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Genome-wide association and pathway analysis of left ventricular function after anthracycline exposure in adults

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Abstract

Background—Anthracyclines are important chemotherapeutic agents, but their use is limited by cardiotoxicity. Candidate gene and genome-wide studies have identified putative risk loci for overt cardiotoxicity and heart failure, but there has been no comprehensive assessment of genomic variation influencing the intermediate phenotype of anthracycline-related changes in left ventricular (LV) function. The purpose of this study was to identify genetic factors influencing changes in LV function after anthracycline chemotherapy.

Methods—We conducted a genome-wide association study (GWAS) of change in LV function after anthracycline exposure in 385 subjects identified from BioVU, a resource linking DNA samples to de-identified electronic medical record data. Variants with p-values $<1 \times 10^{-5}$ were

independently tested for replication in a cohort of 181 anthracycline-exposed subjects from a prospective clinical trial. Pathway analysis was performed to assess combined effects of multiple genetic variants.

Results—Both cohorts were middle-aged adults of predominantly European descent. Among 11 candidate loci identified in discovery GWAS, one single nucleotide polymorphism (SNP) near PR Domain Containing 2, With ZNF Domain (*PRDM2*), rs7542939, had a combined p-value of 6.5×10^{-7} in meta-analysis. Eighteen Kyoto Encyclopedia of Gene and Genomes (KEGG) pathways showed strong enrichment for variants associated with the primary outcome. Identified pathways related to DNA repair, cellular metabolism, and cardiac remodeling.

Conclusions—Using genome-wide association we identified a novel candidate susceptibility locus near *PRDM2*. Variation in genes belonging to pathways related to DNA repair, metabolism, and cardiac remodeling may influence changes in LV function after anthracycline exposure.

Keywords

Anthracycline; cardiotoxicity; genome-wide association study; pathway analysis

INTRODUCTION

Anthracyclines are important chemotherapeutic agents, but their use is limited by cardiotoxicity that characteristically manifests as progressive systolic dysfunction.¹ Many mechanisms for cardiotoxicity have been proposed, including production of reactive oxygen species² and alterations in anthracycline metabolism and transport.^{3,4} More recent data suggest anthracycline-mediated inhibition of topoisomerases, in particular, topoisomerase II β and mitochondrial topoisomerase I, are central to cardiotoxicity, producing double strand DNA breaks, activation of apoptotic pathways, and ultimately impaired mitochondrial function and decreased mitochondrial biogenesis.^{5,6} Anthracyclines also impair sarcomere maintenance, disrupt cell survival signaling, and impair cellular fatty acid metabolism.^{2,7}

Anthracycline cardiotoxicity is dose-dependent at a population level, but substantial inter-individual variability makes it difficult to predict which patients will be affected even when cumulative exposure and clinical parameters are known, indicating influences beyond dose and known risk factors play a substantial role in susceptibility. Several genetic variants influencing development of overt cardiotoxicity and heart failure have been implicated by candidate gene analyses and genome wide association studies (GWAS), primarily in childhood cancers.^{8–13} Nevertheless, known genetic factors do not explain all the observed variability in susceptibility.

Common definitions of anthracycline-induced cardiotoxicity require a significant decrease in cardiac function or clinical evidence of cardiac insufficiency.^{11,13} However, dichotomous classifications have limitations, as more subtle effects may be meaningful, and even severe cardiac dysfunction can be asymptomatic. Indeed, continuous measures of left ventricular (LV) structure and function in the general population are both heritable and associated with important cardiovascular outcomes.¹⁴ The quantitative change in LV function is an intermediate phenotype of anthracycline-induced cardiotoxicity, and, importantly, early

reductions in this metric are associated with later development of overt cardiotoxicity.¹⁵ However, the genomic influences on anthracycline-induced alterations in cardiac function, particularly in adults, have not been studied in a genome-wide, unbiased fashion. Understanding the genomics of this intermediate phenotype may help characterize quantitative, mechanistic aspects of cardiotoxicity and form the basis for identification of at-risk individuals and implementation of measures to minimize or prevent toxicity.

METHODS

Study Populations

Discovery GWAS—The discovery dataset was generated from BioVU, a Vanderbilt resource linking DNA samples to de-identified electronic medical records (EMR). The development, implementation, and use of BioVU as a platform for discovery has been previously described.^{16,17} Briefly, BioVU contains over 215,000 DNA samples extracted from blood leftover after routine clinical care at Vanderbilt University Medical Center.

Subjects with anthracycline exposure were identified using key word searches and automated queries of procedure codes (see Text, Supplemental Digital Content 1). Assessments of left ventricular ejection fraction (LVEF) were extracted retrospectively from clinical reports of imaging modalities including transthoracic echocardiography, radionuclide ventriculography, single-photon emission computed tomography, cardiac computed tomography, and cardiac magnetic resonance imaging. Imaging was performed at the discretion of providers as clinically indicated. Cumulative anthracycline dose, pre- and post-chemotherapy LVEF values, and pre-chemotherapy clinical covariates were extracted by manual review of the EMR. Subjects were excluded if they were not exposed to anthracycline, had no measures of LVEF after chemotherapy initiation, or had insufficient EMR documentation of covariates. LVEF measurements acquired during acute illness (e.g., sepsis) were excluded. Anthracycline exposures considered were doxorubicin, doxorubicin liposomal, daunorubicin, idarubicin, and epirubicin. Doses were converted to doxorubicin equivalents (see Text, Supplemental Digital Content 1)¹⁸, indexed to body surface area, and expressed as doxorubicin equivalents mg/m². In a minority of cases, baseline LVEF and cumulative anthracycline dose were not documented in subjects with otherwise complete information. These data were imputed using multiple imputation implemented in SPSS statistical software version 20 (IBM Corp., Armonk, NY).

Replication—The replication dataset was drawn from subjects enrolled in the “Patients undergoing anthracycline-based chemotherapy to assess the effectiveness of using biomarkers to detect and identify cardiotoxicity and describe treatment” (PREDICT) study (*ClinicalTrials.gov* number NCT01311843). PREDICT is a multicenter study with coordinating sites at MD Anderson Cancer Center, Houston, TX and Vanderbilt University Medical Center, Nashville, TN. The study protocol was approved by the Institutional Review Boards of both institutions. At enrollment, subjects were age ≥ 18 years, had an LVEF ≥ 50%, and were starting a new course of chemotherapy that included an anthracycline. Prior chemotherapy exposure, including anthracyclines, was not an exclusion criteria. Subjects underwent serial assessment of cardiac function with transthoracic echocardiography at

baseline, 6 and 12 months after initiation of chemotherapy, or if indicated clinically. To ensure no subjects were included in both discovery and replication populations, only subjects from outside Vanderbilt were considered for inclusion.

Genotyping and Imputation

BioVU subjects were genotyped in the Vanderbilt Technologies for Advanced Genomics (VANTAGE) Core using the Illumina Omni 1-Quad platform. Pre-imputation quality control filters were applied at the sample level (exclusion criteria: call rate <98%, sex discrepancy, relatedness, duplicate discordance, and HapMap discordance) and single nucleotide polymorphism (SNP) level (exclusion criteria: call rate <98%, replicate discordance, and Mendelian errors). Genotypes were imputed to 1000 Genomes Phase 1 version 3 (April 19, 2012) using IMPUTE2 software¹⁹ and reference panels from all 1000 Genomes populations, and alleles with confidence >0.9 were retained (see Text, Supplemental Digital Content 1). Candidate SNPs with p-values <1×10⁻⁵ in discovery were genotyped in the replication cohort using the Sequenom MassArray genotyping system. Quality control parameters included sample genotyping efficiency >90% and SNP genotyping efficiency >95%.

Statistical Analysis

The primary outcome was maximal change in LVEF from pre-chemotherapy measurement. This intermediate phenotype was chosen because changes in this parameter are used in clinical practice to define and detect anthracycline cardiotoxicity. If LVEF increased from baseline, the maximum positive change and cumulative chemotherapy dose up to that time point was recorded. If LVEF decreased from baseline the maximum negative change and cumulative chemotherapy to that time point was recorded. If both positive and negative changes from baseline LVEF were present, the maximum negative change and cumulative chemotherapy to that time point were recorded.

In discovery GWAS, multiple linear regression, implemented in PLINK, was used to test for association between SNPs and change in LVEF adjusting for age, gender, baseline LVEF, cumulative anthracycline dose at time of outcome LVEF, follow-up time from start of anthracycline, number of cardiac risk factors (hypertension, diabetes, coronary artery disease, dyslipidemia, smoking), and concomitant trastuzumab. All subjects were included regardless of ancestry, and principal components one and two (which explained 28% of the variance in the genetic data) were included to adjust for possible population stratification (see Figures, Supplemental Digital Content 3 and 4). Only SNPs with minor allele frequency (MAF) ≥ 0.05 were included in analyses. *A priori* genome-wide significance was defined as 5×10⁻⁸. Lead SNPs from loci with p-values <1×10⁻⁵ in the discovery GWAS were tested for association in the replication cohort. Analysis again utilized multiple linear regression with clinical covariates as in discovery, with the exception of the substitution of self-reported ancestry for principal components. Analysis was conducted using the R statistical package. The combined p-value from the discovery GWAS and replication for each SNP was determined using inverse variance based meta-analysis (see Text, Supplemental Digital Content 1).

Pathway-based Genome-wide Association Study

Pathway analysis was performed with the Pathway Analysis by Randomization Incorporating Structure (PARIS) software package(23). SNPs were first assigned to gene boundaries based on genomic location. Genes, in turn, were assigned to functional networks, which were tested for overrepresentation of SNPs that were observed to be nominally associated with change in LVEF in the single-SNP GWAS. Since the goal of pathway-based analysis was to extend on the single-SNP GWAS and identify cumulative effects on change in LVEF that related to multiple SNPs, with individually smaller effects, we set a less stringent threshold and significant SNPs were defined as those with p-values ≤ 0.05 from the discovery GWAS. Biological pathways were defined based on the KEGG Pathway Database. (24) A total of 290 KEGG pathways were evaluated for overrepresentation of SNPs significantly associated with change in LVEF. To determine p-values for overall association of pathways, permutation tests were conducted comparing pathways defined in the KEGG database to random pathways generated from regions across the genome having similar linkage disequilibrium (LD) structure (as predicted based on control individuals of European ancestry available in the HapMap population). We chose to generate random pathways for permutation tests using the LD structure predicted for individuals of European ancestry as the majority of the analysis dataset (~84%) that was evaluated in the single-SNP GWAS, from which the p-values for SNPs were derived, represented this ancestral group. The number of SNPs significantly associated with change in LVEF assigned to each evaluated KEGG pathway were compared to the number of significantly associated SNPs in 3,500 randomly generated pathways. Based on this number of permutation tests, we chose a stringent significance threshold of $p < 2.86 \times 10^{-4}$ for pathway-based results, which corresponded to a false discovery rate-corrected p-value of ≤ 0.05 .

To better understand the relationship of associated pathways to risk for change in LVEF, pathways that were significantly associated were further subjected to permutation-based investigations to identify 'core' genes driving overall pathway associations. 'Core' genes were defined as genes whose p-value in the context of the biological pathway was $p < 0.001$.

RESULTS

Discovery and Replication Cohort Identification

Search of BioVU identified 385 subjects with anthracycline exposure, measures of LVEF, and available DNA samples (see Figure, Supplemental Digital Content 4). Subjects were median age 52 years [interquartile range (IQR) 40 to 61 years], 58% female, and 88% white. Non-Hodgkin's lymphoma and breast cancer were the most common malignancies. Median baseline LVEF was 64% [IQR 60 to 68%], and the median change in LVEF from baseline was -6% [IQR 0 to -12%] with a range of 22 to -47% (Figure 1A). The median anthracycline exposure (doxorubicin equivalents) was 240 mg/m² [IQR 180 to 300 mg/m²]. The median time from baseline LVEF to anthracycline exposure was 6 days [IQR 2 to 13 days], and median follow-up between chemotherapy initiation and outcome LVEF was 348 days [IQR 122 to 755 days] (Table 1). Radionuclide ventriculography and transthoracic echocardiography accounted for over 95% of both pre-chemotherapy (74% radionuclide ventriculography; 24% transthoracic echocardiography) and outcome (50% radionuclide

ventriculography; 46% transthoracic echocardiography) LVEF observations. Baseline LVEF and cumulative anthracycline dose were imputed for 74 (19%) and 2 (0.5%) subjects respectively.

The replication cohort included 181 PREDICT subjects with adequate DNA samples and complete phenotype data (see Figure, Supplemental Digital Content 5). Subjects were median age 53 years [IQR 45 to 61 years], 87% female, and 84% white. Breast cancer was the most common malignancy. The median baseline LVEF was 64% [IQR 60 to 68%], and the median change in LVEF was -4% [IQR 3 to -9%] with a range of 16 to -28% (Figure 1B). The median anthracycline dose was 224 mg/m² [IQR 60 to 241 mg/m²], with a median follow-up from start of chemotherapy of 217 days [IQR 180 to 364 days] (Table 2), and XX subjects had prior exposure to chemotherapy

Discovery GWAS and Replication

After imputation and quality control, 4,429,986 markers were included in the discovery GWAS. A single locus on chromosome 1 (1p32.1) reached genome-wide significance with a p-value of 2.7×10^{-8} for the lead SNP rs10443221, which conferred protection ($\beta=4.11$; standard error 0.74) from cardiotoxicity (i.e., change in LVEF was 4.11 points higher for each alternate allele). The nearest characterized gene is *CYP2J2*. Ten other loci had p-values $<1.0 \times 10^{-5}$, including rs7542939 at 1p36.21 (Figure 2; see Figure, Supplemental Digital Content 6 and 7). Lead SNPs from all 11 loci were carried forward for testing in the replication cohort. Results of association testing for discovery and replication are summarized in Table 3. One locus, 1p36.21, successfully replicated with the lead SNP rs7542939 having a p-value of 0.01. This SNP had an overall MAF of 0.15 and conferred increased risk (overall $\beta=-3.82$; standard error 0.77) for a decrease in LVEF. The combined p-value from meta-analysis for this SNP was 6.5×10^{-7} . The nearest annotated gene, *PRDM2*, is approximately 40 kilobases upstream and encodes an H3K9 methyltransferase that regulates gene expression through modification of chromatin at target genes promoters.²² No association between SNP rs10443221 and change in LVEF was observed in the replication cohort ($\beta=-1.2$; standard error 0.80; $p=0.14$).

Pathway-based Genome-Wide Association Study

There were 18 KEGG pathways with strong evidence ($p < 2.86 \times 10^{-4}$) of enrichment for SNPs associated with changes in LVEF (see Table, Supplemental Digital Content 8). Five pathways were directly related to cellular metabolism and mitochondrial function, including: *synthesis and degradation of ketone bodies*, *ubiquinone and other terpenoid-quinone biosynthesis*, *D-Glutamine and D-glutamate metabolism*, *PPAR signaling pathway*, and *aminoacyl-tRNA biosynthesis* (whose association was driven by genes coding for mitochondrial aminoacyl-tRNA synthetases). Associations of five additional pathways, *N-glycan biosynthesis*, *glycosaminoglycan degradation*, *Wnt signaling*, *notch signaling*, and *axon guidance* were driven by genes previously implicated in cardiac remodeling. Also enriched was the DNA repair pathway *base excision and repair*.

DISCUSSION

We conducted a GWAS and pathway analysis of change in LVEF after exposure to anthracycline chemotherapy. Our GWAS results suggest a novel candidate susceptibility locus at chromosome 1p36.21 near *PRDM2*, and pathway analysis highlights the multifactorial nature of anthracycline cardiotoxicity and points toward processes involved in cellular metabolism/energetics, myocardial remodeling, and genome repair as central contributors to susceptibility.

While we cannot say with certainty that the observed association at 1p36.21 is functionally related to *PRDM2*, there are several features that make it a biologically plausible candidate gene. *PRDM2* is critical for BRCA1-dependent repair of DNA double-strand breaks,²³ and impairment of this mechanism exacerbates doxorubicin cardiotoxicity in mice.²⁴ Additionally, *PRDM2* is a transcriptional regulator of heme-oxygenase-1²⁵, which, in addition to its role in protection from oxidative stress^{26,27}, has been shown to facilitate repair of doxorubicin-induced DNA double-strand breaks *in vitro*²⁸ and reduce cardiomyocyte apoptosis in doxorubicin-exposed mice.²⁹

Although the association between rs10443221 and change in LVEF observed in the discovery GWAS was not replicated, *CYP2J2* also has biologic plausibility as a candidate gene. *CYP2J2* converts arachidonic acid to epoxyeicosatrienoic acids (EETs), whose cardiovascular effects include systemic and coronary vasodilation,³⁰ anti-inflammatory actions,³¹ and promotion of angiogenesis.³² Exogenous EETs attenuate doxorubicin-induced mitochondrial damage *in vitro*, and mice with cardiac specific overexpression of human *CYP2J2* show reduced cardiomyocyte apoptosis, lower levels of biomarkers of myocardial injury and oxidative stress, and improved cardiac function after exposure to anthracyclines.³³ These functional data may support the measurement of EET's and *CYP2J2* function in future studies of anthracycline cardiotoxicity.

It was expected that common variants may have cumulative effects on LV function, and that genetic factors contributing smaller effects (OR 1.5) would be overlooked in the typical single-SNP approach to analysis of GWAS data. Pathway-based analysis identified 18 KEGG pathways associated with change in LV function. DNA injury is an important proximal effect of anthracyclines. Therefore, it is not unexpected that associations with pathways involved in DNA repair. Similarly, the heart has very high energy requirements, and heart failure from any cause, particularly when advanced, is associated with myocardial energy depletion, derangement of mitochondrial function and biogenesis, and a general shift in substrate preference from fatty acids to glucose.^{34,35} The observed associations in energetic pathways may suggest that susceptible individuals have less “metabolic reserve” in the face of myocardial injury. Pathway analysis also identified several cellular components and processes previously implicated in myocardial remodeling and response to injury, including glycosylation^{36,37}, constituents of the extracellular matrix (ECM)³⁸, and proteins involved in ECM-receptor interaction.^{39–41} Additionally, two important signaling pathways, wnt and notch, also showed evidence for enrichment. The role of both pathways in cancer and metastasis is well established;^{42,43} they are also important for induction of pathologic hypertrophy, cardiac remodeling, and response to various forms of myocardial injury

including infarction and pressure overload.^{44–48} The diversity of pathways implicated reinforce the complexity of anthracycline cardiotoxicity and may point toward therapeutic targets.

It is interesting that we did not detect associations with many previously implicated susceptibility factors for anthracycline related cardiotoxicity.^{11,13,49} Difficulty replicating associations between candidate variants and important human phenotypes has been described.⁵⁰ It may be that we were underpowered to detect small effect sizes. It is also possible that some published associations are false positives. However, this observation may be related to important differences among studies including population demographics, treatment, comorbidities, follow-up duration, and definition of toxicity.

Results of our study should be interpreted within its limitations. The cohorts used are relatively small, and we were likely underpowered to detect effects of modest size. Additionally, variability in LVEF measurements due to inherent limitations of cardiac imaging and lack of standardized protocols in the discovery cohort (e.g., assessments were clinically indicated and not at pre-specified intervals, contrast was not universally used, LVEF measurements were made using both quantitative and visual estimation methods, and use of different modalities for pre- and post-therapy LVEF determination) could have introduced bias. Finally, we did not perform functional studies to further establish a mechanistic link between the locus tagged by SNP rs7542939 and cardiotoxicity. However, previously published data regarding nearby genes and the availability of an independent replication cohort add confidence to the observed association.

Conclusions

The genomic architecture of anthracycline cardiotoxicity is complex. Using GWAS we identified a novel susceptibility locus near *PRDM2*. Pathway analysis suggested that genome repair, metabolism, and pathways relevant for cardiac remodeling influence susceptibility to anthracycline cardiotoxicity. Our findings extend current understanding by identifying a novel risk locus and emphasizing the multifactorial nature of anthracycline cardiotoxicity. This work helps to frame findings from prior candidate studies into a paradigm of genomic injury followed by progressive metabolic and myocardial remodeling. Finally, our findings provide mechanistic insight into anthracycline cardiotoxicity and suggest testable hypotheses for detection and prevention of this adverse effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Change in ejection fraction from baseline in discovery GWAS and replication cohorts. In each panel, total doxorubicin equivalents per square meter (mg/m²) are shown on the X-axis, and change in left ventricular ejection fraction (LVEF) on the Y-axis. A) Discovery GWAS cohort. B) Replication cohort

Figure 2. Manhattan plot of discovery GWAS of change in left ventricular ejection fraction (LVEF). Results for each variant are organized according to chromosomal position on the X-axis, with $-\log_{10}(p)$ -values for association of genotype to change LVEF from the discovery cohort on the Y-axis. The horizontal line indicates the *a priori* threshold for statistical significance.

Table 1

Demographics for Discovery GWAS Cohort (N=385)

Parameter	N (Percent) or Median (IQR)
Female	223 (58%)
Age at Start of Chemotherapy (years)	52 (40 to 61)
Race	
White	337 (88%)
Black	30 (8%)
Asian	1 (0.3%)
Latino	4 (1%)
Unknown	13 (3%)
Baseline Ejection Fraction (%)	64 (60 to 68)
Time from Chemotherapy to Outcome LVEF* (days)	348 (122 to 755)
Most Frequent Oncologic Diagnosis	
Non-Hodgkin Lymphoma	109 (28%)
Breast cancer	78 (20%)
AML	59 (15%)
Sarcoma	39 (10%)
Hodgkin Lymphoma	28 (7%)
Radiation to Chest	71 (18%)
Cumulative Anthracycline Dose (Doxorubicin equiv. mg/m ²)	240 (180 to 300)
Change in LVEF	−6 (0 to −12) (range 22 to −47)
Cardiovascular Risk Factors	
Hypertension	139 (36%)
Dyslipidemia	76 (20%)
Smoking	
Never	228 (59%)
Former	81 (21%)
Current	76 (20%)
Diabetes	39 (10%)
Coronary artery disease	19 (5%)
BMI [†] (kg/m ²)	28 (24 to 32)
Trastuzumab	26 (7%)

* LVEF = Left ventricular ejection fraction;

[†] BMI = Body mass index

Table 2

Demographics for Replication Cohort (N=181)

Parameter	Percent (N) or Median (IQR)
Female	158 (87%)
Age at Start of Chemotherapy (years)	53 (45 to 61)
Race	
White	152 (84%)
Black	23 (13%)
Asian	3 (2%)
Unknown	3 (2%)
Baseline LVEF (%)	64 (60 to 68)
Time from Baseline LVEF to Anthracycline* (days)	6 (2 to 13 days)
Time from Chemotherapy to Outcome LVEF (days)	217 (180 to 364)
Oncologic Diagnosis	
Breast cancer	145 (80%)
Lymphoma	32 (18%)
Gastrointestinal	3 (2%)
Sarcoma	1 (0.6%)
Radiation to Chest	Not available
Cumulative Anthracycline Dose (Doxorubicin equiv. mg/m ²)	224 (60 to 241)
Change in Ejection Fraction (%)	−4 (3 to −9) [range 16 to −28]
Cardiovascular Risk Factors	
Hypertension	61 (34%)
Dyslipidemia	55 (30%)
Smoking	
Never	106 (59%)
Former	48 (27%)
Current	27 (15%)
Diabetes	25 (14%)
Coronary artery disease	Not available
BMI [†] (kg/m ²)	29 (25–34)
Trastuzumab	25 (14%)

* LVEF = Left ventricular ejection fraction;

[†] BMI = Body mass index

Table 3

Results of Association Testing for Discovery and Replication Cohorts

Locus Details				Discovery GWAS			Replication		
Locus	SNP	Nearest Annotated Gene(s)	Coded Allele	MAF	Beta (SE)	p-value	MAF	Beta (SE)	p-value
1p32.1	rs10443221	HOOK1, CYP2J2, Clorf187	T	0.38	4.11 (0.74)	2.7 e-8	0.34	-1.2 (0.80)	0.14
1p36.21	rs7542939	PRDM2	A	0.15	-4.97 (1.10)	5.9 e-6	0.14	-2.7 (1.08)	0.01
2p12	rs78000710	LRRTM4	C	0.07	-6.60 (1.52)	1.4 e-5	0.08	-0.12 (1.50)	0.94
3q28	rs7635904	TPRG1	G	0.08	-5.82 (1.28)	5.3 e-6	0.12	-0.85 (1.17)	0.47
6p21.1	rs990963	LRRTM4	C	0.39	-3.47 (0.78)	7.8 e-6	0.39	-0.18 (0.79)	0.82
7p21.1	rs7801891	AHR, AGR3	C	0.08	6.21 (1.33)	3.0 e-6	0.06	0.33 (1.50)	0.83
12q24.13	rs11066861	RBM19	A	0.06	-7.10 (1.58)	7.1 e-6	0.06	1.18 (1.64)	0.47
13q31.3	rs34223702	LINC00379	A	0.06	-8.32 (1.72)	1.4 e-6	0.07	-1.11 (1.45)	0.45
15q13.1	rs7179489	FAM189A1	A	0.41	3.29 (0.75)	1.3 e-5	0.40	0.22 (0.78)	0.77
16q24.1	rs55669225	LINC00917	A	0.07	-6.51 (1.47)	9.3 e-6	0.06	-0.86 (1.69)	0.61
19q12	rs12984615	URI1	A	0.49	-3.22 (0.77)	3.0 e-5	0.51	-1.30 (0.73)	0.08