

Whole-Genome Association Analyses of Sleep-disordered Breathing Phenotypes in the NHLBI TOPMed Program

Brian E. Cade^{1,2,3*}, Jiwon Lee¹, Tamar Sofer^{1,2}, Heming Wang^{1,2,3}, Man Zhang⁴, Han Chen^{5,6}, Sina A. Gharib⁷, Daniel J. Gottlieb^{1,2,8}, Xiuqing Guo⁹, Jacqueline M. Lane^{1,2,3,10}, Jingjing Liang¹¹, Xihong Lin¹², Hao Mei¹³, Sanjay R. Patel¹⁴, Shaun M. Purcell^{1,2,3}, Richa Saxena^{1,2,3,10}, Neomi A. Shah¹⁵, Daniel S. Evans¹⁶, Craig L. Hanis⁵, David R. Hillman¹⁷, Sutapa Mukherjee^{18,19}, Lyle J. Palmer²⁰, Katie L. Stone¹⁶, Gregory J. Tranah¹⁶, NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium**, Gonçalo R. Abecasis²¹, Eric A. Boerwinkle^{5,22}, Adolfo Correa^{23,24}, L. Adrienne Cupples^{25,26}, Robert C. Kaplan²⁷, Deborah A. Nickerson^{28,29}, Kari E. North³⁰, Bruce M. Psaty^{31,32}, Jerome I. Rotter⁹, Stephen S. Rich³³, Russell P. Tracy³⁴, Ramachandran S. Vasan^{26,35,36}, James G. Wilson³⁷, Xiaofeng Zhu¹¹, Susan Redline^{1,2,38}, TOPMed Sleep Working Group**

1. Division of Sleep and Circadian Disorders, Brigham and Women's Hospital, Boston, MA 02115, USA
2. Division of Sleep Medicine, Harvard Medical School, Boston, MA 02115, USA
3. Program in Medical and Population Genetics, Broad Institute, Cambridge, MA 02142, USA
4. Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201
5. Human Genetics Center, Department of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, The University of Texas Health Science Center at Houston, Houston, TX 77030 USA
6. Center for Precision Health, School of Public Health and School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX 77030, USA
7. Computational Medicine Core, Center for Lung Biology, UW Medicine Sleep Center, Division of Pulmonary, Critical Care and Sleep Medicine, University of Washington, Seattle WA 98195, USA
8. VA Boston Healthcare System, Boston, MA 02132, USA
9. The Institute for Translational Genomics and Population Sciences, Departments of Pediatrics and Medicine, LABioMed at Harbor-UCLA Medical Center, Torrance, CA 90502, USA
10. Center for Genomic Medicine and Department of Anesthesia, Pain, and Critical Care Medicine, Massachusetts General Hospital, Boston, MA 02114, USA
11. Department of Population and Quantitative Health Sciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA
12. Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
13. Department of Data Science, University of Mississippi Medical Center, Jackson, MS 29216, USA
14. Division of Pulmonary, Allergy, and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA
15. Division of Pulmonary, Critical Care and Sleep Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

16. California Pacific Medical Center Research Institute, San Francisco, CA 94107, USA
17. Department of Pulmonary Physiology and Sleep Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia 6009, Australia
18. Sleep Health Service, Respiratory and Sleep Services, Southern Adelaide Local Health Network, Adelaide, South Australia
19. Adelaide Institute for Sleep Health, Flinders University, Adelaide, South Australia
20. School of Public Health, University of Adelaide, South Australia 5000, Australia
21. Department of Biostatistics and Center for Statistical Genetics, University of Michigan School of Public Health, Ann Arbor, MI 48109, USA
22. Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA
23. Department of Medicine, University of Mississippi Medical Center, Jackson, MS 39216, USA
24. Jackson Heart Study, Jackson, MS 39216, USA
25. Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA
26. Framingham Heart Study, Framingham, MA 01702, USA
27. Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York 10461, USA
28. Department of Genome Sciences, University of Washington, Seattle, WA 98195
29. Northwest Genomics Center, Seattle, WA 98105
30. Department of Epidemiology and Carolina Center of Genome Sciences, University of North Carolina, Chapel Hill, NC 27514, USA
31. Cardiovascular Health Study, Departments of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, WA 98101, USA
32. Kaiser Permanente Washington Health Research Institute, Seattle, WA 98101, USA
33. Center for Public Health Genomics, University of Virginia, Charlottesville, VA 22908, USA
34. Department of Pathology, University of Vermont, Colchester, VT 05405, USA
35. Sections of Preventive Medicine and Epidemiology and Cardiology, Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA
36. Department of Epidemiology, Boston University School of Public Health, Boston, MA 02118, USA
37. Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson MS 39216, USA
38. Division of Pulmonary, Critical Care, and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

*: Please address all correspondence and requests for reprints to:

Brian Cade
Division of Sleep and Circadian Disorders
Brigham and Women's Hospital, Harvard Medical School
221 Longwood Avenue, Boston, MA 02115, USA
1-857-307-0353 (phone) 1-617-278-6946 (fax) bcade@bwh.harvard.edu (e-mail)

** : Full lists of consortium and working group authors are provided in **Supplementary Tables 1 and 2**.

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Cade *et al.*: WGS analyses of sleep-disordered breathing

1 **Abstract**

2 Sleep-disordered breathing (SDB) is a common disorder associated with significant morbidity. Through
3 the NHLBI Trans-Omics for Precision Medicine (TOPMed) program we report the first whole-genome sequence
4 analysis of SDB. We identified 4 rare gene-based associations with SDB traits in 7,988 individuals of diverse
5 ancestry and 4 replicated common variant associations with inclusion of additional samples (n=13,257). We
6 identified a multi-ethnic set-based rare-variant association ($p = 3.48 \times 10^{-8}$) on chromosome X with *ARMCX3*.
7 Transcription factor binding site enrichment identified associations with genes implicated with respiratory and
8 craniofacial traits. Results highlighted associations in genes that modulate lung development, inflammation,
9 respiratory rhythmogenesis and *HIF1A*-mediated hypoxic response.

Cade *et al.*: WGS analyses of sleep-disordered breathing

10 **Introduction**

11 Sleep-disordered breathing (SDB) is a prevalent disorder associated with increased mortality and
12 morbidity [1-4]. The most common type of SDB is obstructive sleep apnea (OSA), characterized by repeated
13 airway collapse leading to intermittent hypoxemia and sleep disruption, that is increased in prevalence with older
14 age and male sex [5]. The disease appears to be multifactorial, reflecting variable contributions of abnormalities in
15 ventilatory control, craniofacial anatomy, and adiposity [5-11]. Due to an incomplete understanding of its
16 pathophysiology, standard OSA treatment only addresses the downstream manifestations of airway collapse
17 through nightly use of pressurized air to the nasopharynx, a therapy that often is poorly tolerated. Therefore, there
18 is a critical need to identify molecular pathways that could provide specific therapeutic targets. The need for
19 overnight studies to phenotype SDB traits has limited the available sample size for genetic analyses, and only
20 several common-frequency genome-wide analysis studies have been reported [11-15]. Increased statistical power
21 may increase the genetic resolution of regions that may not be adequately tagged by current genotyping arrays due
22 to population differences and/or reduced linkage disequilibrium with biologically relevant regions [16].

23 The Trans-Omics for Precision Medicine (TOPMed) program is an NIH National Heart, Lung, and Blood
24 Institute program designed to improve the understanding of the biological processes that contribute to heart, lung,
25 blood, and sleep disorders [17]. TOPMed has generated whole-genome sequencing (WGS) data on over 100,000
26 individuals from multiple cohorts at >30× depth, including seven studies with objective assessment of SDB. A
27 variant imputation server using TOPMed data also allows for high-quality imputation of non-sequenced genotype
28 chip data [18]. A complementary initiative sponsored by the Centers for Common Disease Genomics (CCDG) of
29 the NIH National Human Genome Research Institute has generated sequencing data from additional individuals in
30 two TOPMed cohorts (<https://www.genome.gov/27563570>). These initiatives provide the ability to examine the
31 genetics of SDB at unprecedented detail in African-Americans (AA), Asian-Americans (AsA), European-
32 Americans/Australians (EA), and Hispanic/Latino-Americans (HA).

33 In this first WGS analysis of SDB, we examine the apnea-hypopnea index (AHI), the standard clinic
34 metric of SDB, and four complementary measurements of overnight hypoxemia: average and minimum
35 oxyhemoglobin saturation (SpO₂) during sleep and the percent of the sleep recording with SpO₂ < 90% (Per90);
36 and the average desaturation per hypopnea event. These indices were chosen because of clinical relevance, high
37 heritability, or prior significant GWAS findings [7-9,11,14]. We examined 7,988 individuals with objectively
38 measured SDB and WGS data in conjunction with data from 13,257 individuals with imputed genotype data.

Cade *et al.*: WGS analyses of sleep-disordered breathing

39 **Methods**

40 Each study had a protocol approved by its respective Institutional Review Board and participants
41 provided informed consent. A study overview is provided in **Supplementary Figure 1**. There were two classes of
42 data: “WGS studies” had WGS performed by the TOPMed program and, in some cases, in additional participants
43 by the CCDG program (referred to as “WGS” studies); “Imputed studies” had array-based genotyping later
44 imputed using the TOPMed imputation server (as described below). Some studies with WGS contributed imputed
45 study data from additional array-based genotyped individuals.

46

47 *WGS studies*

48 The Atherosclerosis Risk in Communities Study (ARIC), the Cardiovascular Health Study (CHS), and the
49 Framingham Heart Study Offspring Cohort (FHS) included individuals who participated in the Sleep Heart Health
50 Study (SHHS), who underwent polysomnography (PSG) between 1995 – 1998 using the Compumedics PS-2
51 system [19–22]. These samples included 1,028 EAs from ARIC; 151 AAs and 557 EAs from CHS; and 478 EAs
52 from FHS.

53 The Multi-Ethnic Study of Atherosclerosis (MESA) is investigating the risk factors for clinical
54 cardiovascular disease [23]. PSG was obtained between 2010 – 2013 using the Compumedics Somte system [24].
55 This analysis includes data from 698 EAs, 486 AAs, 456 HAs, and 229 AsAs.

56 The Cleveland Family Study (CFS) was designed to investigate the familial basis of SDB, with four visits
57 occurring from 1990 – 2006 [25]. Sleep was assessed either in a clinical research center using full PSG
58 (Compumedics E series) (visit 4); or in the latest available prior examination using an in-home sleep apnea testing
59 device (Edentrace). Data were analyzed from 505 AAs and 485 EAs (339 AAs and 234 EAs with full PSG data).

60 The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) is studying multiple health
61 conditions in HAs [26,27]. Home sleep apnea testing was performed during the baseline examination (2008 – 2011)
62 using the ARES Unicorder 5.2, a validated device including a forehead-based reflectance oximeter, a nasal
63 pressure cannula and pressure transducer, an accelerometer, and a microphone [28]. 2,339 individuals provided
64 data.

65 The Jackson Heart Study (JHS) is investigating cardiovascular disease in AAs [29]. An in-home sleep
66 study was performed from 2012 – 2016 using a validated Type 3 sleep apnea testing device (Embla Embletta
67 Gold) [30,31]. 575 individuals contributed data.

68

69 *Imputed genotype studies*

Cade *et al.*: WGS analyses of sleep-disordered breathing

70 The Osteoporotic Fractures in Men Study (MrOS) is a multi-center cohort study initially designed to
71 examine the risk factors for osteoporosis, fractures, and prostate cancer in older males [^{32,33}]. An ancillary study
72 (MrOS Sleep; 2003 – 2005) focused on outcomes of sleep disturbances used PSG and nearly identical procedures
73 as in MESA (Compumedics Safiro system) [³⁴]. 2,181 EA individuals were included, with genotyping performed
74 using the Illumina Human Omni 1 Quad v1-0 H array.

75 The Starr County Health Studies (Starr) investigates the risk factors for diabetes in Mexican-Americans
76 [^{35,36}]. An in-home sleep apnea study occurred between 2010 and 2014 using a validated instrument that records
77 finger pulse oximetry, actigraphy, body position, and peripheral arterial tonometry (Itamar-Medical WatchPAT-
78 200) [³⁷]. 782 HA individuals were studied, using Affymetrix 6.0 genotyping data.

79 The Western Australian Sleep Health Study (WASHS) is a clinic-based study focused on the
80 epidemiology and genetics of SDB [³⁸]. PSG was obtained from 1,508 European-ancestry patients (91% referred
81 for SDB evaluation) from 2006 – 2010 (Compumedics Series E). Genotyping was performed using the Illumina
82 Omni 2.5 array.

83 Imputed genotype data were available for additional members of the TOPMed cohorts described above.
84 Study/population combinations with fewer than 100 individuals were excluded. ARIC contributed an additional
85 631 EA individuals (Affymetrix 6.0; dbGaP phg000035.v1.p1). CFS contributed 225 AA and 218 EA individuals
86 (Affymetrix 6.0; Illumina OmniExpress+Exome, Exome, and IBC). CHS contributed 365 individuals (Illumina
87 CNV370 and IBC; phg000135.v1.p1 and phg000077.v1.p1). FHS contributed 192 EA individuals (Affymetrix
88 500k; phg000006.v7). HCHS/SOL contributed 7,155 HA individuals (Illumina Omni 2.5; phg000663.v1).

89

90 *Phenotype and covariate definitions*

91 We examined several SDB measures, including specific measures of OSA: AHI (number of apneas plus
92 hypopneas per hour of sleep, with a minimum 3% desaturation per event) and average oxyhemoglobin
93 desaturation per apnea or hypopnea; and measures of SDB severity [⁷⁻⁹]: average and minimum SpO₂ and the
94 percentage of the night with SpO₂ < 90% (Per90). Apart from WASHS, all sleep data were scored by blinded
95 scorers at one central Sleep Reading Center with high levels of scorer reliability using well-defined procedures
96 [^{39,40}]. We adjusted for age, age², sex, age × sex, body mass index (BMI), and BMI² due to known age and sex
97 effects, some of which are non-linearly associated with outcomes, and our goal of identifying obesity-independent
98 loci. Age and BMI were obtained at the time of the sleep recording. Phenotype analyses were pooled within
99 populations to aggregate very rare variants for testing, and therefore further adjusted for study. Cryptic relatedness
100 and population substructure were controlled for using linear mixed models. Genomic control was applied to
101 population-specific results (or cohort-specific imputed genotype results).

Cade *et al.*: WGS analyses of sleep-disordered breathing

102

103 *WGS and genotyping*

104 Sequence data were derived from the TOPMed Freeze 6a release, jointly called by the TOPMed
105 Informatics Research Center at the University of Michigan (http://github.com/statgen/topmed_variant_calling).
106 The methodology was described elsewhere [17]. In brief, WGS was performed at the Broad Institute (ARIC, FHS,
107 MESA), Baylor College of Medicine (ARIC, CHS, HCHS/SOL), and the University of Washington (CFS, JHS).
108 Additional ARIC and HCHS/SOL WGS funded by CCDG and performed at Baylor College of Medicine were
109 included in the jointly-called data. TOPMed and CCDG calling pipelines have functionally equivalent outcomes
110 despite data processing differences (as detailed in [41]). WGS data were merged and normalized; inferred sequence
111 contamination was identified; and SNPs and small indels were detected (structural variants are not currently
112 available). Lower quality variants were excluded using Mendelian consistency checks. Variants were aligned to
113 Build 38 and annotated using snpEff 4.3t [42]. We excluded variants with $<10\times$ depth or $>5\%$ missingness, leaving
114 152.7 million polymorphic variants in 7,988 individuals with SDB phenotypes.

115 Genotype data were imputed using the TOPMed Imputation Server [18] using a Freeze 5b (Build 38)
116 template. Forward strand checks were performed using the Strand database and the Haplotype Reference
117 Consortium imputation preparation script (<https://www.well.ox.ac.uk/~wrayner/tools/>) and confirmed using
118 Ensembl variant allele checks and internal QC performed on the server. Study-level data were imputed separately.
119 Analyses on variants with r^2 score > 0.5 were therefore performed separately for each study.

120 *Statistical analyses*

121 Single and grouped variant analyses were performed using EMMAX and MMSKAT, both within the
122 EPACTS suite (v3.3, <https://genome.sph.umich.edu/wiki/EPACTS>) [43]. WGS genetic relatedness matrices
123 (GRM) were constructed using autosomal variants (MAF $> 0.1\%$) following a comparison of EPACTS point-wise
124 heritability estimates of the AHI using different minimal MAFs. A grid search identified optimal GRM
125 parameters with imputed data (MAF $> 0.5\%$, $r^2 > 0.90$) using 929 ARIC individuals with imputation and WGS
126 data. \log_{10} P-values using identical association test parameters had a Spearman's ρ correlation of 0.951 between
127 WGS and imputed data. Matrices were constructed separately for each study + population combination (due to
128 potentially differential imputation coverage).

129 Gene-based group sets were constructed with a series of filters considering non-pseudogenes expressed in
130 any GTEx v7 tissue. A variant could be assigned to one or more Ensembl genes based on SNPEff annotations
131 [42,44]. We examined 5_prime_UTR_premature_start_codon_gain_variant, bidirectional_gene_fusion,
132 conservative_inframe_deletion, conservative_inframe_insertion, disruptive_inframe_deletion,
133 disruptive_inframe_insertion, exon_loss_variant, frameshift_variant, gene_fusion, initiator_codon_variant,
134 missense_variant, non_canonical_start_codon, splice_acceptor_variant, splice_donor_variant,

Cade *et al.*: WGS analyses of sleep-disordered breathing

135 splice_region_variant, start_loss, stop_gained, stop_lost, and stop_retained_variant mutations. We also included
136 variants located within experimentally derived promoter regions and Ensembl-derived Tarbase miRNA binding
137 sites; and regulatory variants located within 1000 bases of a particular gene, including ChIP-seq determined
138 transcription factor binding sites (TFBS), and Ensembl-derived CTCF, TFBS, and promoter sites [44-46]. Group set
139 variants were filtered by requiring either a FATHMM-XF score > 0.5 or a CDTS < 1% constrained region score
140 [47,48]. Exonic variants could alternatively have a PrimateAI score > 0.803 or a Havrilla *et al.* < 1% constrained
141 coding region score [49,50].

142 Gene-based tests considered variants in WGS-only data (MAF < 5%). Pooled (across cohort) analyses
143 were performed within each population in order to aggregate information on very rare variants across studies.
144 Combined population results were obtained through meta-analysis of p-values weighted by sample size (due to
145 potentially different MAF spectra driven by population demography). A significance level of $p < 4.51 \times 10^{-8}$ was
146 used, reflecting a Bonferroni adjustment for all genes tested across all phenotype and population configurations.

147 A second set-based analysis was designed to query for TFBS annotation enrichment [51]. We performed
148 250 base-pair sliding window analyses (to improve power by aggregating additional variants beyond an
149 approximate ChIP-seq peak width of 100 base-pairs). We filtered for variants with either a FATHMM-XF score >
150 0.5 or a CDTS 1% score with no MAF cut-offs and meta-analyzed MMSKAT results across the 4 populations,
151 noting windows with p-values < 0.01. These intervals were tested for enrichment of ChIP-seq coordinates with at
152 least 50% physical overlap for up to 437 transcription factors using ReMap 2018 v1.2
153 (<http://tagc.univ-mrs.fr/remap/index.php?page=annotation>) [52].

154 Single-variant EMMAX tests examined common variants (MAF > 0.5%). Meta-analysis across
155 populations (and imputed genotype studies) used METAL with genomic control [53]. We performed bidirectional
156 discovery and replication using the WGS and imputed samples (noting the high genomic resolution in the WGS
157 samples and the higher sample size in the imputed data). We report results including at least 1000 individuals,
158 discovery association p-values < 1×10^{-5} and replication association p-values < 0.05. Significance was defined as
159 $p < 1 \times 10^{-8}$ in joint analyses, reflecting adjustment for five correlated phenotypes (Supplementary Table S3). We
160 performed MetaXcan imputed GTEx gene expression analyses using joint EA results in selected tissues relevant
161 to SDB and GIGSEA pathway analyses of MetaXcan output in whole blood (to maximize power), with empirical
162 p-values incorporating 10,000 permutations [54,55].

163

Cade *et al.*: WGS analyses of sleep-disordered breathing

164 **Results**

165 Study sample

166 A study overview is provided in **Supplementary Figure 1. Tables 1 and 2** provide a summary of the
167 study samples and SDB traits analyzed using WGS and imputed genotypes, respectively. In total, there were
168 21,244 individuals (1,942 AAs; 229 AsAs; 8,341 EAs; and 10,732 HAs). Median AHI levels ranged from mildly
169 to moderately elevated, reflecting the age range and sex distribution of each cohort. Pairwise correlations of
170 phenotypes and covariates are provided in **Supplementary Table 3**.

171

172 Gene-based results

173 Gene-based rare variant results are presented in **Table 3** (for meta-analyzed results across multiple
174 populations) and in **Table 4** (for secondary population-specific results). Collectively, we identified 4 significantly
175 associated genes (Bonferroni $p < 4.51 \times 10^{-8}$). *ARMCX3*, identified in the multiple-population analysis, is an X-
176 linked protein-coding that was associated with average desaturation ($p = 5.29 \times 10^{-8}$). Two protein-coding genes
177 were identified in population-specific analyses of Per90: *MRPS33* ($p = 1.22 \times 10^{-9}$) and *C16orf90* ($p = 1.36 \times 10^{-8}$).
178 We identified 12 suggestively associated genes ($p \leq 4.22 \times 10^{-7}$). Three genes are druggable [^{56,57}]. Nominally
179 significant results ($p < 0.01$) and additional details are presented in **Supplementary Tables 4 and 5**.

180

181 Single-variant results

182 We identified four genome-level significant loci in single-variant analyses ($MAF > 0.5\%$; $p < 1.0 \times 10^{-8}$;
183 **Table 5**). In multiple-population analyses, the 2q12 locus (rs77375846; *IL18RAP*) was associated with average
184 event desaturation in a multiple-population analysis (combined $p = 1.57 \times 10^{-9}$) and minimum SpO₂ (consistent
185 with a previous report [¹⁴]). Two novel population-specific loci were identified. The 8p12 locus (rs35447033,
186 *NRG1*) was associated with AHI in EAs (combined $p = 3.02 \times 10^{-9}$, **Figure 1**). The 5p13 locus (rs28777;
187 *SLC45A2*) was associated with average SpO₂ in EAs (combined $p = 8.08 \times 10^{-10}$, **Figure 2**). In HAs, the 1q32
188 locus (rs116133558; *ATP2B4*) was associated with Per90 (combined $p = 3.51 \times 10^{-10}$) and with average SpO₂ (as
189 previously identified [¹¹]). Twelve additional regions were suggestively associated ($p < 1.0 \times 10^{-7}$).

190 **Supplementary Table 6** provides additional context for all variants in these loci ($p < 1.0 \times 10^{-7}$), including
191 imputation quality, significant eQTLs, and overlap with epigenetic regions [⁵⁸⁻⁶¹]. Manhattan and QQ plots
192 corresponding to the significant associations are provided in **Supplementary Figures 2 – 5**.

193

194 MetaXcan imputed gene expression and GIGSEA pathway analyses

Cade *et al.*: WGS analyses of sleep-disordered breathing

195 We used joint WGS and imputed EA results to impute associations with gene expression levels using a
196 MetaXcan framework for 6 tissues (subcutaneous and visceral omentum adipose, lung, monocytes, skeletal
197 muscle, and whole blood). No individual tests reached Bonferroni significance ($p < 2.60 \times 10^{-7}$; **Supplementary**
198 **Table 7**). Genes that were observed in the top 10 results across the varied analyses (**Supplementary Table 8**)
199 included *ZNF83* (15 instances) and *CHRNE* (13 instances).

200 Whole blood MetaXcan results (with the largest sample size) were further evaluated in GIGSEA-based
201 pathway analyses. KEGG pathway results are shown in **Supplementary Table 9**. The most significantly
202 associated pathway was KEGG_STEROID_HORMONE_BIOSYNTHESIS (average SpO₂ empirical p-value =
203 7.00×10^{-4}). KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY was observed in the top 10 results
204 for 4 of the 5 phenotypes. Gene-centric transcription factor binding site (TFBS) enrichment analysis results are
205 presented in **Supplementary Table 10**. V\$PEA3_Q6 (*ETV4*) was the most significantly associated TFBS
206 (average desaturation empirical p-value = 3.00×10^{-4}) and was the strongest association for AHI and minimum
207 SpO₂ (empirical p-values 0.002 and 0.001, respectively). The most significant miRNA binding site enrichment
208 analysis association was GCATTTG,MIR-105 (average SpO₂ p = 0.002; **Supplementary Table 11**).
209 AGGCACT,MIR-515-3P (the strongest AHI association, p = 0.009) was observed in the top ten results for four
210 phenotypes.

211

212 *ChIP-seq transcription factor binding site interval enrichment*

213 We performed a sliding window analysis to examine enriched intervals containing ChIP-seq derived
214 coordinates for up to 437 transcription factors (**Table 6, Supplementary Table 12**). *FOXP2* TFBS were
215 consistently the most enriched for all phenotypes. Other notable transcription factors in the top 5 included *EGRI*,
216 *KDM4B*, *KDM6B*, and *TP63*. *KDM4B* and *KDM6B* are druggable [^{56,57}]. Leading sliding window results are
217 provided in **Supplementary Table 13**.

218

Cade *et al.*: WGS analyses of sleep-disordered breathing

219 **Discussion**

220 Sleep-disordered breathing (SDB) is associated with increased risk of a wide range of disorders, including
221 atrial fibrillation, cancer, cognitive impairment, diabetes, liver, and interstitial lung diseases, as well as premature
222 mortality [3,4,62–67]. Treatment options, however, are limited by a lack of knowledge of molecular pathways,
223 including those that may be “druggable”. Recent analyses of SDB traits have focused on common variants and
224 identified several preliminary genome-level significant associations using GWAS, admixture mapping, and
225 linkage approaches [11–15], but did not address gene-based or rare variant effects. Ten studies and over 21,000
226 individuals of multiple ancestries with WGS data at unprecedented resolution from the NHLBI TOPMed program
227 combined with densely imputed data from other sources contributed to these results. We identified several variant,
228 gene-based, and pathway-level associations. Analyses adjusted for obesity, a major SDB risk factor, identified
229 loci and genes implicated in pulmonary, inflammatory, and craniofacial pathways. Some associations were
230 population-specific, while others were sex-specific, consistent with population differences and strong sex
231 differences for SDB [24,68–70]. Notably, across multiple ancestral groups, we identified a set-based rare-variant
232 association ($p = 3.48 \times 10^{-8}$) on chromosome X with *ARMCX3*.

233

234 **Gene-based results**

235 Across multiple populations, *ARMCX3* (*ALEX3*) and the RNA anti-sense gene *ARMCX3-AS1* were
236 associated with apnea-hypopnea triggered intermittent hypoxia. *ARMCX3* regulates mitochondrial aggregation
237 and trafficking in multiple tissues and facilitates neuronal survival and axon regeneration [71–73]. Wnt signaling
238 regulates reactive oxygen species (ROS) generation and *ARMCX3*-associated mitochondrial aggregation [72,74].
239 Potential mechanisms for further study include sensitized carotid body chemoreflexes, interaction with
240 inflammatory mechanisms, and neuronal dysfunction within respiratory centers. Sleep apnea and reduced
241 ventilatory drive are enriched in individuals with a primary mitochondrial disorder [75]. Mitochondria are an
242 important source of ROS, which modulate the acute hypoxic ventilatory response. Mitochondria impact *HIF1A*
243 signaling and may contribute to oxygen sensing [76–79]. ROS are required for intermittent hypoxia-induced
244 respiratory long-term facilitation [80,81]. These effects may mitigate the level of hypoxia resulting from recurrent
245 apneas, or conversely, lead to ventilatory instability, promoting apnea occurrence. Mitochondrial ROS also
246 activate the NLRP3 inflammasome in multiple pulmonary diseases, consistent with an inflammation model that
247 includes our IL18-pathway and *HK1* results, ROS-related proinflammatory responses to lung capillary pressure,
248 and evidence of alveolar epithelial injury/SDB interactions [14,82–87]. Our findings suggest value in investigating the
249 mechanisms by which *ARMCX3* predisposes to SDB, and whether these associations are mediated by neuronal
250 dysfunction and/or ROS and carotid body sensitization, and interact with the inflammasome.

251 Additional genes were significantly associated in population-specific analyses, including the

Cade *et al.*: WGS analyses of sleep-disordered breathing

252 mitochondrial ribosomal gene *MRPS33*. Mitochondria are responsible for expression of the 13 essential
253 components of the oxidative phosphorylation system, and a majority of the small subunit proteins have been
254 implicated in disease [88]. The expression of several small and large subunit proteins are altered in a hypoxic
255 environment [89]. *MRPS33* expression varies with oxygen treatment in COPD [90].

256

257 Single-variant results

258 We identified four common frequency associated loci, including multiple-population associations with
259 the *IL18RAP* region. The *IL18RAP* region has been associated with minimum SpO₂ [14], and here we further
260 identify an association with average event desaturation, highlighting a role in an OSA-specific trait. Multiple
261 variants in this region are also GTEx eQTL variants for both interleukin-18 receptor subunits *IL18RAP* and
262 *IL18RI* (Supplementary Table 6) and experimental studies support a role for *IL18* signaling in mediating this
263 association, possibly through effects of pulmonary inflammation on gas exchange (reviewed in [14]).

264 We identified three population-specific loci, including two novel associations in individuals of European
265 ancestry (Figures 1 and 2). 65 variants in the *NRG1* region were associated with the AHI ($p < 1.0 \times 10^{-8}$,
266 Supplementary Table 6). This region was suggestively associated with sleep apnea in a Korean population [91],
267 however the lead signals appear to be independent (rs10097555 Korean $p = 2.6 \times 10^{-6}$, EA $p = 0.91$). *NRG1* is
268 associated with lung development and acute lung injury, and mediates inflammasome-induced alveolar cell
269 permeability [87,92–95]. *NRG1* promotes accumulation of HIF1A and has increased expression in vascular smooth
270 muscle cells following exposure to intermittent hypoxia [96,97]. The lead *SLC45A2* region variant rs28777 (average
271 SpO₂ $p = 8.08 \times 10^{-10}$) has been associated with multiple traits and is in a splicing regulatory element with extreme
272 population differentiation [98]. An association in the *ATP2B4* region with average SpO₂ in HAs [11] has been
273 extended to a second hypoxemia trait at the same variant (Per90 $p = 3.31 \times 10^{-10}$). This gene is the main cellular
274 membrane calcium pump in erythrocytes and also regulates vascular tone [99,100].

275

276 Pathway analyses

277 Several gene pathways were identified in EA individuals using imputed gene expression in whole blood
278 (Supplementary Table 9). KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY (retinoic acid-inducible
279 gene I-like) was the most commonly observed, occurring in the top 10 results for 4 of the 5 phenotypes. This
280 pathway initiates the immune response to RNA virus infection [101], consistent with a role for inflammation at the
281 *NRG1* and *IL18RAP* loci. Steroid hormone biosynthesis (the most significantly associated pathway), PPAR
282 signaling, and metabolism (via ‘starch and sucrose metabolism’) suggest the importance of biological pathways
283 modulating energy homeostasis and balance and metabolic function [102]. In the gene-centric GIGSEA TFBS

Cade *et al.*: WGS analyses of sleep-disordered breathing

284 analysis, V\$PEA3_Q6 (*ETV4*) was the lead association for three phenotypes. *ETV4* influences branching in the
285 developing lung and regulates hypoxia-inducible factor signaling [^{103,104}], a major mechanism influencing
286 ventilatory control.

287

288 Transcription factor binding site enrichment

289 Several transcription factors were identified through interval enrichment of observed TFBS across the
290 genome (Table 6). *FOXP2* was consistently the most enriched transcription factor and is known to regulate gene
291 expression in epithelial lung tissue and response to lung injury through an inflammatory mechanism [^{105,106}].
292 *FOXP2* is also expressed in brainstem respiratory areas including the pre-Bötzinger complex (which is essential
293 for respiratory rhythmogenesis) and impacts airway morphology [^{107,108}]. Two lysine demethylases (*KDM4B* and
294 *KDM6B*) were also identified. *KDM6B* (*JMJD3*) is required for a functional pre-Bötzinger complex [^{109,110}] and
295 reduced KDM6B protein expression was reported in hypoxic OSA patients [¹¹¹]. *Kdm6b* also plays roles in
296 immune function and lung development [^{112–114}]. *Drosophila Kdm4b* knock-outs have increased sleep [¹¹⁵].
297 *KDM4B* (*JMJD2B*) and *KDM6B* are both members of the JmjC protein domain family and are regulated by
298 *HIF1A*, require oxygen as a cofactor and act as oxygen sensors for chromatin in hypoxia [^{116,117}]. *EGRI* mediates
299 hypoxia-induced pulmonary fibrosis [¹¹⁸]. *TP63* is associated with cleft palate in *Tp63* deficient mice, which is
300 associated with an increased prevalence of OSA [^{119,120}], suggesting that its relationship to OSA may be through
301 pathways influencing craniofacial development. Among the leading 250-base pair sliding window results
302 (Supplementary Table 13), 4:105708751-105709001 (Per90 HA $p = 2.72 \times 10^{-9}$) is of note due to regional
303 associations with lung function and expression in human lung [¹²¹].

304

305 Strengths and weaknesses

306 This study is the first genome-wide analysis of objectively measured SDB traits using deep sequencing.
307 Together with improved imputation quality, the TOPMed resource has enabled unprecedented genetic resolution.
308 We examined clinically relevant phenotypes measured using rigorous methodology [^{5,7–10}]. We analyzed data from
309 10 studies of individuals from four population groups that used different ascertainment strategies, which may
310 potentially improve the generalization of our results. While this analysis is among the largest performed for SDB
311 traits to date, our moderate sample size has lower power to detect weaker associations, and data were not
312 available to replicate these first rare variant associations. While there are multiple lines of evidence in the
313 literature to support our findings, additional experimental followup analyses are required.

314

315 Conclusion

Cade *et al.*: WGS analyses of sleep-disordered breathing

316 We have identified the first rare-variant and additional common-variant associations at genome-level
317 significance with objectively measured SDB traits in humans. The results point to biologically relevant pathways
318 for further study, including a novel X-linked association (*ARCMX3*), and a number of associations in genes that
319 modulate lung development, inflammation, respiratory rhythmogenesis and *HIF1A*-mediated hypoxic-response
320 pathways. These associations will motivate future sample collection and follow-up in cell-line and animal
321 validation studies, with potential therapeutic benefit for sleep-disordered breathing and related comorbidities.

Cade *et al.*: WGS analyses of sleep-disordered breathing

322 **Supplementary Data**

323 Supplementary data include 5 figures and 13 tables.

324

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Cade *et al.*: WGS analyses of sleep-disordered breathing

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Cade *et al.*: WGS analyses of sleep-disordered breathing

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Cade *et al.*: WGS analyses of sleep-disordered breathing

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439 Data acquisition: B.E.C., J.L., T.S., M.Z., H.C., S.A.G., D.J.G., J.M.L., J.L., X.L., H.M., S.R.P., S.M.P., R.S.,
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441 Analysis: B.E.C., J.L.

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443 B.E.C. and S.R. had full access to the study data and take responsibility for the integrity of the data and accuracy
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445

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Cade *et al.*: WGS analyses of sleep-disordered breathing

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Cade *et al.*: WGS analyses of sleep-disordered breathing

Figure Legends

Figure 1. Regional plot of the rs35447033 association with AHI in European-ancestry individuals. Joint WGS and imputed results are shown, using Build 38 coordinates.

Figure 2. Regional plot of the rs28777 association with Average SpO₂ in European-ancestry individuals. Joint WGS and imputed results are shown, using Build 38 coordinates.

Cade *et al.*: WGS analyses of sleep-disordered breathing

Tables

Table 1. Sample description for WGS cohorts.

Population	Cohort	N	Age	Percent Female	BMI	Apnea Hypopnea Index 3%	AHI (Percent < 5, 5 – 15, ≥ 15)	Average Desaturation	Average SpO ₂	Minimum SpO ₂	Percent Sleep Under 90% SpO ₂
African-American	CFS*	505	38.65 (18.96)	56.4	32.44 (9.48)	6.85 (22.48)	43.4, 20.6, 36.0	3.62 (1.99)	94.49 (3.91)	84.76 (9.83)	4.79 (13.15)
	CHS	151	75.39 (4.35)	60.3	29.02 (5.08)	9.60 (16.96)	28.5, 36.4, 35.1	2.70 (1.74)	94.82 (2.19)	85.74 (5.35)	3.39 (9.63)
	JHS	575	63.47 (10.94)	64.9	31.8 (6.88)	10.69 (14.42)	24.7, 39.5, 35.8	3.54 (1.72)	94.77 (2.02)	84.30 (6.57)	2.97 (8.91)
	MESA	486	68.81 (9.07)	53.7	30.23 (5.68)	12.67 (20.56)	22.4, 32.9, 44.7	3.42 (2.10)	94.46 (1.99)	83.32 (7.98)	3.89 (9.49)
East Asian-American	MESA	229	67.89 (9.11)	49.8	24.28 (3.3)	14.96 (24.28)	21.8, 28.4, 49.8	3.72 (1.79)	94.92 (1.22)	83.23 (7.58)	2.25 (4.46)
	ARIC	1,028	62.28 (5.67)	53.1	28.72 (5.06)	8.64 (15.62)	34.6, 32.4, 33.0	2.35 (1.29)	94.57 (1.84)	85.95 (5.93)	2.92 (9.24)
European-American	CFS*	485	43.23 (19.49)	50.5	30.81 (8.83)	7.09 (21.90)	44.7, 19.4, 35.9	3.29 (1.86)	93.67 (3.59)	85.55 (9.33)	4.66 (11.87)
	CHS	557	77.90 (4.34)	54.2	27.25 (4.44)	11.42 (15.54)	23.2, 38.1, 38.8	2.58 (1.34)	94.00 (2.00)	84.99 (5.67)	4.77 (12.28)
	FHS*	478	60.09 (8.54)	49.8	28.4 (5.06)	8.10 (14.28)	35.1, 35.1, 29.7	2.35 (1.27)	94.68 (2.04)	85.78 (6.25)	2.96 (9.18)
	MESA	698	68.53 (9.06)	53.2	27.91 (5.1)	12.18 (20.45)	21.6, 35.0, 43.4	3.11 (1.44)	93.96 (1.75)	83.49 (7.50)	4.27 (10.82)
Hispanic/Latino-American	HCHS/SOL	2,339	46.27 (13.86)	60.5	30.23 (6.44)	2.03 (6.30)	68.9, 19.5, 11.6	N/A	96.42 (0.99)	87.04 (5.92)	0.88 (3.63)
	MESA	456	68.49 (9.27)	53.3	30.08 (5.46)	16.31 (22.53)	17.1, 28.3, 54.6	3.62 (2.12)	94.33 (1.60)	81.59 (9.32)	3.80 (7.64)

Seven studies contributed 7,988 individuals with WGS in TOPMed Freeze 6a and objectively measured phenotypes (1,717 African-Americans; 229 Asian-Americans; 3,246 European-Americans; 2,796 Hispanic/Latino-Americans). The overall sample had a mean age of 57.7 and was 56.1% female. Values are displayed as mean (SD), except for the skewed Apnea Hypopnea Index, which is displayed as median (IQR). Sample size N reflects individuals with non-missing AHI and covariate values. *: Family cohort.

Cade *et al.*: WGS analyses of sleep-disordered breathing

Table 2. Sample description for imputed genotype cohorts.

Population	Cohort	N	Age	Percent Female	BMI	Apnea Hypopnea Index 3%	AHI (Percent < 5, 5 – 15, ≥ 15)	Average Desaturation	Average SpO ₂	Minimum SpO ₂	Percent Sleep Under 90% SpO ₂
African-American	CFS*	225	35.46 (20.32)	56.4	29.97 (10.09)	3.99 (10.55)	55.1 / 23.1 / 21.8	2.90 (1.09)	94.65 (4.01)	88.17 (9.6)	5.20 (16.01)
European-American/ Australian	ARIC	631	62.74 (5.72)	49.4	29.15 (5.23)	9.15 (15.02)	29.3 / 37.9 / 32.8	2.50 (1.73)	94.32 (2.15)	85.17 (6.17)	4.12 (11.76)
	CFS*	218	37.57 (18.66)	56.9	28.76 (8.11)	3.4 (10.59)	57.8 / 22.5 / 19.7	2.30 (1.11)	94.09 (3.35)	88.81 (7.8)	3.26 (12.79)
	CHS	365	77.44 (4.65)	64.9	27.10 (4.41)	10.50 (15.14)	25.8 / 39.2 / 35.1	2.63 (1.57)	94.41 (1.91)	84.87 (5.96)	3.93 (11.89)
	FHS*	192	57.45 (9.68)	51.0	28.87 (5.16)	7.30 (14.38)	38.0 / 31.8 / 30.2	2.42 (1.51)	94.73 (1.80)	85.76 (5.46)	2.82 (8.38)
	MrOS	2,181	76.65 (5.60)	0.0	27.21 (3.75)	13.00 (18.00)	18.9 / 36.1 / 45.0	3.54 (1.48)	93.85 (1.73)	84.39 (5.88)	4.40 (9.95)
	WASHS	1,508	52.29 (13.71)	40.9	31.84 (7.93)	7.24 (15.37)	40.1 / 31.1 / 28.8	3.56 (2.00)	94.56 (2.38)	84.61 (7.86)	5.44 (13.82)
Hispanic/ Latino- American	HCHS/ SOL	7,155	46.10 (13.81)	57.8	29.68 (5.86)	2.00 (6.15)	69.1 / 19.3 / 11.6	N/A	96.46 (0.95)	87.06 (6.11)	0.83 (2.99)
	Starr	782	52.34 (11.29)	71.9	32.15 (6.78)	10.35 (17.18)	31.5 / 31.5 / 37.1	N/A	94.65 (2.09)	85.78 (7.50)	2.83 (8.79)

Eight studies contributed 13,257 individuals with genomic data imputed with a TOPMed Freeze 5b reference panel and objectively measured phenotypes (225 African-Americans; 5,095 European-Americans; 7,937 Hispanic/Latino-Americans). ARIC, CFS, CHS, FHS, and HCHS/SOL imputed genomic data reflect individuals without available sequencing in TOPMed Freeze 6. The overall sample had a mean age of 53.7 and was 46.9% female. Values are displayed as mean (SD), except for the skewed Apnea Hypopnea Index, which is displayed as median (IQR). Sample size N reflects individuals with non-missing AHI and covariate values. *: Family cohort.

Cade *et al.*: WGS analyses of sleep-disordered breathing

Table 3. Lead gene-based multiple population results.

Phenotype	Sex	Gene	B38 Positions	P	Populations	N	Variants
Avg Desaturation	All	<i>ARMCX3</i>	X:101,623,082 – 101,625,765	3.48×10^{-8}	AA, AsA, EA, HA	5,222	8, 5, 24, 9
	All	<i>ARMCX3-ASI</i>	X:101,623,082 – 101,625,153	3.49×10^{-8}	AA, AsA, EA, HA	5,222	7, 5, 23, 8
Per90	All	<i>OR5K2</i>	3:98,497,633 – 98,498,634	2.55×10^{-7}	AA, AsA, EA, HA	7,986	4, 2, 1, 1
Per90	Females	<i>ZZEF1</i>	17:4,004,409 – 4,144,018	4.22×10^{-7}	AA, AsA, EA, HA	4,485	85, 16, 87, 131

Lead MMSKAT gene-based results meta-analyzed across populations within one order of magnitude of significance ($p < 4.51 \times 10^{-8}$) are shown. The Populations column indicates which populations had filtered polymorphic variants available for testing, with the number listed in the Variants column. *ARMCX3-ASI* is a RNA gene that is anti-sense to the protein-coding *ARMCX3* gene. Full results for genes with $p < 0.01$, including Ensembl-derived gene biotypes and descriptions, are provided in Supplementary Table 4.

Cade *et al.*: WGS analyses of sleep-disordered breathing

Table 4. Lead gene-based population-specific results.

Phenotype	Model	Gene	B38 Positions	N	Variants	Singletons	P
Per90	HA	<i>LINC01277</i>	6:142,985,371 – 143,010,415	2,803	2	0	5.02×10^{-8}
		<i>OR5K2</i>	3:98,497,633 – 98,498,634	2,803	1	0	2.74×10^{-7}
	AA Females	<i>SI00A16*</i>	1:153,607,528 – 153,616,353	1,009	1	1	2.07×10^{-7}
		<i>CSMD2-ASI</i>	1:33,867,977 – 33,885,456	1,009	1	1	2.07×10^{-7}
	EA Females	<i>MRPS33</i>	7:141,006,422 – 141,014,911	1,702	9	8	1.22×10^{-9}
		<i>LINC01811</i>	3:34,170,921 – 34,558,474	1,702	6	5	9.71×10^{-8}
		<i>NELFCD*</i>	20:58,980,722 – 58,995,761	1,702	12	10	3.32×10^{-7}
		<i>SLC22A8*</i>	11:62,988,399 – 63,015,986	1,702	3	3	3.58×10^{-7}
	HA Females	<i>AL132709.1</i>	14:101,077,452 – 101,077,578	1,660	2	0	1.41×10^{-7}
		<i>EPHX4</i>	1:92,029,443 – 92,063,474	1,660	12	10	3.48×10^{-7}
	HA Males	<i>C16orf90</i>	16:3,493,483 – 3,496,479	1,143	6	3	1.36×10^{-8}
		<i>TVP23B</i>	17:18,781,270 – 18,806,714	1,143	4	4	2.53×10^{-7}
		<i>IPCEF1</i>	6:154,154,536 – 154,356,890	1,143	10	8	4.07×10^{-7}

Lead MMSKAT gene-based population-specific associations within one order of magnitude of significance ($p < 4.51 \times 10^{-8}$) are shown. The Variants column indicates the number of filtered polymorphic variants with minor allele frequency $< 5\%$ available for testing, a portion of which were singletons. *: Druggable gene [^{56,57}]. Full results for genes with $p < 0.01$, including descriptions, are provided in Supplementary Table 5.

Cade *et al.*: WGS analyses of sleep-disordered breathing

Table 5. Lead single-variant analysis results.

Region	Phenotype	Model	SNP	WGS / Imputed N	CAF	WGS Beta (SE)	WGS P	Imputed Beta (SE)	Imputed P	Combined Beta (SE)	Combined P
<i>2q12.1: IL18RAP</i>	Avg desaturation	All	rs77375846 C	4995 / 4838	0.028 – 0.129	-0.152 (0.049)	1.87×10^{-3}	-0.264 (0.049)	5.97×10^{-8}	-0.208 (0.035)	1.57×10^{-9}
<i>2q33.3: PPIAP68</i>	Avg desaturation	All	rs60132122 T	5222 / 4838	0.308 – 0.637	0.062 (0.031)	0.043	0.195 (0.034)	6.26×10^{-9}	0.122 (0.023)	6.49×10^{-8}
<i>11q12.2: MS4A15</i>	Avg SpO ₂	All	rs4939452 C	7929 / 13197	0.347 – 0.524	0.066 (0.023)	4.34×10^{-3}	0.063 (0.014)	3.29×10^{-6}	0.064 (0.012)	4.87×10^{-8}
<i>18q12.3: LINC00907</i>	Avg SpO ₂	All	rs187860354 G	4500 / 7391	0.006 – 0.022	0.442 (0.146)	2.36×10^{-3}	0.432 (0.097)	8.53×10^{-6}	0.436 (0.081)	7.04×10^{-8}
<i>2q12.1: IL18RAP</i>	Min SpO ₂	All	rs138895820 G	7705 / 13194	0.025 – 0.131	0.510 (0.184)	5.58×10^{-3}	0.654 (0.128)	3.36×10^{-7}	0.607 (0.105)	7.93×10^{-9}
<i>10p12.31: NEBL</i>	Min SpO ₂	Females	rs11453507 CA	4450 / 6202	0.138 – 0.514	0.651 (0.140)	3.34×10^{-6}	0.338 (0.102)	8.63×10^{-4}	0.446 (0.082)	5.73×10^{-8}
<i>12q21.2: LINC024064</i>	Min SpO ₂	Females	rs2176909 T	4450 / 6202	0.724 – 0.930	0.828 (0.157)	1.38×10^{-7}	0.319 (0.116)	5.77×10^{-3}	0.498 (0.093)	9.06×10^{-8}
<i>5p13.3: C5orf22</i>	AHI	Males	rs10940956 A	3502 / 7043	0.470 – 0.759	0.930 (0.422)	2.74×10^{-2}	1.430 (0.269)	1.09×10^{-7}	1.285 (0.227)	1.48×10^{-8}
<i>9p22.1: DENND4C</i>	AHI	AA	rs111654000 A	1717 / 225	0.016 – 0.018	-11.240 (2.268)	7.18×10^{-7}	-18.110 (6.724)	7.07×10^{-3}	-11.942 (2.149)	2.74×10^{-8}
<i>1q31.2: AL954650.1</i>	AHI	AA	chr1:191965014_G/A A	1717 / 225	0.286 – 0.301	3.078 (0.641)	1.56×10^{-6}	5.080 (1.759)	3.88×10^{-3}	3.313 (0.602)	3.75×10^{-8}
<i>8p12: AC068672.1, NRG1</i>	AHI	EA	rs35447033 T	3246 / 5095	0.060 – 0.094	2.247 (0.621)	2.95×10^{-4}	2.453 (0.521)	2.54×10^{-6}	2.368 (0.399)	3.02×10^{-9}
<i>5p13.2: SLC45A2</i>	Avg SpO ₂	EA	rs28777 A	3201 / 5024	0.885 – 0.969	-0.526 (0.133)	8.00×10^{-5}	-0.454 (0.096)	2.23×10^{-6}	-0.478 (0.078)	8.08×10^{-10}
<i>1q32.1: ATP2B4</i>	Avg SpO ₂	HA	rs116133558 T	2803 / 7956	0.006 – 0.014	0.371 (0.120)	2.08×10^{-3}	0.294 (0.062)	2.15×10^{-6}	0.310 (0.055)	1.88×10^{-8}
<i>1q23.3: intergenic (RNU6-755P)</i>	Min SpO ₂	HA	rs140743827 A	2803, 7174	0.017 – 0.020	-1.502 (0.593)	1.13×10^{-2}	-1.770 (0.367)	1.42×10^{-6}	-1.696 (0.312)	5.51×10^{-8}
<i>1q32.1: ATP2B4</i>	Per90	HA	rs116133558 T	2803, 7956	0.006 – 0.014	-1.005 (0.450)	2.54×10^{-2}	-1.218 (0.207)	4.15×10^{-9}	-1.181 (0.188)	3.51×10^{-10}
<i>11p11.2: intergenic (AC104010.1)</i>	Avg SpO ₂	HA males	chr11:44652095_T C/T T	1143 / 3024	0.007 – 0.008	0.686 (0.248)	5.65×10^{-3}	0.710 (0.154)	3.83×10^{-6}	0.703 (0.131)	7.25×10^{-8}
<i>10q22.1: HK1</i>	Min SpO ₂	EA males	rs17476364 C	1523 / 3650	0.072 – 0.115	1.215 (0.392)	1.94×10^{-3}	1.099 (0.235)	2.81×10^{-6}	1.129 (0.201)	2.01×10^{-8}
<i>8q23.2: KCNV1</i>	Min SpO ₂	EA males	rs58365105 A	1523, 3650	0.007 – 0.026	-2.878 (0.864)	8.65×10^{-4}	-2.406 (0.540)	8.36×10^{-6}	-2.539 (0.458)	2.96×10^{-8}
<i>2q35: AC019211.1</i>	Per90	EA males	chr2:220369683_G/A A	1540, 187	0.005 – 0.006	12.280 (2.431)	4.38×10^{-7}	17.505 (7.989)	2.85×10^{-2}	12.723 (2.326)	4.48×10^{-8}

Cade *et al.*: WGS analyses of sleep-disordered breathing

Lead EMMAX single-variant associations within one order of magnitude of significance (combined $p < 1.00 \times 10^{-8}$) and with replication evidence ($p < 0.05$) are shown. Full results for all variants in each locus with $p < 1.00 \times 10^{-7}$, including additional associations with secondary models, and metadata and annotations, are provided in Supplementary Table 6.

Cade *et al.*: WGS analyses of sleep-disordered breathing

Table 6. Transcription factor binding site interval enrichment results.

Phenotype	Transcription Factor	# Observed Overlap	# Expected Overlap	-log10 (E-value)
AHI	<i>FOXP2</i>	588	36.20	473.99
	<i>KDM6B</i>	630	51.58	435.29
	<i>THAP1</i>	505	31.89	402.07
	<i>KLF9</i>	745	91.81	395.52
	<i>TP63</i>	997	182.22	383.85
Average Desaturation	<i>FOXP2</i>	493	22.32	460.00
	<i>THAP1</i>	439	19.55	412.76
	<i>UBTF</i>	489	28.20	407.50
	<i>TP63</i>	788	109.36	382.89
	<i>KDM6B</i>	482	30.98	380.39
Average SpO ₂	<i>FOXP2</i>	582	35.87	468.89
	<i>KDM6B</i>	613	51.21	418.65
	<i>EGR1</i>	664	66.76	404.83
	<i>UBTF</i>	574	46.35	399.91
	<i>KDM4B</i>	489	29.56	398.10
Min SpO ₂	<i>FOXP2</i>	561	35.57	445.57
	<i>THAP1</i>	515	31.32	417.89
	<i>KDM6B</i>	569	50.87	373.41
	<i>UBTF</i>	536	45.99	360.56
	<i>EGR1</i>	602	66.25	346.03
Per90	<i>FOXP2</i>	689	39.05	578.42
	<i>KDM6B</i>	739	54.79	539.69
	<i>TP63</i>	1199	193.28	515.44
	<i>THAP1</i>	607	34.47	509.33
	<i>EGR1</i>	786	72.09	507.27

250 base-pair sliding window coordinates with association $p < 0.01$ were queried for interval enrichment of ChIP-seq derived transcription factor binding sites using the ReMap annotation tool. ChIP-seq coordinates were required to have >50% overlap with a sliding window interval. ReMap-derived expected overlaps are obtained from the equivalent number of similarly-sized random regions. E-value indicates the expected value, with a higher log-transformed value indicating greater enrichment. Full results are provided in Supplementary Table 12.



