Breath of Fresh Air: Toward Unraveling the Molecular Underpinnings of Sleep Apnea

Obstructive sleep apnea (OSA) is an important risk factor for multiple diseases (1). A total of 425 million adults aged 30–69 are estimated to have severe OSA, including 65 million in the most affected country, China (2). Positive airway pressure, the primary treatment for OSA, has relatively low adherence (3), emphasizing a continued need for more personalized treatments. OSA and related quantitative traits including the apnea–hypopnea index (AHI) are heritable (4). Genome-wide association analyses (GWAS) are one means of identifying novel molecular processes underlying OSA and potential targets for future interventions in an unbiased manner. Performing OSA GWAS is difficult because of performing overnight polysomnography (PSG) in sample sizes suitably powered for genetic studies. A small but growing number of significant associations with OSA and related quantitative traits have been discovered in the last half-decade, largely on the basis of European- and admixed American-ancestry participants. To date, no strong associations have been identified in East Asians.

In this issue of the *Journal*, Xu and colleagues (pp. 1534–1545) describe the first significant associations with OSA and related traits in a large Han Chinese sample comprised of over 5,000 cases and 15,000 control subjects (5). Highlights of this work include the use of PSG to allow deeper phenotypic assessment of cases in this first large study of an underexamined population and, importantly, efforts to functionally validate key findings. Of the two significantly associated OSA loci and two significantly associated OSA-related quantitative loci, the most biologically interesting region includes *SLC52A3*, a riboflavin (vitamin B2) transmembrane transporter that is known to impact Brown-Vialetto-Van Laere (Online Mendelian Inheritance in Man [OMIM] 211530) and Fazio Londe syndromes (OMIM 211500). Presentation of these rare disorders can include diaphragmatic and facial weakness, nocturnal hypoventilation, respiratory failure, and central sleep apnea (6). The lead variant rs3746804 is a *SLC52A3* coding mutation with potential protein conformation effects and is associated with differences in *SLC52A3* expression in multiple tissues, including lung. *Slc52a3* is known to be highly expressed in the intestine, and whereas evidence for rs3746804-associated regulation in the brain is currently limited, neuropathy is a known consequence of riboflavin transporter disease and is a plausible mechanism of effect (7, 8). The authors demonstrate that the rs3746804 allele associated with an increased AHI is also associated with decreased serum riboflavin (which is not endogenously generated in humans), raising the possibility that riboflavin supplementation may attenuate OSA. Although these effects could be independent of each other, this hypothesis could readily be confirmed by investigators with available data.

There are multiple potential mechanisms in which riboflavin deficiency could impact OSA. Additional riboflavin appears to be required under acute hypoxic conditions (9). Riboflavin is the precursor of flavin adenine dinucleotide, a coenzyme that affects HIF1A stability (10). The authors focus on mitochondrial effects, given known electron transport chain effects in riboflavin-associated neuropathies (8). They demonstrate that the candidate mutation impacts extracellular riboflavin absorption in a cellular assay, and *Slc52a3* inhibition attenuates the increase in mitochondrial activity after chronic intermittent hypoxia exposure. Using CRISPR–Cas9 gene editing, a point mutation knock-in mouse (Slc52a3P258L) was generated that showed higher riboflavin concentrations and downregulation of a number of lipid-associated metabolites in brain tissue, consistent with hyperlipidemia in the livers of *Slc52a3* knockout mice (11). Of note, however, under exposure to chronic intermittent hypoxia, no metabolic changes were seen between wild-type and Slc52a3P258L mice, despite prior gene changes observed under acute hypoxia that were partially rescued with supplements including riboflavin (9).

This evidence substantially adds to a working model suggesting an important role for mitochondria in OSA pathogenesis. Mitochondria are known to impact multiple pulmonary disorders (12). Sleep apnea and reduced ventilatory drive are enriched in individuals with a primary mitochondrial disorder (13). Five genes related to mitochondria have previously been associated with OSA-related quantitative traits in GWAS and whole-genome sequence analyses: *HK1* (hexokinase), *CAV1*, *ARMCX3*, *FECH*, and *MRPS33* (14). Some of these genes may link to the current results. For example, glucose and glucose 6-phosphate, the target and product of *HK1* in the glycolysis cycle, are among the metabolites altered in *Slc52a3*$^{-/-}$ neonatal mice (11, 15). Oxidative stress promotes Cav1 translocation to the mitochondria, and reduced Cav1 leads to degradation of multiple electron transport chain complexes, including complex I, which is particularly sensitive to knockdown of a drosophila *SLC52A3* homolog (8, 16). Additional experimental studies are required to confirm or refute this "mitochondrial" model of OSA pathogenesis, which may enable new therapeutic strategies.

The authors identified a number of other loci significantly and suggestively associated with OSA, OSA-related quantitative traits, and general sleep architecture traits. Less is currently known about the regions containing these remaining associations. One variant, rs2610711, is particularly interesting because of its association with multiple OSA-related quantitative traits, including AHI, AHI in non-rapid eye movement sleep, duration of oxygen saturation below 90%, and oxygen desaturation index. *RNGTT*, the variant's most proximal gene, is involved with mRNA processing. A strong association between rs117733138 (*BTBD9* region) and sleep maintenance efficiency was found, which in combination with prior associations with sleep duration, insomnia, and chronotype, suggests that this region may

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regulate a general sleep parameter in addition to its known link to restless legs syndrome.

In addition to notable strengths, this study is not without limitations. Of note, participants were mostly men, body mass index could be accounted for in only a subset of the cohort, no PSG was available for most of the control subjects, genomic imputation was performed using a low-coverage template on the basis of relatively few samples, and no independent validation was performed.

Nevertheless, this study provides important insights and a novel testable hypothesis that riboflavin supplementation could mitigate OSA severity. Questions remain about the specific tissue and mechanism of effect, which need to be addressed with independent validation, cell-specific targeting, and animal models of OSA. This should not preclude lookups in cohorts with data on OSA and riboflavin in hand. ■

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Brian E. Cade, Ph.D.
Division of Sleep and Circadian Disorders
Brigham and Women's Hospital
Boston, Massachusetts
and
Division of Sleep Medicine
Harvard Medical School
Boston, Massachusetts

Sina A. Gharib, M.D.
Division of Pulmonary, Critical Care, and Sleep Medicine
University of Washington
Seattle, Washington

ORCID IDs: 0000-0003-1424-0673 (B.E.C.); 0000-0002-2480-4367 (S.A.G.).

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