Cognitive Function in Prepubertal Children with Obstructive Sleep Apnea: A Modifying Role for NADPH Oxidase p22 Subunit Gene Polymorphisms?

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Abstract

Pediatric obstructive sleep apnea (OSA) may lead to neurocognitive dysfunction, but not in everyone affected. The frequencies of NADPH oxidase (NOX) polymorphisms in the p22phox subunit were similar between children with OSA and controls, except for rs6520785 and rs4673, the latter being significantly more frequent among the OSA children without deficits than with deficits ($p < 0.02$). Similarly, 8-hydroxydeoxyguanine urine levels and NOX activity were lower among children without cognitive deficits and particularly among those with the rs4673 polymorphism. Thus, polymorphisms within the NOX gene or its functional subunits may account for important components of the variance in cognitive function deficits associated with OSA in children. *Antioxid. Redox Signal.* 16, 171–177.

Introduction

IN CHILDREN, the cumulative evidence strongly suggests that obstructive sleep apnea (OSA) is associated with significant severity-dependent deficits in cognitive function (1). However, at any given level of apnea hypopnea index (AHI), not all children will develop cognitive deficits, suggesting that genetic and environmental factors may be involved. Indeed, differences in the circulating morning levels of high-sensitivity C reactive protein or insulin growth factor appear to be associated with altered cognitive susceptibility to OSA (2). Similarly, the presence of the apolipoprotein E4 allelic variant is associated with an increased probability for the presence of abnormal subtests when assessing neurocognitive function using well-standardized and readily used clinical psychological batteries (5).

Development of a rodent model of OSA allowed for not only confirmation of the presence of memory and learning deficits in the context of this disorder, but has also permitted delineation of several potential mechanisms underlying end-organ injury. Among these mechanisms, increased oxidative stress and activation of NADPH oxidase (NOX) have been shown to mediate neural cell loss in the context of intermittent hypoxia during sleep (7, 9). Further, pharmacological inhibition of NOX with apocynin or, alternatively, transgenic deletion of one the major functional NOX sub-

units was associated with markedly attenuated overall end-organ injury and specifically reduced neuronal apoptosis, when mice were subjected to intermittent hypoxia during sleep (7, 9).

Based on aforementioned considerations, we hypothesized that OSA in children may be associated with NOX gene polymorphisms, particularly regarding the presence or absence of a cognitive-deficit phenotype. In other words, the presence of NOX gene polymorphisms that are associated with reduced NOX activity will be more likely to be present among children with OSA who do not develop neurocognitive deficits. Conversely, children with OSA and with abnormal performances in cognitive function will exhibit increased NOX activity and elevated morning urinary concentrations of 8-hydroxydeoxyguanosine (8-OH-dG), a reliable marker for DNA oxidative stress.

Innovation

Although oxidative stress is involved, the mechanisms underlying the variance in cognitive phenotype in children with obstructive sleep apnea (OSA) remain unclear. The findings of this work show that a significant component of this variance in cognitive morbidity can be accounted for by polymorphisms in the p22 subunit of NADPH oxidase.

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TABLE 1. DEMOGRAPHIC AND POLYSOMNOGRAPHIC CHARACTERISTICS IN 244 CHILDREN WITH OBSTRUCTIVE SLEEP APNEA AND 370 CONTROL CHILDREN

	OSA (n=244)	Control (n=370)	p-Value
Age (years)	6.6±0.5	6.7±0.6	NS
Gender (F:M)	52:48	52:48	NS
African American (%)	30%	30%	NS
BMI (kg/m ²)	18.6±1.3	18.2±0.9	NS
Sleep latency (min)	17.2±8.4	23.7±9.8	NS
REM latency (min)	118.5±27.3	123.5±40.1	NS
TST (h)	8.4±0.7	8.4±0.6	NS
Sleep efficiency (%)	87.7±8.8	85.9±8.3	NS
Stage 1 (%)	10.1±5.4	6.9±6.1	<0.05
Stage 2 (%)	47.4±7.9	45.1±8.7	NS
Slow wave sleep (%)	20.3±7.7	25.1±9.2	<0.04
REM sleep (%)	17.8±7.7	24.6±9.9	<0.02
Spontaneous arousal index (h ⁻¹ TST)	5.3±4.3	8.9±5.8	<0.01
Respiratory arousal index (h ⁻¹ TST)	6.8±2.1	0.1±0.0	<0.0001
AHI (h ⁻¹ TST)	7.9±2.7	0.1±0.0	<0.0001
SpO ₂ nadir	80.6±3.7	95.2±0.9	<0.0001
% TST SpO ₂ <90%	2.1±1.4	0.0±0.0	<0.0001
% TST PETCO ₂ >50 mmHg	21.7±3.9	4.7±4.0	<0.0001

AHI, apnea hypopnea index; BMI, body mass index; h⁻¹ TST, per hour of total sleep time; NS, not significant; OSA, obstructive sleep apnea; REM, rapid eye movement.

NOX Polymorphisms

We recruited a total of 244 children with OSA and 370 age-, gender-, and ethnicity-matched controls. Their demographic characteristics were similar, and as expected, the OSA group exhibited the anticipated differences in respiratory and other sleep measures (Table 1). Of the 18 NOX p22phox single-nucleotide polymorphisms (SNPs) tested, no differences in the frequency of any of the SNPs emerged, except for the frequency

of rs4673 ($p<0.01$) and rs6520785 polymorphisms ($p<0.04$; Supplementary Table S1; Supplementary Data are available online at www.liebertonline.com/ars). Haplotype frequencies and pairwise linkage disequilibrium structures are shown in Figure 1 for both OSA and control children.

Among the children with OSA, we matched children for age, gender, ethnicity, body mass index (BMI), the severity of sleep apnea, and maternal education. This led to identification of 69 children with OSA who exhibited altered neurocognitive performances [NC (+); Table 2] and 47 matched children with OSA who did not have any evidence of neurocognitive deficits [NC (-); Table 2]. Significant differences in the frequency of the NOX allelic variants emerged for the rs4673 polymorphism between these 2 OSA subgroups, and the frequency of rs4673 polymorphism (also known as 242 C>T) was significantly less frequent among the children with cognitive deficits ($p<0.02$; Table 3). Thus, NOX allelic variance appears to segregate among cognitive phenotypes in the context of pediatric OSA.

NOX Activity

To further assess whether NOX activity is increased in the presence of OSA, NADPH oxidase basal and phorbol myristate acetate (PMA)-stimulated activities were examined and found to be elevated in children with OSA compared with children without OSA ($p<0.01$; Fig. 2). However, among the children with OSA, only those with decreased cognitive test performances exhibited increased NOX activity, whereas those with preserved cognitive function had similar NOX activities when compared with controls (Fig. 2). In a subset of 14 children with OSA who harbored the p22 phox rs4673 SNP, NOX activity was significantly lower than among the 22 in whom this allelic variant was absent (Fig. 2).

8-OH-dG Urine Levels

To further ascertain the presence of systemic oxidative stress in OSA, urinary 8-OH-dG levels were measured and emerged as significantly higher in children with OSA

FIG. 1. Pairwise linkage disequilibrium structure and 18 SNPs of the NADPH oxidase (NOX) gene in children with obstructive sleep apnea (OSA) (right panel) and controls (left panel). No significant differences in allelic frequency emerged among the two groups except for rs4673 and rs6520785 polymorphisms.

TABLE 2. DEMOGRAPHIC AND POLYSOMNOGRAPHIC CHARACTERISTICS IN 69 CHILDREN WITH OBSTRUCTIVE SLEEP APNEA AND COGNITIVE DEFICITS AND 47 CHILDREN WITH OBSTRUCTIVE SLEEP APNEA BUT NO COGNITIVE DEFICITS

	OSA (+) NC (n=69)	OSA (-) NC (n=47)	p-Value
Age (years)	6.6±0.5	6.7±0.6	NS
Gender (F:M)	33:36	23:24	NS
African American n (%)	21 (30%)	14 (30%)	NS
BMI (kg/m ²)	18.4±0.8	18.3±0.9	NS
Maternal education	65% college	65% college	NS
Sleep latency (min)	15.1±8.4	14.7±9.8	NS
REM latency (min)	124.5±27.3	120.5±40.1	NS
TST (h)	8.3±0.4	8.4±0.5	NS
Sleep efficiency (%)	86.7±6.8	86.3±7.8	NS
Stage 1 (%)	9.4±5.4	8.2±5.1	NS
Stage 2 (%)	46.4±7.2	47.1±7.7	NS
Slow wave sleep (%)	22.8±6.7	21.1±9.2	NS
REM sleep (%)	21.4±5.7	23.6±5.9	NS
Spontaneous arousal index (h ⁻¹ TST)	5.1±5.3	5.8±5.3	NS
Respiratory arousal index (h ⁻¹ TST)	6.8±2.3	6.1±2.8	NS
AHI (h ⁻¹ TST)	10.3±3.5	11.6±4.4	NS
SpO ₂ nadir	80.6±3.7	83.6±3.9	NS
% TST SpO ₂ <90%	3.1±1.2	2.6±1.0	NS
% TST PETCO ₂ >50 mmHg	21.7±4.9	20.8±5.7	NS

compared with controls (18.8±7.2 ng/mg [range: 5.9–53.7 ng/mg] Cr in OSA *vs.* 11.3±4.1 ng/mg [range: 5.0–21.9 ng/mg] Cr in controls; *p*<0.01). There was significant variability, however, in 8-OH-dG urinary concentrations, and only a weak, albeit significant, association with AHI emerged (*r*: 0.24; *p*<0.001).

Partitioning of OSA children revealed that 8-OH-dG levels were lower among children without cognitive deficits (12.6±4.8 ng/mg Cr) when compared with children with OSA of similar severity but who exhibited the presence of neurocognitive deficits (33.7±11.8 ng/mg Cr; *p*<0.01). Similarly, children with OSA harboring the rs4673 polymorphism in the p22 phox subunit of the NOX gene have lower urinary

8-OH-dG levels ((12.9±5.4 ng/mg Cr) compared with OSA children who did not carry this allelic variant (34.6±10.9 ng/mg Cr; *p*<0.01).

Children with OSA exhibit different frequencies in the distribution of some NOX SNPs compared with healthy children. Further, and as previously shown by several investigative groups, children with OSA are at increased risk for neurocognitive deficits, and such deficits may be present or absent at any level of disease severity. In addition, we report on the increased levels of 8-OH-dG urinary concentrations and NOX activity, both of which are clear indicators of increased oxidative stress in the context of pediatric OSA. More importantly, the dichotomous nature of cognitive outcomes in the context of OSA could be explained, at least in part, by the presence of significantly higher levels of oxidative stress among those affected children in whom cognitive deficits were identified. Exploration of NOX gene p22 phox subunit SNP frequency distribution showed that only 2 of the 18 SNPs tested differed in their frequency in pediatric OSA and further uncovered that the presence of one of these SNPs (*i.e.*, rs4673 or 242 C>T) not only was associated with reduced levels of NOX activity and 8-OH-dG urinary concentrations, but also accounted for at least part of the discrepant phenotypic expression in cognitive functioning in the context of pediatric OSA.

Before we discuss the potential significance of our findings, some technical and methodological remarks are needed. First, our cohort was recruited from the community and does not represent a referral population. As such, it will be definitely important to assess the reproducibility of our findings, not only in other populations, but also to specifically explore the distribution of NOX gene variants and activity levels among symptomatic children being referred for evaluation of habitual snoring. These future studies should elucidate whether specific components of the phenotypic expression of their underlying OSA, such as excessive daytime sleepiness, increased arterial blood pressure, and abnormal endothelial function, are associated with genomic variants in NOX gene or with systemic levels of oxidative stress. Second, we did not assess whether treatment of OSA would lead to reversal or improvements of the oxidative stress markers and, if so, whether these changes would correlate with the degree of cognitive improvements with treatment. Finally, the possibility exists that other

TABLE 3. ALLELE FREQUENCY FOR SINGLE-NUCLEOTIDE POLYMORPHISM rs4673 C>T IN THE p22PHOX SUBUNIT OF THE NADPH OXIDASE GENE AMONG 69 CHILDREN WITH OBSTRUCTIVE SLEEP APNEA AND COGNITIVE DEFICITS AND 47 MATCHED CHILDREN WITH OBSTRUCTIVE SLEEP APNEA BUT NO COGNITIVE DEFICITS

SNP name	SNP ID	Position	Allele/genotype	OSA (-) NC		OSA (+) NC	
				(n=47)		(n=69)	
				Number	Percentage	Number	Percentage
rs4673	C>T	CYBA 242	CC	6	13	22	32
			CT	28	59	24	35
			TT	13	28	23	33
			Allele C	40	42	68	49
			Allele T	54	58	70	51

p<0.02 after adjustment for linkage disequilibrium and multiple comparisons.
SNP, single-nucleotide polymorphism.

FIG. 2. NADPH oxidase basal activity in children with and without OSA (left upper panel), with and without cognitive deficits in the context of OSA (right upper panel), and among the latter, those with and without the p22 phox subunit single-nucleotide polymorphism rs4673 (242 C>T; lower panel).

pro-oxidant and antioxidant genes may contribute to the phenotypic variation in pediatric OSA, and this study examined only a single specific source of oxidant stress that could be activated by OSA. Such coordinated assessments will require more expansive cohort sizes and should be best conducted using multicenter approaches.

As mentioned earlier, OSA in children has now been rather conclusively associated with an increased risk for the presence of neurocognitive and behavioral deficits. However, the large variance in the phenotypic presentation of cognitive dysfunction in the context of sleep-disordered breathing in children suggested that multiple factors other than the severity of the disease *per se* could play a role. Some of these factors would be assumed as intrinsic or as genetically determined factors of susceptibility (5) and have been shown to include thus far inflammatory pathways, lipid membrane transport, and growth factors (5). Additional external factors that have to be yet confirmed in clinical pediatric cohorts would include lifestyle components, such as physical activity, dietary composition, intensity of intellectual activity, and overall literacy (3). The present work adds an additional intrinsic factor, namely NOX genomic variance in its regulatory subunit

p22phox, as an important modulator of cognitive vulnerability in the context of pediatric OSA.

NOX is an important gene that has been primarily studied in the context of the phagocyte oxidative burst, but has since been found to play a multitude of physiological and pathological roles (4, 8). NOX is also a major source of ROS generation in mammalian cells, including the CNS (8). NOX is composed of two membrane-bound subunits (gp91phox and p22phox) and three cytosolic subunits, which include p47phox, p67phox, and Rac, and form functional heterodimers (4, 8). Mutations in the gp91phox and p47phox genes are the most common mutations that cause chronic granulomatous disease, as they disable the NOX complex, thereby preventing production of superoxide. With the increasing understanding on the ubiquitous role of NOX, a large number of allelic variants have been identified for each of the NOX subunits, and some of these variants, particularly those in the p22phox subunit (e.g., 242 C>T or rs4673), have been shown to associate with specific functional implications in several disease states (6). Further, some of the polymorphisms tested in the present study exhibit reduced NOX activity (6), as further confirmed in our cohort and as

evidenced by direct assessment of NOX activity, as well as by indirect assessments of urinary 8-OH-dG levels. Based on present findings, we surmise that polymorphisms within the NOX gene or its functional subunits, such as CYBA 242 C>T (rs4673), may account for important components of the variance in cognitive function deficits associated with OSA in children. Therefore, these polymorphisms and potentially other functionally relevant polymorphisms in the NOX gene complex may operate as disease-modifying outcomes in the context of pediatric OSA.

Concluding Remarks and Future Directions

We present novel information linking the phenotypic heterogeneity of cognitive dysfunction of OSA in children to genetic heterogeneity in the p22 phox subunit of the NOX gene, whereby the presence of the 242 C>T or rs4673 SNP is not only associated with reduced NOX activity levels but also less likely to manifest cognitive deficits despite the presence of OSA. These findings shed further light on the role of oxidative stress in the clinical manifestations and morbidity of a highly prevalent disorder such as OSA. Multicenter studies examining the role of genomic variance in genes underlying oxidative stress pathways and their implications to the phenotypic morbidities associated with pediatric OSA appear warranted (see Supplementary Data for more extensive bibliography).

Notes

Subjects and Methods

The study was approved by the University of Louisville Human Research Committee (protocol No. 474.99), and informed consent was obtained from the legal caregiver of each participant. Consecutive children with a diagnosis of OSA according to polysomnographic criteria and aged between 5 and 10 years were invited to participate in the study. Assent was also obtained from children if they were >6 years of age.

In the first phase of the study, and as part of an ongoing population-based research project, which has been described in greater detail elsewhere (5–7), we prospectively identified 244 nonclinically referred children (aged 5.5–8 years) from the community who were otherwise healthy, who attended public elementary schools, and in whom a questionnaire identified habitual snoring (≥ 3 nights/week) along with polysomnographic evidence of OSA (for criteria, see later). These children underwent a blood draw and a battery of neurocognitive tests the morning after their sleep study.

In parallel, we proceeded to recruit consecutive nonsnoring healthy children ($n=370$) who were matched for age, gender, ethnicity, neighborhood of residence, and level of maternal education and also underwent overnight polysomnography (PSG) to confirm the absence of sleep-disordered breathing and a blood draw the morning after the sleep study. In addition, 30 healthy children were also recruited for a sleep study but did not undergo cognitive function assessments.

Overnight PSG. PSG were conducted and scored as previously reported (5). Central, obstructive, and mixed apneic events were counted. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for a duration of at least 2 breaths. Hy-

popneas were defined as a decrease in oronasal flow of $\geq 50\%$, with a corresponding decrease in SpO_2 of $\geq 4\%$ or more and/or an arousal. The obstructive AHI was defined as the number of obstructive apneas and hypopneas per hour of total sleep time (TST). Arousals were defined according to the American Academy of Sleep Medicine Scoring Manual. OSA was defined by the presence of an obstructive AHI $\geq 1 \text{ h}^{-1}$ TST. Control children were nonsnoring children who had AHI $<1 \text{ h}^{-1}$ TST. Blood was drawn in the morning after the child completed the polysomnographic evaluation and after an overnight fast.

Neurocognitive assessments. The cognitive tests administered the morning after polysomnographic assessment consisted of the Differential Ability Scales (DAS) (2) and the NeuroPsychological Assessment Battery (NEPSY). The DAS (2) is a battery of cognitive tests designed to measure reasoning and conceptual ability in children aged 2 through 17 years. This measure was designed to provide specific information about an individual's strengths and weaknesses across a wide range of intellectual activities. Children were administered either the preschool form or the school-age form of the DAS. The preschool form is divided into a Verbal Cluster (including two subtests) and a Nonverbal Cluster (including two spatial subtests and one nonverbal reasoning subtest) and yields a Global Composite score that is commensurate with an intelligence quotient. The school-age form yields a Spatial Cluster score in addition to the Verbal, Nonverbal, and Global Composite scores. The test was designed so that the Global Composite scores could be examined across forms and throughout the age range. Individual DAS subtests are designed to measure separate and distinct areas of cognitive functioning and thus have high specificity. The ability score for a subtest is expressed as a *T* score with a mean of 50 and a standard deviation of 10. The sum of the core subtest *T* scores is converted to a total standard score, the General Conceptual Ability score, with a mean of 100 and a standard deviation of 15. The NEPSY is a relatively new neurobehavioral test battery and was designed to assess neurobiological development in five functional domains. These include attention/executive functions, language, sensorimotor functions, visuospatial processing, and memory and learning, with a mean score of 100 and SD of 15. All these subtests have good-to-excellent reliability ($r=0.77\text{--}0.91$). As is customary in clinical practice, subjects were considered as being affected if they scored 1 standard deviation below the mean for at least three subtests on either DAS or NEPSY batteries.

Based on this approach, we identified 69 children with OSA and cognitive deficits (OSA+) and were able to identify 47 age-, gender-, ethnicity-, maternal education-, BMI-, and obstructive AHI-matched children who did not present with any cognitive deficit (OSA-). All these 116 children underwent a blood draw the morning after nocturnal polysomnogram under fasting conditions.

DNA isolation. Peripheral blood samples were collected in vacutainer tubes containing EDTA (Becton Dickinson). All DNA samples were extracted using QIAmp DNA blood kit (Qiagen) according to the manufacturer's protocol. The concentration and quality of the DNA were determined using an ND-1000 Spectrophotometer (Nanodrop Technologies). The purity of the DNA were determined by calculating the

ratio of absorbance at 260/280 nm, and all DNA samples had a ratio of 1.8–1.9. The precise length of genomic DNA was determined by gel electrophoresis using 1% agarose gel. All the purified samples were stored at -80°C until further analyses.

Genotyping using real-time PCR. Genotyping was performed using the ABI PRISM 7500 Sequence Detection System for allelic discrimination following the manufacturer's instructions (Applied Biosystems). All 18 polymorphisms were genotyped using TaqMan technology (Applied Biosystems, Inc.). The polymorphisms examined were in the p22 phox subunit, which is the regulatory subunit of NOX complex, and included rs4673 C>T, rs4782308 A>G, rs4595265 G>T, rs6520785 C>G, rs2254073 C>T, rs1049255 A>G, rs1049254 C>T, rs12709102 A>G, rs3794624 A>G, rs9932581 C>T, rs8854 A>G, rs3736112 A>G, rs7059081 C>G, rs4827298 C>T, rs12848910 A>G, rs13306300 A>G, rs34519538 A>G, and rs11547387 A>G.

Two fluorogenic minor groove binder probes were used for each locus using the dyes 6-carboxyfluorescein (excitation: 494 nm) and VIC (excitation: 538 nm), which are easily differentiated in the Applied Biosystems Prism 7500 PCR system. Real-time PCR was performed using 12.5 μl of TaqMan 2 $\times$ universal master mix (Applied Biosystems), 1.25 μl of SNP, 10.25 μl of RNase- and DNase-free water (Ambion), and 1 μl of sample DNA, in a total volume of 25 μl /well reaction. Two wells of a 96-well plate (Applied Biosystems) were used for each sample or SNP. DNase-free water used as nontemplate control was included in each assay run. Assay conditions were 2 min at 50°C , 10 min at 95°C , and 40 cycles of 95°C for 15 s and 60°C for 1 min. Initially, the SNP assay was set up using SDS, version 2.1, software (Applied Biosystems) as an absolute quantification assay, but after assay completion the plate was read using the allelic discrimination settings. Postassay analysis was performed using the SDS software.

NOX activity. Blood samples collected in heparinized tubes (10 IU/ml) were processed within 120 min from blood draw. White blood cells (WBCs) were isolated following lysis of erythrocytes, and 10,000 WBCs were used for NOX activity assays. NOX activity was assessed in WBCs by measuring NADPH-dependent superoxide production. Briefly, duplicate sets of 10,000 cells each from every subject were incubated in 200 μl of assay buffer containing acetylated cytochrome *c* (100 μM) in a 96-well plate at 30°C . NADPH (200 μM) was then added in the absence or presence of superoxide dismutase (SOD, 3 U/ μl) and the reduction of cytochrome *c* was monitored at 550 nm for 10 min. NOX activity was calculated as the SOD-inhibitable reduction of cytochrome *c*. Basal activity and responses to PMA at a concentration of 200 ng/ml were assessed. All chemicals were obtained from Sigma.

Urine 8-OH-dG concentrations. Urine samples were collected in all children and immediately centrifuged at 4°C at 2000 g for 15 min, and a 50 μl aliquot of the supernatant was used for the 8-OH-dG analysis with a commercial ELISA kit (8-OHdG Check; Japan Institute for the Control of Aging). The incubation with the primary antibody (N45.1) was performed

at 37°C , according to the manufacturer's instructions. The 8-OH-dG values from the samples were calculated based on calibration sigmoid plots of the absorbance (450 nm) of an 8-OH-dG standard at various concentrations. Urinary creatinine level was also measured for each sample, and 8-OH-dG concentrations were expressed as per mg creatinine.

Statistical analysis. All analyses were performed using Statistical Package for the Social Sciences software (SPSS), version 18.0 (SPSS, Inc.). Data are presented as means $\pm$ SD unless otherwise indicated. Comparisons of the distribution of demographic factors according to group membership were made with independent *t*-tests (continuous variables) with *p*-values adjusted for unequal variances when appropriate (Levene's test for equality of variances) or chi-square (χ^2) analyses with Fisher's exact test (dichotomous outcomes). For comparisons across groups with and without decreases in cognitive function, an estimate of effect size was conducted using Cohen's *d* values. All *p*-values reported are two tailed, with statistical significance set at $p < 0.05$. The genotypes containing minor (less common) allele (*i.e.*, heterozygous and homozygous minor allele) were grouped and compared with the genotypes consisting of the homozygous major (more common) allele (see Supplementary Data for bibliography pertinent to the Methods).

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References

1. Beebe DW. Neurobehavioral morbidity associated with disordered breathing during sleep in children: a comprehensive review. *Sleep* 29: 1115–1134, 2006.
2. Gozal D, Crabtree VM, Sans Capdevila O, Witcher LA, and Kheirandish-Gozal L. C-reactive protein, obstructive sleep apnea, and cognitive dysfunction in school-aged children. *Am J Respir Crit Care Med* 176: 188–193, 2007.
3. Gozal D, Nair D, and Goldbart AD. Physical activity attenuates intermittent hypoxia-induced spatial learning deficits and oxidative stress. *Am J Respir Crit Care Med* 182: 104–112, 2010.
4. Jiang F, Zhang Y, and Dusting GJ. NADPH oxidase-mediated redox signaling: roles in cellular stress response, stress tolerance, and tissue repair. *Pharmacol Rev* 63: 218–242, 2011.
5. Kheirandish L, and Gozal D. Neurocognitive dysfunction in children with sleep disorders. *Dev Sci* 9: 388–399, 2006.
6. Mehranpour P, Wang SS, Blanco RR, Li W, Song Q, Lassègue B, Dikalov SI, Austin H, and Zafari AM. The C242T CYBA polymorphism as a major determinant of NADPH oxidase activity in patients with cardiovascular disease. *Cardiovasc Hematol Agents Med Chem* 7: 251–259, 2009.
7. Nair D, Dayyat EA, Zhang SX, Wang Y, and Gozal D. Intermittent hypoxia-induced cognitive deficits are mediated by NADPH oxidase activity in a murine model of sleep apnea. *PLoS One* 6: e19847, 2011.
8. Sorce S and Krause KH. NOX enzymes in the central nervous system: from signaling to disease. *Antioxid Redox Signal* 11: 2481–2504, 2009.
9. Zhan G, Serrano F, Fenik P, Hsu R, Kong L, Pratico D, Klann E, and Veasey SC. NADPH oxidase mediates hypersomnolence and brain oxidative injury in a murine

model of sleep apnea. *Am J Respir Crit Care Med* 172: 921–929, 2005.

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Abbreviations Used

8-OH-dG = 8-hydroxydeoxyguanosine

AHI = apnea hypopnea index

BMI = body mass index

Cr = creatinine

DAS = Differential Ability Scales

h⁻¹ TST = per hour of total sleep time

NEPSY = NeuroPsychological Assessment Battery

NOX = NADPH oxidase

OSA = obstructive sleep apnea

PMA = phorbol myristate acetate

SNP = single-nucleotide polymorphism

WBCs = white blood cells