A Candidate Gene Study of Obstructive Sleep Apnea in European Americans and African Americans

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Rationale: Obstructive sleep apnea (OSA) is hypothesized to be influenced by genes within pathways involved with obesity, craniofacial development, inflammation, and ventilatory control.

Objectives: We conducted the first candidate gene study of OSA using family data from European Americans and African Americans, selecting biologically plausible genes from within these pathways.

Methods: A total of 1,080 single nucleotide polymorphisms (SNPs) were genotyped in 729 African Americans and 505 SNPs were genotyped in 694 European Americans. Coding for SNPs additively, association testing on the apnea-hypopnea index (AHI) as a continuous trait, and OSA as a dichotomous trait (AHI ≥ 15) was conducted using methods that account for familial correlations in models adjusted for age, age-squared, and sex, with and without body mass index.

Measurements and Main Results: In European Americans, variants within C-reactive protein (CRP) and glial cell line–derived neurotrophic factor (GDNF) were associated with AHI (CRP: $\beta = 4.6$; SE = 1.1; $P = 0.0000402$) (GDNF: $\beta = 4.3$; SE = 1; $P = 0.0000201$) and with the dichotomous OSA trait (CRP: odds ratio = 2.4; 95% confidence interval, 1.5–3.9; $P = 0.000170$) (GDNF: odds ratio = 2; 95% confidence interval, 1.4–2.89; $P = 0.0000433$). In African Americans, rs9526240 within serotonin receptor 2a (HTR2A: odds ratio = 2.1; 95% confidence interval, 1.5–2.9; $P = 0.00005233$) was associated with OSA.

Conclusions: This candidate gene analysis identified the potential role of genes operating through intermediate disease pathways to influence sleep apnea phenotypes, providing a framework for focusing future replication studies.

Keywords: sleep apnea; body mass index; genetics; candidate gene study

Sleep-disordered breathing (SDB) or its clinical counterpart, obstructive sleep apnea (OSA), is a common disorder characterized by intermittent collapse of the upper airway during sleep-disrupting breathing, and associated with significant morbidity including daytime sleepiness, hypertension, and

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Obstructive sleep apnea has a strong heritable component, although its genetic basis is poorly understood. Existing studies to date have focused on single genes, have been of relatively small samples, or have had limited genetic coverage (i.e., examining known functional variants).

What This Study Adds to the Field

This large-scale candidate gene study of sleep apnea involves systematic coverage of 53 genes representing intermediate disease pathways implicated in this disorder. The results from this study identify significant associations between sleep-disordered breathing and genetic variants for C-reactive protein, glial-derived growth factor, and serotonin 2A receptor genes. Our analyses show that the genetic associations with sleep apnea persist after adjusting for body mass index, suggesting that the underlying genetic mechanism of sleep apnea is not dependent on obesity, a common comorbidity.

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elevated cardiovascular risk. It has been recognized for more than three decades that SDB aggregates within families, suggesting the presence of a genetic basis for this disease (1). Approximately 30–40% of the variance in the most common disease-defining metric for SDB, the apnea-hypopnea index (AHI), has been estimated to be explained by genetic factors (2). Having a first-degree relative with SDB increases one's risk of the disorder by more than 1.5-fold (2). Several genome-wide linkage and association studies have been conducted for SDB-related traits (3–5) (summarized in Riha [6]). The latter have been limited by considering only a handful of polymorphisms in one gene or by consideration of more than a few genes in each given population, and by limited statistical power. To date, no genetic variants have been shown to associate strongly with SDB.

We postulated that identifying genetic variants for SDB would be facilitated by studying polymorphisms in genes in pathways intermediate to SDB and previously established by human or animal work to have a genetic basis, such as obesity, ventilatory control, inflammation, and craniofacial morphology (7). Using knowledge of underlying biology and prior genetic studies, we selected a set of candidate genes and then densely genotyped, in a systematic fashion, each candidate to identify polymorphisms associated with SDB in a family-based study, enriched with SDB. Some of the results of these studies have been previously reported in the form of an abstract (8).

METHODS

See the online supplement for detailed methods. The Cleveland Family Study (CFS) has assembled a collection of 2,534 individuals (43% African American) from 356 families, details of which have been previously published (2). In brief, index cases with laboratory-diagnosed sleep apnea, their family members, and neighborhood control families have been followed longitudinally on as many as four occasions over a period of 16 years and have had measurements made of sleep apnea, anthropometry, and other related phenotypes. DNA samples were available for 711 European Americans and 759 African Americans.

Phenotypes

The main metric of analyses was the AHI, defined as the average number of apneas and hypopneas, each associated with a 3% desatu-

ration, per hour of sleep. The AHI was derived from either portable sleep apnea monitoring (9) (with oxygen saturation, body position, airflow by thermistry, chest wall effort, and heart rate sensors) (Edentrace, Eden Prairie, MN) performed in participants studied before 2000 or by 14-channel attended overnight polysomnography (Compumedics E series, Abottsford, AU) obtained in a clinical research unit after 2000. The details about comparability of the AHI determined from the two recording approaches and how the longitudinal data were used are described in the online data supplement. Body mass index (BMI) was defined as weight divided by the square of height derived from the same examination as the AHI.

Genotyping

DNA was isolated from blood and buccal swabs using Puregene kits (Qiagen, Valencia CA). A total of 52 candidate genes for OSA were

TABLE 1. CANDIDATE GENES

Pathways	Gene Description	Gene	RefSeq	Chr	SNPs in European Americans	SNPs in African Americans
Craniofacial morphology	Fibroblast growth factor receptor 1	FGFR1	NM_023106.1	8	10	15
	Fibroblast growth factor receptor 2	FGFR2	NM_000141.2	10	33	92
	Fibroblast growth factor receptor 3	FGFR3	NM_022965.1	4	2	5
	Muscle segment homeobox 1	MSX1	NM_002448.2	4	2	5
	Muscle segment homeobox 2	MSX2	NM_002449.4	5	2	8
	Paired-like homeobox 2B	PHOX2B	NM_003924.2	4	3	3
	Patched 1	PTCH1	NM_000264.2	9	2	3
	Sonic hedgehog	SHH	NM_000193.2	7	4	6
	Treacle	TCOF1	NM_001008657.1	5	9	17
	Transforming growth factor- β receptor 1	TGFBRI	NM_004612.2	9	4	12
	Cholecystokinin A receptor	CCKAR	NM_000730.2	4	6	15
	C-reactive protein	CRP	NM_000567.2	1	5	7
	Hypocretin receptor 2	HCRTR2	NM_001526.2	6	34	57
Obesity/ inflammation	Insulin-induced gene 2	INSIG2	NM_016133.2	2	13	20
	Leptin	LEP	NM_000230.1	7	4	15
	Leptin receptor	LEPR	NM_002303.3	1	31	111
	Lipoprotein lipase	LPL	NM_000237.1	8	16	38
	Melanocortin 3 receptor	MC3R	NM_019888.2	20	7	8
	Melanocortin 4 receptor	MC4R	NM_005912.1	18	3	5
	Plasminogen activator inhibitor 1	SERPINE1	NM_000602.1	7	6	14
	Tumor necrosis factor	TNF	NM_000594.2	6	4	6
	Uncoupling protein 2	UCP2	NM_003355.2	11	6	8
	Uncoupling protein 3	UCP3	NM_022803.1	11	5	18
	Achaete-scute complex 1	ASCL1	NM_004316.2	12	1	1
	Brain-derived neurotrophic factor	BDNF	NM_170733.2	11	7	17
	Endothelin-converting enzyme 1	ECE1	NM_001397.1	1	19	38
Ventilatory control	Endothelin 1	EDN1	NM_001955.2	6	13	18
	Endothelin 3	EDN3	NM_207032.1	20	8	23
	Endothelin receptor A	EDNRA	NM_001957.1	4	21	57
	Glial cell line-derived neurotrophic factor	GDNF	NM_199234.1	5	21	25
	γ -Glutamyltransferase 1	GGT1	NM_013430.2	22	5	16
	Hypoxia-inducible factor 1 alpha	HIF1A	NM_181054.1	14	8	18
	Necdin	NDN	NM_002487.2	15	1	2
	Nitric oxide synthase 3	NOS3	NM_000603.3	7	6	10
	Rearranged during transfection protooncogene	RET	NM_020630.3	10	13	36
	α_B -Adrenergic receptor	ADRA1B	NM_000679.3	5	18	28
	α_{2A} -Adrenergic receptor	ADRA2A	NM_000681.2	10	4	4
	β_1 -Adrenergic receptor	ADRB1	NM_000684.1	10	3	4
	β_2 -Adrenergic receptor	ADRB2	NM_000024.3	5	4	5
Pleiotropic/ miscellaneous	GABA B receptor 1	GABBR1	NM_001470.1	6	11	17
	Guanine nucleotide-binding protein 3	GNB3	NM_002075.2	12	5	8
	Serotonin receptor 1B	HTR1B	NM_000863.1	6	7	8
	Serotonin receptor 2A	HTR2A	NM_000621.2	13	55	76
	Serotonin receptor 2C	HTR2C	NM_000868.1	X	2	2
	Serotonin receptor 3A	HTR3A	NM_000869.2	11	10	21
	Serotonin receptor 3B	HTR3B	NM_006028.3	11	14	25
	Serotonin receptor 3C	HTR3C	NM_130770.2	3	4	7
	Serotonin receptor 3D	HTR3D	NM_182537.2	3	6	7
	Serotonin receptor 3E	HTR3E	NM_182589.2	3	3	7
	Insulin receptor substrate 1	IRS1	NM_005544.1	2	5	47
	Peripheral myelin protein 22	PMP22	NM_153321.1	17	12	41
	Serotonin transporter	SLC6A4	NM_001045.2	17	8	24

Definition of Abbreviations: Chr = chromosome; RefSeq = reference sequence accession number; SNP = single nucleotide polymorphism.

selected based on putative roles in intermediate pathways (craniofacial morphology, ventilatory control, obesity, and inflammation) hypothesized to be relevant to sleep apnea pathogenesis (Table 1). A total of 522 tagged single nucleotide polymorphisms (SNPs) from European Americans and 1,095 SNPs from African Americans were genotyped using the Illumina Golden Gate Assay.

Quality control procedures included (1) elimination of individuals with call rates less than 90%, (2) visual examination of individual cluster plots and elimination of SNPs with poor call signals or GenTran scores less than 0.20, (3) resolution of mendelian inconsistencies, and (4) evaluation of SNPs for a departure from Hardy-Weinberg equilibrium. A total of 505 SNPs in 694 European Americans and 1,080 SNPs in 729 African Americans from 52 genes met quality control criteria and are included in this analysis (*see online supplement*).

Statistical Methods

The phenotypes modeled were AHI as a continuous variable and OSA defined as an AHI greater than or equal to 15. Because AHI thresholds that define abnormality differ in children, the dichotomous OSA analysis was limited to individuals over the age of 18 years. For continuous traits, linear mixed models were used to account for familial correlations as implemented in the ASSOC procedure of the Statistical Analysis of Genetic Epidemiology program (10). For dichotomous traits, logistic regression was performed by SAS v9.1 (SAS Institute, Research Triangle Park, NC) using generalized estimating equations with an exchangeable correlation structure to account for family relatedness. SNPs were coded additively based on the number of minor alleles present. All models were adjusted for age; age-squared; sex; and an index of racial admixture (percentage European or percentage African ancestry, as described in the online supplement). Secondary models were generated additionally adjusting for BMI (which may be an intermediate trait) as a covariate. To account for multiple comparisons and balance the type I and type II error rates, a Benjamini-Hochberg false discovery rate (FDR) was calculated (11). Thus, in addition to *P* values, we report *q*-values, which can be interpreted as the lowest FDR for which a hypothesis test would be deemed significant.

Haplotype analysis was performed for genes with multiple significant SNPs from the association analysis (*see online supplement*).

RESULTS

Demographic characteristics of the sample are shown in Table 2. The sample included a slight predominance of women, on average was overweight, and included a wide age range. Among the 553 European Americans and 539 African Americans over the age of 18, the prevalence of OSA was 26% and 35%, respectively.

Among the European Americans, three SNPs were associated with the age, age-squared, sex, and ancestry-adjusted dichotomous OSA trait (Table 3), with the strongest association (*q* = 0.02) to rs2808630, a SNP in the 3' untranslated region of the C-reactive peptide (CRP) gene. Each additional minor allele at this SNP was associated with a 2.04-fold increased odds of

OSA. The other two SNPs associated with OSA were in the glial cell-derived neurotrophic factor (GDNF) gene. As shown in Figure 1, SNPs within GDNF were in strong linkage disequilibrium with each other. For the two most significant SNPs (rs2910705 and rs2975100), the pairwise *D'* = 0.977 and *r*² = 0.93.

In analyses of AHI as a continuous trait in European Americans, two SNPs met a 5% FDR threshold and a third met a 10% FDR (Table 4). Each additional risk allele for these SNPs increased the mean AHI by four to five events per hour. The CRP SNP was the same SNP observed with analysis of the dichotomous phenotype. The other two SNPs were both in GDNF with a pairwise *D'* = 1 and *r*² = 0.46. Although these SNPs did not overlap with the two GDNF SNPs that had a *q*-value less than 10% from the OSA analysis, the pairwise *D'* between SNPs ranged from 0.935 to 1.00. Furthermore, as shown in the online supplement, the nominal *P* values for these four SNPs support consistent results between these traits.

Of the five SNPs associated with either OSA or AHI in European Americans, one (rs2808630 in CRP) was associated with SDB in African Americans at a nominal *P* less than 0.05. Each G allele at this SNP increased the risk of OSA by 1.48-fold (95% confidence interval, 1.04–2.11; *P* = 0.029) in African Americans.

Analysis in the African American cohort identified a SNP in the serotonin 2A receptor (HTR2A) to be associated with OSA (Table 5) at a FDR less than 10% (*i.e.*, *q* = 0.05). Each additional minor allele was associated with doubling the risk of OSA. This SNP was not associated with OSA at a *P* less than 0.05 threshold in the European American cohort, although other SNPs within HTR2A were associated with SDB at a nominal 0.05 level (rs6561332, rs1923885, and rs7322347). None of the genotyped SNPs in the African Americans were associated with AHI at a 10% FDR threshold.

In secondary analyses, adjusting for BMI, AHI remained associated with rs2808630 in CRP and rs2973042 in GDNF in European Americans. For every additional G allele in rs2808630, the AHI was increased by 4.09 ± 1.02 (*q* = 0.03) and for every additional C allele in rs2973042, the AHI was increased by 3.52 ± 0.90 (*q* = 0.03). In contrast, after adjusting for BMI in African Americans, no SNPs were associated with either OSA or AHI at a 10% FDR threshold.

Haplotype analyses were performed on GDNF where multiple SNPs were associated with AHI or OSA. In general, haplotypes were not more predictive of OSA status than single SNPs. Haplotype models tested are presented in the online supplement. It should be noted that because of the tag SNP approach used in selecting SNPs for genotyping, few haploblocks contained more than one SNP, limiting this approach.

In addition to the significant results with *q*-values less than or equal to 0.10, we also identified several noteworthy SNPs that

TABLE 2. DEMOGRAPHIC CHARACTERISTICS

	European Americans (<i>n</i> = 694)	African Americans (<i>n</i> = 729)
Number of pedigrees	138	149
Pedigree size	7.2 ± 4.9	6.1 ± 4.4
Age, yr	38.8 ± 19.6, range 3–81	36.4 ± 19.1, range 3–82
Male sex	329 (47%)	307 (42%)
Body mass index, kg/m ²	28.8 ± 8.5, range 13–80	30.8 ± 9.6, range 12–85
Apnea hypopnea index*	2.5 (0.9, 11.6)	3.8 (1.1, 16.7)
Apnea hypopnea index ≥15†	144 (26%)	190 (35%)
Hypertension	173 (25%)	271 (38%)
Diabetes	41 (6%)	80 (11%)

Values expressed as *n* and percentage or mean and SD.

* Median and interquartile range.

† Limited to the 553 European Americans and 539 African Americans over 18 yr of age.

TABLE 3. ADDITIVE MODELS FOR SINGLE NUCLEOTIDE POLYMORPHISMS AND THE RISK OF OBSTRUCTIVE SLEEP APNEA IN EUROPEAN AMERICANS ADJUSTED FOR AGE, SEX, AND EUROPEAN ANCESTRY

Chr	Gene	SNP	Location	Risk Allele	Risk Allele Frequency	Unadjusted for BMI			Adjusted for BMI		
						OR (95% CI)	P Value	Q-Value	OR (95% CI)	P Value	Q-Value
1	CRP	rs2808630	3' UTR	G	0.24	2.04 (1.45–2.87)	0.0000433	0.02	1.94 (1.34–2.81)	0.000416	0.21
5	GDNF	rs2910705	intron	G	0.10	2.44 (1.53–3.89)	0.000170	0.04	2.23 (1.34–3.72)	0.002180	0.22
5	GDNF	rs2975100	5' UTR	A	0.11	2.45 (1.53–3.92)	0.000176	0.04	2.17 (1.29–3.66)	0.003735	0.26

Definition of abbreviations: BMI = body mass index; Chr = chromosome; CI = confidence interval; CRP = C-reactive protein; GDNF = glial cell line–derived neurotrophic factor; OR = odds ratio; SNP = single nucleotide polymorphism; UTR = untranslated region.

did not meet our strict threshold. In European Americans we report an increased risk of AHI at 15 or greater associated with rs5370 of endothelin 1 (odds ratio = 1.77; 95% confidence interval, 1.26–2.48; $P < 0.001$; $q = 0.12$) and rs2071943 in linkage disequilibrium with rs5370 with a similar odds ratio estimate, P value, and q -value. In African Americans we found a haplotype block of nine SNPs in strong linkage disequilibrium (LD) within the leptin receptor with q -values = 0.16 and P values ranging from 0.0005 to 0.002 (rs11208674, rs10493379, rs6693573, rs3790424, rs1343982, rs7413823, rs12042877, rs6676495, and rs12038998). We also note a SNP within hypocretin receptor 2 (rs7768760) was modestly associated with OSA in African Americans (odds ratio = 1.76; 95% confidence interval, 1.28–2.43; $P = 0.0005$; $q = 0.16$).

Although we restricted reporting of our primary findings to associations with an FDR less than 10%, we have provided regression coefficients, standard errors, P values, and q -values for all SNPs in the online supplement to assist researchers in identifying potential opportunities for future replication.

DISCUSSION

SDB is known to aggregate strongly within families. In the Cleveland Family Study, individuals with an affected relative have been shown to have a 1.5-fold greater risk of having OSA themselves (2). The heritability for AHI is 32–36% in both

European Americans and African Americans (4, 5). In this study, we sought to evaluate the role of polymorphisms in 52 candidate genes in explaining the familial aggregation of this disorder. The set of candidate genes was selected based on biologic knowledge of relevant pathways, similarity in phenotype to monogenic diseases, and linkage data from our cohort. Our results support a potential pathogenic role for polymorphisms in GDNF and CRP in European Americans and for a polymorphism in HTR2A in African Americans. The persistence of associations between SDB with CRP and GDNF after BMI adjustment suggests that these genetic variants influence SDB susceptibility through obesity-independent pathways. In contrast, the attenuation of the association between HTR2A and SDB suggests that this association is likely through the influence of HTR2A on weight.

The finding that GDNF variants are associated with SDB phenotypes is especially interesting in light of the likely influences of ventilatory control abnormalities in the pathogenesis of SDB. Ventilatory control abnormalities, such as those that influence the sensing of oxygen or CO_2 or ventilation at sleep state transitions, may predispose to OSA by promoting ventilatory instability (and periodic breathing) (12), impairing the arousal response to airway obstruction (13), or contributing to imbalanced activation of upper airway muscles compared with chest wall muscles (14). GDNF plays a particularly important role in the development of neural pathways vital for normal

Figure 1. Linkage disequilibrium patterns in glial cell–derived neurotrophic factor in European Americans, derived from Haploview v. 4.1 Red squares indicate pairs of single nucleotide polymorphisms (SNPs) with high linkage disequilibrium, evidenced by a high D' and few recombination events ($\text{LOD} \geq 2$). White squares indicate pairs of SNPs for which there is strong evidence for linkage equilibrium. Areas of pink and blue indicate pairs of SNP where $D' < 0.8$ and $\text{LOD} \geq 2$ or $D' = 1$ and $\text{LOD} < 2$, respectively. Haplotype blocks are outlined in heavy black triangular regions.

TABLE 4. ADDITIVE MODELS FOR SINGLE NUCLEOTIDE POLYMORPHISMS AND THE APNEA HYPOPNEA INDEX IN EUROPEAN AMERICANS ADJUSTED FOR AGE, SEX, AND EUROPEAN ANCESTRY

Chr	Gene	SNP	Location	Risk Allele	Risk Allele Frequency	Unadjusted for BMI			Adjusted for BMI		
						Beta (SE)	P Value (empirical P value*)	Q-Value	Beta (SE)	P Value	Q-Value
1	CRP	rs2808630	3' UTR	G	0.24	4.66 (1.13)	0.0000402 (0.00005125)	0.01	4.09 (1.02)	0.0000655	0.03
5	GDNF	rs2973042	Intron	C	0.32	4.26 (1.00)	0.0000201 (0.00001600)	0.01	3.52 (0.90)	0.0000979	0.03
5	GDNF	rs2973041	intron	G	0.17	4.50 (1.30)	0.000535 (0.00075)	0.09	3.36 (1.18)	0.0043630	0.22

Definition of abbreviations: BMI = body mass index; Chr = chromosome; CRP = C-reactive protein; GDNF = glial cell line–derived neurotrophic factor; SNP = single nucleotide polymorphism; UTR = untranslated region.

* See online supplement for calculation of empirical P values.

respiration, specifically influencing the development and differentiation of noradrenergic neurons, including those in the A5 nucleus of the ventrolateral pons, which plays a critical role in respiratory pattern generation (15). The knockout of the GDNF gene results in abnormal central respiratory output. In addition, GDNF seems to play a trophic role for sensory afferent neurons in the carotid body (16), and thus may be important in the development of hypoxic responses. The importance of GDNF in ventilatory control is highlighted by the fact that severe mutations in GDNF are associated with the central congenital hypoventilation syndrome (17). Although reductions in respiratory responses to hypoxia and to inspiratory load challenges among family members of OSA probands have been demonstrated (18–20), this is the first evidence that variants in a gene in a ventilatory control pathway are associated with SDB.

The finding that variants in CRP were associated with SDB provides further evidence that pathways that mediate inflammation may be important in the pathogenesis of SDB. Although most research of inflammation and OSA has considered inflammation as a response to OSA-related stresses (21–23), it is also plausible that abnormalities in inflammatory pathways may contribute to OSA severity by influencing pharyngeal patency through effects on mucosal edema, by contributing to pharyngeal neuropathic changes (24), or possibly through effects on central ventilatory control. Prior small candidate genes studies have suggested that SDB is associated with other inflammatory cytokines, such as tumor necrosis factor- α (25, 26) and IL-6 (27). If replicated, such findings may suggest a role for antiinflammatory therapy in the management of OSA.

Because serotonin plays an important role not only in sleep-wake and appetite regulation but also in upper airway dilator muscle activity through modulation of hypoglossal motor output, serotonergic receptors have gained consideration as candidate genes for SDB. In particular, the serotonin 2A receptor has been found to be the predominant excitatory serotonin receptor subtype at hypoglossal motor neurons (28), and administration of serotonin 2A receptor agonist improves upper airway stability in animal models (29). Although HTR2A may influence SDB through pleiotropic pathways (influencing both airway function and obesity), the association we observed was attenuated with BMI adjustment. It was also stronger in our African American sample, which tended to be more obese.

Thus, further disentangling the influence of this genetic variant on intermediate pathways may require a larger sample with a greater BMI range. In both Chinese and Turkish populations, the A allele in a SNP in the HTR2A promoter region (A-1438G; rs2070040) has been associated with greater OSA severity (30, 31). This SNP is in weak LD ($D' = 0.12$; $r^2 = 0.01$) with the strongest OSA SNP identified for the African American cohort in this study.

In addition to our primary findings (q values ≤ 0.10), we observed suggestive associations between the SDB traits with genetic variants in the leptin receptor (LEPR) in African Americans, hypocretin 2 receptor (HCRTR2) in African Americans, and endothelin-1 (EDN1) in European Americans (lowest q -values of 16, 16, and 12%, respectively) highlighted in the online supplement. Leptin plays a key role in weight homeostasis and has been implicated in influencing hypercapnic ventilatory drive (32). Hypocretin/orexin is a neurotransmitter known to have important effects on sleep/wake regulation, with more recent work implicating hypocretins in influencing upper airway neuromuscular activity (33). Circulating hypocretin levels have been reported to be inversely correlated with OSA severity (34). Prior analysis from European Americans in the CFS found evidence for linkage to AHI at 6p12, the location of the hypocretin receptor 2 (HCRTR2) (3). Endothelin 1 (EDN1), a potent vasoconstrictor implicated in hypertension, is associated with respiratory failure at birth in EDN1 knockout mice and hypoventilation and blunted ventilatory response to hypoxemia and hypercapnia in mice with one functional EDN1 gene (35). EDN1 knockout mice also show marked craniofacial morphologic abnormalities characteristic of disturbed pharyngeal arch development, as seen in humans with OSA caused by Treacher Collins syndrome and Pierre Robin syndrome (36). In a European population, evidence for an increase in the AHI was observed for homozygote carriers of the minor allele for a missense coding SNP (rs5370) in EDN1 (37). The same SNP allele showed an increased risk of AHI greater than or equal to 15 in the current sample of European Americans and can be considered replication at a nominal 0.05 threshold.

It is of interest that modeling the dichotomous and continuously measured traits revealed both overlapping and unique associations. Whereas genetic variants for the OSA trait are most likely to identify susceptibility loci influencing clinical

TABLE 5. ADDITIVE MULTIVARIATE LOGISTIC REGRESSION MODELS FOR SINGLE NUCLEOTIDE POLYMORPHISMS AND THE RISK OF OBSTRUCTIVE SLEEP APNEA IN AFRICAN AMERICANS ADJUSTED FOR AGE, SEX, AND AFRICAN ANCESTRY

Chr	Gene	SNP	Location	Risk Allele	Risk Allele Frequency	Unadjusted for BMI			Adjusted for BMI		
						OR (95% CI)	P Value	Q-Value	OR (95% CI)	P Value	Q-Value
13	HTR2A	rs9526240	intron	A	0.17	2.06 (1.45–2.91)	0.0000523	0.05	1.75 (1.13–2.71)	0.0126	0.49

Definition of abbreviations: BMI = body mass index; Chr = chromosome; CI = confidence interval; HTR2A = serotonin receptor 2A; OR = odds ratio; SNP = single nucleotide polymorphism.

disease, those that are associated with AHI are likely variants that are associated with incremental increases in the AHI across a wide range.

As has been reported for other traits, many of the identified genetic regions associated with OSA differed between the two races. This may reflect differences in allele frequencies for the causal variants or differing linkage disequilibrium patterns. In addition, this may reflect a difference in the relevant mechanisms for OSA pathogenesis between the two groups. For example, the importance of various craniofacial morphologies on OSA risk differs by race (38). Whether relative differences exist in other intermediate phenotypes, such as ventilatory control, is unknown. The fewer number of positive SNPs identified for the African American cohort may also reflect the stricter threshold for significance used in this group. Because of the greater genetic complexity in this population, nearly double the numbers of SNPs were required for genotyping to obtain the same level of coverage for the genes of interest, and so double the number of comparisons for which to account.

Two areas of overlap should be noted across the two races. First, rs2808630 found in the 3' untranslated region of CRP was associated with both OSA and AHI in European Americans after adjustment for multiple comparisons and also met nominal significance criteria for association to OSA in African Americans. In addition, two separate SNPs in EDN1 were suggestive of an association with OSA in European Americans (rs2071943) and African Americans (rs296344).

Given that the genes studied were selected based on biologic plausibility, we highlighted findings that met a threshold FDR of 10%. This threshold corresponds to a nominal P values of 0.0002 and 0.00009 in the European American and African American cohorts, respectively, and is much stricter than prior genetic work in OSA and more conservative than a suggested nominal P value of 0.00005 for candidate gene studies (39). However, even with this approach, the q -value results demonstrate that three of the four primary findings would meet a stricter 5% FDR threshold.

To our knowledge, this is the largest candidate gene study for sleep apnea to date. We were able to assess simultaneously the association between SDB phenotypes and 52 genes that have strong *a priori* evidence for being involved in sleep apnea pathogenesis, and were able to examine associations in two racial groups. Of note, this study includes the only African American cohort to date to have undergone a genetic association study for SDB, despite the fact that this group is at risk for OSA at an earlier age (40). Strengths include the relatively large sample size compared with prior studies in this field and the extensive coverage obtained for each of the genes considered. Although genome-wide association has gained extensive popularity for identifying causal variants for complex diseases, because of the large number of hypotheses being simultaneously tested in such a study design, the sample size required to obtain statistical significance with genome-wide association is substantially larger than with candidate gene studies. This is a particular problem with regards to SDB research given the substantial expense of overnight sleep studies required for phenotyping.

The findings in this study await replication in independent cohorts to confirm the generalizability of the identified associations. Furthermore, more detailed sequencing is needed along with functional assays to identify the causal variants, if any, at each of the regions associated with the SDB phenotypes. Nevertheless, this study identified genetic regions in both European American and African American populations worthy of further investigation as containing risk

alleles. Given the increasingly recognized adverse impact of SDB on diabetes, heart disease, and stroke risk, these variants may prove also to be important in determining cardiovascular risk.

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