

Genetic Associations with Obstructive Sleep Apnea Traits in Hispanic/Latino Americans

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Abstract

Rationale: Obstructive sleep apnea is a common disorder associated with increased risk for cardiovascular disease, diabetes, and premature mortality. Although there is strong clinical and epidemiologic evidence supporting the importance of genetic factors in influencing obstructive sleep apnea, its genetic basis is still largely unknown. Prior genetic studies focused on traits defined using the apnea–hypopnea index, which contains limited information on potentially important genetically determined physiologic factors, such as propensity for hypoxemia and respiratory arousability.

Objectives: To define novel obstructive sleep apnea genetic risk loci for obstructive sleep apnea, we conducted genome-wide association studies of quantitative traits in Hispanic/Latino Americans from three cohorts.

Methods: Genome-wide data from as many as 12,558 participants in the Hispanic Community Health Study/Study of Latinos, Multi-Ethnic Study of Atherosclerosis, and Starr County Health Studies population-based cohorts were metaanalyzed for association with

the apnea–hypopnea index, average oxygen saturation during sleep, and average respiratory event duration.

Measurements and Main Results: Two novel loci were identified at genome-level significance (rs11691765, *GPR83*, $P = 1.90 \times 10^{-8}$ for the apnea–hypopnea index, and rs35424364; *C6ORF183/CCDC162P*, $P = 4.88 \times 10^{-8}$ for respiratory event duration) and seven additional loci were identified with suggestive significance ($P < 5 \times 10^{-7}$). Secondary sex-stratified analyses also identified one significant and several suggestive associations. Multiple loci overlapped genes with biologic plausibility.

Conclusions: These are the first genome-level significant findings reported for obstructive sleep apnea–related physiologic traits in any population. These findings identify novel associations in inflammatory, hypoxia signaling, and sleep pathways.

Keywords: sleep apnea; hypoxia; genetics; GWAS; Prader–Willi syndrome

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At a Glance Commentary

Scientific Knowledge on the

Subject: Previous genetic studies of obstructive sleep apnea (OSA) have largely focused on candidate genes and have not found any associations at commonly accepted levels of significance. Previous studies have also focused on the apnea–hypopnea index, which may not fully reflect information on key OSA physiologic parameters. No studies have systematically examined genetic associations with OSA in Hispanics/Latinos.

What This Study Adds to the

Field: In the largest genetic study of OSA to date, we investigate the genetics of the apnea–hypopnea index, average oxyhemoglobin saturation across the sleep episode, and average apnea/hypopnea event duration length in Hispanic/Latino Americans, a growing population with a high risk of OSA and related comorbidities. We identify three loci significantly associated with OSA traits, and several suggestively associated loci. Several of the identified loci contain genes with plausible OSA-related functions.

Obstructive sleep apnea (OSA) is a common disorder characterized by recurrent episodes of partial or complete upper airway obstruction that result in intermittent hypoxemia and sleep disruption. Individuals with OSA are at increased risk for cardiovascular disease, diabetes, and other disorders that carry significant morbidity and premature mortality, underscoring the need to better understand the etiology of this disorder and to identify targets for novel interventions (1–3). Although there is strong evidence supporting the importance of familial, and specifically genetic factors in influencing OSA susceptibility (4), its genetic basis is not well understood. Moreover, the genetic risk of OSA is known to vary by continental ancestries; a study of 31 global ancestral informative markers in Brazilians has indicated ancestry-related differences of risk for OSA (5). No prior study has studied the genetic associations of OSA in Hispanic/Latino Americans, a growing demographic group with a high prevalence of OSA and associated cardiovascular disease risk factors (6–8).

The physiologic components underlying OSA include contributions from energy balance/obesity, craniofacial structure, upper airway neuronal control, ventilatory control, and inflammation (4). In this paper, we

studied the apnea–hypopnea index (AHI) as the primary measure of OSA severity given its heritability ($h^2 = 0.37$) (9) and widespread clinical use. We also examined two other traits. First, the average percentage of oxyhemoglobin saturation (Sp_{O_2}) during the sleep period, a measure widely available and easily collected in large numbers of individuals using pulse oximetry. Mean sleep Sp_{O_2} correlates with AHI while also providing information on a key physiologic stress associated with OSA. Second, the average respiratory disturbance (apnea or hypopnea) length, a measure reflecting the respiratory arousal threshold, which has been postulated to be a phenotype informative for targeted interventions addressing respiratory stability (10).

In this study, we conducted the largest genome-wide association study (GWAS) of three OSA traits to date. Primary analyses were adjusted for body mass index (BMI) to increase the likelihood of identifying variants that influence OSA through obesity-independent pathways. We focus on Hispanic/Latino Americans, a growing population at high risk for OSA and related comorbidities. We also conducted sex-stratified analyses given differences in OSA clinical manifestations (6).

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Methods

Study Sample

Data were derived from three community-based cohort studies using in-home overnight sleep studies: Hispanic Community Health Study/Study of Latinos (HCHS/SOL), Multi-Ethnic Study of Atherosclerosis (MESA), and the Starr County Health Studies (Starr) (11–13). These studies comprise all known Hispanic/Latino OSA studies with available genome-wide data. Demographic and basic clinical parameters are presented in Table 1. Additional characteristics of each study are provided in the METHODS section of the online supplement.

Phenotype and Covariate Definitions

The quantitative phenotypic outcomes were (1) AHI (defined by events associated with a $\geq 3\%$ desaturation); (2) average hemoglobin oxygen saturation across the sleep period (Sp_{O_2} ; excluding intermittent waking episodes); and (3) average length of apnea/hypopnea events (“event duration,” defined as the average length of respiratory disturbances lasting 10 s or more). The sleep apnea assessments were based on type 3 home sleep apnea testing

monitors for HCHS/SOL (ARES Unicorder 5.2; Advanced Brain Monitoring, Inc. [formerly B-Alert], Carlsbad, CA) and Starr (WatchPAT-200; Itamar Medical Ltd., Caesarea, Israel); and with 14-channel at-home polysomnography for MESA (Compumedics Somte; Compumedics, Ltd., Abbotsford, VIC, Australia). Further descriptions of equipment and more detailed definition of phenotypes are provided in the METHODS section of the online supplement. All sleep records were scored by a central Sleep Reading Center at Brigham and Women’s Hospital with high levels of established reliability (14). Covariates were obtained by questionnaires, direct measurement (BMI), and oximetry (waking oxygen saturation was measured before the sleep recording).

Genotyping and Quality Control

HCHS/SOL participants were assayed with an Illumina Omni 2.5M chip (Illumina, Inc., San Diego, CA). MESA and Starr participants were assayed with an Affymetrix 6.0 chip (Affymetrix, Inc., Santa Clara, CA). Imputation based on the 1000 Genomes Project (15) and quality control is described in the online supplement.

All single-nucleotide polymorphisms (SNPs) and insertion/deletions with an

IMPUTE2 (16) Info score less than 0.88, minor allele frequency less than 1%, or fewer than 20 minor allele counts within a cohort were removed from applicable analyses.

Statistical Analysis

AHI and Sp_{O_2} values were inverse-normal rank-normalized and event duration values were log-transformed (*see* METHODS section of the online supplement) to meet statistical assumptions for normality. Residuals were constructed for each trait using identical covariates. The primary model adjusted for age, age², sex, age \times sex, BMI, and BMI². Population stratification and cryptic relatedness were controlled for using a linear mixed model or population-specific principal components, as described in the online supplement.

GWAS were performed on the residuals using an additive model for SNP effects in linear mixed models (17, 18). A fixed effect, inverse variance weighted metaanalysis was performed using METAL with genomic control applied to each cohort’s results (19). Statistically significant and suggestive thresholds were set at P less than 5.0×10^{-8} and P less than 5.0×10^{-7} , respectively. The choice of P less than 5×10^{-8} reflects a Bonferroni correction

Table 1. Sample Description

	HCHS/SOL	MESA	Starr County
N, AHI/sleep O_2 saturation/event duration	11,317/11,317/9,791	459/458/447	782/782/0
Age, yr, mean (SD)	46.17 (13.79)	68.34 (9.20)	52.34 (11.29)
Percent female	59.1	52.8	71.9
BMI, kg/m ² , mean (SD)	29.79 (6.00)	30.07 (5.52)	32.15 (6.78)
AHI, events/h, median (IQR)	1.97 (0.42–6.62)	17.00 (7.50–30.53)	10.35 (3.60–20.78)
Sleep O_2 saturation, %, mean (SD)	96.45 (0.95)	94.33 (1.56)	94.65 (2.09)
Waking O_2 saturation, %, mean (SD)	96.94 (3.13)	96.12 (1.37)	95.93 (2.43)
Event duration, s, mean (SD)	23.62 (7.98)	21.21 (5.92)	NA
Percent sleep under 90% saturation, mean (SD)	0.85 (3.14)	3.84 (7.35)	2.83 (8.79)
Percent asthma	7.70	5.25	NA
Percent COPD	2.78	0.44	NA
Percent diabetes	19.55	27.47	47.90
Percent hypertensive	27.60	58.30	57.25
Percent Central American	10.37	NA	0.00
Percent Cuban	14.63	3.05	0.00
Percent Dominican	9.44	18.54	0.00
Percent Mexican	39.51	49.77	100.00
Percent Puerto Rican	16.48	14.55	0.00
Percent South American	6.49	NA	0.00
Percent other Hispanic	3.08	14.08	0.00

Definition of abbreviations: AHI = apnea–hypopnea index; BMI = body mass index; COPD = chronic obstructive pulmonary disease; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; IQR = interquartile range; MESA = Multi-Ethnic Study of Atherosclerosis; NA = not available; Starr = Starr County Health Studies.

Sample sizes are listed for AHI (12,558)/average O_2 saturation (12,557)/average event duration (10,238) individuals with genotypes and phenotypes. Event duration sample size is reduced because of availability in Starr County and the exclusion of individuals without an apnea or hypopnea event. Population groups are self-identified for Hispanics/Latinos residing in the United States.

of 1 million independent tests, following a long-standing tradition in genome-wide studies (20, 21). Other choices, including calculating the number of independent linkage disequilibrium blocks, may be less effective when considering differences in admixture patterns. Secondary models were stratified by sex. To reduce the possibility of false-positives caused by small sample sizes, we only report results for SNPs where there was a minimum sample size of 1,000. We defined a locus region to include all SNPs with P less than 1.0×10^{-6} that were within 500 kb of the lead SNP. LocusZoom (22) was used for regional visualization with SNP linkage disequilibrium calculated within HCHS/SOL (comprised of six Hispanic/Latino population background groups).

Results

Study Sample

An overview of sample characteristics is shown in Table 1. As many as 12,558 individuals contributed to the analyses of AHI and average SpO₂ in sleep. Fewer participants contributed to the analysis of average event duration (10,238) because of the exclusion of individuals with no respiratory disturbances and an absence of this phenotype in the Starr County sample. The mean ages across cohorts were 46–68 (HCHS/SOL, 46.1 [SD = 13.8]; Starr, 52.3 [SD = 9.2]; MESA, 68.3 [SD = 11.3]). The percentage of female participants ranged from 53 to 72% (MESA, 52.8%; HCHS/SOL, 59.1%; Starr, 71.9%). The largest demographic subgroup was of Mexican background (including all of the Starr participants), with additional self-identified Caribbean, Central American, and South American backgrounds. Average BMI varied from 29.8 to 32.2 across cohorts (HCHS/SOL,

29.8; MESA, 30.1; Starr, 32.2). Type 2 diabetes was common in the cohorts (HCHS/SOL, 19.6%; MESA, 27.5%; Starr, 47.9%). The median AHI was lower in HCHS/SOL relative to other cohorts (HCHS/SOL, 1.97; Starr, 10.35; MESA, 17.00), likely reflecting the younger age and lower BMI of this cohort.

Pairwise Correlations between OSA Phenotypes

Pairwise correlations between OSA phenotypes, calculated across all three cohorts, are presented in Table 2. AHI was strongly correlated with average sleep SpO₂ and modestly correlated with event duration (pooled Spearman $\rho = -0.608$ and 0.220, respectively). A weak correlation between sleep SpO₂ and event duration was also observed ($\rho = -0.089$). Waking SpO₂ was modestly correlated with average sleep SpO₂ and with AHI ($\rho = 0.303$ and -0.155 , respectively). Both age and BMI were most strongly correlated with AHI, followed by sleep SpO₂ and event duration, respectively. Cohort-specific cross-phenotype correlations results are shown in Table E1 in the online supplement.

GWAS Results

We performed metaanalyses of GWAS for AHI, average sleep SpO₂, and respiratory (apnea and/or hypopnea) event duration. Manhattan and QQ plots for analyses across both sexes and stratified by sex are presented in Figures E1–E3. The QQ plots and genomic inflation factors showed little inflation (range, 0.980–1.015). Lead SNPs for statistically significant or suggestive associations with AHI, sleep SpO₂, and event duration are shown in Tables 3–5.

A significant association with AHI was found at chr 11q21 (lead SNP rs116791765, $P = 1.90 \times 10^{-8}$). The locus included 28 SNPs with P less than 1×10^{-6}

and spanned several genes (*GPR83*, *LINC01171/C11ORF97*, *MRE11A*) (Table 3, Figure 1). A significant association with event duration was found at chr 6q21 (lead SNP rs35424364, $P = 4.88 \times 10^{-8}$). This locus included three SNPs with P less than 1×10^{-6} that spanned the pseudogenes *CCDC162P* and *C6ORF183/LOC100996694* (Table 5, Figure 2; see Table E2).

Additionally, there were one AHI, two SpO₂, and four event duration signals with suggestive evidence of association ($P < 5 \times 10^{-7}$) (Tables 3–5). These signals included SNPs that overlap biologically plausible genes, including rs2743173 (event duration; *PLCB1*) (see Figure E8) and rs116133558 (sleep SpO₂; *ATP2B4/PMCA4B*) (see Figure E6). Additional details and results for SNPs at each locus with P less than 1×10^{-6} are listed in Table E2. Regional association plots are shown in Figures E4–E21. Regional plots for the significant associations are provided in Figures E22–E29. Forest plots for all lead loci effect sizes and standard errors within individual cohorts are shown in Figures E30–E36.

OSA likely reflects genetic factors that operate through BMI-dependent and BMI-independent pathways (9). Results for the top BMI-adjusted model regions were broadly similar using a BMI-unadjusted model (Tables 3–5), suggesting that these novel loci do not contribute to OSA through obesity.

Sex-stratified analyses identified six additional suggestive AHI regions, one suggestive sleep episode SpO₂ region, and one significant and four suggestive event duration regions (Table 6; see Table E3).

For AHI, rs11588454 was marginally above genome-level significance for association in males ($P = 6.97 \times 10^{-8}$). The 190-kb locus region with SNP P values less than 1×10^{-6} is intergenic. *RGS18* is the nearest protein-coding gene (see Figure E13).

Table 2. Pairwise Correlations between OSA Phenotypes and Select Covariates

	AHI	Sleep SpO ₂	Event Duration	Wake SpO ₂	Age
Sleep SpO ₂	—	—	—	—	—
Event duration	−0.608 (−0.619 to −0.596)	−0.089 (−0.109 to −0.068)	—	—	—
Wake SpO ₂	−0.155 (−0.173 to −0.137)	0.303 (0.286 to 0.319)	0.003 (−0.018 to 0.023)	—	—
Age	0.427 (0.411 to 0.442)	−0.391 (−0.406 to −0.375)	0.220 (0.200 to 0.239)	−0.128 (−0.146 to −0.109)	—
BMI	0.375 (0.359 to 0.391)	−0.300 (−0.317 to −0.283)	−0.127 (−0.147 to −0.106)	−0.063 (−0.081 to −0.044)	0.083 (0.064 to 0.101)

Definition of abbreviations: AHI = apnea–hypopnea index; BMI = body mass index; OSA = obstructive sleep apnea; SpO₂ = average percentage of oxyhemoglobin saturation.

Each table cell lists Spearman ρ correlations metaanalyzed using Fisher Z-transformations weighted by sample size. Ninety-five percent confidence intervals are listed in parentheses.

Table 3. Genome-Level Significant and Suggestive Associations with AHI

SNP	B37 Region	SNPs in Region	Genes in Region	N	CAF	β (SE) BMI Adjusted	β (SE) BMI Unadjusted	P Value	Direction
rs116791765 T	11q21:94,074,914-94,242,697	28	GPR83, LINC01171, MRE11A	11,774	0.989/0.988/NA	-0.323 (0.057)	-0.345 (0.061)	1.90×10^{-8} (1.84×10^{-9})	--?
rs999944 A	2p14:65,049,853	1		12,557	0.112/0.106/0.073	-0.095 (0.019)	-0.104 (0.020)	4.53×10^{-7} (2.48×10^{-7})	--+

Definition of abbreviations: AHI = apnea-hypopnea index; BMI = body mass index; CAF = coded allele frequency; NA = not available; SNP = single-nucleotide polymorphism. Lead SNPs for regions with significant ($P < 5.0 \times 10^{-8}$) *P* values. Individual regions were minimally 500 kb apart. Minimum $n = 1,000$. SNPs in region denotes the count of regional SNPs with *P* less than 1.0×10^{-6} . Genes in region indicates overlapping nonpseudogene Ensembl genes within 5 kb of the *P* less than 1×10^{-6} SNPs. *P* values in parentheses were obtained from equivalent BMI-unadjusted model results. Individual regional SNP results are provided in Table E2. BMI-adjusted and unadjusted directions are equivalent except where noted. CAF and direction columns are ordered from left to right as Hispanic Community Health Study/Latinos, Multi-Ethnic Study of Latinos, Multi-Ethnic Study of Atherosclerosis, and Starr County Health Studies.

Table 4. Suggestive Associations with Sleep SpO₂

SNP	B37 Region	SNPs in Region	Genes in Region	N	CAF	β (SE) BMI Adjusted	β (SE) BMI Unadjusted	P Value	Direction
rs75108997 A	1q42:233,947,565-233,954,231	4		11,809	0.013/0.014/NA	-0.291 (0.055)	-0.296 (0.058)	1.40×10^{-7} (3.52×10^{-7})	--?
rs116133558 T	1q32:203,716,843	1	ATP2B4	11,351	0.014/NA/NA	0.291 (0.056)	0.231 (0.059)	2.18×10^{-7} (9.09×10^{-5})	++?

Definition of abbreviations: BMI = body mass index; CAF = coded allele frequency; NA = not available; SNP = single-nucleotide polymorphism; SpO₂ = average percentage of oxyhemoglobin saturation. *P* values in parentheses were obtained from equivalent BMI-unadjusted model results. CAF and direction columns are ordered from left to right as Hispanic Community Health Study/Latinos, Multi-Ethnic Study of Atherosclerosis, and Starr County Health Studies.

Table 5. Genome-Level Significant and Suggestive Associations with Average Event Duration

SNP	B37 Region	SNPs in Region	Genes in Region	N	CAF	β (SE) BMI Adjusted	β (SE) BMI Unadjusted	P Value	Direction
rs35424364 A	6q21:109,584,988–109,643,605	3	C6ORF183, CCDC162P	10,240	0.129/0.097/NA	0.030 (0.006)	0.032 (0.006)	4.88×10^{-8} (1.82×10^{-8})	++
rs74472562 T	11p11:44,762,755	1	RP11-45A12.2, TSPAN18	10,240	0.086/0.092/NA	-0.034 (0.006)	-0.035 (0.006)	1.05×10^{-7} (6.47×10^{-8})	--
rs2743173 T	20p12:8,245,293	1	PLCB1	10,240	0.567/0.570/NA	0.019 (0.004)	0.019 (0.004)	3.85×10^{-7} (2.23×10^{-7})	++
rs72699765 A	15q12:25,809,781–25,829,197	4	AC124997.1	10,240	0.110/0.112/NA	0.029 (0.006)	0.029 (0.006)	3.93×10^{-7} (5.20×10^{-7})	++
rs2033354 T*	2p24:15,212,995–15,218,152	5		10,240	0.784/0.779/NA	-0.022 (0.004)	-0.022 (0.004)	4.77×10^{-7} (3.65×10^{-7})	--

Definition of abbreviations: BMI = body mass index; CAF = coded allele frequency; NA = not available; SNP = single-nucleotide polymorphism. P values in parentheses were obtained from equivalent BMI-unadjusted model results. CAF and direction columns are ordered from left to right as Hispanic Community Health Study/Study of Latinos and Multi-Ethnic Study of Atherosclerosis. *Heterogeneity $P = 0.012$.

Table 6. Suggestive Sex-stratified Associations

Model	SNP	B37 Region	SNPs in Region	Genes in Region	N	CAF	β (SE) BMI Adjusted	β (SE) BMI Unadjusted	P Value	Direction
AHI females	rs199803244 A	12p11:27,645,082–27,659,326	3	SMCO2	6,691	0.945/NA/NA	0.182 (0.035)	0.187 (0.037)	1.64×10^{-7} (4.98×10^{-7})	+??
	rs11897825 A	2p24:21,694,451	1	AC011752.1, AC067959.1	6,691	0.606/NA/NA	-0.085 (0.017)	-0.090 (0.018)	4.34×10^{-7} (5.26×10^{-7})	-??
AHI males	rs11588454 T	1q31:191,618,878–191,808,947	14		5,062	0.750/0.710/0.701	-0.114 (0.021)	-0.124 (0.023)	6.97×10^{-8} (8.04×10^{-8})	--
	rs140743827 A	1q23:164,985,756	1		4,626	0.018/NA/NA	0.392 (0.076)	0.428 (0.083)	2.26×10^{-7} (2.27×10^{-7})	+??
	rs4796285 A	17q12:35,181,996	1		5,062	0.581/0.556/0.593	-0.096 (0.019)	-0.092 (0.020)	3.09×10^{-7} (7.15×10^{-6})	--
	rs111942351 A	5q31:143,073,550	1	CTB-57H20.1	4,842	0.981/0.984/NA	0.363 (0.071)	0.338 (0.078)	3.75×10^{-7} (1.34×10^{-6})	+??
SpO ₂ males	rs11074782 T	16p12:26,505,011	1		5,076	0.230/0.205/0.263	-0.112 (0.022)	-0.113 (0.024)	2.95×10^{-7} (1.55×10^{-6})	--
Event duration females	rs78897171 A	20q13:60,443,251	1	CDH4	5,589	0.023/NA/NA	0.106 (0.020)	0.108 (0.020)	1.63×10^{-7} (1.12×10^{-7})	+?
	rs35329661 T	11q13:74,974,990	1	ARRB1, CTD-2562J17.7, CTD-2562J17.9	5,589	0.013/NA/NA	0.132 (0.026)	0.134 (0.026)	2.80×10^{-7} (2.06×10^{-7})	+?
Event duration males	rs148024591 T	15q26:93,906,988–93,927,482	10	RP11-26608.1	4,414	0.017/0.015/NA	0.119 (0.021)	0.117 (0.022)	2.81×10^{-8} (6.72×10^{-8})	++
	rs4849682 T	2q14:118,911,223–118,942,883	2	AC093901.1	4,414	0.290/0.320/NA	-0.032 (0.006)	-0.030 (0.006)	1.07×10^{-7} (4.56×10^{-7})	--
	rs11610782 A	12q24:126,196,289–126,256,105	2		4,204	0.024/NA/NA	0.109 (0.021)	0.108 (0.021)	3.01×10^{-7} (3.69×10^{-7})	+?

Definition of abbreviations: AHI = apnea-hypopnea index; BMI = body mass index; CAF = coded allele frequency; NA = not available; SNP = single-nucleotide polymorphism; SpO₂ = average percentage of oxyhemoglobin saturation. P values in parentheses were obtained from equivalent BMI-unadjusted model results. CAF and direction columns are ordered from left to right as Hispanic Community Health Study/Study of Latinos, Multi-Ethnic Study of Atherosclerosis, and Starr County. Event duration was not collected in Starr County.

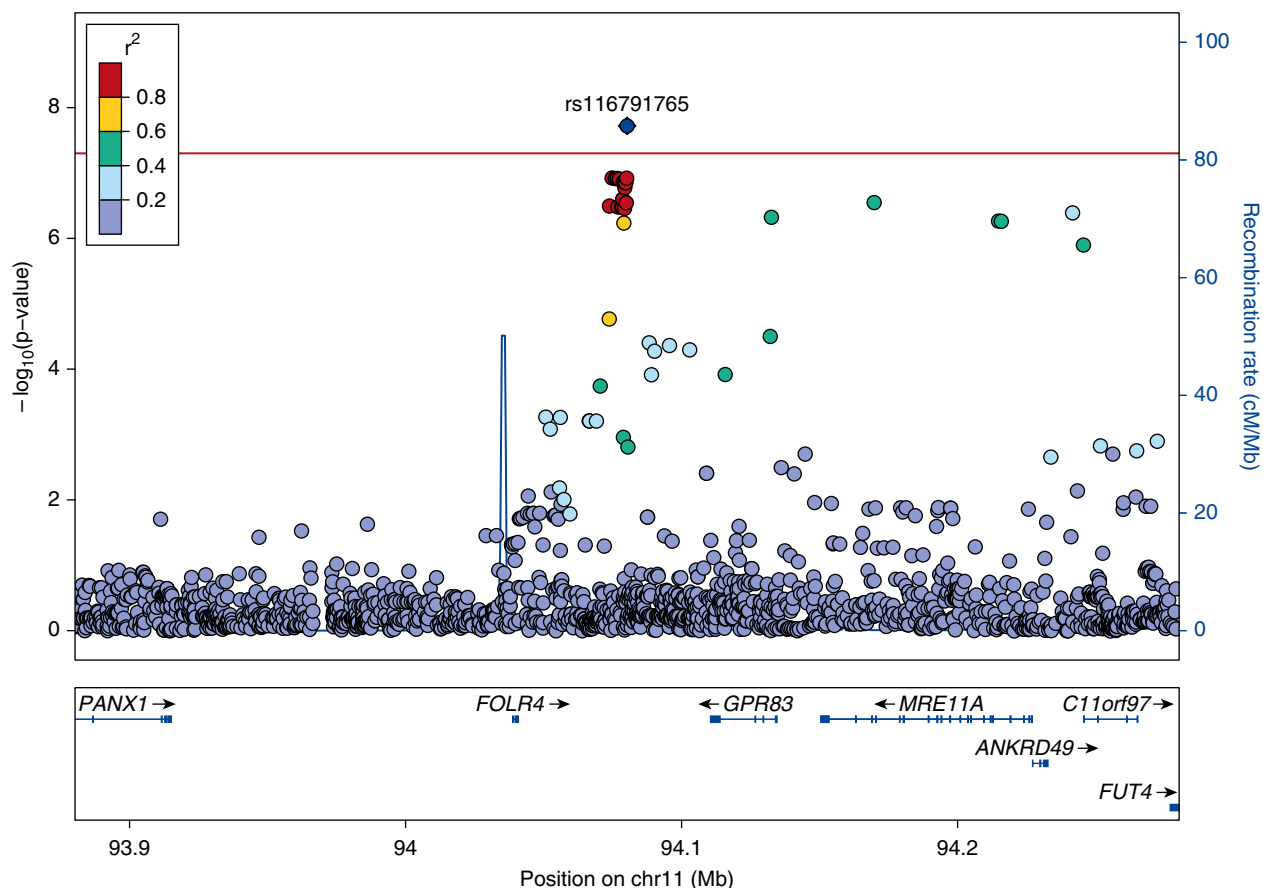


Figure 1. rs116791765 apnea-hypopnea index regional association plot. Physical positions (Build 37 coordinates) are shown on the x-axis. Log-transformed P values are shown on the y-axis. Single-nucleotide polymorphism (SNP) colors indicate the degree of linkage disequilibrium with the lead SNP rs116791765 (based on Hispanic Community Health Study/Study of Latinos genotypes). Regional recombination rates are denoted with the blue line, with recombination hotspots indicated by large increases in the recombination rates. Although rs116791765 is located in an intergenic region, the entire locus with P values $< 1 \times 10^{-6}$ spans three genes (*GPR83*, *MRE11A*, and *ANKRD49*). A fourth gene (*ANKRD49*) is within the region but does not contain any SNPs with P values $< 1 \times 10^{-6}$.

For event duration, rs148024591 was significantly associated in males ($P = 2.81 \times 10^{-8}$; 10 regional SNPs $P < 1 \times 10^{-6}$) (Figure 3). rs148024591 is within the lincRNA gene RP11-226O8.1 (Ensembl ID ENSG00000257060) and 282 kb away from the closest protein-coding gene *RGMA*. Interestingly, among the suggestive loci SNPs in males was rs4849682 ($P = 1.07 \times 10^{-7}$). This SNP is located in the lincRNA AC093901.1 (Ensembl ID ENSG00000226856) and downstream of the candidate gene *INSIG2* (see Figure E20) (23). Among the suggestive loci in females was an association (rs35329661, $P = 2.80 \times 10^{-7}$) near *ARRB1*, a gene known to interact with *HIF1A* (see DISCUSSION). A comparison of sex-stratified results of the lead SNPs identified by combined and stratified analyses is provided in Table E3.

We tested if all 21 loci significantly or suggestively associated with AHI, average Sp_{O_2} , or event duration were associated with the other two OSA traits. Consistent with the crude cross-phenotype correlations reported previously, all lead AHI and sleep Sp_{O_2} SNPs showed the expected inverse effect directionality and significance levels of P less than 0.05 when compared with the estimates for the other OSA trait (see Table E4). The lead event duration SNPs showed no consistent evidence of association for signals identified for the other two traits, with the exception of rs78897171 in females (AHI, $P = 3.28 \times 10^{-3}$; sleep Sp_{O_2} , $P = 5.78 \times 10^{-3}$).

Association Signal Generalizability into Other Populations

To our knowledge, no additional genome-wide studies of OSA in Hispanics/Latinos exist

apart from our cohorts, limiting our ability to replicate. We examined the possible generalizability of our signals into Asian Americans (minimal, $n = 214$), African Americans (minimal, $n = 1,183$), and European Americans (minimal, $n = 5,638$) from the Atherosclerosis Risk In Communities, Cleveland Family Study, Framingham Heart Study, MESA, and Osteoporotic Fractures in Men studies (see METHODS section of the online supplement). No consistent evidence of association in other ethnic groups for the top SNPs was observed (see Table E5). The Asian American and African American sample sizes, however, were relatively small, providing limited ability to assess generalizability.

We also examined generalizability of previously reported AHI candidate gene results to our Hispanic/Latino cohorts (see Table E6) (23–31). Of 17 SNPs tested

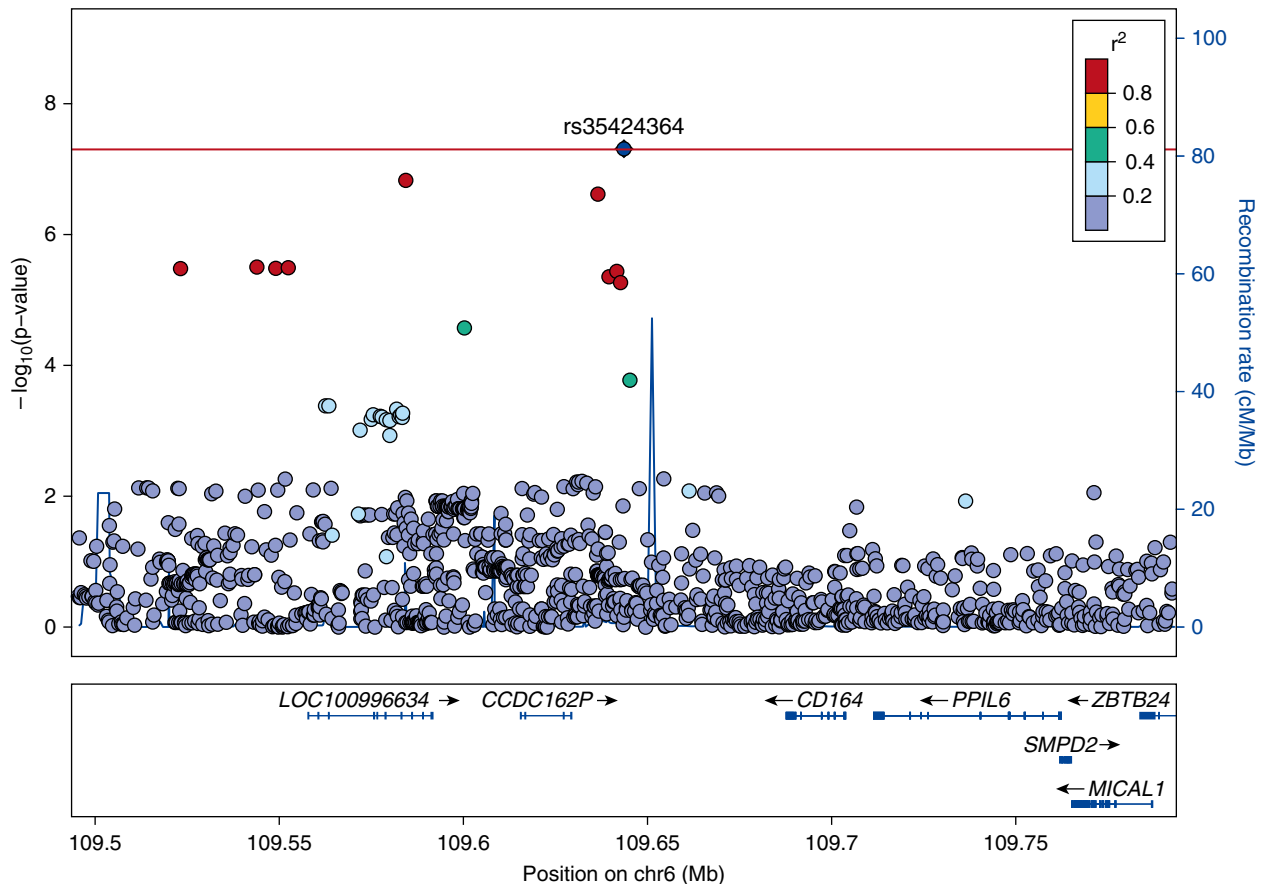


Figure 2. rs35424364 event duration regional association plot.

for association with AHI, only rs10097555 in *NRG1* was nominally significant ($P = 0.034$; A allele, β [SE] = -0.025 [0.012]). rs1421085, a *FTO* region variant that is causative in obesity-related traits (32), was nominally significant using a BMI-unadjusted model ($P = 0.012$; T allele, β [SE] = -0.036 [0.015]).

Bioinformatics Data Mining: Correlated Functional SNPs and Mouse Phenotypes

We searched for SNPs with supporting ENCODE or Roadmap Epigenomics regulatory evidence as calculated by HaploReg (33). A total of 33 SNPs with an association P value less than 1×10^{-6} and located within a significant or suggestive region overlapped promoter, enhancer, or DNase I hypersensitivity regions (see Table E7). Notable SNPs include rs74472562 (event duration, $P = 1.05 \times 10^{-7}$), which overlaps enhancer marks in 60 cell lines; rs4849682 (event duration in men, $P = 1.07 \times 10^{-7}$), which overlaps promoter marks in 82 cell lines and

enhancer marks in 45 cell lines; and rs35329661 (event duration in females, $P = 2.80 \times 10^{-7}$), which overlaps enhancers in 54 cell lines. All three SNPs and 10 others overlapped marks in central nervous system-related cell lines. Two genome-level significant SNPs displayed regulatory evidence: rs116791765 (one promoter mark in embryonic stem cells) and rs35424364 (five enhancer marks in blood cell lines).

Four genes in suggestive regions have been analyzed in ongoing work by the International Knockout Mouse Consortium: *Arrb1*, *Cdh4*, *Smco2*, and *Tspan18* (see Table E8) (34). Potentially relevant phenotypes for these associations were reported for body fat (*Arrb1* and *Smco2*); and fasting glucose, immune system functioning, and mean corpuscular hemoglobin (*Tspan18*).

Discussion

In the present study, we identified several significant genetic associations with OSA-related phenotypes, using the largest

objectively measured sample assembled to date in any ethnic group, and the first in Hispanic/Latino Americans. In total, we detected two significant and nine suggestive regions ($P < 5 \times 10^{-8}$; 5×10^{-7}) in the primary analyses and one significant and 11 suggestive regions in sex-stratified analyses. Multiple genes in associated regions have plausible OSA-related functions, including *GPR83*, *ATP2B4*, *ARRB1*, *INSIG2*, and *PLCB1*.

The genetics of OSA remain largely unknown. Research in this area has been limited by the relatively small numbers of participants that have undergone overnight sleep studies and have genome-wide assays. Prior research has consisted predominantly of candidate gene studies and has had limited power. That body of work identified potential associations of variants in genes including *CRP*, *GDNF*, and *HTR2A*; *LPAR1*, *PLEK*, and *PTGER3*; *PPARGC1B*; *NRG1*; *FTO*; *TRABD2B*; *SLC64A*; *LEPR*; and *TNF* (23–31). To our knowledge, however, no loci have been identified at the generally accepted significance

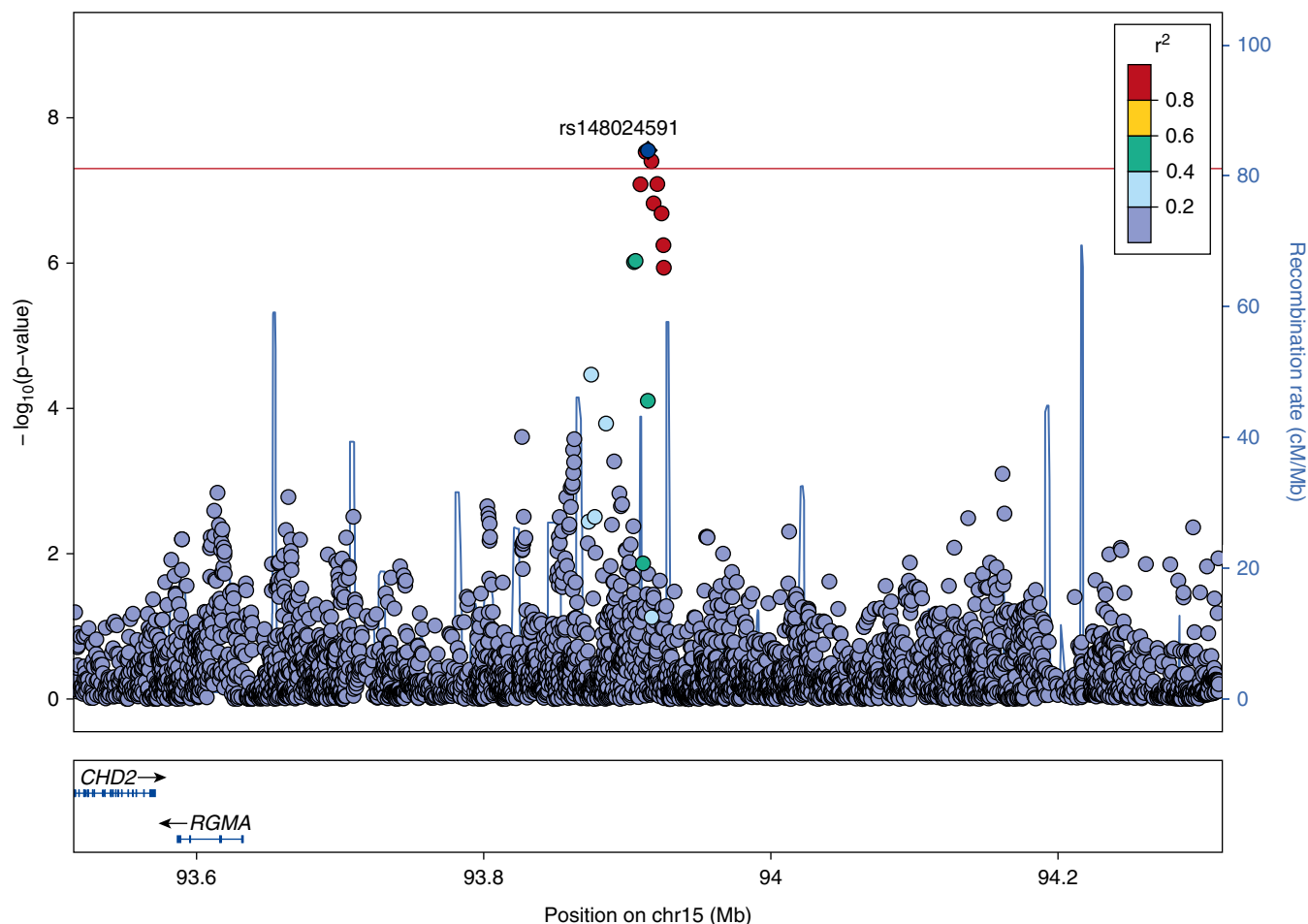


Figure 3. rs148024591 event duration in males regional association plot. Note the two recombination hotspots that bound the P value $< 1 \times 10^{-6}$ region. Although rs148024591 is distant from the closest protein-coding gene, *RGMA*, it is physically located within the lincRNA gene RP11-226O8.1 (Ensembl ID ENSG00000257060).

threshold of 5.0×10^{-8} and most results have not been replicated. Large samples are usually required to identify common variants with individually small effects that have been typical of most complex traits (35).

GWAS can help identify genomic regions containing causal variants or variants influencing the expression of genes outside of the identified region (e.g., enhancers and/or expression quantitative trait loci). Although the current study cannot establish causal mechanisms, several genes in our identified regions have published biologic relationships that suggest prioritization in future studies. The rs116791765 locus on chr 11q21 that associated with AHI includes the G-protein receptor *GPR83* (formerly *Gir*). Rodent studies show that this protein is expressed in multiple brain regions of relevance to OSA, including the hypoglossal nuclei, dorsal motor nucleus of vagus nerve, and

the nucleus of solitary tract (36). *Gpr83* down-regulation in mice leads to decreased core body temperature and increases in weight and circulating adiponectin (37). *Gpr83* knockout mice display differences in expression of the sleep- and wake-related genes *Npy* and *Hcrt* in the hypothalamus (38). GPR83 is also highly expressed in Fox3p regulatory T (Treg) cells, which are important in modulating inflammatory responses, and seem to be important in Treg cell development. Overexpression of a *Gpr83* isoform in T cells interferes with inflammatory responses *in vivo* (39). Hypoxia impacts the relative abundance of Treg cells compared with Th17 cells, both *in vivo* and *in vitro* (40). Alterations in the balance of Treg to Th17 cells, an indication of autoimmunity and inflammation, have been associated with OSA severity. Following tonsillectomy, a significant reduction in the AHI was

accompanied by a significant Treg/Th17 ratio reversal (41, 42). Inflammation may weaken upper airway muscle and exacerbate OSA through effects on muscle fibers and stimulating nerves (43). Hypoxia-induced chemoreceptor responses are muted with antiinflammatory drugs (44).

In addition to AHI, the most common clinical metric of OSA severity, we examined additional measures that provide complementary information on relevant physiologic pathways. We studied average oxygen saturation across the sleep period, an index of hypoxemia, one of the key physiologic stresses associated with OSA. Variation in oxyhemoglobin saturation levels may reflect frequency and duration of respiratory disturbances, the magnitude of work of breathing, baseline oxygen saturation and pulmonary function, and possibly pulmonary vascular and

parenchymal responses to the recurrent “stress” associated with hypoxemia. SpO₂ is measured with a simple oximeter and is easily scalable to the sample sizes required for genetic epidemiologic studies.

The relevance of the average sleep SpO₂ phenotype to genetics of OSA is supported by the high phenotypic correlation between SpO₂ and AHI, and evidence of overlapping genetic signals for this trait and for the AHI (see Table E4). The sleep SpO₂-associated locus at chr 1q32 includes *ATP2B4* (formerly *PMCA4B*), the main cellular membrane calcium pump in erythrocytes (45). *ATP2B4* also negatively regulates nitric oxide bioavailability in endothelial cells (46), impacts blood pressure and regulates vascular tone (47), and thus may have pleiotropic effects relevant to gas exchange during sleep.

Wellman and colleagues have proposed that four physiologic traits affect OSA susceptibility, including the respiratory arousal threshold, defined as the intensity of respiratory stimuli (e.g., negative pharyngeal pressure and increase of carbon dioxide concentration) required to invoke awakening (10, 48). As a surrogate for respiratory arousability, we analyzed average apnea and hypopnea event duration length, with shorter duration events indicating greater arousability (49). This phenotype was only moderately correlated with AHI ($\rho = 0.220$). Analysis of this unique phenotype identified multiple regions containing biologically plausible genes, including *ARRB1*, which physically interacts with and regulates HIF1A, which plays a central role in hypoxia sensing. *ARRB1* affects HIF1A stabilization in normoxia and impacts the expression of *HIF1A* target genes, including *VEGFA* (50). rs35329661, the lead SNP in the region, overlaps enhancer histone marks in 54 cell lines (33). The suggestive locus at 15q12 (see Figure E9) is located within the Prader–Willi syndrome region (15q11–15q13). Patients with Prader–Willi have an altered hypercapnic arousal response. The prevalence of OSA in pediatric patients across 14 studies was 79% (51, 52).

rs4849682, showing suggestive significance with event duration, is located downstream of the insulin signaling gene *INSIG2* (see Figure E20), a candidate OSA gene known to block the actions of sterol

regulatory element binding protein (SREBP), which in turn mediates intermittent hypoxia-induced hyperlipidemia (21, 53, 54). rs4849682 may also impact the lincRNA AC093901.1 (Ensembl ID ENSG00000226856) caused by overlaps with promoter histone marks in multiple cell lines (33).

rs2743173, also showing association with event duration, is located within *PLCB1*. This gene has been shown to positively regulate SREBP signaling and to affect insulin secretion (55, 56). Future work is required to determine if multiple loci invoking SREBP signaling and related pathways may contribute to variation in OSA because of alterations in insulin signaling, fat deposition, or ventilatory control effects. *PLCB1* has also been shown to directly affect sleep. *Plcb1* binds to muscarinic acetylcholine receptors in mice (57), and knockout mice have altered theta rhythms (58). A locus including *PLCB1* was among the strongest (albeit nonsignificant) regions in a GWAS of insomnia in Koreans (59). The lead locus SNP rs6140722 displayed modest association with event duration in our population ($P = 0.0030$).

Several of the SNPs identified in the current analysis are located in known regulatory regions. Other loci overlap RNA genes. Future research is required to understand these regions and the event duration associated region near rs35424364 (with the pseudogenes *CCDC162P* and *C6ORF183/LOC100996694*). This region seems to be biologically active and is associated with red blood cell traits (60).

Our study contains multiple strengths when compared with prior genetic analyses of OSA. Most notably, the sample of 12,558 individuals with objective measures of OSA and genotype data represents the largest study to date on OSA genetics, and is over triple the sample size of our prior largest analysis of objectively phenotyped OSA (24). This boost in power has improved our ability to obtain statistically significant results, in which variants have small effect sizes, in line with other complex phenotypes. We used a stringent imputation quality score threshold to reduce random error. The use of dense imputation allowed us to measure SNPs that were poorly tagged by European-centric gene chip SNPs. For the first time, we have conducted genome-wide studies of sleep SpO₂ and respiratory event duration. These traits may facilitate the genetic

dissection of OSA, providing additional information on OSA-related physiology using relatively easily scalable measurements derived from ambulatory recordings.

Our study also has some weaknesses. Although this is the largest OSA study to date, identification of variants with small effects may require much larger sample sizes. We did not have an independent sample of Hispanic/Latino individuals for replication and were unable to detect generalization of Hispanic/Latino SNP associations in our much smaller sample of African American, Asian American, and European American ancestry samples. These small sample sizes make it difficult to distinguish lack of power from differences in allele frequency and/or mutations private to a subset of continental populations, or true allelic heterogeneity across populations. Opposite directionality may also have been observed because the top SNPs may be in linkage disequilibrium with one or more common or rare causal variants that vary by population and degree of admixture. We also identified multiple loci with opposite directionality among our cohorts. The three cohorts also varied in ancestral backgrounds, including a large proportion of HCHS/SOL individuals with Caribbean ancestry (with higher recent African admixture contributions). This seems to be of particular relevance for our AHI association near *GPR83*, with lead SNPs specifically enriched in African 1,000 Genomes Project populations with rare or nonexistent minor alleles in the American, Asian, and European populations. This metaanalysis focused on common genetic variants. Additional rare variants may be uncovered by future investigations that incorporate exome or whole-genome sequencing, particularly in a family-based context. Future replication is required to validate our findings, which incorporated all known Hispanic/Latino studies with available genotypes and phenotypes.

In summary, we have identified several genome-level significant associations with OSA-related traits in Hispanic/Latino Americans in the largest genetic analysis conducted to date in any population, and also identify the potential utility of two novel OSA phenotypes for use in future genetic studies. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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