Inflammatory cytokines in pediatric obstructive sleep apnea

Yu-Shu Huang, MD\textsuperscript{a}, Christian Guilleminault, DM, MD, DBiol\textsuperscript{b},\textsuperscript{c}, Fang-Ming Hwang, PhD\textsuperscript{d}, Chuan Cheng, MD\textsuperscript{a}, Cheng-Hui Lin, MD\textsuperscript{d}, Hsueh-Yu Li, MD\textsuperscript{d}, Li-Ang Lee, MD\textsuperscript{d}

Abstract
Pediatric obstructive sleep apnea (OSA) is associated with chronic systemic inflammation and with cognitive impairments. This study aimed to investigate the status of proinflammatory cytokines, particularly interleukin 17 (IL-17) and interleukin 23 (IL-23) and cognition in pediatric OSA.

Controls and OSA children participated in the study. Exclusion criteria were adenotonsillectomy, heart, neurological and severe psychiatric diseases, craniofacial syndromes, and obesity. Polysomnogram was followed by serum testing for inflammatory markers and neurocognitive tests such as continuous performance task (CPT) and Wisconsin card sorting test, questionnaires, analyses of plasma high-sensitivity C-reactive protein (HS-CRP), tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6), IL-17, and IL-23.

Seventy-nine, 4 to 12-year-old subjects in 2 groups ended the study: 47 nonobese OSA children (mean age = 7.84 ± 0.56 years, body mass index [BMI] = 16.95 ± 0.47 kg/m\textsuperscript{2}, BMI z-score = 0.15 ± 0.21, and mean apnea–hypopnea index [AHI] = 9.13 ± 1.67 events/h) and 32 healthy control children (mean age = 7.02 ± 0.65 years, with BMI = 16.55 ± 0.58 kg/m\textsuperscript{2}, BMI z-score = −0.12 ± 0.27, and mean AHI = 0.41 ± 0.07 event/h) were enrolled. Serum analyses showed significantly higher levels of HS-CRP, IL-17, and IL-23 in OSA children (P = 0.002, P = 0.024, and P = 0.047). Regression test showed significant influence of HS-CRP, TNF-α, IL-6, IL-17, and specifically IL-23, with the continuous performance test and Wisconsin card sorting test.

OSA children have abnormal levels of IL-17, an interleukin related to T helper 17 cells, a T helper cell involved in development of pediatric OSA; we also found a significant influence of inflammatory cytokines, particularly IL-23, on abnormal neurocognitive testing.

Abbreviations: ADHD = attention deficit–hyperactivity disorder, AHI = apnea–hypopnea index, BMI = body mass index, CPT = continuous performance task, EEG = electroencephalogram, EMG = electromyogram, Hit RT ISI = change hit reaction time interstimulus interval change, Hit SE ISI = change hit standard error interstimulus interval change, Hit-RF = hit reaction time, HS-CRP = high-sensitivity C-reactive protein, IL-1 = interleukin 1, IL-10 = interleukin 10, IL-17 = interleukin 17, IL-23 = interleukin 23, IL-6 = interleukin 6, K-CPT = Conners Kiddie continuous performance test, OSA = obstructive sleep apnea, PSQI = polysomnogram, RDI = respiratory disturbance index, SaO\textsubscript{2} = oxygen saturation, TH17 = T helper 17 cells, TNF-α = tumor necrosis factor alpha, Treg = T-regulatory, WCST = Wisconsin card sorting test, WPPSI-R = Wechsler R intelligence test.

Keywords: inflammatory cytokines, interleukin-17, interleukin-23, neurocognitive functions, pediatric obstructive sleep apnea

1. Introduction
Obstructive sleep apnea (OSA) syndrome affects the sleep and neurocognitive functioning of children, including symptoms of attention deficit–hyperactivity disorder (ADHD).[1–4] Pediatric OSA results in long-term effects on children’s health and development.[5–7] The factors involved in the decrease in cognition, learning, and memory are still incompletely chartered.

1.1. Pediatric OSA and inflammatory cytokines
There is an interaction between OSA and chronic diseases.[8–10] The most acceptable hypothesis associates occurrence of chronic systemic inflammation with OSA.[11–12] Increase in proinflammatory cytokines (C-reactive protein [CRP], tumor necrosis factor alpha [TNF-α], interleukin 6 [IL-6], and interleukin 10 [IL-10]) in adult OSA patients, and high-sensitivity C-reactive protein (HS-CRP) in pediatric OSA patients) supports this hypothesis,[13–14] with a possible association between the apnea–hypopnea index (AHI) and inflammatory cytokine levels. The inflammatory responses may be reversed after OSA treatment.[15–18] In the recent past, advances in our understanding of the precursors of some of the measured cytokines have occurred. Also very recently, the discovery of functional lymphatic vessels lining the dural sinuses and expressing the molecular hallmarks of lymphatic endothelial cells and carrying
fluid and immune cells from the cerebrospinal fluid with connection to the cervical lymphatic nodes has been reported.\(^{139}\)

The proinflammatory cytokines, interleukin 17 (IL-17) and interleukin 23 (IL-23), have been recently emphasized. IL-17 is a proinflammatory cytokine secreted predominantly by T helper 17 cells (TH17) and various cells including innate immune cells and nonimmune cells.\(^{139}\) It is referred to as IL-17A as it is a member of the IL-17 family.\(^{23}\) The IL-17-producing cells secrete IL-17A and another family member, IL-17F, under the stimulation of cytokines such as IL-1, IL-6, and IL-23 secreted by antigen-presenting cells in response to antigen stimulation.\(^{20,21}\) The interaction is as follows: IL-17A and IL-17F form homodimers or heterodimers that bind to the IL-17 receptor complex on inflammation-related cells such as macrophages, epithelial cells, and endothelial cells.\(^{22,23}\) The activated inflammatory cells produce various cytokines including IL-1, IL-6, and TNF-α. The stimulation of these cytokines and inflammatory cells leads to inflammatory responses such as neutrophil recruitment, tissue destruction, and neovascularization. The overreacted immune responses resulted in autoimmune diseases and allergy. During inflammation, expression of IL-17 and IL-17F is upregulated,\(^{22,23}\) with expression of high levels of IL-17 in patients with severe allergy, chronic inflammatory diseases, and autoimmune diseases.\(^{23}\) IL-17 also plays a role in neutrophilic inflammation in the respiratory system,\(^{24}\) and leads to chronic inflammation of the airway;\(^{25}\) for example, there is a high expression level of IL-17F in asthma.\(^{26}\) IL-17 has been linked to adult OSA: there is an upregulated Th17/T-regulatory (Treg) cell ratio and an overexpression of IL-6 and IL-17 in plasma cytokine suggesting that the imbalance of Th17/Treg and the microenvironment created by oversecreted proinflammatory cytokines contribute to the development of OSA.\(^{27}\) In OSA children, cytokine profile obtained from tonsils shows high levels of IL-1β, IL-10, and IL-17A production, indicating a T-cell activation in response to inflammation.\(^{28}\)

IL-23 is a cytokine with immunomodulatory effects.\(^{29}\) It acts on memory-cluster- designation-4 (+) T cells, activates the transcription activator, and stimulates the production of interferon-gamma.\(^{30,31}\) Studies showed that TH17 cells can be regulated by IL-23.\(^{32}\)

Factors leading to cognitive changes in children with OSA are still subject of research. Sleep fragmentation, hypoxemia, hypercapnia, and change in cerebral blood flow might be involved. Inflammatory cytokines may also play a role. We investigated interleukins 17 and 23 and cognition changes in children; we hypothesized that chronic inflammation not only causes cardiovascular diseases in pediatric OSA patient, but also affects cognitive functions and wondered if a correlation between psychometric test and these cytokines could be shown.\(^{13}\) A study found a relationship between abnormal level of C-reactive protein and cognitive dysfunction in school-aged children but investigation of interleukins 17 and 23 will give a much more important view on the inflammatory status present in children with OSA and potential correlations with specific cognitive testing.

We prospectively examined whether the plasma levels of the inflammatory cytokines are altered in children with pediatric OSA related to enlarged T&A and we simultaneously surveyed the changes of neurocognitive tests: we investigated the potential relationship between increase in inflammatory cytokines and neurocognitive functions investigated by psychometric tests,\(^{13}\) correlating the level of CRP, TNF-α, IL-1, IL-2, IL-6, IL-10, IL-17, and IL-23 with polysomnogram (PSG) results and neurocognitive test findings.

### 2. Methods

#### 2.1. Inclusion/exclusion

Children aged 4 to 12 years and their parents were prospectively approached. They were either presenting with complaints and symptoms of pediatric OSA (as defined in the International Classification of Sleep Disorders-2-2005) or had no sleep-related or other symptoms (controls). Subjects were investigated at Chang Gung Memorial University Hospital (CGMH) after approval of the protocol by the institutional review board of CGMH (no. 103-0601C). All caregivers (parents) signed an informed consent. Two groups of participants were collected—group A: normal control (n = 32) and group B (n = 47) pediatric OSA with sleep disturbances.

Obesity, previous adenotonsillectomy craniofacial anomalies, neuromuscular diseases, and other neurological and psychiatric disorders, presence of chronic medical problems, and intelligence quotient (IQ) < 70 defined as mental retardation were the exclusion criteria. In addition, children unable to cooperate with blood withdrawal collection and PSG procedures were eliminated from the study. Obesity was defined based on Taiwan general public health tables.

Inclusion criteria were either present of signs and symptoms evoking OSA for at least 3 months with confirmation by polysomnography-PSG-findings (AHI greater than 1 event/h or respiratory disturbance index (RDI) more than 5 events/h) (OSA group) or absence of complaint, AHI < 1, and presence of a noninflammatory status (absence of asthma, allergies, eczema, or other atopic/autoimmune diseases: normal control).

#### 2.2. Procedures

1. All subjects underwent routine medical history and physical examination by otolaryngologist, craniofacial surgeon, pediatrician, and child psychiatrist assessing comorbidities.
2. Demographic data (age, sex, height, and weight) and all systemic comorbidities were collected on a standardized data sheet.
3. Tonsillar size was graded by specialists following standardized scale from 0 to +4. Adenoid tissue was examined with a lateral x-ray film of the neck and flexible endoscope with amount of obstruction categorized into 4 grades (from grade 0 = 0%–25% to grade 3 = 75%–100%). Allergic rhinitis was confirmed by a specific IgE blood test (ImmunoCAP 100; Phadia, Upsala, Sweden), and duration and persistence of symptoms and comorbidities according to the Allergic Rhinitis and its Impact on Asthma classification.
4. Polysomnography (PSG): The following variables were monitored: electroencephalogram (EEG) (4 leads); eye movement, chin, and leg electromyogram (EMG); electrocardiogram (1 lead); and body position. The respiration was recorded with nasal pressure transducer, mouth thermocouple, chest and abdominal inductive plethysmography bands, neck microphone, diaphragmatic-intercostal muscle EMGs, and pulse oximetry from which both oxygen saturation (SaO₂) and finger plethysmography were derived, data were collected on a 32-channel recording system, (Embla N7000-Covidien, Kanata, Ontario, Canada), with continuous video monitoring. A family member was present during the nocturnal recording. Sleep and wake were scored using international criteria\(^{13}\) with identification of stages 3 and 4. EEG arousal was defined according to the American Sleep Disorders Association.\(^{14}\) Abnormal breathing events during
sleep were analyzed using the definitions of apnea and hypopnea as outlined by the American Academy of Sleep Medicine,[35] and the definition of flow limitation with abnormally increase in respiratory effort leading to arousals as outlined by Guilleminault et al.[36] The AHI and the RDI (number of apneas, hypopneas, and respiratory effort–related arousals per hour of sleep) were calculated. PSG scoring was performed by a technician blinded to the clinical status of the child.

(5) Inflammatory cytokine assessment: Blood samples were collected and allowed to clot for 30 minutes. The samples were then centrifuged, and the serum was frozen at −70°C until assay. All samples were collected the morning after PSG. The serum levels of HS-CRP, TNF-α, IL-1β, IL-6, IL-10, IL-17, and IL-23 were determined by commercially available ultrasensitive enzyme-linked immunosorbent assay kits (R&D systems, Minneapolis, MN) (Table 1). There were duplications of each sample, and the mean was used as the unit of analysis for statistical evaluation of data. The Stem-and-leaf analysis (SPSS, Inc., Chicago, IL) was employed in order to test for extreme outlying cytokine result.

2.3. Questionnaire evaluations and neurocognitive tests

Following PSG, 4 subjective questionnaires were filled out by caregivers to evaluating sleep quality and quality of life of children including the obstructive sleep disorder questionnaire, children’s sleep habits questionnaire, and child behavior checklist.

Evaluation of neurocognitive function was carried out using the Wechsler-R intelligence (WPPSI-R) intelligence test for 3- to 6-year-old children, WPPSI-R for 6- to 16-year-old children to assess IQ score, the Conners 6-year-old children, WPPSI-R for 6- to 16-year-old children to evaluate sleep quality and quality of life of caregivers to evaluating sleep quality and quality of life of children.[35] Following PSG, 4 subjective questionnaires were used to compare the findings in the OSA and control group. Student t tests were used to compare the findings in the OSA and control group. Taking into consideration the size of our group which limits usage of specific statistics such as “a mixed effects model”, we used the “standardized regression test” much better suited which was performed to demonstrate the relationship of cytokines levels with PSG and neurocognitive outcomes after controlling the factors of “asthma, allergy, body mass index (BMI), gender, tonsil hypertrophy, adenoid hypertrophy, turbinate hypertrophy, and nasosseal deviation”. All the reported P values are 2-tailed with statistical significance set at <0.05. Statistics were performed with SPSS version 18.

3. Results

Eighty-two children, 3 to 12 years old, were enrolled; there were 3 dropouts (3.6%). Demographics of the 78 children (mean age 7.4 ± 0.6 year) are in Table 2. The OSA group was significantly different with symptoms of ADHD and enuresis (P<0.001 and 0.036), presence of tonsil and adenoid hypertrophy (P<0.001), BMI, and BMI z-score (P=0.001 and 0.036). The PSGs showed

### Table 1

| ELISA kits | Minimum detection dose for assay |
|------------|----------------------------------|
| R&D systems, Minneapolis, MN | |
| L-1β (DLB80) | Less than 1 pg/mL |
| L-6 (HS600B) | 0.447–9.06 pg/mL |
| L-10 (D1000B) | Less than 3.9 pg/mL |
| L-17 (D1700) | Less than 15 pg/mL |
| L-23 (D2300B) | 2.7–16.3 pg/mL |

ELISA = enzyme-linked immunosorbent assay.

### Table 2

Demographic characteristics of OSA and healthy children.

| Control (n = 32) | OSA (n = 47) | P |
|-----------------|-------------|---|
| Number of males, % | 21 (65.6%) | 30 (63.8%) | 0.428 |
| Age, y | 7.02 ± 0.65 | 7.84 ± 0.56 | 0.366 |
| BMI, kg/m² | 16.55 ± 0.58 | 16.05 ± 0.47 | 0.601 |
| BMI z-score | 0.12 ± 0.27 | 0.15 ± 0.21 | 0.442 |
| AH1 events/h | 0.37 ± 0.06 | 9.13 ± 1.67 | <0.001† |
| PLMI events/h | 0.13 ± 0.10 | 0.93 ± 0.41 | 0.067‡ |
| PLM disorder, % | 0 (0%) | 3 (6.4%) | 0.083‡ |
| Learning disorder, % | 0 (0%) | 1 (2.1%) | 0.461 |
| ADHD, % | 2 (6.2%) | 18 (38.3%) | 0.001‡ |
| Enuresis, % | 4 (12.5%) | 15 (31.9%) | 0.036‡ |
| Other physical comorbidity history | 4 (12.5%) | 6 (12.8%) | 0.561 |
| Asthma, % | 12 (37.5%) | 8 (17.0%) | <0.001‡ |
| Allergic rhinitis, % | 12 (37.5%) | 8 (17.0%) | <0.001‡ |
| Findings of ENT examination | 4 (12.5%) | 32 (68.1%) | <0.001‡ |
| Tonsil hypertrophy (more than Gr. 2), % | 4 (12.5%) | 32 (68.1%) | <0.001‡ |
| Adenoid hypertrophy, % | 3 (9.3%) | 24 (51.1%) | <0.001‡ |
| Turbinate hypertrophy, % | 1 (3.1%) | 6 (12.8%) | 0.158 |
| Nasosseal deviation, % | 0 (0%) | 1 (2.1%) | 0.461 |

† Corrected BMI z-score based on the center for disease control growth charts.
‡ Diagnosed according to the criteria of diagnostic and statistical manual of mental disorders. Fourth edition-text revision.
§ Diagnosed by pediatrics.
¶ P<0.001.
# P<0.05.
$ P<0.01.$
$ P<0.001.$

WCST, and higher score indicates worse performance. “Perseverative response” and “error-T score” are higher in subjects with worse performance of mental flexibility and insight. “Non-perseverative error” reflects difficulty to forming concepts and insight even in flexible answer. Conceptual-level response score indicates the insights in correct principle of card combination. “Learning to learn” depicts the average tendency over successive categories for efficiency to change.

2.4. Statistical analysis

The data are shown as means± standard deviation. Student t tests were used to compare the findings in the OSA and control group. Taking into consideration the size of our group which limits usage of specific statistics such as “a mixed effects model”, we used the “standardized regression test” much better suited which was performed to demonstrate the relationship of cytokines levels with PSG and neurocognitive outcomes after controlling the factors of “asthma, allergy, body mass index (BMI), gender, tonsil hypertrophy, adenoid hypertrophy, turbinate hypertrophy, and nasosseal deviation”. All the reported P values are 2-tailed with statistical significance set at <0.05. Statistics were performed with SPSS version 18.

### Table 2

Demographic characteristics of OSA and healthy children.

| Control (n = 32) | OSA (n = 47) | P |
|-----------------|-------------|---|
| Number of males, % | 21 (65.6%) | 30 (63.8%) | 0.428 |
| Age, y | 7.02 ± 0.65 | 7.84 ± 0.56 | 0.366 |
| BMI, kg/m² | 16.55 ± 0.58 | 16.05 ± 0.47 | 0.601 |
| BMI z-score | 0.12 ± 0.27 | 0.15 ± 0.21 | 0.442 |
| AH1 events/h | 0.37 ± 0.06 | 9.13 ± 1.67 | <0.001† |
| PLMI events/h | 0.13 ± 0.10 | 0.93 ± 0.41 | 0.067‡ |
| PLM disorder, % | 0 (0%) | 3 (6.4%) | 0.083‡ |
| Learning disorder, % | 0 (0%) | 1 (2.1%) | 0.461 |
| ADHD, % | 2 (6.2%) | 18 (38.3%) | 0.001‡ |
| Enuresis, % | 4 (12.5%) | 15 (31.9%) | 0.036‡ |
| Other physical comorbidity history | 4 (12.5%) | 6 (12.8%) | 0.561 |
| Asthma, % | 12 (37.5%) | 8 (17.0%) | <0.001‡ |
| Allergic rhinitis, % | 12 (37.5%) | 8 (17.0%) | <0.001‡ |
| Findings of ENT examination | 4 (12.5%) | 32 (68.1%) | <0.001‡ |
| Tonsil hypertrophy (more than Gr. 2), % | 4 (12.5%) | 32 (68.1%) | <0.001‡ |
| Adenoid hypertrophy, % | 3 (9.3%) | 24 (51.1%) | <0.001‡ |
| Turbinate hypertrophy, % | 1 (3.1%) | 6 (12.8%) | 0.158 |
| Nasosseal deviation, % | 0 (0%) | 1 (2.1%) | 0.461 |

† Corrected BMI z-score based on the center for disease control growth charts.
‡ Diagnosed according to the criteria of diagnostic and statistical manual of mental disorders. Fourth edition-text revision.
§ Diagnosed by pediatrics.
¶ P<0.001.
# P<0.05.
$ P<0.01.$
$ P<0.001.$
TNF-α, IL-10 showed a non-significant increase compared with normal controls. Results of CPT and WCST tests (Table 5) indicated a significant difference between OSA and control in “Hit-RT-Std.-Error” (P = 0.006) and “hit reaction time (Hit-RT)-ISI-change” (P = 0.004).

A standardized regression test was performed to demonstrate the relationship of cytokine levels with PSG and neurocognitive outcomes after controlling the factors of asthma, allergy, BMI, gender, tonsil hypertrophy, adenoid hypertrophy, turbinate hypertrophy, and naso-sensory deviation (Tables 6 and 7). It revealed significant relationship between proinflammatory cytokines and some PSG factors, such as HS-CRP with AHI (β = 0.39, P < 0.05) and IL-17 with AHI severity (β = 0.329, P < 0.05) (Table 6). Higher AI “influenced” serum levels of HS-CRP suggest an impact of inflammatory cytokines on soft tissues hypertrophy. Similarly, higher serum levels of IL-23 was “influenced” by higher AHI (β = 0.403, P < 0.05). Also, lower mean SaO2 “influenced” IL-10 level (β = -0.567, P < 0.01) and

**Table 3**

| Control (n = 32) | OSA (n = 47) | P value |
|-----------------|--------------|---------|
| BMI, kg/m² | 16.55 ± 0.58 | 16.95 ± 0.47 | 0.601 |
| BMI z-score | -0.12 ± 0.27 | 0.15 ± 0.21 | 0.442 |

**Table 4**

| Control (mean ± SD) | OSA (mean ± SD) | P value |
|---------------------|------------------|---------|
| HS-CRP, mg/L | 0.41 ± 0.48 | 1.90 ± 0.44 | 0.002 |
| TNF-α, µg/dL | 12.62 ± 0.94 | 12.58 ± 0.83 | 0.974 |
| IL-1β, pg/mL | 0.42 ± 0.27 | 0.36 ± 0.16 | 0.867 |
| IL-6, pg/mL | 1.10 ± 0.18 | 1.66 ± 0.23 | 0.104 |
| IL-10, pg/mL | 2.10 ± 0.26 | 2.62 ± 0.39 | 0.332 |
| IL-17, pg/mL | 10.20 ± 1.25 | 15.12 ± 1.38 | 0.024 |
| IL-23, pg/mL | 12.29 ± 0.73 | 14.58 ± 0.75 | 0.047 |

**Table 5**

| Control total (n = 32) | OSA total (n = 47) | P value |
|------------------------|---------------------|---------|
| CPT | 39.34 ± 15.91 | 49.51 ± 24.46 | 0.142 |
| Omissions T score | 46.57 ± 5.36 | 52.33 ± 17.93 | 0.074 |
| Commissions T score | 40.50 ± 10.93 | 45.03 ± 13.02 | 0.237 |
| Hit RT T score | 49.94 ± 8.86 | 57.08 ± 13.03 | 0.056 |
| Hit RT std. error T score | 45.79 ± 6.26 | 52.96 ± 12.23 | 0.006 |
| Variability T score | 46.63 ± 7.51 | 61.42 ± 10.74 | 0.006 |
| Detectability T score | 40.87 ± 12.39 | 54.62 ± 15.72 | 0.316 |
| Response style T score | 51.57 ± 14.97 | 53.12 ± 14.69 | 0.732 |
| Persuasions T score | 49.69 ± 7.91 | 55.34 ± 12.25 | 0.104 |
| Hit RT block change T score | 48.99 ± 6.54 | 50.43 ± 6.73 | 0.479 |
| Hit SE block change T score | 49.00 ± 7.71 | 50.07 ± 11.00 | 0.831 |
| Hit RT ISI change T score | 47.80 ± 5.25 | 54.53 ± 10.83 | 0.004 |
| Hit SE ISI change T score | 46.80 ± 8.80 | 51.70 ± 9.06 | 0.077 |
| WCST | 107.20 ± 20.97 | 99.67 ± 24.26 | 0.392 |
| Total errors | 54.80 ± 13.97 | 49.81 ± 16.19 | 0.395 |
| Persuasive responses T scores | 55.10 ± 14.77 | 50.56 ± 16.57 | 0.452 |
| Persuasive errors T scores | 56.30 ± 15.10 | 50.74 ± 16.42 | 0.357 |
| Nonpersuasive errors T scores | 55.60 ± 14.37 | 52.78 ± 16.68 | 0.639 |
| % Conceptual level response T scores | 54.50 ± 4.60 | 50.19 ± 5.31 | 0.487 |
| Learning to learn | -3.49 ± 11.66 | -3.90 ± 9.78 | 0.712 |

P < 0.01. *P < 0.05. **0.05 < P < 0.1.
higher serum levels of TNF-α and IL-1β were “influenced” by higher diastolic pressure (β=0.469 and 0.659, P<0.01). There was a significant relationship between lower performances of CPT test and proinflammatory cytokines as shown in Table 7. The standardized regression test indicated significant findings between proinflammatory cytokines and neurocognitive function tests. The elevated cytokines are related to domains of inattention, vigilance, such as “Hit-RT-ISI-Change T score” and

### Table 6

| Relationships between inflammatory cytokines and PSG findings. |
|---------------------------------------------------------------|
| **HS-CRP** | **TNF-α** | **IL-1β** | **IL-6** | **IL-10** | **IL-17** | **IL-23** |
| AHI severity, † | 0.237 | −0.078 | 0.026 | 0.124 | 0.205 | 0.329 |
| AHI events/h | 0.114 | −0.109 | −0.114 | −0.095 | −0.079 | 0.059 |
| AHREM, events/h | 0.022 | −0.101 | −0.095 | −0.082 | −0.027 | 0.095 |
| AI, events/h | 0.390 | 0.162 | −0.209 | −0.074 | −0.31 | −0.238 |
| Hi, events/h | 0.020 | −0.100 | −0.129 | −0.036 | 0.080 | 0.225 |
| Desaturation index, events/h | 0.156 | 0.076 | −0.151 | −0.088 | −0.147 | 0.001 |
| Sleep efficiency, (%) | −0.037 | 0.041 | −0.090 | 0.050 | −0.150 | −0.126 |
| Awake, (%) | −0.016 | 0.012 | 0.059 | −0.046 | 0.139 | 0.089 |
| REM, % | 0.111 | 0.064 | 0.057 | 0.318 | 0.026 | 0.148 |
| Stage N1, % | 0.022 | −0.168 | 0.076 | −0.011 | 0.139 | −0.086 |
| Stage N2, % | −0.520 | −0.174 | −0.049 | 0.001 | −0.220 | 0.026 |
| Stage N3, % | −0.261 | −0.061 | 0.057 | −0.189 | −0.330 | 0.191 |
| TST | 0.118 | −0.057 | 0.276 | 0.188 | 0.224 | 0.034 |
| Sleep latency, ** | 0.162 | −0.141 | 0.297 | 0.014 | 0.092 | 0.012 |
| PLM index | 0.048 | 0.088 | −0.006 | −0.141 | −0.201 | −0.33 |
| Snore index | 0.079 | −0.145 | 0.133 | −0.052 | 0.066 | 0.157 |
| Mean SaO2, % | 0.040 | −0.159 | 0.012 | −0.123 | −0.57 | −0.242 |
| Systolic pressure | −0.43 | 0.064 | 0.09 | −0.41 | 0.14 | 0.088 |
| Diastolic pressure | −0.15 | 0.47 | 0.66 | 0.18 | −0.10 | −0.06 |

† Standardized regression coefficient. Control factors: asthma, allergy, body mass index, gender, tonsil hypertrophy, adenoid hypertrophy, turicobtip hypotrophy, and nasoseptal deviation.

### Table 7

| Relationships between inflammatory cytokines and neurocognitive function tests. |
|---------------------------------------------------------------|
| **HS-CRP** | **TNF-α** | **IL-1β** | **IL-6** | **IL-10** | **IL-17** | **IL-23** |
| CPT Clinical confidence index | −0.185 | −0.177 | −0.040 | −0.027 | 0.181 | 0.424 |
| Omissions T score | −0.256 | −0.154 | −0.067 | −0.109 | 0.002 | 0.112 |
| Commissions T score | 0.057 | 0.075 | 0.170 | 0.030 | 0.250 | −0.056 |
| Hit RT T score | −0.071 | −0.127 | 0.006 | −0.106 | 0.065 | 0.267 |
| Hit RT std. error T score | −0.207 | −0.216 | 0.083 | −0.103 | 0.182 | 0.294 |
| Variability T score | −0.247 | −0.098 | 0.160 | −0.009 | 0.354 | 0.274 |
| Detectability T score | −0.116 | 0.032 | 0.136 | −0.004 | −0.050 | −0.186 |
| Response style T score | −0.044 | −0.432 | −0.101 | −0.039 | 0.136 | 0.030 |
| Perseverations T score | −0.253 | 0.100 | −0.013 | 0.112 | 0.155 | 0.184 |
| Hit RT block change T score | −0.146 | −0.328 | −0.066 | −0.017 | 0.105 | −0.102 |
| Hit SE block change T score | −0.100 | −0.121 | 0.131 | −0.029 | 0.201 | −0.184 |
| Hit RT ISI change T score | −0.426 | −0.155 | 0.003 | −0.168 | −0.012 | −0.036 |
| Hit SE ISI change T score | −0.389 | −0.119 | 0.174 | 0.159 | 0.049 | 0.114 |
| WCST Total errors standard scores | −0.123 | −0.335 | 0.089 | 0.436 | 0.065 | −0.086 |
| Total errors T scores | −0.124 | −0.345 | 0.082 | 0.433 | 0.068 | −0.083 |
| Perseverative responses T scores | −0.276 | −0.093 | 0.186 | 0.324 | −0.016 | 0.027 |
| Perseverative errors T scores | −0.262 | −0.117 | 0.175 | 0.324 | −0.021 | 0.012 |
| Nonperseverative errors T scores | 0.047 | −0.553 | −0.058 | 0.255 | 0.106 | −0.250 |
| % Conceptual level response T scores | −0.131 | −0.315 | 0.067 | 0.476 | 0.081 | −0.079 |
| Learning to learn | 0.336 | −0.838 | 0.019 | 0.221 | 0.119 | 0.330 |

† Standardized regression coefficient. Control factors: asthma, allergy, body mass index, gender, tonsil hypertrophy, adenoid hypertrophy, turicobtip hypotrophy, and nasoseptal deviation.

P<0.05; P<0.01; CPT = continuous performance task; HI RT ISI = change hit reaction time interstimulus interval change; HI SE ISI = change hit standard error interstimulus interval change; HS-CRP = high-sensitivity C-reactive protein; TNF-α = tumor necrosis factor alpha; WCST = Wisconsin card sorting test.
Findings of ENT examination
Other physical comorbidity
of IL-10 in our subjects: as this cytokine is involved in both pro-
activation of a chain of inflammation, as IL-6 is a crucial cytokine signal in guiding the differentiation of naïve T cells into TH17 cells that release IL-17. But there may be other pathways leading to secretion of IL-17: as for example, IL-23 is a key cytokine and one of its reported activity is to differentiate naïve T cells into IL-17-producing TH17 cells.\(^1\) But IL-17 could also be produced without intervention of IL-23.\(^{137-139}\) Independent of the interaction between the different inflammatory interleukins, the fact remains that IL-17 is abnormally and significantly elevated in children with OSA. Overall, IL-17 acts in synergy with TNF-α, triggering the signaling pathway that upregulates the downstream cytokines, IL-6 and IL-8. In the animal model of autoimmune arthritis,\(^{40,41}\) it was shown that IL-17 itself stimulates the cellular production of IL-1 and TNF-α. In addition, IL-17 ultimately leads to recruitment of inflammatory cells such as neutrophils and other leukocytes.\(^{42}\) In summary, all of the above studies confirmed that IL-17, primarily secreted by TH17, a subset of T helper cells, is a proinflammatory cytokine that acts with the company of other cytokines such as IL-1, TNF-α, and IL-6 to induce systemic inflammatory diseases and plays an important role in the development of autoimmunity. Our study indicates that both IL-17 and IL-23 are elevated in pediatric OSA and could be used as biomarkers of pediatric OSA. They can play a role in the development of secondary health problems noted with OSA.

OSA not only affects cardiovascular functions and growth problem but also causes behavioral and cognitive dysfunction in children.\(^{43}\) But the mechanisms involved are still unknown. Evidence suggests that some peripheral proinflammatory cytokines such as IL-1 and IL-6 can go through the blood–brain barrier.\(^{115,44}\) The new finding in rodent of a direct connection between cerebrospinal fluid and deep neck lymph nodes is also a very important clue.\(^{115}\) These cytokines activate and regulate the vagus nerve system, and this activation affects the function of central nervous system.\(^{44}\) Moreover, chronically rising level of proinflammatory cytokines may also induce neuroinflammation or neurodegeneration and cause impairment of neurocognitive functions.\(^{46,47}\) Other research shows reduction of cognitive function as related to increased level of peripheral IL-6 even in the normal-aging Americans.\(^{48,49}\) Our study shows that higher levels of TNF-α and IL-23 are significantly related to some

Table 8 shows the significant Spearman correlation factors between comorbidities and cytokines such as asthma and IL-6 (\(p=0.261, P=0.026\)); allergic chinitis and HS-CRP (\(p=0.280, P=0.022\)), IL-6 (\(p=0.299, P=0.011\)), and IL-10 (\(p=0.265, P=0.023\)); tonsil hypertrophy and HS-CRP (\(p=0.244, P=0.046\)); adenoid hypertrophy and IL-6 (\(p=0.232, P=0.048\)).

In summary, our study showed that an abnormal increase in interleukins 17 and 23 is present in association with mild-to-moderate OSA in prepubertal children. Lower neurocognitive test results are also demonstrated in the OSA children, and there are significant positive correlations between low scores at cognitive tests (showing decreased alertness and increase in inattention, inability to focus leading to erratic responses, and to appropriately conceptualize) and abnormal level of inflammatory cytokines, more particularly IL-17 and IL-23.

4. Discussion

Plasma levels of proinflammatory cytokines such as HS-CRP, TNF-α, IL-1β, and IL-6 have been previously reported as elevated in children with OSA; and the expression ratio of the IL-10 and IL-6 was elevated in OSA children with recovery after adenotonsillectomy surgery.\(^{117}\) This last finding supported the concept that OSA induces a systemic inflammatory response activating the signal transduction pathway leading to upregulation of inflammatory cytokines and downregulation of anti-inflammatory cytokines.

But the interaction between the different cytokines may not be as clear-cut as thought, the advances in the recognition of the activation of a chain of inflammatory factors allow further understanding. We found a nonsignificant trend toward elevation of IL-10 in our subjects: as this cytokine is involved in both pro- and anti-inflammatory processes, further investigations will be needed.
Our study has limitations: despite the fact that we looked at 79 children, even if a high number for this type of study, this is still an overall low number. Also, our controls were somewhat “hyper-normal”: we eliminated from the study any child who had an indication of abnormal levels of inflammatory cytokines; finally, our children were not all with “severe” OSA (only 17% had AHI > 10), all however presented abnormal PSG findings. In addition, our findings are in line with data showing that even children with low but abnormal AHI have often memory problems, attention problems, and school difficulties. Finally, as the total number of children is relatively limited, despite the usage of “standardized regression test” before performing correlation analyses and the inclusion of all studied children for statistical purpose, the results will need to be confirmed with larger numbers and/or evaluation of change noted with appropriate treatment.

OSA in children impacts brain functioning: cognitive, memory, attention disorders, as well as behavioral and mood problems are much more common than any other listed complications. The impact of inflammatory factors, neuroinflammation, and dysfunction of the neuronal network has been mentioned by many; our study indicates that specific and unreported up-to-now interleukin abnormalities (IL-17 and IL-23) may be present very early in association with sleep disorder breathing; these interleukin cytokines may be potential markers helping in diagnosis and post-treatment follow-up of pediatric OSA.

Acknowledgments

We thank Po-Yu Huang PhD, for help with statistical analysis and Shannon S. Sullivan for reading and editing the manuscript.

References

[1] Lumeng JC, Chevin RD. Epidemiology of pediatric obstructive sleep apnea. Proc Am Thorac Soc 2008;5:242–52.
[2] Tuomilehto H, Peltonen M, Partinen M, et al. Obstructive sleep apnea in children. Laryngoscope 2013;123:1289–93.
[3] Guilleminault C, Winkle R, Korobkin R, et al. Children and nocturnal snoring: evaluation of the effects of sleep related respiratory resistive load and daytime functioning. Eur J Pediatr 1982;139:165–71.
[4] Chevin RD, Archbold KH. Hyperactivity and polysomnographic findings in children evaluated for sleep-disordered breathing. Sleep 2001;24:131–20.
[5] Chevin RD, Archbold KH, Panahi P, et al. Sleep problems seldom addressed at two general pediatric clinics. Pediatrics 2001;107:1375–80.
[6] Uritsch MS, Eimer S, Guenther A, et al. Habitual snoring, intermittent hypoxia, and impaired behavior in primary school children. Pediatrics 2004;114:1041–8.
[7] Suratt PM, Barth JT, Diamond R, et al. Reduced time in bed and obstructive sleep-disordered breathing in children are associated with cognitive impairment. Pediatrics 2007;119:320–9.
[8] Bhattacharjee R, Kheirandish-gozal L, Pillar G, et al. Cardiovascular complications of obstructive sleep apnea syndrome: evidence from children. Prog Cardiovasc Dis 2009;51:416–33.
[9] Gozal D, Kheirandish-gozal L, Bhattacharjee R, et al. Neurocognitive and endothelial dysfunction in children with obstructive sleep apnea. Pediatrics 2010;126:e161–7.
[10] Peppard PE, Young T, Palta M, et al. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000;342:1787–94.
[11] Pai JK, Fischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med 2004;351:2599–610.
[12] Kasasbeh E, Chi DS, Krishnasamy G. Inflammatory aspects of sleep apnea and their cardiovascular consequences. South Med J 2006;99:536–47, quiz 68–69, 81.
[13] Alberi A, Sarchielli P, Golinella F, et al. Plasma cytokine levels in patients with obstructive sleep apnea syndrome: a preliminary study. J Sleep Res 2003;12:305–11.
[14] Punjabi NM, Beamer BA. C-reactive protein is associated with sleep disordered breathing independent of adiposity. Sleep 2007;30:29–34.
[15] Gozal D, Grabbee YM, Sans capdevila O, et al. C-reactive protein, obstructive sleep apnea, and cognitive dysfunction in school-aged children. Am J Respir Crit Care Med 2007;176:188–93.
[16] Yokoe T, Minoguchi K, Matsuo H, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. Circulation 2003;107:1129–34.
[17] Kheirandish-gozal L, Capdevila OS, Tauman R, et al. Plasma C-reactive protein in nonobese children with obstructive sleep apnea before and after adenotonsillectomy. J Clin Sleep Med 2006;2:301–4.
[18] Lee LA, Chen NH, Huang CG, et al. Patients with severe obstructive sleep apnea syndrome and elevated high-sensitivity C-reactive protein need priority treatment. Otologynog Head Neck Surg 2010;143:72–7.
[19] Louveau A, Smirnov I, Keysje T, et al. Structural and functional features of central nervous system lymphatic vessels. Nature 2015;523:337–41.
[20] Iwakura Y, Ishigame H, Saojo S, et al. Functional specialization of interleukin-17 family members. Immunity 2011;34:149–62.
[21] Aggarwal S, Garvey AL. IL-17: prototype member of an emerging cytokine family. J Leukoc Biol 2002;71:1–8.
[22] Zrioual S, Ecochard R, Tournade N, et al. Genome-wide comparison between IL-17A- and IL-17F-induced effects in human rheumatoid arthritis synoviocytes. J Immunol 2009;182:1112–20.
[23] Wright JF, Guo Y, Quazi A, et al. Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. J Cell Biol 2007;282:13447–55.
[24] Iwakura Y, Ishigame H. The IL-2/IL-17 axis in inflammation. J Clin Invest 2006;116:1218–22.
[25] Zhao J, Lloyd CM, Noble A. Th17 responses in chronic allergic airway inflammation abrogate regulatory T-cell-mediated tolerance and contribute to airway remodeling. Mucosal Immunol 2013;6:335–46.
[26] Kawaguchi M, Kokubu F, Fujita J, et al. Role of interleukin-17F in asthma. Inflamm Allergy Drug Targets 2009;9:838–9.
[27] Ye J, Liu H, Zhang G, et al. The treg/h17 imbalance in patients with obstructive sleep apnea syndrome. Mediators Inflamm 2012;2012:815308:1–1.
[28] Anderson ME Jr, Buchwald ZS, Ko J, et al. Patients with pediatric obstructive sleep apnea showed altered T-cell populations with a dominant TH17 profile. Otologynog Head Neck Surg 2014;150:880–6.
[29] Wang M, Zhang W, Shang J, et al. Immunomodulatory effects of IL-23 and IL-17 in a mouse model of allergic rhinitis. Clin Exp Allergy 2013;43:956–66.
[30] Van de vosse E, Lichtenauer-kaligis EG, van dissel JT, et al. IL-17A and IL-17F receptor structure and implications for IL-12 and IL-23 receptor structure and function. Immunogenetics 2003;54:817–29.
[31] Aggarwal S, Ghilardi N, Xie MH, et al. Genetic variation in the interleukin-12/interleukin-23 receptor (beta1) chain, and implications for IL-12 and IL-23 receptor structure and function. Immunogenetics 2005;54:817–29.
[32] Langrish CL, Mckenzie BS, Wilson NJ, et al. IL-12 and IL-23: master regulators of innate and adaptive immunity. Immunol Rev 2004;202:96–105.
[33] Rechtschaffen A, Kales A. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. Los Angeles:UCLA, BISSRI: 1968.
[34] Iber C, Ancoli-Israel S, Chesson AL Jr, et al. American Academy of Sleep Medicine (Addended Berry RB, Brooks R, Gamaldo CE)The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.0. 2012:Darien, IL:American Academy of Sleep Medicine, www.aasmnet.org
[35] American Sleep Disorders Association-ASDA-Atlas Task ForceEFG arousals: scoring rules and examples: a preliminary report from the sleep disorders atlas task force of the American sleep disorders association. Sleep 1992;15:173–84.
[36] Guilleminault C, Li K, Khramtsov A, et al. Sleep-disordered-breathing: surgical outcome in prepubertal children. Laryngoscope 2004;114:132–7.
[37] Veldhoen M, Hocking RJ, Atkins C, et al. TGF-beta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006;24:179–89.
[38] Mangan PR, Harrington LE, O’quinn DB, et al. Transforming growth factor-beta induces development of the Th17 lineage. Nature 2006;441:231–4.
[39] Bettelli E, Carrier Y, Gao W, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441:235–8.
[40] Nakae S, Saijo S, Horai R, et al. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. Proc Natl Acad Sci U S A 2003;100:5986–90.
[41] Cho ML, Kang JW, Moon YM, et al. STAT3 and NF-kappaB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice. J Immunol 2006;176:5652–61.
[42] Lopez kostka S, Dinges S, Griewank K, et al. IL-17 promotes progression of cutaneous leishmaniasis in susceptible mice. J Immunol 2009;182:3039–46.
[43] Beebe DW, Groesz L, Wells C, et al. The neuropsychological effects of obstructive sleep apnea: a meta-analysis of norm-referenced and case-controlled data. Sleep 2003;26:298–307.
[44] Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol Rev 1998;105:83–107.
[45] Guilleminault C, Puyares D, Rosa A, et al. Heart rate variability, sympathetic and vagal balance and EEG arousals in upper airway resistance and mild obstructive sleep apnea syndromes. Sleep Med 2003;4:451–7.
[46] De luigi A, Fragaiconno C, Lucca U, et al. Inflammatory markers in Alzheimer’s disease and multi-infarct dementia. Mech Ageing Dev 2001;122:1985–95.
[47] Smith JA, Das A, Ray SK, et al. Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull 2012;87:10–20.
[48] Marsland AL, Petersen KL, Sathanoori R, et al. Interleukin-6 covaries inversely with cognitive performance among middle-aged community volunteers. Psychosom Med 2006;68:895–903.
[49] Yaffe K, Lindquist K, Penninx BW, et al. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology 2003;61:76–80.