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Rare Genetic Variants Provide Protection for Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) is an extremely common condition
worldwide. OSA was shown many years ago to aggregate in families
(1). Although this has been known for decades, progress in identifying
relevant gene variants has been slow. This likely reflects both
inadequate sample sizes and the etiological heterogeneity of OSA, with
multiple risk factors that are each likely influenced by many genes.

Multiple approaches to identifying gene variants have been used,
including candidate gene studies (which have been very
underpowered [2]), family-based linkage studies (3) (which identified
LOD scores below accepted ranges even for suggestive significance),
and genome-wide association studies. Two different phenotyping
approaches to genome-wide association studies have been used: case-
control analysis on the basis of clinical diagnosis (4) and quantitative
trait analysis using measures from overnight sleep studies (e.g., the
apnea–hypopnea index [AHI]) (5) shown to be heritable (6). The
latter has been facilitated by conducting sleep studies in population-
based cohorts. However, this approach can be challenging because
there may be only a small subset of individuals with clinically
meaningful OSA (5). There are also questions about whether subjects
identified in the general population are representative of individuals
who present clinically (7).

Although these efforts have begun to identify reproducible gene
variants related to OSA, all variants together explain only a small
fraction of the estimated heritability (4). Given that prior analyses
have typically focused on common genetic variants
(e.g., minor allele frequency [MAF] > 5%), one possible explanation
for this “missing heritability” is associations with rare variants
that have a lower prevalence (e.g., MAF < 5%). Although each individual
variant is rare, there are a lot of them. In other complex traits, rare
variants have been shown to explain a proportion of the missing
heritability (8), supporting their potential role in OSA.

To assess the effect of rare variants, the study of Liang and
colleagues (pp. 1271–1280) in this issue of the Journal (9) takes an
interesting and informative multistage approach, leveraging major
resources assembled by the highly innovative Trans-Omics for
Precision Medicine (TOPMed) program sponsored by the NHLBI.
Stage I involved linkage analysis for AHI in 487 European
Americans from 118 families in the CFS (Cleveland Family Study)
(1). The highest linkage peak was a suggestive association (LOD = 2.
31) on chromosome 7q31. The investigators then used a number of
filtering strategies and both gene-based burden tests and sequence
kernel association tests to identify rare variants and implicated
genes in the 20-cM region centered on the linkage peak. Although
a number of different rare variants were identified, the most
significant gene was CAV1 (Caveolin-1), which contained 21

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noncoding variants with MAF \( \leq 0.01 \) associated with reduced AHI on the basis of the burden test (9).

Stage II and stage III analyses focused on quantitative analyses in available cohorts of unrelated individuals. In stage II, the authors analyzed associations for variants within 70 genes implicated in stage I, using whole-genome sequencing data in 2,772 individuals of European ancestry from four cohorts in the TOPMed program. Although analyses restricted to functional rare variants did not identify associated genes, gene-based analyses of noncoding variants identified nominal associations of seven genes (\( \text{CAV1}, \text{TES} \) [testin LIM domain protein], \( \text{ASZ1} \) [ankyrin repeat, SAM and basic leucine zipper domain containing 1], \( \text{PTPRZ1} \) [protein tyrosine phosphatase receptor type Z1], \( \text{SND1} \) [staphylococcal nuclease and Tudor domain containing 1], \( \text{AKRIB1} \) [aldo-keto reductase family 1 member B], and \( \text{POT1} \) [protection of telomeres 1]). Although none of the associations remained significant after correction for multiple comparisons, \( \text{CAV1} \) was associated with lower AHI at this stage. These seven nominally associated genes were then carried forward to stage III using genotype data from 4,449 new individuals. \( \text{CAV1} \) variants were consistently associated with lower AHI in stage III, and gene-based associations remained significant after correcting for multiple comparisons. As further evidence supporting this result, investigators returned to the CFS and showed that individuals carrying any of the 21 noncoding variants in \( \text{CAV1} \) had much lower AHI than noncarriers; carriers were slightly younger, with lower body mass index, neck circumference, and blood pressure. Finally, bioinformatics approaches were used, using RNA sequencing data for 44 tissues in the Genotype-Tissue Expression Project database (10). Identified variants in \( \text{CAV1} \) were associated with higher expression of \( \text{CAV1} \) in skeletal muscle and in T cells in the MESA (Multi-Ethnic Study of Atherosclerosis) cohort (9). Thus, the overall conclusion of this elegant study is that \( \text{CAV1} \) harbors rare, noncoding variants that increase expression of \( \text{CAV1} \) and are protective against OSA.

This study by Liang and colleagues (9) shows the power of using the resources that have been assembled by TOPMed, such as whole-genome sequencing data, coupled with appropriate statistical techniques to answer important questions and advance knowledge. The question now is “Where do we go from here?” First, it is important to see this result replicated in other studies, including in clinical cohorts with real-world patients. The authors failed to replicate results in the UK Biobank study, but as they show, there is likely to be a large number of subjects in the UK Biobank with unrecognized OSA. Fortunately, relevant cohorts are increasingly becoming available (4). Extreme phenotypes of OSA have also been identified (11). “Extreme control subjects” are individuals with high likelihood of OSA on the basis of age, sex, and body mass index but who do not have OSA. Are these rare variants more common in these individuals?

Another question is “Why does \( \text{CAV1} \) protect you from OSA?” There remains the potential for pleiotropy or confounding, as \( \text{CAV1} \) is associated with multiple OSA-related comorbidities. However, the authors also present a number of mechanistic hypotheses, including possible effects on upper airway muscle or cardiopulmonary functions. Ultimately, future functional studies are needed. Although functional studies of genes associated with sleep duration can be performed in multiple model systems, including high-throughput studies in \( \text{Drosophila} \) and zebrafish (12) and more in-depth studies in mice (13), a challenge to functional studies of genes for OSA remains which animal model and phenotype to investigate.

Thus, this study is an important step toward understanding the unexplained heritability of OSA. Although additional replication is needed, it raises a number of important questions about mechanisms. We may finally be turning the corner in the search for gene variants that affect OSA risk, and the next few years are set to be a highly productive era of investigation in this area.

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