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Genetic analysis of candidate SNPs for metabolic syndrome in obstructive sleep apnea (OSA)

Antonio Grilo, MD, PhD[1], **Elena S. Ruiz-Granados, MD**[2], **Concha Moreno-Rey, BS**[3], **Jose M. Rivera, MD**[1], **Agustin Ruiz, MD, PhD**[3], **Luis M. Real, PhD**[3], and **Maria E. Sáez, PhD**[3,4,*]

[1]Unidad de Hipertensión, Servicio de Medicina Interna. Hospital Universitario de Valme. Carretera de Cádiz S/N. 41014 Seville, Spain

[2]Hospital Infanta Elena. Ctra. Sevilla-Huelva s/n, 21080 Huelva, Spain

[3]Neocodex. Avda. Charles Darwin 6, Acc. A. 41092 Seville, Spain

[4]Centro Andaluz de Estudios Bioinformáticos (CAEBI). Avda. Leonardo da Vinci 18, Planta 1, Mod. 7A. 41092, Seville, Spain

Abstract

Obstructive sleep apnea (OSA) is a common disorder characterized by the reduction or complete cessation in airflow resulting from an obstruction of the upper airway. Several studies have observed an increased risk for cardiovascular morbidity and mortality among OSA patients. Metabolic syndrome (MetS), a cluster of cardiovascular risk factors characterized by the presence of insulin resistance, is often found in patients with OSA, but the complex interplay between these two syndromes is not well understood. In this study, we present the results of a genetic association analysis of 373 candidate SNPs for MetS selected in a previous genome wide association analysis (GWAS). The 384 selected SNPs were genotyped using the Illumina VeraCode Technology in 387 subjects retrospectively assessed at the Internal Medicine Unit of the “Virgen de Valme” University Hospital (Seville, Spain). In order to increase the power of this study and to validate our findings in an independent population, we used data from the Framingham Sleep study which comprises 368 individuals. Only the rs11211631 polymorphism was associated with OSA in both populations, with an estimated OR=0.57 (0.42-0.79) in the joint analysis ($p=7.21 \times 10^{-4}$). This SNP was selected in the previous GWAS for MetS components using a digenic approach, but was not significant in the monogenic study. We have also identified two SNPs (rs2687855 and rs4299396) with a protective effect from OSA only in the abdominal obese subpopulation. As a whole, our study does not support that OSA and MetS share major genetic determinants, although both syndromes share common epidemiological and clinical features.

Keywords

Obstructive sleep apnea; Metabolic syndrome; Polymorphisms; Genome wide association analysis

[*]Coresponding autor: María Eugenia Sáez (mesaez@caebi.es). Centro Andaluz de Estudios Bioinformáticos (CAEBi). Avda. Leonardo da Vinci 18, Planta 1, Mod. 7A. 41092, Seville, Spain. Phone: +34 668 883 773.

1. INTRODUCTION

Obstructive sleep apnea (OSA) is a common disorder characterized by the reduction or complete cessation in airflow resulting from an obstruction of the upper airway. This obstruction results in repetitive breathing pauses during sleep. As a consequence, the architecture of sleep is disrupted: there is a decrease in REM sleep as well as deeper stages of non-REM sleep (Berry et al., 1998).

Several studies have observed an increased risk for cardiovascular morbidity and mortality among OSA patients (Peppard et al., 2000; Marin et al., 2005; Marshall et al., 2008). Metabolic syndrome (MetS), a cluster of cardiovascular risk factors characterized by the presence of insulin resistance (Grundy et al., 2004), is often found in patients with OSA, but the exact nature of this relationship is still controversial (Ip et al., 2002; Coughlin et al., 2004; Reichmuth et al., 2005; Gruber et al., 2006). Repetitive hypoxias have been shown to cause insulin resistance (Braun et al., 2001) and the use of continuous positive airway pressure (CPAP) has been evaluated in OSA patients in relation with mortality (Campos-Rodriguez et al., 2012) and insulin sensitivity. Again the results are still conflicting: whereas some studies reported a better metabolic profile after CPAP treatment (Brooks et al., 1994; Lam et al., 2010; Sharma et al., 2011), other reports failed to identify improved insulin sensitivity (Smurra et al., 2001; West et al., 2007) or a reduction in blood pressure levels (Iellamo and Montano, 2006; Campos-Rodriguez et al., 2007). Regarding the prevalence of the individual MetS components in OSA patients, it is particularly high for hypertension and obesity. Some authors have even reported a dose dependent effect between blood pressure and OSA severity (Nieto et al., 2000; Barcelo et al., 2005).

In the pathogenesis of OSA, genetic factors play also an important role, explaining up to 40% of the variance in the apnea hypopnea index (AHI), a quantitative measure of OSA (Strohl et al., 1978; Pillar and Lavie, 1995; Redline et al., 1995; Palmer et al., 2004) Nevertheless, there are few data regarding specific genes associated with OSA. The candidate gene approach has been the standard for identifying genes associated with most common diseases. In the case of OSA, it has been focused in genes affecting upper airway and ventilator control (such as serotonin-2 receptors of transcription or the transcription factor Phox2b) and, particularly in genes related to metabolic syndrome components (Riha et al., 2009; Kent et al., 2010) such as genes encoding the angiotensin converting enzyme (ACE), endothelin (ET-1), leptin and its receptor (LEP, LEPR), tumor necrosis factor alpha (TNFα) or apolipoprotein E (APOE). Unfortunately none of them have been consistently replicated. Recently published meta-analyses have investigated the role of APOE, TNFα and ACE gene polymorphisms in OSA pathogenesis, but they only found a statistical significant association for the TNFα gene (Lee et al., 2011; Varvarigou et al., 2011; Huang et al., 2012).

As the techniques of genome wide analysis have become available, they have been also applied to the study of the genetic causes of OSA. To date, three whole-genome analyses have been published (Palmer et al., 2003a; Palmer et al., 2004; Relf et al., 2010). As a result, new candidate chromosomal regions for OSA have been identified, some of them including genes associated with inflammatory responses.

In a previous study, we performed a genome wide analysis in general population aimed at identifying genes associated with metabolic syndrome and its endophenotypes (Gayan et al., 2010). We selected the 384 markers with the best score for association using both a monogenic and digenic approach. In this paper, we present the results of the results of the monogenic association analysis of these selected polymorphisms with OSA in a cohort of 387 hypertensive patients with polysomnographic data available. We also explore the role of several demographic, anthropometric and biochemical values in the development of OSA.

2. MATERIAL AND METHODS

2.1. Patients

The genetic association study of OSA includes 387 subjects retrospectively assessed at the Internal Medicine Unit of the "Virgen de Valme" University Hospital (Seville, Spain). All of them are hypertensive patients. All participants gave their written consent to participate in the study. The study protocol was approved by the Ethics Committee of the "Virgen de Valme"Hospital (Seville, Spain).

In order to increase the power of this study and to validate our findings in an independent population, we used data from the Framingham Sleep study. This subpopulation of the population-based Framingham study comprises 368 individuals with recorded AHI values.

2.2. Interventions

For the OSA study, AHI was measured by overnight unattended limited channel polysomnography. OSA was defined as an AHI of 5 events per hour or more.

Other phenotype measurements determined are body mass index (BMI), waist circumference (WC), systolic and diastolic blood pressures (SBP, DBP) fasting glucose, 2-hours glucose (OGTT, oral glucose tolerance test), fasting insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides (TGs).

SBP and DBP were measured by ABMP (using a non invasive portable validated recorder Spacelab 90207; Spacelabs Medical; Redmond, WA).

After an overnight fasting period, 20 ml of blood were obtained from an antecubital vein without compression. Plasma glucose was determined in duplicate by a glucose-oxidase method adapted to an Autoanalyzer (Hitachi 704, Boehringer Mannheim, Germany). Total cholesterol, triglycerides and HDL cholesterol were determined by enzymatic methods using commercial kits (Boehringer Mannheim, Germany). LDL cholesterol was calculated by the Friedewald formula.

The metabolic syndrome status was established according to ATPIII definition as it was recently modified: presence of at least three components between abdominal obesity (WC ≥ 102 cm men, 88 cm women), hypertriglyceridemia (TGs ≥ 150 mg/dl), hypertension (≥ 85/130 mmHg), HDL-c (< 40 mg/dl men, < 50 mg/dl women) and fasting glucose ≥ 100 mg/dl (Grundy et al., 2004).

2.3. Genotyping and quality control

The 384 SNPs genotyped in this study were selected after performing a monogenic and a digenic genome wide association study (GWAS) on metabolic syndrome components. For this study, we used Affymetrix 250k data from 801 Caucasian subjects derived from cross-sectional population- based epidemiological survey in Spain aimed at investigating the prevalence of metabolic syndrome and related components (Lorenzo et al., 2001; Martinez-Larrad et al., 2005; Gayan et al., 2010).

We obtained 5 ml of peripheral blood from all patients and controls to isolate germline DNA from leukocytes. DNA extraction was performed in a MagNa Pure LC Instrument (Roche Diagnostics) according to the manufacturer's instructions.

The 384 selected SNPs were genotyped using the Illumina VeraCode Technology and the BeadXpress Reader according to manufacturer's instructions.

All SNPs were subjected to quality control filters. Specifically we only selected SNPs successfully genotyped in at least 95% of individuals and with a p-value for Hardy-Weinberg equilibrium (HWE) larger than 10^{-4}. Finally, 373 SNPs were used in the genetic association study of OSA.

2.4. Statistical analyses

In order to analyze the associations between OSA and demographic/clinical data, we used regression procedures. To select the independent determinants of OSA we used a stepwise linear regression model using AHI as dependent variable; those traits associated with $p<0.05$ in this model, were used as covariables for the genetic association study of OSA. These analyses were performed using SPSS software.

We used unconditional logistic regression models for the statistical genetic analysis. We performed independent analyses in both populations (i.e. Valme and Framingham) and a joint analysis controlling by population effect (included as a covariate in the model). Joint analysis has been described more powerful than meta-analysis (Skol et al., 2006). We also performed a meta-analysis in order to compare the consistency of both approaches. For these genetic association analyses, we used PLINK software (Purcell et al., 2007).

We carried out a Breslow-Day test to investigate the independence of these associations with metabolic syndrome (ATPIII definition) and abdominal obesity. In instances when evidence of heterogeneity between-cluster was found, we run stratified analysis. We also tested the effect of hypertension in the Framingham population.

3. RESULTS

The clinical characteristics of the study population are summarized in table 1.

We first analyzed the individual effect of different demographic and clinical traits on OSA phenotype (table 2). The strongest predictor of OSA in both populations is WC ($10^{-14} < p < 10^{-8}$), followed by sex (lower prevalence in women). In the stepwise regression model WC,

sex, age, and use of antihypertensive and glucose lowering medication remain in the equation. Therefore we selected these traits as covariates in the analyses

In the independent analysis for each population, we failed to identify any SNP associated with OSA with $p< 10^{-4}$ as determined by the Bonferroni correction ($p=0.05/373 \approx 10^{-4}$). None but the rs11211631 polymorphism was associated with OSA with $p<0.5$ in both populations with the same direction of effect. In the joint analysis (table 3), this SNP is associated with OSA with $p=7.21 \times 10^{-4}$, which is in the range of Bonferroni correction. This is also the only SNP associated in the meta-analysis with $p<10^{-3}$ ($p=9 \times 10^{-4}$). At this locus, the presence of the A allele reduces the risk of OSA presentation with an estimated OR for both populations of 0.57. The rs11211631 polymorphism is also associated with the severity of OSA with p=0.022 in the joint analysis.

To test the independence of these associations with metabolic syndrome (ATPIII definition), we performed a Breslow-Day test and a stratified analysis. These results are shown in table 4. We have identified two SNPs with a statistically significant effect among individuals with MetS: rs2687855 (p=0.0004) on chromosome 3 and rs4299396 (p=0.0005) on 20p12.3.

We have also investigated the independence of these associations with WC, the strongest predictor of the occurrence of OSA in our population study. For sixteen markers, we found suggestive ($p<0.05$) heterogeneity between individuals with and without abdominal obesity (ATPIII criteria) but we could not identify associations with a p value $< 10^{-3}$ in any strata.

4. DISCUSSION

This study shows an association genetic analysis of OSA syndrome with 373 candidate gene variants for MetS and related traits. These candidate SNPs were selected in a GWAS for MetS in a population-based sample using monogenic and digenic genome wide SNP selection approaches). Some of them were not associated with MetS in the monogenic association analysis, but they were significant in the digenic study. The rs11211631 polymorphism, which our study has pointed out as a possible prognostic marker for the occurrence of OSA, was selected for conferring increased risk for MetS in combination with the rs17334288 polymorphism (OR=5.84). The genetic association study of this pair of polymorphism with OSA was not significant (OR=1.57 [0.97-2.54], p=0.06), although a trend for significance is observed. These results may suggest that the effect of this combined genotype on OSA, if exits, is lower than in MetS.

As a whole, our study does not support that OSA and MetS share major genetic determinants. None of the thirteen polymorphisms selected in the previous GWAS study, that we have independently validated in a large meta-analysis with more than 14,000 individuals (data not shown), is associated with OSA in this study. We have investigated if the presence of hypertension in our population, a well known risk factor for OSA, was acting as a confounding factor in our study. The Breslow-Day test performed in the Framingham population based sample did not show evidence of heterogeneity affecting our results (p=0.5019 for the rs11211631 polymorphism)

Other studies and three recent meta-analyses have also failed to confirm the role of other candidate genes for MetS with the exception of TNFα (Lee et al., 2011; Varvarigou et al., 2011; Huang et al., 2012). On the other hand, both syndromes are largely complex diseases determined by a large number of genes, and some of them are probably the same. To study these complex interactions, polygenic studies in large populations would be very useful for our better understanding of the interplay between these high prevalence conditions.

It is clear from epidemiological studies that OSA and MetS share clinical determinants. In this study, we have observed that WC is the best predictor of SAOS. We carried a Breslow-Day test to investigate the effect of the presence of abdominal obesity and we identified two SNPs (rs2687855 and rs4299396) with a strong protective effect from OSA among the obese group. Interestingly, these SNPs are located in chromosomal regions linked to phenotypes related to airflow. The rs2687855 polymorphism on chromosome 3q24 lies within the chronic obstructive pulmonary disease QTL 14, and in particular with the phenotype post-BD FEV1 (post-bronchodilator forced expiratory volume at 1 second) minus pre-BD FEV1, a measure of chronic airflow obstruction (Palmer et al., 2003b). The rs4299396 polymorphism on 20p12.3 is located within the binding site for CEBP, an important transcriptional activator in the regulation of genes involved in immune and inflammatory responses, in a chromosomal region between 1-phosphatidylinositol-4 5-bisphosphate phosphodiesterase beta-1 and beta-4 encoding genes (PLCb1 and PLCB4); this chromosomal region is part of the allergic/atopic asthma related QTL 25 (Xu et al., 2001) determined by the post-BD FEV1 phenotype, a measure of reversible airflow obstruction.

In conclusion, we have not found evidence of common genetic factors between OSA and MetS after the monogenic analysis of 373 candidate SNPs for MetS components. Only one variant, rs11211631, seems to be consistently associated with the occurrence of OSA in the two independent populations analyzed although its effect in OSA would need independent validations. WC is the strongest predictor of OSA syndrome in our study and two SNPs (rs2687855 and rs4299396) showed association with OSA only in the abdominal obese subpopulation, but again these results must be confirmed in independent populations. The later illustrates how the presence MetS traits can be confounding factors for the identification of genetic factors for OSA.

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Table 1

Clinical characteristics of the study population.

CONTINOUS TRAITS	MEN			WOMEN		
	MEAN	N	SD	MEAN	N	SD
AGE (years)	56,44	225	10,77	59,08	161	10,53
BMI (kg/m2)	33,72	225	5,67	38,82	162	9,15
WC (cm)	112,42	218	12,21	114,03	157	15,07
SBP (mmHg)	135,45	224	14,44	138,81	159	18,43
DBP(mmHg)	80,88	224	10,40	81,91	158	13,56
GLC (mg/dl)	116,29	220	35,90	125,83	160	44,66
TGs (mg/dl)	174,87	225	112,75	157,85	160	72,92
CHOL (mg/dl)	210,31	225	44,76	215,18	160	40,59
HDL-c (mg/dl)	47,35	224	11,41	54,61	157	11,74
LDL-C (mg/dl)	130,59	217	39,58	129,85	156	36,37
AHI (apnea/min)	42,40	225	24,51	32,81	162	28,67

DICHOTOMIC TRAITS	N	%	N	%
OSA (AHI≥5)	218 (225)	96,9	133 (162)	82,1
OBESITY (BMI≥30)	170 (225)	75,6	147 (162)	90,7
ABDOMINAL OBESITY	175 (218)	80,3	150 (157)	95,5
MetS (ATP3)	168 (224)	75	137 (157)	87,3
ALCOHOL CONSUMERS	130 (222)	59,1	15 (158)	9,5
SMOKERS	173 (225)	76,9	25 (162)	15,4
Under antiHT treatment	221 (225)	98,2	162 (162)	100
Under lipid-lowering treatment	117 (225)	52	66 (162)	40,7

BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; GLC, basal glucose; TGs, triglycerides; CHOL, total cholesterol; HDL-c, HDL cholesterol; LDL-c, LDL-cholesterol; AHI, apnea-hypoapnea index. (CC> 102cm male, CC> 88 cm female); MS: metabolic syndrome.

Table 2

Effect of several determinations in AHI values.

	JOINT ANALYSIS		SPANISH POPULATION		FRAMINGHAM POPULATION	
	BETA	**p**	**BETA**	**p**	**BETA**	**p**
WC	0.511 (0.349)	1.76 E-46 (4.38 E-18)	0.282 (0.272)	2.93 E-8	0.410 (0.410)	8.39 E-14 (9.21 E-14)
Antihypertensive medication use	0.423 (0.210)	4.14 E-34 (3.75 E-8)	-0.044	0.383	0.177	0.001
BMI	0.405	3.36 E-31	0.147	0.004	0.351	4.45 E-12
MetS (ATPIII definition)	0.331	2.41 E-20	0.083	0.105	0.222	2.40 E-5
Oral antidiabetic drugs	0.274 (0.088)	1.68 E-14 (0.013)	0.086	0.090	0.080	0.123
Fasting glucose	0.249	4.94 E-12	0.108	0.036	0.162	0.002
Tobacco use	0.245	8.48 E-12	0.077	0.129	-0.080	0.128
Lipid lowering medication use	0.212	4.03 E-9	0.004	0.938	0.111	0.034
Sex	-0.209 (-0.131)	7.24 E-9 (7.67 E-5)	-0.177 (-0.211)	4.56 E-4 (2.45 E-5)	-0.254	8.12 E-7
SBP	0.204	1.69 E-8	-0.035	0.500	0.115	0.028
TGs	0.172	2.25 E-6	0.088	0.084	0.154	0.003
DBP	0.124	0.001	-0.137	0.007	0.104	0.046
Alcohol consumption	-0.082	0.025	0.074	0.152	0.073	0.164
HDL-c	-0.081	0.028	-0.110	0.032	-0.177	0.001
Age	0.075	0.039	0.161 (0.147)	0.001 (0.003)	0.112	0.032
Cholesterol	0.053	0.148	-0.034	0.501	0.078	0.136
LDL-c	0.012	0.740	-0.049	0.348	0.076	0.145

The numbers in brackets are the results of the stepwise regression analysis (only those with a p value < 0.05 are shown). BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; GLC, basal glucose; TGs, triglycerides; CHOL, total cholesterol; HDL-c, HDL cholesterol; LDL-c, LDL-cholesterol; AHI, apnea-hypoapnea index

Table 3

Results from the monogenic genetic association analysis of OSA.

Chr	SNP	A1	Joint analysis		Spanish population		Framingham population	
			OR (L95-U95)	p	OR (L95-U95)	p	OR (L95-U95)	p
1	rs11211631	A	0,57 (0,42-0,79)	7,21 E-4	0,48 (0,26-0,90)	0,02	0,61 (0,41-0,90)	0,01
1	rs12401728	T	0,65 (0,44-0,97)	0,04	0,90 (0,46-1,77)	0,77	0,54 (0,32-0,92)	0,02
2	rs7573770	T	1,80 (1,07-3,05)	0,03	2,56 (0,94-6,96)	0,07	1,63 (0,87-3,05)	0,13
3	rs2687855	G	0,67 (0,48-0,92)	0,02	0,71 (0,40-1,26)	0,25	0,63 (0,41-0,95)	0,03
3	rs3114672	A	0,68 (0,46-1,00)	0,05	0,97 (0,50-1,87)	0,93	0,53 (0,32-0,88)	0,01
4	rs13122418	G	0,67 (0,47-0,97)	0,03	0,72 (0,38-1,37)	0,31	0,65 (0,41-1,01)	0,06
5	rs958625	C	1,63 (1,03-2,57)	0,04	1,33 (0,64-2,74)	0,44	1,80 (1,01-3,23)	0,05
5	rs16899057	G	1,53 (1,04-2,25)	0,03	1,86 (0,87-3,98)	0,11	1,29 (0,81-2,06)	0,28
5	rs288028	C	0,67 (0,48-0,95)	0,02	0,67 (0,37-1,23)	0,20	0,70 (0,46-1,06)	0,09
7	rs12540806	T	0,70 (0,51-0,96)	0,03	0,69 (0,41-1,18)	0,17	0,72 (0,48-1,07)	0,10
8	rs1967103	G	1,41 (1,02-1,96)	0,04	1,88 (1,03-3,42)	0,04	1,19 (0,79-1,79)	0,40
9	rs10756216	T	0,66 (0,46-0,95)	0,02	0,68 (0,37-1,25)	0,22	0,67 (0,42-1,05)	0,08
9	rs10980526	A	0,68 (0,47-0,99)	0,04	0,68 (0,36-1,28)	0,23	0,68 (0,43-1,09)	0,11
11	rs12274522	G	0,69 (0,48-1,00)	0,05	0,99 (0,54-1,83)	0,98	0,60 (0,38-0,96)	0,03
15	rs558290	T	0,64 (0,46-0,88)	0,01	0,70 (0,40-1,22)	0,20	0,66 (0,44-0,99)	0,04

CHR: chromosome; A1: minor allele; OR: odds ratio; L95, U95: lower and upper 95% CI (confidence interval).

Table 4

Results from the Breslow-Day test and stratified analysis by the presence of MetS.

SNP	A1	MAF	Breslow-Day test	Individuals free of MetS			Individuals with MetS		
			P	N	OR (L95-U95)	P	N	OR(L95-U95)	P
rs3924486	G	0,49	0,0296	269	0,96 (0,58-1,60)	0,8845	410	0,86 (0,55-1,36)	0,5283
rs2554297	G	0,23	0,0036	269	0,60 (0,36-1,02)	0,0604	409	2,56 (1,37-4,79)	0,0033
rs4852175	A	0,49	0,0030	268	0,94 (0,60-1,47)	0,7794	409	0,49 (0,30-0,79)	0,0037
rs7573770	T	0,12	0,0053	268	1,20 (0,60-2,40)	0,6066	409	3,68 (1,41-9,61)	0,0078
rs1402246	G	0,38	0,0093	269	0,48 (0,29-0,79)	0,0042	409	2,38 (1,41-4,02)	0,0011
rs9856633	A	0,21	0,0435	269	1,21 (0,65-2,26)	0,5415	410	0,81 (0,48-1,37)	0,4358
rs9845651	T	0,26	0,0302	269	1,04 (0,62-1,75)	0,8702	410	0,67 (0,41-1,09)	0,1046
rs2687855	**G**	**0,34**	**0,0126**	**269**	**1,00 (0,63-1,61)**	**0,9865**	**410**	**0,43 (0,26-0,68)**	**0,0004**
rs11762595	C	0,48	0,0073	269	0,76 (0,48-1,19)	0,2291	410	0,92 (0,60-1,43)	0,7188
rs4921657	C	0,39	0,0291	269	0,96 (0,60-1,55)	0,8731	410	1,64 (0,97-2,76)	0,0639
rs10511746	G	0,14	0,0152	269	1,86 (0,91-3,78)	0,0870	410	0,42 (0,23-0,75)	0,0035
rs11139400	A	0,36	0,0235	267	0,82 (0,50-1,34)	0,4231	409	0,70 (0,44-1,10)	0,1200
rs11001808	C	0,34	0,0009	269	1,18 (0,72-1,94)	0,5143	410	1,26 (0,77-2,06)	0,3544
rs10842447	C	0,36	0,0254	269	1,38 (0,82-2,32)	0,2273	410	1,35 (0,81-2,25)	0,2509
rs2026165	A	0,23	0,0195	269	1,59 (0,87-2,90)	0,1286	410	1,44 (0,79-2,63)	0,2310
rs7174474	A	0,29	0,0166	269	1,35 (0,80-2,29)	0,2643	410	0,52 (0,33-0,83)	0,0067
rs10520583	A	0,38	0,0199	269	0,91 (0,55-1,50)	0,7022	410	0,82 (0,52-1,27)	0,3709
rs7189322	T	0,25	0,0067	269	0,84 (0,49-1,44)	0,5360	408	2,10 (1,13-3,91)	0,0196
rs8092640	A	0,24	0,0056	268	0,56 (0,32-0,96)	0,0366	410	1,57 (0,93-2,66)	0,0941
rs4299396	**T**	**0,27**	**0,0107**	**269**	**1,23 (0,74-2,03)**	**0,4275**	**410**	**0,40 (0,24-0,67)**	**0,0005**

BP: base pair; A1: minor allele; MAF: minor allele frequency;X2: chi squared; N: number of subjects; OR: odds ratio; SE: standard error; L95, U95: lower and upper 95% CI (confidence interval).