gene-based analyses

Genes	Number of variants	Variants	Distance to index SNP (bp)	*P*	$P_{conditional}$*
EFEMP2	5	p.Pro9Thr, p.Thr312Ala, p.His292Tyr, p.Glu261Lys, p.Ile259Val,	61271	0.0021	0.0020
FBXO18	5	p.Val15Leu, p.His33Arg, p.Pro88Leu, p.Val552Ile, p.Ile981Phe	50305	0.0082	0.0082
KLHL26	3	p.Val109Leu, p.Arg115Gln, p.Tyr531Phe	207391	0.0303	0.0260
OR2A12	6	p.Tyr34His, p.Thr76Ala, p.Met181Thr, p.Val183Ile, p.Ala223Thr, p.Ser264Asn	−282629	0.0498	NA
RNASEH2C	1	p.Arg145Leu	−86985	0.0389	0.0398
GFBR2	8	p.Ser46Arg, p.Val191Ile, p.Arg193Trp, p.Thr206Met, p.Asp247Val, p.Arg313Gln, p.Thr315Met p.Arg403His,	−18225	0.0447	NA
TRIP13	4	p.Val82Ile, p.Ile99Val, p.Gly173Glu, p.Ile196Val	−384737	0.0238	0.0237
VTI1A	2	p.Asn40Asp, p.Leu104Pro	−475838	0.0429	0.0424

*Conditional to the corresponding GWAS index SNP.