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ASSOCIATION STUDIES ARTICLE

Admixture mapping identifies novel loci for obstructive sleep apnea in Hispanic/Latino Americans

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Abstract

Obstructive sleep apnea (OSA) is a common disorder associated with increased risk of cardiovascular disease and mortality. Its prevalence and severity vary across ancestral background. Although OSA traits are heritable, few genetic associations have been identified. To identify genetic regions associated with OSA and improve statistical power, we applied admixture mapping on three primary OSA traits [the apnea hypopnea index (AHI), overnight average oxyhemoglobin saturation (SaO_2) and percentage time $SaO_2 < 90\%$] and a secondary trait (respiratory event duration) in a Hispanic/Latino American population study of 11 575 individuals with significant variation in ancestral background. Linear mixed models were performed using previously inferred African, European and Amerindian local genetic ancestry markers. Global African ancestry was associated with a lower AHI, higher SaO_2 and shorter event duration. Admixture mapping analysis of the primary OSA traits identified local African ancestry at the chromosomal region 2q37 as genome-wide significantly associated with AHI ($P < 5.7 \times 10^{-5}$), and European and Amerindian ancestries at 18q21 suggestively associated with both AHI and percentage time $SaO_2 < 90\%$ ($P < 10^{-3}$). Follow-up joint ancestry-SNP association analyses identified novel variants in *ferrochelatase* (*FECH*), significantly associated with AHI and percentage time $SaO_2 < 90\%$ after adjusting for multiple tests ($P < 8 \times 10^{-6}$). These signals contributed to the admixture mapping associations and were replicated in independent cohorts. In this first admixture mapping study of OSA, novel associations with variants in the iron/heme metabolism pathway suggest a role for iron in influencing respiratory traits underlying OSA.

Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of upper airway obstruction during sleep resulting in oxygen desaturation and sleep fragmentation (1). OSA affects more than 10% of adults (2) and increases risk of adverse health outcomes, including hypertension and cardiovascular disease as well as increased mortality (3–5). Candidate gene studies have identified associations between the apnea hypopnea index (AHI), the chief disease defining metric, measured continuously or dichotomized, with variants in genes associated with inflammation, serotoninergic pathways and ventilatory control (6–8). We have recently identified the first genome-wide significant associations with objectively measured OSA traits (9,10). However, the findings are limited by the statistical power with the modest sample size of the available datasets.

One approach for increasing power while being robust to allelic heterogeneity is admixture mapping (11,12), a powerful analytic tool that can be applied to recently admixed populations whose ancestral populations were from isolated continents. This method assumes that the ancestral populations have different disease prevalence/severity and different allele frequencies

(AFs), reflecting adaptations to different environments. Under this assumption, local ancestry can be correlated with a phenotype and such association can be detected using regression analysis. With the ability to accurately infer local ancestries, admixture mapping has successfully identified risk genetic variants associated with several complex disorders (11,13–15).

The potential for admixture mapping to identify genetic association with OSA is supported by epidemiological studies of population differences: compared to European-Americans (EAs), OSA prevalence is higher in Hispanic/Latinos (16), African-American (AA) children and young adults (17–19) and Asians (20,21). A genetic linkage study revealed differences in estimated heritability and genomic regions associated with OSA traits in AAs compared to EAs (22).

To explore these differences, we conducted admixture analyses of three primary OSA phenotypes [AHI, overnight average oxyhemoglobin saturation (SaO_2) and percentage time $SaO_2 < 90\%$], and a secondary heritable OSA measure (average respiratory event duration) (22) in a large Hispanic/Latino sample. To our knowledge, this is the first admixture mapping study of OSA phenotypes.

Results

Sample characteristics

This study included 11 575 individuals from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) with self-identified Hispanic/Latino background (including Central American, Cuban, Dominican, Mexican, Puerto Rican and South American) (23,24) (Materials and Methods). The sample characteristics varied across background groups (Table 1). The Caribbean Islanders (Cubans, Dominicans and Puerto Ricans) had higher average African ancestry proportion and lower average Amerindian ancestry proportion compared to the mainlanders (Mexicans, Central Americans and South Americans) (25). OSA (defined as an AHI > 5) was present in 31% of the sample. Cubans had the highest prevalence of OSA.

The correlations among the traits are provided in Supplementary Material, Table S1. As expected, AHI increased with age, male sex and body mass index (BMI), and was negatively correlated with average SaO_2 ($\rho = -0.69$) and positively correlated with time% $SaO_2 < 90\%$ ($\rho = 0.77$). Event duration was weakly correlated with the other OSA traits (Supplementary Material, Table S1).

Since the four OSA traits were skewed, they were rank normalized and adjusted for age, sex, BMI, population structure and relatedness in this study (Materials and Methods). The overall estimated heritability for the OSA traits varied from 11 to 17%.

Global ancestry associations

The associations between rank-normalized OSA traits and global African, Amerindian and European ancestries adjusted for demographic variables and BMI are shown in Table 2. Global African ancestry percentage was associated with lower AHI ($\beta = -0.0005$, $P = 0.023$) and higher average SaO_2 ($\beta = 0.0006$, $P = 0.015$), suggesting a protective effect for OSA. European ancestry percentage was associated with lower average SaO_2 ($\beta = -0.0007$, $P = 8.50 \times 10^{-4}$). Respiratory event duration was shorter in African and European ancestries ($\beta = -0.0019$ and -0.0008, $P = 7.52 \times 10^{-9}$ and 6.20×10^{-4}), and longer in Amerindian ancestry ($\beta = 0.0015$, $P = 6.20 \times 10^{-4}$). Secondary analyses using European ancestry as a reference group present consistent estimated effects for African and Amerindian ancestries (Supplementary Material, Table S2).

Admixture mapping

We performed admixture mapping analyses for each ancestry group separately across the genome using linear mixed regression models, excluding the regions within 2Mb around the centromere and chromosomal boundary (Materials and Methods) (26). OSA traits were rank normalized, adjusted for age, sex, BMI (and interactions and non-linear terms as appropriate), top five principal components (PCs) and kinship coefficient matrix (Materials and Methods). The admixture mapping results are presented in Supplementary Material, Figs S1–4. Previous permutation analyses suggested the genome-wide significance level was 5.7×10^{-5} for admixture mapping analysis in HCHS/SOL data (27) (Materials and Methods). Our primary analysis of AHI and hypoxemia traits identified African ancestry at chromosome 2q37 genome-wide significantly associated with AHI ($\beta = -0.079$, $P = 3.7 \times 10^{-5}$) and 15 additional regions with suggestive admixture mapping evidence including chromosome 18q21, with similar ancestry associations for both AHI and percentage time $SaO_2 < 90\%$ ($P < 10^{-3}$; Supplementary Material, Table S3). European ancestry was positively associated with both traits ($\beta = 0.048$ and 0.049, $P = 2.74 \times 10^{-4}$ and 2.01×10^{-4}, for AHI and percentage time $SaO_2 < 90\%$, respectively), whereas Amerindian ancestry was negatively associated with both traits ($\beta = -0.052$ and -0.058, $P = 4.46 \times 10^{-4}$ and 9.15×10^{-5}, for AHI and percentage time $SaO_2 < 90\%$, respectively). Secondary analysis using respiratory event duration additionally identified one genome-wide significant region ([chromosome 16q12-21], $P = 6.4 \times 10^{-6}$) associated with African ancestry and seven suggestive regions ($P < 10^{-3}$; Supplementary Material, Table S3).

Fine-mapping analyses

We then followed up 2q37, 18q21 and 16q12–21 by single SNP association and joint SNP-ancestry analyses (Materials and Methods). Since 18q21 was associated with two correlated OSA traits, we further performed a combined trait analysis incorporating their phenotypic correlation (Materials and Methods). Significance level for this analysis was determined as $P < 8 \times 10^{-6}$ accounting for multiple comparisons in multiple traits (13,28) (Materials and Methods).

At chromosome 2q37, single SNP association analysis identified two SNPs in linkage disequilibrium (LD) associated with AHI, led by rs6750391[T] [*AC011286.1*, effect AF (EAF) = 0.898, $\beta = 0.09$, $P = 1.78 \times 10^{-6}$] (Table 3 and Supplementary Material, Table S4, Supplementary Material, Fig. S5). The association effect directions were positive across background groups (Fig. 1). The T allele had a lower frequency in African ancestry populations (AF = 0.49) relative to Amerindian (AF = 0.93) and European ancestry populations (AF = 0.95) (Supplementary Material, Table S5). The association *P*-value between local African ancestry and AHI was more significant when only local African ancestry was included in the regression model ($P = 3.7 \times 10^{-5}$) than when both rs6750391 and local African ancestry were included ($P = 2.8 \times 10^{-2}$) (Table 4, Fig. 2A), suggesting that rs6750391 partially accounted for the admixture mapping evidence.

Table 1. Sample characteristics of the analyzed samples from HCHS/SOL

	All	Cuban	Dominican	Puerto Rican	Mexican	Central American	South American
N	11 575	2105	1066	2031	4298	1246	829
African Ancestry%, mean (SD)	13.8 (16.1)	15.2 (0.7)	44 (1.5)	21.8 (1.6)	4.3 (0.5)	10.6 (0.9)	6.4 (0.7)
Amerindian Ancestry%, mean(SD)	30.5 (23.7)	5.8 (0.5)	6.5 (0.9)	13.7 (1.6)	49.1 (1.1)	45.4 (1.3)	45.3 (1.4)
European Ancestry%, mean (SD)	55.7 (20.9)	79 (0.9)	49.5 (1.6)	64.5 (2)	46.5 (1.1)	44 (1.3)	48.3 (1.5)
Male%	41	46.9	34.6	42.4	39.5	40.4	39.8
Age, years, mean (SD)	46.09 (13.86)	48.68 (13.33)	45.25 (14.31)	47.57 (14.26)	44.66 (13.81)	44.71 (13.37)	46.46 (13.28)
BMI, kg/m^2, mean (SD)	29.82 (6.06)	29.32 (5.89)	29.34 (5.64)	30.81 (6.96)	29.86 (5.91)	30.06 (5.83)	28.66 (5.24)
AHI, events/h, median (IQR)	2.00 (0.41–6.62)	2.42 (0.53–8.33)	1.46 (0.32–5.35)	2.34 (0.51–7.37)	1.86 (0.38–6.35)	1.87 (0.39–6.31)	1.74 (0.42–5.69)
Average SaO$_2$, %, mean (SD)	96.45 (0.94)	96.37 (0.97)	96.56 (0.78)	96.33 (1.07)	96.51 (0.9)	96.49 (0.98)	96.44 (0.88)
Percentage time SaO$_2$ < 90%, mean (SD)	0.82 (3.05)	1.05 (3.45)	0.65 (2.45)	0.86 (3.37)	0.74 (2.73)	0.96 (3.64)	0.69 (2.62)
Average event duration, s, mean (SD)	23.65 (7.97)	23.92 (8.03)	23.3 (8.21)	22.76 (7.86)	23.89 (7.95)	23.31 (7.48)	24.78 (8.33)

Table 2. Associations between OSA traits and individual global ancestry percentage, adjusting for sex, age, age^2, age × sex, BMI and BMI^2

	African Ancestry			Amerindian Ancestry			European Ancestry		
	Estimate per 1% change of ancestry	SE	P	Estimate per 1% change of ancestry	SE	P	Estimate per 1% change of ancestry	SE	P
AHI	−0.0005	0.0002	0.023	0.0003	0.0002	0.09	−0.00004	0.0002	0.847
Average SaO$_2$	0.0006	0.0003	0.015	0.0002	0.0002	0.204	−0.0007	0.0002	8.50×10^{-4}
Percentage time SaO$_2$ < 90%	0.0001	0.0003	0.658	−0.0001	0.0002	0.607	0.0005	0.0002	0.805
Average event duration	−0.0019	0.0003	7.52×10^{-9}	0.0015	0.0002	5.96×10^{-12}	−0.0008	0.0003	6.20×10^{-4}

We identified 20 significant SNPs in strong LD overlapping *NARS* (*asparaginyl-tRNA synthetase*) and *FECH* (*ferrochelatase*) in the 18q21 region, led by rs57403733[G] (EAF = 0.803, associated with the combined AHI and percentage time SaO$_2$ < 90% traits (P = 3.2×10^{-7}) (Table 3 and Supplementary Material, Table S4, Supplementary Material, Fig. S6). Rs57403733[G] is positively associated both with AHI and percentage time SaO$_2$ < 90% (β = 0.066 and 0.078, P = 1.47×10^{-5} and 3.98×10^{-7}). The association effect directions were highly consistent across background groups (Fig. 1). The G allele had a lower frequency in Amerindian ancestry populations (EAF = 0.79) relative to African (EAF = 0.99) and European ancestry populations (EAF = 0.96) (Supplementary Material, Table S5). Conditional on rs57403733,

the association P-values between Amerindian/European ancestries and the AHI and percentage time SaO$_2$ < 90% became less significant (P > 10^{-2}) (Table 4, Fig. 2B and C).

At chromosome 16q12-21, single SNP association analysis identified four SNPs in *RP11-96H17.1* associated with our secondary trait, event duration (Table 3 and Supplementary Material, Table S4). Rs74021948[A] (EAF = 0.984, β = 0.302, P = 3.07×10^{-7}) was in weak LD with rs12935339[G] (EAF = 0.308, β = −0.074, P = 3.44×10^{-6}) and two other SNPs (r^2 < 0.2) (Supplementary Material, Fig. S7). Therefore, we investigated rs74021948 and rs12935339 separately. The association directions were consistent among background groups for both SNPs (Fig. 1). Conditioning on rs74021948 or rs12935339, the

Table 3. Significant independent SNPs ($P < 8 \times 10^{-6}$) under the candidate regions associated with OSA traits in HCHS/SOL

SNP	B37 position	Nearest gene(s)	Alleles (E/A)	EAF	Info	Trait	β (se)	P
rs6750391	2:237635300	*AC011286.1*	T/C	0.898	0.998	AHI	0.090 (0.019)	1.78×10^{-6}
rs74021948	16:63065908	*RP11-96H17.1*	A/C	0.984	0.956	Event duration	0.302 (0.059)	3.07×10^{-7}
rs12935339	16:63132772	*RP11-96H17.1*	G/A	0.308	0.999	Event duration	−0.074 (0.016)	3.44×10^{-6}
rs57403733	18:55281709	*NARS, FECH*	G/A	0.803	0.999	AHI	0.066 (0.015)	1.47×10^{-5}
						Percentage time $SaO_2 < 90\%$	0.078 (0.015)	3.98×10^{-7}
						Combined[a]		3.20×10^{-7}

CPASSOC was performed to combine the effect of AHI and percentage time $SaO_2 < 90\%$.

Table 4. Comparison between ancestry-OSA association model with and without adjusting for lead SNPs in the three significant regions

Genomic region (Mb)	Ancestry	Trait	SNP covariate	Est	SE	P
2:237.54–238.39	African	AHI	No SNP (baseline)	−0.079	0.019	3.70×10^{-5}
			rs6750391	−0.047	0.021	2.80×10^{-2}
18:51.81–57.12	Amerindian	AHI	No SNP (baseline)	−0.052	0.015	4.46×10^{-4}
			rs57403733	−0.032	0.016	4.27×10^{-2}
	European	AHI	No SNP (baseline)	0.048	0.013	2.74×10^{-4}
			rs57403733	0.032	0.014	2.02×10^{-2}
	Amerindian	Percentage time $SaO_2 < 90\%$	No SNP (baseline)	−0.058	0.015	9.15×10^{-5}
			rs57403733	−0.024	0.018	1.63×10^{-1}
	European	Percentage time $SaO_2 < 90\%$	No SNP (baseline)	0.049	0.013	2.01×10^{-4}
			rs57403733	0.027	0.014	5.46×10^{-2}
16:51.14–64.12	African	Average event duration	No SNP (baseline)	−0.113	0.025	6.42×10^{-6}
			rs74021948	−0.094	0.025	2.11×10^{-4}
			rs12935339	−0.095	0.025	2.00×10^{-4}
			rs74021948 and rs12935339	−0.080	0.026	1.82×10^{-3}

significance of association between local African ancestry and event duration decreased from $P = 6.4 \times 10^{-6}$ to $P = 2 \times 10^{-4}$. When conditioning on both SNPs, the significance level further decreased ($P = 1.82 \times 10^{-3}$) (Table 4, Fig. 2D).

SNP level replication generalization and bioinformatics analysis

SNPs detected by admixture mapping and follow-up association analyses were tested in 1935 AAs, 6490 EAs and 1240 Hispanic/Latino Americans (HAs) from the replication cohorts (described in Supplementary Table S6). In the *NARS/FECH* region on chromosome 18, 12 of the 20 significant SNPs had replication P-value <0.05 for percentage time $SaO_2 < 90\%$ in EAs. The smallest replication P-value was 1.6×10^{-3} for rs3745063 (Supplementary Material, Table S7).

Rs57403733 and rs3745063 are intronic variants of *NARS*. Rs57403733 is associated with the expression of the gene *FECH* in transformed fibroblasts cells. Rs3745063 is an expression quantitative trait locus (eQTL) for *FECH* in lymphoblastoid (29), monocytes and whole blood cells and overlaps promoter histone

marks in five tissues (eight cell lines) and enhancer histone marks in 14 tissues (51 cell lines).

We further observed a significant negative association between *FECH* expression and average SaO_2 in 203 HAs from MESA ($\beta = -0.552$, $P = 0.003$), adjusting for age, sex and BMI.

Associations of SNPs at *AC011286.1 or RP11-96H17.1* were not replicated.

Discussion

In this first admixture mapping analysis of sleep apnea traits, we leveraged information on diverse ancestral backgrounds available in the largest cohort with objectively measured sleep apnea phenotypes and genome-wide data, identifying several novel signals in regions that differ in local ancestry. Primary analyses identified a genome-wide significant region at chromosome 2q37 that showed a negative association between African ancestry and AHI. Amerindian ancestry and European ancestry at chromosome 18q21 were also suggestively associated with both AHI and degree of hypoxemia during sleep. Secondary analysis identified that African ancestry at
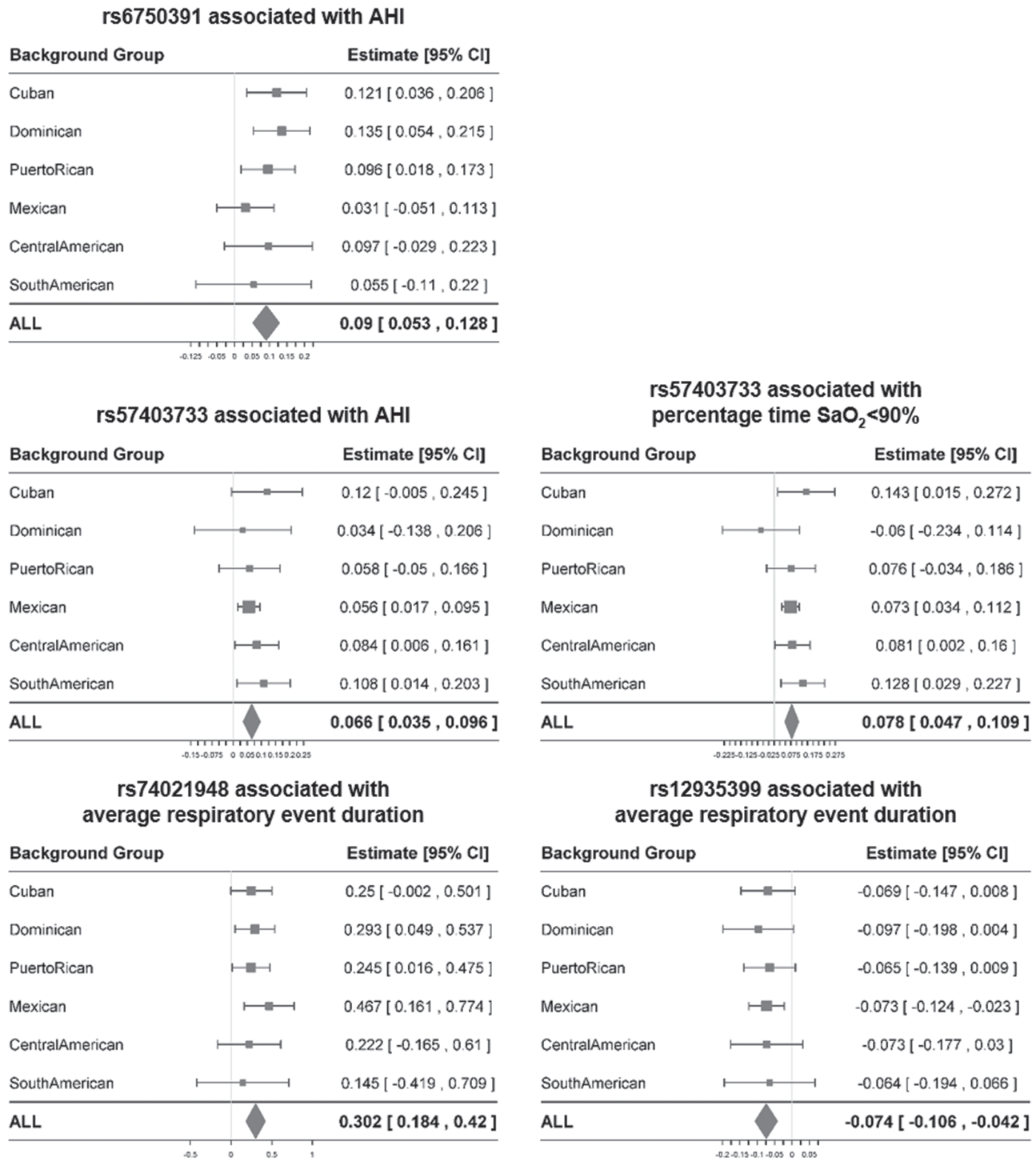
Figure 1. Associations between identified independent variants and OSA traits among background groups.

chromosome 16q12-21 was also negatively and significantly associated with respiratory event duration. Follow-up joint association analyses in three of the regions which were associated with OSA traits identified variants in *AC011286.1*, *NARS/FECH* and *RP11-96H17.1* ($P < 8 \times 10^{-6}$) that explained part of the admixture mapping evidence. The association of *NARS/FECH* for percentage time SaO$_2$ < 90% was replicated in independent European cohorts. Notably, bioinformatics and expression data

indicated that the variants in chromosome 18 associated with AHI and hypoxemia are also associated with expression of *FECH*. Furthermore, analysis of available expression data showed that increased *FECH* expression is associated with lower nocturnal SaO$_2$. Since *FECH* catalyzes the insertion of iron as part of heme B biosynthesis, these data suggest a novel biological pathway linking OSA with pathways associated with iron/heme metabolism.
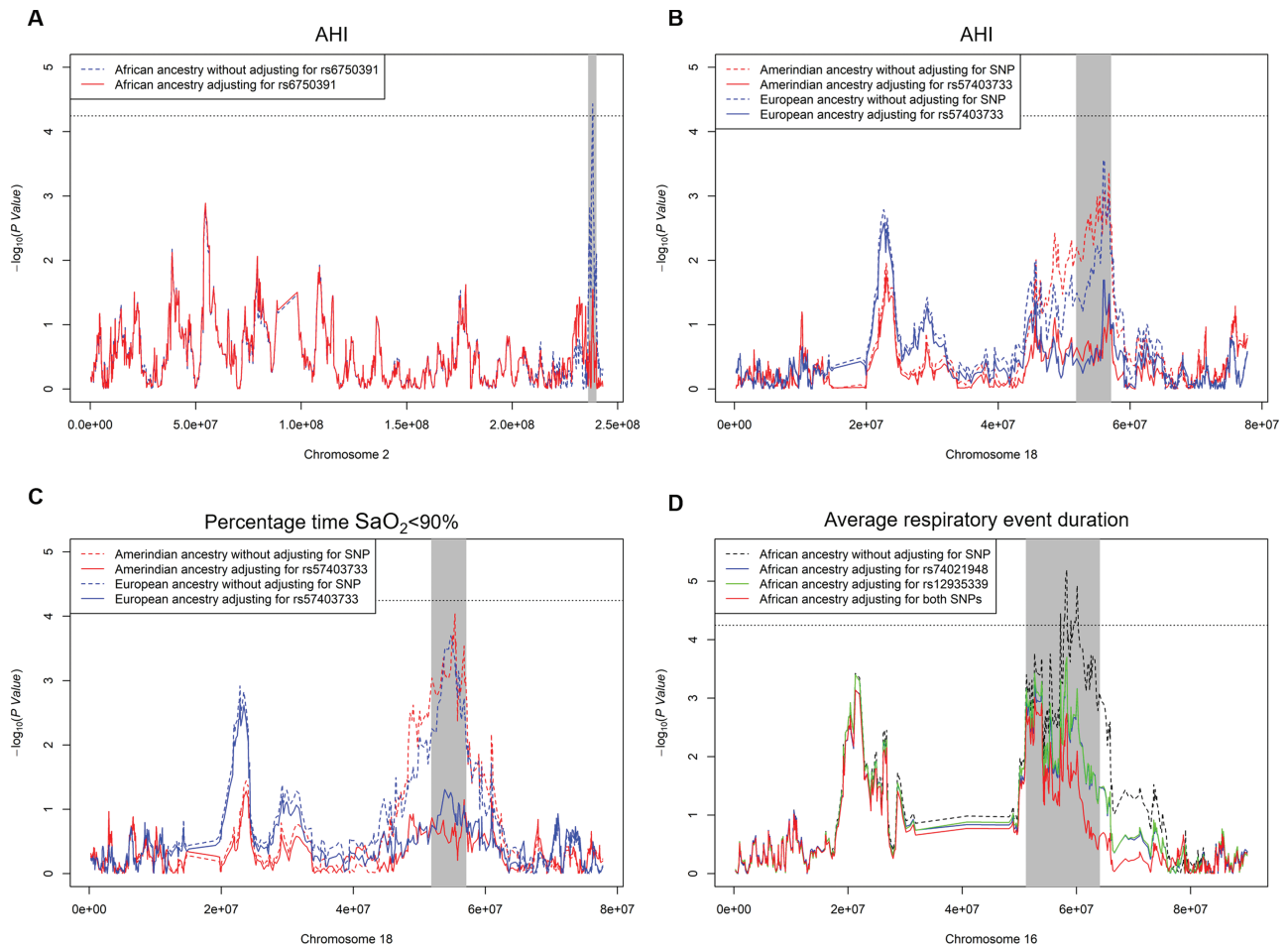
Figure 2. Admixture mapping results of significant ancestry and OSA traits with and without adjusting for target SNPs.

We used local ancestry information sampled across the genome to estimate global ancestry as well as to detect risk genomic regions using admixture mapping. Two previous studies have used information on genetic ancestry to study sleep-related traits in recently admixed populations. A study from an admixed Brazilian population identified a negative association between African genetic ancestry and OSA (30), consistent with our results. Halder *et al* (31) also reported an association between African ancestry and fragmented sleep. This finding also is consistent with our findings that shorter event duration—a marker of low arousal threshold—associated with African ancestry. However, these studies only investigated the association with average individual ancestry proportions (global ancestries) calculated from ancestral informative markers and did not perform admixture mapping or fine mapping to identify specific genetic variants.

Studies of OSA genetics have lagged behind those of other diseases. This partly reflects a need for objectively measuring sleep traits in large samples that are genotyped. Studies have been relatively small and have mostly examined candidate genes and focused on the AHI or the dichotomous trait defined by AHI thresholds, reporting associations with a variety of genes in inflammatory, metabolic and other pathways, that largely were not replicated (6,7,32–39). A genome-wide association analysis including HCHS/SOL identified associations between *GPR83* and AHI, and between *C6ORF183/CCDC162P* and event duration

(9); however, independent samples were limited and did not permit replication.

Admixture mapping analysis provided a strategy for improving the power to detect genetic variants associated with OSA, including those that may underlie population differences. The novel association for AHI on chromosome 2q37 maps to an area implicated in a rare disorder, the 2q37 deletion syndrome. This disorder is characterized by hypotonia, facial dysmorphisms and obesity, traits often observed with OSA (40). About half of the patients with this syndrome have sleep disturbances, including one child with sleep apnea (40,41). Several regulatory genes reside in this area, including ones that influence development, such as *HDAC4* and *TWIST2*. In our analysis, two variants within *AC011286.1*, a linc *RNA* gene, in this region were associated with AHI. *AC011286.1* is expressed in esophageal mucosa (Supplementary Material, Fig. S8), a tissue that shares development origins with the pharynx, which may differentially impact airway collapsibility.

In addition to analyzing the AHI, we also examined two measures of overnight oxygen saturation, which are key features of OSA, that are heritable and predict, and likely mediate, its associated cardiovascular risk and mortality (42–44). We combined information on two highly correlated traits, AHI and percentage time $SaO_2 < 90\%$, to further improve power in a region associated with both traits. We also explored associations with average event duration, another OSA trait that varies across

ancestral backgrounds, is highly heritable, but not strongly correlated with the AHI (22).

In the region on chromosome 18q21, we detected 20 variants on *NARS/FECH* associated with both AHI and percentage time $SaO_2 < 90\%$, with 12 also associated in independent cohorts. Many variants are eQTLs for *FECH* in multiple tissues, suggesting that this might be the relevant gene under this admixture mapping and association peak. Furthermore, using available expression data from monocytes, we found that increased *FECH* expression was associated with lower nocturnal SaO_2. *FECH*, the last enzyme of the heme biosynthesis pathway which inserts the iron atom into the porphyrin IX ring and generates heme B, is largely expressed in whole blood (Supplementary Material, Fig. S8) (45). Hemoproteins such as hemoglobin myoglobin have diverse biological functions involved in oxygen transport and oxygen storage (46,47). Intracellular free heme is toxic and is degraded to carbon monoxide, biliverdin and iron via heme oxygenase. Heme degradation and its products (e.g. carbon monoxide) play key roles in many biological systems, including glucose metabolism and carotid body oxygen sensing, a mechanism implicated in ventilatory control and sleep apnea (48–50). Iron homeostasis influences redox balance, inflammatory response and energy metabolism (51). Iron chelation influences *HIF1-α (Hypoxia Inducible Factor)* accumulation and may influence oxygen sensing in the carotid body (52,53), where chemoreceptors play a key role in ventilatory stability and the pathogenesis of sleep apnea (54). Together, our admixture/association findings, supported by bioinformatics and our own expression data, suggest a role for iron/heme in influencing clinically important OSA traits. Future research is needed to determine if our associations reflect variations in hemoglobin and oxygen carrying capacity or effects of iron on ventilatory control or metabolism.

In secondary analyses, we also examined respiratory event duration, which is shorter in AAs than EAs, and partially reflects respiratory arousability and chemoreflex sensitivity, which when abnormal can lead to sleep fragmentation (55–57). In the region of chromosome 16q12-21, we detected four variants on *RP11-96H17.1* associated with event duration. *RP11-96H17.1* is largely expressed in the brain (Supplementary Material, Fig. S8).

This analysis provides insight into differences in OSA susceptibility across ancestral groups. Notably, African ancestry was associated with a lower AHI and shorter respiratory disturbances. Shorter events, and the reduced physiological tolerance of ventilatory disturbances it reflects, may contribute to sleep fragmentation and sleepiness for any given AHI level, consistent with epidemiological studies reporting shorter sleep duration and sleepiness in AAs (21). Although BMI-adjusted AHI levels are higher in AA children and young adults compared to EAs, this difference dissipates with advancing age (17).

Our study has several strengths. It was conducted on the largest cohort of individuals with Hispanic/Latino background, a highly admixed sample, with objective sleep recording. We focused our analyses on clinically important phenotypes that vary across populations. To gain power we also analyzed two highly correlated traits (AHI and percentage time $SaO_2 < 90\%$) by combining their summary statistics (58). Admixture mapping can be more powerful than traditional genome-wide association analyses when trait distributions vary by ancestry. High resolution local ancestries were used, enhancing the accuracy of ancestral estimation. Follow-up two-step fine-mapping analyses (single SNP association and joint SNP-ancestry association analyses) efficiently targeted the variants both associated with the trait and contributing to admixture mapping. Although our replication samples were only modest in size, we were able to

replicate 12 of 26 variants in European cohorts, providing the first replicated genetic association finding for OSA traits in a multi-ethnic population.

This study also has several limitations. In-laboratory polysomnography was not performed in the HCHS/SOL, precluding our assessment of sleep architecture. The overall ancestry-specific sample sizes were only modest. Despite this, several top findings replicated in cohorts studied with full polysomnography. Although multiple variants in *NARS/FECH* were replicated in EAs, the SNP-level replication power was limited by modest sample sizes in other replication cohorts. The complex admixture history and genetic diversity among Hispanic/Latinos present challenges in identifying ideal replication cohorts.

In summary, we identified three novel genomic regions associated with OSA traits in a large Hispanic/Latino cohort using admixture mapping methods. Novel variants and genes detected in those regions were identified that likely explain some of the difference in OSA across ancestral groups. Further investigation of the role of iron/heme pathways, a topic of growing interest due to associations with diabetes, obesity, liver disease and metabolism, and gene regulatory areas on chromosomes 2 and 16 may provide further insight into mechanisms underlying OSA.

Materials and Methods

Sample

The primary sample was the HCHS/SOL, a community-based study in the US, which includes 16 415 adults aged 18–74 with self-identified Hispanic/Latino background (23,24). Individuals were recruited from randomly selected households near four centers in Miami, San Diego, Chicago and the Bronx area of New York. Most participants identified themselves from one of six ethnic background groups, including 1730 Central Americans, 2348 Cubans, 1460 Dominicans, 6471 Mexicans, 2728 Puerto Ricans and 1,068 South Americans. A total of 14 400 individuals had overnight home sleep apnea tests (HSATs) that met study quality criteria and reported no regular use of overnight oxygen or sleep apnea treatment devices. Of these, 11 575 had available genotyping and comprised the primary study sample. The study was approved by the Institutional Review Boards at each participating institution and participants provided written informed consent.

Phenotypes and covariates

During the baseline HCHS/SOL examination (2008–2011), physical measures and questionnaires, blood and HSATs were collected as previously described (24). Age, sex and background were self-reported. BMI was derived from measured weight (kg) and height (cm). Sleep apnea assessments were obtained with the ARES Unicorder 5.2 (B-Alert, Carlsbad, CA) as described before (59). The monitor measures airflow using a nasal cannula and pressure transducer; blood hemoglobin oxygen saturation (SaO_2) and pulse rate using a forehead-based reflectance oximeter; body position and movement by an actigraph and snoring by a microphone. Sleep records were transmitted to a central Sleep Reading Center where they were manually scored as described before (60). Respiratory events (apneas or hypopneas) were identified when airflow declined by $\geq 50\%$ for ≥ 10 s. Since thermistry was not collected, apneas were not distinguished from hypopneas. In this paper, only respiratory

events associated with ≥3% oxyhemoglobin desaturation were analyzed. The AHI was computed as the number of respiratory events divided by the estimated sleep time; this measure highly correlates with polysomnography-derived AHI ([61]). The average SaO_2 level and percent time when SaO_2 was <90% used oximetry-based SaO_2 values measured continuously during sleep. The average respiratory disturbance event duration was derived by measuring the time between the first breath that was reduced by ≥50% to the termination of the respiratory disturbance event (in seconds).

Genotypes

Genotyping was conducted using an Illumina Omni2.5M SNP array with additional customized content, including 2 536 661 SNPs. Local African, European and Amerindian ancestries were inferred from quality controlled SNPs across the genome using RFMix software ([62],[63]). The global African, Amerindian and European ancestries for each individual, defined as the proportion of each genome inherited from each ancestry, were calculated by the average values of local ancestries at 14 815 uniformly distributed loci on autosomal chromosomes. OSA trait heritability was estimated for the pooled sample and individual background groups using the unbiased Haseman–Elston regression ([64]).

Global ancestry association analysis

We performed association analyses between OSA traits with each of the global African, Amerindian and European ancestry percentage using linear regression model. Since the OSA traits were skewed, they were rank normalized. Sex, age, age^2, age × sex, BMI and BMI^2 were adjusted as fixed-effect covariates. We further used global European ancestry as a reference and tested the association between each OSA trait with global African and Amerindian ancestry percentage in one regression model as a secondary analysis. This analysis was less powerful because of larger standard errors introduced by multicollinearity (high correlation between African and Amerindian ancestries).

Admixture mapping analysis

We performed admixture mapping analyses for each ancestry group separately across the genome using a linear mixed regression model (GENESIS package in R). Regions within 2 Mb around the centromere and chromosomal boundary were excluded because of lower local ancestry inference accuracy ([26]). OSA traits were rank normalized and then adjusted for sex, age, age^2, age × sex, BMI and BMI^2 as fixed-effect covariates. PCs and kinship coefficients were estimated iteratively, robust to ancestry and population and family structure and admixture, as described before ([25],[65],[66]). The top five PCs separated the background group genetic structure well in this sample ([25]). Population stratification was controlled for by adjusting for fixed effects of five PCs, and family structure was allowed for by adding a variance component proportional to the kinship coefficient matrix. We performed another set of analyses additionally adjusting for sampling weight, households and census blocks. We did not observe significant change from our primary model (Supplementary Material, Figs. S1–4). Admixture mapping reduces the number of comparisons across the genome; therefore, a significance level of $5 × 10^{-8}$ for traditional GWAS is not applicable. Based on previous permutation analyses using HCHS/SOL data, the significance level was determined to

be $5.7 × 10^{-5}$, corresponding to a family-wise error rate at 0.05 level ([27]). We defined a suggestive region as admixture mapping signals of $P < 10^{-3}$.

Single SNP association analysis

We further evaluated three regions with significant admixture signals to identify SNPs in those regions that were significantly associated with OSA traits, using genotyped and 1000G imputed SNPs with minor AF >0.01 and imputation information scores >0.4 ([67]). The boundary of those significant regions was defined with admixture mapping $P < 10^{-3}$. Linear mixed models were used with the same set of covariates as in the primary admixture mapping analysis. We estimated the number of independent tests in admixture mapping and the single SNP association analyses in each region (accounting for one trait at 2q37 and 16q12-21, and two traits at 18q21) using Li and Ji's method ([13],[28]), resulting in a Bonferroni significance level of $P = 8 × 10^{-6}$. For 18q21 region significantly associated with AHI and percentage time $SaO_2 < 90\%$, we performed multiple trait analysis by combining the summary statistics for each trait and incorporating the trait correlation using CPASSOC software ([58]), which provides a single test P-value of association for multiple traits.

Joint SNP and local ancestry analysis

To examine whether an SNP within a candidate region could explain the admixture mapping signal, we performed a joint SNP and local ancestry analysis, simultaneously modeling local ancestry and SNP association. We compared the association P-value between the local ancestry and the OSA trait with and without adjusting for that SNP. This analysis was able to indicate whether a SNP contributes to the admixture signal.

Replication analysis

Single SNP replication analysis was conducted using eight independent cohorts comprising individuals of EA, AA and HA (Supplementary Methods). Inverse-variance fixed-effect meta-analyses were performed to combine the results in each ancestry group.

Epigenetic annotation and eQTL analysis

We used Haploreg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) to examine the epigenetic annotations of SNPs ([68]). eQTL evidence and gene expression were queried using the GTEx Portal (https://www.gtexportal.org/home/) ([45]). We further tested the association for OSA traits on the gene expression level of the genes associated with leading SNPs (*NARS* and *FECH*) using Multi-ethnic Study of Atherosclerosis (MESA) data (online supplement), adjusting for age, sex and BMI in each population group.

Supplementary Material

Supplementary Material is available at *HMG* online.

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