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Common *INSIG2* polymorphisms are associated with age-related changes in body size and high-density lipoprotein cholesterol from young adulthood to middle age

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Abstract

Insulin-induced gene 2 (*INSIG2*) plays an important role in the regulation of cholesterol and fatty acids synthesis. A polymorphism, rs7566605, located 10 kilobases upstream of the *INSIG2* gene, was identified in a genomewide association study of obesity. We conducted an association study of 12 *INSIG2* tag–single nucleotide polymorphisms with longitudinal measures of body size (body mass index and waist circumference) and lipid metabolism (plasma high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides levels). We investigated their interaction with age in 4304 Coronary Artery Risk Development in Young Adults participants (49.5% blacks, 50.5% whites) followed prospectively for 20 years. rs7566605 was not associated with variation in body size or lipid metabolism at any age in either racial group. However, rs1352083 and rs10185316 were associated with age-related decline in high-density lipoprotein cholesterol in whites ($P = .0005$ and .04, respectively). A similar trend was observed in blacks who consistently maintained a body mass index less than 25 kg/m^2 over the study period. These data support a role of *INSIG2* sequence variation in the regulation of cholesterol metabolism.

1. Introduction

Insulin-induced gene 2 (*INSIG2*) encodes a protein of the endoplasmic reticulum that prevents the proteolytic processing of sterol regulatory element-binding proteins (SREBPs) into active transcription factors, which regulate cholesterol and fatty acid synthesis [1]. A study integrating quantitative trait loci (QTL) mapping using a comprehensive single nucleotide polymorphism (SNP) map and gene expression profiling analyses identified *INSIG2* as a susceptibility gene influencing plasma cholesterol levels in mice [2]. However, few studies have examined the association between sequence variation in the *INSIG2* gene and measures of cholesterol metabolism in humans [3].

In a genomewide association study, a SNP (rs7566605) upstream of the *INSIG2* gene was recently identified, which was associated with increased body mass index (BMI) in 923 individuals from the Framingham Heart study [4]. This association was replicated in several but not all cohorts of varying ethnicity and age [5]. Many additional studies have failed to reproduce this finding [6-11]. Because the functional relevance of the rs7566605 polymorphism is uncertain and linkage disequilibrium (LD) patterns may vary across populations, investigation of additional polymorphisms in the *INSIG2* gene may provide further information about the relationship of this gene with metabolic risk factors, including obesity-related phenotypes and plasma lipids. Moreover, it has been suggested that age may play an

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important role in modifying the association between *INSIG2* gene variation and BMI [5]. To address these issues, we conducted an association study of 12 tagSNPs of the *INSIG2* gene, selected based on publicly available LD information, with longitudinal measures of body size (waist circumference and BMI) and plasma lipid levels (high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, and triglycerides); and we investigated their interaction with age in black and white Coronary Artery Risk Development in Young Adults (CARDIA) study participants followed prospectively for 20 years.

2. Material and methods

2.1. Study sample and data collection

Details about the CARDIA study design have been previously published [12]. Briefly, 5115 black and white men and women, 18 to 30 years of age, were initially recruited from the total community in Birmingham, AL; from selected census tracts in Chicago, IL, and Minneapolis, MN; and from the Kaiser-Permanente health plan membership in Oakland, CA. The initial study population was approximately balanced with respect to race, age, sex, and education groups. From the time of initiation of the study in 1985-1986, participants have completed 7 sequential examinations in years 0 (baseline), 2, 5, 7, 10, 15, and 20. Retention rates for the follow-up examinations were 90%, 86%, 81%, 79%, 74%, and 72%, respectively. Written informed consent was obtained from the participants at each examination, and all study protocols were approved by the institutional review boards of the participating institutions.

Each participant's age, race, and sex were self-reported during the recruitment phase and verified during the baseline clinic visit. Body weight, height, and waist circumference were assessed at each examination. Body weight was measured to the nearest 0.1 kg, using a calibrated scale, with the participant in light clothing without shoes. Height was measured to the nearest 0.5 cm with a vertical ruler. Body mass index was computed as body weight/height^2 (kilograms per square meter). Blood samples were drawn after an overnight fast at each examination. Total plasma cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels were measured according to standardized methods [13]. Participants eligible for the current study included 4304 individuals, including 2129 blacks and 2175 whites, who consented to isolation of genomic DNA from a blood sample obtained at the year-10, -15, or -20 examination. Individuals without genotype data were more likely to be black and male and were slightly younger and less educated, but were otherwise similar to those with genotype data (not shown).

2.2. Polymorphism selection and genotyping

Single nucleotide polymorphisms were chosen from publicly available genotype data on individuals of African and European ancestry from the HapMap project [14,15]. Within each race, a minimal set of tagSNPs was selected based on pairwise LD relationships (r^2), as described by Carlson et al [16] and implemented in the Tagger algorithm [17]. Briefly, bins of SNPs are created based on a specified r^2 threshold; and then one SNP is selected to represent the remainder of SNPs in that bin. In this study, we used an r^2 threshold of 0.8 and minimum allele frequency of 0.05. A total of 12 SNPs spanning a 28-kilobase region around the *INSIG2* gene were genotyped in the sample of CARDIA black and white participants using the TaqMan assay (Applied Biosystems, Foster City, CA) as previously described [18]. Primer and probes are available from the authors upon request. Polymorphism genotyping in the CARDIA study adheres to a rigorous quality control program, which includes barcode identification of samples, robotic sample handling, and blind replicate genotype assessment on 5% of the total sample (n = 219). The overall genotyping rate was 97%, and the concordance rate for blind duplicates was greater than 99%.

2.3. Statistical analyses

Genotype frequencies were estimated in each racial group by direct counting. Agreement of the genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using a χ^2 goodness-of-fit test.

To examine the association of *INSIG2* polymorphisms with 20-year measures of body size (BMI) and lipid metabolism (plasma HDL cholesterol, LDL cholesterol, and triglycerides levels), we first performed serial cross-sectional analyses on the subset of the CARDIA participants who completed all 7 examinations from year 0 to year 20. In each racial group, associations between each phenotype and individual tagSNPs were assessed using multiple linear regression models adjusting for baseline age, sex, and field center. Additional models adjusting for baseline BMI and current use of lipid-lowering medication were estimated in the analyses of plasma lipid levels. Triglyceride levels were log-transformed to reduce skewness. For each polymorphism, genotypes were coded as the number of copies (0, 1,

Table 1
Baseline characteristics of the CARDIA participants genotyped for *INSIG2* tagSNPs

Characteristic	Total cohort (n = 4304)	Constant cohort (n = 2705)
Age (y)	25.0 (3.6)	25.3 (3.5)
Male (%)	44.3	43.4
Black (%)	49.5	42.5
Education (y)	13.9 (2.2)	14.2 (2.2)
Current smoking (%)	29.1	24.1
BMI (kg/m^2)	24.5 (5.1)	24.4 (4.8)
LDL cholesterol (mg/dL)	109.5 (31.1)	109.8 (30.6)
HDL cholesterol (mg/dL)	53.1 (13.1)	53.3 (12.7)
Log-triglycerides (mg/dL)	4.15 (0.48)	4.14 (0.47)
Diabetes (%)	0.6%	0.6%

Table 2
Allele frequencies and *P* value for test of significance of departure from Hardy-Weinberg equilibrium expectations for 12 *INSIG2* tagSNPs in each racial group

Polymorphism	Chromosomal position	Gene location	Allele 1	Allele 2	Blacks (n = 2129)		Whites (n = 2175)	
					Freq (allele 1)	HWE *P*	Freq (allele 1)	HWE *P*
rs7566605	118552495	5′ Flanking	C	G	0.26	.15	0.33	.32
rs10185316	118560948	5′ Flanking	G	C	0.30	.40	0.32	.66
rs4848492	118561741	5′ Flanking	C	T	0.20	.59	0.10	.61
rs13428113	118563355	Intron 1	C	T	0.41	.24	0.58	.65
rs1352083	118564311	Intron 1	T	C	0.27	.70	0.25	.82
rs12464355	118566320	Intron 1	G	A	0.01	1.00	0.09	.11
rs17528324	118572626	Intron 2	A	G	0.01	1.00	0.05	.67
rs889904	118576941	Intron 2	G	A	0.40	.96	0.52	.28
rs10490624	118578962	Intron 3	G	A	0.11	.14	0.09	.59
rs13409050	118580240	Intron 3	A	G	0.25	.48	0.07	.63
rs9308762	118580344	Intron 3	C	T	0.14	.06	0.17	.75
rs17047764	118585051	3′ Flanking	C	G	0.39	.37	0.16	.81

HWE indicates Hardy-Weinberg equilibrium.

or 2) of the reference allele. Additive models have been shown to perform well even when the underlying inheritance model is recessive or dominant [19,20]. Significance of allelic effects on each trait was determined empirically by permutation-derived *P* values. Both pointwise and family-wise (ie, corrected for multiple tests) permutation-derived *P* values were estimated.

To examine the age-dependent effects of *INSIG2* polymorphisms on measures of body size and lipid metabolism independently from secular trends or other age-unrelated changes in the phenotypes, we performed longitudinal analyses using generalized estimating equations. In each racial group, models were estimated with *INSIG2* genotype (coded as previously), time-dependent age, and genotype × time-dependent age interaction as independent variables and adjusting for sex, field center, and examination. Additional models adjusting for time-dependent BMI and lipid-lowering medication use were estimated for the analyses of plasma lipids.

3. Results

Baseline characteristics of the CARDIA participants included in this study are shown in Table 1. Of the 4304 CARDIA participants with available DNA, there were 2705 individuals who participated in all 7 examinations. Compared with the total cohort, individuals in the constant cohort were more likely to be female, white, and slightly better educated and were less likely to smoke. Allele frequencies of the 12 *INSIG2* polymorphisms are shown in Table 2 by race. Genotype frequency distributions of all polymorphisms were in agreement with Hardy-Weinberg equilibrium expectations in each racial group. Pairwise LD between the *INSIG2* SNPs

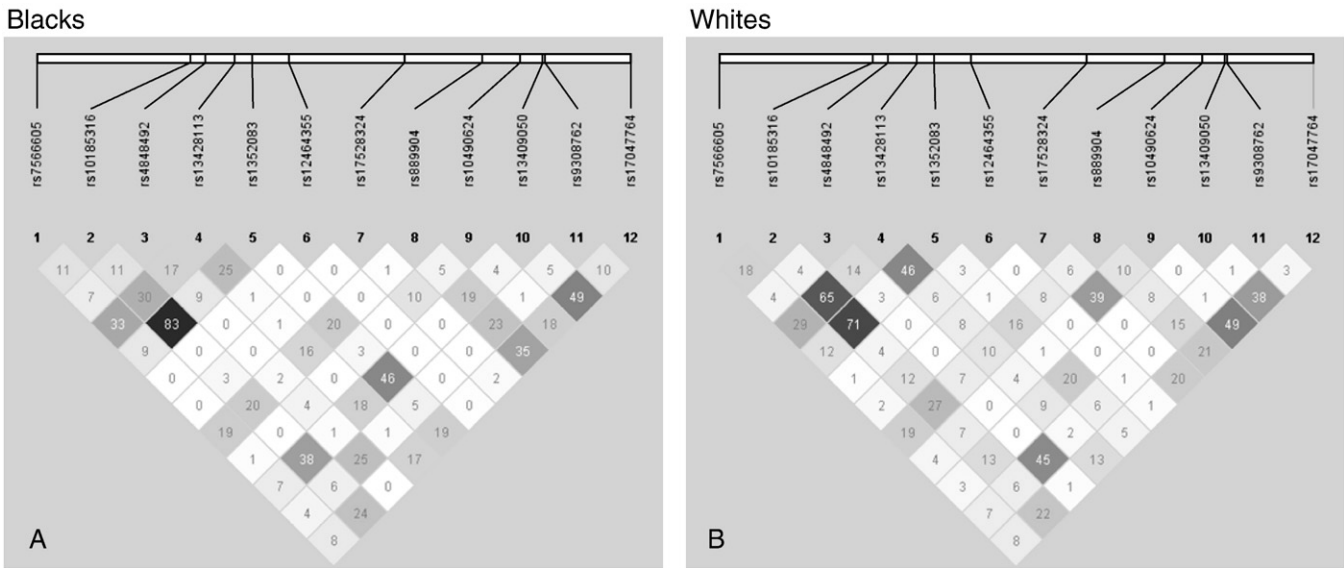

Fig. 1. Pairwise LD (r^2) among 12 *INSIG2* tagSNPs by racial group.

is shown in Fig. 1. As expected based on our tagSNP selection strategy, there was little LD between the selected SNPs, with the exception of rs10185316 and rs1352083 in blacks ($r^2 = 0.83$).

We first performed serial cross-sectional analyses of association between each *INSIG2* SNP and lipid levels and measures of body size in the constant cohort by race. In blacks, rs13409050 was associated with variation in BMI at all examinations; and both significance of association and magnitude of the effect tended to increase over time. At baseline, individuals carrying the common rs13409050_G allele had a 0.60-kg/m^2–higher BMI per copy ($P = .03$). At year 20, these individuals had a 1.1-kg/m^2–higher BMI per copy ($P = .01$) (Table 3). Similar results were obtained in the association of the rs13409050 polymorphism with waist circumference. At baseline, individuals carrying the common rs13409050_G allele had a 1.1-cm–higher waist circumference per copy ($P = .05$). At year 20, these individuals had a 2.1-cm–higher waist circumference per copy ($P = .01$). In blacks from the CARDIA study, there was no significant association of *INSIG2* polymorphisms with plasma HDL cholesterol levels (Table 4) at any of the 7 examinations, similarly for plasma LDL cholesterol and triglyceride levels (not shown).

In whites, none of the *INSIG2* polymorphisms were significantly associated with variation in body size at any of the 7 examinations (Table 3). However, 2 polymorphisms in moderate LD ($r^2 = 0.7$), rs10185316 and rs1352083, showed similar associations with variation in HDL cholesterol (Table 4). At baseline, individuals carrying the variant allele rs10185316_G had a 0.84-mg/dL–lower plasma HDL cholesterol level per copy ($P = .05$) compared with those who did not carry this allele. The magnitude and statistical significance of this difference increased over time. At year 20, these individuals had a 1.8-mg/dL–lower plasma HDL cholesterol per copy ($P = .003$). A similar trend was observed for the rs1352083_T variant, although both the magnitude and statistical significance of the difference in plasma HDL cholesterol between variant and wild-type allele were slightly less than for rs10185316. Adjusting for BMI and current use of lipid-lowering medication did not significantly affect these associations (Table 4). There was no significant association of *INSIG2* polymorphisms with plasma LDL cholesterol or triglycerides levels at any of the 7 examinations (not shown).

Table 3
Serial cross-sectional association of *INSIG2* tagSNPs with BMI in the constant cohort by race

SNP	Ref allele	Year 0		Year 2		Year 5		Year 7		Year 10		Year 15		Year 20	
		Difference[a] (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b
African Americans (n = 1149)															
rs7566605	C	0.05 (0.27)	.86	0.02 (0.30)	.96	0.04 (0.31)	.89	−0.05 (0.32)	.87	0.04 (0.33)	.90	0.38 (0.35)	0.28	0.20 (0.36)	.56
rs10185316	G	−0.46 (0.25)	.07	−0.50 (0.27)	.07	−0.6 (0.28)	.03	−0.60 (0.30)	.04	−0.54 (0.31)	.08	−0.57 (0.33)	.08	−0.58 (0.33)	.09
rs4848492	C	0.30 (0.29)	.32	0.32 (0.32)	.32	0.34 (0.34)	.33	0.30 (0.35)	.39	0.36 (0.36)	.32	0.09 (0.38)	.80	0.15 (0.39)	.69
rs13428113	C	−0.07 (0.23)	.75	0.002 (0.25)	.98	0.01 (0.26)	.96	0.004 (0.28)	.99	−0.03 (0.28)	.90	0.21 (0.30)	.48	0.20 (0.31)	.49
rs1352083	T	−0.45 (0.26)	.07	−0.56 (0.29)	.05	−0.64 (0.30)	.04	−0.70 (0.31)	.02	−0.62 (0.32)	.06	−0.66 (0.34)	.05	−0.73 (0.35)	.05
rs12464355	G	0.05 (0.97)	.95	0.19 (1.06)	.85	0.21 (1.13)	.85	0.12 (1.16)	.91	−0.70 (1.21)	.59	−0.63 (1.28)	.63	−0.58 (1.30)	.66
rs17528324	A	−1.71 (0.96)	.08	−1.20 (1.07)	.23	−1.81 (1.11)	.08	−1.17 (1.15)	.33	−0.96 (1.19)	.40	−1.38 (1.27)	.27	−0.75 (1.31)	.57
rs889904	G	0.12 (0.24)	.61	0.13 (0.26)	.63	0.07 (0.27)	.81	−0.07 (0.28)	.83	−0.03 (0.30)	.89	0.01 (0.31)	.95	0.18 (0.32)	.58
rs10490624	G	0.02 (0.37)	.95	0.12 (0.41)	.78	0.43 (0.43)	.31	0.55 (0.45)	.22	0.66 (0.46)	.15	0.22 (0.49)	.65	0.66 (0.50)	.21
rs13409050	G	0.60 (0.27)	.03	0.68 (0.30)	.02	0.80 (0.31)	.02	0.83 (0.33)	.01*	0.81 (0.34)	.02	1.0 (0.36)	.005†	0.95 (0.34)	.01*
rs9308762	C	0.19 (0.34)	.58	0.24 (0.37)	.51	0.07 (0.39)	.87	0.04 (0.41)	.93	−0.04 (0.42)	.93	−0.08 (0.45)	.87	−0.17 (0.46)	.70
Whites (n = 1556)															
rs7566605	C	−0.12 (0.15)	.43	−0.10 (0.16)	.46	−0.03 (0.17)	.86	0.02 (0.19)	.90	0.002 (0.20)	.99	−0.08 (0.22)	.72	−0.21 (0.26)	.43
rs10185316	G	0.14 (0.15)	.33	0.11 (0.16)	.48	0.17 (0.17)	.33	0.09 (0.19)	.66	0.17 (0.20)	.37	0.14 (0.22)	.54	−0.06 (0.25)	.82
rs4848492	C	0.17 (0.23)	.47	0.09 (0.25)	.74	0.01 (0.27)	.97	−0.01 (0.30)	.96	−0.05 (0.32)	.83	−0.26 (0.36)	.50	−0.13 (0.40)	.73
rs13428113	T	0.19 (0.14)	.15	0.15 (0.15)	.32	0.17 (0.17)	.30	0.08 (0.18)	.64	0.14 (0.19)	.46	0.05 (0.22)	.81	−0.09 (0.25)	.73
rs1352083	T	−0.001 (0.16)	.99	−0.04 (0.17)	.81	0.11 (0.19)	.53	0.07 (0.21)	.72	0.09 (0.22)	.65	0.08 (0.24)	.75	−0.19 (0.28)	.50
rs12464355	G	−0.03 (0.25)	.90	−0.01 (0.27)	.96	−0.03 (0.29)	.90	0.13 (0.32)	.68	0.07 (0.34)	.84	0.33 (0.38)	.38	0.38 (0.44)	.36
rs17528324	A	0.32 (0.30)	.28	0.33 (0.32)	.32	−0.001 (0.35)	.99	−0.11 (0.39)	.79	0.16 (0.41)	.67	.25 (0.46)	.60	0.26 (0.53)	.61
rs889904	A	0.22 (0.14)	.12	0.22 (0.15)	.12	0.26 (0.16)	.10	0.24 (0.18)	.19	0.30 (0.19)	.10	0.41 (0.21)	.06	0.35 (0.24)	.15
rs10490624	G	0.07 (0.24)	.77	0.06 (0.26)	.80	−0.02 (0.28)	.94	−0.11 (0.31)	.75	0.04 (0.33)	.89	0.11 (0.37)	.76	0.06 (0.42)	.87
rs13409050	G	−0.46 (0.27)	.09	−0.30 (0.30)	.31	−0.74 (0.32)	.02	−0.70 (0.35)	.05	−0.81 (0.38)	.04	−0.91 (0.41)	.03	−0.74 (0.48)	.14
rs9308762	C	0.17 (0.19)	.36	0.07 (0.20)	.72	0.02 (0.22)	.92	−0.02 (0.25)	.91	−0.08 (0.26)	.73	−0.07 (0.29)	.83	0.21 (0.33)	.54

Models were adjusted for age, sex, and field center. Ref indicates reference; SE, standard error.

[a] Expressed in kilograms per square meter.

[b] Pointwise empirical *P* value derived from permutation.

* Familywise empirical *P* value <.10.

† Familywise empirical *P* value <.05.

Table 4
Serial cross-sectional association of *INSIG2* tagSNPs with HDL cholesterol levels in the constant cohort by race

SNP	Ref allele	Year 0		Year 2		Year 5		Year 7		Year 10		Year 15		Year 20	
		Difference[a] (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b	Difference (SE) in trait value per copy of ref allele	P^b
African Americans (n = 1149)															
rs7566605	C	0.41 (0.61)	.53	0.52 (0.69)	.44	0.01 (0.68)	.98	−0.08 (0.68)	.90	0.27 (0.66)	.68	0.11 (0.66)	.87	0.84 (0.76)	.29
rs10185316	G	0.65 (0.57)	.26	0.77 (0.64)	.24	0.56 (0.64)	.39	0.93 (0.63)	.14	0.42 (0.62)	.49	0.18 (0.62)	.77	0.05 (0.72)	.95
rs4848492	C	1.12 (0.66)	.10	−0.76 (0.76)	.31	0.93 (0.76)	.21	0.57 (0.74)	.44	0.76 (0.72)	.31	0.67 (0.73)	.38	0.47 (0.84)	.56
rs13428113	C	−0.98 (0.52)	.06	−0.21 (0.59)	.71	−1.22 (0.59)	.03	−0.95 (0.58)	.10	−0.70 (0.57)	.22	−0.40 (0.57)	.47	−0.24 (0.66)	.73
rs1352083	T	0.50 (0.60)	.41	0.58 (0.67)	.38	0.54 (0.67)	.39	1.15 (0.66)	.08	0.44 (0.64)	.47	0.36 (0.65)	.58	−0.15 (0.75)	.81
rs12464355	G	1.91 (2.21)	.38	1.75 (2.45)	.45	−2.97 (2.52)	.22	−1.25 (2.45)	.61	−1.19 (2.39)	.63	1.50 (2.40)	.51	−0.46 (2.79)	.87
rs17528324	A	0.57 (2.23)	.80	−0.38 (2.52)	.87	0.52 (2.49)	.84	−0.78 (2.46)	.75	−0.23 (2.44)	.93	−0.70 (2.46)	.77	1.17 (2.81)	.66
rs889904	G	−0.57 (0.54)	.29	−0.57 (0.61)	.34	−0.73 (0.62)	.21	−0.54 (0.61)	.37	0.18 (0.59)	.76	−0.15 (0.59)	.81	0.46 (0.69)	.51
rs10490624	G	0.75 (0.85)	.36	0.21 (0.96)	.83	0.52 (0.96)	.57	−0.19 (0.93)	.83	0.11 (0.92)	.91	0.25 (0.93)	.79	0.02 (1.0)	.98
rs13409050	G	−0.84 (0.62)	.19	−0.90 (0.70)	.20	−1.12 (0.71)	.11	−1.81 (0.69)	.01*	−1.07 (0.68)	.11	−1.20 (0.68)	.08	−0.91 (0.79)	.24
rs9308762	C	0.56 (0.77)	.45	−1.27 (0.89)	.15	0.16 (0.88)	.85	0.28 (0.86)	.74	0.49 (0.85)	.55	0.28 (0.84)	.75	0.60 (0.99)	.53
Whites (n = 1556)															
rs7566605	C	0.06 (0.45)	.90	0.21 (0.49)	.66	0.12 (0.49)	.81	−0.03 (0.49)	.96	0.25 (0.48)	.62	0.15 (0.50)	.77	0.28 (0.58)	.60
rs10185316	G	−0.84 (0.45)	.05	−1.1 (0.49)	.04	−1.1 (0.49)	.03	−1.2 (0.49)	.02	−1.6 (0.48)	.004†	−1.5 (0.51)	.005†	−1.8 (0.58)	.003†
		−0.71 (0.43)[c]	.09	−0.80 (0.47)[c]	.10	−0.72 (0.46)[c]	.10	−0.91 (0.47)[c]	.05	−1.3 (0.46)[c]	.005†	−1.3 (0.49)[c]	.005†	−1.6 (0.56)[c]	.004†
rs4848492	C	0.04 (0.71)	.96	−0.21 (0.78)	.79	0.33 (0.77)	.64	0.89 (0.78)	.27	0.74 (0.77)	.33	1.33 (0.81)	.10	1.3 (0.92)	.17
rs13428113	T	−0.71 (0.43)	.10	−0.94 (0.47)	.05	−0.60 (0.46)	.19	−0.91 (0.47)	.19	−1.0 (0.46)	.04	−0.85 (0.48)	.07	−1.07 (0.55)	.06
rs1352083	T	−0.3 (0.48)	.74	−0.3 (0.53)	.74	−0.6 (0.52)	.27	−0.7 (0.53)	.27	−1.2 (0.53)	.03	−1.3 (0.55)	.02	−1.8 (0.62)	.007†
		−0.15 (0.46)[c]	.74	−0.16 (0.51)[c]	.75	−0.34 (0.50)[c]	.49	−0.54 (0.51)[c]	.29	−1.0 (0.50)[c]	.04	−1.1 (0.53)[c]	.03	−1.6 (0.60)[c]	.01*
rs12464355	G	−1.0 (0.76)	.19	0.85 (0.83)	.32	−0.15 (0.81)	.85	0.34 (0.82)	.66	0.05 (0.82)	.93	−0.57 (0.85)	.52	−1.0 (0.97)	.30
rs17528324	A	−1.9 (0.92)	.05	−2.44 (1.0)	.01	−1.71 (0.99)	.10	−1.6 (1.0)	.10	−1.7 (0.99)	.09	−1.67 (1.04)	.12	−0.99 (1.19)	.41
rs889904	A	−0.71 (0.43)	.10	−0.51 (0.47)	.27	−0.52 (0.46)	.24	−0.36 (0.47)	.45	−0.58 (0.46)	.22	−1.10 (0.48)	.02	−1.21 (0.55)	.03
rs10490624	G	−0.82 (0.73)	.27	−0.93 (0.81)	.25	−0.67 (0.79)	.38	−0.23 (0.81)	.76	−0.87 (0.79)	.28	−1.08 (0.83)	.20	−0.23 (0.95)	.79
rs13409050	G	−0.71 (0.83)	.40	0.64 (0.91)	.49	0.06 (0.89)	.95	1.04 (0.91)	.25	0.85 (0.89)	.34	0.95 (0.93)	.32	1.18 (1.06)	.24
rs9308762	C	0.21 (0.58)	.71	0.16 (0.63)	.79	0.28 (0.62)	.66	0.65 (0.63)	.32	0.53 (0.63)	.41	1.33 (0.65)	.05	1.10 (0.74)	.15

Models were adjusted for age, sex, and field center.
[a] Expressed in milligrams per deciliter.
[b] Pointwise empirical *P* value derived from permutation.
[c] Model further adjusted for baseline BMI and current use of lipid-lowering medication.
* Familywise empirical *P* value <.10.
† Familywise empirical *P* value <.05.

We next examined the association of *INSIG2* polymorphisms with age-related changes in measures of body size and plasma lipid levels using generalized estimating equations models in the total cohort. Consistent with results of the serial cross-sectional analyses, we observed a significant association of rs13409050 with variation in BMI in blacks; and this association was age dependent. At age 18 years, individuals carrying the common allele of rs13409050 (G) had a slightly higher BMI per copy (0.4 kg/m^2, P = .07) (Table 5) and slightly larger waist circumference (0.7 cm, P = .09) compared with individuals who did not carry this allele. However, with increasing age, these individuals had a significant increase in BMI (0.02 kg/m^2 per year of age and per copy of the allele, P = .02) (Table 5) and waist circumference (0.05 cm per year of age and per copy of the allele). In whites, we also observed an age-dependent association between *INSIG2* SNPs and HDL cholesterol levels, consistent with results from the serial cross-sectional analyses (Table 6). At age 18 years, individuals carrying the variant allele rs10185316_G had a slightly lower plasma HDL cholesterol level per copy (0.7 mg/dL, P = .05) compared with those who did not carry this allele. With increasing age, these individuals tended to have increasingly lower plasma HDL cholesterol levels (0.03 mg/dL per year of age and per copy of the allele, P = .08). Similarly for rs1352083, individuals aged 18 years carrying the variant rs1352083_T allele had slightly, although not significantly, lower HDL cholesterol levels than those who did not carry this allele. However, as they got older, carriers of the rs1352083_T allele had a greater decline in HDL cholesterol levels (0.07 mg/dL per year of age and per copy of the allele, P = .002). Further adjusting for BMI and use of lipid-lowering medication did not significantly modify these age-dependent associations (Table 6).

We also examined the association of *INSIG2* sequence variants with plasma lipid levels in a subset of the CARDIA participants who consistently maintained their BMI at less than 25 kg/m^2 over the 20-year study period (n = 246 blacks, 615 whites). In whites who were consistently lean, rs1352083_T and rs10185316_G were significantly associ-

Table 5
Age-dependent association of selected *INSIG2* tagSNPs with BMI in the total cohort by race

SNP	Reference allele	Difference[a] in trait value (95% CI) at age 18 y per copy of reference allele	*P*	Change[a] in trait value (95% CI) per year of age per copy of reference allele	*P*
African Americans (n = 2129)					
rs7566605	C	−0.03 (−0.46; 0.39)	.88	0.004 (−0.015; 0.023)	.65
rs10185316	G	0.02 (−0.37; 0.41)	.92	−0.006 (−0.024; 0.011)	.49
rs4848492	C	0.01 (−0.48; 0.51)	.95	−0.008 (−0.029; 0.013)	.44
rs13428113	C	−0.003 (−0.36; 0.36)	.99	0.009 (−0.008; 0.025)	.32
rs1352083	T	−0.06 (−0.45; 0.34)	.77	−0.011 (−0.029; 0.007)	.23
rs12464355	G	0.47 (−1.44; 2.39)	.62	−0.03 (−0.11; 0.05)	.51
rs17528324	A	−0.16 (−1.83; 1.50)	.85	0.003 (−0.063; 0.069)	.92
rs889904	G	0.10 (−0.27; 0.47)	.60	0.001 (−0.016; 0.018)	.93
rs10490624	G	−0.04 (−0.62; 0.54)	.89	0.011 (−0.017; 0.039)	.44
rs13409050	G	0.4 (0.03; 0.80)	.07	0.02 (0.01; 0.03)	.02
rs9308762	C	0.12 (−0.43; 0.68)	.66	−0.006 (−0.031; 0.018)	.61
Whites (n = 2175)					
rs7566605	C	0.19 (−0.09; 0.47)	.20	−0.003 (−0.019; 0.012)	.69
rs10185316	G	0.10 (−0.17; 0.37)	.48	−0.005 (−0.019; 0.010)	.51
rs4848492	C	0.20 (−0.30; 0.69)	.44	−0.014 (−0.043; 0.015)	.35
rs13428113	C	−0.18 (−0.45; 0.08)	.17	0.009 (−0.005; 0.023)	.18
rs1352083	T	0.07 (−0.22; 0.37)	.62	−0.003 (−0.018; 0.013)	.75
rs12464355	G	−0.26 (−0.68; 0.16)	.23	0.017 (−0.010; 0.044)	.22
rs17528324	A	−0.07 (−0.56; 0.42)	.77	−0.005 (−0.032; 0.023)	.74
rs889904	G	−0.004 (−0.26; 0.26)	.98	−0.008 (−0.021; 0.006)	.26
rs10490624	G	−0.15 (−0.54; 0.23)	.44	0.004 (−0.018; 0.027)	.70
rs13409050	G	0.002 (−0.47; 0.48)	.99	0.023 (−0.002; 0.048)	.08
rs9308762	C	0.08 (−0.31; 0.46)	.69	−0.006 (−0.027; 0.014)	.55

Models were adjusted for examination, sex, and field center. CI indicates confidence interval.
[a] Expressed in kilograms per square meter.

ated with a greater age-related decrease in HDL cholesterol (0.13 and 0.11 mg/dL per year of age and per copy of the allele, $P = .0004$ and .003, respectively). In blacks, a similar trend was observed, although statistical significance was not reached (0.08 and 0.10 mg/dL per year of age and per copy of the allele, $P = .10$ and .12, respectively).

In an effort to determine whether the identified SNPs had potential functional consequences, we screened the 2 tagSNPs against a set of previously reported expression-associated SNPs in lymphoblastoid cell lines [21]. Neither polymorphism (or their proxy) was associated with variation in *INSIG2* gene expression levels in cell lines.

4. Discussion

In the CARDIA cohort of young adults of African and European ancestry, sequence variants of the *INSIG2* gene were significantly associated with BMI and HDL cholesterol; and these associations were age dependent.

We failed to reproduce an association of rs7566605 with measures of body size, including BMI at any age, in either blacks or whites, consistent with our previous report [11] and similar to many studies [7-10]. Although it was previously suggested that this polymorphism was associated with an increased BMI in overweight individuals [10], additional analyses of a subgroup of CARDIA participants who were consistently overweight over the 20-year study period (n = 537 blacks, 475 whites) did not reveal a significant association of rs7566605 with body size (not shown). Our results also did not support an earlier suggestion of an interaction of this polymorphism with age, namely, a stronger effect on BMI in younger individuals and a decreasing effect size with increasing age [5]. A recent report also showed suggestive evidence of an association of this polymorphism with BMI in children [22]. Nonetheless, we report a significant association of the common allele of rs13409050—a tagSNP located in intron 3 of the *INSIG2* gene—with a greater BMI and waist circumference in blacks. We also provide evidence of an interaction of this polymorphism with age on body size, specifically an increasing effect size with increasing age. These associations were not observed in whites, possibly because of differences in allele frequency, in LD patterns, or in genetic or environmental contexts. The mechanisms that underlie the association of *INSIG2* variants and increased BMI remain unclear. *INSIG2* is ubiquitously expressed, and adipocyte differentiation is characterized by an enhanced *INSIG2* expression [23]. It has been recently suggested that *INSIG2* may regulate adipogenesis via the SREBP1 [23,24]. A recent study in Hispanic individuals also reported an association of *INSIG2* gene variants with computed tomography–measured adiposity [25]. Future characterization of functional variation in the *INSIG2* gene

Table 6
Age-dependent association of selected *INSIG2* tagSNPs with HDL cholesterol levels in the total cohort by race

SNP	Reference allele	Difference[a] in trait value (95% CI) at age 18 y per copy of reference allele	*P*	Change[a] in trait value (95% CI) per year of age per copy of reference allele	*P*
African Americans (n = 2129)					
rs7566605	C	−0.07 (−0.93; 0.80)	.88	0.006 (−0.043; 0.055)	.81
rs10185316	G	0.27 (−0.53; 1.08)	.51	−0.024 (−0.068; 0.020)	.29
rs4848492	C	0.21 (−0.76; 1.18)	.67	0.02 (−0.03; 0.07)	.43
rs13428113	C	−0.47 (−1.22; 0.28)	.22	0.008 (−0.030; 0.047)	.66
rs1352083	T	0.05 (−0.79; 0.88)	.91	−0.015 (−0.061; 0.030)	.50
rs12464355	G	−0.48 (−3.84; 2.87)	.78	0.02 (−0.12; 0.16)	.78
rs17528324	A	1.01 (−1.63; 3.65)	.45	0.03 (−0.14; 0.20)	.76
rs889904	G	−0.65 (−1.43; 0.13)	.10	0.025 (−0.017; 0.066)	.24
rs10490624	G	0.50 (−0.76; 1.77)	.44	−0.012 (−0.076; 0.051)	.70
rs13409050	G	0.72 (−0.16; 1.60)	.11	0.014 (−0.036; 0.063)	.59
rs9308762	C	−0.41 (−1.48; 0.67)	.46	−0.012 (−0.068; 0.043)	.66
Whites (n = 2175)					
rs7566605	C	−0.20 (−0.93; 0.53)	.59	−0.002 (−0.042; 0.037)	.90
rs10185316	G	−0.7 (−1.4; 0.01)	.05	−0.03 (−0.07; 0.01)	.09
		−0.6 (−1.3; 0.06)[b]	.07	−0.04 (−0.07; 0.00)[b]	.05
rs4848492	C	0.22 (−1.03; 1.47)	.73	0.06 (−0.005; 0.118)	.07
rs13428113	C	0.56 (−0.14; 1.26)	.11	0.013 (−0.025; 0.051)	.49
rs1352083	T	−0.19 (−0.98; 0.59)	.62	−0.07 (−0.11; −0.02)	.002
		−0.14 (−0.88; 0.60)[b]	.71	−0.07 (−0.11; −0.03)[b]	.0007
rs12464355	G	0.99 (−0.46; 2.43)	.18	−0.046 (−0.115; 0.023)	.19
rs17528324	A	−1.48 (−3.06; 0.10)	.06	0.07 (−0.01; 0.15)	.09
rs889904	G	0.11 (−0.56; 0.80)	.74	0.02 (−0.01; 0.06)	.27
rs10490624	G	−0.74 (−1.91; 0.44)	.22	0.012 (−0.051; 0.076)	.70
rs13409050	G	0.13 (−1.28; 1.54)	.85	−0.09 (−0.16; −0.02)	.01
rs9308762	C	0.09 (−0.83; 1.02)	.84	0.053 (0.004; 0.103)	.03

Models were adjusted for examination, sex, and field center.
[a] Expressed in milligrams per deciliter.
[b] Also adjusted for time-dependent BMI and use of lipid-lowering medication.

may shed light on the relationship of this gene with obesity-related traits.

We provide evidence of an association of 2 *INSIG2* variants, rs1352083 and rs10185316, with age-dependent decline in HDL cholesterol in the 20-year period from young adulthood to middle age. This association was observed in both black and white individuals who maintained a normal weight (BMI <25 kg/m^2) over the study period, although statistical significance was reached only for whites. rs10185316 is located in the promoter region of the gene, and rs1352083 is located in intron 1. These variants have similar allele frequencies in the 2 racial groups and are in LD. The relationship between *INSIG2* and cholesterol metabolism has been well documented in experimental studies. In the presence of sterols, *INSIG2* prevents the proteolytic activation of SREBPs by Golgi enzymes and, hence, blocks cholesterol metabolism [1]. Moreover, *INSIG2* has been shown to regulate the degradation of hydroxymethylglutaryl–coenzyme A reductase, a rate-limiting enzyme in cholesterol synthesis [26,27]. However, few studies have investigated the association of *INSIG2* polymorphism with plasma lipid levels. No significant association of rs7566605 was reported with plasma levels of total, HDL, or LDL cholesterol in middle-aged individuals from European ancestry [3] or Japanese ancestry [28]. However, none of these studies examined the relationship of other *INSIG2* gene variants with longitudinal profiles of plasma lipid levels.

Despite the clear strengths of our study, including a well-characterized biracial sample of young adults followed prospectively for 20 years, and a more comprehensive tagSNP strategy in the *INSIG2* gene, some limitations must be acknowledged. First, we have not examined possible interactions of *INSIG2* polymorphisms with environmental or other genetic factors known to influence body size and plasma lipid levels. Therefore, we cannot exclude that the associations (or lack thereof) reported here may vary in different environmental contexts, such as physical activity or dietary fat intake, or in the presence of other susceptibility genes [29]. Second, association studies cannot distinguish the causal SNP (or combination of SNPs) among those in LD. Therefore, as the functional relevance of the identified SNPs is uncertain, we cannot exclude that a polymorphism (s) unrelated to *INSIG2* function may be responsible for the effects observed in this study. Finally, the novel SNP associations reported here require validation through analysis in other cohorts. In particular, replication in other African American cohorts taking into account genetic measures of ancestry is warranted to rule out any spurious findings due to population stratification.

In summary, common variants of the *INSIG2* gene are associated with age-dependent increase in body size and

decrease in plasma HDL cholesterol in the period of young adulthood to early middle age. The molecular mechanisms that mediate these relationships remain to be investigated. In particular, characterization of functional variants in the *INSIG2* gene will contribute toward a better understanding of the role of this gene in susceptibility to metabolic and cardiovascular dysfunction.

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