Leveraging pleiotropy to discover and interpret GWAS results for sleep-associated traits

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Abstract

Genetic association studies of many heritable traits resulting from physiological testing often have modest sample sizes due to the cost and invasiveness of the required phenotyping. This reduces statistical power to discover multiple genetic associations. We present a strategy to leverage pleiotropy between traits to both discover new loci and to provide mechanistic hypotheses of the underlying pathophysiology, using obstructive sleep apnea (OSA) as an exemplar. OSA is a common disorder diagnosed via overnight physiological testing (polysomnography). Here, we leverage pleiotropy with relevant cellular and cardio-metabolic

phenotypes and gene expression traits to map new risk loci in an underpowered OSA GWAS. We identify several pleiotropic loci harboring suggestive associations to OSA and genome-wide significant associations to other traits, and show that their OSA association replicates in independent cohorts of diverse ancestries. By investigating pleiotropic loci, our strategy allows proposing new hypotheses about OSA pathobiology across many physiological layers. For example we find links between OSA, a measure of lung function (FEV_1/FVC), and an eQTL of desmoplakin (*DSP*) in lung tissue. We also link a previously known genome-wide significant peak for OSA in the hexokinase (*HK1)* locus to hematocrit and other red blood cell related traits. Thus, the analysis of pleiotropic associations has the potential to assemble diverse phenotypes into a chain of mechanistic hypotheses that provide insight into the pathogenesis of complex human diseases.

Introduction

Genome-wide association studies of human phenotypes ranging from gene expression to human diseases are now routine. Cumulatively, the data indicate that complex traits are highly polygenic, [1,2] and genetic correlation between these traits indicates abundant pleiotropy. [3–5] Interpreting the plethora of results raises two major challenges: first, generating testable mechanistic hypotheses about the underlying pathophysiology; and second, increasing statistical power to identify associations in traits with small or moderate sample sizes. Leveraging pleiotropy can help address both of these challenges. Previous work has demonstrated that including many correlated traits in association studies increases power to detect associations common to multiple traits. [4,6] This approach is untried in genetic investigations of obstructive sleep apnea (OSA). Here, we demonstrate that using shared associations between correlated traits can identify effects in under-powered studies of

obstructive sleep apnea (OSA), and that leveraging molecular and physiological endophenotypes in this way also generates clear and testable biological hypotheses.

OSA is characterized by recurrent episodes of partial or complete obstruction of the pharyngeal airway resulting in multiple physiological disturbances, including sympathetic nervous system activation, increased energy cost of breathing, intermittent hypoxemia, and wide swings in intrathoracic pressure. This disorder is highly prevalent in the general population, affecting more than 15% of middle-aged adults, with increased prevalence observed with aging, obesity, and cardiometabolic disease, and is more common in men.[7] OSA leads to sleep disruption, particularly increased sleep fragmentation and decreased proportion of restorative stages of sleep, resulting in daytime sleepiness, impaired quality of life and cognitive deficits. [8] Moreover, OSA is associated with increased rates of hypertension, incident heart disease, stroke, diabetes, depression certain cancers and overall mortality. [9] [10,11] [12–18] Despite the large number of epidemiological studies indicating that OSA is closely associated with these outcomes, there appears to be subgroup differences in susceptibility, e.g., middle-aged individuals and men are more likely to experience OSA-related cardiovascular disease in some studies than older individuals and women, respectively. This underscores gaps in our knowledge of the pathophysiological pathways linking OSA to other diseases. [19,21] Pathophysiological pathways linking OSA to other diseases and factors that influence individual differences in susceptibility are poorly understood. While there are several effective treatments for OSA, including continuous positive airway pressure (CPAP), there appears to be substantial variation in overall clinical response and attenuation of cardiometabolic consequences, suggesting heterogeneity in both the etiology of the disease and susceptibility to its physiological disturbances.

Indices of OSA, including the apnea hypopnea index (AHI; the number of breathing pauses per hour of sleep), apneic event duration, indices of overnight hypoxemia, habitual snoring, and excessive daytime sleepiness, show substantial heritability in family studies. [20] Past studies have identified only a handful of associations with a variety of OSA related traits. We have previously described a GWAS of OSA traits measured by overnight polysomnography in multi-ethnic cohorts totaling ~20,000 individuals. [22] In that study, we found two genome-wide significant multiethnic associations: variants in a locus on 10q22 were associated with indices of average and minimum SpO_2 and percentage of sleep with SpO_2 < 90%, and variants in a locus on 2q12 were associated with minimum oxygen saturation (SpO_2). In another study we identified a locus in 17p11 with a male specific effect on AHI. [23] Furthermore, in an admixture mapping study in Hispanic/Latino Americans, we identified a locus on 2q37 associated with AHI and one in a locus on 18q21 associated with AHI and SpO_2< 90%.[24]

The low number of genetic associations reported to date only explains a small fraction of OSA trait heritability. This relative paucity of findings is driven primarily by modest sample sizes, a reflection of the expense and difficulty of measuring physiological phenotypes by overnight polysomnography. This also limits our ability to fine-map associations down to causal variants and thus identify relevant genes. Overnight polysomnography is logistically difficult and expensive, so data on hundreds of thousands of individuals – sample sizes at which GWAS designs are well-powered to detect tens of loci and, aided by additional experiments, fine-map some of them – have yet to be collected and may never be available. [1,2] Biological interpretation of available genetic associations is further complicated by the observation that most GWAS effects localize to enhancer regions and other regulatory elements and are often distal to physiologically relevant genes. [25,26]

Now that GWAS of massive sample sizes have been accumulated for various comorbid conditions and endophenotypes related to OSA, we hypothesize that analysis of shared associations across correlated traits can identify effects in under-powered studies of OSA and generate clear and testable biological hypotheses. A number of computational methods increase power for discovering genetic associations by capitalizing on pleiotropy between disease phenotypes or between a disease and a molecular trait such as gene expression. One common approach takes advantage of the genetic correlation among phenotypes. [6,27] This class of methods gains substantial additional power by pooling association signals across traits. However, such methods require large sample sizes and suffer from power loss when genetic correlation is limited to a subset of loci. An alternative approach analyzes individual loci to detect pleiotropic alleles, with no regard to overall genetic correlation.[28–33,4,34–37] Only a handful of existing methods account for the possibility that the apparent pleiotropy is driven by the linkage disequilibrium (LD) between two distinct causal variants each of which drives only one phenotype. [4,31,32,34,36]

In this study, we apply a joint likelihood mapping method (JLIM) to detect shared associations between OSA and other related traits. JLIM has high specificity when rejecting apparent pleiotropy driven by distinct causal variants in LD, and does not depend on estimates of genetic correlation which necessitate large sample sizes. We have previously applied this approach to identify shared associations between gene expression traits and autoimmune disease risk. [35] However, JLIM's rationale generalizes beyond gene expression to any potential intermediate trait or biological marker that might also mediate OSA pathogenesis. Here, in a novel application of JLIM, we reverse our strategy to ask whether highly significant associations with traits that

plausibly influence OSA measures, also have pleiotropic effects on OSA parameters, as a way to discover and simultaneously interpret new associations to sleep apnea traits in an underpowered study.

We focus on a set of well powered intermediate traits which have previously been implicated in the pathobiology of OSA. Given prior GWAS studies suggesting the involvement of inflammatory genes in OSA, [38–40] case-control and cohort studies reporting high levels of inflammation, including elevations in neutrophils and monocytes in OSA, [41,42] we included leukocyte and platelet related traits in our pleiotropic comparisons. Similarly, we also included red blood cell related traits given prior GWAS implicating iron metabolism [24] and erythrocyte function. [22] We will refer to these as clinical traits. In addition, OSA is associated with lung, [43,44] obesity and cardiovascular-related pathologies, [42,45–47] and we have included clinical traits that reflect that, together with gene expression traits in tissues implicated in such pathologies.

By linking different clinical and gene expression traits to OSA at specific loci, our analysis leads to new hypotheses about OSA pathobiology across many physiological layers, in addition to finding new associations.

Materials and Methods

OSA Cohorts

To study pleiotropic associations underlying the risk of OSA, we prepared two sets of cohorts: the discovery cohorts to identify pleiotropic variants and independent replication cohorts to validate their associations to OSA traits. For the discovery cohorts, we used individual-level

genotype data in order to determine the significance of pleiotropy by permutation (JLIM). At the replication stage, we do not carry out any pleiotropy analysis, we only check for genetic association to OSA, so summary-level association statistics were sufficient. In addition, we restricted the genetic ancestry of GWAS discovery cohorts to that of European ancestry, to match GWAS of clinical traits. This was in order to avoid potential issues due to mismatch of LD patterns in our pleiotropy analysis. In contrast, we did not require the replication cohorts to have any specific ancestry. Thus, our replication cohorts included all available ethnicities.

The discovery cohorts included the subset of samples of European ancestry from the following five cohorts: the Atherosclerosis Risk in Communities Study (ARIC),[48] Osteoporotic Fractures in Men (MrOS) Study,[49] Multi-Ethnic Study of Atherosclerosis (MESA),[50] Cardiovascular Health Study (CHS),[51] and the Western Australian Sleep Health Study (WASHS).[52] ARIC is a study that investigates atherosclerosis and cardiovascular risk factors. It is one of the cohorts included in the Sleep Heart Health Study, which collected polysomnography and genotype data.[53] Genotype data were obtained through dbGaP (phg000035.v1.p1). MESA is a population-based study focused on cardiovascular risk factors, which included participants of four ethnicities: African-, Asian-,European- and Hispanic/Latino-Americans ranging from ages of 45 to 86 years old. We only included samples from European-Americans in the discovery cohort. Polysomnography data measuring sleep related traits was later obtained from individuals who did not use overnight oxygen, CPAP or an oral device for sleep apnea.[54] MrOS is a multi-center prospective epidemiological cohort assembled to examine osteoporosis, fractures and prostate cancer in older males.[55] An ancillary study (MrOS Sleep) measured sleep disturbances and related outcomes.[56] CHS is a cohort aimed to study coronary heart disease and stroke in individuals aged 65 and older, and genotype data was obtained through dbGaP (Illumina CNV370 and IBC;

phg000135.v1.p1 and phg000077.v1.p1). WASHS is a clinic-based study designed to examine OSA and its associated genetic risk factors in patients referred to a sleep clinic in Western Australia. Not all individuals had measurements for the four OSA related traits of interest. Details on genotyping, imputation and QC procedures have been previously reported [22].See Table S8 for details.

The replication cohorts were: the Hispanic Community Health Study/Study of Latinos (HCHS/SOL),[57,58] Starr County Health Studies (Starr),[59] Cleveland Family Study (CFS)[60] and Framingham Heart Study (FHS),[61] in addition to non-European samples of CHS and MESA. HCHS/SOL is a population-based study to examine protective and risk factors for many health conditions among Hispanic/Latinos living in four urban areas within the USA (Chicago IL, Miami FL, San Diego CA and Bronx NY). Starr is a cohort collected to study risk factors for diabetes in a population of Mexican-Americans in Texas, later phenotyped for sleep traits.[62] CFS is a family-based study, which recruited patients with OSA, their relatives, and neighborhood control families to study the familial and genetic basis of sleep apnea (356 families of African American or European American ancestry). We included only unrelated individuals from CFS. FHS is an epidemiological cohort established to study cardiovascular disease risk factors, using follow-up medical examinations every two years for the population of European Ancestry in Framingham, MA. Data from the first Sleep Heart Health Study was obtained between 1994-1998. Genotype data was obtained through dbGaP (Affymetrix 500k; phg000006.v7). See Table S9 for the details of each cohort.

We examined the following four OSA related traits in the discovery and replication cohorts: minimum and average oxygen saturation (SpO_2), apnea-hypopnea index (AHI), and event

duration. Briefly, the minimum and average SpO_2 were calculated from oximetry-based SpO_2 measurements over the entire recorded sleep interval excluding occasional waking periods. AHI was scored by counting the number of episodes of complete (apnea) or partial (hypopneas) airflow reduction associated with $\geq$ 3% desaturation per hour of sleep. The event duration was measured for the average length of apneas and hypopneas, from the nadir of the first reduced breath to the nadir following the last reduced breath (in seconds). The full description of phenotyping protocols is present in the original study which first reported their genetic analysis in the context of OSA.[22–24] We rank-normalized all OSA traits, separately in each cohort, in order to obtain normally distributed phenotypes.

Clinical Trait Data

For clinical traits, we used GWAS summary statistics calculated for various traits in the UK Biobank,[63,64] blood cell related phenotypes in a general UK population,[65] and cardio-metabolic phenotypes in individuals of European Ancestry.[66] There is no sample overlap between clinical trait GWAS data and our discovery or replication cohorts. The full list of clinical traits is shown in Table S1. The GWAS summary statistics for UK Biobank traits and blood cell counts were downloaded from their websites. The summary statistics of cardio-metabolic traits from[66] were obtained directly from the authors.

This research was approved by Partners in Healthcare IRB (protocol #2010P001765).

Identifying pleiotropic variants affecting both clinical traits and OSA

We applied Joint Likelihood Mapping (JLIM version 2.0) [35] to test whether the association signals of two traits were driven by a shared genetic effect. We ran JLIM only on the loci in which there was strong evidence of association to a clinical trait (genome-wide significant) and a suggestive association to the OSA trait ($p < 0.01$). In these loci, JLIM compares the likelihood of observed association signals under the following three competing possibilities: the OSA trait has no causal variant in the locus ("H_0"), the same OSA causal variant is shared with the clinical trait ("H_1") or the OSA causal variant is distinct from the clinical trait causal variant ("H_2") shown in Figure S1. Briefly, JLIM calculates the ratio between the likelihood of the data under H_1 compared to that under H_2 and evaluates the significance of this statistic by permuting the phenotypes simulating the lack of causal effect under H_0. The false positives due to H_2 are indirectly controlled by the conservative behavior of JLIM: the expected value of JLIM statistic is lower under H_2 than under H_0. JLIM assumes that only up to one causal variant is present for each trait in a locus. However, simulations showed that the accuracy of JLIM remained robust in the presence of multiple causal variants in a locus (Figure S7).

To run JLIM, we used the genetic association statistics of OSA traits calculated over all SNPs in a 200kb analysis window around the focal SNP (the lead SNP in the clinical trait). We derived these statistics from our discovery cohorts by combining association signals of each cohort using an inverse-variance weighted meta-analysis approach. The association statistics were calculated in individual cohorts by linear regression adjusting for age, sex, BMI and the top three principal components. The principal components were calculated from genome-wide genotype data in each cohort separately. We used mean imputation for missing covariate values. Multi-allelic SNPs and variants with minor allele frequencies (MAF) below 0.05 were excluded from

the analysis. We only used variants present in all of the discovery cohorts. We only ran JLIM in the discovery cohorts.

We used the same pipeline to generate permuted association statistics for JLIM. OSA phenotypes were randomly shuffled in each cohort separately. For each permutation, the association statistics were calculated in the same way including all the covariates. Then, the cohort-level association statistics calculated on permuted data were combined across cohorts by meta-analysis. This permutation procedure was repeated for up to 100,000 times, adaptively, to estimate JLIM p-values.

Replication of OSA associations in independent samples

We validated the OSA associations identified in the discovery cohorts by replicating them in out-of-sample multi-ethnic replication cohorts (Table S9). There was no sample overlap between our discovery and replication cohorts. We combined the *p*-values of associations across the six replication cohorts by applying an inverse variance-weighted meta-analysis technique. We defined the *p*-value of association < 0.05 as nominal evidence of replication and the *p*-value $< 0.05/65$ as a more stringent Bonferroni-corrected replication cutoff, given that 65 loci were uncovered for their pleiotropic associations to OSA in the first analysis (Table 1, Table S3).

Identifying pleiotropic variants affecting both gene expressions and OSA

We used *cis*-expression quantitative trait loci (eQTLs) from the Gene-Tissue Expression project (GTEx release v7) [67] and BLUEPRINT epigenome project (release 20151109), [68] to examine pleiotropy between the variation in gene expression levels and OSA phenotypes. Among the

GTEx datasets, we only considered liver (N = 133), spleen (N = 116), skeletal muscle (N = 420) and lung (N = 333) tissues for our analysis, based on the potential relevance of these tissues to OSA and their sample sizes. Again, we included only samples of European ancestry for this analysis. Genetic ancestry was identified by the first two axes in principal component analysis (Figure S9,S10). The GTEx post-QC genotype data were obtained from dbGAP (phs000424.v7) along with the sample covariates. The post-QC normalized RNA levels were downloaded from the GTEx portal. For the analysis of immune-cell eQTLs, we used BLUEPRINT datasets which consisted of genotypes of participants and expression profiles of $CD14^+$ monocytes (N = 194), neutrophils (N = 196) and $CD4^+$ T cells (N = 169). The RNA transcripts of BLUEPRINT samples were derived from unstimulated primary cells collected from healthy individuals of European ancestry. The genotype data of BLUEPRINT participants were downloaded from the European Genome-phenome archive (EGA; EGAC00001000135). The QCed and normalized gene expression levels were obtained from the BLUEPRINT project.

We focused this analysis on the 65 loci with putative pleiotropic associations between OSA and clinical traits (Table S3). Using GTEx and BLUEPRINT eQTLs, we scanned for pleiotropy between eQTLs and clinical or OSA traits. We considered all protein-coding genes whose transcription start sites (TSS) were less than 1Mb away from the focal SNP of a clinical trait. The protein-coding genes were defined by Ensembl annotation (release 92). The genes with eQTL association *p*-value > 0.01 at the focal SNP were excluded due to weak evidence of association to gene expression. Multiallelic SNPs and variants with MAF < 0.05, were excluded from the analysis. The default parameters were used for JLIM. The number of permutations to estimate the *p*-value of JLIM statistic were adaptively increased up to 100,000 starting from the minimum of 1,000.

Since JLIM requires permutation of eQTL associations to generate the null distribution of its statistic, we re-calculated the eQTL association statistics from the original data and generated permutation statistics from the same pipeline by randomly shuffling the phenotypes. Specifically, for each tested gene, we used normalized gene expression values as a phenotype and ran a linear regression for each SNP in a 200kb JLIM analysis window surrounding the focal SNP. Similarly as in the original study, when we calculated the eQTL association statistics for GTEx tissues, we included the top three principal components (PC), 15 PEER factors,[69] sex and platform (Illumina HiSeq 2000 or HiSeq X) as covariates. For BLUEPRINT, we generated the eQTL association statistics including the covariates of the top three PCs, age and sex. The same set of covariates were also used to generate permutation data for JLIM.

Simulated datasets

To compare the accuracy of JLIM to other methods, we simulated genetic loci with pleiotropic associations under different scenarios with unbalanced sample sizes. For one of two traits, we simulated a well-powered GWAS of a quantitative trait with sample sizes of 100,000, 150,000 and 200,000. For the other trait, we simulated an under-powered GWAS with much smaller sample sizes of 5,000, 10,000 and 15,000. To generate datasets of realistic LD backgrounds, we used real genotypes of 80 randomly picked loci across the genome. The genotype data of 15,000 individuals were obtained by subsampling from six cohorts of European Ancestry (MESA, ARIC, MrOS, CHS, CFS, FHS and WASHS) and further down-sampled to a target cohort size as needed. Each locus was 200 kb in length. Chromosome 6 and the sex chromosomes were excluded from our simulations due to the difference in LD patterns from the rest of genome. We removed multiallelic sites and variants with MAF < 0.05.

In each of these simulated loci, the SNP in the midpoint of the genomic segment was chosen as the causal variant for the well-powered trait (focal SNP). We fixed the true genetic effect β^F of the focal SNP to be large enough to explain 0.05% of the variance in the trait. This value was chosen to represent typical effect sizes of genome-wide significant association peaks in a well-powered study. In GWAS of sample sizes of 100,000, 150,000 and 200,000, the median *p*-values of association at such a focal SNP are 1.5 x 10^{-12}, 4.7 x 10^{-18} and 1.5 x 10^{-23}, respectively. JLIM only requires GWAS summary statistics for the well-powered trait. Therefore in each locus, we generated summary statistics by sampling the association statistics $\widehat{\boldsymbol{Z}}$ of the locus from the following multivariate normal distribution [70]:

$$\widehat{\boldsymbol{Z}} \sim \mathcal{N}\left(\sqrt{N_1}\,\boldsymbol{\Sigma}\boldsymbol{\beta}, \boldsymbol{\Sigma}\right),$$

where N_1 is the GWAS sample size, m is the number of markers in the locus, $\boldsymbol{\Sigma}$ is the $m \times m$ LD matrix, and $\boldsymbol{\beta}$ is the m-dimensional vector of true causal effects (on standardized genotype values) at all SNPs in the locus. $\boldsymbol{\beta}$ was set to β^F at the focal SNP and to 0 at all other SNPs. The *p*-values of association were calculated from $\widehat{\boldsymbol{Z}} = \left(\hat{Z}_1, \dots, \hat{Z}_j, \dots, \hat{Z}_m\right)^T$ as follows:

$$p_j = 2 \times \Phi(-|\hat{Z}_j|),$$

where $\hat{Z}_j$ is the association statistic at SNP j, and Φ is the standard normal cumulative distribution function. Because of sampling noise, in a minority of simulations, all SNPs in the locus failed to pass the genome-wide significance threshold. When this happened, we re-simulated the locus until it reached the target threshold. This was done to mimic our data

analysis, where we only included loci in which the clinical trait association p-value was genome-wide significant.

For underpowered GWAS datasets, we simulated loci with no causal effect (H_0), the same causal effect at the focal SNP (H_1) or a causal effect at a distinct variant from the focal SNP (H_2). For simulations of H_0, we assumed that all SNPs had the causal effect size of 0. For simulations of H_1 and H_2, the true causal effect size was fixed to β^F, but the causal variant was chosen to be the focal SNP in case H_1 or to be a distinct SNP in case of H_2. The distinct SNPs were chosen from an LD window relative to the focal SNP, selecting one in each of the following LD ranges: low ($|r| \leq 0.3$), intermediate ($0.3 < |r| \leq 0.6$) and high ($0.6 < |r| \leq 0.8$). Since JLIM requires individual-level genotype data, for the underpowered trait, we simulated phenotypes for all individuals and calculated the association statistics by linear regression, instead of sampling the summary statistics from a multivariate normal distribution. The genotypes were generated by down-sampling from a total of 15,000 samples to the target cohort size. For H_0, we simulated baseline phenotype values by sampling from the standard normal distribution. For H_1 and H_2, we added the genetic effect β^F to the baseline phenotypes, depending on the genotypes of the simulated causal SNP. The simulated causal effect size of β^F is expected to produce median association *p*-values of p= 0.11, 0.025, and 0.0062 , with GWAS sample sizes of 5,000, 10,000 and 15,000, respectively. Each parameter set was simulated 100 times per locus.

Next, we examined the robustness of our method when the true effect sizes were only weakly correlated between two traits. Here, we assumed that the causal effect sizes between two traits were coupled and the degree of this coupling was governed by the correlation coefficient $\rho_{1,2}$.

Specifically, we modeled the causal effect sizes $\left(\beta_F^{(1)}, \beta_F^{(2)}\right)$ at the focal SNP F for the two traits as distributed according to the following bivariate normal distribution:

$$\begin{pmatrix} \beta_F^{(1)} \\ \beta_F^{(2)} \end{pmatrix} \sim \mathcal{N}\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_1^2 & \rho_{1,2}\sigma_1\sigma_2 \\ \rho_{1,2}\sigma_1\sigma_2 & \sigma_2^2 \end{pmatrix}\right),$$

where σ_1^2 and σ_2^2 are the variance of per-SNP effect sizes for trait 1 and 2, respectively. We assumed that $\sigma_1^2 = h_1^2/fM_1$ and $\sigma_2^2 = h_2^2/fM_2$. Here, h_1^2 and h_2^2 are the heritability of each trait, both set to 0.5. M_1 , M_2 are the numbers of independent causal SNPs in the genome for each trait, both set to 1,000,000 in our simulation, and f is the fraction of SNPs expected to be causal in each trait, set to 0.01 in our simulation. We considered the correlation coefficient $\rho_{1,2}$ to be 0, 0.35 or 0.7. In such a scenario, given a fixed value of $\beta_F^{(1)}$, the conditional distribution of $\beta_F^{(2)}$ follows the normal distribution such that:

$$\beta_F^{(2)} \mid \beta_F^{(1)} \sim \mathcal{N}\left(\rho_{1,2}\frac{\sigma_2}{\sigma_1}\beta_F^{(1)}, \sigma_2^2\left(1-\rho_{1,2}^2\right)\right).$$

Using this conditional normal distribution, we kept the effect size of the focal SNP for the well-powered trait $\beta_F^{(1)}$ to be the constant β^F, explaining 0.05% of trait vFariation, and sampled the causal effect size $\beta_F^{(2)}$ of the underpowered trait to differ from, but be correlated to that of the well-powered trait.

Last, we also evaluated the accuracy of our method under the presence of additional causal variants that were not shared across two traits. Here, we randomly selected two additional SNPs

for each trait in a locus. The effect sizes of these four extra non-pleiotropic variants were sampled from a normal distribution with variance of $\sigma_1^2/5$ and $\sigma_2^2/5$ for each corresponding trait. We simulated the effect sizes of focal SNP for the well-powered trait and main causal SNP for the under-powered trait in the same manner as the previous set of simulations.

Simulated benchmark of coloc and eCAVIAR

We ran coloc (version 3.1)[31] with summary statistics of GWAS for clinical and OSA traits as inputs. The sample sizes of tested GWAS cohorts were provided along with allele frequencies and *p*-values of association at all SNPs. We used the default prior settings, where the frequency of causal SNPs (p_1 and p_2) was set to 10^{-4} for both traits, and the frequency of colocalization ($p_{1,2}$) was set to 10^{-5}. The pleiotropic variants were identified based on the posterior probability of colocalization (PP4).

We ran eCAVIAR (version 2.2)[34] with summary statistics of both traits and reference LD matrix. The default parameter setting was used. As a prior, eCAVIAR assumes that each variant in the locus analyzed is causal for a particular trait with a probability of 0.01, independently of other variants and phenotypes. It then computes the posterior probability of each variant being causal for both traits. By default, eCAVIAR considered up to two causal variants per trait in each locus. We used the top posterior probability that an identical SNP is causal for both traits (called “the maximum CLPP score”) to find the pleiotropic variants.

Since the posterior probabilities of coloc and eCAVIAR were measured in relative scales, we needed to calibrate the cutoffs of these scores before comparing their accuracies with JLIM. For

this, we used null simulations of no causal effect for underpowered trait (H_0) to find the score cutoffs corresponding to the 1% false positive rate. To calculate precision, we mixed a randomly sampled subset of positive cases with all of our negative cases at a specified ratio. This step was iterated for 500 rounds, and then the average precision was reported.

Results

Creating a framework to identify associations in underpowered GWAS through pleiotropy

We used JLIM [35] to identify pleiotropic loci, where a genetic effect drives association to two traits. First, we selected genome-wide significant loci (association $p < 5 \times 10^{-8}$) in our well-powered trait (here, a clinical trait), and from these we selected the subset where the lead (focal) SNP also shows nominal association to OSA traits ($p < 0.01$). We then used JLIM to directly evaluate if the association to the two traits was consistent with the same underlying effect, indicating a pleiotropic effect. This two-step strategy allowed us to distinguish between cases where there was association only in the clinical trait; where there was a shared association in both traits; and if there was distinct associations in both traits stemming from different underlying effects (Figure S1).

Simulations

We first assessed our strategy in simulated data, varying sample size, LD and the presence of multiple conditionally independent associations in a locus. For reference, we also compared JLIM results to those from two other popular pleiotropy detection methods, which unlike JLIM

are Bayesian: coloc [31] and eCAVIAR. [34] We simulated statistics for pairs of traits where the same variant is causal (positive cases), and where two different variants in LD are driving each of the associations at a locus (negative cases), and assessed the capacity of each method to discriminate between them. All three methods directly contrast the likelihood of true pleiotropy to that of the alternative driven by two distinct causal variants, although coloc does not explicitly account for the effect of LD in summary statistics.

We used genotypes from European ancestry samples as a reference from which we simulated quantitative traits in 5,000; 10,000; and 15,000 samples as surrogates for the OSA GWAS. To simulate summary statistics from a well powered GWAS (representing our clinical traits), we sampled values from a multivariate normal (MVN) distribution using the local LD matrix as a variance-covariance parameter [70] with clinical trait samples sizes of 100,000; 150,000; and 200,000 samples. We found that coloc was the most sensitive method (Figure 1A), though this came at a substantial false positive rate and drop in precision (Figure 1B and C). Overall, we found that JLIM has high specificity at discriminating pleiotropic associations from cases where two distinct variants in LD drive associations in the same locus in different traits (Figure S1). We explored a range of other parameters, including sample size for each trait, the correlation between effect sizes in both traits, the LD between causal variants in negative cases, and the level of allelic heterogeneity in the loci, detailed in supplemental section (Figures S2-S7).

Identifying pleiotropic associations between clinical traits and sleep apnea-related traits

Based on clinical relevance [71] and heritability, [20] we focused on four OSA-related traits measured in five European-ancestry cohorts: the apnea-hypopnea index (AHI), [23] average respiratory event duration, [24] minimum and average oxygen saturation (SpO_2). [22] We used summary statistics from the remaining multiethnic cohorts in our replication effort.

We assembled a collection of GWAS summary statistics for a total of 55 candidate intermediate traits from across these physiological areas: erythroid, leukocyte and platelet counts and function, from a study combining the UK Biobank and INTERVAL datasets (170,000 individuals of European ancestry); [65] cardiovascular, metabolic and respiratory traits from the UK Biobank (380-450,000 European ancestry participants),[64,72] and cardio-metabolic traits (36,000 European ancestry participants). [66] We then compared associations in each of these clinical traits to our OSA traits (6,781 European ancestry participants; Table S8), to identify potential associations in the latter. A complete list of clinical traits we considered is presented in Table S1

We tested for directional causal effects of the selected clinical traits on our OSA related traits using Mendelian Randomization (MR).[73] Due to the low sample sizes in OSA traits, no comparison reached statistical significance after multiple test correction (Table S11). After excluding the extended MHC region and the sex chromosomes, we identified 3,191 genome-wide significant associations ($p < 5 \times 10^{-8}$) in the 55 clinical traits, of which 221 had a corresponding suggestive OSA association at the lead SNP ($p < 0.01$; Table S2). We then explicitly tested for evidence of pleiotropy between clinical and OSA traits using JLIM. We found evidence that in 65/221 of these regions the OSA and clinical trait associations are consistent with a shared, pleiotropic underlying causal variant (false discovery rate (FDR) < 0.05; Table 1, Table S3). FDR values were computed based on the JLIM p-values obtained, using the total number of trait comparisons tested (Table S2).

To independently validate our 65 putative OSA trait associations from the discovery stage, we compiled summary statistics for the same traits in 15,594 individuals of Asian, African American, European and Hispanic ancestries (Table S9). These individuals do not overlap with those from

the cohorts used in our JLIM analysis. We do not attempt to replicate pleiotropic associations; we only replicate the OSA association statistics. JLIM relies on local LD patterns being preserved between clinical and OSA trait cohorts, so we cannot use multi-ethnic data in our discovery analysis. We found that 2/65 variants in Table S3 show significant association with the same OSA trait as the initial observation, after Bonferroni correction for the number of tests performed (which we consider more appropriate than FDR for out-of-sample testing). The variant in SNP rs17476364 (Figure 2A) links every single one of the red blood cell related clinical traits analyzed with average SpO_2. It is an intronic variant in the hexokinase 1 (*HK1*) region in chromosome 10, and has been previously reported, as it reached genome-wide significance in association to minimum and average SpO_2.[22] The variant in SNP rs2277339 is a missense coding variant in DNA primase subunit 1 (*PRIM1*) in chromosome 12. It links both plateletcrit and mean corpuscular volume to AHI. In the UK biobank, it has documented significant associations to height, waist to hip-ratio, age at menopause and multiple red blood cell related traits.[74] A further 10 variants, shown in Table 1, were below nominal association p values < 0.05 in the replication set, but did not survive multiple test correction.

We performed sensitivity analyses to assess whether our discovery analysis was generating false positive associations due to our selection criteria, or our replication data was generating false negatives due to low statistical power. We randomly selected 100,000 SNPs (excluding the HLA region and sex chromosomes) from our initial OSA GWAS in individuals of European ancestry, giving us 400,000 association statistics across four OSA traits. We found that 4,162 of these would have been selected for our JLIM analysis, in line with expectation for our in-sample threshold of association $p < 0.01$. Out of these, 223 had a significant out-of-sample nominal replication (association $p < 0.05$). In comparison, our pleiotropy analysis results are 3.5-fold

enriched at this threshold (12/65 hits), suggesting the presence of true associations to sleep traits (one-sided Fisher exact $p = 0.00019$; Figure 2).

JLIM has higher statistical power to detect pleiotropy when the underlying association to OSA is stronger, raising a concern that loci with stronger OSA associations in the discovery set are driving the out-of-sample replication rate and are overrepresented among loci with significant evidence of pleiotropy. To address this, we compare the out-of-sample nominal replication after correcting for OSA associations in the discovery sample The rate of nominal replication remained significantly higher among the 65 pleiotropic associations compared to random controls when we controlled for the bias due to the difference in strength of association to OSA (Combined Fisher exact $p = 0.00434$, 3.23-fold enrichment; Table S10). In fact, the 156/221 associations that did not show significant evidence of pleiotropy were also slightly enriched for nominal replication relative to the set of randomly selected variants (one-sided Fisher exact $p = 0.076$, 1.7-fold enrichment; Figure 2). This suggests that the FDR cutoff we applied to our JLIM results is conservative, and additional pleiotropic effects – and therefore true OSA associations – remain to be discovered. In both cases where we could clearly replicate an OSA association in our multiethnic cohort data, we detected pleiotropy with multiple erythrocyte traits.

Incorporating gene expression to construct molecular hypotheses of sleep apnea physiology

Non-coding regions with evidence of gene regulatory activity carry a large proportion of heritability in most traits analyzed in large GWAS. [75] We reasoned that some OSA causal variants would reside in such regulatory regions, and thus act on gene regulation. We therefore sought shared associations between gene expression traits and the clinical traits for which we identified a pleiotropic association in the 65 loci in Table 1 and Table S3. To do so, we compiled expression quantitative trait loci (eQTL) data for protein-coding genes expressed in lung, liver,

spleen and skeletal muscle from individuals with European ancestry from the GTEx Project, [67] and monocyte, T cell and neutrophil populations in individuals from BLUEPRINT. [76] We chose these tissues for potential relevance to OSA pathology: the lung is involved in OSA-related hypoxemia; [43,44] previous GWAS associations have implicated the neuromuscular junction in overnight SpO_2 levels; [22] the spleen and liver are known to mediate filtration of erythrocytes and iron homeostasis; and leukocytes are key modulators of inflammation. We calculate FDR based on JLIM p-values over the 167 comparisons shown in Table S4, where we include these eQTL analyses.

We were able to identify shared associations between eQTL and clinical traits in 34/65 loci (Table 2, Table S5). This includes several notable examples, including a locus on chromosome 1, where eQTL for both *PSEN2* (presenilin 2) and *COQ8A* (coenzyme Q8A) levels in neutrophils are pleiotropic with the percentage of neutrophils in white blood cells, which in turn is pleiotropic with AHI. Another example is a locus on chromosome 6 where we find that a known eQTL for desmoplakin expression in lung tissue is pleiotropic with a measure of lung function (FEV_1/FVC, the ratio of forced expired volume per second to forced vital capacity), which in turn is pleiotropic with an association with AHI (Figure 3). Thus, in these and other loci we can attribute associations to gene expression in specific cell types, clinical and sleep apnea traits to the same genetic variant, and thus construct specific biological hypotheses of the pathophysiology underlying OSA.

We also looked for pleiotropy between gene expression traits and OSA in the three loci harboring known genome-wide significant OSA associations in the discovery sample (Table S6). In each locus, we compared OSA trait summary statistics to eQTLs for genes within 1Mb from the most associated variant, where the OSA focal variant had an eQTL association *p*-value <

0.01 (Table 3). Given the low number of comparisons, we calculated the FDR values by pooling results from these three loci with the set of clinical to eQTL comparisons in Table S4. We replicated a previously found pleiotropic effect in a locus on chromosome 17, [22] where minimum oxygen saturation (SpO_2) co-localizes with expression of the epsilon subunit of the nicotinic receptor (*CHRNE*), in various tissues, including lung, neutrophils, monocytes and muscle (Figure 4).

Discussion

In our comparison of clinical (respiratory, cardiometabolic, inflammatory) traits to OSA related traits, the strongest finding lies in an intronic region of hexokinase 1 (*HK1*) and is associated with average overnight oxygen saturation level (SpO_2). This locus is pleiotropic with all red blood cell related traits tested (Figure 2A) and corresponds to one of the most significant genome wide associations we had previously reported from this data. [22] Prior to this analysis, two alternative hypotheses for the etiology of this signal had been proposed: that *HK1* acted by modulating inflammation, or that it affected OSA by altering erythrocyte function. Our results provide evidence that is consistent with the erythrocyte related pathway. Mutations in *HK1* have been implicated in anemia, together with severe hemolysis and marked decreases in red blood cells.[77] As discussed previously,[22] it is possible that *HK1* affects the Rapoport-Luebering shunt through glycolytic pathway intermediates, which in turn mediates oxygen carrying in mature erythrocytes. Factors that influence arterial oxygen levels can lead to a more severe OSA phenotype (i.e., lower average levels of oxygen saturation predispose to greater hypoxemia with each breathing obstruction). Lowered oxygen carrying capacity and thus more tissue hypoxia could also contribute to breathing instability (and thus apneas) via Hypoxia-Inducible Factor-1

(HIF-1) and enhanced carotid body sensitivity and chemoreflex activation, or through long-term respiratory facilitation and plasticity. [78,79]

The analysis of pleiotropy can be used to concatenate more than one phenotype to create candidate "causal chains," which by linking eQTLs to well-powered traits to sleep apnea related traits can hint at promising biological targets. Among the most significant results for this multicomponent model is desmoplakin (*DSP*), a gene whose expression in lung tissue is affected by an eQTL that co-localizes with a lung function phenotype (FEV_1/FVC) which itself co-localizes with AHI. This same co-localizing SNP (rs2076295) has been implicated both in Interstitial Lung Disease (ILD) and *DSP* gene expression a separate study. [80] It is also the lead SNP in FEV_1/FVC trait at that locus in the UK biobank GWAS used in our analysis. Desmoplakin is a key component of desmosomes, which have a role in cell-cell adhesion, suggesting a role in epithelial integrity in lung pathology, which, in turn, could have a downstream effect on OSA. OSA is highly prevalent in ILD as well as associated with subclinical markers of ILD. While prior research suggested that OSA may have been causally related to interstitial lung injury, the current data suggest a common causal pathway. [43]

We also found that *PSEN2* (presenilin 2) and *COQ8A* (coenzyme Q8A) eQTLs in neutrophils colocalize with associations to the neutrophil percentage of white cells and AHI. Previous studies suggest that sleep disturbances can trigger increased hematopoiesis of neutrophils in the bone marrow. [46] Patients with OSA often present with elevated levels of circulating neutrophils, which may contribute to the pathogenesis of OSA through effects on upper airway and respiratory muscle inflammation, and possibly by effects on brain centers influencing breathing and sleep-wake organization. Other data implicate inflammation as a downstream consequence of OSA-related hypoxemia and sleep fragmentation that mediates increased

cardiovascular risk.[41] The association with *PSEN2* is also particularly interesting given that mutations of this gene result in increased production of amyloid-beta proteins, elevations of which are a hallmark of Alzheimer's disease, which is associated with OSA.[81] While these findings suggest a mechanistic link across sleep, neutrophils, cognition and cardio-respiratory disease, we did not specifically test respiratory, brain or cardiovascular traits for genetic associations at this locus.

Another interesting result is an eQTL in the epsilon subunit of the nicotinic acetylcholine receptor *CHRNE* that co-localizes with a genome-wide significant association in minimum oxygen saturation. This receptor is present at neuromuscular junctions and mutations in this subunit are known to cause congenital myasthenic syndrome in humans that can result in progressive respiratory impairment. [82]

From a methodological perspective, the analysis of pleiotropy has become an important tool in the analysis of complex trait genetics. Most complex traits are highly polygenic, implying that many variants associated with a single trait will also be associated with other traits or will be in LD with such variants. Different computational methods are required for different applications and for different genetic architectures. If the goal is to increase power to detect an association and the genetic correlation is broadly dispersed over many loci, methods explicitly capitalizing on the broad genetic correlation are capable of producing large power gains. [6,27] This family of methods remains inaccessible for small GWAS studies, where estimates of genetic correlation from small samples are too noisy to be useful. We tested the genetic correlation between the four OSA traits and the clinical traits, and no genetic correlation estimate reached statistical significance after multiple test correction (Table S7).

In cases where genetic correlation varies across the genome, power can still be increased with the help of other methods that leverage pleiotropy to reduce multiple testing burden.[28] Development of another group of approaches was motivated by the need to link genetic associations to genes via eQTL data [31,34,35] but, as shown here, these methods can be easily adapted to the analysis of other traits. Because of the abundance of association signals, especially for cellular and molecular traits, distinguishing between true pleiotropy due to the same underlying causal variants and different causal variants in LD is important for all the applications. Therefore, in our study of OSA we elected a method that explicitly models LD structure in the locus. The drawback of this choice is the need to restrict the discovery sample to a demographically homogeneous subset while using the available multi-ethnic cohort for replication.

Pleiotropy does not necessarily imply a causal relationship between phenotypes. Nonetheless, as we demonstrate here, a shared genetic basis between OSA and organismal, cellular and molecular traits can reveal new aspects of the underlying biology. This will likely be of benefit for other clinically relevant traits that are difficult to study at the scale required for GWAS. Traits that are burdensome or expensive to phenotype, rare diseases that are hard to sample and diseases that affect under-represented populations could all lead to underpowered genetic studies, which are unlikely to get dramatically higher sample sizes in the near future. Therefore, there is an unmet need to optimize the signals we can extract from small GWAS and the strategy presented here should help in achieving this goal.

Supplemental Data

Supplemental Data include ten figures and eleven tables and can be found with this article online at:

Declaration of Interests

The authors declare no competing interests.

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Web Resources

JLIM 2.0: http://genetics.bwh.harvard.edu/wiki/sunyaevlab/jlim2.0 and https://github.com/cotsapaslab/jlim/

GWAS summary statistics in the UK Biobank: http://data.broadinstitute.org/alkesgroup/UKBB/

GWAS summary statistics of blood cell traits: http://www.bloodcellgenetics.org/

GTEx portal (post-QC normalized gene expression levels): http://gtexportal.org/home/

Post-QC normalized gene expression levels for BLUEPRINT:

ftp://ftp.ebi.ac.uk/pub/databases/blueprint/blueprint_Epivar/Pheno_Matrix/

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Figures

Figure 1. JLIM has higher precision than competing methods.

Examples of simulated data with increasing LD between the causal SNPs for the clinical and OSA traits. For each method (coloc, JLIM and eCAVIAR). The threshold of detection for each method was selected such that 1% of null simulations (H_0) are accepted as positives. For each set, negative and positive cases were simulated 100 times in 80 loci. The positive cases (H_1 in Figure S1) have the same SNP causing an effect on the clinical trait (N_1=150,000) and the OSA trait (N_2=10,000). As for the negative cases (H_2 in Figure S1), we randomly selected a causal SNP for the OSA trait within an LD window in a specified range. This SNP would have low (0.0 < | r | < 0.3), medium (0.3 < | r | < 0.6) and high (0.6 < | r | < 0.8) linkage to the causal SNP in the clinical trait. All causal SNPs had the same effect on the traits. We show **A** the sensitivity (fraction of true positives detected), **B** the false positive rate (fraction of false negatives among all negatives, also equal to 1-specificity) and **C** the precision (fraction of true positives among all cases detected) under a simulation where 1% of cases are positive. LD in negative cases does not affect sensitivity, given that it is computed from positive cases exclusively.

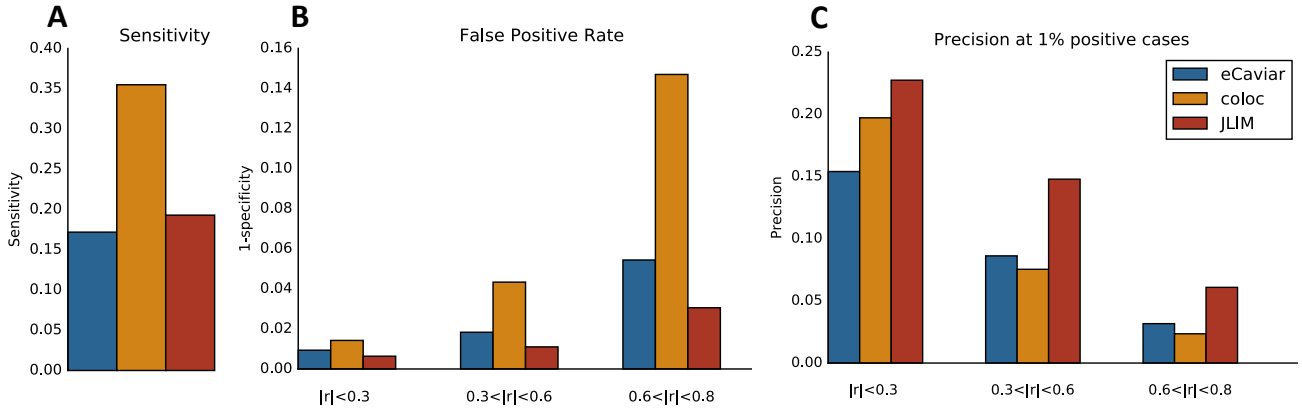

Figure 2. Pleiotropy at HK1 locus and fractions of loci that replicate out of sample.

A) Putatively pleiotropic locus linking a clinical trait (hematocrit) with an OSA trait (average SpO_2). The JLIM p-value which tests for pleiotropy between both traits at the locus is p=0.008. This SpO_2 association replicates out of sample ($p = 8.61 * 10^{-5}$.). **B)** Plots showing the fractions of randomly selected and putative pleiotropic loci with OSA associations that replicated in the multiethnic cohorts (out of sample). All loci included passed a discovery sample (European ancestry) 0.01 p-value threshold. JLIM significant loci (FDR<0.05) were more likely to have significant out of sample OSA association p-values than randomly selected cohorts (OR=4.00 Fisher exact p=0.00019).

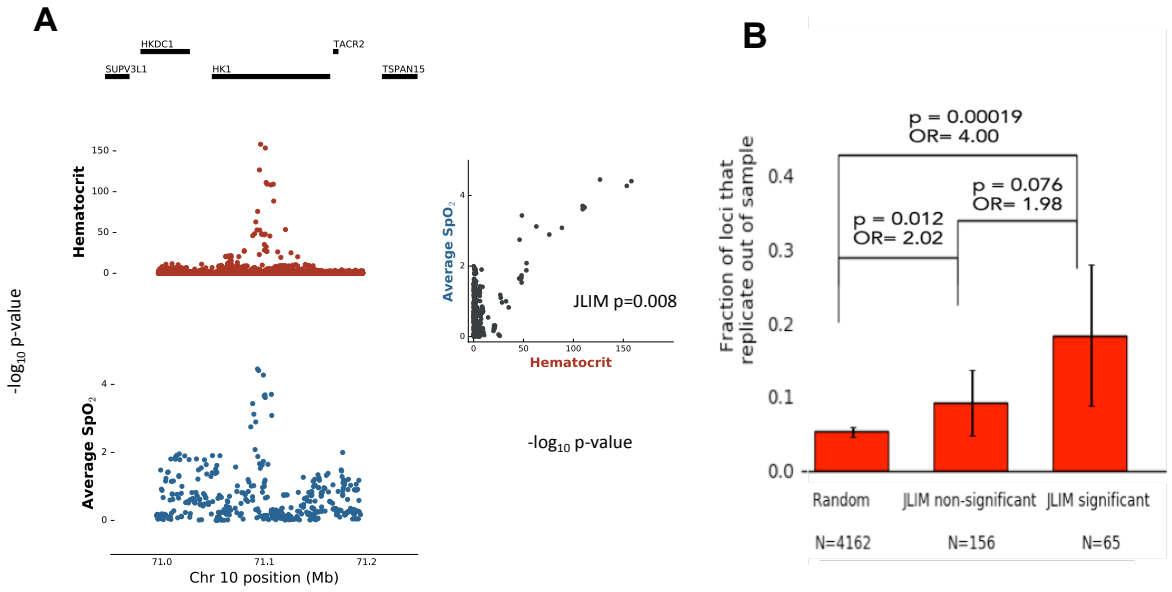

Fig 3. Candidate causal chains linking a clinical trait (red), an OSA related trait (blue) and an eQTL (yellow).

A) A candidate association in chromosome 1 with putative pleiotropic associations between the clinical trait neutrophil percentage of white cells (red), AHI (blue) and expression of *PSEN2* (presenilin 2) in neutrophils (yellow). Gene expression and the appropriate gene in the locus are shown in yellow. Pairwise comparisons of $-\log_{10}$(p-values) between associated traits are shown on the right with matching colors in axis labels. **B)** A candidate association in chromosome 6 with putative pleiotropic associations between the clinical trait FEV_1/FVC (red), AHI (blue) and expression of *DSP* (desmoplakin) in lung (yellow).

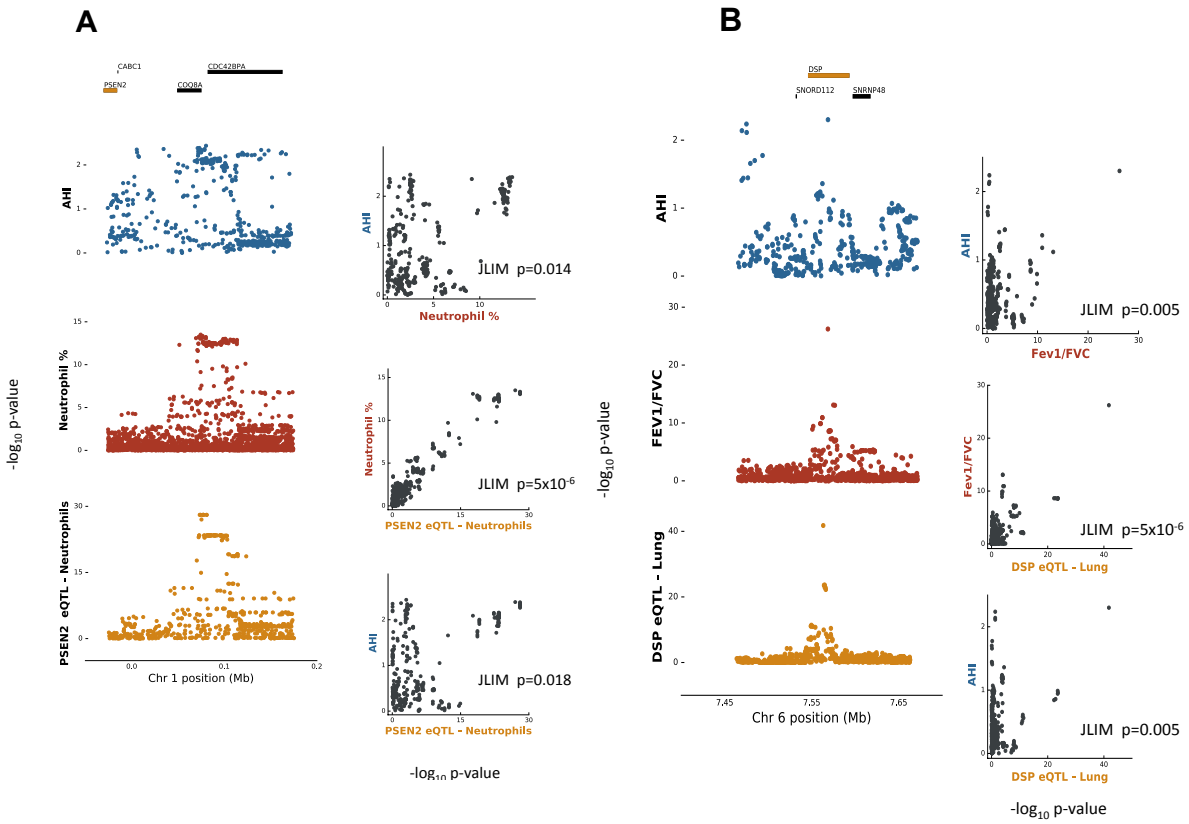

Fig 4. Pleiotropic locus linking gene expression and an OSA related trait.

A locus in chromosome 17 has associations between minimum oxygen saturation (blue) and expression of *CHRNE* (cholinergic receptor nicotinic epsilon subunit) in lung (yellow). Gene expression trait p-values and the appropriate gene in the locus are shown in yellow. Pairwise comparisons of $-\log_{10}$(p-values) between associated traits are shown on the right with matching colors in axis labels. The JLIM p-value which tests for pleiotropy between both traits at the locus is $p = 5 * 10^{-6}$.

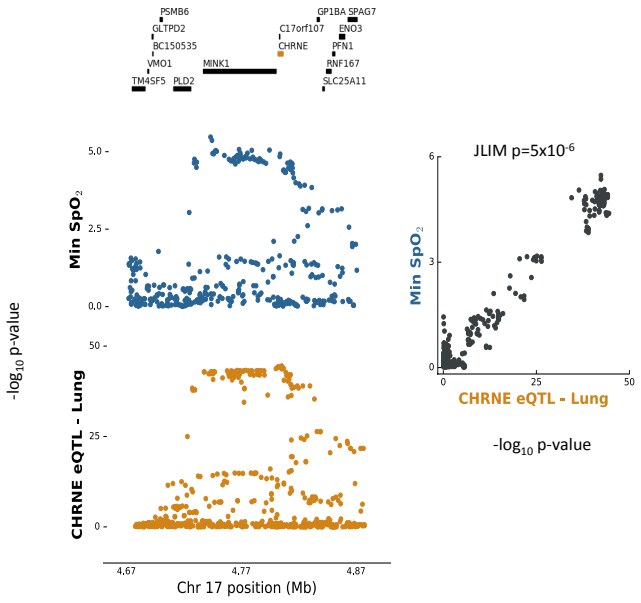

Table 1. Loci with significant pleiotropic associations between a clinical trait and an OSA trait with nominally significant replication.

SNP	Coordinate	Clinical phenotype	Clinical p-value	JLIM p-value	OSA phenotype	OSA phenotype p-value	Replication p-value
rs17476364 *†	chr10.71094504	Hematocrit	7.65×10^{-159}	0.008	Average O2 saturation	0.000156	8.61×10^{-5}
		Reticulocyte count	1.90×10^{-96}	0.008			
		Red blood cell count	1.75×10^{-48}	0.008			
rs2277339 †	chr12.57146069	Plateletcrit	1.13×10^{-10}	0.009	AHI	0.00642	0.000629
		Mean corpuscular volume	1.07×10^{-9}	0.011			
rs11187838	chr10.96038686	Systolic B.P	9.10×10^{-46}	0.015	Average O2 saturation	0.00516	0.00419
rs34211119	chr2.60720318	High light scatter reticulocyte count	3.15×10^{-9}	0.002	Event Duration	0.00283	0.00423
		High light scatter reticulocyte percentage of red cells	4.81×10^{-9}	0.002			
		Immature fraction of reticulocytes	9.48×10^{-13}	0.002			
14:103566835	chr14.103566835	Mean platelet volume	7.18×10^{-70}	0.007	AHI	0.00724	0.00511
		Platelet count	4.80×10^{-39}	0.008			
		Platelet distribution width	2.40×10^{-23}	0.006			
rs17010961	chr4.86723103	Systolic B.P	7.90×10^{-24}	0.00078	Average O2 saturation	2.74×10^{-5}	0.00744
rs4711750	chr6.43757082	Reticulocyte fraction of red cells	6.68×10^{-11}	0.009	AHI	0.00529	0.0111
		High light scatter reticulocyte percentage of red cells	5.42×10^{-11}	0.01			
		Myeloid white cell count	6.71×10^{-9}	0.009			
rs17476364 *	chr10.71094504	Hematocrit	7.65×10^{-159}	0.00013	Minimum O2 saturation	6.81×10^{-7}	0.0206
		Red blood cell count	1.75×10^{-48}	0.00013			
		Reticulocyte count	1.90×10^{-96}	0.00013			
rs2595105	chr4.111552761	Basal metabolic rate	7.00×10^{-13}	0.004	Minimum O2 saturation	0.00481	0.0356
rs11172113	chr12.57527283	FEV1/FVC	8.90×10^{-30}	0.012	Average O2 saturation	0.00469	0.0405
rs9825482	chr3.188448320	Sum eosinophil basophil counts	3.62×10^{-13}	0.002	AHI	0.000202	0.0450
rs542618547*	chr3.188446663	Eosinophil percentage of white cells	7.56×10^{-15}	0.003	AHI	0.000193	0.0453
		Eosinophil count	1.03×10^{-14}	0.002			
		Eosinophil percentage of granulocytes	7.55×10^{-14}	0.003			

Each row denotes one SNP and its corresponding associations to clinical and OSA traits. Each SNP may be associated with more than one clinical trait. In SNPs marked with (*) redundant clinical traits were removed for clarity. Here we only show variants with nominally significant replication p-values, the full table of 65 loci/sleep associations including non-significant replication p-values is included in Table S3. Two variants had a significant out of sample replication p-value after Bonferroni correction (0.05/65) and are marked with the symbol (†). AHI stands for Apnea-hypopnea index. Coordinates correspond to hg19. The clinical p-value column refers to the association p-value of the SNP to the clinical phenotype. Clinical phenotypes and their corresponding datasets are listed in table S1. The OSA phenotype p-value refers to the association p-value of the SNP to the OSA trait when including the five European ancestry cohorts (sample sizes in Table S8). The replication p-value refers to the association p-value of the SNP to the OSA trait when including all replication cohorts (sample sizes in Table S9). The JLIM p-value tests for pleiotropy between the clinical and OSA phenotypes.

Table 2. Candidate causal chains.

SNP	Coordinate	OSA phenotype	OSA-clinical JLIM p-value	Clinical phenotype	eQTL-clinical JLIM p-value	Gene	eQTL p-value	Tissue/Cell type
rs6426558	chr1.227175367	AHI	0.014	Neutrophil percentage of white cells	5.00×10^{-6}	*PSEN2* (presenilin 2)	9.80×10^{-28}	Neutrophils
					5.00×10^{-6}	*COQ8A* (coenzyme Q8A)	3.07×10^{-37}	Neutrophils
9:136139907	chr9.136139907	Average O_2 saturation	0.00077	IL6	5.00×10^{-6}	*BARHL1* (BarH like homeobox 1)	3.85×10^{-29}	Neutrophils
rs2076295	chr6.7563232	AHI	0.005	FEV1/FVC	5.00×10^{-6}	*DSP* (desmoplakin)	1.68×10^{-42}	Lung
rs34233420	chr17.38004929	AHI	0.00273	Lymphocyte count	2.00×10^{-6}	*GSDMA* (gasdermin A)	3.16×10^{-15}	T-Cells
rs11653357	chr17.33923607	Event Duration	0.015	Platelet distribution width	3.00×10^{-6}	*SLFN12L* (schlafen family member 12 like)	6.55×10^{-8}	T-Cells
					6.00×10^{-6}	*SLFN13* (schlafen family member 13)	6.63×10^{-5}	T-Cells
rs1693551	chr8.101675584	Average O_2 saturation	0.011	Diastolic B.P.	0.0001	*SNX31* (sorting nexin 31)	4.72×10^{-6}	Lung
rs66538782	chr1.46596236	Event Duration	0.008	Sum neutrophil eosinophil counts	0.0001	*TMEM69* (transmembrane protein 69)	5.61×10^{-6}	T-Cells
rs2277339	chr12.57146069	AHI	0.011	Mean corpuscular volume	0.00031	*ZBTB39* (zinc finger and BTB domain containing 39)	0.000283	Neutrophils
					0.00044	*B4GALNT1* (beta-1 4-N-acetyl-galactosaminyltransferase 1)	0.00134	Muscle
rs7162943	chr15.89615275	Average O_2 saturation	0.00016	Mean platelet volume	0.00047	*DET1 (COP1* ubiquitin ligase partner)	0.000235	Lung
rs35259020	chr9.136950919	AHI	0.004	Reticulocyte count	0.00064	*STKLD1* (serine/threonine kinase like domain containing 1)	0.00482	Monocytes
rs14667194	chr9.136934203	AHI	0.00191	Red cell distribution width	0.00071	*STKLD1* (serine/threonine kinase like domain containing 1)	0.00482	Monocytes
					0.00077	*BRD3* (bromodomain containing 3)	0.000373	Spleen

These SNPs link an OSA trait, a clinical trait and gene expression, with each association passing an FDR 0.05 threshold. The full set of putative causal chains is shown in Table S5. Here we only show chains with an eQTL-clinical JLIM p<0.001. AHI stands for Apnea-hypopnea index. Coordinates correspond to hg19. The eQTL p-value refers to the association p-value of the SNP to the gene expression trait of the gene indicated, measure in the tissue/cell type indicated. The OSA-clinical JLIM p-value refers to a pleiotropy

test between the OSA and clinical traits, while the eQTL-clinical JLIM p-value corresponds to a test of pleiotropy between gene expression and the clinical trait.

Table 3. Genome wide significant loci in OSA traits colocalizing with eQTL.

SNP	Coordinate	OSA phenotype	OSA p-value	Gene	Tissue/Cell type	eQTL p-value	JLIM p-value
rs12150370	chr17.4777634	Minimum O_2 saturation	3.37×10^{-8}	*CHRNE* (cholinergic receptor nicotinic epsilon subunit)	Lung	6.63×10^{-43}	5.00×10^{-6}
					Neutrophils	1.14×10^{-10}	2.00×10^{-5}
					Monocytes	3.98×10^{-11}	4.00×10^{-5}
					Muscle	3.64×10^{-6}	5.00×10^{-5}
				C17orf107 (chromosome 17 open reading frame 107)	Neutrophils	1.98×10^{-5}	8.00×10^{-5}
					Lung	6.27×10^{-12}	0.006
				INCA1 (inhibitor of CDK cyclin A1 interacting protein 1)	Liver	0.000246	0.005
				ALOX15 (arachidonate 15-lipoxygenase)	Monocytes	0.000122	0.018
rs16926246	chr10.71093392	Average O_2 saturation	2.46×10^{-8}	*SRGN* (serglycin)	Monocytes	0.000469	0.00047
				TYSND1 (trypsin domain containing 1)	Neutrophils	0.00185	0.003
				SUPV3L1 (Suv3 like RNA helicase)	Liver	0.00376	0.01

SNPs shown are genome wide significant in the OSA trait and are pleiotropic to eQTL, where the JLIM p-value passed an 0.05 FDR significance threshold, and the eQTL p-value passed a 0.01 nominal p-value threshold. The eQTL p-value refers to the association p-value of the SNP to the gene expression trait of the gene indicated, measured in the tissue/cell type indicated. The JLIM p-value refers to a pleiotropy test between the OSA and gene expression traits. Full corresponding set of comparisons tested with JLIM in Table S6.