Published in final edited form as:

J Pediatr. 2011 January ; 158(1): 81–86. doi:10.1016/j.jpeds.2010.07.032.

TNF- α Gene Polymorphisms and Excessive Daytime Sleepiness In Pediatric Obstructive Sleep Apnea

Abdelnaby Khalyfa, PhD^{1,2}, Laura D. Serpero, PhD², Leila Kheirandish-Gozal, MD^{1,2},
Oscar Sans Capdevila, MD^{2,3}, and David Gozal, MD^{1,2}

¹Section of Pediatric Sleep Medicine, Department of Pediatrics, Pritzker School of Medicine, University of Chicago, Chicago, Illinois, USA

²Kosair Children's Hospital Research Institute and Division of Pediatric Sleep Medicine, Department of Pediatrics, University of Louisville, Louisville, KY, USA

³Pediatric Sleep Unit, Division of Neurophysiology, Department of Neurology, Hospital Sant Joan de Déu, Barcelona, Spain

Abstract

Objective—To assess sleepiness, TNF- α plasma levels and genomic variance in the TNF- α gene in children with obstructive sleep apnea (OSA).

Study design Children being evaluated for OSA (n=60) and matched controls (n=80) were assessed with a modified Epworth scale (ESS) questionnaire and underwent a blood draw the morning after NPSG. TNF-plasma concentrations were assayed using ELISA and genomic DNA was extracted. Genotyping and allelic frequencies were determined for 4 TNF- α single nucleotide polymorphisms using real time PCR genotyping assays.

Results—Morning TNF- α levels and ESS scores were increased in the presence of OSA but substantial variability was present. Although TNF- α plasma concentrations were globally increased in OSA, most of the variance was attributable to the presence or absence of TNF- α –308G gene polymorphism.

Conclusions—TNF- α levels are increased in a subset of children with OSA, particularly among those harboring the TNF- α –308G single nucleotide polymorphism. Among the latter, significant increases in excessive daytime sleepiness symptoms are also present. The relatively high variability of excessive daytime sleepiness in pediatric OSA may be related to underlying TNF- α gene polymorphisms, particularly –308G.

Obstructive sleep apnea (OSA) is a prevalent condition in children that has recently emerged as a major cause of neurocognitive and behavioral dysfunction, and cardiovascular and metabolic morbidity (1). In adults affected with OSA, excessive daytime sleepiness is extremely frequent, and constitutes one of the most important symptoms prompting medical referral for evaluation and treatment. However, excessive daytime sleepiness has not been

© 2010 Mosby, Inc. All rights reserved.

Corresponding author: David Gozal, M.D., Department of Pediatrics, Comer Children's Hospital, The University of Chicago, 5721 S. Maryland Avenue, MC 8000, Suite K-160, Chicago, IL 60637. Tel: (773) 834-1483; (773) 702-4523 – FAX; dgozal@uchicago.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare no conflicts of interest.

reported as frequently in children with OSA, possibly reflecting the subjective perceptions of caretakers because children are unlikely to verbalize vague symptoms (2). Recent studies using excessive daytime sleepiness-oriented questionnaires suggested that excessive daytime sleepiness is present among 20–50% of all children (3), and similar findings emerge from studies using the multiple sleep latency test as a more objective indicator of sleep propensity (4–6).

TNF- α is one of the most important cytokines involved in sleep regulation (7). TNF- α levels are elevated in adults patients with OSA, and circulating morning TNF- α levels were also increased in children with OSA and were strongly correlated with the degree of sleep fragmentation (8). However, substantial variability in TNF- α levels was apparent such that, at any given level of OSA severity, children with OSA had either elevated or normal morning plasma TNF- α levels, suggesting the possibility that genomic variations in the TNF- α gene may account for such disparate findings. Indeed, increased production of TNF- α levels both *in vitro* and *in vivo* has been reported to be associated with a functional TNF- α gene polymorphism, consisting of a guanine (G) to adenine (A) substitution at position –308 in the promoter region (9,10).

We therefore conducted the present case-control study to assess whether variances in TNF- α gene polymorphisms may account for the differences in TNF- α levels among children with OSA, and whether excessive daytime sleepiness symptoms would correlate with the TNF- α genotype and plasma levels of this inflammatory cytokine.

Methods

The study was approved by the University of Louisville Human Research Committee, and informed consent was obtained from the legal caretaker of each participant. Assent was also obtained from children if they were >6 years of age. Consecutive habitually snoring children with polysomnographic evidence of OSA (see below) were enrolled in the study. To enable better separation between OSA and control children, we excluded children with habitual snoring who did not fulfill the polysomnographic criteria of OSA. Additional exclusion criteria included the presence of genetic disorders, cerebral palsy, neuromuscular diseases, or any underlying systemic diseases or acute infectious processes. In addition, we included 80 control children who were age, sex, and ethnicity-matched who attended the same schools, had no history of snoring, and who also had normal polysomnographic findings (see below). Blood was drawn between 7:00 and 8:00 AM, the morning after the child underwent a standard polysomnographic evaluation in the sleep laboratory at the University of Louisville Pediatric Sleep Laboratory.

The Epworth Sleepiness Scale (ESS) is a commonly used tool used to assess the general level of daytime sleepiness in adults (11). It consists of an 8-item questionnaire on the likelihood of falling asleep during commonly encountered situations, and as such scores can range from 0 to 24, with an ESS score >10 suggested as indicative of increased daytime sleepiness. We employed a previously modified ESS tool for children in which the mention of alcohol was omitted and another question indicated that the subject was a passenger in the car (5).

TNF- α Plasma Level Assay

Blood was centrifuged within <5 min of collection in a cooled centrifuge and plasma was then immediately stored in multiple aliquots at –80°C until assay within <2 weeks from collection. Plasma or supernatant TNF- α levels were examined using commercial ELISA kits (R&D systems, Minneapolis, MN; cat # QTA00B). This method has a minimum detection level of 0.74 pg/ml with intra-assay and inter-assay coefficients of variability of

7.4% and 7.8% respectively, and a dynamic linear range between 2.2 and 7,500 pg/ml. To ensure consistency and to prevent protein degradation, particular care was taken to standardize all steps of plasma sample processing and to minimize thawing more than once for each aliquot.

DNA Extraction

Peripheral blood samples were collected in vacutainer tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ, USA). All DNA samples were extracted using QIAmp DNA blood kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. The concentration and quality of the DNA were determined using a ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). 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). Four TNF- α single nucleotide polymorphisms, namely rs1800629 (–308), rs361525 (–238), rs2228088 (256), and rs30993665 (948) were examined in this study (Applied Biosystems). TNF –308 (also known as TNF2), lies on the extended haplotype HLA-A1-B8-DR3-DQ2, and is associated with autoimmunity and high TNF alpha production (12). Table I shows the minor allele frequencies in the general population.

All polymorphisms were genotyped using TaqMan technology (Applied Biosystems, Inc.). Two fluorogenic minor groove binder probes were used for each locus using the dyes 6-carboxyfluorescein (FAM; excitation, 494 nm) and VIC (excitation, 538 nm) which are easily differentiated in PCR system. Real-time PCR reaction was performed using 12.5 μl of TaqMan 2 $\times$ universal master mix (Applied Biosystems, CA), 1.25 μl of SNP, 10.25 μl of RNase- and DNase-free water (Ambion, Austin, TX), and 1 μl of sample DNA, in a total volume of 25 μl per single well reaction. Two wells of a 96 well-plate (Applied Biosystems, CA) were used for each sample for each of the 4 single nucleotide polymorphisms. DNase-free water used as non-template 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, CA) as an absolute quantification assay, but after assay completion the plate was read using the allelic discrimination settings. Post-assay analysis was performed using the SDS software.

Overnight Polysomnography

A standard overnight multichannel polysomnographic evaluation was performed in the sleep laboratory (13). Briefly, chest and abdominal wall movement were monitored by respiratory impedance or inductance plethysmography, heart rate by ECG, air flow was assessed with a sidestream end-tidal capnograph which also provided breath-by-breath assessment of end-tidal carbon dioxide levels (PETCO₂; BCI SC-300, Menomonee Falls, WI), nasal pressure catheter, and an oronasal thermistor. Arterial oxygen saturation (SpO₂) was assessed by pulse oximetry (Nellcor N 100; Nellcor Inc., Hayward, CA), with simultaneous recording of the pulse waveform. The bilateral electro-oculogram (EOG), 8 channels of electroencephalogram (EEG), chin and anterior tibial electromyograms (EMG), and analog output from a body position sensor (Braebon Medical Corporation, NY) were also monitored. All measures were digitized using a commercially available polysomnography systems (Rembrandt, MedCare Diagnostics, Amsterdam, The Netherlands, or Stellate,

Montreal, Canada). Tracheal sound was monitored with a microphone sensor (Sleepmate, VA) and a digital time-synchronized video recording was performed.

Sleep architecture was assessed by standard techniques (14). The proportion of time spent in each sleep stage was expressed as percentage of total sleep time (%TST). 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 duration of at least two breaths (13). Hypopneas were defined as a decrease in oronasal flow of $\geq 50\%$ on either the thermistor or nasal pressure transducer signal with a corresponding decrease in SpO_2 of $\geq 3\%$ or arousal (13,15). The obstructive apnea/hypopnea index (AHI) was defined as the number of apnea and hypopneas per hour of TST. The diagnostic criteria for OSA in this study consisted of an obstructive AHI $\geq 2/\text{hrTST}$ in the presence of snoring during the night, and a nadir oxyhemoglobin saturation $< 92\%$. Control children were defined as non-snoring children with AHI $< 1/\text{hrTST}$. Arousals were defined according to the American Academy of Sleep Medicine Scoring Manual (15).

Body Mass Index

Height and weight were obtained from each child using standard techniques. BMI was then calculated ($\text{body mass}/\text{height}^2$). BMI Z scores for age and sex were determined from Centers of Disease Control and Prevention growth charts (National Center for Health & Statistics. CDC Growth Charts. US Department of Health & Human Services, 2000). Children with BMI z-scores exceeding 1.65 were classified as fulfilling the criteria for obesity.

Data Analysis

Data are presented as means $\pm$ SE unless otherwise indicated. All analyses were conducted using SPSS software (version 17.5; SPSS Inc., Chicago, Ill.). Comparisons of demographics and other measures were made with independent t-tests, non-parametric tests, or analysis of variance (ANOVA) followed by post-hoc Bonferroni corrections, with p values adjusted for unequal variances when appropriate (Levene test for equality of variances), or chi square (χ^2) analyses with Fisher Exact Test (dichotomous outcomes). In addition conditional logistic regression was conducted to assess the potential association of any given genotype (single nucleotide polymorphism) and condition (OSA, control) regarding TNF- α levels and excessive daytime sleepiness. All p -values reported are 2-tailed with statistical significance set at < 0.05 .

Results

A total of 60 children with OSA and 80 age-, sex-, ethnicity-, and BMI z score-matched controls were enrolled in the study out of 154 potential candidates. The 14 children who refused to participate (6 children with OSA and 8 controls) did not differ from the other participants, and refused to have a blood draw. Table II shows the major demographic and polysomnographic characteristics of the two groups, as well as the mean morning TNF- α levels and ESS scores for both groups. Table III illustrates the latter 2 measures for each of the groups depending on the presence or absence of the specific single nucleotide polymorphism for the TNF- α gene. Of note, there were no significant differences in age, sex, ethnic group distribution or BMI among the 4 sub-groups.

Globally, both TNF- α levels and ESS scores were higher in children with OSA compared with controls ($p < 0.02$; Table II). However, when groups were partitioned based on the presence or absence of the minor alleles concerning the four single nucleotide polymorphisms, significant differences emerged in TNF- α levels among OSA children who harbored the -308 minor allele, compared with those children without it. Indeed, TNF- α

levels were markedly higher in the presence of -308 ($p < 0.0003$), but only among children with OSA, such that those differences were not apparent in controls (Table II). For all other 3 TNF- α polymorphisms tested, no differences emerged in their corresponding morning TNF- α levels (Table II). Furthermore, ESS scores were markedly increased in children with the TNF- α -308 polymorphism (Table III), but were within the range measured in controls when this specific polymorphism was absent ($p < 0.0001$; Table III).

Discussion

The present study confirms our previous findings showing that moderate to severe OSA in children leads to significantly increased circulating morning TNF- α plasma concentrations (8). However, the present study further elucidates the specific contribution of the TNF- α $-308G$ gene polymorphism to the increases in plasma TNF- α levels when OSA is present, and links the susceptibility to the presence of symptoms of excessive daytime sleepiness to increased TNF- α levels and the TNF- α $-308G$ gene polymorphism. Taken together, we believe that morning plasma TNF- α measurements may provide a reliable marker for the presence of excessive daytime sleepiness among habitually snoring sleepy children, and that those children with the TNF- α $-308G$ gene polymorphism are more likely to exhibit excessive daytime sleepiness when OSA is present.

TNF- α , a member of TNF super-family, has a wide range of physiological effects including a central role in inflammation, and the gene is located on chromosome 6p21.31 within the major histocompatibility complex (MHC) (16). Several polymorphisms have recently been identified in the promoter region of the TNF- α gene that may be responsible for the variations in TNF- α in plasma. Wilson *et al.* (17) identified a biallelic G to A polymorphism located at position -308 in the TNF- α promoter. Several polymorphisms in the 5'-flanking region of TNF- α gene have also been connected to differences in TNF- α expression, with -308 and -238 polymorphisms being the most frequently examined (18). The TNF- α allele ($-308G$) has been shown to increase promoter activity and has been linked to several immune-mediated diseases (19,20). Therefore, the -308 polymorphism could potentially modify and modulate the cell-type and stimulus specific regulation of TNF- α synthesis at the transcriptional level (20). Several studies on the -308 G/A polymorphism showed that it not only affected gene expression, but also that the rare A allele resulted in higher TNF production *in vitro* (21,22). Similar studies involving the TNF- α -238 G/A polymorphism showed the presence of an effect on the putative regulatory sequence, and that the common G allele was associated with higher TNF- α production (23,24). Also, the TNF- α $-238A$ allele has been associated with significantly decreased transcriptional activity *in vitro* (25).

The present study reproduces previously reported findings of global elevations in morning plasma TNF- α levels in a large cohort of children with varying degrees of sleep-disordered breathing (2–6,8). Thus, OSA is clearly a disorder associated with systemic inflammation as illustrated by the increased plasma levels of CRP, IL-6, and many other inflammatory mediators that have been reported by several groups of investigators (26–34). We should also note that plasma TNF- α concentrations in pediatric OSA have yielded inconsistent findings (35–38), which may be due to methodological differences in sampling and processing (2–6,8).

Similar to adults, not all children with OSA displayed increased TNF- α concentrations. The theory that such variability in the TNF- α plasma level phenotype would be explained by the presence of specific polymorphisms in the TNF- α gene was corroborated and implicated the TNF- α $-308G$ gene polymorphism as the primary contributor to the variance in TNF- α and ESS scores in pediatric OSA. Similar findings implicating TNF- α $-308G$ in the variance of TNF- α concentrations in the context of obesity and adult and pediatric OSA have been

recently published (39–43), thereby lending further credence to the concept supporting a genotype-phenotype interaction in OSA-associated morbidity.

Based on these findings, it appears justified to further explore the role of additional cytokine gene polymorphisms in pediatric OSA and their link to specific end-organ injury such as cognitive deficits (29) or cardiovascular (44,45) or metabolic dysfunction (46).

Sleep apnea in children is associated with increased daytime sleepiness in a subset of children, whether the sleepiness is examined subjectively or objectively using the multiple sleep latency test (MSLT; 2–6,8). The present study further confirms these studies, and especially the conclusions of Melendres et al who used a modified ESS questionnaire, and reported substantial variability in ESS scores among children with OSA (5). Of note, the ESS values in OSA patients and controls were slightly lower in our cohort, and may reflect cultural and population-related differences, rather than imply that true differences in biological sleepiness are indeed present. In addition, although the validity of the ESS questionnaire has not been critically assessed, a correlation between ESS and mean sleep latency values derived from the MSLT has been reported in children (3).

In summary, pediatric OSA leads to increases in morning plasma TNF- α concentrations, that are particularly prominent among those children harboring the TNF- α –308G gene polymorphism. The morning levels of TNF- α paralleled ESS scores, such that highest ESS scores were clustered in the TNF- α –308G positive group. This group of children appears therefore to be at risk for increased end-organ morbidity in the context of OSA, and seems particularly prone to exhibit symptoms of daytime sleepiness. Morning TNF- α levels appear to be a reliable and accurate measure of symptoms of excessive sleepiness in children with OSA. Based on such conclusions, we posit that evidence of sleepiness may serve as an indicator of more diffuse end-organ injury as a consequence of OSA.

Thus, identification of those children at higher risk for developing morbid consequences from OSA could ultimately lead to different management regimens whereby the cut-off AHI for adenotonsillectomy in children at risk, i.e., those with TNF alpha –308 polymorphisms for example, may be lower than the one routinely advocated for children who appear to be at lesser risk for long-term complications from OSA.

Acknowledgments

Supported by National Institutes of Health (grant HL-65270 to D.G.).

References

1. Capdevila OS, Kheirandish-Gozal L, Dayyat E, Gozal D. Pediatric obstructive sleep apnea: complications, management, and long-term outcomes. *Proc Am Thorac Soc* 2008;5(2):274–282. [PubMed: 18250221]
2. Carroll JL, McColley SA, Marcus CL, Curtis S, Loughlin GM. Inability of clinical history to distinguish primary snoring from obstructive sleep apnea syndrome in children. *Chest* 1995;108:610–618. [PubMed: 7656605]
3. Chervin RD, Weatherly RA, Ruzicka DL, Burns JW, Giordani BJ, Dillon JE, Marcus CL, Garetz SL, Hoban TF, Guire KE. Subjective sleepiness and polysomnographic correlates in children scheduled for adenotonsillectomy vs other surgical care. *Sleep* 2006;29:495–503. [PubMed: 16676783]
4. Gozal D, Wang M, Pope DW Jr. Objective sleepiness measures in pediatric obstructive sleep apnea. *Pediatrics* 2001;108:693–697. [PubMed: 11533338]
5. Melendres MC, Lutz JM, Rubin ED, Marcus CL. Daytime sleepiness and hyperactivity in children with suspected sleep-disordered breathing. *Pediatrics* 2004;114:768–775. [PubMed: 15342852]

6. Gozal D, Kheirandish-Gozal L. Obesity and excessive daytime sleepiness in prepubertal children with obstructive sleep apnea. *Pediatrics* 2009;123:13–18. [PubMed: 19117855]
7. Krueger JM. The role of cytokines in sleep regulation. *Curr Pharm Des* 2008;14:3408–3416. [PubMed: 19075717]
8. Gozal D, Serpero LD, Kheirandish-Gozal I, Sans Capdevila O, Khalyfa A, Tauman R. Sleep measures and morning plasma TNF- α levels in children with sleep-disordered breathing. *Sleep* 2010;33:319–325. [PubMed: 20337189]
9. Louis E, Franchimont D, Piron A. Tumor necrosis factor (TNF) gene polymorphism influences TNF- α production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998;113:401–406. [PubMed: 9737669]
10. Kroeger KM, Steer JH, Joyce DA, et al. Effects of stimulus and cell type on the expression of the -308 tumor necrosis factor promoter polymorphism. *Cytokine* 2000;12:110–119. [PubMed: 10671295]
11. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991;14:540–545. [PubMed: 1798888]
12. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* 1997;94:3195–3199. [PubMed: 9096369]
13. Montgomery-Downs HE, O'Brien LM, Gulliver TE, Gozal D. Polysomnographic characteristics in normal preschool and early school-aged children. *Pediatrics* 2006;117(3):741–753. [PubMed: 16510654]
14. Rechtschaffen, A.; Kales, A. Brain Information Services/Brain Research Institute. Los Angeles: University of California; 1968. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects.
15. Iber C, Ancoli-Israel S, Chesson AL Jr, Quan SF. The AASM manual for the scoring of sleep and associated events. American Academy of Sleep Medicine. 2007
16. Tracey KJ, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol* 1993;9:317–343. [PubMed: 8280464]
17. Wilson AG, di Giovine FS, Blakemore AIF, Duff GW. Single base change in the human tumor necrosis factor alpha (TNF- α) gene detectable by *NcoI* restriction of PCR product. *Human Mol Genet* 1992;1:353–358. [PubMed: 1363876]
18. Hajeer AH, Hutchinson IV. Influence of TNF-alpha gene polymorphisms on TNFalpha production and disease. *Hum Immunol* 2001;62:1191–1199. [PubMed: 11704281]
19. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol* 1997;34:391–399. [PubMed: 9293772]
20. Abraham LJ, Kroeger KM. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J Leukoc Biol* 1999;66:562–566. [PubMed: 10534109]
21. Bouma G, Crusius JB, Oudkerk Pool M, Kolkman JJ, von Blomberg BM, Kostense PJ, Giphart MJ, Schreuder GM, Meuwissen SG, Peña AS. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 1996;43:456–463. [PubMed: 8668926]
22. Louis E, Franchimont D, Piron A, Gevaert Y, Schaaf-Lafontaine N, Roland S, Mahieu P, Malaise M, De Groote D, Louis R, Belaiche J. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998;113:401–406. [PubMed: 9737669]
23. Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. *Hepatology* 1997;26:143–146. [PubMed: 9214463]
24. Huizinga TW, Westendorp RG, Bollen EL, Keijsers V, Brinkman BM, Langermans JA, Breedveld FC, Verweij CL, van de Gaer L, Dams L, Crusius JB, García-Gonzalez A, van Oosten BW, Polman CH, Peña AS. TNF-alpha promoter polymorphisms, production and susceptibility to

- multiple sclerosis in different groups of patients. *J Neuroimmunol* 1997;72:149–153. [PubMed: 9042107]
25. Kaluza W, Reuss E, Grossmann S, Hug R, Schopf RE, Galle PR, Maerker-Hermann E, Hoehler T. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. *J Invest Dermatol* 2000;114:1180–1183. [PubMed: 10844563]
 26. Tauman R, Ivanenko A, O'Brien LM, Gozal D. Plasma C-reactive protein in children with sleep-disordered breathing. *Pediatrics* 2004;113:e564–e569. [PubMed: 15173538]
 27. Kheirandish-Gozal L, Sans Capdevila O, Tauman R, Gozal D. Plasma C-reactive protein in non-obese children with obstructive sleep apnea before and after adenotonsillectomy. *J. Clin. Sleep Med* 2006;2:301–304. [PubMed: 17410279]
 28. Tauman R, O'Brien LM, Gozal D. Hypoxemia and obesity modulate plasma C-reactive protein and interleukin 6 levels in snoring children. *Sleep & Breathing* 2007;11:77–84. [PubMed: 17171553]
 29. Gozal D, McLaughlin Crabtree V, Sans Capdevila O, Witcher LA, Kheirandish-Gozal L. C reactive protein, obstructive sleep apnea, and cognitive dysfunction in school-aged children. *Am J Respir Crit Care Med* 2007;176:188–193. [PubMed: 17400731]
 30. Gozal D, Serpero LD, Sans Capdevila O, Kheirandish-Gozal L. Systemic inflammation in non-obese children with obstructive sleep apnea. *Sleep Med* 2008;9:254–259. [PubMed: 17825619]
 31. Khalyfa A, Sans Capdevila O, Boazza M, Serpero LD, Kheirandish-Gozal L, Gozal D. Genome-wide gene expression profiling in children with obstructive sleep apnea. *Sleep Med* 2009;10:75–86. [PubMed: 18261956]
 32. Goldbart AD, Tal A. Inflammation and sleep disordered breathing in children: a state-of-the-art review. *Pediatr Pulmonol* 2008;43:1151–1160. [PubMed: 19009600]
 33. Larkin EK, Rosen CL, Kirchner HL, Storfer-Isser A, Emancipator JL, Johnson NL, Zambito AM, Tracy RP, Jenny NS, Redline S. Variation of C-reactive protein levels in adolescents: association with sleep-disordered breathing and sleep duration. *Circulation* 2005;111:1978–1984. [PubMed: 15837952]
 34. Li AM, Chan MH, Yin J, So HK, Ng SK, Chan IH, Lam CW, Wing YK, Ng PC. C-reactive protein in children with obstructive sleep apnea and the effects of treatment. *Pediatr Pulmonol* 2008;43:34–40. [PubMed: 18041751]
 35. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* 1997;82:1313–1316. [PubMed: 9141509]
 36. Waters KA, Mast BT, Vella S, de la Eva R, O'Brien LM, Bailey S, Tam CS, Wong M, Baur LA. Structural equation modeling of sleep apnea, inflammation, and metabolic dysfunction in children. *J Sleep Res* 2007;16:388–395. [PubMed: 18036084]
 37. Li AM, Lam HS, Chan MH, So HK, Ng SK, Chan IH, Lam CW, Wing YK. Inflammatory cytokines and childhood obstructive sleep apnoea. *Ann Acad Med Singapore* 2008;37:649–654. [PubMed: 18797557]
 38. Tsaoussoglou M, Bixler EO, Calhoun S, Chrousos GP, Sauder K, Vgontzas AN. Sleep-disordered breathing in obese children is associated with prevalent excessive daytime sleepiness, inflammation, and metabolic abnormalities. *J Clin Endocrinol Metab* 2010;95:143–150. [PubMed: 19926716]
 39. Riha RL, Brander P, Vennelle M, McArdle N, Kerr SM, Anderson NH, Douglas NJ. Tumour necrosis factor-alpha (-308) gene polymorphism in obstructive sleep apnoeahypopnoea syndrome. *Eur Respir J* 2005;26:673–678. [PubMed: 16204600]
 40. Popko K, Gorska E, Potapinska O, Wasik M, Stoklosa A, Plywaczewski R, Winiarska M, Gorecka D, Sliwinski P, Popko M, Szwed T, Demkow U. Frequency of distribution of inflammatory cytokines IL-1, IL-6 and TNF-alpha gene polymorphism in patients with obstructive sleep apnea. *J Physiol Pharmacol* 2008;59 Suppl 6:607–614. [PubMed: 19218687]
 41. Bhushan B, Guleria R, Misra A, Luthra K, Vikram NK. TNF-alpha gene polymorphism and TNF-alpha levels in obese Asian Indians with obstructive sleep apnea. *Respir Med* 2009;103:386–392. [PubMed: 19022640]

42. Kanbay A, Kokturk O, Ciftci TU, Tavit Y, Bukan N. Comparison of serum adiponectin and tumor necrosis factor- α levels between patients with and without obstructive sleep apnea syndrome. *Respiration* 2008;76:324–330. [PubMed: 18487876]
43. Aouizerat B, Dodd M, Lee K, West C, Paul S, Cooper B, Wara W, Swift P, Dunn L, Miaskowski C. Preliminary evidence of a genetic association between tumor necrosis factor α and the severity of sleep disturbance and morning fatigue. *Biol Res Nurs* 2009;11:27–41. [PubMed: 19419979]
44. Gozal D, Kheirandish-Gozal L, Serpero LD, Sans Capdevila O, Dayyat E. Obstructive sleep apnea and endothelial function in school-aged nonobese children: effect of adenotonsillectomy. *Circulation* 2007;116:2307–2314. [PubMed: 17967978]
45. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology; American Heart Association Stroke Council; American Heart Association Council on Cardiovascular Nursing; American College of Cardiology Foundation. Sleep apnea and cardiovascular disease: an American Heart Association/American College Of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). *Circulation* 2008;118:1080–1111. [PubMed: 18725495]
46. Gozal D, Capdevila OS, Kheirandish-Gozal L. Metabolic alterations and systemic inflammation in obstructive sleep apnea among nonobese and obese prepubertal children. *Am J Respir Crit Care Med* 2008;177:1142–1149. [PubMed: 18276939]

Table 1

TNF-alpha polymorphisms minor allele frequency.

	SNPs	Positions	Alleles	Minor Allele	Frequency
1	rs2228088	256	G/T	T	5% (AA)
2	rs30993665	948	A/C	C	3% (C), 9% (AA)
3	rs1800629	-308	A/G	A	14% (Chinese)
4	rs361525	-238	A/G	A	5% (C), 7% (AA)

AA: African American
C: Caucasian

Table 2

Demographic, polysomnographic, modified Epworth scores and TNF- α levels in 60 children with OSA and 80 matched controls.

		OSA (n=60)	Control (n=80)	P value
Age (years)		7.2 $\pm$ 0.2	7.2 $\pm$ 0.3	NS
Sex (% males)		50	50	NS
African American (%)		21.6	21.2	NS
BMI z score (%obese)		1.23 $\pm$ 0.10 (36.6)	1.16 $\pm$ 0.09 (25.6)	NS
TST (min)		463 $\pm$ 46	476 $\pm$ 45	NS
Sleep Efficiency (%)		87 $\pm$ 5	86 $\pm$ 7	NS
NREM Sleep (%TST)	Stage 1	7.5 $\pm$ 6.6	5.9 $\pm$ 3.7	<0.05
	Stage 2	46.1 $\pm$ 8.2	43.1 $\pm$ 7.0	NS
	Stage 3+4	26.9 $\pm$ 6.3	29.4 $\pm$ 7.4	NS
REM Sleep (%TST)		19.5 $\pm$ 3.9	21.6 $\pm$ 4.2	<0.05
OAHI (/hrTST)		8.9 $\pm$ 2.7 (range: 4.2–34.9)	0.5 $\pm$ 0.2 (range: 0.1–0.87)	<0.0001
SpO2 Nadir (%)		85.3 $\pm$ 4.2	94.6 $\pm$ 1.8	<0.0001
RAI (/hrTST)		4.3 $\pm$ 1.4	0.1 $\pm$ 0.1	<0.0001
% PETCO2 > 55 mmHg		19.4 $\pm$ 3.4	2.6 $\pm$ 1.7	<0.0001
TNF-α (pg/ml)		459.8 $\pm$ 63.2	295.6 $\pm$ 37.0	<0.02
ESS		6.5 $\pm$ 1.2	4.05 $\pm$ 0.5	<0.02

BMI z– body mass index, Z score; NS – not significant; TST – total sleep time; NREM – non rapid eye movement; REM – rapid eye movement; OAHI – obstructive apnea hypopnea index; SpO2 – oxygen saturation measured by pulse oxymetry; RAI – respiratory arousal index; PETCO2 – end tidal carbon dioxide tension; ESS – Epworth sleepiness score

Table 3

TNF- α Gene Polymorphisms and TNF- α plasma levels (in pg/ml) and ESS scores in 60 children with OSA and 80 matched controls.

	CONTROLS (N=80)		OSA (N=60)		P value	
	(+) (n)	(-) (n)	(+) (n)	(-) (n)	(+) vs (-) in CONTROLS	(+) vs (-) in OSA
SNP -308						
	369.6 $\pm$ 87.5 (N=19)	272.5 $\pm$ 40.1 (N=61)	893.1 $\pm$ 34.8 (N=15)	315.4 $\pm$ 17.8 (N=45)	NS	<0.00003
ESS	4.2 $\pm$ 0.6	4.0 $\pm$ 0.7	12.3 $\pm$ 1.3	4.6 $\pm$ 0.7	NS	<0.0001
SNP -238						
	351.9 $\pm$ 142.3 (n=6)	291.0 $\pm$ 38.6 (N=74)	561.5 $\pm$ 170.8 (N=11)	437.0 $\pm$ 67.8 (n=49)	NS	NS
SNP -256						
	260.6 $\pm$ 77.0 (n=15)	303.7 $\pm$ 42.1 (n=65)	478.3 $\pm$ 112.8 (n=20)	450.6 $\pm$ 77.2 (n=40)	NS	NS
SNP -948						
	n/a (n=2)	298.5 $\pm$ 37.8 (n=78)	n/a (N=3)	476.6 $\pm$ 65.8 (n=57)		

SNP – single nucleotide polymorphism; for all SNP: (+) indicates the presence of the minor allele for each polymorphism; TNF- α plasma levels are in pg/ml