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Gene-based analysis identified the gene *ZNF248* is associated with late-onset asthma in African Americans

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Abstract

Background—Late-onset asthma (LOA) has distinct characteristics and its pathogenesis might rely on unique pathways. Although current studies are focused primarily on childhood asthma, more research is needed to show the mechanisms underlying LOA.

Objective—To conduct genomewide association analysis and gene-based analysis to identify single-nucleotide polymorphisms and genes associated with LOA.

Methods—The Women's Health Initiative (WHI) observational cohort and the Multi-Ethnic Study of Atherosclerosis (MESA) were used to identify subjects with LOA. The association between LOA and body mass index and smoking was evaluated. In the discovery stage of the genetic analysis, 1,218 African American subjects from WHI with genotype data (271 cases and 947 controls) were used for single-nucleotide polymorphism and gene-based association analyses. Significant or suggestive results were subsequently investigated in an independent African American population from MESA (38 cases and 806 controls).

Results—In WHI, the relative odds for LOA in obese vs normal-weight subjects was 2.55 (95% confidence interval 1.74–3.76). Ever smokers also had greater odds for LOA compared with never smokers (odds ratio 1.59, 95% confidence interval 1.21–2.09). The same trends were observed in MESA. In WHI, 6 single-nucleotide polymorphisms were associated with LOA at a genomewide-suggestive significance level ($P < 1.0 \times 10^{-5}$). The gene *ZNF248* was associated with LOA and reached genomewide significance ($P = 4.0 \times 10^{-7}$). In MESA, the association between *ZNF248* and LOA was successfully replicated ($P = .015$).

Conclusion—Smoking and obesity are risk factors for LOA. *ZNF248* confers increased susceptibility to LOA in African Americans.

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Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anai.2016.05.005.

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Introduction

Asthma is a heterogeneous pulmonary disorder that can affect individuals across the entire age spectrum. Multiple cluster analysis has shown that age of asthma onset is important to distinguish between asthma endotypes, and late-onset asthma (LOA; ie, asthma that first presents in older adults) has distinct characteristics.[1–4] Compared with childhood-onset asthma, LOA is less likely to be allergic and is potentially driven by the T-helper cell type 1 response, because neutrophils are predominant in the local tissue.[5–8]

Asthma has a strong genetic component, and genomewide association studies (GWASs) have successfully identified more than 100 asthma genes and loci and shed light on the underlying disease mechanisms.[9] However, most studies have focused solely on those with early-onset asthma or grouped together those with early-onset asthma and those with LOA. LOA genetics has been largely understudied, and the mechanisms underlying LOA are not clear.[8] Recently, the association between asthma and variants near the HLA complex group 22 gene (*HCG22*) has been shown to increase with older age of onset in a Japanese population.[10] This suggests that the genetic contribution to asthma might differ according to age of onset. To enhance the ability to identify susceptibility loci and uncover potentially unique molecular pathways, additional research specifically focused on LOA is needed. Novel findings could lead to effective asthma prevention and personalized asthma treatment in the elderly.

The present study investigated genetic factors associated with LOA. The Women's Health Initiative (WHI) observational cohort and the Multi-Ethnic Study of Atherosclerosis (MESA) were used to identify subjects with LOA. The WHI cohort consists of 2 racial groups with genotype data, African Americans and Hispanics. To increase detection power, we focused on the larger population, African Americans, in the discovery stage. Genomewide association analysis and gene-based analysis were conducted in 1,218 African American subjects (271 cases and 947 controls). The top single-nucleotide polymorphisms (SNPs) and genes were subsequently investigated in an independent African American population from MESA (38 cases and 806 controls).

Methods

Subjects and LOA Case Definition

Data from WHI were used for discovery. WHI is a long-term national health study focusing on strategies for disease prevention in postmenopausal women.[11] Subject recruitment and screening were completed in 1998, and participants were followed for 8 years by 6 follow-up examinations (conducted in 2001, 2002, 2003, 2004, 2005, and 2006, respectively) for first-time asthma diagnosis by a physician. For the present analysis, LOA cases were defined as those who reported never having had an asthma diagnosis at the time of recruitment and who reported an asthma diagnosis for the first time during follow-up. Control subjects were those who never reported an asthma diagnosis during any examination.

Data from MESA were used to investigate the top SNPs and genes identified in WHI. MESA recruited subjects 45 to 84 years old who were free of clinical cardiovascular disease

and other serious clinical conditions across 6 US field centers.[12] The screening examination took place in 2000 and was followed by 4 examinations (conducted in 2002, 2004, 2005, and 2010, respectively). LOA cases and controls were defined as previously described for WHI. Subject exclusion and inclusion procedures in WHI and MESA are shown in eFigure 1.

Data for WHI and MESA were obtained from the database of Genotypes and Phenotypes (dbGaP; WHI accession number: phs000200.v10.p3; MESA accession number: phs000209.v12.p3). This study was approved by the human investigation committee of Yale University (New Haven, Connecticut).

Genotyping and Quality Control

In WHI and MESA, genomic DNA was isolated from peripheral blood samples and genotyped using the Affymetrix Genomewide Human SNP Array 6.0 chip containing 909,622 SNPs. Quality control (QC) procedures and subsequent genetic analyses were performed using PLINK v1.07.[13] Subjects were excluded based on low overall call rate (<95%) and/or if they had a failed sex check. SNPs were excluded based on low call rate in the case or control group (<98%), low minor allele frequency (<1%) in all subjects, and/or significant deviation from Hardy-Weinberg equilibrium in controls ($P < 1 \times 10^{-7}$). A subset of linkage disequilibrium (LD)-pruned SNPs was generated using pairwise $r^2 > 0.5$ in a window of 50 SNPs and shifted by 5 SNPs at each step for genetic QC procedures including identity-by-descent (IBD) and principal component (PC) analyses. The pairwise IBD matrix was calculated to assess cryptic relatedness (pi-hat > 0.2 in the IBD test). One subject from each pair was randomly removed. PC analysis was conducted using default parameters in EIGENSTRAT v3.0[14]; the resulting PCs were used as covariates in logistic regression models to control for population stratification. Outliers were identified after 5 iterations and were excluded from subsequent analysis.

QC procedures for WHI and MESA are presented in eTable 1. After exclusions, 1,218 WHI subjects (271 cases and 947 controls) and 844 MESA subjects (38 cases and 806 controls) were analyzed.

Statistical Analysis

Associations between LOA and potential risk factors, such as weight and smoking status, were evaluated at baseline. Weight status was ascertained by body mass index (BMI; kilograms per meter squared), which was calculated from objective height and weight measurements. Subjects were grouped into normal-weight (BMI <25 kg/m^2), overweight (BMI ≥25–<30 kg/m^2), and obese (BMI ≥30 kg/m^2) categories. Mean BMI values between subjects with and without LOA were tested with the *t* test. Weight status and “ever-smoking” prevalence between subjects with and without LOA were compared using the χ^2 test.

Odds ratios (ORs) were calculated to compare the likelihood of developing LOA in subjects of different weight and ever-smoking status. A logistic regression model was built according to the WHI statistical results. Age was retained in the models, regardless of the statistical significance of its association with LOA.

Genetic Analysis

For genetic analysis, SNPs were coded assuming an additive genetic model (0, 1, and 2, indicating the number of minor alleles). Genomewide association analysis was conducted for 1,218 WHI subjects with 864,525 SNPs that passed QC procedures. PCs were added into the logistic models, and the first one was sufficient to adjust for population stratification by calculating the genomic inflation factor ($\lambda = 1.01$). Quantile-quantile plots based on P values from the genomewide association analysis in WHI are shown in eFigure 2. The logistic regression model was adjusted for age, weight status (normal weight, overweight, or obese), dichotomously coded ever-smoking status, and first PC. A Bonferroni correction was used to adjust for multiple comparisons ($P < 5.78 \times 10^{-8} = .05/864{,}525$). The genomewide-suggestive P value was set at $P = 1.0 \times 10^{-5}$. Then, the 6 SNPs that were genomewide suggestive were tested in MESA, adjusting for the same covariates plus sex.

The Versatile Gene-Based Association Study (VEGAS) algorithm was applied to assign variants to genes and conduct gene-based analysis for WHI and MESA, respectively.[15] The VEGAS algorithm used all GWAS SNPs as input and assigned them to each gene according to positions on the UCSC Genome Browser hg18 assembly. The present study used the default gene boundary definition, which is 50 kb plus the translated regions, to capture regulatory regions and SNPs in LD. However, in general, different gene boundaries produce robust results from the VEGAS algorithm.[16] By using simulation based on the LD structure of the HapMap phase 2 population (in the present study, the Yoruba population in Ibadan, Nigeria), the VEGAS algorithm takes into account LD between markers in a gene. The top 10 genes from the WHI analysis were examined in MESA.

Results

Subject Summary and Statistics

Table 1 presents WHI and MESA subject characteristics stratified by status of LOA. Subjects with and without LOA were similar in age within and across the 2 datasets. However, mean BMI, smoking prevalence, and weight status were significantly associated with LOA status in WHI. On average, subjects with LOA in WHI had significantly higher BMI (32.10 ± 7.12 kg/m^2) than those without LOA (29.10 ± 5.90 kg/m^2; $P = 5.93 \times 10^{-10}$). The prevalence of ever smokers was significantly higher in subjects with LOA in WHI than in those without LOA (62% vs 50%; $P = 1.22 \times 10^{-3}$). Subjects with LOA in WHI also were more likely to be obese than those without LOA (55% vs 36%; $P = 8.78 \times 10^{-8}$). These differences also were observed in MESA but were not statistically significant.

Table 2 presents the unadjusted associations between subject characteristics and LOA. Compared with subjects of normal weight, obese subjects had significantly greater odds of developing LOA (OR 2.55, 95% confidence interval [CI] 1.74–3.76). Compared with never smokers, ever smokers also had greater odds of developing LOA (OR 1.59, 95% CI 1.21–2.09). These results are consistent with those of previous studies showing smoking and obesity as risk factors for LOA.[17]

Genomewide Single SNP Association Analysis

The overall SNP analysis results for WHI are shown in the Manhattan plot in Figure 1. No SNP was significantly associated with LOA based on a Bonferroni-corrected *P* value of 5.78×10^{-8}. However, 6 SNPs were suggestively associated with LOA ($P < 1 \times 10^{-5}$). Then, a genomewide association analysis was performed in MESA, adjusting for the same covariates as in WHI plus sex. Quantile-quantile plots based on *P* values from the genomewide association analysis in MESA are shown in eFigure 3. The 6 genomewide-suggestive SNPs from WHI were checked for an association in MESA. However, no SNPs reached a *P* value less than .05 (Table 3).

Gene-Based Analysis

To increase the power to detect genomic regions associated with LOA, a gene-based analysis was conducted using the VEGAS algorithm. After applying the VEGAS algorithm to WHI, the SNPs were shown to occupy 17,734 genes. Gene association *P* values were evaluated against a Bonferroni-corrected significance threshold ($0.05/17{,}734 = 2.82 \times 10^{-6}$). Table 4 lists the top 10 associated genes from WHI and their respective *P* values in MESA. Associations reaching nominal significance ($P < .05$) in MESA were considered potentially replicated. *ZNF248* was the most significantly associated gene in WHI ($P = 4.0 \times 10^{-7}$). This association also was observed in MESA and was nominally significant ($P = .015$). A plot for this region made in LocusZoom is shown in Figure 2.[18] The SNP with the lowest *P* value within each gene was reported by the VEGAS algorithm. In WHI, the best SNP within *ZNF248* was rs1208661 (OR 2.01, 95% CI 1.18–3.43; $P = .01$). In MESA, the best SNP within *ZNF248* was rs200944 (OR 1.89, 95% CI 1.18–3.04; $P = .008$).

Discussion

In this study, we used 2 prospective cohorts, WHI and MESA, to identify LOA cases among postmenopausal African Americans. Our study focused solely on LOA and verified that obesity and smoking are risk factors for LOA. We conducted a GWAS study in WHI, which identified 6 genomewide-suggestive SNPs associated with LOA. Furthermore, we performed gene-based analyses and identified a genomewide significant gene, *ZNF248*, that was associated with LOA in WHI and MESA.

In the GWAS, we identified 6 SNPs with genomewide-suggestive *P* values less than 1×10^{-5} in WHI. The genes harboring these SNPs have not been previously associated with asthma. However, a connection between some of these genomic regions (eg, rs1976063, which lies in the intronic region of *FRMD4B*, and rs10800888, which is 2 kb upstream of *ADIPOR*) is biologically plausible. Previous GWASs have associated *FRMD4B* with celiac disease, a T-cell–mediated immune disease caused by dietary gluten intolerance in genetically susceptible individuals.[19,20] Studies have shown that celiac disease and asthma are comorbid conditions, suggesting a shared genetic predisposition.[21,22] *ADIPOR1* is adiponectin receptor 1, which can stimulate ceramidase to degrade ceramide to sphingosine.[23] Phosphorylated sphingosine 1 (sphingosine-1 phosphate) has been shown to be involved in the pathogenesis of airway inflammation and asthma by promoting contraction of airway smooth muscle cells and regulating the activation and function of mast cells, eosinophils,

and dendritic cells.[24,25] Further investigation of these regions is warranted because the GWAS findings from WHI could not be replicated in MESA.

Compared with WHI, MESA had fewer LOA cases, possibly because MESA recruited relatively healthier individuals at the time of enrollment. Therefore, MESA might not have had enough power to replicate the WHI SNP findings. To increase detection power, we conducted a gene-based analysis, which collapses evidence of association across SNPs in genes rather than testing hundreds of thousands of SNPs separately. MESA successfully replicated the significant results from WHI in the gene-based analysis. *ZNF248* is located at 10p11.[26,27] It is a zinc finger gene that encodes a protein that is 579 amino acids in length and is expressed in a wide range of adult tissues.[27] *ZNF248* contains the Kruppel-associated box (KRAB) domain, which functions as a regulatory transcription factor.[27] Although the physiologic function of *ZNF248* has not been investigated, many ZNF proteins containing the KRAB domain have been shown to play an important role in the regulation of the immune system.[28] For example, mice experiments have demonstrated that *ZNF271* negatively regulate T-helper cell type 2 cell proliferation and control T-helper cell type 2–dependent diseases, such as allergic asthma.[29]

Moreover, to elucidate whether the *ZNF248* association is specific to LOA, we conducted a post hoc gene-based analysis for *ZNF248* and non-LOA in WHI and MESA. Non-LOA cases were defined as subjects who reported an asthma diagnosis during the screening examination. Controls were the same as those used in the LOA analysis. *ZNF248* was tested for the association with non-LOA in 1,684 subjects (736 non-LOA cases and 948 controls) in WHI and 993 subjects (187 non-LOA cases and 806 controls) in MESA. Despite increased detection power, the association between *ZNF248* and non-LOA was much weaker (eTable 2; $P = .002$ in the WHI, $P = .94$ in MESA). The results indicate that this association is dependent on age of asthma onset because *ZNF248* is associated only with LOA.

In this study, we used self-report of a physician diagnosis of asthma to define our phenotype. Self-reported asthma is commonly used in population-based studies, has been rigorously evaluated, and displays high sensitivity and specificity.[30–32] Nevertheless, we acknowledge that it would have been ideal to define the asthma phenotype using objective data (eg, pulmonary function test data). However, such data were not available in WHI or MESA, and, as a result, we cannot discard the possibility that our study was susceptible to some degree of misclassification of the outcome variable. Nonetheless, it is unlikely that misclassification was differential with respect to subject genotype. Therefore, any resulting bias would be toward the null (ie, would lead to an underestimation of the association). Given the intermittent nature of asthma, participants might have started to have asthma symptoms and to obtain treatment before the baseline examination but had not yet received a physician's diagnosis at that time. These subjects would likely get diagnosed during the follow-up and be misclassified as having LOA. We attempted to limit misclassification of these subjects by examining data on asthma medication available from MESA. Specifically, MESA subjects who were using asthma medication but did not report physician-diagnosed asthma during the baseline examination were excluded to avoid classifying those with

potential non-LOA as having LOA. No such data on asthma medication were available for WHI subjects.

Previous studies have shown that older patients frequently exhibit features that overlap between asthma and chronic obstructive pulmonary disease and the coexistence of asthma and emphysema and/or chronic bronchitis.[33,34] To minimize the effect of these comorbid conditions and decrease potential heterogeneity, we added a variable that captured self-report of a physician's diagnosis of at least emphysema, chronic bronchitis, and/or chronic obstructive pulmonary disease in WHI and MESA as a covariate in the logistic regression model to test the association of the SNPs in gene *ZNF248* with LOA and subsequently conducted a gene-based analysis (eTable 3). By adding this covariate to the regression model, the association between *ZNF248* and LOA in the 2 cohorts was strengthened (*P* values changed from 4.0×10^{-7} and .015 to 2.0×10^{-7} and .002 in WHI and MESA, respectively). A stronger signal was most likely achieved by decreasing heterogeneity in the phenotype, but this would need to be replicated in a dataset with better information on these comorbidities.

In conclusion, this is the first attempt to detect genetic risk factors associated with LOA in African Americans. A novel association between LOA and *ZNF248* was detected and replicated in 2 independent African American populations. This novel LOA-associated gene suggests a potential unique mechanism underlying LOA and could help elucidate specific disease pathways. Additional studies are clearly needed to explore the genetics of LOA and contribute to asthma prevention and therapy in elder populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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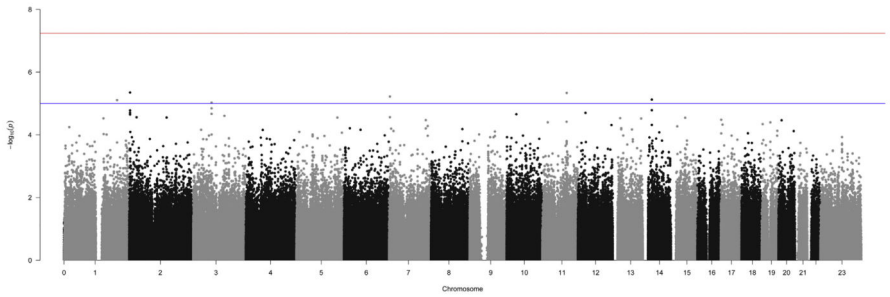

Figure 1.

Manhattan plot of the Women's Health Initiative genomewide association study results. The Manhattan plot was generated based on logistic regression analysis adjusted for age, smoking, weight status, and the first principal component in the Women's Health Initiative. The red line represents the genomewide-significant *P* value of 5.78×10^{-8}. The blue line represents the genomewide-suggestive *P* value of 1×10^{-5}.

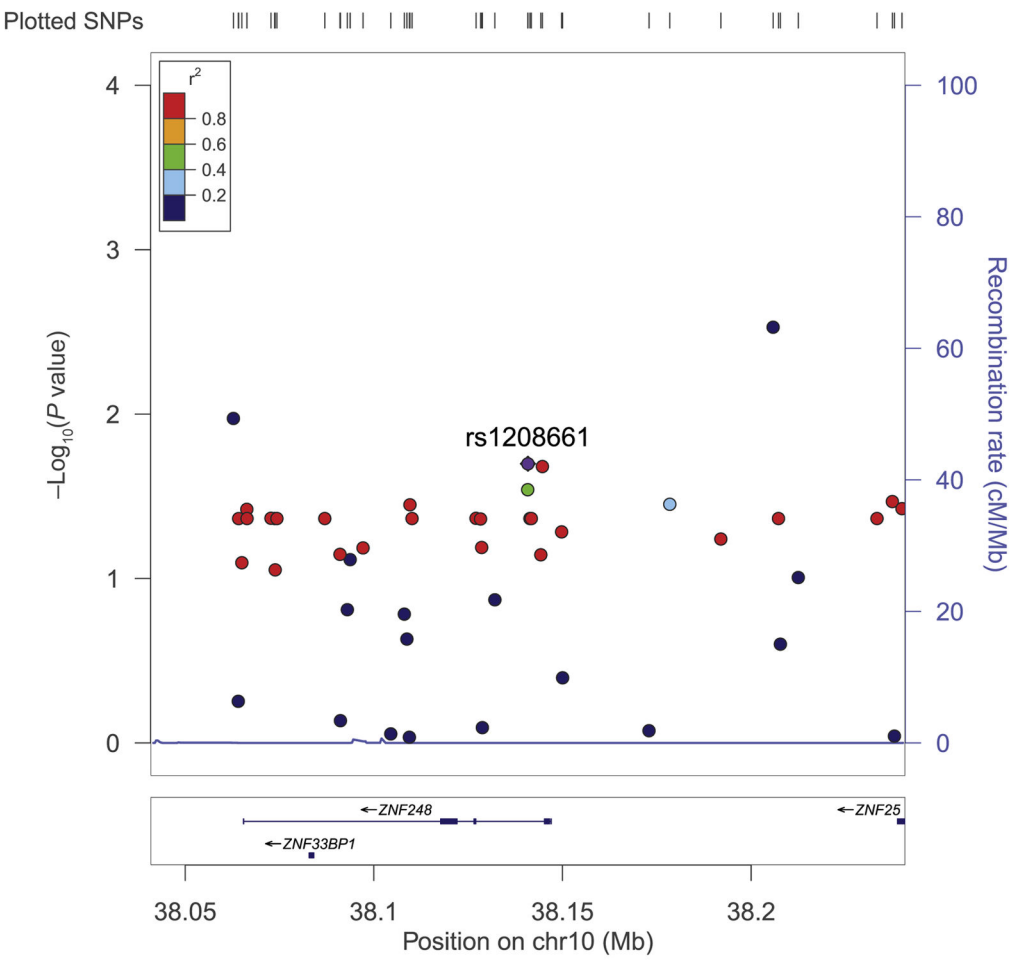

Figure 2.

Regional plot for the *ZNF248* locus. (Top) A regional plot was generated for *ZNF248* using the hg19/1000 Genomes AFR genome build as reference. *P* values on the $\log_{-10}$ scale are displayed on the y axis, and chromosome positions are displayed on the x axis. This plot shows the best single-nucleotide (rs1208661; purple) with a 100-kb flanking region on each side. The pairwise linkage disequilibrium pattern with rs1208661 is shown. (Bottom) Name, position, and transcription direction of genes in this region.

Table 1

Summary of subjects in the genetic analysis[a]

Characteristics	WHI (n = 1,218)			MESA (n = 844)		
	LOA (n = 271)	No asthma (n = 947)	*P* value[b]	LOA (n = 38)	No asthma (n = 806)	*P* value[b]
Age (y)[c]	61 ± 7	61 ± 7	.14	61 ± 9	61 ± 9	.93
Sex						.13
Men	0	0		13 (34%)	388 (48%)	
Women	271 (100%)	947 (100%)		25 (66%)	418 (52%)	
Smoking status			1.22×10^{-3}			.14
Never smoker	104 (38%)	471 (50%)		10 (26%)	320 (40%)	
Ever smoker	167 (62%)	476 (50%)		28 (74%)	486 (60%)	
BMI (kg/m^2)	32.10 ± 7.12	29.10 ± 5.90	5.93×10^{-10}	30.31 ± 5.72	30.02 ± 5.57	.76
Weight status			8.78×10^{-8}			.56
Normal weight	40 (15%)	235 (25%)		4 (11%)	139 (17%)	
Overweight	81 (30%)	367 (39%)		16 (42%)	319 (40%)	
Obesity	150 (55%)	345 (36%)		18 (47%)	348 (43%)	

Abbreviations: BMI, body mass index; LOA, late-onset asthma; MESA, Multi-Ethnic Study of Atherosclerosis; WHI, Women's Health Initiative.

[a] Values are presented as number (percentage) for categorical variables and mean ± SD for continuous variables.

[b] *P* values were calculated using *t* tests for continuous variables and χ^2 tests for categorical variables.

[c] Age was based on the screening examination in WHI and MESA.

Table 2

Unadjusted odds ratios for development of late-onset asthma for different populations in the Women's Health Initiative

	OR	95% CI	*P* value
Weight status			
Normal weight	1.00	reference	—
Overweight	1.30	0.86, 1.96	.22
Obese	2.55	1.74, 3.76	1.94×10^{-6}
Smoking status			
Never smoker	1.00	reference	—
Ever smoker	1.59	1.21–2.09	1×10^{-3}

Abbreviations: CI, confidence interval; OR, odds ratio.

Table 3

Genomewide suggestive SNPs in WHI and their replication in MESA

SNP	Chr	Position	Gene	Min/maj	WHI		MESA	
					OR (95% CI)	*P* value[a]	OR (95% CI)	*P* value[a]
rs11692425	2	3391612	*TRAPPC12*	T/C	0.56 (0.44–0.72)	4.44×10^{-6}	1.53 (0.94–2.49)	.09
rs10741446[b]	11	92824476	*FAT3*	C/T	0.55 (0.42–0.71)	4.58×10^{-6}		
rs1074119	7	3156700	*LOC100129603*	C/T	0.63 (0.51–0.77)	5.96×10^{-6}	1.00 (0.63–1.60)	.10
rs12437203[c]	14	34313130		G/A	1.59 (1.30–1.94)	7.48×10^{-6}	1.12 (0.70–1.80)	.63
rs10800888	1	202958899	*ADIPOR1*	A/G	1.93 (1.45–2.57)	7.75×10^{-6}	0.39 (0.14–1.1)	.08
rs1976063	3	69274129	*FRMD4B*	C/G	1.71 (1.35–2.17)	9.35×10^{-6}	1.42 (0.82–2.46)	.21

Abbreviations: Chr, chromosome; CI, confidence interval; min/maj, minor/major alleles; MESA, Multi-Ethnic Study of Atherosclerosis; OR, odds ratio; SNP, single-nucleotide polymorphism; WHI, Women's Health Initiative.

[a] *P* values were determined by logistic regression analysis, with age, smoking, weight status, and the first principal component as covariates in WHI or with sex, age, smoking, weight status, and the first principal component as covariates in MESA.

[b] SNP rs10741446 did not pass quality control in MESA.

[c] SNP rs12437203 did not map to any gene.

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Table 4

Top 10 genes from gene-based analysis in WHI and their replication in MESA

Gene	Chr	WHI					MESA				
		Gene-P[a]	SNPs, n	Best SNP	OR (95% CI)	SNP-P[b]	Gene-P[a]	SNPs, n	Best SNP	OR (95% CI)	SNP-P[b]
ZNF248	10	4.0×10^{-7}	29	rs1208661	2.01 (1.18–3.43)	.01	.015	28	rs200944	1.89 (1.18–3.04)	.01
FUT7	9	1.2×10^{-5}	5	rs2049040	0.64 (0.49–0.83)	6.04×10^{-4}	.09	5	rs13301354	1.66 (1.04–2.63)	.03
NPDC1	9	2.1×10^{-5}	7	rs2049040	0.64 (0.49–0.83)	6.04×10^{-4}	.15	7	rs13301354	1.66 (1.04–2.63)	.03
C9orf139	9	2.5×10^{-5}	7	rs2049040	0.64 (0.49–0.83)	6.04×10^{-4}	.18	7	rs13301354	1.66 (1.04–2.63)	.03
NKX2-8	14	4.6×10^{-5}	60	rs7144325	1.72 (1.30–2.29)	1.54×10^{-4}	.85	56	rs11622332	1.40 (0.88–2.22)	.15
TTC15	2	4.9×10^{-5}	58	rs11692425	0.56 (0.44–0.72)	4.44×10^{-6}	.30	58	rs10193454	0.49 (0.27–0.86)	.01
FAM136A	2	7.6×10^{-5}	30	rs1058256	0.21 (0.08–0.53)	9.92×10^{-4}	.93	26	rs2862185	2.68 (0.76–9.45)	.13
ENTPD2	9	8.7×10^{-5}	8	rs2049040	0.64 (0.49–0.83)	6.04×10^{-4}	.22	8	rs13301354	1.66 (1.04–2.63)	.03
SNRPG	2	1.1×10^{-5}	29	rs1058256	0.21 (0.08–0.53)	9.92×10^{-4}	.92	25	rs2862185	2.68 (0.76–9.45)	.13
UPF2	10	1.8×10^{-5}	38	rs10795917	0.56 (0.39–0.79)	9.71×10^{-4}	.81	38	rs7079388	2.67 (0.95–7.45)	.06

Abbreviations: Chr, chromosome; CI, confidence interval; Gene-P, gene *P* value; MESA, Multi-Ethnic Study of Atherosclerosis; OR, odds ratio; SNP, single-nucleotide polymorphism; SNP-P, SNP *P* value; WHI, Women's Health Initiative.

[a]Gene-P was based on gene-based analysis using the Versatile Gene-Based Association Study algorithm.

[b]SNP-P was based on a genomewide association study.