Title

Multiethnic Meta-Analysis Identifies RAI1 as a Possible Obstructive Sleep Apnea—related Quantitative Trait Locus in Men

Journal

American Journal of Respiratory Cell and Molecular Biology, 58(3)

ISSN

1044-1549

Authors

Chen, Han

Cade, Brian E

Gleason, Kevin J

et al.

Publication Date

2018-03-01

DOI

10.1165/rcmb.2017-0237oc

Peer reviewed

ORIGINAL RESEARCH

Multiethnic Meta-Analysis Identifies *RAI1* as a Possible Obstructive Sleep Apnea–related Quantitative Trait Locus in Men

Han Chen[1,2,3*], Brian E. Cade[4,5*], Kevin J. Gleason[4,6], Andrew C. Bjonnes[7,8], Adrienne M. Stilp[9], Tamar Sofer[4,5,9], Matthew P. Conomos[9], Sonia Ancoli-Israel[10], Raanan Arens[11], Ali Azarbarzin[4,5], Graeme I. Bell[12], Jennifer E. Below[2,13], Sung Chun[7,14], Daniel S. Evans[15], Ralf Ewert[16], Alexis C. Frazier-Wood[17], Sina A. Gharib[18], José Haba-Rubio[19], Erika W. Hagen[20], Raphael Heinzer[19], David R. Hillman[21], W. Craig Johnson[9], Zoltan Kutalik[22,23], Jacqueline M. Lane[4,5,8,24], Emma K. Larkin[25], Seung Ku Lee[26], Jingjing Liang[27], Jose S. Loredo[28], Sutapa Mukherjee[29], Lyle J. Palmer[30], George J. Papanicolaou[31], Thomas Penzel[32], Paul E. Peppard[20], Wendy S. Post[33], Alberto R. Ramos[34], Ken Rice[9], Jerome I. Rotter[35], Scott A. Sands[4,5], Neomi A. Shah[36], Chol Shin[37], Katie L. Stone[15], Beate Stubbe[16], Jae Hoon Sul[7,14,38], Mehdi Tafti[19,39], Kent D. Taylor[35], Alexander Teumer[40], Timothy A. Thornton[9], Gregory J. Tranah[15], Chaolong Wang[1,41], Heming Wang[4,5,27], Simon C. Warby[42], D. Andrew Wellman[4,5], Phyllis C. Zee[43], Craig L. Hanis[2], Cathy C. Laurie[9], Daniel J. Gottlieb[4,5,44], Sanjay R. Patel[45], Xiaofeng Zhu[27], Shamil R. Sunyaev[7,14,24,46], Richa Saxena[4,5,8,24], Xihong Lin[1], and Susan Redline[4,5,47]

[1]Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, Massachusetts; [2]Human Genetics Center, Department of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health and [3]Center for Precision Health, School of Public Health & School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, Texas; [4]Division of Sleep and Circadian Disorders, Brigham and Women's Hospital, Boston, Massachusetts; [5]Division of Sleep Medicine, Harvard Medical School, Boston, Massachusetts; [6]Department of Public Health Sciences, University of Chicago, Chicago, Illinois; [7]Division of Genetics, Brigham and Women's Hospital, Boston, Massachusetts; [8]Center for Genomic Medicine and Department of Anesthesia, Pain, and Critical Care Medicine, Massachusetts General Hospital, Boston, Massachusetts; [9]Department of Biostatistics, University of Washington, Seattle, Washington; [10]Departments of Medicine and Psychiatry, University of California, San Diego, California; [11]the Children's Hospital at Montefiore, Division of Respiratory and Sleep Medicine, Albert Einstein College of Medicine, Bronx, New York; [12]Section of Adult and Pediatric Endocrinology, Diabetes, and Metabolism, the University of Chicago, Chicago, Illinois; [13]Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Nashville, Tennessee; [14]Division of Medical Sciences, Harvard Medical School, Boston, Massachusetts; [15]California Pacific Medical Center Research Institute, San Francisco, California; [16]Internal Medicine B, University Medicine Greifswald, Greifswald, Germany; [17]Children's Nutrition Research Center, Baylor College of Medicine, Houston, Texas; [18]Computational Medicine Core, Center for Lung Biology, University of Washington Medicine Sleep Center, Division of Pulmonary, Critical Care, and Sleep Medicine, University of Washington, Seattle, Washington; [19]Center of Investigation and Research on Sleep, Lausanne University Hospital, Lausanne, Switzerland; [20]Department of Population Health Sciences, University of Wisconsin, Madison, Wisconsin; [21]Department of Pulmonary Physiology and Sleep Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia; [22]Institute of Social and Preventive Medicine, University Hospital of Lausanne, Lausanne, Switzerland; [23]Swiss Institute of Bioinformatics, Lausanne, Switzerland; [24]Program in Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts; [25]Department of Medicine, Division of Allergy, Pulmonary, and Critical Care, Vanderbilt University Medical Center, Nashville, Tennessee; [26]Institute of Human Genomic Study, College of Medicine, Korea University Ansan Hospital, Jeokgum-ro, Danwon-gu, Ansan-si, Gyeonggi-Do, Republic of Korea; [27]Department of Epidemiology and Biostatistics, School of Medicine, Case Western Reserve University, Cleveland, Ohio; [28]Division of Pulmonary Critical Care and Sleep Medicine, Department of Medicine, University of California San Diego School of Medicine, La Jolla, California; [29]Adelaide Institute for Sleep Health, Flinders Centre of Research Excellence, Flinders University, Adelaide, South Australia, Australia; [30]School of Public Health, University of Adelaide, Adelaide, South Australia, Australia; [31]Division of Cardiovascular Sciences, National Heart, Lung, and Blood Institute, Bethesda, Maryland; [32]University Hospital Charité Berlin, Sleep Center, Berlin, Germany; [33]Division of Cardiology, Johns Hopkins University, Baltimore, Maryland; [34]Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida; [35]Institute for Translational Genomics and Population Sciences, Los Angeles BioMedical Research Institute and Department of Pediatrics at Harbor–University of California Los Angeles Medical Center, Torrance, California; [36]Division of Pulmonary, Critical Care, and Sleep, Icahn School of Medicine at Mount Sinai, New York, New York; [37]Department of Pulmonary, Sleep, and Critical Care Medicine, College of Medicine, Korea University Ansan Hospital, Jeokgum-ro, Danwon-gu, Ansan-si, Gyeonggi-do, Republic of Korea; [38]Department of Psychiatry and Biobehavioral Sciences, University of California Los Angeles, Los Angeles, California; [39]Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland; [40]Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany; [41]Computational and Systems Biology, Genome Institute of Singapore, Singapore; [42]Department of Psychiatry, University of Montreal, Montreal, Quebec, Canada; [43]Department of Neurology and Sleep Medicine Center, Northwestern University, Chicago, Illinois; [44]Veterans Affairs Boston Healthcare System, Boston, Massachusetts; [45]Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; [46]Department of Biomedical Informatics, Harvard Medical School, Boston, Massachusetts; and [47]Division of Pulmonary, Critical Care, and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts

ORCID ID: 0000-0002-9510-4923 (H.C.).

Abstract

Obstructive sleep apnea (OSA) is a common heritable disorder displaying marked sexual dimorphism in disease prevalence and progression. Previous genetic association studies have identified a few genetic loci associated with OSA and related quantitative traits, but they have only focused on single ethnic groups, and a large proportion of the heritability remains unexplained. The apnea–hypopnea index (AHI) is a commonly used quantitative measure characterizing OSA severity. Because OSA differs by sex, and the pathophysiology of obstructive events differ in rapid eye movement (REM) and non-REM (NREM) sleep, we hypothesized that additional genetic association signals would be identified by analyzing the NREM/REM-specific AHI and by conducting sex-specific analyses in multiethnic samples. We performed genome-wide association tests for up to 19,733 participants of African, Asian, European, and Hispanic/Latino American ancestry in 7 studies. We identified rs12936587 on chromosome 17 as a possible quantitative trait locus for NREM AHI in men ($N = 6{,}737$; $P = 1.7 \times 10^{-8}$) but not in women ($P = 0.77$). The association with NREM AHI was replicated in a physiological research study ($N = 67$; $P = 0.047$). This locus overlapping the *RAI1* gene and encompassing genes *PEMT1*, *SREBF1*, and *RASD1* was previously reported to be associated with coronary artery disease, lipid metabolism, and implicated in Potocki–Lupski syndrome and Smith-Magenis syndrome, which are characterized by abnormal sleep phenotypes. We also identified gene-by-sex interactions in suggestive association regions, suggesting that genetic variants for AHI appear to vary by sex, consistent with the clinical observations of strong sexual dimorphism.

Keywords: obstructive sleep apnea; genetics; genome-wide association studies; multiethnic; sexual dimorphism.

Clinical Relevance

We identified an association with apnea–hypopnea index during non–rapid eye movement sleep in men in a region that includes a strong biological candidate gene, but not in women. Biological pathways involved in the etiology of obstructive sleep apnea are likely different in men and women, and understanding sex-specific genetic associations may yield novel insights into the pathogenesis of this complex condition.

Obstructive sleep apnea (OSA) is a complex chronic condition that affects more than 10% of the population, and is associated with cardiometabolic and behavioral morbidity (1–3). The prevalence of OSA is particularly high in minority racial/ethnic groups, such as those with African, Asian, and Hispanic ancestry (4–7). Moreover, OSA is approximately threefold more prevalent in men as compared with women (8). In women, OSA severity is less likely to worsen in the supine compared with other sleeping positions (9), and more likely to worsen in rapid eye movement (REM) sleep, when neuromuscular tone and chemoreflexes are reduced (9, 10). These differences have been attributed to sex differences in airway collapsibility, related to both differences in anatomy and respiratory chemosensitivity (11). An increase in OSA severity in women after menopause also suggests a role for sex hormones in influencing this disorder (12).

The severity of OSA is most often characterized by the apnea–hypopnea index (AHI), defined as the number of apnea and hypopnea events per hour of sleep. AHI levels are highly heritable in African Americans and European Americans, with 30%–40% of the variance explained by genetic factors (13, 14). Previous genetic studies have identified several genetic variants associated with AHI, although these findings were based on modest sample sizes or single ethnic groups, and largely have not been replicated across populations (15–18).

Large-scale genome-wide association studies (GWAS) have identified sexual

(Received in original form June 30, 2017; accepted in final form October 24, 2017)

*These authors contributed equally to this work.

The views expressed in this article are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

Author Contributions: H.C., B.E.C., K.J.G., A.C.B., A.M.S., T.S., M.P.C., A.A., G.I.B., J.E.B., S.C., D.S.E., R.E., A.C.F.-W., S.A.G., J.H.-R., E.W.H., R.H., D.R.H., W.C.J., Z.K., J.M.L., E.K.L., S.K.L., J.L., S.M., L.J.P., G.J.P., W.S.P., A.R.R., K.R., J.I.R., S.A.S., N.A.S., B.S., J.H.S., K.D.T., A.T., T.A.T., C.W., H.W., S.C.W., D.J.G., and S.R. participated in data acquisition and analysis of data; S.A.-I., R.A., J.S.L., L.J.P., T.P., P.E.P., C.S., K.L.S., M.T., G.J.T., D.A.W., P.C.Z., C.L.H., C.C.L., D.J.G., S.R.P., X.Z., S.R.S., R.S., X.L., and S.R. participated in study conception and design; all authors participated in interpretation of analysis results, manuscript drafting, and manuscript editing for intellectual content.

Correspondence and requests for reprints should be addressed to Han Chen, Ph.D., Human Genetics Center, Department of Epidemiology, Human Genetics and Environmental Sciences, Center for Precision Health, School of Public Health, School of Biomedical Informatics, The University of Texas Health Science Center at Houston, 1200 Pressler Street, RAS E-517, Houston, TX 77030. E-mail: han.chen.2@uth.tmc.edu.

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

Am J Respir Cell Mol Biol Vol 58, Iss 3, pp 391–401, Mar 2018

Originally Published in Press as DOI: 10.1165/rcmb.2017-0237OC on October 27, 2017
Internet address: www.atsjournals.org

dimorphism in genetic loci for traits associated with OSA, such as body fat distribution, particularly waist circumference and waist-to-hip ratio, each adjusted for body mass index (BMI) (19, 20). Furthermore, measures of adiposity, such as waist phenotypes, have been shown to be regulated by sexually dimorphic genes (19, 21). Animal models suggest that both gonadal hormones and X chromosome dose influence lipid levels (22). Despite strong clinical and epidemiological evidence for sex differences in OSA, prior genetic association studies were not sufficiently powered to study consistent sex differences in OSA in multiethnic samples (15–17).

We conducted GWAS in multiethnic samples from seven cohorts to identify genetic variants with sex-specific association for AHI. Given differences in the physiological bases for OSA in REM and non-REM (NREM) sleep (23), we performed analyses for AHI calculated for each sleep state (REM, NREM). Although BMI is a significant risk factor for OSA, only 40% of the genetic variance for OSA is shared with BMI (14). Therefore, we adjusted for BMI to discover genetic loci acting independently of BMI, which may provide insights into novel etiological mechanisms. We focused on association signals that show concordant direction of effects across African, Asian, Hispanic/Latino, and European Americans through BMI-independent pathways.

Methods

Study Subjects

We included seven cohorts in the discovery analyses: the ARIC (Atherosclerosis Risk in Communities Study; $n = 1{,}463$ European Americans), the CFS (Cleveland Family

This work was supported by National Institutes of Health (NIH) grants R01-HL113338, P01-CA134294, and R35-CA197449 (H.C.), T32-HL007901 and R01-HL113338 (B.E.C.), and R01-HL113338 and R35-HL135818 (S.R.). The Sleep Reading Center of Brigham and Women's Hospital was supported by NIH grants 5-R01-HL046380-15 and 5-KL2-RR024990-05; the Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute (NHLBI) contracts HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C; the Framingham Heart Study is conducted and supported by NHLBI in collaboration with Boston University contract N01-HC-25195; funding for SHARe Affymetrix genotyping was provided by NHLBI contract N02-HL- 64278; SHARe Illumina genotyping was provided under an agreement between Illumina and Boston University; funding support for the Framingham Sleep Heart Health Study was provided by NIH/NHLBI grant U01 HL 53941; the baseline examination of the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) was carried out as a collaborative study supported by contracts from the NHLBI to the University of North Carolina (N01-HC65233), University of Miami (N01-HC65234), Albert Einstein College of Medicine (N01-HC65235), Northwestern University (N01-HC65236), and San Diego State University (N01-HC65237). The following institutes/centers/offices contributed to the first phase of HCHS/SOL through a transfer of funds to the NHLBI: National Institute on Minority Health and Health Disparities, National Institute on Deafness and Other Communication Disorders, National Institute of Dental and Craniofacial Research (NIDCR), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Neurological Disorders and Stroke (NINDS), and NIH Institution-Office of Dietary Supplements. The Genetic Analysis Center at the University of Washington was supported by NHLBI and NIDCR contracts HHSN268201300005C AM03 and MOD03. Provision of genotyping services was supported in part by the National Center for Advancing Translational Sciences (NCATS) Clinical and Translational Science Institute (CTSI) grant UL1TR000124 and NIDDK Diabetes Research Center (DRC) grant DK063491. The Multi-Ethnic Study of Atherosclerosis (MESA) is conducted and supported by the NHLBI in collaboration with MESA investigators, and was supported by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, and N01-HC-95169 from the NHLBI and by grants UL1-TR-000040 and UL1-TR-001079 from National Center for Research Resources (NCRR). Funding for SHARe genotyping was provided by NHLBI contract N02-HL-64278. Genotyping was performed at Affymetrix and the Broad Institute of Harvard and Massachusetts Institute of Technology. Funding support for the Sleep Polysomnography dataset was provided by NHLBI grant HL56984. Provision of genotyping services supported in part by NCATS CTSI grant UL1TR000124 and NIDDK DRC grant DK063491. The Osteoporotic Fractures in Men Study (MrOS) is supported by NIH funding. The following institutes provide support: the National Institute on Aging (NIA), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NCATS, and NIH Roadmap for Medical Research under grants U01 AG027810, U01 AG042124, U01 AG042139, U01 AG042140, U01 AG042143, U01 AG042145, U01 AG042168, U01 AR066160, and UL1 TR000128. The NHLBI provides funding for the MrOS Sleep ancillary study "Outcomes of Sleep Disorders in Older Men" under grants R01 HL071194, R01 HL070848, R01 HL070847, R01 HL070842, R01 HL070841, R01 HL070837, R01 HL070838, and R01 HL070839. The NIAMS provides funding for the MrOS ancillary study "Replication of candidate gene associations and bone strength phenotype in MrOS" under grant R01 AR051124. The NIAMS provides funding for the MrOS ancillary study "GWAS in MrOS and SOF" under grant RC2 AR058973. The Starr County Health Studies are supported in part by NIH grants R01 DK073541, U01 DK085501, R01 AI085014, and R01 HL102830, and funds from The University of Texas Health Science Center at Houston. G.I.B. was supported in part by grant P30 DK020595 and a gift from the Kovler Family Foundation. The KoGES_Ansan study was provided with biospecimens and data from the Korean Genome Analysis Project (4845-301), the Korean Genome and Epidemiology Study (2009-E71002-00, 2010-E71001-00, 2011-E71004-00, 2012-E71005-00, 2013-E71005-00, and 2014-E71003-00), and Korea Biobank Project (4851-307) that were supported by the Korea Centers for Disease Control & Prevention, Republic of Korea. The Cardiovascular Health Study research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, and R01HL130114, with additional contribution from NINDS. Genotyping among the African American cohort was supported in part by HL085251 from NHLBI. Additional support was provided through NIA grant R01AG023629. The provision of genotyping data was supported in part by NCATS CTSI grant UL1TR000124 and NIDDK DRC grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The HypnoLaus and CoLaus/HypnoLaus study was supported by research grants from GlaxoSmithKline, the Faculty of Biology and Medicine of Lausanne, the Swiss National Science Foundation grants 3200B0-105993, 3200B0-118308, 33CSCO-122661, 33CS30-139468, and 33CS30-148401, the Leenaards Foundation, and the Vaud Pulmonary League (Ligue Pulmonaire Vaudoise). The Study of Health in Pomerania is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by Federal Ministry of Education and Research grants 01ZZ9603, 01ZZ0103, and 01ZZ0403, Competence Network Asthma/ COPD grant FKZ 01GI0881-0888, the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg–West Pomerania, and the network "Greifswald Approach to Individualized Medicine" funded by Federal Ministry of Education and Research grant 03IS2061A. The polysomnography was additionally funded by the German Restless Legs Association. Funding for the Western Australian Sleep Health Study was obtained from the Sir Charles Gairdner and Hollywood Private Hospital Research Foundations, the Western Australian Sleep Disorders Research Institute, and the Centre for Genetic Epidemiology and Biostatistics at the University of Western Australia. Funding for the genome-wide association studies (GWAS) genotyping was obtained from the Ontario Institute for Cancer Research and a McLaughlin Centre Accelerator Grant from the University of Toronto. The Wisconsin Sleep Cohort (WSC) Study phenotype data collection was supported by NIH grants R01HL62252 and 1UL1RR02501. GWAS typing of the WSC was conducted thanks to private donations and NIH-23724 to Dr. Emmanuel Mignot, Psychiatry and Behavioral Sciences, Stanford Center for Sleep Sciences and Medicine.

Study; $n = 731$ African Americans and 702 European Americans), the FHS (Framingham Heart Study; $n = 646$ European Americans), the HCHS/SOL (Hispanic Community Health Study/Study of Latinos; $n = 11,317$ Hispanic/Latino Americans), the MESA (Multi-Ethnic Study of Atherosclerosis; $n = 490$ African Americans, 228 Asian Americans, 707 European Americans, and 458 Hispanic/Latino Americans), the MrOS (Osteoporotic Fractures in Men Study; $n = 2,209$ European Americans), and Starr (the Starr County Health Studies; $n = 782$ Hispanic/Latino Americans) (Table 1). An additional six cohort studies and data from one physiological research study were analyzed to examine for generalizability across samples. Details about the study subjects are provided in the data supplement.

Phenotypes and Covariates

OSA was quantified using the AHI, defined as the number of episodes of complete (apnea) or partial (hypopnea) cessations of airflow per hour of sleep (or recording time). In this study, sleep data from all seven discovery studies were scored in our Sleep Reading Center. Details of the sleep testing and scoring procedures for each cohort are provided in the data supplement. The primary phenotype was the AHI calculated across the total sleep (or recording) period (AHI-total; AHI-T). All studies used a hypopnea definition that required a 3% or greater event-related desaturation. Covariates include age, sex, and BMI. AHI measured during REM (AHI-R) and NREM (AHI-N) sleep periods also were analyzed where available (ARIC, CFS, FHS, MESA, and MrOS).

Genotyping and Quality Control

Study participants in ARIC, MESA, and Starr were genotyped using the Affymetrix 6.0 array; CFS participants were genotyped using the Illumina OmniExpress, Affymetrix 6.0, and the ITMAT-Broad-CARe (IBC) (24) arrays; FHS participants were genotyped using the Affymetrix 500K mapping array and Illumina Omni5 array; HCHS/SOL participants were genotyped using the Illumina Omni 2.5M array with custom content; and MrOS participants were genotyped using the Illumina Omni 1M array. Data from CFS, FHS, MESA, MrOS, and Starr were phased using SHAPEIT (25) and imputed using IMPUTE2 (26), and a 1000 Genomes Project phase 3 background (version 5; all populations, which contains haplotypes on 2,504 samples for a total of approximately 81.2 million polymorphic markers); ARIC and HCHS/SOL were imputed using a 1000 Genomes Project phase 1 background. SNP strands were checked in Ensembl and with 1000 Genomes data in SHAPEIT. SNPs with an IMPUTE2 Info score less than 0.88, or a minor allele frequency less than 1% in each study cohort, were excluded from analyses.

Statistical Analysis

Rank-normalized age and sex-adjusted residuals were analyzed using linear mixed models with a genetic relatedness matrix in GEMMA (27) to control for population stratification and relatedness, adjusting for BMI and BMI^2. Multiethnic meta-analyses were performed using the inverse variance–weighted fixed-effects approach in METAL (28). Details on statistical analyses are provided in the data supplement.

Results

Demographics

Key characteristics of each cohort are presented in Table 1, with additional details in the data supplement. Across the seven distinct cohorts, data were available for 19,733 individuals, including 10,113 women. Participants were, on average, middle-aged to elderly, and were overweight to obese. The proportion with moderate to severe sleep apnea (AHI $\geq$ 15 events/h) ranged from 11.7% to 54.8%. In general, the AHI varied with the mean age of the cohort (higher in the older cohorts). Overall, the sample ancestry was 29.0% European, 6.2% African, 63.6% Hispanic, and 1.2% Asian.

Sex-combined and Sex-stratified Analyses

The top results of the multiethnic meta-analyses are shown in Table 2. In sex-combined results, eight loci showed suggestive association ($P < 1.0 \times 10^{-6}$) with AHI-T, five loci with AHI-N, and two loci with AHI-R. These regions included rs146579140, where variation was associated with AHI-N at an almost significant level ($P = 8.8 \times 10^{-8}$). In sex-stratified results, 6 loci showed suggestive association with AHI-T, 3 loci with AHI-N, and 3 loci with AHI-R in women; 11 loci showed suggestive association with AHI-T, 3 loci with AHI-N, and 2 loci with AHI-R in men. In addition, there was one locus significantly associated with AHI-N in men on chromosome 17 (Figure 1), with a lead SNP rs12936587 ($P = 1.7 \times 10^{-8}$). This locus overlapped with the gene, *RAI1* (Figure 1C), which codes retinoic acid induced 1 that has been implicated in Smith-Magenis syndrome (SMS) (29). This lead SNP also showed suggestive association with AHI-N in sex-combined analysis, although the findings reflected associations in men and not women. Figure 2 shows that, compared with men with a homozygous genotype of the ancestral allele (A), men with more risk alleles (G) had a higher age- and BMI-adjusted AHI-N on average, but there was no such pattern in women.

Gene-by-Sex Interaction

We performed gene-by-sex interaction analyses for top loci in Table 2 and identified 13 gene-by-sex interactions in multiethnic meta-analyses, after Bonferroni correction to control for a family-wise significance level of 0.05. Of these 13 gene-by-sex interactions, 12 had significant or suggestive association in men, but not in women (including rs12936587 with AHI-N; interaction $P = 2.6 \times 10^{-5}$), and one had suggestive association in sex-combined results, but neither in men nor in women (although the P value in men was still several orders of magnitude lower than in women). These results suggest that there might be different genetic mechanisms for OSA in women and men.

Expression Quantitative Trait Loci Databases

We examined our most significant SNPs in the *RAI1* region (National Center for Biotechnology Information build 37 locations: chr17:17531709–17644364; $P < 1 \times 10^{-7}$) in expression quantitative trait loci (eQTLs) databases that associate SNPs with gene expression in specific cell lines and tissues (*see* Table E1 in the data supplement). Five of the eight genes associated with the *RAI1* locus SNPs had a minimum eQTL P value less than 1×10^{-6}: *PEMT* (whole-blood $P = 2.1 \times 10^{-20}$), *SREBF1* (whole-blood $P = 5.0 \times 10^{-20}$), *RASD1* (monocyte $P = 6.8 \times 10^{-12}$), *RAI1* (lymphoblastoid $P = 3.4 \times 10^{-7}$), and *TOM1L2* (pituitary $P = 9.4 \times 10^{-7}$).

Table 1. Sample Description of Study Subjects in Discovery Cohorts

Ethnic Group	Cohort	*n*	Age (*Yr*)	% Female	BMI (kg/m^2)	AHI	% OSA	AHI-N	AHI-R	AHI-R/AHI-N
African Americans	CFS*	731	37.84 (19.44)	56.2	31.63 (9.69)	5.85 (19.70)	31.7	2.47 (12.19)	9.23 (30.87)	2.89 (8.70)
	MESA	490	69.13 (9.10)	54.3	30.42 (5.71)	13.34 (21.16)	46.5	8.64 (20.1)	30.97 (39.83)	2.64 (5.09)
Asian Americans	MESA	228	68.13 (9.19)	50.4	24.08 (3.19)	13.97 (23.90)	47.4	11.61 (24.31)	21.77 (34.19)	1.68 (2.64)
European Americans	ARIC	1,463	62.43 (5.69)	51.5	28.83 (5.13)	8.70 (15.50)	32.5	5.75 (13.78)	16.46 (27.68)	2.58 (4.69)
	CFS*	702	41.59 (19.45)	52.7	30.24 (8.66)	5.59 (18.99)	31.1	2.07 (13.14)	7.06 (21.20)	2.28 (7.40)
	FHS*	646	59.38 (8.97)	50.0	28.49 (5.01)	8.18 (14.51)	30.0	5.25 (13.66)	15.25 (23.60)	2.48 (4.98)
	MESA	707	68.52 (9.10)	53.6	27.99 (5.21)	12.62 (20.67)	44.0	9.61 (20.45)	22.31 (30.65)	1.80 (2.97)
	MrOS	2,209	76.68 (5.66)	0.0	27.22 (3.74)	12.73 (18.11)	43.7	10.93 (19.58)	18.30 (24.17)	1.53 (2.42)
Hispanic/Latino Americans	HCHS/SOL	11,317	46.17 (13.79)	59.1	29.79 (6.00)	1.97 (6.20)	11.7	NA	NA	NA
	MESA	458	68.34 (9.20)	52.8	30.07 (5.52)	16.94 (23.05)	54.8	12.16 (23.12)	30.00 (36.09)	2.13 (3.50)
	Starr	782	52.34 (11.29)	71.9	32.15 (6.78)	10.35 (17.18)	37.1	NA	NA	NA

Definition of abbreviations: AHI = apnea–hypopnea index; AHI-N = non–rapid eye movement–specific AHI; AHI-R = rapid eye movement-specific AHI; ARIC = Atherosclerosis Risk in Communities Study; BMI = body mass index; CFS = Cleveland Family Study; FHS = Framingham Heart Study; HCHS/SOL = Hispanic Community Health Study/Study of Latinos; MESA = Multi-Ethnic Study of Atherosclerosis; MrOS = Osteoporotic Fractures in Men Study; NA = not applicable; OSA = obstructive sleep apnea; Starr = the Starr County Health Studies.
Seven studies included 19,733 individuals with genotypes and phenotypes (1,221 African Americans, 228 Asian Americans, 5,727 European Americans, and 12,557 Hispanic/Latino Americans). Non–rapid eye movement– and rapid eye movement–specific data are only available in a sample of the CFS data, and were not collected in HCHS/SOL and Starr. Mean (SD) are listed for age and BMI, and median (interquartile range) are listed for AHI, AHI-N, AHI-R, and AHI-R/AHI-N. OSA is defined as an AHI of 15 or greater.
*Family cohorts.

Table 2. Significant and Suggestive Multiethnic Meta-Analysis Results for Apnea–Hypopnea Index (Total), Apnea–Hypopnea Index–Non–Rapid Eye Movement, and Apnea–Hypopnea Index–Rapid Eye Movement

Trait	SNP	Chr	Locus	Alleles	Sex Combined			Women			Men			Sex Difference
					n	Effect	*P* Value	*n*	Effect	*P* Value	*n*	Effect	*P* Value	*P* Value
AHI-T	rs76321756	1		C/T	2,381	0.130	9.7×10^{-5}	1,289	−0.017	7.0×10^{-1}	1,092	0.244	2.3×10^{-7}	2.7×10^{-6}
AHI-T	rs11897825	2	AC011752.1; AC067959.1	G/A	15,364	0.026	2.2×10^{-2}	7.650	0.080	3.3×10^{-7}	7,714	−0.014	3.6×10^{-1}	2.8×10^{-3}
AHI-T	rs999944	2		G/A	19,733	0.072	9.7×10^{-7}	10,113	0.063	2.0×10^{-3}	9,620	0.077	2.6×10^{-4}	5.5×10^{-3}
AHI-T	rs35520189	2		C/A	19,733	0.052	6.1×10^{-7}	10,113	0.064	5.8×10^{-6}	9,620	0.042	5.3×10^{-3}	5.9×10^{-1}
AHI-T	rs72149316:AATAA	2	*SCN3A*	GATAA/AATAA	1,679	0.428	6.0×10^{-7}	919	0.326	3.7×10^{-3}	764	0.606	5.9×10^{-7}	2.3×10^{-3}
AHI-T	rs34526934	2	HOXD-AS2;HOXD3; HOXD4;MIR10B	A/T	19,505	0.060	1.1×10^{-6}	9,998	0.024	1.7×10^{-1}	9,507	0.087	3.6×10^{-7}	1.8×10^{-3}
AHI-T	rs62189527	2	HDAC4	C/T	12,665	0.174	4.6×10^{-5}	7,384	0.067	2.3×10^{-1}	5,281	0.320	7.4×10^{-7}	9.7×10^{-4}
AHI-T	rs35001935:CGTGTGT	3	C3orf67; RP11-147N17.1	CGTGT/CGTGTGT	1,679	0.251	7.7×10^{-7}	919	0.091	1.9×10^{-1}	760	0.339	1.3×10^{-6}	3.3×10^{-4}
AHI-T	chr3:166153534:D	3		A/AAC	2.461	0.249	3.4×10^{-5}	1,481	0.376	6.1×10^{-7}	980	0.157	9.4×10^{-2}	3.2×10^{-1}
AHI-T	rs34188544	4		G/A	2,135	0.522	3.6×10^{-7}	1,264	0.333	1.4×10^{-2}	871	0.728	2.1×10^{-6}	8.2×10^{-3}
AHI-T	rs73352871	5		C/A	12,996	0.082	2.4×10^{-3}	7,610	−0.025	4.8×10^{-1}	5,386	0.203	5.9×10^{-7}	2.2×10^{-6}
AHI-T	rs79987021	7		T/C	12,265	0.256	6.2×10^{-7}	7,199	0.186	5.4×10^{-3}	5,066	0.316	7.7×10^{-5}	1.5×10^{-2}
AHI-T	rs117004340	8	DLC1	T/A	19,504	0.079	2.2×10^{-5}	9,997	−0.001	9.7×10^{-1}	9,507	0.136	1.8×10^{-7}	9.2×10^{-4}
AHI-T	rs3736021	8	*ENTPD4*	T/G	19,732	0.065	7.2×10^{-7}	10,112	0.057	1.1×10^{-3}	9,620	0.071	3.4×10^{-4}	1.1×10^{-1}
AHI-T	rs35857674	8	*SNTG1*	A/G	19,732	0.024	2.2×10^{-2}	10,112	0.070	7.9×10^{-7}	9,620	−0.016	2.8×10^{-1}	5.2×10^{-3}
AHI-T	rs117169866	10		G/A	18,512	0.150	5.6×10^{-7}	9,436	0.116	7.0×10^{-3}	9,396	0.158	1.1×10^{-4}	6.7×10^{-2}
AHI-T	rs1387259	12	RP11-370I10.2; *ZNF641*	A/G	19,733	0.052	2.3×10^{-6}	10,113	0.031	4.1×10^{-2}	9,620	0.078	3.7×10^{-7}	2.3×10^{-2}
AHI-T	rs9600832	13	NBEA	C/T	19,733	0.064	1.2×10^{-5}	10,113	0.012	5.4×10^{-1}	9,620	0.104	5.6×10^{-7}	7.8×10^{-5}
AHI-T	rs75900232	13		G/A	16,337	0.159	2.1×10^{-4}	8,942	−0.037	5.2×10^{-1}	8,199	0.307	6.5×10^{-7}	4.9×10^{-5}
AHI-T	rs115432071	16	C16orf62	T/A	12,996	0.197	8.5×10^{-6}	7,610	0.055	3.4×10^{-1}	5,386	0.346	3.0×10^{-7}	1.6×10^{-4}
AHI-T	rs4787347	16	*HS3ST4*	G/A	19,733	0.029	3.3×10^{-3}	10,113	0.067	7.2×10^{-7}	9,620	0.002	8.8×10^{-1}	1.9×10^{-1}
AHI-T	rs112190082	17	*STX8*	G/T	13,905	0.099	1.0×10^{-3}	8,188	0.200	3.6×10^{-7}	5,717	0.013	7.7×10^{-1}	3.4×10^{-2}
AHI-T	rs113724004	17		T/C	19,505	0.069	5.9×10^{-5}	9,998	0.020	4.0×10^{-1}	9,507	0.123	8.0×10^{-7}	3.9×10^{-4}
AHI-T	rs142002225	20		T/C	19,733	0.055	2.8×10^{-4}	10,113	0.108	3.2×10^{-7}	9,620	0.022	3.1×10^{-1}	3.4×10^{-1}
AHI-N	rs146579140	2		C/T	6,737	0.206	8.8×10^{-8}	2,412	0.092	1.6×10^{-1}	4,212	0.237	5.2×10^{-7}	1.3×10^{-2}
AHI-N	rs10474877	5	*ANKRD33B*	T/C	6,737	0.039	1.1×10^{-1}	2,412	0.203	2.1×10^{-7}	4,212	−0.015	6.2×10^{-1}	3.7×10^{-2}
AHI-N	rs73686127	7		A/T	1,300	0.508	2.6×10^{-7}	719	0.509	4.7×10^{-5}	367	0.464	4.6×10^{-3}	2.2×10^{-1}
AHI-N	rs10968431	9	LINGO2	G/A	1,300	0.239	5.3×10^{-4}	719	−0.030	7.5×10^{-1}	581	0.474	9.7×10^{-7}	1.3×10^{-5}
AHI-N	rs79697311	11		C/A	6,029	0.215	5.2×10^{-3}	1,295	0.719	6.9×10^{-7}	3,885	0.176	6.9×10^{-2}	4.2×10^{-1}
AHI-N	rs34174435	11	*OR5AN1*	GA/G	5,304	0.101	6.4×10^{-7}	1,673	0.105	4.4×10^{-3}	3,631	0.086	3.3×10^{-4}	1.1×10^{-1}
AHI-N	rs75482679	11	CNTN5	T/C	6,737	0.128	1.0×10^{-5}	2,412	−0.019	6.9×10^{-1}	4,325	0.176	8.7×10^{-7}	1.3×10^{-4}
AHI-N	rs72986876	11	*PDGFD*	A/T	6,511	0.223	7.8×10^{-7}	2,299	0.194	1.3×10^{-2}	4,212	0.217	7.9×10^{-5}	4.4×10^{-1}
AHI-N	rs116696666	16		A/T	1,300	0.290	1.6×10^{-3}	719	0.608	5.9×10^{-7}	581	0.136	3.1×10^{-1}	2.5×10^{-1}
AHI-N	rs12936587	17	RAI1	G/A	6,737	0.083	6.9×10^{-7}	2,412	−0.008	7.7×10^{-1}	4,325	0.116	1.7×10^{-8}	2.6×10^{-5}
AHI-R	chr1:74135031	1	RP4-788P17.1	A/T	1,546	0.310	9.8×10^{-7}	858	0.208	1.0×10^{-2}	688	0.403	5.2×10^{-5}	1.2×10^{-1}
AHI-R	rs72956768	4	TMEM154	G/T	1,290	0.254	6.4×10^{-4}	714	0.020	8.3×10^{-1}	576	0.563	5.2×10^{-7}	1.5×10^{-4}
AHI-R	rs35077018	7	*HUS1*; *PKD1L1*	G/GCTAGTGCGTGCATG AACTAGTTGGTCTGC AAGTACAAGATGTAT AAATATACAGGGGAA AAAACATC	1,290	0.198	5.5×10^{-4}	714	0.357	8.2×10^{-7}	576	0.049	5.9×10^{-1}	3.4×10^{-2}
AHI-R	rs9297743	8	RP11-89K10.1	G/A	6,700	0.127	5.5×10^{-4}	2,400	−0.037	5.1×10^{-1}	4,300	0.241	9.2×10^{-7}	2.6×10^{-3}
AHI-R	rs79697311	11		C/A	5,998	0.173	1.8×10^{-2}	1,289	0.692	4.4×10^{-7}	3,867	0.068	4.7×10^{-1}	4.6×10^{-2}
AHI-R	rs201360344	12	*C12orf55*	G/GA	924	0.478	5.5×10^{-7}	498	0.516	3.2×10^{-5}	426	0.329	3.1×10^{-2}	4.1×10^{-1}
AHI-R	rs41408454	15		G/A	1,290	0.287	1.1×10^{-3}	714	0.523	8.8×10^{-7}	576	0.019	8.9×10^{-1}	1.2×10^{-2}

Definition of abbreviations: AHI = apnea–hypopnea index; AHI-N = non–rapid eye movement sleep–specific AHI; AHI-R = rapid eye movement sleep-specific AHI; AHI-T = AHI-total; Chr = chromosome.

SNPs with significant ($P < 5.0 \times 10^{-8}$) and suggestive ($P < 1.0 \times 10^{-6}$) *P* values. SNPs that were present in only one ethnic group were excluded. Alleles: effect allele/noneffect allele.

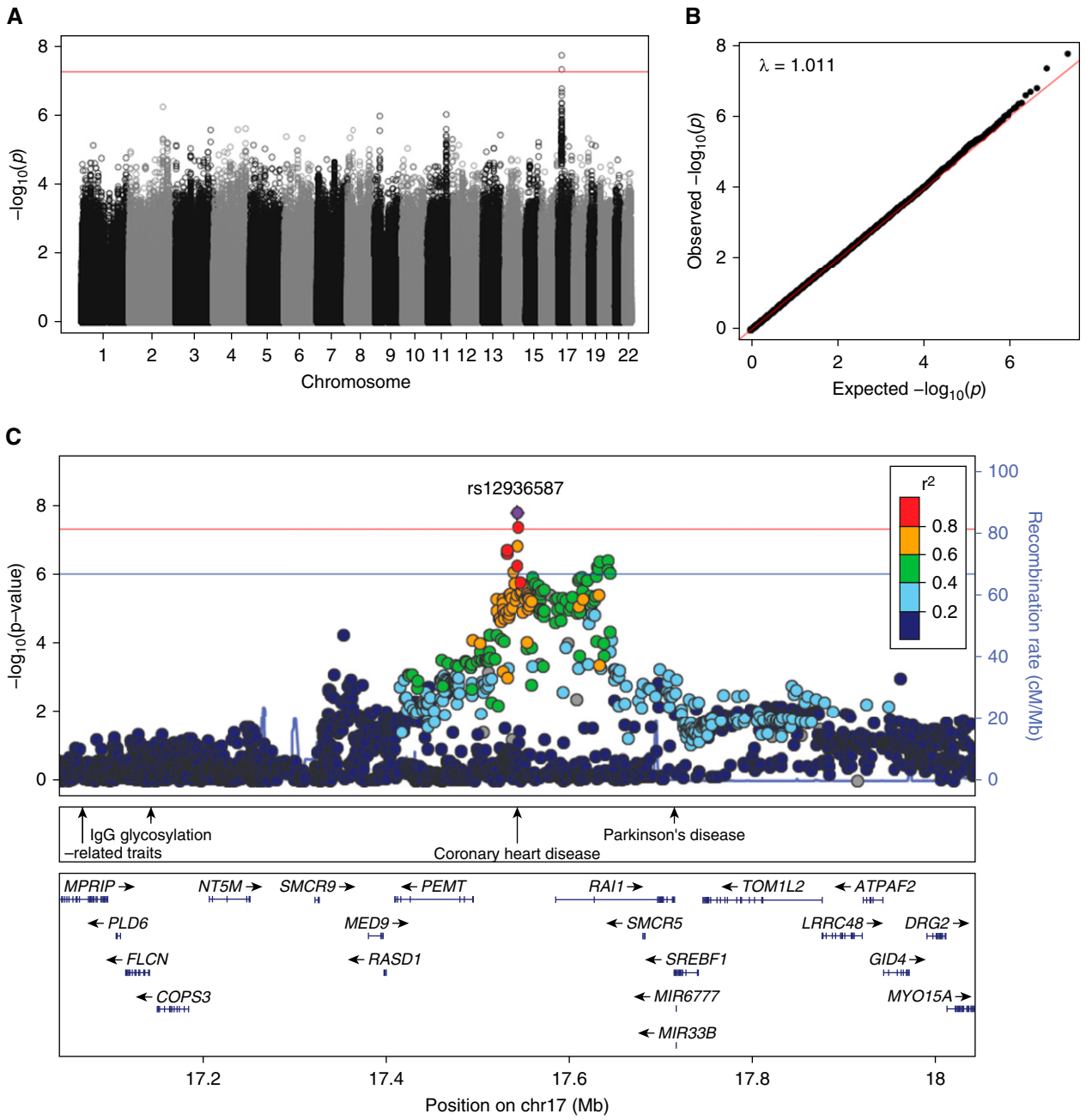

Figure 1. Manhattan, quantile–quantile (Q-Q), and regional association plots of multiethnic meta-analysis results for apnea–hypopnea index during non–rapid eye movement sleep (AHI-N) in men. (*A*) The Manhattan plot shows −log10 *P* values against genomic coordinates (National Center for Biotechnology Information build 37), and consecutive chromosomes were colored in black and gray alternately. (*B*) The Q-Q plot shows observed −log10 *P* values against expected values under no association. (*C*) The regional association plot of multiethnic meta-analysis results for AHI-N in men near the *RAI1* gene on chromosome 17.

Assessment of Generalizability in Independent Samples

In summary data provided by replication cohort studies (Table E2), we found no evidence for association with AHI-N for *RAI1* in men ($P = 0.34$). However, a consistent direction of association was found in the CHS (Cardiovascular Health Study), the only replication cohort in which sleep studies were scored by the same sleep reading center that scored data in the discovery cohorts.

We replicated the association with rs12936587 in an independent physiological research study of 67 individuals (70% male; Table E3) studied with in-laboratory polysomnography for the purposes of elucidating the physiology of OSA. Details about the study subjects are provided in the data supplement. The sample included 55 patients with moderate to severe OSA without other significant comorbidities and 12 healthy control subjects. In this well-phenotyped sample,

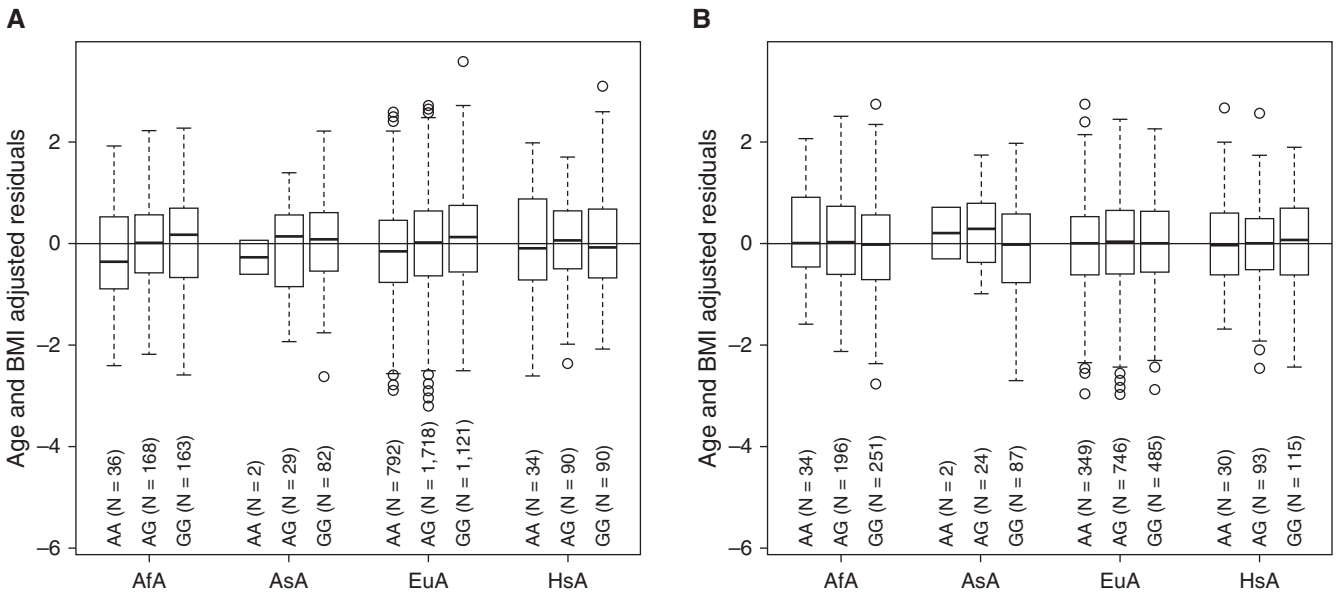

Figure 2. Sex differences in the distribution of body mass index (BMI) and BMI^2-adjusted residuals for study subjects with different genotypes (AA, AG, and GG) of rs12936587. The phenotype is rank-normalized sex-stratified residuals of apnea–hypopnea index during non–rapid eye movement sleep adjusting for age and age^2. (*A*) Men and (*B*) women. AfA = African Americans; AsA = Asian Americans; EuA = European Americans; HsA = Hispanic/Latino Americans.

after adjusting for age, sex, and BMI, the risk G allele of rs12936587 was associated with increasing AHI-N ($P = 0.047$). The association was stronger when we restricted the analysis to AHI-N in the supine position ($P = 0.017$), when airway collapsibility is high.

Discussion

To our knowledge, this is the largest genome-wide analysis of AHI and the only multiethnic sex-specific AHI meta-analysis to date. It is also the first human genetic epidemiological study that has examined AHI-R and AHI-N. Analyses of rigorously collected quantitative sleep data and genome-wide genotype data identified several novel genetic regions with at least suggestive association evidence with each AHI measure. The most significant findings emerged from sex-specific and sleep state–specific analyses. Across all cohorts and race/ethnic groups, the most significant finding was for an association between a locus in *RAI1* in men for AHI-N sleep. Our results identify several biologically plausible candidates for future functional studies, and highlight genetic variants that may specifically influence OSA propensity in REM versus NREM sleep, which may have different associations in men and women. The finding of multiple significant gene-by-sex interactions further provides statistical evidence of distinct genetic mechanisms influencing OSA in men and women.

RAI1 is a promising candidate gene for OSA. It encodes a protein that is highly expressed in neuronal tissues and is involved in early neural differentiation and transcriptional regulation of circadian clock components. Haploinsufficiency of the *RAI1* gene has been implicated in SMS (29), a complex neurobehavioral disorder that is characterized by multiple craniofacial abnormalities, sleep disturbances, and obesity (30). The craniofacial features include a brachycephalic head form and midface hypoplasia, which are anatomic risk factors for OSA (30, 31). A majority of individuals with SMS have significant sleep difficulties and disturbed sleep architecture and circadian rhythms (32), and excessive daytime sleepiness. Speech abnormalities, a hoarse voice, and airway hypotonia are also reported (33), suggesting a role of *RAI1* in influencing upper airway function. Abnormalities in *RAI1* also have been implicated in Potocki-Lupski syndrome (PTLS) (34–36). Patients with PTLS often have developmental delay and mild dysmorphic facial features (34, 35), and can exhibit multiple neurological and cardiovascular abnormalities. Eight of the nine patients with PTLS in the initial study displayed central and/or OSA (36). Both SMS and PTLS appear to involve the *RAI1* gene on the short arm of chromosome 17 (37). A *de novo RAI1* mutation has been reported in a boy with rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation. The individual displayed an AHI of 10 at age 5 years and 27 at age 8 years, hypercholesterolemia, and macrocephaly (38). In mice, *Rai1* haploinsufficiency is associated with hyperphagia and obesity and abnormal expression of multiple genes in the hypothalamus, including *BNDF* (associated with behavioral and psychiatric morbidities) and *WNT9B* (associated with midfacial development) (39). Although the authors suggested the value in further investigating the role of *RAI1* in growth, adiposity and behavior, our results also suggest value in considering sleep apnea as a relevant *RAI1* phenotype.

RAI1 and other genes in the locus may be involved in OSA etiology. Multiple SNPs in the locus overlap epigenetic and/or eQTLs evidence that may indicate regulatory effects. The SNP rs12938840 ($P = 3.97 \times 10^{-7}$) overlaps enhancer regions in 127 Roadmap Epigenomics and

ENCODE cell lines, and in a further 129 samples of brain regions (40–43). Lead SNPs are associated with expression of five genes (minimum eQTL $P < 1 \times 10^{-6}$; Table E1), including *PEMT*, *SREBF1*, *RAI1*, *RASD1*, and *TOM1L2* (44–47). *SREBF1* (formerly *SREBP1*), an important cholesterol biosynthesis regulator, is activated in mice subjected to intermittent hypoxia, leading to hyperlipidemia (48). Activation of *Srebf1* in mouse type 2 alveolar cells leads to lipotoxicity, chronic pulmonary inflammation, and alveolar remodeling (49). *Pemt*, also involved with lipid metabolism, displays sex-specific effects in regulation of high-density lipoproteins and very-low-density lipoproteins in mice (50). A waist:hip ratio GWAS association with rs4646404 at the *PEMT* locus was largely sex specific (21). *RAI1* and *RASD1* (formerly *DEXRAS1*) regulate circadian rhythm (32, 51, 52). *Rai1* haploinsufficiency in mice leads to sex-specific differences in subcutaneous and abdominal fat distributions (39). The lead SNP rs12936587 (*RAI1*) is also significantly associated with coronary artery disease. A sex-stratified analysis indicated that this result was almost entirely due to an association in men (53), providing an exciting avenue for investigating sex differences in not only OSA, but also in the association between OSA with coronary artery disease (reported to be stronger in men compared with women) (2). These results also support the importance of future assessment of pleiotropy, specifically the influence of genetic variants that influence both OSA and cardiometabolic disease and other co-occurring traits.

There are several possible explanations for stronger associations between the *RAI1* locus and AHI-N in men compared with women. Men are more likely to have a higher AHI-N than women (9), which has been attributed to poorer neuromuscular compensatory mechanisms. Thus, genetic variants that further reduce airway patency or ventilatory stability in sleep may have stronger effects in men due to underlying anatomic or physiological risk factors. Conversely, factors that protect women in NREM sleep from recurrent apneas, such as sex hormone–mediated modulation of respiratory chemosensitivity in NREM sleep, may attenuate effects of some genetic variants. It is also possible that sex steroids interact with genetic variants in *RAI1* to differentially affect the development of the brain or craniofacial structures, or otherwise interact with genes regulated by sex hormones. *RAI1* is up-regulated by retinoic acid, which can interact with sex steroids. In the western mosquitofish, *Gambusia affinis*, retinoic acid controls sex-specific development of motor neurons within the spinal cord (54). Furthermore, it has been reported that male *Rai1*-transgenic mice are more growth retarded than are female transgenic mice (55). *Rai1* haploinsufficiency in mice leads to sex-specific differences in adiposity, with greater abdominal fat in females compared with males (39).

This study has several strengths, including the rigorous phenotyping for all discovery cohorts by a central sleep reading center of the sleep studies to ensure high degrees of quality control. Participants in five of the seven cohorts were studied using almost identical equipment and scoring techniques. Consistency of findings for our most significant finding was observed across the five distinct discovery cohorts with available data on AHI-N, as well as across all ethnic/racial groups, even when using data from alternative sleep apnea testing devices. The inclusion of multiple ethnic/race groups allowed leveraging different linkage disequilibrium structures across populations to identify genetic variants consistently associated with the phenotypes across multiple ethnic/race groups. Genome-wide genotype data were available for the largest sample with OSA phenotypes to date.

The AHI was defined using standard approaches that are used commonly, are reproducible, and show heritability. Hypopneas minimally required a 3% or greater oxyhemoglobin desaturation. Although AHI levels are highly correlated regardless of hypopnea definition (56), it is possible that associations may have varied because of use of different measurement approaches. The strongest findings for the AHI-N may not only reflect the specificity of this phenotype, but also the greater accuracy of AHI measures scored from polysomnograms that include electroencephalography recording. The power for replication was limited due to modest sample size (particularly for stage-specific results), although associations in the CHS European Americans, which had undergone identical phenotyping as several of the discovery cohorts, provided evidence consistent with the discovery finding in the *RAI1* region. In addition, in an independent in-laboratory physiological research study of carefully phenotyped individuals that explicitly recruited known cases of OSA without other significant comorbidities, the association with AHI-N in the *RAI1* region was replicated in sex-combined analysis. This sample, however, was too small to test for sex-specific differences in associations. Although this observation needs to be cautiously interpreted, it is of interest that the strongest finding was for AHI-N sleep in the supine position. Men have a significantly higher proportion of apneas in NREM sleep than women, likely due to the occurrence of greater breathing instability in NREM sleep in men compared with women. Men also have more severe sleep apnea in the supine compared with the nonsupine position, attributed to the effects of positional-dependent airway collapsibility. In contrast, women show a REM-predominant pattern and less positional dependency (57). In other words, the lead SNP associated most strongly with a phenotype subtype most characteristic of "male" sleep apnea. The lack of significant association for this phenotype in our sex-combined discovery sample may reflect differences in the spectrum of sleep apnea in the physiological study compared with the predominant community-based samples, where sleep apnea in women tends to be mild.

Our study, although identifying novel genetic pathways that may influence OSA, was not designed to identify specific mechanisms. In particular, we were not able to assess the extent to which the genetic associations with AHI could be explained by craniofacial features, differences in body fat distribution (particularly neck circumference), or physiological traits due to lack of information on specific intermediate phenotypes in most of the study samples.

In conclusion, we have identified from multiethnic meta-analyses several interesting biological candidates for sex-specific and sleep state–specific associations with AHI, the most widely used clinical measure for OSA. The approach underscores the value of sex-specific analyses in a trait, such as OSA, for which there are significant differences in

presentation and pathogenesis between men and women. It is widely recognized that the overall AHI likely reflects a heterogeneous set of phenotypes. The analysis of sleep state–specific (REM, NREM) findings allowed assessment of more specific OSA phenotypes (i.e., operating in the background of different levels of neuromuscular control) than the overall AHI. Further investigation of the *RAI1* regional association is particularly promising given its role in at least three congenital syndromes associated with sleep abnormalities and its influence on metabolic and physiological traits closely associated with OSA. However, future large-scale studies are warranted for replication and refinement of signals. These studies could lead to important insights into the underlying pathogenesis of the disorder, resulting in targeted treatments, as well as inform screening and risk stratification. Additional insights into the genetic bases for OSA may be gleaned from further detailed phenotyping, including assessments of neuromuscular control of the airway. ■

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

Acknowledgment: The authors thank the staff and participants of the ARIC (Atherosclerosis Risk in Communities Study), CFS (Cleveland Family Study), FHS (Framingham Heart Study), HCHS/SOL (Hispanic Community Health Study/Study of Latinos), MESA (Multi-Ethnic Study of Atherosclerosis), MrOS (Osteoporotic Fractures in Men Study), Starr (the Starr County Health Studies), KoGES_Ansan, CHS (Cardiovascular Health Study), CoLaus/HypnoLaus, SHIP-TREND (Study of Health in Pomerania), WASHS (Western Australian Sleep Health Study), and WSC (Wisconsin Sleep Cohort) studies for their important contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. A full list of principal CHS investigators and institutions can be found at http://chs-nhlbi.org. SHIP is part of the Community Medicine Research (CMR) net of the University of Greifswald, Germany. The University of Greifswald is a member of the Caché Campus program of the InterSystems GmbH. The CMR encompasses several research projects that share data of the population-based SHIP (http://ship.community-medicine.de). This article was not prepared in collaboration with investigators of ARIC, CHS, or FHS, and does not necessarily reflect the opinions or views of ARIC, CHS, FHS, or Boston University.

References

1. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea–hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 2005;365:1046–1053.
2. Gottlieb DJ, Yenokyan G, Newman AB, O'Connor GT, Punjabi NM, Quan SF, *et al*. Prospective study of obstructive sleep apnea and incident coronary heart disease and heart failure: the sleep heart health study. *Circulation* 2010;122:352–360.
3. Kendzerska T, Gershon AS, Hawker G, Tomlinson G, Leung RS. Obstructive sleep apnea and incident diabetes: a historical cohort study. *Am J Respir Crit Care Med* 2014;190:218–225.
4. Alkhazna A, Bhat A, Ladesich J, Barthel B, Bohnam AJ. Severity of obstructive sleep apnea between black and white patients. *Hosp Pract (1995)* 2011;39:82–86.
5. Redline S, Sotres-Alvarez D, Loredo J, Hall M, Patel SR, Ramos A, *et al*.; The Hispanic Community Health Study/Study of Latinos. Sleep-disordered breathing in Hispanic/Latino individuals of diverse backgrounds. *Am J Respir Crit Care Med* 2014;189:335–344.
6. Pensuksan WC, Chen X, Lohsoonthorn V, Lertmaharit S, Gelaye B, Williams MA. High risk for obstructive sleep apnea in relation to hypertension among southeast Asian young adults: role of obesity as an effect modifier. *Am J Hypertens* 2014;27:229–236.
7. Chen X, Wang R, Zee P, Lutsey PL, Javaheri S, Alcántara C, *et al*. Racial/ethnic differences in sleep disturbances: the Multi-Ethnic Study of Atherosclerosis (MESA). *Sleep* 2015;38:877–888.
8. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S. The occurrence of sleep-disordered breathing among middle-aged adults. *N Engl J Med* 1993;328:1230–1235.
9. Mohsenin V. Effects of gender on upper airway collapsibility and severity of obstructive sleep apnea. *Sleep Med* 2003;4:523–529.
10. Koo BB, Patel SR, Strohl K, Hoffstein V. Rapid eye movement–related sleep-disordered breathing: influence of age and gender. *Chest* 2008;134:1156–1161.
11. Wimms AJ, Ketheeswaran S, Armitstead JP. Obstructive sleep apnea in women: specific issues and interventions. Sydney, Australia: ResMed Science Center; 2014.
12. Hachul H, Frange C, Bezerra AG, Hirotsu C, Pires GN, Andersen ML, *et al*. The effect of menopause on objective sleep parameters: data from an epidemiologic study in São Paulo, Brazil. *Maturitas* 2015;80:170–178.
13. Redline S, Tishler PV, Tosteson TD, Williamson J, Kump K, Browner I, *et al*. The familial aggregation of obstructive sleep apnea. *Am J Respir Crit Care Med* 1995;151:682–687.
14. Patel SR, Larkin EK, Redline S. Shared genetic basis for obstructive sleep apnea and adiposity measures. *Int J Obes* 2008;32:795–800.
15. Larkin EK, Patel SR, Goodloe RJ, Li Y, Zhu X, Gray-McGuire C, *et al*. A candidate gene study of obstructive sleep apnea in European Americans and African Americans. *Am J Respir Crit Care Med* 2010;182:947–953.
16. Patel SR, Goodloe R, De G, Kowgier M, Weng J, Buxbaum SG, *et al*. Association of genetic loci with sleep apnea in European Americans and African-Americans: the Candidate Gene Association Resource (CARe). *PLoS One* 2012;7:e48836.
17. Yue W, Liu H, Zhang J, Zhang X, Wang X, Liu T, *et al*. Association study of serotonin transporter gene polymorphisms with obstructive sleep apnea syndrome in Chinese Han population. *Sleep* 2008;31:1535–1541.
18. Cade BE, Chen H, Stilp AM, Gleason KJ, Sofer T, Ancoli-Israel S, *et al*. Genetic associations with obstructive sleep apnea traits in Hispanic/Latino Americans. *Am J Respir Crit Care Med* 2016;194:886–897.
19. Randall JC, Winkler TW, Kutalik Z, Berndt SI, Jackson AU, Monda KL, *et al*.; DIAGRAM Consortium; MAGIC Investigators. Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet* 2013;9:e1003500.
20. Winkler TW, Justice AE, Graff M, Barata L, Feitosa MF, Chu S, *et al*.; CHARGE Consortium; DIAGRAM Consortium; GLGC Consortium; Global-BPGen Consortium; ICBP Consortium; MAGIC Consortium. The influence of age and sex on genetic associations with adult body size and shape: a large-scale genome-wide interaction study. *PLoS Genet* 2015;11:e1005378.
21. Shungin D, Winkler TW, Croteau-Chonka DC, Ferreira T, Locke AE, Mägi R, *et al*.; ADIPOGen Consortium; CARDIOGRAMplusC4D Consortium; CKDGen Consortium; GEFOS Consortium; GENIE Consortium; GLGC; ICBP; International Endogene Consortium; LifeLines Cohort Study; MAGIC Investigators; MuTHER Consortium; PAGE Consortium; ReproGen Consortium. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015;518:187–196.
22. Link JC, Chen X, Prien C, Borja MS, Hammerson B, Oda MN, *et al*. Increased high-density lipoprotein cholesterol levels in mice with XX versus XY sex chromosomes. *Arterioscler Thromb Vasc Biol* 2015;35:1778–1786.
23. Siddiqui F, Walters AS, Goldstein D, Lahey M, Desai H. Half of patients with obstructive sleep apnea have a higher NREM AHI than REM AHI. *Sleep Med* 2006;7:281–285.

24. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, *et al*. Concept, design and implementation of a cardiovascular gene-centric 50 K SNP array for large-scale genomic association studies. *PLoS One* 2008;3:e3583.
25. Delaneau O, Marchini J, Zagury JF. A linear complexity phasing method for thousands of genomes. *Nat Methods* 2011;9:179–181.
26. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5:e1000529.
27. Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. *Nat Genet* 2012;44:821–824.
28. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010;26:2190–2191.
29. Elsea SH, Girirajan S. Smith-Magenis syndrome. *Eur J Hum Genet* 2008;16:412–421.
30. Smith AC, Dykens E, Greenberg F. Sleep disturbance in Smith-Magenis syndrome (del 17 p11.2). *Am J Med Genet* 1998;81:186–191.
31. Cakirer B, Hans MG, Graham G, Aylor J, Tishler PV, Redline S. The relationship between craniofacial morphology and obstructive sleep apnea in whites and in African-Americans. *Am J Respir Crit Care Med* 2001;163:947–950.
32. Williams SR, Zies D, Mullegama SV, Grotewiel MS, Elsea SH. Smith-Magenis syndrome results in disruption of CLOCK gene transcription and reveals an integral role for RAI1 in the maintenance of circadian rhythmicity. *Am J Hum Genet* 2012;90:941–949.
33. Gropman AL, Duncan WC, Smith AC. Neurologic and developmental features of the Smith-Magenis syndrome (del 17p11.2). *Pediatr Neurol* 2006;34:337–350.
34. Brown A, Phelan MC, Patil S, Crawford E, Rogers RC, Schwartz C. Two patients with duplication of 17p11.2: the reciprocal of the Smith-Magenis syndrome deletion? *Am J Med Genet* 1996;63: 373–377.
35. Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, *et al*. Molecular mechanism for duplication 17p11.2- the homologous recombination reciprocal of the Smith-Magenis microdeletion. *Nat Genet* 2000;24:84–87.
36. Potocki L, Bi W, Treadwell-Deering D, Carvalho CM, Eifert A, Friedman EM, *et al*. Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. *Am J Hum Genet* 2007;80:633–649.
37. Lupski JR, Stankiewicz P. Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genet* 2005;1:e49.
38. Thaker VV, Esteves KM, Towne MC, Brownstein CA, James PM, Crowley L, *et al*. Whole exome sequencing identifies RAI1 mutation in a morbidly obese child diagnosed with ROHHAD syndrome. *J Clin Endocrinol Metab* 2015;100:1723–1730.
39. Burns B, Schmidt K, Williams SR, Kim S, Girirajan S, Elsea SH. Rai1 haploinsufficiency causes reduced Bdnf expression resulting in hyperphagia, obesity and altered fat distribution in mice and humans with no evidence of metabolic syndrome. *Hum Mol Genet* 2010;19: 4026–4042.
40. Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, *et al*.; Roadmap Epigenomics Consortium. Integrative analysis of 111 reference human epigenomes. *Nature* 2015;518:317–330.
41. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;489:57–74.
42. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;40: D930–D934.
43. Vermunt MW, Reinink P, Korving J, de Bruijn E, Creyghton PM, Basak O, *et al*.; Netherlands Brain Bank. Large-scale identification of coregulated enhancer networks in the adult human brain. *Cell Reports* 2014;9:767–779.
44. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, *et al*. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* 2013;45: 1238–1243.
45. Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, *et al*. Genetics and beyond—the transcriptome of human monocytes and disease susceptibility. *PLoS One* 2010;5:e10693.
46. Lappalainen T, Sammeth M, Friedländer MR, 't Hoen PA, Monlong J, Rivas MA, *et al*.; Geuvadis Consortium. Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* 2013; 501:506–511.
47. GTEx Consortium. Human genomics: the Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015;348:648–660.
48. Li J, Nanayakkara A, Jun J, Savransky V, Polotsky VY. Effect of deficiency in SREBP cleavage-activating protein on lipid metabolism during intermittent hypoxia. *Physiol Genomics* 2007;31:273–280.
49. Plantier L, Besnard V, Xu Y, Ikegami M, Wert SE, Hunt AN, *et al*. Activation of sterol-response element-binding proteins (SREBP) in alveolar type II cells enhances lipogenesis causing pulmonary lipotoxicity. *J Biol Chem* 2012;287:10099–10114.
50. Noga AA, Vance DE. A gender-specific role for phosphatidylethanolamine N-methyltransferase–derived phosphatidylcholine in the regulation of plasma high density and very low density lipoproteins in mice. *J Biol Chem* 2003;278:21851–21859.
51. Boone PM, Reiter RJ, Glaze DG, Tan DX, Lupski JR, Potocki L. Abnormal circadian rhythm of melatonin in Smith-Magenis syndrome patients with RAI1 point mutations. *Am J Med Genet A* 2011;155A: 2024–2027.
52. Cheng HY, Dziema H, Papp J, Mathur DP, Koletar M, Ralph MR, *et al*. The molecular gatekeeper Dexras1 sculpts the photic responsiveness of the mammalian circadian clock. *J Neurosci* 2006; 26:12984–12995.
53. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, *et al*.; CARDIoGRAMplusC4D Consortium.Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013;45:25–33.
54. McCaffery PJ, Adams J, Maden M, Rosa-Molinar E. Too much of a good thing: retinoic acid as an endogenous regulator of neural differentiation and exogenous teratogen. *Eur J Neurosci* 2003;18: 457–472.
55. Girirajan S, Patel N, Slager RE, Tokarz ME, Bucan M, Wiley JL, *et al*. How much is too much? Phenotypic consequences of Rai1 overexpression in mice. *Eur J Hum Genet* 2008;16:941–954.
56. Redline S, Kapur VK, Sanders MH, Quan SF, Gottlieb DJ, Rapoport DM, *et al*. Effects of varying approaches for identifying respiratory disturbances on sleep apnea assessment. *Am J Respir Crit Care Med* 2000;161:369–374.
57. Lozo T, Komnenov D, Badr MS, Mateika JH. Sex differences in sleep disordered breathing in adults. *Respir Physiol Neurobiol* 2017;245: 65–75.