UNIVERSIDADE DE LISBOA
Faculdade de Medicina

Sleep, Sleep-disordered-breathing, Cognition and Prematurity

Yu-Shu Huang

Orientadores: Prof. Doutora Maria Teresa de Aguiar dos Santos Paiva
Prof. Doutor Christian Guilleminault

Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, especialidade em Psiquiatria e Saúde Mental

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2017

Dedication:

This work is dedicated to my parents who believe that I should do what I wanted and could do it. It is also dedicated to my daughter Lulu Pei-Ju Huang who accepted my work in the evenings and nights and my absences while growing-up performing my duties and who accepted the demands placed on a woman academic physician wanting to pursue a career in her field.

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* Dr **Jen-Fu Hsu,** Associate Division of Pediatric Neonatology, Department of Pediatrics, Chang Gung Memorial Hospital and Medical College, Taoyuan, Taiwan

Índex

I-1. Summary:

Prematurity leads to many handicaps, some of them are only recognized later in life and may impact the individuals for the rest of their life. The delivery date compared to the full term delivery time will be a measure of "indication of risks" of post-natal handicap risk, but this is only one of the many measurements that can be looked at. Many studies have investigated development of premature infants, based on different criteria at entry in the considered study. Our investigations are only a limited contribution to the investigation of premature infants. We included infants born as young as 24 weeks of gestational-age [GA] but none of the infants had major neurological syndromes recognized at birth. "Normal infants" defined as infants with more than 37 weeks of GA, birth-weight >2500g and absence of any indication of health problems born in the same hospital maternity at same time as premature infants were also recruited to serve as normal controls.

As most newborn infants spend a large amount of time asleep, all the presented studies include investigation of sleep, and once sleep-time occurred mostly during the nocturnal period, it focused on the polygraphic monitoring of the nocturnal sleep.

The premature cohort study was a longitudinal study and parents who signed informed consent approved by the Chang Gung Hospital and Medical College Ethic Committee, were asked to come back on a yearly basis for at least 5 years. This is an on-going study and not every child has been followed for such time. Furthermore, as in any longitudinal study, loss of patients occurred as parents did not bring children back. At entry 400 parents signed the informed consent, currently at 5 years follow-up 150 children have ended the follow-up period and about 215 at 4 years. The sample is a non-random convenience sample of children selected based on the parents` willingness to participate in the protocol and obtained with the help of neonatologist physicians in our NICUs.

Most of the studies presented in the thesis come-out of this longitudinal study. The studies asked specific questions, particularly looking at development of abnormal obstructive breathing during sleep. But some of our studies looked also at children of older age as some of the findings that we observed in our premature cohort needed a different investigative approach, and prior validation on older children-premature and in non-premature infants.

We have included these studies in our narrative as they become part of our research, and they are part of a general research program on "sleep- breathing-and-cognition" in children.

The thesis has the following organization:

1. Summary in English and Portuguese

   The preamble explains major sleep problems of premature infants and the work done

2. Introduction with background and objectives as follows:

   (1) the understanding of the development of abnormal upper airway in premature infants and abnormal breathing during sleep.

   (2) the understanding the craniofacial development and impact on occurrence of pediatric SDB.

   (3) to evaluate the differences in development between premature and full-term infants.

   (4) finally investigating, from our cohort-data, the relationship between the SDB and the developmental problems that we could observed in our premature infants.

To perform these tasks she had to:

(1) Develop an "infant sleep questionnaires" in Chinese version to use through our studies

(2) Develop pediatric obstructive sleep apnea questionnaires in Chinese version to use in our study.

(3) To investigate the possible actions of pediatric SDB on neurocognitive dysfunctions in our children and is behind the protocols presented in our studies.

(4) To develop the biomarkers for diagnosis and selection for treatment of pediatric SDB

(5) To improve treatments of pediatric OSA.

3. Literature review including Sleep disordered breathing in infants and children, the problem of obesity, the issues related to craniofacial development, type of breathing, facial configuration and the lingual frenulum, the pro inflammatory cytokines, neurodevelopmental and neurocognitive dysfunctions of SDB in infants and children.

4. The methodology used which includes the set-up of the Sleep Lab, the multidisciplinary team and the development of Chinese-version of Infant Sleep Questionnaire: "Brief Infant Sleep Questionnaire-Chinese version"
5. The results which are based on the published articles
6. The discussion in which she integrates the achieved results in the international literature.

The following research projects were undertaken:

Study #1The Chinese version of the obstructive-sleep-apnea questionnaire-18

Our first study involved defining another tool for our research considering the parents' reluctance to come back, year after year to the sleep-laboratory for follow-up investigation. Not to have too many "absence of data", we had to validate another tool: a questionnaire that parents would be willing to fill- in, even at home if necessary. This validation involved monitoring of children during sleep.

Our study validate the CBISQ, but it is also the first study targeting the difference between premature and full-term infants through a reliable and valid screening questionnaire.

Study #2 - Sleep and Breathing in premature infants at 6 months post-natal age –

Sleep and sleep breathing disturbances were investigated in premature infants at 6 months of age.

Premature infants are presenting with a more narrow and high-arched palate, more SDB-related sleep problems, and more neurodevelopmental deficits than normal full-term infants.

Till 2 years of age, premature infants with narrow and high-arched palate have more SDB-related sleep problems and more neurodevelopmental deficits than those without.

These findings support our hypothesis that high and narrow-arched palate in premature infants plays a role in the development of sleep problems and neurodevelopmental delays.

Study #3 - Early detection of minor neurodevelopmental dysfunctions at age 6 months in prematurely born neonates.

The infants involved in our longitudinal studies were selected as free of clear neurological signs and symptoms. However, these children are considered to have clear neurological risks. We

investigated such possibility assessing subtle neuromotor dysfunctions, such as difficulties with gross motor skill, social contact, or learning.

Premature neonates, even those born at 33 to 36 weeks, are found to have MNDs (Minor Neurodevelopmental Dysfunction) as early as 6 months corrected age by BSID-II and DDST, with risk increasing as gestation decreases.

Moreover, we used multivariate logistic regression to evaluate the relationships between neonatal factors and the presence of MNDs. After multivariate logistic regression adjusted for GA and BBW (Birth Body Weight), MND was independently associated with postnatal corticosteroid use and cholestasis. Besides, neonates with BBW less than 1000g were significantly associated with MNDs when compared with those with BBW more than 2000g.

Study#4 Pediatric Obstructive Sleep Apnea and the critical role of oral-facial growth (a review of our evidences placed in an historical context)

We looked to the critical role of facial growth in the development of SDB.

Currently at 4 years of age, 77% of our premature infants have OSA and a high and narrow hard palate. There is a relationship between gestational age(GA) at birth and presence of problems which increase with lower GA at birth. But, this is not the only factor. As found, children with normal palate and AHI may progressively deteriorate during the first post-natal months. Preliminary data indicate that normal functioning of suction, swallowing, mastication and nasal breathing post-birth are key factors in the switch overtime to abnormal breathing and high and narrow hard palate.

Study #5 ***Short nasal lingual frenulum, mouth breathing and abnormal oral facial growth***.

This study was performed to investigate the role of abnormal oral facial functioning in children and development of OSA.

Short lingual frenulum may lead to abnormal orofacial growth early in life, a risk factor for development of SDB.

Careful surveillance for abnormal breathing during sleep should occur in the presence of short lingual frenulum.

Study #6 Inflammation, cytokines and mouth breathing

From of our longitudinal study on premature children we had found out that mouth breathing was frequent and that tonsils were not initially enlarged in children that developed OSA, but become enlarged. We had shown that children with enlarged tonsils and adenoids (T&A) treated with adenotonsillectomy present relapse of OSA within 3 years. We questioned if inflammatory factors could not play a role in the development of OSA and if persistence of inflammatory factors could not be involved in relapse of OSA. This study is only the first step in our investigation but it shows that specific inflammatory factors, currently very much looked at: ie interleukines(IL) 17 and 23 are clearly abnormal in OSA children, supporting our hypothesis and opening the field for further studies. We do not have post-surgery results as the study takes time to be completed, but we demonstrated the validity of our hypothesis and the need to pursue this line of research.

By Regression analysis the following conclusions were achieved: significant relationships between pro-inflammatory cytokines and PSG scores with higher AHI score and OSA severity, such as HS-CRP (High Sensitivity- C Reactive Protein) (β=0.390, $P<0.05$) and IL-17(β=0. 329, $P<0.05$. Higher AI "influenced" serum levels of HS-CRP suggesting an impact of inflammatory cytokines on soft tissues hypertrophy. Higher serum levels of IL-23 (β=0.403, $P<0.05$) were "influenced" by higher AI. Lower mean SaO_2 (O2 saturation) "influenced" IL-10 level (β= -0.567, $P<0.01$), and higher serum levels of TNF-α (tumor necrosis factor alpha) 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 pro-inflammatory cytokines. Significant Spearman's correlation factors between pro-inflammatory cytokines and clinical findings such as asthma and IL-6 (ρ= 0.261, P=0.026*); allergic rhinitis and HS-CRP (ρ= 0.280, P=0.022*), IL-6(ρ= 0.299, P=0.01*) and IL-10(ρ= -0.265, P=0.023*) ; Tonsil hypertrophy and HS-CRP(ρ= 0.244, P=0.046*) ; Adenoid hypertrophy and IL-6 (ρ=0.232, P=0.048*) were observed.

● **Key words:** prematurity, sleep, obstructive breathing, cognition

I-2. Sumário:

A prematuridade leva a muitas deficiências, algumas das quais são apenas reconhecidas mais tarde, podendo afetar os indivíduos para o resto das suas vidas. À data do parto, a comparação com o tempo de gestação duma criança prematura com o de uma criança de termo será uma medida de "indicação de riscos", nomeadamente de risco de deficiência pós-natal. Muitos estudos têm investigado o desenvolvimento do bebé prematuro, com base em critérios diferentes. As nossas investigações são apenas uma contribuição limitada à investigação de bebés prematuros. Incluímos bebês nascidos com apenas 24 semanas de idade gestacional (IG), mas nenhuma das crianças incluídas tinha síndromes neurológicas graves reconhecidas na altura do nascimento. As "Crianças normais" foram definidas como tendo mais de 37 semanas de IG, peso ao nascer > 2500g e ausência de qualquer indicação de problemas de saúde, e foram recrutadas para servir como controlos normais.

Como a maioria dos recém-nascidos passa uma grande quantidade de tempo dormindo, todos os estudos apresentados incluem a investigação de sono durante o período noturno, designadamente registo poligráfico do sono noturno.

O estudo de coorte de prematuros foi um estudo longitudinal e os pais que assinaram o consentimento livre e esclarecido aprovado pela Comissão de ética da Faculdade de Medicina e do Chang Gung Hospital, foram convidados a voltar anualmente, pelo menos durante 5 anos. Este é um estudo que continua a decorrer e nem todas as crianças foram seguidas por este período de tempo. Além disso, como em qualquer estudo longitudinal, a perda de pacientes ocorreu quando os pais não trouxeram as crianças para re-observação. Na entrada 400 pais assinaram o consentimento informado, atualmente há follow-up de 5 anos para 150 crianças e cerca de 215 têm follow-up aos 4 anos. A coorte resulta de uma amostra de conveniência não-aleatória das crianças selecionadas com base na disponibilidade dos pais para participar do protocolo, os quais foram obtidos com a ajuda de médicos neonatologistas no nosso Hospital.

A maioria dos estudos apresentados na tese resultam deste estudo longitudinal. Também foram feitos estudos com perguntas específicas, particularmente os que se referem ao desenvolvimento de disfunção respiratória de tipo obstrutivo durante o sono. Mas alguns dos estudos também olharam para as crianças de idade mais avançada, no sentido de validar algumas das conclusões

que observámos na nossa coorte prematura. Estes estudos são parte de um programa de investigação sobre "sono-respiração-e-cognição" em crianças.

A tese tem a seguinte organização:

1. Sumário em Inglês e Português
2. Preâmbulo
3. Introdução com justificativo e objetivos:
    (1) compreensão do desenvolvimento anormal das vias aéreas superiores em recém-nascidos prematuros e da respiração anormal durante o sono.
    (2) compreensão do desenvolvimento craniofacial e seu impacto na ocorrência de apneia pediátrica.
    (3) avaliação da diferença no desenvolvimento de recém-nascidos de termo e prematuros.
    (4) investigação, na coorte de dados, a relação entre a disfunção respiratória do sono e os problemas do desenvolvimento em prematuros.

Para executar essas tarefas, teve que:

    (1) Desenvolver um "questionário de sono infantil" em versão chinesa
    (2) Desenvolver um questionário para apneia obstrutiva pediátrica do sono em versão chinesa.
    (3) Investigar os efeitos da apneia pediátrica em disfunções neurocognitivos
    (4) Identificar biomarcadores para diagnóstico e seleção do tratamento da SAOS (Síndrome da Apneia Obstrutiva do Sono) pediátrica
    (5) Melhorar os tratamentos de SAOS pediátrica.

4. Revisão de literatura, incluindo disfunção respiratória do sono em bebês e crianças, o problema da obesidade, os problemas relacionados ao desenvolvimento craniofacial, tipo de respiração, configuração facial e o freio lingual, o citoquinas pro-inflamatórias, desenvolvimento neurológico e neurocognitivo, e disfunções da SAOS em bebês e crianças.
5. A metodologia utilizada, incluiu a estruturação de um laboratório de sono, uma equipa multidisciplinar e a validação de questionários em chinês (Questionário Breve de Sono do Recém-nascido.

6. Os resultados que foram publicados em revistas indexadas
7. A discussão que integra os resultados obtidos com os da literatura internacional.

As investigações feitas foram as seguintes:

Estudo # 1 - Versão chinesa do questionário---apneia obstrutiva 18

Desenvolvemos uma ferramenta para nossa pesquisa. Considerando a relutância dos pais em voltar, ano após ano ao laboratório de sono, e para evitar a "ausência de dados", tivemos que validar uma outra ferramenta: um questionário que os pais estariam dispostos a preencher, mesmo em casa, se necessário. Esta validação implicava o acompanhamento das crianças durante o sono.

O nosso estudo validou o CBISQ, (Chinese Brief Infant Sleep Questionnaire) e é o primeiro estudo que avalia a diferença entre crianças prematuras e de termo, através de um questionário de triagem fiável e válido.

Estudo #2 - Sono e respiração em bebés prematuros com 6 meses de idade post-natal –

O sono e os distúrbios respiratórios do sono foram investigados em recém-nascidos prematuros com 6 meses de idade.

Os bebés prematuros que apresentam um palato mais estreito e mais arqueado, têm mais problemas de sono relacionados com a respiração e mais déficits de desenvolvimento neurológico do que os recém-nascidos de termo.

Estes resultados suportam a nossa hipótese que o palato estreito e arqueado em bebés prematuros desempenha um papel no desenvolvimento de problemas de sono e atrasos de desenvolvimento neurológico.

Estudo #3- Deteção precoce de disfunções minor do neurodesenvolvimento (DMND) aos 6 meses de idade em recém-nascidos prematuros.

As crianças envolvidas em nossos estudos longitudinais foram selecionadas como não tendo sinais e sintomas neurológicos na altura do nascimento. No entanto, estas crianças são consideradas ter riscos neurológicos claros. Nós investigámos esta possibilidade avaliando disfunções subtis, tais como motilidade fina, contato social, ou de aprendizagem.

Prematuros, mesmo aqueles que nasceram com 33 a 36 semanas, são encontrados para ter DMND logo aos 6 meses corrigidos idade; o risco aumenta com a diminuição da idade de gestação

Usando a regressão logística multivariada para avaliar as relações entre fatores Neonatais e a presença de DMND, verificou-se, após ajustamento para IG e PN (Peso à Nascença), que a presença de DMND tinha uma associação independente com o uso de corticosteroides no período pós-natal e a presença de colestase. Além disso, recém-nascidos com PN inferior a 1000g tiveram significativamente mais DMND quando comparados com aqueles com PN superior a 2000 g.

Estudo #4 Papel crítico de crescimento oral-facial na apneia obstrutiva do sono pediátrica (uma revisão das nossas evidências num contexto histórico)

Olhámos para o papel crítico de crescimento facial no desenvolvimento da SAOS

Aos 4 anos de idade, 77% dos nossos bebés prematuros têm SAOS e um palato duro alto e estreito. Existe uma relação entre a idade gestacional e presença de problemas Crianças com o palato normal e IAH (Índice de Apneia Hipopneia) podem deteriorar progressivamente durante os primeiros meses pós-parto. Dados preliminares indicam que o normal funcionamento da sucção, deglutição, mastigação e respiração nasal são fatores-chave na prorrogação respiração anormal.

Estudo n º 5 ***Freio lingual, respiração bucal e crescimento oro-facial anormal***. Este estudo foi realizado para investigar o papel do funcionamento orofacial no desenvolvimento de SAOS Um freio lingual curto pode levar a um crescimento orofacial anormal no início da vida, sendo um fator de risco para o desenvolvimento de DRS (Distúrbio Respiratório do Sono). A vigilância cuidadosa da respiração durante o sono deve ocorrer nestas condições

Estudo #6, Inflamação, citocinas e respiração oral

A partir de nosso estudo longitudinal em crianças prematuras descobrimos que a respiração oral era frequente e que as amígdalas não estavam necessariamente aumentadas em crianças que desenvolveram SAOS. Crianças com hipertrofia das amígdalas e adenoides apresentavam recidivas de SAOS após adenoamigdalectomia ao fim de 3 anos. Questionámos se fatores

inflamatórios poderiam ter um papel no desenvolvimento da SAOS e se a persistência de fatores inflamatórios poderia estar envolvida na recaída. Este estudo é apenas o primeiro passo em nossa investigação, mas mostra que fatores inflamatórios específicos, as interleucinas 17 e 23 estão claramente anormais em crianças com SAOS. Não temos ainda resultados post cirurgia, mas demonstramos a validade da nossa hipótese e a necessidade de prosseguir nesta linha de pesquisa. Por análise de regressão, as conclusões foram: há uma relação significativa entre citocinas pró-inflamatórias e valores da PSG, designadamente de IAH elevado e severidade da SAOS, tais como PCR-as (Proteína C reativa de alta sensibilidade) ($\beta = 0.390$, $P < 0,05$) e IL-17(β=0. 329, P<0.05). IA (Índice de Apneia) e níveis séricos de PCR mais elevados-sugerem um impacto de citocinas inflamatórias na hipertrofia de tecidos moles. Níveis séricos de IL-23 mais elevados ($\beta = 0.403$, $P < 0,05$) associaram-se a um IA mais elevado. $SatO_2$ médias (Saturações médias de O2) baixas associaram-se a IL-10 (β =-0.567, $P < 0,01$), e níveis mais elevados de FNT-α (Fator de necrose tumoral alfa) e IL-1 β foram "associaram-se" a pressão diastólica mais elevada (β = 0.469 e 0.659, $P < 0,01$). As citocinas pró-inflamatórias correlacionaram-se positivamente com dados clínicos tais como asma e IL-6 ($\rho = 0,261$, $P = 0,026$); rinite alérgica e PCR-as (ρ= 0.280, P=0.022*), IL-6(ρ= 0.299, P=0.01*) e IL-10(ρ=-0.265, P=0.023*); Hipertrofia de amígdalas e PCR-as (ρ= 0.244, P=0.046); Hipertrofia dos adenoides e IL-6 ($\rho = 0.232$, $P = 0,048$*).

Palavras chave: prematuridade, sono, respiração obstrutiva, cognição

● II. Introduction

Sleep is essential to human life and developmentally involves both physiologic and mental processes. During infancy, humans spend a majority of time asleep. Sleep is recognized not only as a resting state, but also as a state of intense brain development during which neurotransmitters, specific for each sleep stage, impact brain maturation. Therefore, we will discuss some important issues for pediatric sleep especially pediatric sleep-disordered breathing in the early years of life in our thesis.

II-1. Background and Rationale

Abnormal breathing during sleep in children may affect up to 7 to 10% of pre-pubertal children [1]. The consequences of sleep disordered breathing-SDB- are not only tiredness, fatigue, but the syndrome may also lead to cardio-vascular and metabolic changes. In children, behavioral problems are also common with daytime hyperactivity, inattention, difficulties in learning, aggressiveness against peers, and at night nocturnal disrupted sleep, parasomnias- more particularly night terrors and sleepwalking-, bruxism and even insomnia [2]. Persistence of obstructive sleep apnea-OSA- in children after adenotonsillectomy has been reported over the years by different groups [1-5]. In one of our studies [6], four factors were identified as significantly related to persistence of SDB after adenotonsillectomy: 1) enlarged nasal inferior turbinates; 2) nasal septal deviation; 3) retro-placement of the mandible; and 4) a Mallampati-scale grade 3 or 4 airway [7]. Mallampati-scale scores of 3 and 4 are associated with risk for difficulty with intubation and may also indicate a narrow upper airway (see figure1). Anatomically small upper airway was similarly suggested as a cause for persistent SDB by

Tauman et al [2]. Studies have suggested that children with SDB and residual problems after adenotonsillectomy, had certain facial features that were hypothesized to play a role in the development of OSA in children. These facial features involve the nasomaxillary complex and the mandible [6,8]. Based on this hypothesis, some patients have undergone orthodontic-treatments such as rapid maxillary expansion or bimandibular expansion that targeted widening of the maxilla and mandible respectively; these approaches have been successful to some degree in eliminating residual obstructive sleep apnea in children [8-12]. But the factors behind the findings of anatomical involvement in development of SDB are still very much unknown. It has been shown that SDB and OSA apnea may be seen in different family members and a genetic influence behind occurrence of these syndromes has been suggested [13-18].

Environmental factors are also suspected: experimental studies on new-born monkeys [19-22] have shown that creation at birth of increase in nasal resistance by placing a ligature restricting the size of the nares results in concomitant mouth breathing, increased facial height, abnormal maxillary and mandibular development with mandibular retrusion and abnormal mandibular growth, and secondary small upper airway. In children, nasal allergies leading to nasal turbinate enlargement, deviated nasal septum, enlarged adeno-tonsils have similar effects emphasizing the role of environmental factors in the development of small upper airway leading to SDB [23,24]. A recent retrospective study performed at Stanford University on 400 children showed that 373 (93.3%) had craniofacial features considered to be risk-factors for SDB, including small mandible and/or high and narrow hard palate associated with a narrow nasomaxillary complex [25]. It seems also that premature infants are at greater risk to present these anatomical risks factors [25] and to develop SDB with its behavioral consequences.

Sleep is important for pediatric development: Premature neonates bear less mature organs and higher incidence of multiple morbidities, as well as poorer developmental outcomes later during their infancy and childhood. SDB encompasses varieties of respiratory disorders that occurs or are exacerbated exclusively during sleep; and the breathing event may be defined as central, obstructive, or mixed based on polysomnographic recording. Conditions that disrupt respiration and sleep in premature neonates and infants include apnea of prematurity, central apnea, bronchopulmonary dysplasia, etc.

Obstructive SDB is characterized by partial or complete upper airway obstruction and includes a spectrum of conditions ranging from primary snoring (PS) to upper airway resistance syndrome (UARS) to evident apneas with repeated arousals and intermittent hypoxia, as seen in obstructive sleep apnea syndrome (OSAs). Since the first identification of sleep apnea in children by Guilleminault et al. in 1976 [26], SDB and OSA have been found to be associated with cardiovascular and metabolic (e.g., hypertension) [27], growth (e.g., failure to thrive) [28], neurocognitive (e.g., low academic performance) [29], and neurobehavioral (e.g., inattention, hyperactivity, impulsivity, aggressivity, and poor executive functions, communication, and adaptive skills) [30,31] morbidities during childhood or adolescence. Even primary snoring itself, without other symptoms or polysomnographic findings, has been shown to be associated with adverse neurocognitive and neurobehavioral outcomes as well as cardiovascular sequelae that are linked with systemic inflammation [31]. Recently, preterm birth has been recognized as a risk factor for both sleep disordered breathing (group-age 8 – 11) and obstructive sleep apnea (group-age 2.5 – 6) in prepubertal children [32,33].

Anatomical factors contributing to upper airway obstruction include: nasal septum deviation, allergic rhinitis and chronic nasal obstruction, craniofacial anomalies, adenotonsillar hypertrophy, obesity, cleft palate following pharyngeal flap surgery, etc.

Adenotonsillar hypertrophy is the first-line treatment target for childhood SDB and OSA. However, craniofacial anomalies, instead of adenotonsillar hypertrophy, are important factors that caused of OSA in infants [33-35]. Craniofacial anomalies are hypoplasia or displacement of the maxilla or mandible, such as midface hypoplasia, micrognathia (small mandible), and glossoptosis (posterior tongue displacement), which commonly contribute to OSA in infants with Down syndrome [36] or Pierre Robin sequence [37] as examples. High and narrow-arched palate associated with narrow nasomaxillary complex is also an important craniofacial abnormality that gives rise to OSA [25,38], as seen in Apert syndrome [38] Reduced nasal breathing is accompanied by open mouthed breathing, which exposes the tonsils to abnormal stimulation and subsequently leads to their local inflammation and hypertrophy as hypothesized. Furthermore, mouth breathing and abnormal tongue positioning may give rise to impairment of maxillomandibular growth [38,39].

The first 4 – 6 years of life are critical for maxillomandibular growth as 60% of the adult face is built during that period [39]. The consequent impairment of maxillomandibular growth further increases the risk of SDB.

In 1998, Gozal first reported high prevalence of sleep disordered breathing (18.1%) among 279 low performance (< 10 percentile) first-grade elementary school children [29]. To date, there is an abundant literature demonstrating the association of pediatric SDB and OSA with cognitive (mental) and behavioral problems [30,40-42]. But how sleep problems and early

developmental deficits interact in premature infants and young toddlers is still not extensively studied. We hypothesize that abnormal craniofacial development and narrow-high -arched palate in premature infants may play a major role in the development of SDB and may subsequently worsen any neurodevelopmental deficits. In this prospective study, we focused on the craniofacial development, presence of sleep problems, and neurodevelopment, trying to note the evolution of these three factors and their interaction during the post-natal growth of premature individuals.

II-2. Study Purpose

Our research aims at:

1. Contributing to:
    (1) the understanding the development of abnormal upper airway in premature infants and abnormal breathing during sleep.
    (2) the understanding the craniofacial development and impact on occurrence of pediatric SDB.
    (3) evaluate the difference in development of full-term infants versus premature individuals.
    (4) finally investigating, from our cohort-data, the relationship between the SDB and the developmental problems that we could observe in our premature infants.
2. To perform these tasks we had firstly to:
    (1) Develop an “infant sleep questionnaires” in Chinese version to use through our studies
    (2) Develop pediatric obstructive sleep apnea questionnaires in Chinese version to use in our study.
    (3) Our ultimate goal was to investigate the possible actions of pediatric SDB on neurocognitive dysfunctions in our children and is behind the protocols presented in our studies. Based on our findings we hope:

(4) To develop the biomarker for diagnosis and selection of treatment of pediatric SDB

(5) To improve treatments of pediatric OSA.

III. Literature Review

III-1. Pediatric Sleep-Disordered-Breathing (SDB)

Pediatric obstructive sleep apnea (OSA) was initially described in 1976 [26], and in 1981 Guilleminault et al published a review of 50 pediatric patients [43] emphasizing that pediatric OSA was different from the clinical presentation reported in adults. The authors emphasized that these children had more disturbed nocturnal sleep than excessive daytime sleepiness, and presented more behavioral problems, particularly school problems related to attention deficit, poor school performance, hyperactivity, all symptoms classified as "attention-deficit-hyperactivity syndrome", nocturnal enuresis, sleep-terrors, sleep-walking, confusional arousals: symptoms classified as "NREM hypersomnias", depression, insomnia and psychiatric problems. Cardiology-related symptoms were infrequent but tachybradycardia was regularly noted. Adenotonsillectomy (T&A) was performed and was successful in some but not all children, as shown well by follow-up studies; finally, a small group of children presented an abnormal weight increase post-T&A. These children presented apnea and hypopneas closely following the current polysomnographic definition.

However, a year later, Guilleminault et al published a new report indicating that children may present the same chronic symptoms, but polysomnographic investigations performed with these children using esophageal pressure manometry showed absence of apnea and hypopnea, but presence of abnormal upper airway (UA) resistance with snoring of variable intensity [44]. In 1982, many of the features presented today in reports on pediatric SDB were already clearly indicated but some of the raised issues still need further research, including recurrence post-T&A, weight increase also post-T&A and the issue of having "sleep-disordered-breathing" with similar complaints, symptoms and clinical findings at evaluation associated with and without snoring with very different patterns of abnormal breathing at the PSG evaluation.

In the 1990s, the obesity epidemic started in the industrialized world and added a level of complexity. Two different syndromes were observed: a) obesity per se could lead to the same complaints and symptoms as OSA syndrome in a normal-weight child; b) obesity could lead to the development of OSA as a co-morbidity due to the deposit of fat in the tongue tissues and other UA muscles. The obese presentation could lead to a "chest-bellow syndrome" when supine, related to the abdominal fat deposit, and it could worsen the symptoms seen in a slim OSA child.

To attribute to obesity and OSA their respective responsibilities in the clinical presentation was difficult, particularly due to the fact that the children were often not seen early at time of development of the health problem but only after several years of evolution.

III-2. Obesity and SDB

Obesity is a complex disorder leading to worsening supine ventilation secondary to restrictive chest-bellows syndrome [45]. Obesity also leads to progressive fatty infiltration of the neck and UA. MRI studies have shown that a progressive fatty infiltration of the genio-hyoid and genio-glossal muscles occurs along with dissociation of muscle fibers with fat cells [46]. Certain ethnicities, particularly African-American children, have a stronger association between obesity and SDB [47].

Obesity is associated with a progressive dysfunction of the adipocytes. Pre-adipocytes differentiate into mature adipocytes and form adipose tissue in response to a positive energy balance. Adipose tissue not only stores energy, but also acts as a dynamic endocrine organ, vital for hormone and cytokine (adipokine) secretion. White adipose tissue (WAT), located in abdominal and subcutaneous deposits in mammals, performs the majority of energy storage and adipokine secretion [48]. Brown adipose tissue (BAT) mediates the non-shivering thermogenesis, well known to protect infants from cold exposure. Genetics play a role in the control and development of WAT and BAT.

Dysfunction of adipocytes leads to stimulation of adipokines, particularly TNF-alpha and interleukins 6 and 1. These defects lead to pivotal inflammatory responses, both local and general, in addition to abnormal secretion of peptides found not only in the adipocyte, but also in the gut and brain. Peptides such as leptin, adinopectin, obesin, etc., are involved, and dysfunction of the adipocytes leads to leptin resistance and ghrelin dysfunction. These two peptides are crucial to food intake, insulin resistance, and dysregulation of glucose and lipid control [48]. Overweight and obese individuals, with or without SDB, will develop these dysfunctions. The consequences of these abnormalities affect the cardiovascular, respiratory, metabolic, and cerebral systems. Sleep fragmentation, which occurs with abnormal breathing, will cause changes in metabolic controls in part through the process of epigenetics, by which environmental events trigger a genetic cascade that would not have otherwise occurred. Obesity

along with fatty infiltration of the UA will always lead to SDB from simple flow limitation to frank OSA.

III-3. Non-overweight children and SDB: Why does the upper airway collapse during sleep in non-overweight children?

The upper airway (UA) is a collapsible tube and the muscles that constitute its borders are inserted on bones that are part of the oral facial region. The muscles forming the limits of the UA are controlled by reflexes, and the reflex-loops call upon sensory receptors, sensory nerve-fibers, brainstem neurons integrators, and a motor loop to act on these muscles. During sleep, it was shown that many of these reflexes are attenuated or even non-functional at times, particularly during Rapid-Eye-Movement (REM) sleep. This leads to an increase in the risk of collapse during sleep as compared to wakefulness. Studies looking at the laws of physics that govern the airflow in the UA have determined that fluid-dynamics-physic-laws can be applied to investigate the changes in UA airflow [49].

One of the features impacting the UA is its dynamic airway collapsibility. The abnormal collapsibility in both children and adults has been related to the different stages of sleep, which cause fundamental modifications to the pharyngeal muscle tone and reflex responses. Other factors have also been considered, including one's position during sleep.

Given that sleep usually occurs in a recumbent position, both intrinsic and extrinsic factors affect its collapsibility: The upper airway (UA) has an intrinsic collapsibility that is studied via evaluation of the "critical pressure", [50,51] while extrinsic factors may lead to increased overall collapsibility.

Three external factors that impact the retropalatal and retroglossal space of the UA have been firmly established: (a) UA fat deposit, (b) non-fat related hypertrophy of UA tissues in which chronic inflammation is a participant, and (c) craniofacial features impacting UA size, and possibly related to genetic and environmental factors.

- **Monkey Experimental Investigation**

Historically, orthodontists performed fundamental experimental studies in the 1980s on new-born Rhesus monkeys [20,22,52]. These experimenters placed a soft hollow cone silicon plug filling the nares and held in position by a silk ligature. The emphasis of the study was on the

orthodontic changes, and sleep was not monitored. However, it became evident that the great increase in nasal resistance had a dramatic impact on the naso-maxillary and mandibular skeleton leading to a halt in the growth and development of abnormal maxilla and mandible, and to adaptive changes in soft tissues that were associated with deviation in jaw posture and tongue activity. Systematic recording of orofacial muscles, including the genio-glossus and genio-hyoid muscles, demonstrated that such abnormal nasal resistance led to abnormal electro-myographic (EMG) activity with induction of an abnormal rhythmic discharge pattern compared to control animals. This pattern was slowly reversible once the nasal resistance was eliminated. The experiment showed that in these growing monkeys, the nose is progressively occluded with increase in nasal resistance, and an adverse effect is seen on the morphology of maxilla and mandible. Moreover, this adverse effect is associated with changes in the EMG activity of orofacial muscles [20,22,52].

- **Cranio-facial Growth**

The earliest form of the face appears in the **fourth week** of life of fetal development. Migration of cranial neural crest cells into developing facial prominences is an important step in fetal development and the family of Homeobox or HOX genes (n=39) play a major role in the development of the end tissue [38]. By the **ninth week** of fetal development, the initial cartilaginous facial skeleton is well established and by the **twelfth week** of fetal growth, areas of ossification appear and bone rapidly replace the cartilaginous template forming the early cranial base. At the same time, the bones of the cranial vault and of the mandible and maxilla develop through intramembranous ossification [53,54]. Post-natal development is rapid. The head that represent nearly a quarter of the child's length at birth decreases to about 12% at adulthood. 60% of the adult face is developed by 6 years of age, with maximum growth between birth and 2 years of age.

During infancy and early childhood, the cranial base increases in length through endochondral ossification that occurs at important growth sites called synchondroses (growth centers). Two of these growth centers, the" intermaxillary synchondrosis" and "alveolo-dental ligament", are active until close to the end of puberty. The growth of the cranial-base is the initial engine of the facial growth through enchondral ossification. The maxilla and mandible are pulled down and forward by the soft tissues on which they are attached. However, if the maxilla benefits

from growth at the mid-palatal suture and from growth of the alveolar process that accompanies tooth eruption, the mandible lacks an open suture and grows mostly through enchondral ossification at the condyles. The dental-alveolar structure develops with eruption of the teeth, and the maintenance of the occlusal contact is an important element related to vertical ramus growth [55-57].

- **Nasal Breathing and Mouth Breathing**

At birth, an infant is an obligatory nose breather. Beginning in the 1960s, important attention was given to the development of nasal breathing early in life. Planas [58] indicated that the normal airflow through the nose can be considered a "praxia" that develops very early in life. That is, nasal ventilation provides direct feedback on the thoracic ventilatory movements. The nasal airflow-thoracic-ventilatory movements involved a complex series of reflexes with engraving in the motor cortex. The lack of normal nasal breathing coupled with thoraco-abdominal ventilation leads to deficiencies in the development of normal breathing and lack of learning to adjust between the amplitude of the thoraco-abdominal ventilatory movements and the nasal resistance. It was found that normal nasal ventilation is critical for the normal development of the sinuses. But investigation of a deviated septum or presence of enlarged adenoids close from birth showed that nasal breathing has an impact on skeletal oral-facial development [59-63]. This is particularly important because, as mentioned above, the face grows extensively between birth and 2 years of age.

The skeletal changes noted involved the anterior part of the nasal fossae. In this anterior portion, a slight elevation of the floor of the nasal cavity was found to be related to normative bone resorption and an abnormal narrowness to the transversal lower part of the pyriform aperture, which impacts the anterior part of the maxilla. This impact on the maxilla, in turn, leads to a malposition of the superior incisive, and such malposition has a negative feedback on the growth of the anterior part of the maxilla and the nasal fossae. Abnormal nasal flow also leads to a dysfunction of the deciduous canine that may lead to a malposition of the permanent canines.

The skeletal changes also involve the posterior part of the nasal fossae. The impairment of the nasal flow impacts the normal periosteum resorption and this absence of resorption limits the normal lowering of the inferior part of the nasal fossae. Finally, abnormal nasal flow also leads to a narrowness of the transversal section of the nasal fossae and to an abnormal sagittal growth

of the maxilla (i.e., an inconstant impairment of the development and expansion of the maxillary sinuses). The narrowness of this transversal section may have an impact on the normal development of the 3rd molar later on [59-63].

Abnormal nasal airflow was shown to affect the palate and its maxillary-alveolo-dental development [59,62,63]. The development of the palate is impacted in 3 dimensions:

First, there is an abnormal vertical development with appearance of a high, ogival vault. **Secondly**, a narrowness occurs whereby the palate forms an extreme V-shape and the narrowness involves both the part of the palate at the level of the nasal fossae and the lower part located under the sinus. It was noted that if the nasal obstruction is predominant on one side, there is an asymmetry of the palate vault with deviation of the ogival arch toward the hypoplastic nasal fossa. Such changes have an impact on teeth orientation: with an oblique teeth direction on one side with the least impairment and a vertical development on the other side [59].

Finally, there is a sagittal impact leading to development of a small maxilla. Such changes interfere, as mentioned above, with the maxillary dental arch growth, which will disturb the mandibular dental arch development secondarily. In particular, the changes lead to the disappearance of the diastasis (or interspace) between the deciduous incisive teeth, which in turn interferes with the placement of the permanent teeth.

- **Other early-in-life functions involving the oral cavity**

Nasal breathing is not the only function that has a very important role on the oral-facial development. Coordination between nasal breathing and sucking must also develop very early in life. This is very apparent with breastfeeding, but also necessary with bottle-feeding. Sucking and swallowing are very coordinated activity that starts during the last trimester of gestation, and appropriate nasal breathing is important for these activities. Such coordinated actions (e.g., breathing and sucking) play a role in the stimulation of the structures involved in maxillary growth early in life. Mastication between 6 to 12 months of age is an added stimulus for such growth and involve a cortico-geniculum pathway and development of “active swallowing” on the top of the “swallowing reflex” involving only brain-stem neuronal networks. Anomalies in these functions will increase the risk of abnormal development of the bone structures supporting the UA leading to an increased risk of collapsibility of the UA during sleep [64].

To maintain a UA lumen that avoids the risk of collapsibility during sleep means appropriate orofacial development during childhood.

We question if specific risks factors for the occurrence of SDB exist early during postnatal development, and if these risk factors can be identified in children developing OSA.

- **Short lingual frenulum and oral-facial development**

We have already reported several factors that impact normal growth of the oral-facial structures leading to the development of OSAS in both children and adults. Another risk factor which has not yet been linked to the development of OSAS is "**a short lingual frenulum** ". Normally at birth, the tongue is placed high in the palate, and its continuous activity related to sucking, swallowing and masticating induces stimulation of the intermaxillary synchondrosis [65], which is active until 13–15 years of age, leading to normal oral-facial growth. Normal nasal breathing is associated with this tongue position. A short lingual frenulum has been associated with sucking and swallowing difficulties early in life, leading to "clipping" of the frenulum in the newborn [66-69]. In older children speech difficulties have been related to an untreated short frenulum [69-71]. It was also shown to lead to mouth breathing with modification of the position of the tongue and secondary orthodontic impacts resulting in an anterior and posterior crossbite, a disproportionate growth of the mandible and an abnormal growth of the maxilla [69,71,72]. All these anatomical changes impact the size of the upper airway and increase the risk of its collapse during sleep. But the association between a short lingual frenulum and OSAS is currently often unrecognized. "Clipping" of the short lingual frenulum is still proposed when difficulties are recognized during very early infancy, but if a simple clipping is performed after the first few months of life, the long-term results are reported as unpredictable, with persistence of an abnormal short lingual frenulum due to fibrosis occurring on the clipped abnormal vestigial tissue. [Our study presented here, investigated the association between a short lingual frenulum and OSAS in children and results of limited treatment.]

In summary: To maintain a UA lumen that avoids the risk of collapsibility during sleep means appropriate orofacial development during childhood. Moreover, early recognitions of these factors may potentially lead to early interventions aimed at preventing OSA from occurring.

III-4. Neurodevelopmental dysfunctions in prematurely born neonates

The prevalence and risk factors of neurodevelopmental sequelae, such as cerebral palsy, kernicterus, hearing loss, and cognitive deficiencies are the major topics of premature infants [73-75]. However, prematurely born neonates without major neurological deficits have been proven to be at higher risk of developing subtle neuromotor dysfunctions, such as difficulties with gross motor skill, social contact, or learning [76]. The frequency of these minor neurodevelopmental dysfunctions (MNDs) is usually assessed in early childhood (age 2-6 years) [73,75-77], and assessment of the quality of general movement during this period is found to be a powerful instrument to predict later neurological and behavioral developmental difficulties at school age [78-80]. Given the fact that the most common disability of premature infants at 2 years of age is developmental and cognitive impairments and these assume great significance at their school years, it is imperative to understand the prevalence and risk factors of these disabilities. Besides, few studies have provided data related to neurodevelopmental outcomes of late-preterm infants [81-83], and it is unknown whether these MNDs can be detected at earlier age of young infancy.

The aim our present study was to determine the prevalence of MNDs and cognitive and motor functions at 6 months corrected age in a cohort of premature neonates, and also to investigate which neonatal factors are associated with MNDs, and to figure out if MNDs in these high-risk neonates is associated with pediatric SDB.

III-5. Neurocognitive function and pediatric SDB

OSA not only affects cardiovascular functions and growth problem but also causes behavioral and cognitive dysfunction in children [84]. But the mechanisms involved are still unknown. OSA syndrome affects the sleep and neurocognitive functioning of children [44,85-87] including symptoms of attention deficit/hyperactivity disorder [44,87]. Pediatric OSA results in long-term effects on children's health and development [88-90]. The factors involved in the decrease in cognition, learning and memory are still incompletely chartered. In our study we investigated some of the pathophysiology of the cause and the effect of cognitive dysfunction and pediatric SDB.

(1). Pediatric OSA and inflammatory cytokines

There is an interaction between OSA and chronic diseases [91-93]. The most acceptable hypothesis associates occurrence of chronic systemic inflammation with OSA [94-96]. Increase in pro-inflammatory cytokines (C reactive protein (CRP), tumor-necrotic-factor (TNF-α), interleukines (IL-6, and IL-10) in adult OSA patients and high-specific C reactive-protein (HS-CRP) in pediatric OSA patients) supports this hypothesis [96-98], with a possible association between the apnea-hypopnea-index (AHI) and inflammatory cytokine levels. The inflammatory responses may be reversed after OSA treatment [99,100]. 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 [101].

(2). The pro-inflammatory cytokines IL-17 and IL-23

The pro-inflammatory cytokines IL-17 and IL-23 have been recently emphasized. IL-17 is a pro-inflammatory cytokine secreted predominantly by T helper 17 cells (TH 17) and various cells including innate immune cells and non-immune cells [98]. It is referred to as IL-17A as it is a member of the IL-17 family [102]. 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 (APC) in response to antigen stimulation [102,103]. The interaction is as follow: IL-17A and IL-17F form homodimers or heretodimers that bind to the IL-17 receptor complex on inflammation-related cells such as macrophages, epithelial cells and endothelial cells [104,105]. 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 [104,105], with expression of high levels of IL-17 in patients with severe allergy, chronic inflammatory diseases and autoimmune diseases [105,106]. IL-17 also takes part in neutrophilic inflammation in the

respiratory system [106,107], and leading to chronic inflammation of the airway [107]; as example there is high expression level of IL-17F in asthma [108]. IL-17 has been linked to adult OSA: there is an up-regulated 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 over-secreted pro-inflammatory cytokines contribute to the development of OSA [109]. In OSA children, cytokine profile obtained from tonsils shows high levels of IL-1b, IL-10 and IL-17A production, indicating a T cell activation in response to local inflammation [110].

IL-23, is a cytokine with immunomodulatory effects [111]. It acts on memory-cluster –designation-4(+) T-cells, activates the transcription activator, and stimulates the production of interferon-gamma [112,113]. Studies showed that TH17 cells can be regulated by IL-23 [114].

Factors leading to cognitive changes in children with OSA are still subject of research: sleep fragmentation, hypoxemia, hypercapnia, change in cerebral-blood-flow may 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 affect cognitive functions and we wondered if a correlation between psychometric test and these cytokines could be shown [98]. A previous study had found a relationship between abnormal level of C-reactive protein and cognitive dysfunction in school age 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, 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.

● IV. Methodology

IV-1. Study Process:

Ethics:

The study was approved by the Chang Gung Hospital and Medical College Ethic Committee. All parents of the included infants signed an informed consent.

Subjects:

(1) Number of subjects: A total of 400 infants (300 preterm and 100 full term infants) were eligible for inclusion in this study. The study included in the conditions of a total of 300 premature infants. (Effect = 0.25, a = 0.05, power = 0.95, total sample size = about 180. Considering the long-term drop-out rate is about 40%, so 300 premature infants were included).

(2) Inclusion criteria:

(a). Study group A: All neonates born in our hospital before 37 completed gestational weeks, without presentation of exclusion criteria (temporary intubation of premature children can still be included in the study) comprised the preterm group.

(b). This group of premature babies will be divided into two groups according to the “high and narrow hard palate”, one group is "high and narrow hard palate" group A1, the other group is no "high and narrow hard jaw" group A2.

(c). Normal full term group: We also included 100 neonates that delivered at 37 to 40 weeks of gestational age with birth body weight of more than 2500 grams, without presentation of exclusion criteria comprised the normal “full-term-infants group”.

(d). All parents had to sign consent form during the study period.

(3) Exclusion criteria:

(a). The neonates with severe physical impairments (such as severe congenital heart diseases, DiGeorge syndrome, congenital hydrocephalus and kernicterus) due to perinatal insults or hypoxic ischemic encephalopathy.

(b). We also excluded bronchopulmonary dysplasia or requirement of oxygen support (nasal canula) after discharge.

(c). Neonates with confirmed severe congenital malformations were excluded.

(e). Parents that cannot sign the informed consent and cannot be involved in systematic following.

(f). Subjects judged by the investigator to be inappropriate as a subject of the study.

Duration: 2009.01.09 to 2016.01.09.

Procedure:

(1). Visit 1: Infants who meet the inclusion criteria had an oral photography within 48 hours of birth (included the photo of upper hard and soft jaw, tongue and uvula part). Physical (PE) and neurological (NE) examination was performed at the same time by experienced neonatal pediatrician.

(2). Visit 2: The same procedure for photography, PE and NE was followed at 3 months after birth. In the same time Parents were asked to complete the Children's Sleep Questionnaires.

(3). Visit 3 to 14: The same procedure of photography, PE and NE was followed at
6 months, 12 months, 18 months, 24 months, 30 months, 36 months, 42 months, 48 months, 54 months, 60 months, 66 months and 72 months after birth.

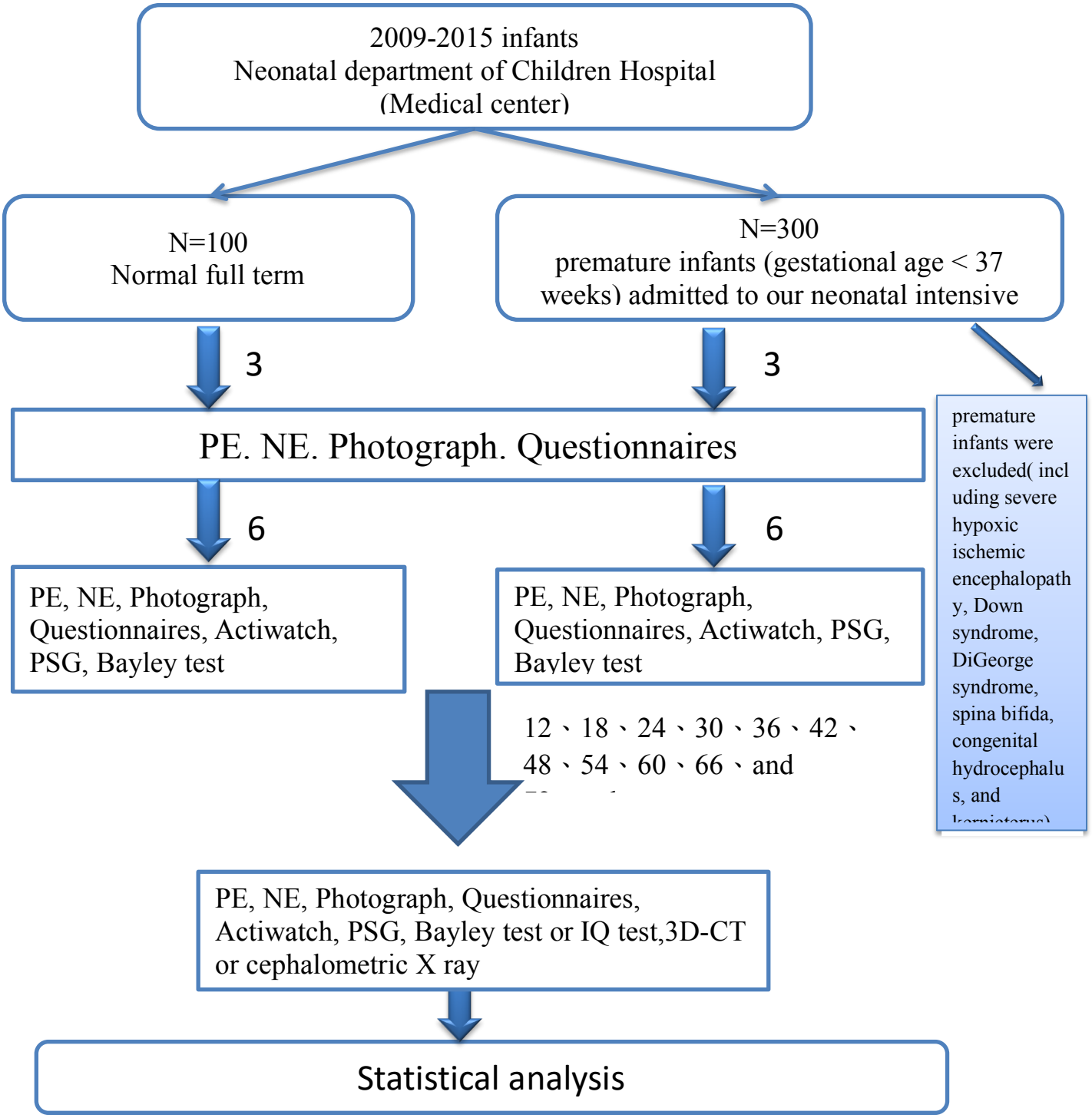
Parents were asked to complete the Children's Sleep Questionnaires, Sleep Log and developmental, emotional and behavioral questionnaires. In the same time, experienced pediatric psychiatrist routinely performed the developmental assessment (such as DDST), and pediatric psychologists performed Bayley test (or WPPSI and WISC test) every year.

(4). Sleep assessment: sleep focused interview, portable actigraphy-watch, night polysomnography (PSG) were performed every year.

(5). Craniofacial 3D-CT or cephalometric X ray was performed at the age of four, five and six years old.

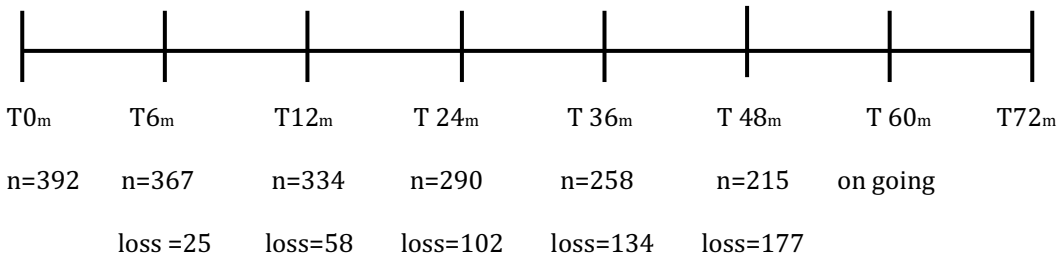
(6). The collected craniofacial and oral pictures were sent to Stanford Sleep Center and the Department of Craniofacial Surgery (in a blind) to score blindly the palate and craniofacial profile.

IV-2. Framework:



IV-3. Study Flux diagram:

The following diagram shows the number of children, both included and lost for follow up in each evaluation time point



IV-4. Research Instruments:

(1). Infant Polysomnography: Development of a Pediatric Sleep Lab in Taiwan

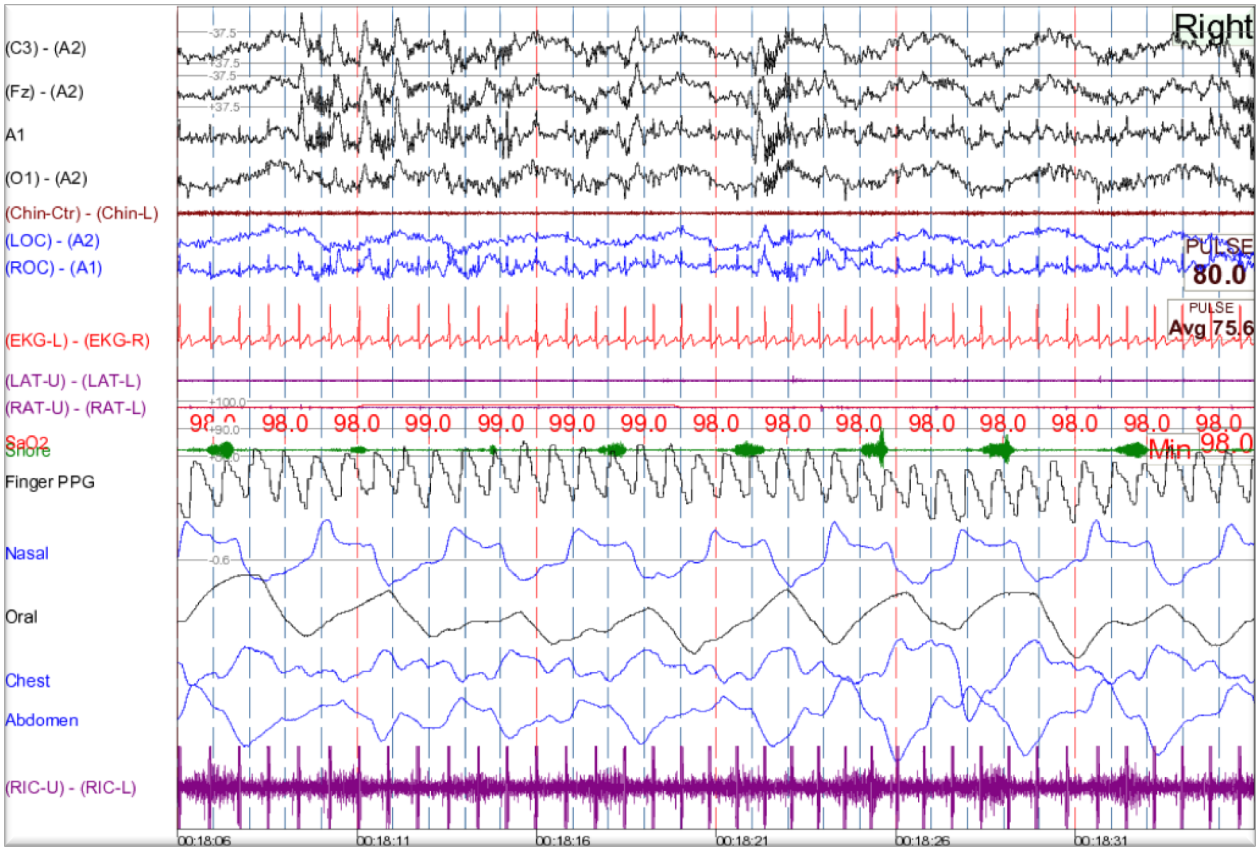
All our studies involved monitoring of sleep, a particularly arduous task the younger and the more premature the child is, and the presented data took several years to collect and to process, and implied the development from scratch of a Pediatric Sleep Lab in Taiwan.

The recording protocol was the same over-time:

Monitoring of sleep means that polysomnography –PSG-was systematically performed in the laboratory.

PSG was always performed following the recommendations of the American Academy of Sleep Medicine-AASM [115]. The following variables were systematically monitored: 4 EEG leads referred to the contra-lateral mastoid: F1-M2, C3-M2, C4-M1, O1-M2 (2 extra channels were placed- F2-M1 and O2-M1- to be able to monitor these brain-regions if the initial recordings showed technical problems during the long-sleep related-recording) 2 eye-leads (E1-M2, E2-M1) 3 chin-EMG (right, central and left) with possibility of monitoring chin EMG between 2 of these electrodes. We also monitored leg (right and left) muscle contractions (tibial anterior EMG) and intercostal/diaphragmatic activity. Respiration was monitored using nasal cannula-pressure transducer, mouth thermistor, thoracic and abdominal respiratory inductive plethysmography

bands, finger-oximetry, and a neck sound recording device. Children were continuously video monitored during the recording; mother were always present during recordings.



Example of a 30 seconds of a PSG recording during stage 2 NREM sleep in a snoring child with a montage as indicated above.

(2). **Actigraphy:**

All infants were set up with an actigraph (Philips Respironics actiwatch 2, with a small size well-suited for use with younger subjects or those sensitive to wrist-worn devices) on the left leg of the non-dominant side. The equipment measured body movements and light exposure. It was placed on the infant at the time of the visits and kept for 7 days, and was analyzed with commercially available software with one point every 2 minutes and indicated activity/non-activity. The equipment was correlated with a log simultaneously kept by care-givers. Therefore, actigraphy data can be analyzed in many ways including estimation of PSG endpoints such as:

total sleep time, wake after sleep onset, sleep latency sleep efficiency, and movement during the night. Moreover the daytime activity and circadian rhythms can also be evaluated [116].

(3). Development of Chinese-version of Infant Sleep Questionnaire: "Brief Infant Sleep Questionnaire-Chinese version," (CBISQ): (published article # 1)

(a). The Brief Infant Sleep Questionnaire (BISQ) [117] was developed by Avi Sadeh. The original BISQ had 13 items distributed in 3 categories, evaluating sleep duration, night awakenings, and method of falling asleep of infants aged 29 months or younger. It was found to be correlated significantly with sleep measures derived from actigraphy and sleep diaries. BISQ measures (number of night wakings and nocturnal sleep duration) were the best predictors for distinguishing between clinical and control samples. High test-retest correlations ($r > 0.82$) were demonstrated for BISQ measures for a subsample of 26 infants. The study demonstrated that BISQ measures derived from a large Internet survey provided developmental and sleep ecology-related findings that corresponded to the existing literature findings on sleep patterns in early childhood. [117].

(b). To validate the Chinese version of the BISQ:

First, we translated and then back-translated the BISQ from English to Chinese. Following this, it was translated back to English simultaneously by two bilingual individuals until the versions were considered completely interchangeable conceptually and linguistically.

2nd step, we originally signed up 229 premature infants from the PICU, but only 191 (83.41%) completed the study when they were 6 months old. The 191 6-month-old premature infants in the preterm infants group comprised 99 boys and 92 girls with an average birth body weight of 1646.66 g. The average gestational age was 31.52 weeks, with a maximum of 36 weeks and minimum of 24 weeks. There were 68 6-month-old infants in the full-term infants group, including 34 boys and 34 girls with an average birth body weight of 3169.69 grams and average gestational age of 38.35 weeks. (The small group ($n = 68$) of full-term infants (the "controls") whose parents signed the consent form underwent a similar evaluation and was studied the same way as the premature infants). Then we did the test-retest study for Chinese version of the BISQ to check the reliability of CBISQ.

3rd step, all parents completed the BISQ-Chinese version and sleep diaries. At the same time, all premature infants were submitted to one night of polysomnography (PSG) in the sleep laboratory and also were set up with an actigraph kept for 7 days.

4th step, to confirm CBISQ is a reliable and valid tool for sleep measurement, the statistical software package SPSS, Version 18 was used for data analysis. Variables are presented as either mean ± standard deviation (SD) or frequency. We used the t-test and Chi-square test for evaluation of differences between these 2 groups. The Pearson correlation coefficient was used in analysis of correlation between questionnaire and sleep Lab data. The statistical significance was defined at the 0.05 level.

(4). Development of Chinese-version Pediatric Obstructive Sleep Apnea Questionnaire: Chinese version OSA-18 (published article # 2)

To develop a questionnaire with good reliability that allows early detection of symptoms and is easy to use in the follow-up of pediatric OSA is clearly important, but such an instrument in Chinese is very few. Therefore, to validate empirically the Chinese version of the OSA-18 and to evaluate its ability to screen the effect of treatment of OSAS children is important. Moreover, we aim at analyzing the frequency of symptoms and finding the difference in symptom-reports of OSA between Asian and Caucasian children.

(a). The OSA-18 is a questionnaire evaluating pediatric SDB that has been shown to possess satisfactory test retest reliability and internal consistency in English-speaking groups [118,119]. The survey consists of 18 items grouped into 5 domains: sleep disturbance (4 items), physical suffering (4 items), emotional distress (3 items), daytime problems (3 items), and caregiver concerns (4 items). Items are scored on a 7-point ordinal scale and have excellent test-retest reliability (R _ 0.74). The overall survey score is calculated as the mean of the 18 items, which correlates significantly with the respiratory distress index (R _ 0.43) and adenoid size (R _ 0.43). It also provides a direct global rating of SDB-related quality of life (QOL) via a 10-point visual analogue scale with specific semantic anchors. Therefore OSA-18 is designed for screening OSA syndrome and it has good test-retest reliability and construct validity and can be used for screening OSA syndrome [118,119].

(b). To validate the Chinese version of the OSA-18:

First step, we got the permission to translate the OSA-18 into Chinese before the start of the study. English version into Mandarin Chinese by one of the investigators. Following this, it was

translated back to English simultaneously by two bilingual individuals until the versions were considered completely interchangeable conceptually and linguistically.

2nd step: The participants in the study were recruited from kindergarten and school settings. We contacted the school counseling centers and explained to teachers the purpose of the study and necessary procedures. All eligible children and their respective parents were informed that participation in the survey was completely voluntary, and thereafter parental approval and signed informed consent were obtained. In all, 191 children were recruited. A package of questionnaires, including the OSA-18 and basic demographic questions, was completed by the parents (or caregivers). The OSA-18 was given again 4 weeks later to obtain re-test data. Participants with missing data or who did not complete the questionnaire twice were excluded from the study (n=28); 163 children (school group) completed the study. The ratio of gender had no significant difference either. 3rd step, the clinical participants included 88 children with OSA (apnea–hypopnea index (AHI) >1/hour) who were treated with TA and were followed up. The participants and their respective parents were informed that participation was completely voluntary, and thereafter parental approval and signed informed consent were obtained. The patients were recruited from a sleep disorder center in a children's hospital, and were diagnosed by a pediatric psychiatrist and a pediatric department of otolaryngology, both with expertise in pediatric sleep medicine. OSA syndrome was diagnosed based on clinical interview and clinical evaluation followed by one nocturnal PSG recording. Using the International Classification of Sleep Disorders Second Edition (ICSD II) diagnostic criteria, 88 children presented symptoms and clinical features associated with OSA and had an AHI >1/h associated with breathing-related arousals or oxygen desaturation in one nocturnal PSG recording. Once diagnoses were confirmed, a research assistant administered to the participants' parents a package of questionnaires consisting of the OSA-18 and demographic questions. The OSA-18 was administered again to those treated with TA at the 6-month post-treatment follow-up visit. To establish the construct validity of a measure, nomological validity was taken into consideration. We also used nomological validity to analyze the relation between QOL and the constructs for OSA-18 in the pretest and post-test.

(5). Neurodevelopmental assessments:

(a). The Bayley Scales of Infant and Toddler Development (Bayley II and III) Test: Bayley test is the most widely used developmental assessment tools in preschool-aged children. It is considered to be an integrative developmental assessment that borrows from different areas of child development. The Bayley is now being used extensively in both clinical and research paradigms to diagnose the developmental delays and to determine subsequent qualification for early intervention services. The Bayley Scales of Infant Development-2nd Edition (BSID-II) consists of a mental score, which yields a Mental Developmental Index (MDI) and a motor score, which yields a Psychomotor Developmental Index (PDI). In 2006, the third edition, the Bayley-III (3), was published which has separate cognitive and language scales. There are three domains "Cognitive ", "language" and "motor "in Bayley-III. The cognitive and language scores <85 or CB-III scores <80 provide the best correspondence with Bayley-II MDI scores <70, it means developmental delay [120,121].

(b). Denver Developmental Screening Test (DDST): DDST is used for developmental measurements in preschool children and consists of four subscales: personal-social contact, fine motor, language and gross motor. Each of them is scored as either passed or failed. For each category in the overall assessment, patients were considered to be developmentally delayed if they failed two or more considered to be developmentally delayed if they failed two or more test items that 75 to 90% of children of their age could pass or if they failed one or more test items that more than 90% of children younger than their age could pass. Otherwise, the development of the children was considered to be normal [122].

IV-4. Analytical Methods

Statistical analysis:

• SPSS 20th Edition for data analysis and processing was used. All p values are two-sided, and those less than 0.05 were considered statistically significant.

• Demography data analysis of the gestation age, body weight and height, sex, endotracheal intubation, presence of feeding difficulties, tongue location, presence of "high and narrow palate", developmental problems, sleep problems and other characteristics of preterm infants

were present as a percentage. The Chi-Square test or Fisher exact tests were used for categorical data, and Student t test was used for continuous variables with normal distributions. The Wilcoxon/Mann-Whitney U test or the Kruskal-Wallis test were used for continuous variables without a normal distribution.

• The relationship between developmental delay, neurological cognitive dysfunction and pediatric sleep problems was assessed by the Pearson correlation coefficient.

• The relationship between the craniofacial profile such as" high and narrow hard palate" and the age and the sleep pediatric SDB was assessed by the Pearson correlation coefficient.

• Multivariate analysis of gender, gestation age, craniofacial characteristics and other variables with "high and narrow hard palate" was performed to understand their interactions on the impact of pediatric SDB and also was used to determine the independent risk factors (Simple main effect mode, interactive mode analysis).

• ANOVA was used to compare different variables in different groups in preterm infants and full term group.

● V. Results

We created in Taipei (Taiwan) a prospective cohort of 300 infants born between 25 and 37 weeks of gestational age. Three hundred children involved in the cohort have been followed until 24 months of age at the minimum. The cohort study is still on going. According to the preliminary data, we organize into several important reports.

V-1. Development of the " Brief Infant Sleep Questionnaire-Chinese version" and investigation of " Sleep and breathing" in premature infants at 6 months post-natal age (# 1 article was published in BMC Pediatrics, 2014)

(A) Background

Poor sleep contributes to the developmental problems seen in preterm infants. We evaluated sleep problems in preterm infants 6 months of post-gestational age using the subjective Brief Infant Sleep Questionnaire (BISQ) and objective sleep tests. Therefore, we had to develop a "Brief Infant Sleep Questionnaire-Chinese version" (CBISQ) questionnaire first.

(B) Method

The study included 68 6-month-old full-term healthy infants and 191 premature infants born at <37 weeks gestation. All parents completed the BISQ-Chinese version and sleep diaries. At the same time, all premature infants were submitted to one night of polysomnography (PSG) in the sleep laboratory and also were set up with an actigraph kept for 7 days. Statistical analyses were performed using correlation coefficients and the t-test with SPSS version 18 to compare questionnaire responses with other subjective and objective measures of sleep.

(C) Results:

(1) Demographic data

We originally signed up 229 premature infants from the PICU, but only 191 (83.41%) completed the study when they were 6 months old. The **191** 6-month-old premature infants in the preterm infants group comprised 99 boys and 92 girls with an average birth body weight of 1646.66 g. The average gestational age was 31.52 weeks, with a maximum of 36 weeks and minimum of

24 weeks. There were **68** 6-month-old infants in the full-term infants group, including 34 boys and 34 girls with an average birth body weight of 3169.69 grams and average gestational age of 38.35 weeks (Table 1).

Table 1 Demographic data of the study subjects

		Preterm infants (n = 191)	Full-term infants (n = 68)	P value
Gender	Male	n = 99 (51.83%)	n = 34 (50%)	
	Female	n = 92 (48.17%)	n = 34 (50%)	
Birth body weight (g)		1646.66 ± 588.60	3169.69 ± 410.58	<0.001*
Birth body height (cm)		39.96 ± 5.11	49.61 ± 1.88	<0.001*
Head circumference (cm)		28.88 ± 3.33	34.25 ± 1.24	<0.001*
Gestational age (weeks)		31.52 ± 3.21	38.35 ± 1.43	<0.001*

Birth body weight, Birth body height and Head circumference were all assessed at birth.

(2) The test–retest reliability of CBISQ:

The original BISQ had 13 items distributed in 3 categories, evaluating sleep duration, night awakenings, and method of falling asleep of infants aged 29 months or younger. To validate the Chinese version of the BISQ, we first translated and then back-translated the BISQ from English to Chinese. The test–retest reliability of the CBISQ was acceptable. There was significant correlation between the repeated sleep measures for location of sleep (r = 0.678*), preferred body position (r = 0.796*), nocturnal sleep duration (r = 0.534*), method of falling asleep (r = 0.848*), difficulty in falling asleep (r = 0.785*), number of night awakenings (r = 0.439*), daytime sleep duration (r = 0.455*), and subjective consideration of sleep problems (r = 0.663*). In order to evaluate respiratory sleep problems compared with actigraphy and PSG, we added 3 questions to the BISQ to create the CBSIQ version; these included “time spent with mouth breathing” (r = 0.568*), “severity of loud-noisy breathing” (0.760*), and “time spent crying during the night” (r = 0.206).

(3) Validation of CBISQ (table 2): We compared the subjective CBISQ to other objective data obtained from sleep diaries, actigraphy and PSG data. These items of CBISQ showed sleep measures of “nocturnal sleep duration, number of night awakenings, daytime sleep duration and loud-noisy breathing” were significantly correlated with sleep diary, actigraphy and PSG. Items such as “mouth breathing” and “loud-noisy breathing” correlated with “total number of

obstructive apnea during sleep" in PSG. Longer "Daytime sleep duration" also correlated with AHI >1; We were interested in these correlations as "mouth breathing" and "Loud-noisy breathing" are common symptoms associated with pediatric obstructive sleep apnea. Moreover, higher AHI means severe OSA will interrupt sleep and increase night awakenings and then induce daytime sleepiness. Therefore, the trend in the correlation between "AHI" and "number of obstructive apnea" in PSG and "Daytime sleep duration" in CBISQ were important and meaningful. The additional questions in the CBISQ pertaining to respiratory sleep problems also were shown to correlate with PSG.

The PSG data showed that 80.6% of 6-month-old premature infants had an apnea–hypopnea index (AHI) >1 event/hour (mean AHI = 3.63 ± 3.24), mean SaO2 97.01 ± 1.00%, total sleep time 368.83 ± 55.05 mins, sleep efficiency 82.43 ± 14.66%, and REM 24.03 ± 6.43% at polysomnography.

Table 2 Subjective and objective sleep problems of premature infants

Questionnaire	Objective sleep measure	r	P value
CBISQ	**Criterion of Sleep diary**		
Nocturnal sleep duration	Nocturnal sleep duration	0.757	0.011*
Number of night awakenings	Nocturnal awake time	0.632	0.002*
	Numbers of night awakening	0.580	0.001*
	Nocturnal sleep efficiency	−0.644	<0.001*
Daytime sleep duration	Daytime sleep duration	0.630	0.002*
Loud-noisy breathing	Daytime sleep duration	0.348	0.064+
CBISQ	**Actigraphy Criterion**		
Time spent with mouth breathing	Awakening time during sleep	0.514	0.042*
	Nocturnal sleep efficiency	−0.255	0.013*
CBISQ	**PSG Criteria**		
Nocturnal sleep duration	Nocturnal sleep duration	0.545	0.002*
Number of night awakenings	Desaturation index	0.636	0.003*
Daytime sleep duration	AHI in sleep	0.767	0.075+
	Awakening after sleep onset	0.350	0.080+
Time spent with mouth breathing	Obstructive apnea count	0.509	0.026*
Loud-noisy breathing	Arousal count	0.401	0.089+
	Obstructive apnea count	0.535	0.018*

*: P value < 0.05. +: P value < 0.10.
AHI: Apnea-Hypopnea Index (events/hour); Desaturation index: desaturation events/hour; “Arousal count ” means total number of arousal during sleep ; “Obstructive apnea count” means total number of obstructive apnea during sleep.

(4) Comparison of the differences between full-term and premature infants (table 3)

Since our CBISQ was shown to be a reliable and valid tool for sleep measurement, we used the data to compare the differences in sleep between the preterm-infant and full-term-infant groups. The results revealed, as expected, a significant difference between the 2 groups: The premature group preferred a side body position and being held when falling asleep, had more night awakenings, greater subjective mention of presence of a sleep problem by caregivers, louder noisy breathing and more time spent crying during the night. Also, the premature infants had longer nocturnal and daytime sleep duration.

Table 3 Comparison of the preterm-infants group and the full-term-infants group using the CBISQ

CBISQ Sleep measure		Preterm infants	Full-term infants	P value
Location of sleep	Infant crib in a separate room	1.0%	0.0%	-
	Infant crib in parents' room	33.0%	33.3%	0.965
	In parents' bed	64.9%	66.6%	0.828
	Infant crib in room with sibling	1.0%	0.0%	-
Preferred body position	On his/her back	52.2%	51.0%	0.876
	On his/her side	37.7%	31.4%	0.397
	On his/her belly	10.1%	17.6%	0.193
Nocturnal sleep duration (minutes, mean ± SD)		544.87 ±81.93	490.71 ±134.48	0.027*
Nocturnal sleep-onset time# (mean ± SD)		2.61 ± 1.31	2.53 ± 1.02	0.782
Method of falling asleep	While feeding	19.8%	23.1%	0.555
	Being rocked	23.4%	21.5%	0.752
	Being held	24.8%	23.1%	0.769
	In bed alone	12.2%	12.3%	0.986
	In bed near parent	19.8%	20.0%	0.969
Difficulty falling asleep# (mean ± SD)		2.47 ± 1.93	2.16 ± 1.71	0.494
Number of night awakenings# (mean ± SD)		2.28 ± 0.93	1.72 ± 0.67	0.014*
Daytime sleep duration (minutes, mean ± SD)		364.07 ±152.1	271.67 ±133.16	0.014*
Subjective consideration of sleep problems# (mean ± SD)		1.53 ± 0.69	1.21 ± 0.54	0.024*
Time spent with mouth breathing# (mean ± SD)		1.47 ± 0.57	1.20 ± 0.42	0.077+
Loud-noisy breathing# (mean ± SD)		1.88 ± 0.69	1.44 ± 0.51	0.010*
Time spent with crying during night# (mean ± SD)		1.64 ± 0.71	1.26 ± 0.45	0.003*

*: P value < 0.05. +: P value < 0.10.
#: These questions were evaluated by severity, and scored from 1 to 4.

(D). Summary of the study # 1:

(1) CBISQ was shown to be a reliable and valid tool for infant's sleep measurement.

(2) Premature infants have more sleep problems than full-term infants (such as "nocturnal sleep duration", "being held to fall asleep", "number of nighttime awakenings", "daytime sleep duration"," loud-noisy breathing", and "duration spent crying during the night"), also including the known risk of "abnormal breathing during sleep" at 6 months corrected age.

(3) PSG confirmed the presence of a very high percentage (80.6%) of premature infants with AHI > 1 event/hour at 6 months corrected age.

V-2. Minor neurodevelopmental dysfunctions at age 6 months in prematurely born neonates (#2 article published in Early Human Development,2013)

(A) Background

To investigate the 6-month neurodevelopmental outcomes of prematurely born neonates and find the determining neonatal factors of minor neurological dysfunctions (MNDs).

(B) Method

We examined data collected prospectively on 151 infants born before 37th week of gestation in 2010 who were assessed at 6 months corrected age with the Bayley Scales of Infant Development-2nd Edition (BSID-II) and the Denver Developmental Screening Test (DDST). According to gestational age, the study subjects were analyzed separately into three cohorts: very preterm (GA ≦ 28 weeks), preterm (GA 29-32 weeks), and late-preterm (GA 33-36 weeks). The Chi-Square test or Fisher exact tests were used for categorical data, and Student t test was used for continuous variables with normal distributions. The Wilcoxon/Mann-Whitney U test or the Kruskal-Wallis test was used for continuous variables without a normal distribution. All p values are two-sided, and those less than 0.05 were considered to be statistically significant. Multivariate logistic regression was used to determine the independent risk factors of MNDs in preterm neonates. The following social and demographic characteristics were included in the analysis: mother's nationality (foreign spouses, Taiwanese or aboriginal residences), maternal educational level (university level or not), SES, and mother's age (<20, 20-29, or ≧30 years). Neonatal characteristics were determined from medical records as described above. Potential risk factors found individually significant at p ≦ 0.05 were entered together into a set of backward selection for choosing the most significant indicators. These variables were finally analyzed by multivariate logistic regression model adjusted for GA and birth body weight (BBW) after their collinear coefficients were checked by Collinearity Diagnostics. All statistics were performed using the commercially available software SPSS 18.0 for Windows.

(C) Results

(1) Demographic data (table 1)

Of all premature neonates whose parents were approached and signed the informed consent to participate this study, 151 neonates were completely assessed at 6 months corrected age. Seventy-eight percent (118/151) of the neonates had neurodevelopmental assessments between 5.5 to 6.5 months corrected age; of the other 33 children, most were assessed before 7 months of corrected age. The neonatal and family characteristics of three cohorts, defined according to the gestational age, were summarized in Table 1. It worth noticing that most of the mothers were moderately educated and 80% of them were older than 30 years. Approximately one-fourth of children in the study were multiple births (twin, triplet, or higher order). Nearly 8.6% of the cohort was SGA and the rest was all appropriate for gestational age (AGA). The rate of neonates with an Apgar score ≦ 7 at 5 minutes was 29.1%.

Table 1. Characteristics of 151 premature infants assessed at 6 months corrected age: total cohort, very preterm, preterm, and late-preterm infants

	Total cohort (n=151)	Very preterm cohort (GA ≦ 28 wks, n=37)	Preterm cohort (GA 29-32 wks, n=53)	Late preterm (GA 33-36 wks, n=61)
Gestational age (wks), median (range)	32 (24-36)	26 (24-28)	31 (29-32)	34.5 (33-36)
Birth body weight (g), median (range)	1515 (582-3935)	890 (582-1595)	1430 (700-2620)	2135 (1085-3935)
Gender (male: female)	82: 69	19: 18	27: 26	36: 25
Mode of delivery (C/S, n [%])	110 (72.8)	19 (51.4)	40 (75.5)	51 (83.6)
Maternal age				
< 20 y/o, n (%)	3 (2.0)	1 (2.7)	0 (0)	2 (3.3)
20-29 y/o, n (%)	27 (17.9)	9 (24.3)	10 (18.9)	8 (13.1)
≧30 y/o, n (%)	121 (80.1)	27 (73.0)	43 (81.1)	51 (83.6)
Small for gestational age, n (%)	13 (8.6)	1 (2.7)	5 (9.4)	7 (11.5)
5 min Apgar score ≦ 7, n (%)	44 (29.1)	26 (70.3)	12 (22.6)	6 (9.8)
Prenatal betamethasone use, n (%)	106 (70.2)	35 (94.6)	50 (94.3)	21 (34.4)
Postnatal dexamethasone use, n (%)	12 (7.9)	9 (24.3)	3 (5.7)	0 (0)
Multiple births, n (%)	40 (26.5)	7 (18.9)	17 (32.1)	16 (26.2)

Socio-economic status				
High, n (%)	12 (7.9)	3 (8.1)	4 (7.5)	5 (8.2)
Middle, n (%)	113 (74.8)	26 (70.2)	41 (77.4)	46 (75.4)
Low, n (%)	26 (17.2)	8 (21.6)	8 (15.1)	10 (16.4)
Maternal educational level				
Master or higher, n (%)	8 (5.3)	3 (8.1)	1 (1.9)	4 (6.6)
University or Collage, n (%)	49 (32.5)	9 (24.3)	20 (37.7)	20 (32.8)
High school, n (%)	89 (58.9)	23 (62.2)	30 (56.6)	36 (59.0)
Below elemental school, n (%)	5 (3.3)	2 (5.4)	2 (3.8)	1 (1.6)
Maternal Nationality				
Normal residences, n (%)	142 (94.0)	33 (89.1)	50 (94.3)	59 (96.7)
Foreign spouses*, n (%)	5 (3.3)	2 (5.4)	2 (3.8)	1 (1.6)
Aboriginal residences, n (%)	4 (2.6)	2 (5.4)	1 (1.9)	1 (1.6)

*Including woman from Mainland China

C/S: Caesarean section delivery

(2). Neonatal morbidity of premature infants: (table 2)

The majority of the infants had respiratory difficulties at birth and required respiratory support during hospitalization in our NICU (table 2). 11.3% (17/151) of the neonates did not need any respiratory support. RDS, TTNB, and PPHN were diagnosed in 31.8%, 29.1%, and 3.3% of our neonates, respectively. BPD developed in one-fourth of infants, and 9.3% of neonates still required oxygen support (nasal canula) at discharge. As expected, the distributions of these respiratory difficulties were highly associated with their gestational ages. PDA was found near one-fifth of neonates, and three-fourth of those with PDA were treated surgically. Cranial ultrasound showed IVH in 25 (16.3%) neonates, and severe intracranial lesions (IVH grade III/IV or PVL) in 9 (6.0%) neonates. More severe ROP (stage II with plus disease or ≧ stage III) was present in 9.3% of the infants, and 7.3% of them required laser therapy.

Table 2. Neonatal morbidity of 151 premature infants during NICU admission

	Total cohort (n=151)	Very preterm cohort (GA ≦ 28 wks, n=37)	Preterm cohort (GA 29-32 wks, n=53)	Late preterm (GA 33-36 wks, n=61)
Length of hospitalization (d), median (IQR)	45 (24-74.5)	96.5 (73.7-123)	53 (39-65)	22.5 (14.5-30.8)
Duration of ventilation (d), median (IQR)	21 (5-51)	75 (53.5-97)	28 (12-44)	4.5 (0-12)
Diagnosis of respiratory diseases				
Respiratory distress syndrome, n (%)	48 (31.8)	28 (75.7)	17 (32.1)	3 (4.9)
TTNB, n (%)	20 (13.2)	1 (2.7)	12 (22.6)	7 (11.5)
Bronchopulmonary dyplasia, n (%)	38 (25.2)	27 (73.0)	11 (20.7)	0 (0)
PPHN, n (%)	5 (3.3)	2 (5.4)	1 (1.9)	2 (3.3)
Nasal nanula required at discharge, n (%)	14 (9.3)	11 (29.7)	3 (5.7)	0 (0)
Congenital heart disease				
PDA (indomethacin/surgery), n (%)	23 (15.2)/7 (4.6)	15 (40.5)/5 (13.5)	6 (11.3)/2 (3.8)	2 (3.3)/0 (0)
ASD ± VSD, n (%)	16 (10.6)	1 (2.7)	6 (11.3)	9 (14.8)
Complicated CHD, n (%)	1 (0.7)	0 (0)	1 (1.9)	0 (0)
IVH (Grade≦II/Grade III&IV), n (%)	19 (12.3)/6 (4.0)	8 (21.6)/4 (10.8)	5 (9.4)/2 (3.8)	6 (9.8)/0 (0)
PVL, n (%)	4 (2.6)	2 (5.4)	2 (3.8)	0 (0)
NEC (probable/definite), n (%)	4 (2.6)/3 (2.0)	2 (5.4)/2 (5.4)	2 (3.8)/1 (1.9)	0 (0)/0 (0)
ROP				
Stage II with plus disease or ≥ stage III, n (%)	11 (7.3)	4 (10.8)	7 (13.2)	0 (0)
Laser treatment required, n (%)	14 (9.3)	13 (35.1)	1 (1.9)	0 (0)
History of neonatal sepsis				
Early-onset neonatal sepsis, n (%)	1 (0.7)	1 (2.7)	0 (0)	0 (0)
Late-onset neonatal sepsis, n (%)	26 (17.2)	12 (32.4)	10 (18.9)	4 (6.6)
Rule out sepsis, n (%)	37 (24.5)	15 (40.5)	17 (32.1)	5 (8.2)
Use of TPN, n (%)	108 (71.5)	37 (100)	48 (90.5)	23 (37.7)
Cholestasis, n (%)	17 (11.3)	11 (29.7)	3 (5.7)	3 (4.9)

IQR: interquartile range, TTNB: transient tachypnea of newborn, PDA: patent ductus arteriosus, ASD: atrial septal defect, VSD: ventricular septal defect, CHD: congenital heart disease, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia, NEC: necrotizing enterocolitis, ROP: retinopathy of prematurity, TPN: total parenteral nutrition

(3). The developmental evaluation results:

In table 3 the median BSID-II scores and the results of DDST are presented. A MDI and a PDI of < 70 were noted in 6 (4.0%) and 20 (13.2%) of our study subjects, respectively. All six neonates with MDI < 70 were found to have PDI < 70. There are obvious trends of mental delay and psychomotor delay as gestation decreases, although the difference did not reach statistical significance. Extremely low birth weight (ELBW, BBW < 1000g) neonates accounted for half of those with psychomotor delay and the majority of mental delay (4/6, 67%) in our study population. By DDST, 22 (14.6%) infants had at least one of four categories of delay at 6-months-old assessments; 2 had delay in all four categories, 5 had delay in two categories, and 15 had delay in one category. The most common category of delay was gross motor skills, which was noted in approximately 13.2% infants. The results of DDST gross motor subscale and PDI were found to be highly corrected; among those who failed in the DDST gross motor subscale by examiners, all had a PDI below 70. No significant difference in the rate of neurodevelopmental delay was found between SGA children and AGA children.

We determined which of the maternal sociodemographic or neonatal factors were associated with MND in the three cohorts. After combination of BSID-II and DDST, a total of 20 infants were found to have MNDs. None of the mothers' social and demographic characteristics (as recorded at birth) were found to be significantly associated with MND.

Table 3. Bayley Scales of Infant Development-II: psychomotor and mental index scores and Denver Development Screening Test in preterm neonates at 6 months of corrected age

	GA ≦ 28 weeks (n= 37)	GA 29-32 weeks (n= 53)	GA 33-<37 weeks (n= 61)
BSID-II index			
MDI, median (IQR)	98.0 (90.0-103.5)	97.0 (89.8-102.8)	96.5 (91.0-105.0)
MDI < 70 (n, %)	4 (10.8)	1 (1.9)	1 (1.6)
PDI, median (IQR)	85.0 (73.0-101.5)	88.0 (79.0-97.0)	91.5 (85.0-104.0)
PDI < 70 (n, %)	8 (21.6)	7 (13.2)	5 (8.2)
DDST			
Any delay, n (%)	8 (21.6)	9 (17.0)	5 (8.2)
Personal-Social delay, n (%)	2 (5.4)	0 (0)	0 (0)
Fine motor delay, n (%)	2 (5.4)	2 (3.8)	1 (1.6)
Language delay, n (%)	4 (10.8)	2 (3.8)	0 (0)
Gross motor delay, n (%)	8 (21.6)	7 (13.2)	5 (8.2)

BSID-II: Bayley Scales of Infant Development-II (Bayley 1993), DDST: Denver Development Screening Test, IQR: interquartile range, MDI: Mental Developmental Index, PDI: Psychomotor Developmental Index

(4). The relationships between neonatal factors and the presence of MNDs:

Table 4 shows the univariate analyses and the final multivariate models that explain the relationships between neonatal factors and the presence of MNDs. Several neonatal factors, including BBW, low Apgar score at 5 minutes and acute fetal distress in the delivery room, confirmed neonatal sepsis, chronic lung disease, cholestasis, length of mechanical ventilation, postnatal corticosteroid use, and the presence of ROP were found to be associated with MNDs of neonates at 6 months old. After multivariate logistic regression adjusted for GA and BBW, MND was independently associated with postnatal corticosteroid use (odds ratio [OR], 11.2; 95% confidence interval [CI], 1.9-66.0, P= 0.008) and cholestasis (OR, 6.2; 95% CI, 1.16-33.1, P=0.033). Besides, neonates with BBW less than 1000g were significantly associated with MNDs when compared with those with BBW more than 2000g (OR, 51.4; 95% CI, 1.9-1369, P=0.019).

Table 4. Neonatal characteristics associated with psychomotor delay in 6-month-old neonates born preterm, with multivariate analysis comparing normal vs psychomotor developmental delay

Characteristic	Neonates, No. (%)		Univariate Analysis		Multivariate Analysis	
	Normal (n=131)	MND (n=20)	Crude OR (95% CI)	P value	AOR (95% CI)	P value
GA, (weeks)						
≦28	29 (22.1)	8 (40.0)	3.1 (0.9-10.3)	0.066	10.0 (0.8-127)	0.075
29-32	46 (35.1)	7 (35.0)	1.7 (0.5-5.7)	0.389	17.4 (0.9-347)	0.062
33-36	56 (42.7)	5 (25.0)	1 (references)		1 (references)	
Birth body weight, (g)						
<1000	18 (13.7)	10 (50.0)	22.2 (2.6-186.9)	0.004	51.4 (1.9-1369)	0.019
1000-1499	42 (32.1)	4 (20.0)	3.8 (0.4-35.6)	0.241	5.8 (0.4-79.8)	0.190
1500-1999	31 (23.7)	5 (25.0)	6.5 (0.7-58.1)	0.096	10.2 (0.9-106)	0.052
≧2000	40 (30.5)	1 (5.0)	1 (references)		1 (references)	
SGA	12 (9.2)	1 (5.0)	0.5 (0.1-4.2)	0.543		
Male gender	68 (51.9)	14 (70.0)	2.2 (0.8-6.0)	0.137		
Caesarean section delivery	96 (73.3)	14 (70.0)	0.9 (0.3-2.4)	0.759		
Multiple births	35 (26.7)	4 (20.0)	0.6 (0.2-2.1)	0.722		
Antenatal steroids	63 (48.1)	13 (65.0)	1.8 (0.7-4.8)	0.242		
Apgar score ≦ 7 at 5 min	33 (25.2)	11 (55.0)	3.4 (1.3-8.9)	0.014		
Acute fetal distress requiring resuscitation in the delivery room*	7 (5.3)	4 (20.0)	4.2 (1.1-15.9)	0.036		
Postnatal corticosteroid therapy	10 (7.6)	12 (60.0)	20.1 (5.3-77.0)	<0.001	11.2 (1.9-66.0)	0.008
Bronchopulmonary dysplasia	28 (21.4)	10 (50.0)	3.5 (1.3-9.2)	0.012		
Length of mechanical ventilation (d) per additional 10 days increase**	-	-	1.3 (1.2-1.6)	<0.001		
Confirmed neonatal sepsis	19 (14.5)	8 (40.0)	3.7 (1.3-10.3)	0.012		
Retinopathy of prematurity#	16 (12.2)	9 (45.0)	5.6 (2.0-15.5)	0.001		
Cholestasis	9 (6.9)	8 (40.0)	8.6 (2.8-26.4)	<0.001	6.2 (1.16-33.1)	0.033
Intraventricular hemorrhage (Grade III or IV)	4 (3.1)	2 (10.0)	3.4 (0.6-19.7)	0.179		
Periventricular leukomalacia (PVL)	4 (3.1)	0 (0)	-	0.549		
Oxygen requirement at discharge¶	10 (7.6)	4 (20.0)	2.9 (0.8-10.3)	0.104		
Breast feeding at discharge	113 (86.3)	18 (90.0)	1.3 (0.3-4.4)	0.578		

*Including those required cardiac massage or epinephrine through endotracheal tube in the delivery room
**Including conventional ventilation and high frequency oscillatory ventilator use
#Including those with stage II with plus disease or ≧ stage III or those required laser therapy

(D) Summary of study # 2:

(1). Premature neonates, even those born at 33 to 36 weeks, are found to have MNDs as early as 6 months corrected age by BSID-II and DDST, with risk increasing as gestation decreases.

(2). Moreover, we used multivariate logistic regression to evaluate the relationships between neonatal factors and the presence of MNDs. After multivariate logistic regression adjusted for GA and BBW, MND was independently associated with postnatal corticosteroid use and cholestasis. Besides, neonates with BBW less than 1000g were significantly associated with MNDs when compared with those with BBW more than 2000g.

V-3. Sleep-disordered breathing, Craniofacial development and Neurodevelopment in Premature Infants: A 2 years following Study (This article is going to be submitted/ unpublished)

(A) Background

Premature infants are reported to be at greater risk of developing sleep disordered breathing and obstructive sleep apnea has been reported not only to be more severe in adults with past history of prematurity, but also to be associated with clear narrow hard palate and very narrow upper airway. How does that risk develops, what is the frequency of such risk, is it associated with greater risk of poor sleep in premature children and does the association of SDB early on impact early development of premature infants are the questions that have not been previously responded. The relationship between specific findings and pathophysiology is also still unclear. Our study aims at bringing further information on the development of SDB in premature infants, its relations with anatomic development of the oro-pharynx based on clinical information and the potential impact on neuro-cognitive development. To investigate these issues we created a prospective cohort of premature infants recruited at birth and followed up to 2 years of age collecting information on general development and sleep and mental development, presence of obstructive SDB and association with narrow upper airway.

(B) Method

Neonates of gestational age less than 37 weeks were enrolled. Basic obstetric and birth data were collected and participants were followed up at 6, 12, 18, and 24 months of corrected age. The assessments (1) craniofacial assessment by inspection and photo documentation of the shape of the hard palate or cephalometric x ray and water view, (2) sleep recording by Chinese Brief Infant Sleep Questionnaire (CBISQ), sleep diary, actigraphy, and night-time polysomnography, and (3) development assessment by the Bayley-Scales of Infant Development (BSID)–II and the Denver Developmental Screening Test (DDST) were done at each visit. Demographic data are calculated. Paired-sample t test is performed to compare follow-up data with their initial values. Statistics are performed on the software PASW Statistics (SPSS) 18.

(C) Results

(1) Demographic data (table 1)

244 premature and 30 full term infants were enrolled. The demographic and initial data at time of enrollment are presented in table 1. Premature infants are more frequently with high-and-narrow palate (HNP) at birth (62.3%) than term infants (10.0%). Premature infants are further divided into 2 groups according to presence of HNP. Individuals with HNP have smaller body size figures, especially head circumference, and are at higher risk of intubation and difficult feeding than those without.

Table 1 Demographic data of premature infants, with and without high-and-narrow palate (HNP) at birth.

	Full term infants (n = 30)	**Premature infants**			
		Total (n = 244)	**HNP (n = 152, 62.3%)**	**No HNP (n = 92, 37.7%)**	***p* value**
Male gender, n (%)	15 (50.0)	139 (57.0)	84 (55.3)	55 (59.8)	0.490
Birth history					
Gestational age (weeks), mean ± SD	39.27 ± 1.01	31.54 ± 3.22**	31.23 ± 3.31	32.03 ± 3.01	0.060
Body weight (grams), mean ± SD	3131.0 ± 390.0	1691.9 ± 593.9**	1665.0 ± 625.3	1736.3 ± 538.2	0.365
Body height (cm), mean ± SD	49.38 ± 2.03	40.54 ± 5.26**	40.19 ± 5.56	41.11 ± 4.72	0.194
Head circumference (cm), mean ± SD	34.07 ± 1.21	29.41 ± 3.42**	29.07 ± 3.53	29.97 ± 3.18	0.050*
Cesarean section, n (%)	9 (30.0)	172 (70.5)††	104 (68.4)	68 (73.9)	0.424
Intubation, n (%)	2 (6.7)	134 (54.9)††	91 (59.9)	43 (46.7)	0.062
Difficult feeding, n (%)	3 (10.0)	114 (46.7)††	80 (52.6)	34 (37.0)	0.025†
HNP at birth, n (%)	3 (10.0)	152 (62.3)††	-	-	-
Polysomnographic data					
AHI (events/hour), mean ± SD	n/a	3.00 ± 2.95	3.52 ± 3.32	2.02 ± 1.73	0.000**

*Significant difference between groups (independent *t* test $p < 0.05$)
**Highly significant difference (independent *t* test $p < 0.001$)
†Significant difference between groups (Pearson's χ^2 test $p < 0.05$)
††Highly significant difference between groups (Pearson's χ^2 test $p < 0.001$)
AHI, apnea-hypopnea index.

(2). Polysomnographic data and neurodevelopment is different between premature infants with and without high-and-narrow palate (HNP)

With regard to sleep problems: the mean apnea-hypopnea index (AHI) obtained from PSG is significantly higher in HNP group (3.52* ± 3.32) than in the non-HNP group (2.02* ± 1.73) (Figure 1). Table 2 displays the detailed PSG findings of premature infants, showing significantly higher proportion of infants with sleep problem (defined by AHI > 1) in the HNP group (84.7%) than in the non-HNP group (68.7%).

Table 2a Polysomnographic characteristics in premature infants, with and without high-and-narrow palate (HNP) at birth.

Variables	Total **(n = 181)**	**HNP: 65.2% (n = 118)**	**No HNP:34.8% (n = 63)**	***p* value**
AHI (1/hour) in sleep, mean ± SD	3.00 ± 2.95	3.52 ± 3.32	2.02 ± 1.73	0.000**
AHI > 1, n (%)	143 (79.0)	100 (84.7)	44 (68.7)	0.011†
AI (1/hour), mean ± SD	1.03 ± 1.05	1.03 ± 1.00	1.01 ± 1.15	0.924
HI (1/hour), mean ± SD	2.21 ± 2.73	2.62 ± 3.10	1.44 ± 1.58	0.001**
RDI (1/hour), mean ± SD	3.99 ± 3.76	4.30 ± 4.05	3.37 ± 3.07	0.163
RERA (counts), mean ± SD	3.41 ± 6.73	3.30 ± 7.35	3.62 ± 5.31	0.786
Sleep latency (min), mean ± SD	15.51 ± 27.06	15.39 ± 22.84	15.80 ± 33.52	0.934
Sleep efficiency (%), mean ± SD	83.9 ± 13.8	83.3 ± 14.5	84.8 ± 12.4	0.485
N1 sleep (%), mean ± SD	15.3 ± 8.9	15.5 ± 9.2	15.0 ± 8.4	0.716
N2 sleep (%), mean ± SD	40.3 ± 10.6	39.1 ± 10.8	42.7 ± 9.7	0.027*
N3 sleep (%), mean ± SD	18.2 ± 11.4	19.2 ± 12.7	16.3 ± 8.2	0.107
REM sleep (%), mean ± SD	23.7 ± 6.5	24.0 ± 6.9	23.1 ± 5.6	0.400
PLMS index (1/hour), mean ± SD	3.19 ± 7.97	3.89 ± 9.18	1.96 ± 5.17	0.294
Mean SaO_2 (%), mean ± SD	97.60 ± 0.84	97.54 ± 0.92	97.70 ± 0.66	0.152

*Significant difference (independent *t* test $p < 0.05$)
**Highly significant difference (independent *t* test $p < 0.001$)
†Significant difference (Pearson's χ^2 test $p < 0.05$)
‡Only 181 of the 244 infants received PSG examination.
AHI, apnea-hypopnea index; AI, apnea index; HI, hypopnea index; RDI, respiratory disturbance index; RERA, respiratory-effort related arousal; PLMS index, periodic leg movements in sleep index; Mean SaO2, mean oxygen saturation.

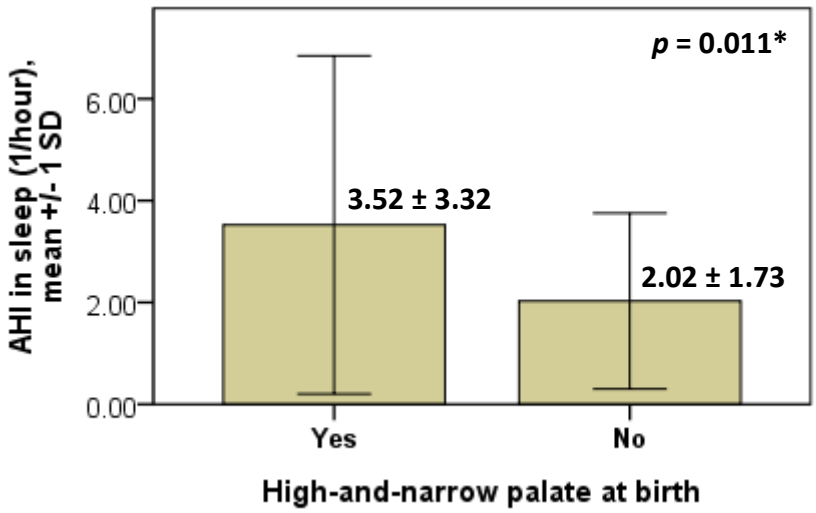


Table 2b PSG data following 2 years in premature infants

Corrected age, years (n =181)	**0.5 year**	**1 year**	**1.5year**	**2 year**
AHI, mean ± SD	5.61 ± 3.75	5.41 ± 4.16	3.54 ± 4.98	4.03 ± 1.67
AHI > 1/hr, n (%)	99.5%	97.2%	86.2%	88.2%
AI, mean ± SD	1.84 ± 1.77	1.59 ± 1.63	1.29 ± 1.56	1.49 ± 0.85
HI, mean ± SD	4.25 ± 4.25	3.78 ± 3.72	2.37 ± 3.94	1.58 ± 1.55
RDI, mean ± SD	8.10 ± 4.59	6.70 ± 5.38	5.07 ± 6.89	4.39 ± 4.42
Efficiency%, mean ± SD	85.75 ± 7.16	83.71 ± 9.19	82.17 ± 14.99	86.5 ± 10.78
Awake%, mean ± SD	12.09 ± 5.94	12.19 ± 7.77	12.83 ± 15.27	8.30 ± 8.60
REM%, mean ± SD	26.03 ± 5.36	25.43 ± 5.90	26.29 ± 14.11	24.15 ± 3.79
TST, mean ± SD	384.4 ± 35.3	378.2 ± 34.6	371.0 ± 61.4	400.3 ± 45.3
PLMS Index, mean ± SD	4.39 ± 7.01	3.03 ± 6.07	5.00 ± 10.85	2.50 ± 5.74
MeanSaO2%, mean ± SD	98.39 ± 0.84	97.58 ± 1.45	97.62 ± 0.82	97.59 ± 0.66
High and narrow palate (+),n (%)	118(65.2%)	101(55.8%)	108(59.7%)	96(53.0%)

Table 3 shows proportions of premature infants with developmental delays, identified with Denver-II and BSID-II at follow-ups. The 244 infants were sorted into 4 groups, according to presentation of HNP at birth and subsequent alternations till 2 years corrected age. Infants with persistent presence and absence of HNP are classified as group 1 (n = 110) and group 2 (n = 68), respectively. In group 3 (n = 42) are Infants with HNP initially but who later improved, and in

group 4 (n = 24) are those infants born without but who later developed HNP. [We have not analyzed the data of group 3 and 4 in this article].

Comparing group 1 and group 2, a higher proportion of group 1 infants are with developmental delays identified with all Denver-II items at all of the time points. Figure 2 representatively shows proportions of infants with Denver-II total score (i.e., any delay)-defined delays in group 1 and 2. Regarding BSID-II, using 75 as cutoff, higher proportion of group 1 infants has low psychomotor development index (PDI); but proportions with low mental development index (MDI) render no difference.

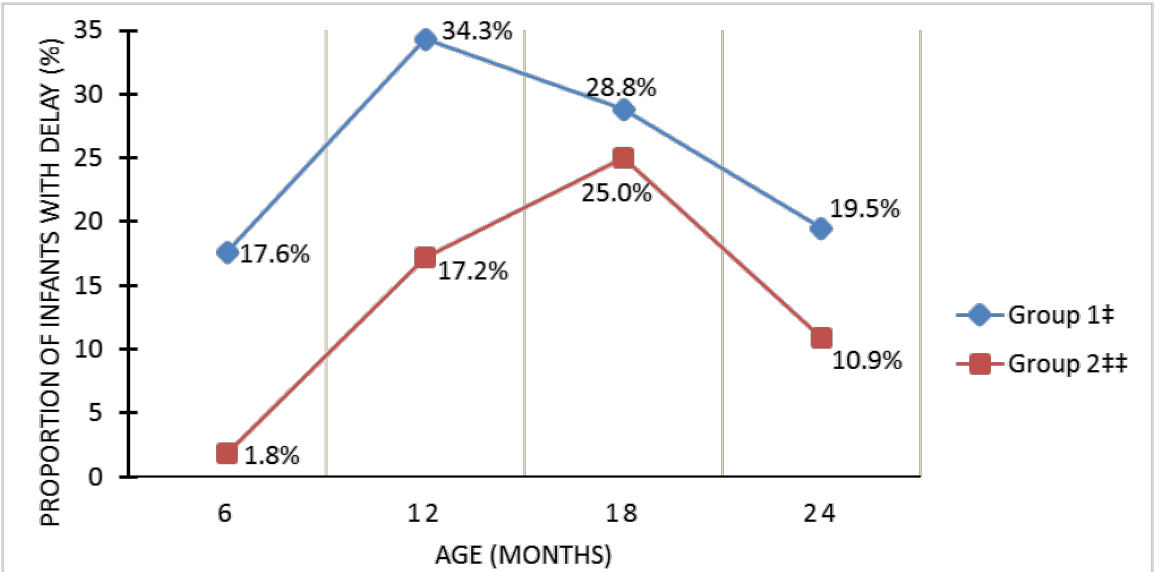
Table 3 Relationship between HNP and Developmental delays (DDST and Bayley–II)

Age	6 months			12 months		
(N = 244)	Total	Group 1‡	Group 2‡‡	Total	Group 1	Group 2
Denver–II						
Any delay	14.8%	17.6%	1.5%	27.8%	34.3%	17.5%
Personal-social delay	2.5%	1.9%	1.5%	10.3%	10.5%	7.9%
Fine-motor adaptive delay	2.5%	3.7%	0.0%	5.1%	8.6%	1.6%
Language delay	2.9%	1.9%	0.0%	20.9%	28.6%	9.5%
Gross motor delay	12.3%	16.7%	1.5%	15.4%	16.2%	7.9%
BSID–II						
MDI < 75	6.4%	4.8%	5.4%	9.7%	8.2%	8.7%
PDI < 75	19.1%	25.8%	2.7%	18.5%	24.6%	8.7%
Age	18 months			24 months		
	Total	Group 1	Group 2	Total	Group 1	Group 2
Denver–II						
Any delay	28.3%	28.8%	25.4%	16.6%	19.5%	11.1%
Personal-social delay	8.1%	10.6%	3.4%	5.5%	9.1%	0.0%
Fine-motor adaptive delay	6.3%	9.6%	1.7%	5.5%	7.8%	2.2%
Language delay	23.8%	25.0%	18.6%	11.7%	10.4%	11.1%
Gross motor delay	10.8%	12.5%	8.5%	8.0%	11.7%	0.0%
BSID–II						
MDI < 75	20.0%	13.8%	13.3%	19.4%	18.2%	18.8%
PDI < 75	7.3%	6.9%	0.0%	8.3%	9.1%	0.0%

‡Group 1: children with persistent presence of high and narrow palate till 2 years corrected age.
‡‡Group 2: children with persistent absence of high and narrow palate till 2 years corrected age.
Denver–II, Denver Developmental Screening Test–II; BSID–II, Bayley Scales of Infant Development–II; MDI, mental development index; PDI, psychomotor development index.

Figure 2

The relationship between developmental delay (Denver-II: any delay) and HNP



‡Group 1: children with persistent presence of high and narrow palate till 2 years corrected age.
‡‡Group 2: children with persistent absence of high and narrow palate till 2 years corrected age.

Different sleep problems of premature infants in the following years were showed in Table 4.

Table 4. CBISQ data in premature infants following 2 years

Corrected age (N=244)	0.5 years (N=244)	1 year (N=226)	P value	1.5 years (N=203)	P value	2 years (N=151)	P value
BISQ1_a, n (%)	3(1%)	6(2.4%)	-	5(0.9%)	-	4(2.7%)	-
BISQ1_b, n (%)	94(32.9%)	54(10.2%)	-	45(22.6%)	-	21(14.3%)	-
BISQ1_c, n (%)	178(62.2%)	185(74.6%)	-	148(74.4%)	-	101(68.7%)	-
BISQ1_d, n (%)	2(0.7%)	2(0.8%)	-	0(0%)	-	7(4.8%)	-
BISQ2_a, n (%)	173(60.7%)	96(38.7%)	-	77(38.7%)	-	46(31.3%)	-
BISQ2_b, n (%)	117(41.1%)	153(61.7%)	-	107(53.8%)	-	90(61.2%)	-
BISQ2_c	33(11.6%)	76(30.6%)	-	86(43.2%)	-	51(34.7%)	-
BISQ3min	523.92±107.7	537.89±84.77	.019	548.97±88.51	.009	555.30±70.39	.006
BISQ3_1	2.67±1.38	2.44±1.38	.019	2.16±1.16	.000	2.34±1.32	.007
BISQ3_2_a	77(28.4%)	67(27.1%)	-	31(15.7%)	-	22(15%)	-
BISQ3_2_b	92(17.3%)	39(15.8%)	-	21(10.6%)	-	8(5.4%)	-
BISQ3_2_c	113(41.5%)	41(16.6%)	-	26(13.1%)	-	5(3.4%)	-
BISQ3_2_d	44(16.2%)	38(15.4%)	-	54(27.3%)	-	27(18.4%)	-
BISQ3_2_e	74(27.2%)	124(50.2%)	-	111(56.1%)	-	108(73.5%)	-
BISQ3_3	2.55±1.91	2.12±1.75	.044	1.80±1.63	.000	1.85±1.53	.000
BISQ3_3_1	2.34±2.42	2.79±4.04	.075	2.63±5.03	.857	3.07±6.28	.314
BISQ3_4	4.31±2.93	3.77±3.03	.249	3.21±2.60	.000	2.72±2.36	.000
BISQ3_5	2.05±0.94	1.97±0.98	.158	1.81±0.98	.090	1.65±0.77	.000
BISQ3_5_1	1.85±1.35	1.78±1.42	.780	1.42±1.26	.003	1.18±0.70	.000
BISQ3_6	1.62±1.10	1.70±1.49	.394	1.33±0.96	.046	1.23±0.73	.005
BISQ3_6_1	2.54±2.70	2.86±4.32	.824	3.40±6.07	.405	3.30±6.75	.716
BISQ4_1	1.84±1.79	1.60±1.41	.557	1.45±1.31	.126	1.42±1.05	.012
BISQ4_2	1.61±2.43	1.94±3.65	.067	2.55±5.63	.511	2.68±6.44	.606
BISQ5min	276.34±135.0	223.42±99.70	.000	198.49±97.90	.000	172.96±89.18	.000
BISQ6	1.53±0.72	1.55±0.73	.161	1.48±0.72	.117	3.10±19.55	.326
BISQ7	1.41±0.54	1.49±0.60	.219	1.55±0.62	.510	1.56±0.63	.004
BISQ8	1.58±0.67	1.47±0.59	.004	1.47±0.63	.021	1.46±0.60	.895
BISQ9	1.59±0.67	1.48±0.64	.050	1.40±0.65	.000	1.40±0.60	.011

(D) Summary of the study # 3:

These data showed that:

(1). Premature infants are presenting with a more narrow and high-arched palate, more SDB-related sleep problems, and more neurodevelopmental deficits than normal full-term infants.

(2). Till age 2 years, premature infants with narrow and high-arched palate have more SDB-related sleep problems and more neurodevelopmental deficits than those without.
(3). These findings support our hypothesis that high and narrow-arched palate in premature infants plays a role in the development of sleep problems and neurodevelopmental delays.

V-4. Development of Chinese-version Pediatric Obstructive Sleep Apnea Questionnaire(OSA-18) and To find the common symptoms of Pediatric OSA in Taiwan (# 4 article was published in Psychiatry and Clinical Neurosciences, 2016)

(A) Background

The OSA-18 is designed to screen OSA and has good reliability and validity. The goal of this study is to validate the Chinese version of the OSA-18, and to analyze the frequency of symptoms and find the common symptoms of OSA in Taiwanese children.

(B) Method

We validated the OSA-18 on an ethnic Chinese group and compared the treatment outcomes to show the sensitivity of the questionnaire. The caregivers completed the questionnaire twice at an interval of 4 weeks to test reliability. In the validation study, we included 88 OSA children. The OSA-18 and follow-up PSGs were performed before and 6 months post-adenotonsillectomy.

(C) Results

(1) Demographic data:

We enrolled 163 students (89 boys and 74 girls, mean age: 9.5+1.7 years) whose parents completed the questionnaire twice were included in **study 1** (the school group). The clinical participants for **study 2** included 88 subjects (72 boys and 16 girls, mean age: 8.9+2.7 y/o, BMI=19.54 + 4.64) with OSA who had TA surgical treatment (the OSA group).

The PSG data of the OSA children showed an AHI= 13.54+7.23 events/hour (range: 1.9 to 19.7 events/hour). The reliability statistics showed the Cronbach's alpha of the Chinese-language version of the OSA-18 questionnaire to be 0.897, meeting the significance level.

(2). The validation of Chinese version of the OSA-18

The study results showed a well-fitting model for the sample (CFI = 0.97, RMSEA = 0.073, SRMR = 0.079). Standardized coefficients are reported in Table 1. Factor loadings of the “Sleep Disturbance items” of the pretest ranged from 0.57 to 0.70, and those of the post-test ranged from 0.66 to 0.76 and were significant. Loadings of the “Physical Suffering items” of the pretest ranged from 0.40 to 0.85, and those of the post-test ranged from 0.50 to 0.84 and were significant. Loadings of the “Emotional Distress items” of the pretest ranged from 0.77 to 0.88, and those of the post-test ranged from 0.79 to 0.85 and were significant. Loadings of the “Daytime

Situation items" of the pretest ranged from 0.51 to 0.88, and those of the post-test ranged from 0.58 to 0.86 and were significant. Loadings of the "Caregiver Concern items" of the pretest ranged from 0.77 to 0.82, and those of the post-test ranged from 0.79 to 0.84 and were significant. There was a demonstration of convergent validity for these 10 latent factors (Figure 2). Correlations of the 5 latent factors for the pretest ranged from 0.40 to 0.75, and those of the post-test ranged from 0.52 to 0.85 and were not higher than 0.85, indicating discriminate validity for the pretest and the post-test. The factor intraclass correlations of "Sleep Disturbance", "Physical Suffering", "Emotional Distress", "Daytime Situation", and "Caregiver Concern" were 0.91, 0.78, 075, 0.76, and 0.89, respectively (see Table 1), demonstrating the presence of sufficient test-retest reliability. The composite reliability coefficients for these 10 factors were acceptable (the Sleep Disturbance of the pretest: CR = 0.72; the Physical Suffering of the pretest: CR = 0.83; the Emotional Distress of the pretest: CR = 0.86; the Daytime Situation of the pretest: CR = 0.73; the Caregiver Concern of the pretest: CR = 0.88; the Sleep Disturbance of the post-test: CR = 0.79; the Physical Suffering of the post-test: CR = 0.84; the Emotional Distress of the post-test: CR = 0.86; the Daytime Situation of the post-test: CR = 0.75; the Caregiver Concern of the post-test: CR = 0.89). The Chinese version of the OSA-18 demonstrated good reliability.

Table 1 Correlation matrix of latent factors

	SD_1	**PS_1**	**ED_1**	**DS_1**	**CC_1**	**SD_2**	**PS_2**	**ED_2**	**DS_2**
PS_1	0.71								
ED_1	0.45	0.40							
DS_1	0.56	0.50	0.75						
CC_1	0.75	0.61	0.62	0.60					
SD_2	**0.91**	0.57	0.43	0.49	0.64				
PS_2	0.61	**0.78**	0.34	0.47	0.59	0.67			
ED_2	0.56	0.43	**0.75**	0.64	0.61	0.63	0.52		
DS_2	0.56	0.48	0.66	**0.76**	0.71	0.62	0.59	0.85	
CC_2	0.68	0.53	0.48	0.52	**0.89**	0.72	0.66	0.67	0.79

Note: SD_1: Sleep Disturbance of the pretest; PS_1: Physical Suffering of the pretest;ED_1: Emotional Distress of the pretest; DS_1; Daytime Situation of the pretest; CC_1; Caregiver Concern of the pretest; SD_2:Sleep Disturbance of the post-test; PS_2: Physical Suffering of the post-test; ED_2: Emotional Distress of the post-test; DS_2; Daytime Situation of the post-test; CC_2; Caregiver Concern of the post-test; The bold numbers are the test-retest reliability.

To establish the construct validity of a measure, nomological validity should be taken into consideration. Nomological validity generally deals with whether the constructs under investigation are related to other constructs in a way that is theoretically meaningful. In this study, we presented 2 kinds of nomological network. One is the relation of the constructs of the OSA-18 with QOL, and the other is the difference shown by the 2 subject groups (AHI>1 and AHI≦1) in the scores of the constructs for OSA-18. There was a presence of substantial correlation between QOL and the constructs for OSA-18 in the pretest and post-test (Tables 2a and 2b); these results demonstrated the strong nomological validity of the Chinese version of the OSA-18. The results also showed an improvement in the QOL score, from 5.12+1.58 to 6.05+1.76 (p=0.001) after AT.

Table 2a. Correlation of the constructs of OSA with QOL

	SD_1	**PS_1**	**ED_1**	**DS_1**	**CC_1**
QOL_pre	-0.40***	-0.35***	-0.41***	-0.38***	-0.57***
	SD_2	**PS_2**	**ED_2**	**DS_2**	**CC_2**
QOL_post	-0.44***	-0.37***	-0.37***	-0.48***	-0.61***

Note: The correlation coefficients are derived from CFA.
***p<0.001; QOL: quality of life

In terms of discriminating pediatric OSA from normal controls using the OSA-18, the area under the curve (AUC) for differentiating pediatric OSA from normal controls was 0.856. The value of 0.50 was not included within a 95% confidence interval of the AUC (0.80–0.96), strongly suggesting that the discriminative capability of the Chinese version of the OSA-18 is statistically sound. The ROC analysis included sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV), with cut-off scores ranging from 65 to 66 (Table 3).

Table 3 - ROC analysis for discrimination of OSA and school groups in Chinese version of the OSA-18 (receiver operator characteristic)

Cut-off point	*sensitivity*	*specificity*	*PPV*	*NPV*
55 score	0.87	0.64	0.80	0.74
59 score	0.84	0.64	0.80	0.70
60 score	0.84	0.64	0.80	0.70
63 score	0.84	0.64	0.80	0.70
64 score	0.84	0.64	0.80	0.67
65 score	0.82	0.71	0.84	0.68
66 score	0.79	0.77	0.86	0.68

PPV: positive predictive value; NPV: negative predictive value

(2) The common symptoms of pediatric OSA in Taiwanese children:

AHI and SaO2 significantly improved after AT without significant change in BMI (Table 4). There was improvement in all items of the OSA-18 6 months' post-TA (Table 4). Moreover, the most common symptoms of pediatric OSA were: + Poor attention span (93.1%), + Loud snoring (88.0%), +Caregiver worries about child's health(87.1%), +Difficulty awakening (86.1), and +mouth breathing (79.2%) (Table 4). After TA surgery, the most improved symptoms were "Daytime drowsiness and Poor attention span" (Table 5).

Table 4 PSG data and the clinical characteristic data pre- and 6 months post-TA

Variable (N=88)	Pre-surgery	Post-surgery (6 months)	P value
Male (%)	N=72(81.8%)	N=72(81.8%)	-
Female (%)	N=16(18.2%)	N=16(18.2%)	
Age (y/o) (mean±SD)	8.9 ± 2.7	9.6 ± 2.53	0.73
BMI (Kg/m^2) (mean±SD)	19.54 ±4.64	19.31 ±4.17	0.778
Tonsil hypertrophy (more than grade 2)	97.7%	0	<0.001
Adenoid hypertrophy	84.1%	0	<0.001
PSG findings(n=88)			
AHI (events/hr)	13.54+7.23	3.47 ±2.01	.001
AI (events/hr)	4.06 ±3.69	1.03 ±0.70	.023
PMLI (events/hr)	1.58 ±3.65	1.02 ±1.19	.878
REM (%)	11.14±6.05	14.00±6.31	.021
Stage 1 (%)	19.09 ±8.65	15.39 ±7.58	.072
Stage 2 (%)	35.11 ±12.71	26.11 ±4.48	.069
Stage 3+Stage 4 (%)	35.54 ±6.01	44.53 ±10.10	.034
Mean SaO2	96.20 ± 2.10	96.92 ± 1.56	.032
sleep efficiency (%)	87.26 ±12.72	88.73 ± 10.52	.395
Sleep latency (mins)	18.97 ± 9.36	15.02 ±7.41	.040
Total sleep time (mins)	425.79 ± 35.26	429.21 ± 36.48	.287

BMI: Body Mass Index, BW: body weight AHI: Apnea-Hypopnea Index, AI: Apnea Index; mean SaO2: mean oxygen saturation; PLMI: Periodic Leg Moment Index

Table 5 Change in OSA-18 Chinese version questionnaire scores after adenotonsillectomy (n=88)

Item	Pre-surgery		Post-surgery 6 months		P value
	score (No. 1 to No. 3)	**score (No. 4 to No. 7)**	**score (No. 1 to No. 3)**	**score (No. 4 to No. 7)**	
Item 1	12.0%	88.0%	24.8%	75.2%	.001
Item 2	38.4%	61.6%	88.2%	11.8%	.001
Item 3	49.0%	51.0%	88.4%	11.6%	.001
Item 4	37.6%	62.4%	76.8%	23.2%	.001
Item 5	24.8%	79.2%	76.7%	23.3%	.001
Item 6	31.7%	68.3%	73.1%	26.9%	.001
Item 7	27.7%	72.3%	56.5%	43.3%	.001
Item 8	23.0%	77.0%	72.8%	27.2%	.001
Item 9	36.6%	63.4%	58.0%	42.0%	.001
Item 10	51.5%	48.5%	69.6%	30.4%	.001
Item 11	39.4%	60.6%	60.9%	39.1%	.001
Item 12	28.0%	72.0%	71.2%	28.8%	.001
Item 13	6.9%	93.1%	42.0%	58.0%	.001
Item 14	13.9%	86.1%	40.6%	59.4%	.001
Item 15	12.9%	87.1%	55.1%	44.9%	.001
Item 16	24.8%	75.2%	66.2%	33.8%	.001
Item 17	51.5%	48.5%	73.9%	26.1%	.001
Item 18	51.4%	48.6%	53.6%	46.4%	.001
	(scale 1 to 5)	(scale 6 to 10)	(scale 1 to 5)	(scale 6 to 10)	
QOL item	61.2%	38.8%	38.2%	61.8%	.001

Item 1 Loud snoring; 2 Breath holding/pauses; 3 Choking or gasping; 4 Fragmented sleep; 5 Mouth breathing; 6 Frequent colds or URIs; 7 Rhinorrhea; 8 Dysphagia; 9 Mood swings or tantrums; 10 Aggression/hyperactivity; 11 Discipline problems; 12 Daytime drowsiness; 13 Poor attention span; 14 Difficulty awakening; 15 Caregiver worried about child's health; 16 Caregiver concerned child does not have enough air; 17 Caregiver missed activities; 20 Caregiver frustration. (scale 1 means " non of the time"; scale 4 means "some of the time"; scale 7 means "all of the time"). QOL item: quality of life (QOL) via a 10-point visual analogue scale. (The higher scale comes with higher quality of life).

We also analyzed the correlation between OSA-18 and AHI, tonsil size and adenoid hypertrophy. The results showed a statistically significant correlation between OSA-18 total score and AHI ($r=0.291^*$, $p<.05$) and the hypopnea index ($r=0.29^*$, $p<.05$). Also, there was a significant correlation ($r=.532^*$, $p<.01$) between adenoid hypertrophy and OSA-18 total score. However, tonsil size did not correlate with OSA-18 total score.

(D) Summary of the study # 4:

(1). The Chinese-language version of the OSA-18 questionnaire for pediatric OSA is a reliable and valid instrument. It is a suitable screening tool and outcome measure for OSA in Taiwanese children.

(2). The common symptoms in non-obese OSA children were "Poor attention span, Loud snoring, Caregiver worries about child's health, Difficulty awakening, and mouth breathing". The symptom of "mouth breathing" was the most improvement after TA in all symptoms. As we know the "mouth breathing" is associated with the pediatric orofacial development.

V-5. Sleep-Disordered Breathing and oral-facial development in the premature infant (# 5 article was published in Frontier in Neurology, 2013)

(A) Background

From the previous researches and our premature infant study, we hypothesize that "pediatric OSA in non-obese children is a disorder of oral facial growth". Therefore, we review of evidence in support of an oral-facial growth impairment in the development of pediatric sleep apnea in non-obese children.

(B) Review of findings

(1). Review of early historical data in the orthodontic literature indicating the abnormal oral-facial development associated with mouth breathing and nasal resistance.

(2). Review of the progressive demonstration of sleep disordered breathing in children who underwent incomplete treatment of OSA with adenotonsillectomy, and demonstration of abnormal oral-facial anatomy that must often to be treated in order to achieve the resolution of OSA.

(3). Review of long term recurrence data on OSA and indication of oral-facial myofunctional dysfunction in association with the recurrence of OSA.

Since the report of obstructive sleep apnea syndrome in children in 1976 (Guilleminault et al), recognition of abnormal breathing during sleep has progressed. Follow- up of the first cases of children treated by tracheostomy, then by home nasal CPAP that was not well accepted in these teen-agers, led to usage of more specific surgery oriented toward treatment of the upper airway, performing maxilla-mandibular surgery (Powell et al., 1983) with follow-up for more than 25 years in one case indicating complete long term resolution of the OSAS.

(a) Lessons from OSA treatment with adenotonsillectomy :

Despite the widespread use of techniques with limited ability to identify the complete cessation of abnormal breathing and its effects during sleep, studies progressively reported an absence of complete elimination of sleep-disordered-breathing despite significant initial improvement in many tests. Early on two studies showed that prepubertal adolescents who were considered to be completely cured from OSA presented with OSA again as teenagers (Guilleminault et al., 1989). Interestingly, these subjects had a narrowing behind the base of tongue and orofacial

anatomical abnormalities that either did not exist initially or had not been recognized at its earlier stage. Tasker et al (2002) also confirmed the presence of an abnormal upper airway and SDB twelve years after adenotonsillectomy. In more recent studies with larger number of patients, this point was made very clear: Despite a clear improvement of clinical symptoms, Guilleminault et al (2004) reported a complete resolution of OSA following adenotonsillectomy in only 51% of the non-obese prepubertal children they successively studied with systematic PSG at approximately 3 months post-surgery. Tauman et al (2006), Guilleminault et al (2007) and Huang et al (2013) confirmed these findings. In a more recent multi-center study (Bhattacharjee et al., 2010), though 50% of the 500 recruited children had been obese, adenotonsillectomy again improved clinical symptoms and PSG results but did not cure the syndrome in about 70% of the enlisted children. Recently, Chen et al (2012) reported on a systematic prospective study of prepubertal children treated with adenotonsillectomy, who were then considered to have normal PSG according to AASM 2007 scoring criteria (Iber et al., 2007). All children whose parents signed informed consent were included in the prospective follow-up study, which spanned a 5-year period, and included not only systematic PSGs and clinical evaluations, but also attentional neurocognitive testing performed during the daytime that was interpreted blindly by the same trained psychologist and 3D-CT of the upper airway that was also interpreted blindly by the same cranial-facial specialist. The follow-up evaluations performed at 3- and 6- months showed normal PSG (AASM criteria) and neurocognitive scores. However, further follow-up showed once again the simultaneous presence of abnormal breathing during sleep (AASM criteria) and abnormal neurocognitive test results in 40% of the prepubertal children. Moreover, 3D-CT analysis showed abnormal oral-facial development in these children. Kim and Guilleminault (2011) also reported on a systematic investigation of the presentation of anatomical oral-facial abnormalities noted in 400 prepubertal children diagnosed with OSA and enlarged adenotonsillectomy before otolaryngological treatment. It came out that nearly all the children with OSA had at least one type of oral-pharyngeal abnormality from the list of pre-defined potential problems. As expected based on previous findings, clinical symptoms and abnormal PSG persisted in some children at 3-months follow-up after adenotonsillectomy. However, the study showed that many children had one of the many anatomical oral-facial abnormality investigated before surgery and scored based on systematic pre-established evaluation scales: this non-specific scoring was insufficient to predict complete

or incomplete results from adenotonsillectomy. Further analyses produced more specific clinical examination findings that better predicted persistence of abnormal PSG results post adenotonsillectomy. Similar to an analysis performed by Guilleminault and his coworkers (2007), the clinical findings included the presence of a Mallampati (or Friedman-Mallampati) scale score of 3 or 4 (Mallampati et al., 1985), the presence of a deviated septum, and the presence of a small mandible. The Mallampati scale does not identify one particular anatomical abnormality, but an abnormal score represents a combination of deficiencies involving both the nasomaxillary complex and the position of the mandible.

In summary: The findings suggest the need to assess the upper airway on anatomic elements more related to oral facial growth and possibly more generalized oral-pharyngeal growth impairment rather than looking at many anatomic deviations from what is considered the norm.

(b) Lessons from orthodontia and the experimental infant monkey model

European orthodontists showed that abnormal nasal resistance induced by enlarged adeno-tonsils in children were associated with mouth breathing and led to important cranio-facial changes (Haas, 1961; Linder-Aronson, 1969; 1970; Wertz, 1970; Timms, 1974; Gray, 1975; Hershey et al., 1976;McNamara, 1981;Timms, 1984). Ablation of the adeno-tonsils led to cessation of the mouth breathing and progressive return to normal facial development facilitated by orthodontia use in follow-up studies. Other orthodontists stricken by narrowness of the maxilla and its very negative impact on teeth position and facial growth during prepubertal development had performed “rapid maxillary expansion” (RME), and reported that such treatment had also made an impact on a sleep-related complaint. For example, treated children experienced elimination of their nocturnal enuresis. However, the most important findings were obtained on the infant monkeys, when the important role of abnormal nasal resistance during the developmental period was demonstrated.

Between 1970 and 1980, a number of very important experiments on newborn rhesus monkeys were performed, whereby a small silicone head was placed within the nostrils of infant monkeys and held by a thin thread to the nostrils in order to induce a clear nasal resistance for the first 6 months of life, and subsequent removal of the nasal obstruction thereafter (Harvold et al., 1981;Vargervik et al., 1984). As reported by Harvold and his team (1981), the blockade of the nasal passage led to narrowing of dental arches, decrease in maxillary arch length, anterior

cross bite, maxillary over-jet and increase in anterior face height. These results were well illustrated, but the most significant findings in our view, was the report by Vargervik et al (1984) and Miller et al (1984). These researchers from the same team showed that experimentally induced abnormal nasal resistance led to systematic changes in the orofacial muscles. The changes were noted in the systematic recording of different muscles, more particularly the geniohyoid, the genioglossal muscles that constitute the tongue, and also, in the suprahyoid dorsal tongue fibers – the upper lip elevators, and the digastric muscles.

EMG changes were reported to show abrupt induction of rhythmic discharge patterns. This is contrary to normal firing, which is near continuous and desynchronized in any normal individual. Tonic EMG discharges changed back to the normal pattern when nasal breathing was restored at the end of the 6-month experiment. These alterations were entirely related to the abnormal nasal breathing accompanying the increase in nasal resistance. Increase in nasal resistance has a dramatic effect on the maxillomandibular skeleton, halting growth (Harvold et al., 1981) and bringing about adaptive changes in the soft tissues that are associated with deviation in jaw posture and tongue activity (Miller et al., 1984;Vargervik et al., 1984). That is, the nasal obstruction induces functional changes in the nasomaxillary complex and on the mandible. The consequences of the experiments were as follows: There was an absence of development, which impacted the maxilla and restricted the nose and upper jaw; there was a displacement of the mandible leading to mouth breathing and; oral breathing developed in association with increase in nasal resistance, leading to mouth opening and mouth breathing that occurred day and night. These led to the narrowing of the cranial skeleton (Harvold et al., 1981;Miller et al., 1984;Vargervik et al., 1984;Rubin, 1987;Vargervik and Harvold, 1987). Further follow-up showed that these changes were reversible if the experimental nasal resistance was withdrawn while the infant monkey was still in its developmental phase.

In summary: (1) In growing animals in which the nasal airway is gradually occluded, there is an adverse effect on the morphology of the nasomaxillary complex, mandible, and pharyngeal airway space. (2) The morphometric changes are induced by altered functioning of the muscles, with changes in muscle firing that are triggered by abnormal nasal resistance. Obstructive sleep apnea syndrome was mostly unknown at the time of this investigation and no sleep recording was performed on the experimental animals.

(c) Application of work in orthodontia in the field of SDB

More recently, findings that support the incomplete resolution of abnormal oral-pharyngeal growth by adenotonsillectomy have led to the usage of orthodontic techniques to help treat pediatric SDB. Several studies were performed over time, based on reports demonstrating the important role of the mesio-palatine suture in the nasomaxillary complex growth and examining its ossification process in depth. Cartilage is a connective tissue made of chondrocytes embedded in a matrix rich in collagen (particularly type II), associated with proteoglycans in hyaline cartilage that strengthens it, and often elastin-(depending on the type of cartilage). Hyaline cartilage is the forerunner of skeletal bones in the fetus, and endochondral ossification is the process leading to formation of the nasomaxillary complex.

Rapid Maxillary Expansion- RME-(Pirelli et al., 2004) is a procedure applying orthopaedic forces on the mid-palatal sutures using the first molars and permanent premolars as anchor teeth; while in deciduous dentition, the second primary molars are selected as long as they can provide the required firmness. The device is composed of a central expansion screw with four arms: 2 front arms and 2 back arms; the bone distraction at suture level enables an effective enlargement of the maxillary skeletal base. Enlargement is visually appreciable as the bone distraction leads to an inter-incisive space (a diastema), and with X-rays (as the gain appears as a radiotransparency corresponding to the visually seen space). The procedure usually last 3 to 4 weeks with daily turning of a mid-line screw that allows enlargement (distraction) of the space at the level of mid-line suture: The transpalatal force, that exceeds the orthodontic one, produces an orthopaedic force that opens the mid-palatal suture leading to maxillary movement without tipping teeth. Once the needed extension is obtained (end of the activation phase), the midline screw is blocked, and the device is kept in place for at least 4 to 6 months more, to let the newly formed bone strengthen. Unfortunately, such important cartilage is missing from the mandible. Nevertheless, manipulation and verticalization of teeth can stimulate mandibular growth and such bimaxillary distraction is often needed in OSA children. In addition, maxillary widening also seems to independently impact mandibular growth. One negative element of RME is in its anteroposterior lengthening capabilities. That is, it is a limited approach when anteroposterior lengthening is needed. In the past, appliances such as the Herbs appliance or its equivalent were thought to be capable of producing anterior-posterior growth in prepubertal children. However, while such appliances may protrude the lower jaw forward, there is no evidence to date that

more growth than expected with age is attained. Distraction osteogenesis may be performed in these cases, but while such approach is performed in children with clear malformations at birth, it has not been recommended in non-syndromic children with OSA till oral-facial growth is well advanced (Guilleminault and Li, 2004). Normally, 60% of the facial growth is obtained by 6 years of age and about 90% near 11 to 12 years. Thus, distraction osteogenesis is not usually performed before approximately 14 years of age in non-syndromic children with OSA. At that time, there is always a question of whether the anteroposterior advancement will be sufficient on its own or whether the teenager will need both the anteroposterior and the lateral extension. If the latter is need, as is most commonly the case, MMA (Holty and Guilleminault, 2010) is the best recommendation. On the other hand, distraction osteogenesis may be useful in certain cases, such as in the elimination of residual OSA.

In summary*:* (1) The studies presented here indicate that adenotonsillectomy in non-obese children does not "cure" OSA in many prepubertal children, and that oral-facial anatomical problems are clearly involved in the development of OSA in children. Moreover, these anatomical problems may be amenable to orthodontic treatment but not in all subjects. (2) Several studies have shown that RME or bimaxillary distraction have a clear impact on pediatric OSA and may resolve the residual problem that is seen post adenotonsillectomy. The combination of adenotonsillectomy and RME may potentially resolve OSA symptoms completely and a small prospective follow-up study indicates that results may be sustained 36 months' post treatment (Villa et al., 2011).

(d) Interaction between adenotonsils and orofacial growth and evidences from our premature infants

Swedish investigators suggested that children first become mouth breathers, and then their tonsils become subjected to repetitive abnormal stimulations related to mouth breathing, which leads to an inflammatory reaction (Zettergreen et al., 2002). The resulting tonsilar enlargement will involve inflammatory factors such as leukotriene.

<u>Our Premature infants study</u>

Method: Our prospective cohort of 300 infants born between 25 and 37 weeks of gestational age. These infants are evaluated shortly after birth (within the first week) and at 3 months, 6

months, 12 months, 18 months, and 24 months post-birth. Children received clinical evaluations that included developmental scales, and neurological evaluation that included feeding behaviors, actigraphy, PSG, and systematic photographs of the face (frontal and lateral) and of oral regions. Fiber optic illumination is used in the photographs of the oral regions to evaluate the size and presentation of the hard palate, and photos are scored blindly by a specialist who is not involved in the clinical evaluations.

Preliminary results: Three hundred children involved in the cohort have been followed until 24 months of age at the minimum. For this review of findings, infants who had nasal or mouth tube placed at birth were eliminated from evaluation.

(1) The current results showed that all infants below 34 weeks of age present a **high and narrow hard palate**. The width of the palates was between 27 and 31 mm in infants born at 37weeks GA and in 35% of infants born at 36 weeks GA, it was smaller than 27mm in all other infants with smaller width the more premature the infant was. Feeding behavior problems were present in infants 36 weeks and younger. All infants that had to stay in hospital due to premature birth were bottle-fed. Only older infants were breast fed. Moreover, in this series of studies, any infant kept in the hospital immediately after birth was thus separated from their mothers and was bottle-fed with either formula or expressed breast milk.

(2) Currently, 82% of the studied infants (n=207/252) present with a high and narrow hard palate, as well as apnea and/or hypopnea during sleep. Mouth breathing is noted in these children and became more apparent with age, particularly noted at the 1st follow-up visit at +3months post birth.

(3) All children at 35 weeks and younger exhibited limb hypotonia during the general neurological evaluation, older infants demonstrated also hypotonia but in lower number with longer pregnancy duration, hypotonia was indicated by the persistence of a positive “scarf” sign and this positive finding was noted even at 37 weeks of gestational age (Korobkin and Guilleminault, 1979).

(4) At 6 months after birth (i.e., with different degree of maturation due to different gestational age), the 207 mentioned above children without history of intubation showed no sign of an abnormally enlarged tonsil at the clinical evaluation and at the photographic evaluation, but all of them presented with a high and narrow hard palate.

(5) In summary: Enlarged tonsils when present were only noted at later examination after mouth breathing earlier documentation of high and narrow hard palate and mouth breathing during sleep. The hypothesis that the enlargement of tonsils occurs only after the growth and presence of the high narrow hard palate and the development of mouth breathing is supported by evidence from this cohort. However, no valid study of adenoids could be done with the study design as no optic-scopy at the back of the nose was performed. Nevertheless, documentation of a high and narrow hard palate present at birth indicates the presence of abnormal oral-facial feature existing at birth in most cases.

(6) Development of abnormal hard palate after birth: The most interesting cases are the small fraction of subjects, 9% (n=23), that had a normal hard palate at birth, but then presented with an abnormal hard palate during the investigation at 6 months of age. These children were all in the 36 weeks and older age group. None of them had ICU hospitalization, but they were considered to show positive scarf signs indicative of the presence of some degree of hypotonia. Also, all of these children were quickly bottle-fed due to difficulties with breast feeding. At the 6-month evaluation, their tongue was placed flat and low on all photos and at examination: the presentation is similar to that of a hypotonic tongue. These children had normal breathing during sleep at birth evaluation but developed SDB as documented by sleep recording during the follow-up period.

(7) Infants with normal palate at follow-up: In this study only nine per cent of subjects (n=22) had a completely normal hard palate, normal breathing during sleep and normal development. Most were in the 36 weeks and older age group and had normal breast feeding. However, there was a pair of twins who belonged to the 34 weeks gestational age group. These twins were followed by a special myofunctional reeducation team applying tongue reeducation techniques to strengthen tongue and oral muscles very early after birth And they were bottle-fed but a special “hard” nipple, with hardness and size adjusted overtime, was used to elicit more effort from their tongue muscles when feeding.

(8) Hard and narrow hard palates were seen at birth in most premature infants and SDB was recorded in most of them; mouth breathing and sleep-disordered breathing were noted at follow-up; enlargement of tonsils was not observed when hard and narrow hard palate was documented; adenoids were not studied. But a small number of children (9%) had normal hard palates including two early premature twins treated with myofunctional techniques

recommended to strengthen oral-facial muscles. Also, a small group of children with normal palate at birth and normal breathing during sleep, there was a concomitant appearance of SDB, development of hard and narrow palate, mouth breathing and clinically noted tongue hypotonia at follow-up indicative of development of oral-facial growth problem and post-natal SDB.

In summary: Premature infants and some full-term infants may present with abnormal oral-facial presentation, particularly a high and narrow hard palate. Such presentation is associated with oral facial hypotonia. Systematic follow-up up to 36 months of post birth-age indicates persistence of abnormal tongue position and of abnormal breathing with presence of mouth breathing during PSG.

(D) Summary of the findings from study # 5:

(1) The different data accumulated over time on SDB children and the experimental data obtained from the infant monkeys, years ago all lean toward the same direction: There is a strong association between "normal oral-facial muscle tone" and "normal development of the nasomaxillary complex and the mandible". Presence of abnormal muscle tone, either experimentally induced by creation of abnormal nasal resistance or due to premature birth, is quickly associated with mouth breathing particularly during sleep, abnormal placement of the tongue, and either development –as shown in the rhesus monkey and in some infants- or worsening of the oral-facial anatomy. In humans, SDB is noted in association with the pathological hypotonia of the tongue muscles: In a small group of infants seen at birth with a normal hard palate, development of a high and narrow hard palate and SDB was documented in children who also presented oral-facial hypotonia. When high and narrow hard palate was noted at birth, hypotonia was present and SDB was noted. In rare cases efforts to counteract oral-muscle hypotonia and high and narrow hard palate very early in life may bring normal development and absence of SDB at follow-up.

(2) As suggested by Swedish investigators, enlargement of tonsils appears to be a secondary phenomenon, but it further impacts nasal resistance (no information on adenoids has been obtained in our infant studies, but it was obtained in the long-term follow-up of older children with 3D-CT scans). Adenotonsillectomy is often insufficient for the complete resolution of breathing problems.

(3) Interruption of normal development with premature birth may explain the frequency of breathing problems during sleep in premature infants, but it may be seen in full term infants and may have very negative consequences (Rambaud and Guilleminault, 2012). Could the abnormality leading to oral-facial hypotonia begin in utero? It is possible: Investigation of facial expression and movements shows that beginning in early pregnancy, the fetus exhibits regular movements in the mouth and face; For example, the most frequent movement during the second trimester is sucking (Kurjak et al., 2005). Abnormal pregnancy and/or impairment of these movements may impede normal muscle activity at birth.

V-6. Short lingual frenulum, mouth breathing and abnormal oral facial growth (# 6 article was published in Int J Pediatr Res ,2015)

(A) Background

In our follow-up investigation we observed that some of the children in our cohort had a short lingual frenulum. No data was available on the role of short frenulum and abnormal breathing during sleep. The Stanford University group engaged in the study on the association of presence of short lingual frenulum and OSA, our goal was more modest and complimentary with this Stanford study: It was to appreciate if simple clipping of the short lingual frenulum at the age of recognition in our cohort was sufficient investigating oral facial functioning in children and impact on OSA.

(B) Method

We performed a retrospective study of children and teenagers referred for suspicion of sleep-disordered breathing (SDB) during the last five years.

The de-identified charts of children, age 2 to 17 years, referred for suspicion of SDB were selected. Obese and syndromic children were eliminated from the review. To be included in the analysis, charts contained information on initial complaints leading to consult with usage of Pediatric Sleep Questionnaire , report of clinical evaluation of orofacial findings indicating investigation of tonsils using the Friedman scale , investigation of subjective upper airway opening using the Mallampati-Friedman scale , evaluation of inferior nasal turbinates, dental crowding, presence of over jet and/or overbite , and determination of "facial harmony" with frontal measurements. If nasal allergies were suspected, a consult with an allergist for treatment was obtained, and if orthodontic problems were suspected, results of evaluation by a specialist were available. Indication of short lingual frenulum and its potential association with speech, swallow, or mastication problems were outlined. All patients had in-laboratory diagnostic polysomnography (PSG). The conclusion of the testing was abnormal breathing during sleep with indication of the treatment plan. Post treatment follow-up was available indicating: (a) Selected treatment (b) Changes compared to baseline, including questionnaire data (c) Post-treatment PSG findings Based on the results obtained, a follow-up decision contained any further treatment recommendations and subsequent follow up information including PSG data.

The data were collected and organized on spreadsheets. Sleep and respiratory scoring of PSGs followed the pediatric scoring guidelines, according to the American Academy of Sleep Medicine (AASM- 2007) [115] . The presence of nasal flow limitation was determined using criteria published by Guilleminault et al. and Palombini et al. The time spent mouth-breathing during sleep was also calculated as a percentage of total sleep time . Data were analyzed using t-test for repeated measures.

(C) Results

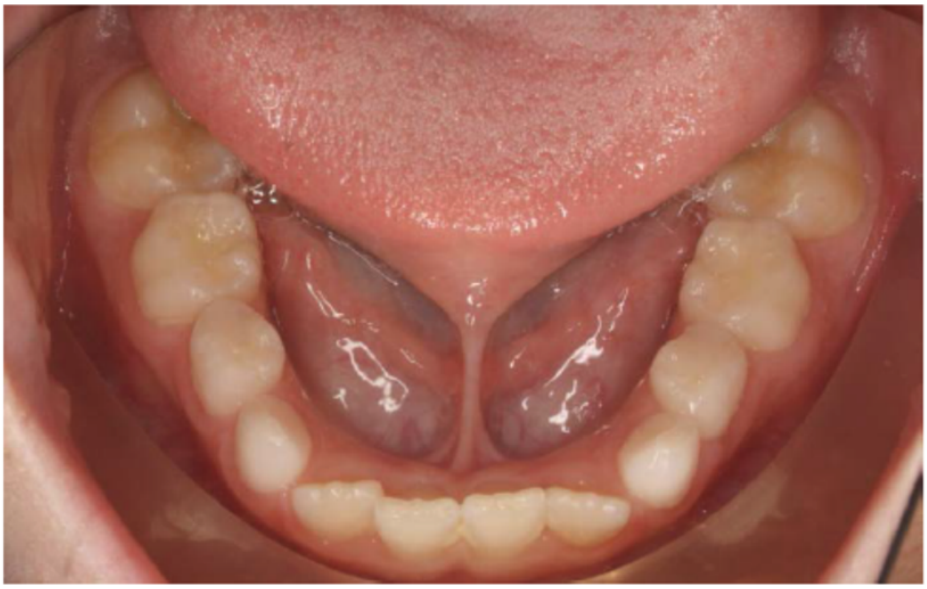
(1) Demographic data:

We identified 27 patients with association of short lingual frenulum and SDB who met entry criteria (Figure 1). The mean age was 11.4 years (range: 2 to 16 years). Children presented with symptoms of SDB (Table 1) such as snoring, poor sleep, and fatigue but also a history of symptoms associated with short lingual frenulum such as problems with speech, swallowing or suction, particularly early in life. Children with speech problems may have been sent to speech therapy, but none of the children had frenulum treatment. As can be seen (Table 1), 10 children had enlarged tonsils (Friedman scores of 3 and 4), five children had been recommended to consult an orthodontist due to evident crowding of dentition, 22 children presented with an abnormal Mallampati-Friedman score of 3 or 4 .

Table 1. Disease characteristics at entry and after first treatment

	At entry		After 1st treatment	
	n	(%)	n	(%)
Demographics (n=27) Boys Mean Age (years) (SD) ***Disease characteristics***	18 (63%) 11.4 ± 5.2			12.3 ± 4.6
Overall symptoms	27	(100)	9	(90)
Fatigue	27	(100)	10	(37)
EDS	9	(35)	1	(4)
Poor sleep	18	(67)	9	(33)
Snoring	20	(74)	2	(7.5)
Speech problems+	13	(48)	2	(7.5)
Swallowing problems+	7	(26)	0	(0.0)
Chewing problems+	6	(22)	1	(3.7)
Tonsil scale 0/1 2 3 4 Mouth breathing *PSG findings* AHI, mean ±SD SaO2nadir, mean ±SD Flow limitation, mean ±SD) Mouth breathing (%TST)	 8(30) 9(33) 5(18.5) 5(18.5) 27(100) 12 ± 4.6 89 ± 2.5 73 ± 11 78 ± 14		 18(66.6) 9(33) 0 (0.0) 0 (0.0) 25 (92.5) 3 ± 2 94 ± 1.6 31 ± 9 61 ± 16	

" +" indicates that symptom was reported during pre-pubertal period but not present necessarily at time of evaluation.



(2) Short lingual frenulum had associated with anatomic problems and high and narrow hard palate

In all cases, there was presence of a high and narrow hard palate and distortion of the "harmonic face" with either a longer lower third of the face or reduction of the middle third compared to the other thirds. All children presented with abnormal orofacial anatomy and reduced oral cavity size. All children had PSG s with an abnormal apnea-hypopnea-index (AHI) and abnormal nadir of oxygen saturation during sleep. Results are presented in Table 1.

On initial presentation, children with a short lingual frenulum had associated anatomic problems linked to abnormal breathing during sleep, such as adenotonsillar hypertrophy, but all presented with orofacial features favoring collapse of the upper airway during sleep with presence of a high and narrow palatal vault . Finally, detailed family history revealed that one parent had a short frenulum in 6 out of our 27 cases, and short frenulum was present in three siblings of the patients. Following recognition of abnormal breathing during sleep, some children (n=10) had been referred to ear-nose-throat specialists (ENT) for T&A and frenectomy when large tonsils were present, and 8 children with normal sized tonsils (confirmed by nasopharyngoscopy and/or lateral radiographs) were also referred to ENT for frenectomy. Nine children were referred to orthodontists for rapid maxillary expansion (RME) and need for frenectomy was also mentioned. Children were asked to have post-treatment follow-up by sleep medicine including investigation with PSG.

(3) Follow-up post treatment#1

The sleep medicine follow-up occurred between three and four months post T&A with frenectomy or isolated frenectomy; and about one month after rapid maxillary expansion, with expander in place, for children sent to orthodontists. None of the children sent for orthodontia treatment had frenectomy and two children had T&A without frenectomy. In summary: 13 children had frenectomy either isolated (n=5) or in association with T&A (n=8), but frenectomy was not performed in 14 children despite recommendation to perform such treatment.

Table 1 presents the results obtained post treatment #1, independent of its type. There was an overall improvement by clinical evaluation and PSG including children in which frenectomy had not been performed; parents reported, however, persistence of some symptoms particularly of "fatigue" and "poor sleep" in about one third of the cases. The AHI was significantly decreased, and oxygen saturation nadir was significantly improved ($p=0.01$ for each condition, paired t-test); flow limitation was also improved ($p=0.05$ paired t-test) but to a lesser degree. There was persistence of mouth-breathing: only two children, treated by both T&A and frenectomy spent less than 10% of their sleep mouth breathing, a percentage considered as normal . All others had abnormal mouth-breathing during sleep, including the five children that underwent isolated frenectomy. The conclusion of this first follow-up was that children recognized with OSA were clearly improved after either T&A or orthodontics, performed with or without frenectomy. However, 92.5% of the treated children still had an abnormal amount of mouth-breathing during sleep. After collection of data, children without frenectomy were referred back to ENT and recommended to undergo treatment (n=16). Considering the prior finding of persistence of mouth-breathing despite frenectomy, all children with residual AHI, flow limitation, and mouth-breathing were recommended to have myofunctional treatment with a specialist after surgery (n=25).

(4) Follow-up post treatment #2

Eleven patients came back for further follow-up, six months after the first post treatment investigation. They had treatment with myofunctional therapy for 4 to 6 months . These included those who underwent frenectomy with orthodontia (n=5), isolated frenectomy (n=4)), and children post T&A with new frenectomy subsequent to the first follow up visit (n= 2). None of these 11 patients or their parents had clinical complaints. At follow-up PSG, these cases had a

mean AHI of 0.8 ± 0.9, a mean oxygen saturation nadir of 97.2 ± 1.0%, a mean flow limitation during sleep of 7.5 ± 6%, and time spent mouth breathing during sleep of 4 ± 4.1%.

(D) Summary of the findings from study # 6:

(1) Short lingual frenulum may lead to abnormal orofacial growth early in life, a risk factor for development of SDB.

(2) Careful surveillance for abnormal breathing during sleep should occur in the presence of short lingual frenulum.

V-7 Inflammation, Cytokines, Neurocognition and Pediatric sleep-disordered-breathing (# 7 article was published in Medicine J, 2016)

(A) Background

Pediatric OSA results in long-term effects on children's health and development. It also affects the sleep and neurocognitive functioning of children [1-4] including symptoms of attention deficit/hyperactivity disorder. The factors involved in the decrease in cognition, learning and memory are still incompletely chartered. Previous researches showed there was an interaction between OSA and chronic diseases. The most acceptable hypothesis associates occurrence of chronic systemic inflammation with OSA. Therefore, we hypothesized that chronic inflammation not only causes cardiovascular diseases in pediatric OSA patient, but also affect cognitive functions and wondered if a correlation between psychometric test and these cytokines could be shown. This study aimed to investigate the status of pro-inflammatory cytokines, particularly IL-17and IL 23 and cognition in pediatric OSA.

(B) Method

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 (WCST), questionnaires, analyses of plasma high-sensitivity C- reactive- protein (HS-CRP), tumor-necrosis-factor-alpha (TNF-α), interleukins 1 (IL-1), 6 (IL-6), 17 (IL-17) and 23 (IL-23).

(C) Results

(1) Demography data :

Eighty-two children, 4-12 years old, were enrolled; there were 3 drop-outs (3.6%). Demographic of the 78 children (mean age 7.43 $\pm$ 0.6 year) are in table 1. The OSA group was significantly different with symptoms of ADHD and enuresis ($p=0.001$ and $p=0.036$), presence of tonsil and adenoid hypertrophy ($p<0.001$), body-mass-index [BMI], BMI z score ($p=0.001$ and 0.036).

Table 1 Demographic characteristics of OSA and healthy children

	Control (n=32)	OSA (n=47)	*p* value
Number of males (%)	21 (65.6%)	30 (63.8%)	0.428
Age (years)	7.02±0.65	7.84±0.56	0.366
BMI (kg/m^2)	16.55±0.58	16.95±0.47	0.601
BMI z score [a]	-0.12±0.27	0.15±0.21	0.442
AHI(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 (%)[b]	0(0%)	1(2.1%)	0.461
ADHD (%)[b]	2(6.2%)	18(38.3%)	0.001**
Enuresis (%)[b]	4(12.5%)	15(31.9%)	0.036*
Other Physical Comorbidity History			
Asthma (%) [c]	4(12.5%)	6(12.8%)	0.561
Allergic rhinitis (%) [c]	4(12.5%)	23(48.9%)	<0.001***
Findings of ENT examination			
Tonsil hypertrophy (more than Gr. 2) (%) [c]	4(12.5%)	32(68.1%)	<0.001***
Adenoid hypertrophy (%)[c]	3(9.3%)	24(51.1%)	<0.001***
Turbinate hypertrophy (%) [c]	1(3.1%)	6(12.8%)	0.158
Nasoseptal deviation (%) [c]	0(0%)	1(2.1%)	0.461

†$0.05 \leqq P < 0.1$. *$P<0.05$. **$P<0.01$. ***$P<0.001$. BMI, body mass index; ENT, ear, nose and throat; ADHD, attention deficient hyperactivity disorder; PLM, periodic limb movement. [a]Corrected BMI z score based on the Center for Disease Control (CDC) growth charts. [b]Diagnosed according to the criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition-Text Revision. [c]Diagnosed by pediatricians.

(2) PSG data:

The PSGs showed (see table 2) significantly higher AHI, AHI in REM, AI, Desaturation Index [DI] and snore index in OSA children ($p<0.001$, $p<0.001$, $p=0.001$, $p<0.001$ and $p=0.002$ respectively).

Table 2 Comparison of polysomnographic findings in OSA and healthy children

	Control (n=32)	OSA (n=47)	*p* value
BMI (kg/m^2)	16.55±0.58	16.95±0.47	0.601
BMI z score	-0.12±0.27	0.15±0.21	0.442
Polysomnographic findings			
AHI(events/h)	0.37±0.06	9.13±1.67	<0.001***
AHI/REM (events/h)	0.65±0.18	16.25±3.68	<0.001***
AI(events/h)	0.18±0.05	2.12±0.51	0.001**
Desaturation Index(events/h)	0.41±0.06	7.27±1.59	<0.001***
Sleep efficiency (%)	89.70±1.32	83.65±2.28	0.131
Awake (%)	6.74±1.42	10.90±2.36	0.305
REM (%)	18.62±1.406	19.11±1.16	0.820
Stage N1 (%)	10.46±1.70	10.24±1.44	0.935
Stage N2 (%)	41.65±2.67	42.56±2.74	0.853
Stage N3 (%)	28.28±2.71	30.40±1.28	0.434
TST (mins)	405.26±10.96	383.34±11.15	0.274
Sleep latency(mins)	17.67±3.84	20.86±3.74	0.629
PLM Index(events/h)	0.13±0.10	0.93±0.41	0.067†
Snore Index(events/h)	30.41±15.28	156.02±35.62	0.002**
Mean SaO_2 (%)	95.97±1.12	90.12±3.61	0.332
Systolic pressure	100.80±17.20	106.76±19.40	0.387
Diastolic pressure	60.00±22.36	66.79±11.86	0.141

†$0.05 \leqq P < 0.1$. *$P<0.05$. **$P<0.01$. ***$P<0.001$. BMI, body mass index; AHI, apnea-hypopnea index; AHI/REM, AHI during REM; AI, apnea index; REM, rapid eye movement; TIB, Time in Bed; SPT, Sleep Period Time; TST, Total Sleep Time; WASO, Wake time after sleep onset; PLM, Periodic Limb Movement; Mean SaO_2, mean oxygen saturation; Lowest SaO_2, lowest oxygen saturation.

(3) Inflammatory cytokines result:

In addition, (see table 3), the expression of inflammatory cytokines IL-17, IL-23 and HS-CRP was significantly elevated in children with OSA (p=0.002, p=0.024, p=0.047 respectively). Plasma levels of TNF-α, IL-1, IL-6 and IL-10 showed a non-significant elevation comparing with normal control.

Table 3 - Comparison of inflammatory cytokines in healthy and OSA children

	Control(n=32) mean±SD	OSA(n=47) mean±SD	*p* value
HS-CRP mg/l	0.41±0.48	1.90±0.44	0.002**
TNF-αug/dl	12.62±0.94	12.58±0.83	0.974
IL-1βpg/ml	0.42±0.27	0.36±0.16	0.857
IL-6 pg/ml	1.10±0.18	1.66±0.23	0.104
IL-10 pg/ ml	2.10±0.28	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*

*$P<0.05$.**$P<0.01$.

HS-CRP, high sensitivity-C reactive protein; TNF-α, tumor necrosis factor alpha; IL-1β, Interleukins 1 beta; IL-6, Interleukins 6; IL-10, Interleukins 10; IL-17, Interleukins 17; IL-23, Interleukins 23.

(4) The neurocognitive test results:

Results of CPT and WCST tests (see table 4) indicate significant differences between OSA and control in "Hit- RT- Std.- Error"(p=0.006) and "Hit-RT-ISI-Change" (p=0.004).

Table 4 - Comparison of CPT and WCST findings in OSA children

	Control total(n=32)	OSA total(n=47)	*p* value
CPT (Continuous performance task)			
Clinical, Confidence Index	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	46.43±7.31	51.42±10.57	0.099†
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
Perseverations 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	49.40±7.71	50.07±11.00	0.831
Hit RT ISI Change T score	47.88±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			
Total Errors Standard scores	107.20±20.97	99.67±24.26	0.392
Total Errors T scores	54.80±13.97	49.81±16.19	0.395
Perseverative Responses T scores	55.10±14.77	50.56±16.57	0.452
Perseverative Errors T scores	56.30±15.10	50.74±16.42	0.357
Non Perseverative Errors T scores	55.60±14.37	52.78±16.66	0.639
% Conceptual Level Response T scores	54.50±4.60	50.19±3.31	0.487
Learning to Learn	-3.49±11.66	-3.90±9.78	0.712

The result of CPT score is presented in T-scores. According to the Conners' CPT Computer Program User's Manual, high T-scores are designed to indicate an attention problem. Any T-score above 60 is considered abnormal. The Confidence Index presents the summary of the CPT. The Omissions reveals the number of targets which the person did not respond to. The Commission reveals the number of times when the person responds to a non-target. Hit RT, Hit reaction time; which reflects the mean response time. Hit RT std. Error, Hit reaction time standard error; which measures the speed consistency. The variability, also a measure of response time consistency, which calculates the standard deviation of the 18 standard error values calculated for each sub-block. The detectability is a measure of discriminative power.

The higher response style T score indicates that the person act more cautiously to avoid commission error, and the lower score indicates that the person respond more freely to make sure they answer most of the target. The perseverations T score shows the frequency when responding time is lower than 100ms. Hit RT block change, Hit reaction time block change. The Hit RT block change shows the change in reaction time over the 6 time blocks; the higher Hit RT block change T scores indicates a slowing of reaction time as the test progress. Hit SE block change, Hit standard error block change, which indicates the consistency the person react to the targets as test progress. Hit RT ISI Change, Hit reaction time inter-stimulus interval change, reflects the change in reaction time over three inter-stimulus intervals (1,2 and 4 seconds.) Higher score reflects slowing of reaction time as the intervals between targets increased. Hit SE ISI Change, Hit reaction time inter-stimulus interval change. Higher score reflects the person became more erratic as the time between targets increased.

WCST, Wisconsin Card Sorting Test. The total errors scores is an overall score of WCST test, and the higher raw score indicates worse performance. The perseverative response and error raw score are higher in the person with worse performance of mental flexibility and insight. The non-perseverative error reflects difficulty to forming concepts and insight even in flexible answer. The conceptual level response score indicates the insights in correct principle of the card combination. Learning to learn depicts the average tendency over successive categories for efficiency to change.

(5) Relationships between inflammatory cytokines and PSG data

A Standardized Regression Test was performed to demonstrate the relationship of cytokines levels with PSG and neurocognitive outcomes after controlling the factors of "asthma, allergy, BMI, gender, tonsil hypertrophy, adenoid hypertrophy, turbinate hypertrophy, and naso-septal deviation" (see tables 5 and 6).

It revealed significant relationships between pro-inflammatory cytokines and some PSG factors, such as HS-CRP with AI (β=0.390, P<0.05) and IL-17 with AHI severity (β=0. 329, P<0.05) (see table 6). Higher AI "influenced" serum levels of HS-CRP suggesting an impact of inflammatory cytokines on soft tissues hypertrophy. Similarly, higher serum levels of IL-23 was "influenced" by higher AI (β=0.403, P<0.05). Also, lower mean SaO2 "influenced" IL-10

level (β= -0.567, P<0.01), and higher serum levels of TNF-α and IL-1β were "influenced" by higher diastolic pressure (β=0.469 and 0.659, P<0.01).

Table 5 Relationships between inflammatory cytokines and PSG findings

	HS-CRP	TNF-α	IL-1β	IL-6	IL-10	IL-17	IL-23
Polysomnographic data							
AHI severity [a,c]	0.237	-0.078	0.026	0.124	0.205	0.329*	0.184
AHI(events/h) [a]	0.114	-0.109	-0.114	-0.095	-0.079	0.059	0.163
AHIREM (events/h) [a]	0.022	-0.101	-0.095	-0.082	-0.027	0.095	0.331†
AI (events/h) [a]	0.390*	0.162	-0.209	-0.074	-0.31†	-0.238	0.403*
HI (events/h) [a]	0.020	-0.100	-0.129	-0.036	0.080	0.225	0.276
Desaturation Index (events/h) [a]	0.156	0.076	-0.151	-0.088	-0.147	0.001	0.317†
Sleep efficiency (%)[a]	-0.037	0.041	-0.090	0.050	-0.150	-0.126	-0.077
Awake (%)[a]	-0.016	0.012	0.059	-0.046	0.139	0.089	0.054
REM (%)[a]	0.111	0.064	0.057	0.318*	0.026	0.148	-0.090
Stage N1 (%)[a]	0.022	-0.168	0.076	-0.011	0.139	-0.086	0.183
Stage N2 (%)[a]	-0.520	-0.174	-0.049	0.001	-0.220	0.026	-0.234
Stage N3 (%)[a]	-0.261	-0.061	0.057	-0.189	0.330*	0.191	-0.206
TST [a]	0.118	-0.057	-0.27†	0.188	-0.224	0.034	0.067
Sleep latency [a]	0.162	-0.141	0.297*	0.014	0.092	0.012	0.133
PLM Index [a]	-0.048	0.088	-0.006	-0.141	-0.201	-0.33*	0.230
Snore Index [a]	-0.079	-0.145	0.133	-0.052	0.066	0.157	-0.052
Mean SaO_2 (%)[a]	0.040	-0.159	0.012	-0.123	-0.57**	-0.242	-0.023
Systolic pressure [a]	-0.43†	0.064	0.09	-0.41†	-0.14	0.088	-0.56*
Diastolic pressure [a]	-0.15	0.47**	0.66**	-0.18	-0.10	-0.10	-0.06

[a]Standardized regression coefficient. Control factors: Asthma, allergy, BMI, gender, Tonsil hypertrophy, Adenoid hypertrophy, Turbinate hypertrophy, Nasoseptal deviation. †0.05≦$P<0.1$. *$P<0.05$. **$P<0.01$. AHI, apnea-hypopnea index; [c]AHI severity: 5≧AHI>1 events/h (mild), 10≧AHI>5 events/h (moderate), AHI>10 events/h (severe); AI, apnea index; HI, hypopnea index; REM, rapid eye movement; TST, Total Sleep Time.

(6) Relationships between inflammatory cytokines and neurocognitive function

There was a significant relationship between *lower performances* of CPT test and pro-inflammatory cytokines as shown in table 6. The Standardized-Regression-Test indicated significant findings between pro-inflammatory cytokines and neurocognitive-function tests. The

elevated cytokines are related to domains of inattention, vigilance, such as "Hit-RT-ISI-Change T- score" and HS-CRP (β= -0.426, P<0.05); "Response-Style T-score" and TNF-α (β= -0.432, P<0.05); "Hit RT ISI Change T score" and "Hit SE ISI Change T score" with IL23 (β= -0.545, -0.526, P<0.01); and higher "Confidence-Index" with IL17 (β= 0.424, P<0.05). When looking at the influence between inflammatory cytokines and WCST, the results indicate that elevated cytokines, such as TNF-α and IL6 are related to decrease of executive functions: such as "non-Perseverative- Errors T-scores" (β= -0.553, P<0.05) ; "Learning-to-Learn" (β= -0.838, P<0.05) ; and "Percent-Conceptual-Level-Response- T-scores" (β= 0.476, P<0.05); especially IL-23 with significant poor performance of "non-Perseverative-Errors T- scores" (β= -0.729, P<0.01).

Table 6 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 [a]	-0.185	-0.177	-0.040	-0.027	0.181	0.424*	-0.317
Omissions T score [a]	-0.256	-0.154	-0.067	-0.109	0.002	0.112	-0.336†
Commissions T score [a]	0.037	0.075	0.170	0.030	0.250	-0.056	0.045
Hit RT T score [a]	-0.071	-0.127	0.006	-0.106	0.065	0.267	-0.249
Hit RT Std.Error T score [a]	-0.207	-0.216	0.083	-0.103	0.182	0.294†	-0.322†
Variability T score [a]	-0.247	-0.098	0.160	-0.009	0.354†	0.274	-0.291
Detectability T score [a]	-0.116	0.032	0.136	-0.004	-0.050	-0.186	0.034
Response Style T score [a]	-0.044	-0.432*	-0.101	-0.039	0.136	0.309†	-0.129
Perseverations T score [a]	-0.253	0.109	-0.013	-0.112	0.155	0.184	-0.203
Hit RT Block Change T score [a]	-0.146	-0.328†	-0.066	-0.017	0.105	-0.102	-0.051
Hit SE Block Change T score [a]	-0.100	-0.121	0.131	-0.029	0.201	-0.184	-0.051
Hit RT ISI Change T score [a]	-0.426*	-0.155	0.003	-0.168	-0.012	-0.036	-0.545**
Hit SE ISI Change T score [a]	-0.389†	-0.192	0.174	-0.159	0.049	0.114	-0.526**
WCST							
Total Errors Standard scores [a]	-0.123	-0.335	0.089	0.436†	0.065	-0.086	-0.443†
Total Errors T scores [a]	-0.124	-0.345	0.082	0.433†	0.068	-0.083	-0.446†
Perseverative Responses T scores [a]	-0.276	-0.093	0.186	0.324	-0.016	0.027	-0.179
Perseverative Errors T scores [a]	-0.262	-0.117	0.175	0.324	-0.021	0.012	-0.197
nonPerseverative Errors T scores [a]	0.047	-0.553*	-0.058	0.255	0.106	-0.250	-0.729**
% Conceptual Level Response T scores [a]	-0.131	-0.315	0.067	0.476*	0.081	-0.079	-0.404†
Learning to Learn [a]	0.336	-0.838*	0.019	0.221	0.119	0.330	0.295

[a]Standardized regression coefficient. Control factors: Asthma, allergy, BMI, gender, Tonsil hypertrophy, Adenoid hypertrophy, Turbinate hypertrophy, Nasoseptal deviation., †$0.05 \leqq P < 0.1$. *$P<0.05$. **$P<0.01$. WCST, Wisconsin Card Sorting Test; CPT, Continuous performance task

(D) Summary of the findings in study # 7

(1) Our study showed that an abnormal increase in HS-CRP, interleukins 17 and 23 is present in association with mild to moderate OSA children.

(2) Lower neuro-cognitive test results are also demonstrated in the OSA children, and there are significant positive correlation between low scores at cognitive tests (showing decrease 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.

VI. Discussion

VI-1. Sleep and breathing in premature infants

(1) Our study validated the CBISQ; furthermore, it was the first study targeting the difference between premature and full-term infants through a reliable and valid screening questionnaire. Based on our results, premature infants, rather than full-term infants, preferred a side body position and being held when falling asleep. Apart from this, premature infants had longer nocturnal sleep duration, more night awakenings, and longer daytime sleep duration. The caregivers of premature infants noted that their children had more sleep problems. A significant difference between preterm and full-term infants is nocturnal sleep duration and loud-noisy breathing was noted. The number of night awakenings and time spent with mouth-breathing also varied between the 2 groups. These questionnaire findings indicated a higher prevalence of sleep-breathing disorders , and correlated with those obtained with PSG that showed a very high percentage (80.6%) of premature infants with AHI greater than 1 event/hour. These disturbances impact nocturnal sleep duration and quality.

(2) Our results provide new insights into the difference between premature and full-term infants regarding sleep problems. During the first year of life, infancy-sleep is a rapid maturational process , so the settling time, daytime sleep duration and time spent crying during the night may vary widely both among infants and between questionnaires repeated at several occasions. Infancy sleep maturation may impact the maturation of the central nervous system, overall functioning, and future cognitive, motor, and temperament development [123].

(3) Moreover, even till 2 years of age, premature infants with narrow and high-arched palate have more SDB-related sleep problems and more neurodevelopmental deficits than those without. These findings support our hypothesis that high and narrow-arched palate in premature infants plays a role in the development of sleep problems and subsequent neurodevelopmental delays.

(4) **Limitations:** There are some methodological limitations that should be noted when interpreting our findings. First, the sample sizes of the 2 groups in our study were very different, so some of the differences between the premature and full-term infants may not

be apparent in the CBISQ sleep measurement. Second, 16.59% of our study group (premature infants) did not complete the CBISQ assessment and sleep examination when they were 6 months old, but our follow-up study had more than 70% of the initially signed-up children.

(5) **Strengths:** Our study monitored simultaneously objective and subjective data investigating sleep problems of the premature. Actigraphy provides only integrated sleep data, not detailed parameters of the sleep status, so the validity testing of the CBISQ sleep measure with actigraphy, although providing quantitative biological parameters, is not totally satisfactory. Therefore we added PSG: , it is difficult to perform PSG in premature infants because of uncooperative child, night feeding and size of premature infant with too small limbs and torso for some equipments. However, our premature group was large, and actigraphy and PSG data were both obtained from a large number of premature infants.

VI-2. Neurodevelopmental dysfunctions in prematurely born neonates

(1) Our study shows that 13.2% of the preterm neonates have MNDs at the corrected age of 6 months and even 8.6% of neonates born late-preterm (GA 33-36 weeks) have MNDs. Furthermore, as GA decreases, the proportion of children with MNDs confirmed by both PDI below 70 and failure of DDST gross motor subscale increases. Although several neonatal factors are found to be associated with MNDs, the multivariate model for these infants showed that postnatal corticosteroid use and cholestasis were independent predictors of MNDs at their 6 months corrected age.

(2) It is difficult to compare our study with previous studies because our subjects were assessed at much earlier age. The proportion of MND in very preterm neonates and preterm neonates (GA ≦ 28 weeks and GA 29-32 weeks) is similar to other studies [27-29], but 28.6% (6/21) of our neonates with a birth weight ≦ 750g had MND, which is slightly higher than a recent study conducted by Claas MJ et al . The percentage of ELBW children with a MDI or a PDI of < 70 assessed with the BSID-II at or around 2 years of age ranged from 10.6% to 50%; this variability in the obtained results relates to the different designs used and to selection bias of the different studies. For example, some studies evaluated infants with presumably fine motor function impairments and avoided those with moderate or severe dysfunctions;

others evaluated their results after an individualized developmental care. In our study group, 4 of the 28 ELBW infants (14.2%) had a MDI of < 70, whereas 10/28 (35.7%) neonates assessed with the BSID-II had a PDI of < 70. Given the fact that comparable percentages of low PDI and MDI in each study subgroups were usually noted , our results strongly suggest that early detection of psychomotor delay maybe more feasible than mental developmental delay by the current screening tools.

(3) In all cases the postnatal corticosteroid use in our infants corresponded to dexamethasone in low dose (0.05mg/kg/day) intravenously for chronic lung disease, which is proven effective to facilitate extubation without significant short-term side effects . Several human studies have found that postnatal dexamethasone exposure was associated with increased risk of long-term neurological and motor dysfunctions, but their steroid dosages were much higher (0.5mg/kg/day) and the administration period longer. A larger, randomized controlled trial is required to further assess the long-term effects of low dose dexamethasone. The prenatal steroid is inevitably used for prevention of RDS in premature neonates, which had no significant impact on the long-term neurological outcomes , in spite of the fact that most corticosteroids, including dexamethasone or betamethasone, are capable of transfusing to the fetus through placenta. Therefore, the real pathophysiological mechanisms of postnatal corticosteroid use and its effects upon the developing brain of premature infants are still unknown and deserve further in vitro and in vivo studies. Cholestasis, was another independent predictor of MNDs in our study; it may be an indicator of severe sickness in the high risk and very preterm neonates, rather than a direct cause of the MNDs at 6 months corrected age.

(4) Several neonatal factors, including multiple birth, severe abnormalities on early cranial ultrasonography, breast feeding, treatment methods of PDA, chronic lung disease and SGA have been reported to independently influence the neurodevelopmental outcomes at 2 to 5 years of age. The significant predictors of a low MDI and a low PDI are usually considered together, but the pathogenesis of developmental delay in psychomotor and mental domains are supposed to be different and supported by some evidences. The associations of ultrasound-defined lesions of the brain and developmental delays have been well documented in recent publications. Cerebral white matter injury, caused by systemic inflammatory response, transient hypoxia or prolonged mechanical ventilations during

hospitalization may also predispose to low MDI and low PDI scores. Our study, after excluding infants with cerebral palsy and major neurological deficits, focused on MNDs of grossly normal premature infants and can not conclude similar findings.

(5) Although the Bayley test is the gold standard in screening developmental delay of early childhood, it is most reliable when performed at or around 24 months corrected age. Thus, DDST was applied as the supplemental tool to confirm the MNDs in our study. We found the results of BSID-II PDI < 70 and failure in DDST motor subscale were highly correlated; the NMDs in our study are defined as at least two abnormal measures, which decrease the possibility of false positivity.

(6) **Limitations**: This study has some limitations. Not all premature neonates born during the study period were enrolled, because their parents declined to participate in the study. We do not know whether those refusing to participate were significantly different from our study group, and whether this potential difference could have influenced our results. This study is also limited by its inadequate sample size, for which the drop out cases of the longitudinal follow-up and repeated examinations also contributed.

VI-3. Sleep-Disordered Breathing and oral-facial development in the premature infant

(1) Currently at 4 years of age, 77% of our premature infants have OSA and a high and narrow hard palate. There is a relationship between gestational age-GA- at birth and the presence of problem which increases with younger GA of the subject at birth. But, this is not the only factor. As found, children with normal palate and AHI may progressively deteriorate during the first post-natal months. Preliminary data indicates that normal functioning of suction, swallow, mastication and nasal breathing post-birth are key factors in the switch overtime to abnormal breathing and high and narrow hard palate.

(2) **Limitation**: As any longitudinal study we have lost patients along successive years, however our sample to date is over 250 prematures at 4 years, the group with a transition from normal to abnormal is small (n=39); eventually some other factors are involved in this negative evolution; a larger study focusing only on this question is needed.

VI-4. Inflammation, Neurocognition and Pediatric sleep-disordered-breathing

(1) From of our longitudinal study on premature children we had found out that mouth breathing was in frequent association with snoring and sleep-disordered-breathing and that tonsils were not initially enlarged in children that developed OSA, but become enlarged later on [see study #3]. We showed that children with enlarged tonsils and adenoids (T&A) treated with adenotonsillectomy may present relapse of OSA within 3 years. Inflammatory factors have been mentioned as potential factors in the development of co-morbidity in adult OSA [124, 125]. We question if inflammation could not play a role in the development of pediatric OSA and if persistence of inflammatory factors could not be abnormally elevated in pediatric OSA. The world of inflammatory factors has become complicated as many of the usually monitored interleukines such as interleukin1,6, 10 have been showed to be under the control of other interleukines more recently identified such as interleukines 17 and 23. In the search of biological markers of OSAS in children Kallikrein-1, uromodulin, urocotin-3, and orosomucoid-1 in the urine are eventually accurate as OSA diagnostic test in children [125] Furthermore, fundamental research has shown that functional lymphatic vessels expressing all of the molecular hallmarks of the lymphatic endothelial cells are able to carry both fluid and immune cells from the cerebro-spinal-fluid and are connected to the deep lymphatic cervical ganglions , demonstrating a direct interaction with brain structures. Obesity is a further variable linking OSAS, Cognition and inflammation [126]; in our studies we have shown that OSA could occur in non-obese children, Taken altogether these considerations imply further research.

(2) Children with OSA have neurological dysfunction, presenting symptoms of inattention and difficulty learning, this is a known co-morbidity of pediatric OSA. Abnormal inflammatory cytokines may be involved in the difficulties that children present when performing neuro-cognitive testing. Our study evaluated the importance of the SDB, of the cytokines abnormalities and of neurofunctional cognition, questioning if a correlation could be found between these different abnormalities. If we have support for our hypothesis, this will lead later on to a second investigation looking at the impact of SDB treatment on the same variables.

(3) **Limitations**: Our study has limitations: despite the fact that we looked at 79 children- 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 that 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: Our findings are in line with data showing that even children with low but abnormal AHI have often memory, 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 are considered preliminary andrequire confirmation with larger numbers subjects and/or evaluation of change after an appropriate treatment is used.

VII. Conclusion and Recommendations

Our work had a marked impact in Pediatric Sleep Research in Taiwan, with special reference to our Hospital, in which a Pediatric Sleep Laboratory has been developed and a multidisciplinary team was organized.

Furthermore, we organized an observation cohort of preterm infants, in which sleep, as a main human body function, is measured together with other biological, neurodevelopmental data.

It is still a work in progress.

We are following our premature cohort, and despite the attrition that we expected, we see a persistence of abnormal breathing during sleep, with apnea and hypopnea in 77% of our group at 4 years of age. Premature infants have more learning and attention problems than control full-term children but the children, and particularly premature infants, with sleep-disordered-breathing –SDB-have more dysfunctions than those without SDB.

Some of the premature as full term infants have short lingual frenulum , an anatomical presentation not checked in premature infants and often difficult to recognize in early premature, but that should be systematically checked with further growth. “Clipping” of the lingual frenulum may not be possible but dis-insertion of this vestigial abnormally placed fibrous tissue can be done.

Many premature infants have generalized hypotonia at birth and this is particularly true the more premature the infant is. In our studies we selected infants that were “healthy premature” and not in need of tube feeding, intubation, ventilation support as example. Despite this initial status, our follow-up studies show that the long-term prognosis of these children is associated with abnormal cognition, abnormal oral facial development, abnormal sleep-wake and abnormal breathing during sleep. In the recent past, attention has focused on the role of some basic functions such as sucking, swallowing, chewing, nasal breathing, that are impaired in premature infants bellow 35 weeks of GA. Our findings indicate the defect, but have not address really the potential solutions. In the recent past the field of “myofunctional treatment”-MFT- has surged, but there is little objective information concerning-MFT- in infants, its easiness of application, and its outcome. However, the problem is important: the World Health Organization indicated that 15% of all pregnancies ended with a premature birth. Technological advances have allowed

survival of younger and younger premature, but to obtain survival is not enough: currently many survivors present handicaps that can be already demonstrated by 6 months post-birth. New treatment avenues must be developed, new approaches involving families have to be found, MFT is one of these new fields that may lead to improvement of the overall long term status of the premature infant at some level, but the current data are very limited. Our work outlined only some of the challenges that are currently faced.

The involvement of inflammatory cytokines with SDB is also clear but much more work needs to be done in this area. Further works may indicate if a treatment is successful performing a blood test and not having to perform systematic PSG. But the field of cytokines is getting always more complex and the selection of which cytokine(s) to systematically follow is still uncertain, and much more work is needed in this area . The interaction between inflammatory cytokines and neuro-cognitive functions is also a wide-open field for further research

Sleep is always affected in children with health problems, but sleep and investigation of vital variables during sleep is an important test in order to understand the development and worsening of SDB, and despite its challenges, it should be a test used much more in clinical practice when daytime problems cannot be easily resolved. Our contribution took several years to acquire, but raises many more questions than at the start of this work. Many fields will have to collaborate to cope with the challenges that prematurity brings to society.

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VIII. Articles List

Huang YS, Hwang FM, Lin CH, Lee LA, Huang PY, Chiu ST. Clinical manifestations of pediatric obstructive sleep apnea syndrome: Clinical utility of the Chinese-version Obstructive

Sleep Apnea Questionaire-18. Psychiatry Clin Neurosci. 2015 Dec;69(12):752-62. (SCI; IF=1.64; Psychiatry 91/140).

Huang YS, Paiva T,Hsu TF,Kuo1 MC , Guilleminault C*. Sleep and breathing in premature infants at 6 months post-natal age. BMC Pediatrics 2014, 14:303. (SCI； IF=1.91).

Huang YS. Early detection of minor neurodevelopmental dysfunctions at age 6 months in prematurely born neonates. Early Human Development 2013,89: 87-93 (SCI, IF: 2.046).

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Huang YS, Guilleminault C, Hwang FM, Cheng C, Lin CH, Li HY, Lee LA. Inflammatory Cytokines in Pediatric Obstructive Sleep Apnea. Medicine 2016; 95:40-49.

IX. Articles Facsimile

Psychiatry and Clinical Neurosciences 2015; 69: 752–762 doi:10.1111/pcn.12331

Regular Article

Clinical manifestations of pediatric obstructive sleep apnea syndrome: Clinical utility of the Chinese-version Obstructive Sleep Apnea Questionaire-18

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Aims: Childhood obstructive sleep apnea syndrome (OSA) affects not only the children's physical health, but also their mental development, behavioral problems and learning difficulties. Therefore, an early diagnosis is important. However, the assessment tools of polysomnography are demanding. The Obstructive Sleep Apnea Questionnaire-18 (OSA-18) is designed to screen OSA and has good reliability and validity. The goal of this study was to validate the Chinese version of the OSA-18, to analyze the frequency of symptoms and find the most common symptoms of OSA in Taiwanese children.

Methods: We validated the OSA-18 in an ethnic Chinese group and compared the treatment outcomes to show the sensitivity of the questionnaire. The caregivers completed the questionnaire twice at an interval of 4 weeks to test reliability. In the validation study, we included 88 OSA children. The OSA-18 and follow-up polysomnography were performed before and 6 months after adenotonsillectomy.

Results: Results showed the excellent test–retest reliability ($r = 0.84^{**}$) of the OSA-18. There was a statistically significant correlation between the OSA-18 and, respectively, the Apnea–Hypopnea Index ($r = 0.29^{*}$), and the Hypopnea Index ($r = 0.29^{*}$). Quality of life showed a significant correlation with the Apnea Index ($r = 0.43^{**}$), central apnea count ($r = 0.50^{***}$), and mixed apnea count ($r = 0.36^{*}$). The cut-off point of the OSA-18 total scores for detecting pediatric OSA in children aged 6–12 years was 66. The common symptoms of pediatric OSA were poor attention span, loud snoring, caregiver worried about child's health, difficulty awakening, and mouth breathing.

Conclusions: Our results show that the Chinese version of the OSA-18 is a reliable and valid instrument. The questionnaire also showed improvement in the quality of life of OSA children post-adenotonsillectomy.

Key words: adenotonsillectomy, pediatric obstructive-sleep apnea, polysomnography, questionnaire, validation.

SLEEP-DISORDERED BREATHING (SDB) is a common disorder in children and adolescents.[1,2] It is characterized by repeated episodes of prolonged partial or complete upper airway obstruction during sleep.[2,3] SDB without alterations in alveolar ventilation or sleep architecture is called primary snoring and affects up to 12% of young children. In contrast, SDB with associated apneas or hypopneas affects between 2% and 5% of children and is called obstructive sleep apnea (OSA) syndrome.[3,4]

The symptoms of OSA in children and teenagers are different from those noted in adults. The symp-

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toms most commonly reported in pediatric OSA are mouth-breathing, nocturnal snoring, stopping of breathing, nocturnal sweating, enuresis, sleep fragmentation and restless sleep, and parasomnia.[3,4] The most negative consequences affecting children and teenagers, secondary to SDB, are daytime sleepiness, impaired school performance, possibly depression, behavioral problems, and neurocognitive dysfunction, including symptoms of attention deficit/hyperactivity disorders (ADHD).[1,5–9] In recent years, research on snoring, SDB and OSA has included case-series studies and other clinical designs showing more externalized behavioral problems in patients who snore or have OSA.[2,10] Guilleminault *et al.* and Chervin *et al.* found that snoring or pediatric OSA is also related to hyperactivity and inattention.[7,8] However, more and more research indicates that ADHD symptoms and behavioral and cognitive functioning impairment of children with OSA will improve after adenotonsillectomy (AT).[11–16]

Childhood SDB affects children's physical health, but it also affects their mental development, behavioral presentation, learning, and school performance.[14–16] Therefore, it is important to make the diagnosis early. Standardized procedures, such as polysomnography (PSG), have been developed for the assessment of SDB. However, the use of these procedures is limited due to the requirement of equipment and the demands on time and labor. The Obstructive Sleep Apnea Questionnaire-18 (OSA-18) is designed for screening OSA syndrome; the questionnaire has good test–retest reliability and construct validity and can be used for screening OSA syndrome.[17–19]

Another issue is the recurrence and persistence of pediatric SDB after TA.[19–21] More and more studies examining the efficacy and outcomes of AT in pediatric OSA have shown an incomplete resolution of OSA after surgery.[20–24] Therefore, sleep specialists suggested that children diagnosed with OSA undergo long-term follow up, even if there is demonstrable improvement post-TA.[20,22,25] To develop a questionnaire with good reliability that allows early detection of symptoms and is easy to use in the follow up of pediatric OSA is clearly important, but such an instrument in Chinese has not been available. The goal of this study was to empirically validate the Chinese version of the OSA-18 and to evaluate its ability to screen the effect of treatment of elementary OSA children. The secondary goal was to validate the Chinese version of the OSA-18, to analyze the frequency of symptoms and to find the most common symptoms of OSA in Taiwanese children.

METHODS

Scale–Chinese version

The OSA-18 is a questionnaire evaluating pediatric SDB that has been shown to possess satisfactory test–retest reliability and internal consistency in English-speaking groups (Fig. 1).[17,18] The survey consists of 18 items grouped into 5 domains[17,18]: sleep disturbance (four items), physical suffering (four items), emotional distress (three items), daytime problems (three items), and caregiver concerns (four items). Items are scored on a 7-point ordinal scale and have excellent test–retest reliability (R _ 0.74). The overall survey score is calculated as the mean of the 18 items, which correlates significantly with the respiratory distress index (R _ 0.43) and adenoid size (R _ 0.43). It also provides a direct global rating of SDB-related quality of life (QOL) via a 10-point visual analogue scale with specific semantic anchors.

Permission to translate the OSA-18 into Chinese was obtained before the start of the study. The Chinese version of the OSA-18 was initially translated from the English version by Rosenfeld *et al.* into the Mandarin Chinese version by one of the investigators. Following this, it was translated back to English simultaneously by two bilingual individuals until the versions were considered completely interchangeable conceptually and linguistically.

Participants and procedures

Study 1

The participants in study 1 were recruited from school settings. The Institutional Review Board approved the study, and informed consent was obtained from all subjects prior to implementation. We contacted the school counseling centers and explained to teachers the purpose of the study and necessary procedures. All eligible students and their respective parents were informed that participation in the survey was completely voluntary, and thereafter parental approval and signed informed consent

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OSA-18 Quality of Life Survey
Evaluation of Sleep-Disordered Breathing

Instructions. For each question below, please circle the number that best describes how often each symptom or problem has occurred during the past *4 weeks* (or since the last survey if sooner). Thank you.

	None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time
SLEEP DISTURBANCE							
During the past *4 weeks*, how often has your child had...							
...loud snoring?	1	2	3	4	5	6	7
...breath holding spells or pauses in breathing at night?	1	2	3	4	5	6	7
...choking or gasping sounds while asleep?	1	2	3	4	5	6	7
...restless sleep or frequent awakenings from sleep?	1	2	3	4	5	6	7
PHYSICAL SUFFERING							
During the past *4 weeks*, how often has your child had...							
...mouth breathing because of nasal obstruction?	1	2	3	4	5	6	7
...frequent colds or upper respiratory infections?	1	2	3	4	5	6	7
...nasal discharge or runny nose?	1	2	3	4	5	6	7
...difficulty in swallowing foods?	1	2	3	4	5	6	7
EMOTIONAL DISTRESS							
During the past *4 weeks*, how often has your child had...							
...mood swings or temper tantrums?	1	2	3	4	5	6	7
...aggressive or hyperactive behavior?	1	2	3	4	5	6	7
...discipline problems?	1	2	3	4	5	6	7
DAYTIME PROBLEMS							
During the past *4 weeks*, how often has your child had...							
...excessive daytime drowsiness or sleepiness?	1	2	3	4	5	6	7
...poor attention span or concentration?	1	2	3	4	5	6	7
...difficulty getting out of bed in the morning?	1	2	3	4	5	6	7
CAREGIVER CONCERNS							
During the past *4 weeks*, how often have the above problems...							
...caused you to worry about your child's general health?	1	2	3	4	5	6	7
...created concern that your child is not getting enough air?	1	2	3	4	5	6	7
...interfered with your ability to perform daily activities?	1	2	3	4	5	6	7
...made you frustrated?	1	2	3	4	5	6	7

OVERALL, HOW WOULD YOU RATE YOUR CHILD'S QUALITY OF LIFE AS A RESULT OF THE ABOVE PROBLEMS?
(Circle one number)

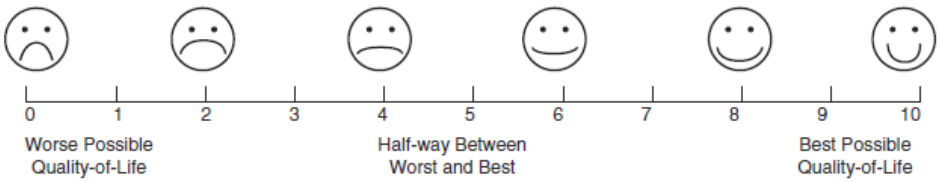

Figure 1. English version of Obstructive Sleep Apnea Questionnaire-18 (OSA-18).

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were obtained. In all, 191 students (aged 6–12 years) were recruited from schools. A package of questionnaires, including the OSA-18 and basic demographic questions, was completed by the parents (or caregivers). The OSA-18 was given again 4 weeks later to obtain re-test data. Participants with missing data or who did not complete the questionnaire twice were excluded from the study ($n = 28$); 163 students (school group) completed the study. The mean age and sex ratio were not significantly different between the 163 students who completed the study and the 28 excluded students who did not complete the questionnaire twice. The 28 excluded students were distributed randomly in our study group.

Study 2

The clinical participants in study 2 included 88 children (aged 6–12 years) with OSA (Apnea–Hypopnea Index [AHI] > 1/h) who were treated with TA and were followed up. The participants and their respective parents were informed that participation was completely voluntary, and thereafter parental approval and signed informed consent were obtained. The patients were recruited from a sleep disorder center in a children's hospital, and were diagnosed by a pediatric psychiatrist and a pediatric department of otolaryngology, both with expertise in pediatric sleep medicine. OSA syndrome was diagnosed based on clinical interview and clinical evaluation followed by one nocturnal PSG recording. Using the International Classification of Sleep Disorders Second Edition (ICSD II) diagnostic criteria,[26] 88 children presented symptoms and clinical features associated with OSA and had an AHI > 1/h associated with breathing-related arousals or oxygen desaturation in one nocturnal PSG recording. The ear, nose and throat exam was performed by an otolaryngologist and a craniofacial surgeon. Tonsillar size was graded as follows: (i) small tonsils confined to the tonsillar pillars; (ii) tonsils that extended just outside the pillars; (iii) tonsils that extended outside the pillars but did not meet at the midline; and (iv) large tonsils that met at the midline.[27] Adenoid tissue was examined with a lateral X-ray film of the neck or a flexible fiberoptic endoscope. The amount of obstruction was categorized into 4 grades (grade 0 = 0–25%, grade 1 = 25–50%, grade 2 = 50–75%, and grade 3 = 75–100%). Allergic rhinitis was confirmed by a specific IgE blood test (ImmunoCAP 100; Phadia, Uppsala, Sweden), and duration and persistence of symptoms and comorbidities according to the Allergic Rhinitis and its Impact on Asthma classification.

Once diagnoses were confirmed, a research assistant administered to the participants' parents a package of questionnaires consisting of the OSA-18 and demographic questions. The OSA-18 was administered again to those treated with TA at the 6-month post-treatment follow-up visit.

Statistical analysis

Statistical analyses were conducted using SPSS 17.0 (SPSS, Chicago, IL, USA) and LISREL 8.80 (Scientific Software International, Skokie, IL, USA). Confirmatory factor analysis (CFA) was used in this study to test the validity of the dyadic data (pretest and post-test) ($n = 163$). CFA combines the factor structures of the dyads into a model testing convergent validity and discriminant validity. The item intraclass covariance can be dealt with by correlating the errors of the same two indicators for the pretest and the post-test. The correlation of latent factors for the same construct of the pretest and the post-test can also be used as factor-intraclass-correlation (for repeated measures, this is test–retest reliability).

The hypothesized model in our study had 10 latent factors, including Sleep Disturbance with four items, Physical Suffering with four items, Emotional Distress with three items, Daytime Situation with three items, and Caregiver concern with four items for the pretest and post-test, respectively. For the pretest and post-test, the errors across the same items were correlated. As we had a treatment for some of the participants, we did not set the corresponding intercepts and factor loading equally for the pretest and post-test in our model.

Several criteria were used in determining the overall fit to the data for the hypothesized model. These included the comparative fit index (CFI), which must meet or exceed 0.90,[28] the root mean square error of approximation (RMSEA), where values <0.05 are indicative of a good fit, those between 0.05 and less than 0.08 are a reasonable fit, and those over 0.1 are a poor fit,[29] and the standard root mean square residual (SRMR), where values less than 0.08 indicate an acceptable fit.[30]

The composite reliability (CR) of a latent factor is calculated as:

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$$CR = \left(\sum \text{standardized loading}\right)^2 \Big/ \left(\left(\sum \text{standardized loading}\right)^2 + \sum (\text{measurement errors})\right).$$

Composite reliability is equivalent to the internal reliability coefficient alpha. In general, the value of composite reliability should exceed 0.70 for acceptable reliability. Receiver–operator curve (ROC) analyses were also performed to examine the clinical utility of the OSA-18 in distinguishing pediatric OSA subjects from normal controls.

To establish the construct validity of a measure, nomological validity will be taken into consideration. We also used nomological validity to analysis the relation between QOL and the constructs for OSA-18 in the pretest and post-test.

RESULTS

In all, 163 students (89 boys and 74 girls, mean age: 9.5 ± 1.7 years) whose parents completed the questionnaire twice were included in study 1 (the school group). The clinical participants for study 2 included 88 subjects (72 boys and 16 girls, mean age: 8.9 ± 2.7 years, body mass index [BMI] = 19.54 ± 4.64) with OSA who had TA surgical treatment.

The PSG data of the OSA children showed an AHI = 13.54 + 7.23 events/h (range: 1.9–19.7 events/h). The reliability statistics showed the Cronbach's alpha of the Chinese-language version of the OSA-18 questionnaire to be 0.897, meeting the significance level.

The study results showed a well-fitting model for the sample (CFI = 0.97, RMSEA = 0.073, SRMR = 0.079). Standardized coefficients are reported in Fig. 1 and Table 1. Factor loadings of the 'Sleep Disturbance items' of the pretest ranged from 0.57 to 0.70, and those of the post-test ranged from 0.66 to 0.76 and were significant. Loadings of the 'Physical Suffering items' of the pretest ranged from 0.40 to 0.85, and those of the post-test ranged from 0.50 to 0.84 and were significant. Loadings of the 'Emotional Distress items' of the pretest ranged from 0.77 to 0.88, and those of the post-test ranged from 0.79 to 0.85 and were significant. Loadings of the 'Daytime Situation items' of the pretest ranged from 0.51 to 0.88, and those of the post-test ranged from 0.58 to 0.86 and were significant. Loadings of the 'Caregiver Concern items' of the pretest ranged from 0.77 to 0.82, and those of the post-test ranged from 0.79 to 0.84 and were significant. There was a demonstration of convergent validity for these 10 latent factors (Fig. 2). Correlations of the five latent factors for the pretest ranged from 0.40 to 0.75, and those of the post-test ranged from 0.52 to 0.85 and were not higher than 0.85, indicating discriminate validity for the pretest and the post-test. The factor intraclass correlations of 'Sleep Disturbance', 'Physical Suffering', 'Emotional Distress', 'Daytime Situation', and 'Caregiver Concern' were 0.91, 0.78, 075, 0.76, and 0.89, respectively (see

Table 1. Correlation matrix of latent factors

	SD_1	PS_1	ED_1	DS_1	CC_1	SD_2	PS_2	ED_2	DS_2
PS_1	0.71								
ED_1	0.45	0.40							
DS_1	0.56	0.50	0.75						
CC_1	0.75	0.61	0.62	0.60					
SD_2	**0.91**	0.57	0.43	0.49	0.64				
PS_2	0.61	**0.78**	0.34	0.47	0.59	0.67			
ED_2	0.56	0.43	**0.75**	0.64	0.61	0.63	0.52		
DS_2	0.56	0.48	0.66	**0.76**	0.71	0.62	0.59	0.85	
CC_2	0.68	0.53	0.48	0.52	**0.89**	0.72	0.66	0.67	0.79

The bold numbers are the test–retest reliability.
SD_1: Sleep Disturbance of the pretest; PS_1: Physical Suffering of the pretest; ED_1: Emotional Distress of the pretest; DS_1; Daytime Situation of the pretest; CC_1; Caregiver Concern of the pretest; SD_2: Sleep Disturbance of the post-test; PS_2: Physical Suffering of the post-test; ED_2: Emotional Distress of the post-test; DS_2; Daytime Situation of the post-test; CC_2; Caregiver Concern of the post-test.

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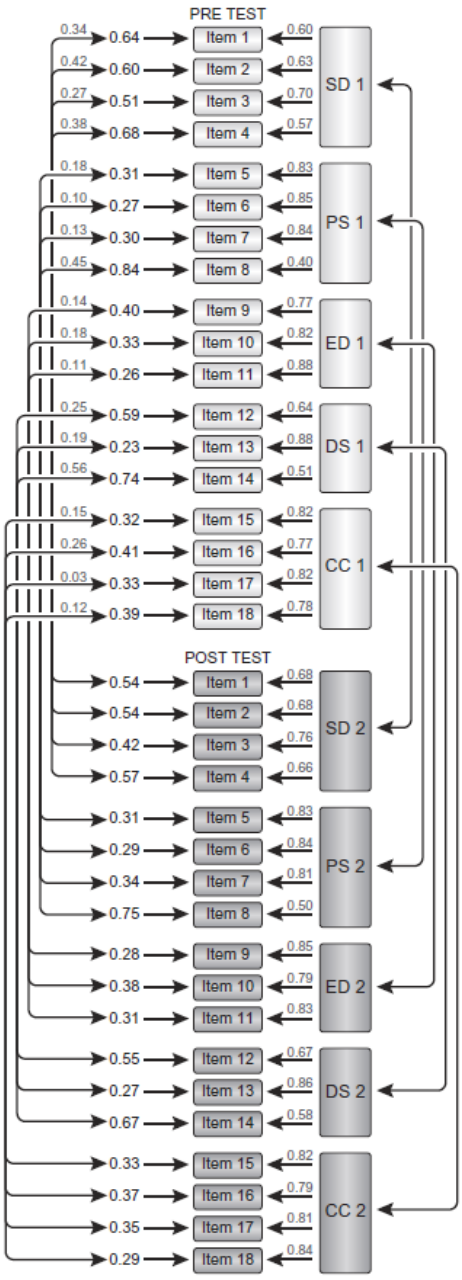

Figure 2. Confirmatory factor analysis of the pretest and post-test.1. SD_1: Sleep Disturbance of pretest; PS_1: Physical Suffering of pretest; ED_1: Emotional Distress of pretest; DS_1: Daytime Situation of pretest; CC_1: Caregiver Concern of pretest; SD_2: Sleep Disturbance of post-test; PS_2: Physical Suffering of post-test; ED_2: Emotional Distress of post-test; DS_2: Daytime Situation of post-test; CC_2: Caregiver Concern of post-test.2. Factor loadings of the 'Sleep Disturbance items' of the pretest ranged from 0.57 to 0.70, and those of the post-test ranged from 0.66 to 0.76 and were significant. Loadings of the 'Physical Suffering items' of the pretest ranged from 0.40 to 0.85, and those of the post-test ranged from 0.50 to 0.84 and were significant. Loadings of the 'Emotional Distress items' of the pretest ranged from 0.77 to 0.88, and those of the post-test ranged from 0.79 to 0.85 and were significant. Loadings of the 'Daytime Situation items' of the pretest ranged from 0.51 to 0.88, and those of the post-test ranged from 0.58 to 0.86 and were significant. Loadings of the 'Caregiver Concern items' of the pretest ranged from 0.77 to 0.82, and those of the post-test ranged from 0.79 to 0.84 and were significant. There was a demonstration of convergent validity for these 10 latent factors. Correlations of the five latent factors for the pretest ranged from 0.40 to 0.75, and those of the post-test ranged from 0.52 to 0.85 and were not higher than 0.85, indicating discriminate validity for the pretest and the post-test.

Table 1), demonstrating the presence of sufficient test–retest reliability. The composite reliability coefficients for these 10 factors were acceptable (the Sleep Disturbance of the pretest: CR = 0.72; the Physical Suffering of the pretest: CR = 0.83; the Emotional Distress of the pretest: CR = 0.86; the Daytime Situation of the pretest: CR = 0.73; the Caregiver Concern of the pretest: CR = 0.88; the Sleep Disturbance of the post-test: CR = 0.79; the Physical Suffering of the post-test: CR = 0.84; the Emotional Distress of the post-test: CR = 0.86; the Daytime Situation of the post-test: CR = 0.75; and the Caregiver Concern of the post-test: CR = 0.89). The Chinese version of the OSA-18 demonstrated good reliability.

To establish the construct validity of a measure, nomological validity should be taken into consideration. Nomological validity generally deals with whether the constructs under investigation are related to other constructs in a way that is theoretically meaningful. In this study, we presented two kinds of nomological network. One is the relation of the constructs of the OSA-18 with QOL, and the other is the difference shown by the two subject groups (AHI > 1 and AHI ≦ 1) in the scores of the constructs for OSA-18. There was a presence of substantial correlation between QOL and the constructs for OSA-18 in the

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Table 2a. Correlation of the constructs of obstructive sleep apnea syndrome with QOL

	SD_1	PS_1	ED_1	DS_1	CC_1
QOL_pre	−0.40***	−0.35***	−0.41***	−0.38***	−0.57***
	SD_2	PS_2	ED_2	DS_2	CC_2
QOL_post	−0.44***	−0.37***	−0.37***	−0.48***	−0.61***

***$P < 0.001$.
The correlation coefficients are derived from CFA.
QOL, quality of life.

pretest and post-test (Tables 2a and 2b); these results demonstrated the strong nomological validity of the Chinese version of the OSA-18. The results also showed an improvement in the QOL score, from 5.12 ± 1.58 to 6.05 ± 1.76 ($P = 0.001$) after AT. AHI and mean oxygen saturation significantly improved after AT without significant change in BMI (Table 3). There was improvement in all items of the OSA-18 6 months' post-TA (Table 4). Moreover, the most common symptoms of pediatric OSA were poor attention span (93.1%), loud snoring (88.0%), caregiver worried about child's health (87.1%), difficulty awakening (86.1), and mouth-breathing (79.2%) (Table 4). After TA surgery, the most improved symptoms were daytime drowsiness and poor attention span (Table 4).

In terms of discriminating pediatric OSA from normal controls using the OSA-18, the area under the curve (AUC) for differentiating pediatric OSA from normal controls was 0.856. The value of 0.50 was not included within a 95% confidence interval of the AUC (0.80–0.96), strongly suggesting that the discriminative capability of the Chinese version of the OSA-18 is statistically sound. The ROC analysis included sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV), with cut-off scores ranging from 65 to 66 (Table 5).

We also analyzed the correlation between OSA-18 and AHI, tonsil size and adenoid hypertrophy. The results showed a statistically significant correlation between OSA-18 total score and AHI ($r = 0.291$*, $P < 0.05$) and the Hypopnea Index ($r = 0.29$*, $P < 0.05$). Also, there was a significant correlation ($r = 0.532$*, $P < 0.01$) between adenoid hypertrophy and OSA-18 total score. However, tonsil size did not correlate with OSA-18 total score.

DISCUSSION

The goal of the present study was to examine the psychometric properties of the OSA-18 Chinese version, and to evaluate the clinical utility of the OSA-18 in screening children with non-obese SDB. Moreover, we also used the questionnaire to analyse the common symptoms of pediatric OSA and tried to infer the possible pathophysiology of pediatric OSA.

Table 2b. Comparison of the mean change after the treatment of children with obstructive sleep apnea syndrome

$n = 88$	Pre-surgery	Post-surgery 6 months	Sig. (2-tailed)
Sleep disturbance (M ± SD)	16.25 ± 4.76	9.59 ± 4.57	0.001
Physical suffering (M ± SD)	15.27 ± 4.71	11.15 ± 4.76	0.005
Emotional distress (M ± SD)	11.14 ± 4.7	9.39 ± 4.00	0.013
Daytime problems (M ± SD)	12.35 ± 3.43	10.0 ± 3.64	0.001
Caregiver concern (M ± SD)	17.76 ± 5.10	12.06 ± 4.60	0.001
QOL (M ± SD)	5.12 ± 1.58	6.05 ± 1.76	0.001

Significant difference showed the good sensitivity of the OSA-18 in evaluating the improvement in symptoms.
M ± SD, mean ± SD of total score; QOL, quality of life.

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Table 3. PSG data and the clinical characteristic data pre- and 6 months post-TA

Variable ($n = 88$)	Pre-surgery	Post-surgery (6 months)	*P*-value
Age (years) (mean ± SD)	8.9 ± 2.7	9.6 ± 2.53	0.73
Sex (male/female) (%)	$n = 72$ (81.8%)/$n = 16$ (18.2%)	$n = 72$ (81.8%)/$n = 16$ (18.2%)	–
BMI (Kg/m^2) (mean ± SD)	19.54 ± 4.64	19.31 ± 4.17	0.778
Tonsil hypertrophy (more than grade 2)	97.7%	0	0.000
Adenoid hypertrophy	84.1%	0	0.000
PSG findings ($n = 88$)			
AHI (events/h)	13.54 + 7.23	3.47 ± 2.01	0.001
AI (events/h)	4.06 ± 3.69	1.03 ± 0.70	0.023
PMLI (events/h)	1.58 ± 3.65	1.02 ± 1.19	0.878
REM (%)	11.14 ± 6.05	14.00 ± 6.31	0.021
Stage 1 (%)	19.09 ± 8.65	15.39 ± 7.58	0.072
Stage 2 (%)	35.11 ± 12.71	26.11 ± 4.48	0.069
Stage 3 + Stage 4 (%)	35.54 ± 6.01	44.53 ± 10.10	0.034
Mean SaO_2	96.20 ± 2.10	96.92 ± 1.56	0.032
Sleep efficiency (%)	87.26 ± 12.72	88.73 ± 10.52	0.395
Sleep latency (min)	18.97 ± 9.36	15.02 ± 7.41	0.040
Total sleep time (min)	425.79 ± 35.26	429.21 ± 36.48	0.287

AHI, Apnea–Hypopnea Index; AI, Apnea Index; BMI, body mass index; BW, bodyweight; PLMI, Periodic Leg Moment Index; PSG, polysomnography; SaO_2, oxygen saturation.

The OSA-18 is not only a discriminative measure of SDB severity, but also a measure evaluating longitudinal change in SDB status.[17,18] The Chinese version of the OSA-18 demonstrated good validity in clinical settings. Children and young adolescents with OSA had higher scores using the OSA-18 than children without sleep complaints. Moreover, there was a significant reduction in OSA-18 total scores post-TA in our children, indicating that the OSA-18 Chinese-language version can be used as an outcome measure for the treatment of OSA children.

Although more and more cohort studies examining the efficacy and outcomes of AT in pediatric OSA have shown the recurrence and persistence of pediatric SDB after TA.[19–24] Our 3-year follow-up study also showed the recurrence of SDB 1 year after TA.[20] However, the AHI improved a lot during the 6 months after TA. This finding is the same result as those in the studies by Marcus *et al.* and Katz *et al.*, which showed that pediatric SDB improved 7 months after TA.[31,32] Therefore we selected 6 months post-TA in this study as adequate for assessment.

When considering the use of the OSA-18 as a screening tool for 6–12-year-old pediatric OSA, ROC analysis showed that with a cut-off score of 66, the OSA-18 had good sensitivity (0.79) in detecting OSA in children; it also demonstrated fair specificity (0.77), meaning that it could avoid selecting too many false positive cases. Its PPV (0.86) and NPV (0.68) were also good.

Consistent with US studies,[17,18,33] QOL showed significant correlation with Apnea Index ($r = 0.428$**), central apnea count ($r = 0.497$***), and mixed apnea count ($r = 0.362$*). Overall, between-group difference was significant in the OSA-18 ($F = 22.67$***), and in its subscales ($F = 5.498$* –32.714***), and also showed excellent results in discriminant analysis. Our study showed that QOL significantly improved after TA treatment. Therefore, consistent with previous studies using the English version of the OSA-18, the current results show that the Chinese version can be a useful measure across different cultures and nationalities.

There were some symptoms in our children with OSA, such as inattention and poor attention span, mouth-breathing, and difficulty awakening, that were frequently reported. Although excessive daytime sleepiness (EDS) is commonly considered

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Table 4. Change in OSA-18 Chinese version questionnaire scores after adenotonsillectomy ($n = 88$)

	Pre-surgery		Post-surgery 6 months		
Item[†]	Score (No. 1 to No. 3)	Score (No. 4 to No. 7)	Score (No. 1 to No. 3)	Score (No. 4 to No. 7)	*P*-value
Item 1	12.0%	88.0%	24.8%	75.2%	0.001
Item 2	38.4%	61.6%	88.2%	11.8%	0.001
Item 3	49.0%	51.0%	88.4%	11.6%	0.001
Item 4	37.6%	62.4%	76.8%	23.2%	0.001
Item 5	24.8%	79.2%	76.7%	23.3%	0.001
Item 6	31.7%	68.3%	73.1%	26.9%	0.001
Item 7	27.7%	72.3%	56.5%	43.3%	0.001
Item 8	23.0%	77.0%	72.8%	27.2%	0.001
Item 9	36.6%	63.4%	58.0%	42.0%	0.001
Item 10	51.5%	48.5%	69.6%	30.4%	0.001
Item 11	39.4%	60.6%	60.9%	39.1%	0.001
Item 12	28.0%	72.0%	71.2%	28.8%	0.001
Item 13	6.9%	93.1%	42.0%	58.0%	0.001
Item 14	13.9%	86.1%	40.6%	59.4%	0.001
Item 15	12.9%	87.1%	55.1%	44.9%	0.001
Item 16	24.8%	75.2%	66.2%	33.8%	0.001
Item 17	51.5%	48.5%	73.9%	26.1%	0.001
Item 18	51.4%	48.6%	53.6%	46.4%	0.001
	(scale 1 to 5)	(scale 6 to 10)	(scale 1 to 5)	(scale 6 to 10)	
QOL item[‡]	61.2%	38.8%	38.2%	61.8%	0.001

[†]Item 1. Loud snoring; 2. Breath holding/pauses; 3. Choking or gasping; 4. Fragmented sleep; 5. Mouth breathing; 6. Frequent colds or URI; 7. Rhinorrhea; 8. Dysphagia; 9. Mood swings or tantrums; 10. Aggression/hyperactivity; 11. Discipline problems; 12. Daytime drowsiness; 13. Poor attention span; 14. Difficulty awakening; 15. Caregiver worried about child's health; 16. Caregiver concerned child does not have enough air; 17. Caregiver missed activities; 20. Caregiver frustration. Scale 1 means 'none of the time'; scale 4 means 'some of the time'; scale 7 means 'all of the time'.
[‡]QOL item: quality of life via a 10-point visual analogue scale (the higher scale comes with higher quality of life).

to be a primary feature of OSA in adults, the studies by Gozal *et al.* found that fewer children with OSA reported EDS than adults with OSA, with the notable exception of obese children.[6,34] They found that in the presence of OSA of similar severity, obese children were at an increased risk of EDS.[34] In our study, we found that mouth-breathing is a common symptom associated with OSA in non-obese children. Guilleminault *et al.* and Huang *et al.* demonstrated that abnormal nasal resistance early in life leads to mouth-breathing associated with abnormal muscle tone, oral–facial hypotonia, and secondary changes in maxillary–mandibular growth.[35,36] Our premature infants study presented with abnormal oral–facial features, particularly a high and narrow hard palate with presence of mouth-breathing and sleep apnea demonstrated on

Table 5. Receiver–operator curve analysis for discrimination of OSA and school groups in Chinese version of the OSA-18

Cut-off point	Sensitivity	Specificity	PPV	NPV
55 score	0.87	0.64	0.80	0.74
59 score	0.84	0.64	0.80	0.70
60 score	0.84	0.64	0.80	0.70
63 score	0.84	0.64	0.80	0.70
64 score	0.84	0.64	0.80	0.67
65 score	0.82	0.71	0.84	0.68
66 score	0.79	0.77	0.86	0.68

NPV, negative predictive value; OSA, obstructive sleep apnea syndrome; OSA-18, Obstructive Sleep Apnea Questionnaire-18; PPV, positive predictive value.

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PSG.[35–37] This study's finding proves that open-mouth-breathing is a very important symptom of non-obese pediatric OSA.

There are some methodological limitations that should be noted when interpreting our findings. First, the sample sizes of the two groups in our study were very different: the clinical participants for study 2 included only 39 subjects. Furthermore, the sample sizes of the two groups were small. Second, the questionnaire was completed by parents, not children. Some children do not sleep with their parents, so some findings may have been overlooked. However, this study is the first to analyze the symptoms of SDB in Taiwanese children before and after AT using a subjective questionnaire and objective PSG.

Conclusion

The Chinese-language version of the OSA-18 questionnaire for pediatric OSA is a reliable and valid instrument. It is a suitable screening tool and outcome measure for OSA in Taiwanese children. The common symptoms in non-obese OSA children were poor attention span, loud snoring, caregiver worried about child's health, difficulty awakening, and mouth-breathing. The questionnaire also showed improvement in the QOL of OSA children post-TA.

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RESEARCH ARTICLE Open Access

Sleep and breathing in premature infants at 6 months post-natal age

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Abstract

Background: Poor sleep contributes to the developmental problems seen in preterm infants. We evaluated sleep problems in preterm infants 6 months of post-gestational age using the subjective Brief Infant Sleep Questionnaire (BISQ) and objective sleep tests. We also compared the sleep of premature infants with that of full-term infants.

Methods: The study included 68 6-month-old full-term healthy infants and 191 premature infants born at <37 weeks gestation. All parents completed the BISQ-Chinese version and sleep diaries. At the same time, all premature infants were submitted to one night of polysomnography (PSG) in the sleep laboratory and also were set up with an actigraph kept for 7 days. Statistical analyses were performed using correlation coefficients and the t-test with SPSS version 18 to compare questionnaire responses with other subjective and objective measures of sleep.

Results: The sleep problems indicated in the subjective questionnaire for the premature infants, particularly: "the nocturnal sleep duration, number of night awakenings, daytime sleep duration, duration of time with mouth breathing, and loud-noisy breathing" had significant correlations with sleep diaries, actigraphy and PSG results. The BISQ showed that duration of infant's sleeping on one side, nocturnal sleep duration, being held to fall asleep, number of nighttime awakenings, daytime sleep duration, subjective consideration of sleep problems, loud-noisy breathing, and duration spent crying during the night were significantly different between the premature infants and the term infants. PSG confirmed the presence of a very high percentage (80.6%) of premature infants with AHI > 1 event/hour as indicated by the questionnaire.

Conclusion: Premature infants have more sleep problems than full-term infants, including the known risk of abnormal breathing during sleep, which has been well demonstrated already with the BISQ-Chinese (CBISQ).

Keywords: Sleep questionnaire, Sleep-disordered breathing, Prematurity, Full-term infant

Background

Sleep is essential to human life and developmentally involves both physiologic and mental processes. During infancy, humans spend a majority of time in sleep [1-4]. Sleep is recognized not only as a resting state, but also as a state of intense brain development during which neurotransmitters specific for each sleep stage impact brain maturation [2,5-7].

Numerous studies have shown differences in sleep quality between premature and full-term infants [3-6,8-12]. Premature infants often have many problems during sleep, and the more premature they are the more problems are seen [6,8]. However, when sent home, the infant is considered by the pediatrician to have passed any life-threatening situations. Also, it seems that premature infants develop sleep-wake cycles differently from full-term infants, but that maturation of sleep is related to both time since birth and gestational age [9,12]. Many maturational events occur during sleep. Some parental reports indicated that 21% of children born prematurely are habitual snorers [13-15]. Premature infants have the well-known risk of abnormal control of breathing during sleep just after birth, and sleep apnea of prematurity has been well described [13-15]. Their presence usually leads premature infants to stay in the hospital setting till judged to have normal control of breathing during sleep. Many functions are altered in premature infants, and more so the more premature

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the infant is. Often there is generalized hypotonia, well known by specific neurological exams including regular follow-up of the presence of the "scarf-sign" [16], but there is also immaturity of many functions, including poor sucking and poor swallowing, and often lack of coordination of sucking, swallowing and breathing, leading to important and well documented oxygen saturation drops during feeding when such dysfunctions exist [17,18]. The sleep breathing problems also may be due to a lack of tone in the upper airway followed by collapse and obstruction or diaphragmatic movement dysfunction with immaturity of reflexes. Therefore, sleep-disordered breathing (SDB) is often noted in premature infants [14,15]. In the recent past, we made the preliminary observation that premature infants may have oral-facial developmental growth problems [18]. Such difficulties may lead to specific breathing problems during sleep and impair the sleep of the infants.

Our study focuses on the sleep of premature infants at 6 months of post-natal age who were born between 24 and 36 weeks of gestational age but had been considered to be in sufficient good health to be sent home without any treatment, and who were having only regular post-natal visits scheduled with the pediatrician. We compared our findings to those simultaneously obtained in a small group of full-term infants born during the same period.

Methods

Subjects

During the years 2010–2012, women delivering before 37 weeks of gestational age were asked to enroll their neonates prior to discharge from the neonatal intensive care unit of Chang Gung Memorial Hospital, a medical center with a pediatric department in Taiwan as part of the preterm infant group. Both boys and girls were included. The full-term infant group, with a gestational age ranging from 37 to 40 weeks and birth body weight of more than 2500 grams, was enrolled from the neonatal outpatient clinic in our hospital.

Inclusion criteria

- All neonates born in our hospital before 37 completed gestational weeks, without presentation of exclusion criteria and with a signed parental consent form during the study period comprised the "preterm-infants group".
- All neonates delivered at 37 to 40 weeks of gestational age with birth body weight of more than 2500 grams, without presentation of exclusion criteria and with a signed parental consent form during the study period comprised the "full-term-infants group".

Exclusion criteria

- Neonates with severe physical impairments (such as severe congenital heart diseases, DiGeorge syndrome, congenital hydrocephalus, and kernicterus) due to perinatal insults or hypoxic ischemic encephalopathy. We also excluded bronchopulmonary dysplasia or still required oxygen support (nasal cannula) after discharge.
- Neonates with confirmed severe congenital malformations.

Methods

(1) After obtaining written parental informed consent to take part in an institutionally approved human subject protocol (Chang Gung Memorial Hospital IRB: 98-2670C), the infants meeting the inclusion criteria began participation in our prospective growth study during the first year of life.

(2) Infants had a pediatric evaluation at a regular 6-month post-natal visit and a sleep clinical evaluation. As part of this sleep evaluation, all premature infants underwent one night of PSG (Embla N7000 PSG recording-sleep- system) in the pediatric sleep laboratory. The following variables were monitored: electroencephalography, electromyography, electrocardiography and electro-oculography. Respiration was monitored with a nasal-cannula-pressure transducer, oral thermistor, thoracic and abdominal inductive plethysmography bands and pulse-oximetry. The infants were continuously video-monitored. For PSG scoring, the recommendations of the AASM-2007 were followed [7].

(3) All infants were set up with an actigraph (Philips Respironics actiwatch 2, with a small size well-suited for use with younger subjects or those sensitive to wrist-worn devices.) on the left leg of the non-dominant side. The equipment measured body movements and light exposure. It was placed on the infant at the time of the visit and kept for 7 days, and was analyzed with commercially available software with one point every 2 minutes and indicated activity/non-activity. The equipment was correlated with a log simultaneously kept by care-givers.

(4) At the same time, the parents were asked to fill out a validated questionnaire, the "Brief Infant Sleep Questionnaire-Chinese version", (CBISQ), at the regular 6-month and 7-month visits to check the consistency of responses. The CBISQ is derived from the Brief Infant Sleep Questionnaire (BISQ) [19] of Avi Sadeh Additional file 1. The original BISQ had 13 items distributed in 3 categories, evaluating sleep duration, night awakenings, and method of falling

asleep of infants aged 29 months or younger. To validate the Chinese version of the BISQ, we first translated and then back-translated the BISQ from English to Chinese. The test–retest reliability of the CBISQ was acceptable. There was significant correlation between the repeated sleep measures for location of sleep (r = 0.678*), preferred body position (r = 0.796*), nocturnal sleep duration (r = 0.534*), method of falling asleep (r = 0.848*), difficulty in falling asleep (r = 0.785*), number of night awakenings (r = 0.439*), daytime sleep duration (r = 0.455*), and subjective consideration of sleep problems (r = 0.663*). In order to evaluate respiratory sleep problems compared with actigraphy and PSG, we added 3 questions to the BISQ to create the CBSIQ version; these included "time spent with mouth breathing" (r = 0.568*), "severity of loud-noisy breathing" (0.760*), and "time spent crying during the night" (r = 0.206).

(5) The small group (n = 68) of full-term infants (the "controls") whose parents signed the consent form underwent a similar evaluation and was studied the same way as the premature infants.

Analysis

Pediatrician notes of the 6-month follow-up evaluation for both the premature and full-term infants were reviewed. Complaints with specific emphasis on sleep were collected. The responses to the CBSIQ were then tabulated. Responses of premature infants one month later to the same questionnaire were also tabulated, and actigraphy and PSG data were analyzed. The statistical software package SPSS, Version 18 was used for data analysis. Variables are presented as either mean ± standard deviation (SD) or frequency. We used the t-test and Chi-square test for evaluation of differences between these 2 groups. The Pearson correlation coefficient was used in analysis of correlation between questionnaire and sleep Lab data. The statistical significance was defined at the 0.05 level.

Results

Demographic data

We originally signed up 229 premature infants from the PICU, but only 191 (83.41%) completed the study when they were 6 months old. The 191 6-month-old premature infants in the preterm infants group comprised 99 boys and 92 girls with an average birth body weight of 1646.66 g. The average gestational age was 31.52 weeks, with a maximum of 36 weeks and minimum of 24 weeks. There were 68 6-month-old infants in the full-term infants group, including 34 boys and 34 girls with an average birth body weight of 3169.69 grams and average gestational age of 38.35 weeks (Table 1).

The subjective and objective sleep problems of the premature infants (Table 2)

We compared the subjective CBISQ to other objective data obtained from sleep diaries, actigraphy and PSG data. These items of CBISQ showed sleep measures of "nocturnal sleep duration, number of night awakenings, daytime sleep duration and loud-noisy breathing" were significantly correlated with sleep diary, actigraphy and PSG. Items such as "mouth breathing" and "loud-noisy breathing" correlated with "total number of obstructive apnea during sleep" in PSG. Longer "Daytime sleep duration" also correlated with AHI >1; We were interested in these correlations as "mouth breathing" and "Loud-noisy breathing" are common symptoms associated with pediatric obstructive sleep apnea. Moreover, higher AHI means severe OSA will interrupt sleep and increase night awakenings and then induce daytime sleepiness. Therefore, the trend in the correlation between "AHI" and "number of obstructive apnea" in PSG and "Daytime sleep duration" in CBISQ were important and meaningful. The additional questions in the CBISQ pertaining to respiratory sleep problems also were shown to correlate with PSG.

The PSG data showed that 80.6% of 6-month-old premature infants had an apnea–hypopnea index (AHI) >1 event/hour (mean AHI = 3.63 ± 3.24), mean SaO2 97.01 ± 1.00%, total sleep time 368.83 ± 55.05 mins, sleep efficiency 82.43 ± 14.66%, and REM 24.03 ± 6.43% at polysomnography.

Comparison of the differences between full-term and premature infants (Table 3)

Since our CBISQ was shown to be a reliable and valid tool for sleep measurement, we used the data to compare the differences in sleep between the preterm-infant and

Table 1 Demographic data of the study subjects

		Preterm infants (n = 191)	Full-term infants (n = 68)	P value
Gender	Male	n = 99 (51.83%)	n = 34 (50%)	
	Female	n = 92 (48.17%)	n = 34 (50%)	
Birth body weight (g)		1646.66 ± 588.60	3169.69 ± 410.58	<0.001*
Birth body height (cm)		39.96 ± 5.11	49.61 ± 1.88	<0.001*
Head circumference (cm)		28.88 ± 3.33	34.25 ± 1.24	<0.001*
Gestational age (weeks)		31.52 ± 3.21	38.35 ± 1.43	<0.001*

Birth body weight, Birth body height and Head circumference were all assessed at birth.

Table 2 Subjective and objective sleep problems of premature infants

Questionnaire	Objective sleep measure	r	P value
CBISQ	**Criterion of sleep diary**	0.757	0.011*
Nocturnal sleep duration	Nocturnal sleep duration		
Number of night awakenings	Nocturnal awake time	0.632	0.002*
	Numbers of night awakening	0.580	0.001*
	Nocturnal sleep efficiency	−0.644	<0.001*
Daytime sleep duration	Daytime sleep duration	0.630	0.002*
Loud-noisy breathing	Daytime sleep duration	0.348	0.064+
CBISQ	**Actigraphy criterion**		
Time spent with mouth breathing	Awakening time during sleep	0.514	0.042*
	Nocturnal sleep efficiency	−0.255	0.013*
CBISQ	**PSG criteria**		
Nocturnal sleep duration	Nocturnal sleep duration	0.545	0.002*
Number of night awakenings	Desaturation index	0.636	0.003*
Daytime sleep duration	AHI in sleep	0.767	0.075+
	Awakening after sleep onset	0.350	0.080+
Time spent with mouth breathing	Obstructive apnea count	0.509	0.026*
Loud-noisy breathing	Arousal count	0.401	0.089+
	Obstructive apnea count	0.535	0.018*

*P value < 0.05. +P value < 0.10.
AHI: Apnea-Hypopnea Index (events/hour); Desaturation indec desaturation events/hour; "Arousal count" means total number of arousal during sleep; "Obstructive apnea count" means total number of obstructive apnea during sleep.

full-term-infant groups. The results revealed, as expected, a significant difference between the 2 groups: The premature group preferred a side body position and being held when falling asleep, had more night awakenings, greater subjective mention of presence of a sleep problem by caregivers, louder noisy breathing and more time spent crying during the night. Also, the premature infants had longer nocturnal and daytime sleep duration.

Discussion

Based on the results described above, we consider the CBISQ a reliable and valid tool for the measurement of sleep problems in infants. It can be used to measure nocturnal sleep duration, number of night awakenings, daytime sleep duration, time spent breathing with the mouth and loud-noisy breathing during sleep. These measures may indicate the presence of underlying infancy sleep problems and sleep-disordered-breathing. Finally, to make the CBISQ an internationally available tool, it was submitted to a Chinese–English back-translation.

Not only did our study validate the CBISQ, but it is also the first study targeting the difference between premature and full-term infants through a reliable and valid screening questionnaire. Based on our results, premature infants, rather than full-term infants, preferred a side body position and being held when falling asleep. Apart from this, premature infants had longer nocturnal sleep duration, more night awakenings, and longer daytime sleep duration. The caregivers of premature infants noted that their children had more sleep problems. A significant difference between preterm and full-term infants in nocturnal sleep duration and loud-noisy breathing was noted. The number of night awakenings and time spent with mouth-breathing also varied between the 2 groups. These questionnaire findings indicated a higher prevalence of sleep-breathing disorders [20-22], and correlated with those obtained with PSG that showed a very high percentage (80.6%) of premature infants with AHI greater than 1 event/hour. These disorders then impact nocturnal sleep time and quality.

As a pilot study in this field, our results provide new insights into the difference between premature and full-term infants with regard to sleep problems. During the first year of life, infancy-sleep is a rapid maturational process [23,24], so the settling time, daytime sleep duration and time spent crying during the night may vary widely between repeated questionnaires. Infancy sleep maturation may impact the maturation of the central nervous system, overall functioning, and future cognitive, motor, and temperament development [25].

There are some methodological limitations that should be noted when interpreting our findings. First, the sample sizes of the 2 groups in our study were very different, so some of the differences between the premature and full-term infants may not be apparent in the CBISQ sleep

Table 3 Comparison of the preterm-infants group and the full-term-infants group using the CBISQ

CBISQ Sleep measure		Preterm infants	Full-term infants	P value
Location of sleep	Infant crib in a separate room	1.0%	0.0%	-
	Infant crib in parents' room	33.0%	33.3%	0.965
	In parents' bed	64.9%	66.6%	0.828
	Infant crib in room with sibling	1.0%	0.0%	-
Preferred body position	On his/her back	52.2%	51.0%	0.876
	On his/her side	37.7%	31.4%	0.397
	On his/her belly	10.1%	17.6%	0.193
Nocturnal sleep duration (minutes, mean ± SD)		544.87 ± 81.93	490.71 ± 134.48	0.027*
Nocturnal sleep-onset time[‡] (mean ± SD)		2.61 ± 1.31	2.53 ± 1.02	0.782
Method of falling asleep	While feeding	19.8%	23.1%	0.555
	Being rocked	23.4%	21.5%	0.752
	Being held	24.8%	23.1%	0.769
	In bed alone	12.2%	12.3%	0.986
	In bed near parent	19.8%	20.0%	0.969
Difficulty falling asleep[‡] (mean ± SD)		2.47 ± 1.93	2.16 ± 1.71	0.494
Number of night awakenings[‡] (mean ± SD)		2.28 ± 0.93	1.72 ± 0.67	0.014*
Daytime sleep duration (minutes, mean ± SD)		364.07 ± 152.1	271.67 ± 133.16	0.014*
Subjective consideration of sleep problems[‡] (mean ± SD)		1.53 ± 0.69	1.21 ± 0.54	0.024*
Time spent with mouth breathing[‡] (mean ± SD)		1.47 ± 0.57	1.20 ± 0.42	0.077+
Loud-noisy breathing[‡] (mean ± SD)		1.88 ± 0.69	1.44 ± 0.51	0.010*
Time spent with crying during night[‡] (mean ± SD)		1.64 ± 0.71	1.26 ± 0.45	0.003*

*P value < 0.05. +P value < 0.10.
[‡]These questions were evaluated by severity, and scored from 1 to 4.

measurement. Second, 16.59% of our study group (premature infants) did not complete the CBISQ assessment and sleep examination when they were 6 months old, but our follow-up study had more than 70% of the initially signed-up children. Third, actigraphy provides only integrated sleep data, not detailed parameters of the sleep status, so the validity testing of the CBISQ sleep measure with actigraphy was not ideal. Fourth, it is difficult to perform PSG in premature infants because of uncooperative child, night feeding and size of premature infant with too small limbs and torso for some equipments. However our premature group was large, and actigraphy and PSG data were both obtained from this large number of premature infants. But our study monitored simultaneous objective and subjective data investigating sleep problems of the premature.

Using this reliable and validated tool, the CBISQ, longitudinal studies surveying premature infants using sleep measurement and mental development every 3 months from birth can be designed to address the issue of delayed development in premature infants and how this correlates with sleep-breathing disorders or poor nocturnal sleep quality. Use of the CBISQ may help clinicians follow the evolution of sleep problems overtime and schedule sleep studies when needed.

Conclusion

Infancy sleep maturation may impact the maturation of the central nervous system, overall functioning, and future cognitive, motor, and temperament development. Premature infants have more sleep problems than full-term infants, including the known risk of abnormal breathing during sleep, which has been well demonstrated already with the BISQ-Chinese (CBISQ).The longitudinal studies surveying premature infants using sleep measurement and mental development every 3 months from birth can be designed to address the issue of delayed development in premature infants and how this correlates with sleep-breathing disorders or poor nocturnal sleep quality.

Additional file

Additional file 1: Brief Infant Sleep Questionnaire-Chinese version (CBISQ). The CBISQ is derived from the Brief Infant Sleep Questionnaire (BISQ) [19] of Avi Sadeh. We translated the BISQ from English to Chinese.

Abbreviations

BISQ: Brief infant sleep questionnaire; CBISQ: Brief infant sleep questionnaire-Chinese version; PSG: Polysomnography; AHI: Apnea-Hypopnea Index; PICU: Premature intensive care unit.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Y-SH: designed and organized the study, collected and conducted the data analysis and drafted the manuscript. CG: scientific advisor, co-designed the study, supervised the research process and strictly revised the manuscript. M-CK: participated in the study design and data analysis, and rechecked the data-related issues. TP: participated in study design and revision of the manuscript. J-FH: participated in the data collection. All authors read and approved the final manuscript.

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Early detection of minor neurodevelopmental dysfunctions at age 6 months in prematurely born neonates

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ABSTRACT

Objective: To investigate the 6-month neurodevelopmental outcomes of prematurely born neonates and find the determining neonatal factors of minor neurological dysfunctions (MNDs).
Study design: We examined data collected prospectively on 151 infants born before 37th week of gestation in 2009–2010 who were assessed at 6 months corrected age with the Bayley Scales of Infant Development—2nd Edition (BSID-II) and the Denver Developmental Screening Test (DDST).
Results: Of 151 neonates born before 37 weeks, 20 (13.2%) had MNDs at 6 months corrected age. These proportions were 21.6%, 13.2%, and 8.2% for neonates born before 28 weeks, 29 weeks to 32 weeks, and 33 weeks to 36 weeks, respectively. Half of neonates with MNDs have a birth body weight of less than 1000 g. BSID-II and DDST are highly correlated in assessing the MNDs of premature neonates at 6 months corrected age. MND was independently associated with postnatal corticosteroid use (odds ratio [OR], 11.2; 95% confidence interval [CI], 1.9–66.0, $P=0.008$) and cholestasis (OR, 6.2; 95% CI, 1.16–33.1, $P=0.033$).
Conclusions: Premature neonates, even those born at 33 to 36 weeks, are found to have MNDs as early as 6 months corrected age by BSID-II and DDST, with risk increasing as gestation decreases.
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1. Introduction

Improved obstetric and neonatal care, including use of intra-partum antibiotics, successful resuscitation in the delivery room and advanced progress of mechanical ventilation have resulted in increasing survival of premature infants [1–3]. The prevalence and risk factors of severe neurodevelopmental sequelae, such as cerebral palsy, kernicterus, hearing loss, and cognitive deficiencies are the major topics of recent studies [4–7]. However, prematurely born neonates without major neurological deficits have been proven to be at higher risk of developing subtle neuromotor dysfunctions, such as difficulties with gross motor skill, social contact, or learning [8]. The frequency of these minor neurodevelopmental dysfunctions (MNDs)[1] is usually assessed in early childhood (age 2–6 years) [3,4,8,9], and assessment of the quality of general movement during this period is found to be a powerful instrument to predict later neurological and behavioral developmental difficulties at school age [10–12].

Given the fact that the most common disability of premature infants at 2 years of age is developmental and cognitive impairments and these assume great significance at their school years, it is imperative to understand the prevalence and risk factors of these disabilities. Besides, few studies have provided data related to neurodevelopmental outcomes of late-preterm infants [13–15], and it is unknown whether these MNDs can be detected at earlier age of young infancy. The aim of the present study was to determine the prevalence of MNDs and cognitive and

[1] Keynote: Minor neurodevelopmental dysfunctions (MNDs) can be detected as early as 6 months corrected age in prematurely born neonates, even those born late-preterm at 33 to 36 weeks. Postnatal corticosteroid use and cholestasis are found to be independently associated with MNDs by multi-factors analysis adjusted to birth body weight and gestational age. The high rate of MNDs at 6 months old justifies the requirement of early intervention for these preterm children.

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motor functions at 6 months corrected age in a cohort of premature neonates, to investigate which sociodemographic or neonatal factors are associated with MNDs, and to figure out if MNDs in these high risk neonates can be precisely detected at early infancy.

2. Methods

2.1. Study population

During the years 2009–2010, women delivering before 37 completed weeks in our institution were asked to enroll in the study before their neonates were discharged from our neonatal intensive care units (NICUs). After written parental informed consent of an institutionally approved human subjects' protocol, infants participated in a prospective growth study during the first year of life with developmental assessment protocols performed every three months. All these participants were prematurely born with a gestational age of ≧24 and less than 37 completed weeks. They were a non-random convenience sample of children selected for their willingness to participate in the protocol and obtained from some neonatologist physicians in our NICUs. Since the current study aimed to detect MNDs, neonates with cerebral palsy, severe mental or sensory impairments due to perinatal insults or hypoxic ischemic encephalopathy were excluded from this study. We also excluded children with confirmed chromosome anomalies and severe congenital malformations.

2.2. Data collection and definitions

The obstetric and perinatal data were collected by reviewing the medical charts. Gestational age (GA) was based on the last menstrual period and early prenatal ultrasonography. Small for gestational age (SGA) and large for gestational age (LGA) were defined as birth weight <10th percentile and >90th percentile for gestational age, respectively [16]. The neonatal respiratory morbidities, including respiratory distress syndrome (RDS), pneumothorax, transient tachypnea of newborn (TTNB), bronchopulmonary dysplasia (BPD), and persistent pulmonary hypertension of newborn (PPHN) were recorded and according to the standard diagnostic criteria [17]. Patent ductus arteriosus (PDA) with its treatments and other congenital heart disease were documented by cardiac ultrasound. Cholestasis was defined according to the definition of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition [18,19]. Periventricular leukomalacia (PVL) and intraventricular hemorrhage (IVH) were graded according to previous publications [20,21]. Antenatal betamethasone and postnatal dexamethasone use was registered. Ophthalmologists examined the eyes of the infants according to protocol and definitions of retinopathy of prematurity (ROP) were those endorsed by the international Committee for Classification of Retinopathy of Prematurity [22]. Socioeconomic status (SES) was determined using Hollingshead Two-Factor Index of Social Position [23] from paternal occupation and education. Three social classes were defined as follows: SES scores 11–29, lower class; SES scores 30–40, middle class; and SES scores 41–55, upper class.

2.3. Neurodevelopmental assessments

The assessment at 6-months' corrected age included the Bayley Scales of Infant Development—2nd Edition (BSID-II) and Denver Developmental Screening Test (DDST). The BSID-II consists of a mental score, which yields a Mental Developmental Index (MDI) and a motor score, which yields a Psychomotor Developmental Index (PDI) [24]. The mean ± standard deviation (SD) of the standardization sample is 100 ± 15, and a score of less than 70, which is more than 2 SDs below the mean, is defined as a significant delay for the BSID-II Mental and Motor scales [24,25]. The DDST is used for developmental measurements in preschool children and consists of four subscales: personal–social contact, fine motor, language, and gross motor [26]. Each test item was scored as either pass or fail. For each category in the overall assessment, patients were considered to be developmentally delayed if they failed two or more test items that 75 to 90% of children of their age could pass or if they failed one or more test items that more than 90% of children younger than their age could pass. Otherwise, the development of the children was considered to be normal.

The BSID-II were administered by certified examiners who are experienced clinicians specifically trained for these two test procedures. Examiners were aware of the infant's enrollment in the study but were not informed of child's medical or hospitalization history. The DDST was administered by one well-experienced pediatric psychiatrist (the corresponding author) who specializes in pediatric psychiatry and sleep medicine. To obtain a single outcome measure, four subscales of DDST, MDI and PDI were combined. When at least two of these six outcome measures were abnormal in an infant, this subject was considered as having MNDs.

2.4. Statistical analysis

According to gestational age, the study subjects were analyzed separately into three cohort: very preterm (GA≦28 weeks), preterm (GA 29–32 weeks), and late-preterm (GA 33–36 weeks). The Chi-square test or Fisher exact tests were used for categorical data, and Student *t* test was used for continuous variables with normal distributions. The Wilcoxon/Mann–Whitney *U* test or the Kruskal–Wallis test was used for continuous variables without a normal distribution. All *P* values are two-sided, and those less than 0.05 were considered to be statistically significant.

Multivariate logistic regression was used to determine the independent risk factors of MNDs in preterm neonates. The following social and demographic characteristics were included in the analysis: mother's nationality (foreign spouses, Taiwanese or aboriginal residences), maternal educational level (university level or not), SES, and mother's age (<20, 20–29, or ≧30 years). Neonatal characteristics were determined from medical records as described above. Potential risk factors found individually significant at $P \leqq 0.05$ were entered together into a set of backward selection for choosing the most significant indicators. These variables were finally analyzed by multivariate logistic regression model adjusted for GA and birth body weight (BBW) after their collinear coefficients were checked by Collinearity Diagnostics. All statistics were performed using the commercially available software SPSS 13.0 for Windows (SPSS®, Chicago, IL).

3. Results

Of all premature neonates whose parents were approached and signed the informed consent to participate this study, 151 neonates were completely assessed at 6 months corrected age (Fig. 1). Seventy-eight percent (118/151) of the neonates had neurodevelopmental assessments between 5.5 and 6.5 months corrected age; of the other 33 children, most were assessed before 7 months of corrected age. The neonatal and family characteristics of three cohorts, defined according to gestational age, were summarized in Table 1. It worth noticing that most of the mothers were moderately educated and 80% of them were older than 30 years. Approximately one-fourth of children in the study were multiple births (twin, triplet, or higher order). Nearly 8.6% of the cohort was SGA and the rest was all appropriate for gestational age (AGA). The rate of neonates with an Apgar score≦7 at 5 min was 29.1%.

The majority of the infants had respiratory difficulties at birth and required respiratory support during hospitalization in our NICU (Table 2). 11.3% (17/151) of the neonates did not need any respiratory support. RDS, TTNB, and PPHN were diagnosed in 31.8%, 29.1%, and 3.3% of our neonates, respectively. BPD developed in one-fourth of infants, and 9.3% of neonates still required oxygen support (nasal canula) at discharge. As expected, the distributions of these respiratory difficulties were highly

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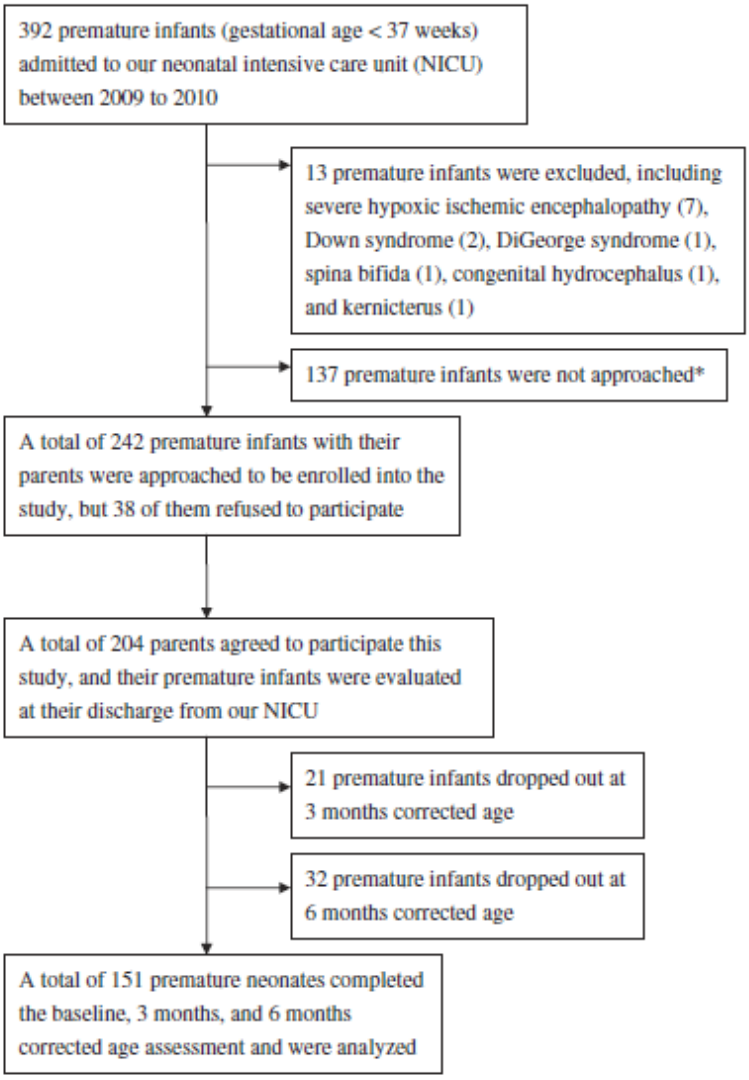

Fig. 1. The flow chart describing the enrollment of the premature infants from our NICU to complete baseline, 3 months, and 6 months corrected age assessments. *not approached because their attending physicians didn't participate this study.

associated with their gestational ages. PDA was found near one-fifth of neonates, and three-fourth of those with PDA were treated surgically. Cranial ultrasound showed IVH in 25 (16.3%) neonates, and severe intracranial lesions (IVH grade III/IV or PVL) in 9 (6.0%) neonates. More severe ROP (stage II with plus disease or ≧stage III) was present in 9.3% of the infants, and 7.3% of them required laser therapy.

In Table 3 the median BSID-II scores and the results of DDST are presented. A MDI and a PDI of <70 were noted in 6 (4.0%) and 20 (13.2%) of our study subjects, respectively. All six neonates with MDI<70 were found to have PDI<70. There are obvious trends of mental delay and psychomotor delay as gestation decreases, although the difference did not reach statistical significance. Extremely low birth weight (ELBW, BBW<1000 g) neonates accounted for half of those with psychomotor delay and the majority of mental delay (4/6, 67%) in our study population. By DDST, 22 (14.6%) infants had at least one of four categories of delay at 6-months-old assessments; 2 had delay in all four categories, 5 had delay in two categories, and 15 had delay in one category. The most common category of delay was gross motor skills, which was noted in approximately 13.2% infants. The results of DDST gross motor subscale and PDI were found to be highly corrected; among those who failed in the DDST gross motor subscale by examiners, all had a PDI below 70. No significant difference in the rate of neurodevelopmental delay was found between SGA children and AGA children.

We determined which of the maternal sociodemographic or neonatal factors were associated with MND in the three cohorts. After combination of BSID-II and DDST, a total of 20 infants were found to have MNDs. None of the mothers' social and demographic characteristics (as recorded at birth) were found to be significantly associated with MND. Table 4 shows the univariate analyses and the final multivariate models that explain the relationships between neonatal factors and the presence of MNDs. Several neonatal factors, including

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Table 1
Characteristics of 151 premature infants assessed at 6 months corrected age: total cohort, very preterm, preterm, and late-preterm infants.

	Total cohort (n=151)	Very preterm cohort (GA≦28 weeks, n=37)	Preterm cohort (GA 29–32 weeks, n=53)	Late preterm (GA 33–36 weeks, n=61)
Gestational age (weeks), median (range)	32 (24–36)	26 (24–28)	31 (29–32)	34.5 (33–36)
Birth body weight (g), median (range)	1515 (582–3935)	890 (582–1595)	1430 (700–2620)	2135 (1085–3935)
Gender (male:female)	82:69	19:18	27:26	36:25
Mode of delivery (C/S, n [%])	110 (72.8)	19 (51.4)	40 (75.5)	51 (83.6)
Maternal age				
<20 y/o, n (%)	3 (2.0)	1 (2.7)	0 (0)	2 (3.3)
20–29 y/o, n (%)	27 (17.9)	9 (24.3)	10 (18.9)	8 (13.1)
≧30 y/o, n (%)	121 (80.1)	27 (73.0)	43 (81.1)	51 (83.6)
Small for gestational age, n (%)	13 (8.6)	1 (2.7)	5 (9.4)	7 (11.5)
5 min Apgar score≦7, n (%)	44 (29.1)	26 (70.3)	12 (22.6)	6 (9.8)
Prenatal betamethasone use, n (%)	106 (70.2)	35 (94.6)	50 (94.3)	21 (34.4)
Postnatal dexamethasone use, n (%)	12 (7.9)	9 (24.3)	3 (5.7)	0 (0)
Multiple births, n (%)	40 (26.5)	7 (18.9)	17 (32.1)	16 (26.2)
Socio-economic status				
High, n (%)	12 (7.9)	3 (8.1)	4 (7.5)	5 (8.2)
Middle, n (%)	113 (74.8)	26 (70.2)	41 (77.4)	46 (75.4)
Low, n (%)	26 (17.2)	8 (21.6)	8 (15.1)	10 (16.4)
Maternal educational level				
Master or higher, n (%)	8 (5.3)	3 (8.1)	1 (1.9)	4 (6.6)
University or college, n (%)	49 (32.5)	9 (24.3)	20 (37.7)	20 (32.8)
High school, n (%)	89 (58.9)	23 (62.2)	30 (56.6)	36 (59.0)
Below elemental school, n (%)	5 (3.3)	2 (5.4)	2 (3.8)	1 (1.6)
Maternal nationality				
Normal residences, n (%)	144 (95.4)	34 (91.9)	50 (94.3)	60 (98.4)
Foreign spouses[a], n (%)	5 (3.3)	2 (5.4)	2 (3.8)	1 (1.6)
Aboriginal residences, n (%)	2 (1.3)	1 (2.7)	1 (1.9)	0 (0)

C/S: Caesarean section delivery.
[a] Including woman from Mainland China.

BBW, low Apgar score at 5 min and acute fetal distress in the delivery room, confirmed neonatal sepsis, chronic lung disease, cholestasis, length of mechanical ventilation, postnatal corticosteroid use, and the presence of ROP were found to be associated with MNDs of neonates at 6 months old. After multivariate logistic regression adjusted for GA and BBW, MND was independently associated with postnatal corticosteroid use (odds ratio [OR], 11.2; 95% confidence interval [CI], 1.9–66.0, $P=0.008$) and cholestasis (OR, 6.2; 95% CI, 1.16–33.1, $P=0.033$). Besides, neonates with BBW less than 1000 g were significantly associated with MNDs when compared with those with BBW more than 2000 g (OR, 51.4; 95% CI, 1.9–1369, $P=0.019$).

4. Discussion

Our study shows that 13.2% of the neonates born preterm have MNDs at the corrected age of 6 months and even 8.6% of neonates born late-preterm (GA 33–36 weeks) have MNDs. Furthermore, as GA decreases, the proportion of children with MNDs confirmed by both PDI below 70 and failure of DDST gross motor subscale increases. Although several neonatal factors are found to be associated with MNDs, the multivariate model for these infants shows that postnatal corticosteroid use and cholestasis are independent predictors of MNDs at their 6 months corrected age.

It is difficult to compare our study with previous studies because our subjects were assessed at much earlier age. The proportion of MND in very preterm neonates and preterm neonates (GA≦28 weeks and GA 29–32 weeks) is similar to other studies [27–29], but 28.6% (6/21) of our neonates with a birth weight≦750 g had MND, which is slightly higher than a recent study conducted by Claas MJ et al. [30]. The percentage of ELBW children with a MDI or a PDI of <70 assessed with the BSID-II at or around 2 years of age ranged from 10.6% to 50% [27–32], which varied because of different designs or some selection bias of different studies. For example, some studies restricted their study subjects to infants with presumably fine motor function impairments and avoided those with moderate or severe dysfunctions [29], while others evaluated the results after individualized developmental care [27,28]. In our study group, 4 of the 28 ELBW infants (14.2%) had a MDI of <70, whereas 10/28 (35.7%) neonates assessed with the BSID-II had a PDI of <70. Given the fact that comparable percentages of low PDI and MDI in each study subgroups were usually noted [27–29], our results strongly suggest that early detection of psychomotor delay maybe more feasible than mental developmental delay by the current screening tools.

All the postnatal corticosteroid uses in our infants was low dose dexamethasone (0.05 mg/kg/day) intravenously for chronic lung disease, which is proven effective to facilitate extubation without significant short-term side effects [33]. Several human studies have found postnatal dexamethasone exposure to be associated with increased risk of long-term neurological and motor dysfunctions [8,34–36], but their dosage of steroid were much higher (0.5 mg/kg/day) or may be longer. A larger, randomized controlled trial is required to further assess the long-term effects of low dose dexamethasone. The prenatal steroid is inevitably use for prevention of RDS in premature neonates, which has been found no significant impact on the long-term neurological outcomes [8,37,38], although most corticosteroids, including dexamethasone or betamethasone, are capable of transfusing to the fetus through placenta. Therefore, the real pathophysiological mechanisms of postnatal corticosteroid use to influence the developing brain of premature infants are still unknown and deserve further *in vitro* and *in vivo* studies. Cholestasis, concluded as another independent predictor of MNDs in our study, may be an indicator of severe sickness in the high risk and very preterm neonates, rather than a direct cause of the MNDs at 6 months corrected age.

Several neonatal factors, including multiple birth, severe abnormalities on early cranial ultrasonography, breast feeding, treatment methods of PDA, chronic lung disease and SGA have been reported to independently influence the neurodevelopmental outcomes at 2

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Table 2
Neonatal morbidity of 151 premature infants during NICU admission.

	Total cohort (n = 151)	Very preterm cohort (GA≦28 weeks, n = 37)	Preterm cohort (GA 29–32 weeks, n = 53)	late preterm (GA 33–36 weeks, n = 61)
Length of hospitalization (d), median (IQR)	45 (24–74.5)	96.5 (73.7–123)	53 (39–65)	22.5 (14.5–30.8)
Duration of ventilation (d), median (IQR)	21 (5–51)	75 (53.5–97)	28 (12–44)	4.5 (0–12)
Requirement of mechanical ventilation[A], n (%)	134 (88.7)	37 (100)	51 (96.2)	46 (75.4)
Diagnosis of respiratory diseases				
Respiratory distress syndrome, n (%)	48 (31.8)	28 (75.7)	17 (32.1)	3 (4.9)
TTNB, n (%)	44 (29.1)	1 (2.7)	16 (30.2)	27 (44.3)
Bronchopulmonary dysplasia, n (%)	38 (25.2)	27 (73.0)	11 (20.7)	0 (0)
PPHN, n (%)	5 (3.3)	2 (5.4)	1 (1.9)	2 (3.3)
Nasal cannula required at discharge, n (%)	14 (9.3)	11 (29.7)	3 (5.7)	0 (0)
Congenital heart disease				
PDA (surgery/indomethacin), n (%)	23 (15.2)/7 (4.6)	15 (40.5)/5 (13.5)	6 (11.3)/2 (3.8)	2 (3.3)/0 (0)
ASD ± VSD, n (%)	16 (10.6)	1 (2.7)	6 (11.3)	9 (14.8)
Complicated CHD, n (%)	1 (0.7)	0 (0)	1 (1.9)	0 (0)
IVH (Grade≦II/Grades III and IV), n (%)	19 (12.3)/6 (4.0)	8 (21.6)/4 (10.8)	5 (9.4)/2 (3.8)	6 (9.8)/0 (0)
PVL, n (%)	4 (2.6)	2 (5.4)	2 (3.8)	0 (0)
NEC (probable/definite), n (%)	4 (2.6)/3 (2.0)	2 (5.4)/2 (5.4)	2 (3.8)/1 (1.9)	0 (0)/0 (0)
ROP				
Stage II with plus disease or ≥stage III, n (%)	11 (7.3)	4 (10.8)	7 (13.2)	0 (0)
Laser treatment required, n (%)	14 (9.3)	13 (35.1)	1 (1.9)	0 (0)
History of neonatal sepsis				
Early-onset neonatal sepsis, n (%)	1 (0.7)	1 (2.7)	0 (0)	0 (0)
Late-onset neonatal sepsis, n (%)	26 (17.2)	12 (32.4)	10 (18.9)	4 (6.6)
Rule out sepsis, n (%)	37 (24.5)	15 (40.5)	17 (32.1)	5 (8.2)
Use of TPN, n (%)	108 (71.5)	37 (100)	48 (90.5)	23 (37.7)
Cholestasis, n (%)	17 (11.3)	11 (29.7)	3 (5.7)	3 (4.9)

IQR: interquartile range, TTNB: transient tachypnea of newborn, PDA: patent ductus arteriosus, ASD: atrial septal defect, VSD: ventricular septal defect, CHD: congenital heart disease, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia, NEC: necrotizing enterocolitis, ROP: retinopathy of prematurity, TPN: total parenteral nutrition.
[A] Mechanical ventilation includes intubation with any form of ventilator support, nasal intermittent mandatory ventilator (N-IMV), and nasal continuous positive airway pressure(N-CPAP).

to 5 years old [8,30–32,39–44]. The significant predictors of a low MDI and a low PDI are usually considered together, but the pathogenesis of developmental delay in psychomotor and mental domains are supposed to be different and supported by some evidences [29,41]. The associations of ultrasound-defined lesions of the brain and developmental delays have been well documented in recent publications [40,41,45]. Cerebral white matter injury, caused by systemic inflammatory response, transient hypoxia or prolonged mechanical ventilations during hospitalization may also predispose to low MDI and low PDI scores [29,42,43]. Our study, after excluding infants with cerebral palsy and major neurological deficits, focused on MNDs of grossly normal premature infants and can not conclude similar findings.

Table 3
Bayley Scales of Infant Development—II: psychomotor and mental index scores and Denver Development Screening Test in preterm neonates at 6 months of corrected age.

	GA≦28 weeks (n = 37)	GA 29–32 weeks (n = 53)	GA 33–<37 weeks (n = 61)
BSID-II index			
MDI, median (IQR)	98.0 (90.0–103.5)	97.0 (89.8–102.8)	96.5 (91.0–105.0)
MDI<70 (n, %)	4 (10.8)	1 (1.9)	1 (1.6)
PDI, median (IQR)	85.0 (73.0–101.5)	88.0 (79.0–97.0)	91.5 (85.0–104.0)
PDI<70 (n, %)	8 (21.6)	7 (13.2)	5 (8.2)
DDST			
Any delay, n (%)	8 (21.6)	9 (17.0)	5 (8.2)
Personal–social delay, n (%)	2 (5.4)	0 (0)	0 (0)
Fine motor delay, n (%)	2 (5.4)	2 (3.8)	1 (1.6)
Language delay, n (%)	4 (10.8)	2 (3.8)	0 (0)
Gross motor delay, n (%)	8 (21.6)	7 (13.2)	5 (8.2)

BSID-II: Bayley Scales of Infant Development—II [24], DDST: Denver Development Screening Test, IQR: interquartile range, MDI: Mental Developmental Index, PDI: Psychomotor Developmental Index

Our study did not conclude any maternal or family characteristics to be independently associated with MNDs in our premature infants. However, several studies have found low family SES or maternal education level as a significant predictor of suboptimal MDI or delayed cognitive development in ELBW neonates [31,46–49]. The possible explanations include poor recognition, unavailable resources or support to enhance development, and potential genetic problems of these families with low social class [46–49]. Our inability to demonstrate this relationship conclusively may result from the relatively small number of infants from low SES and small study sample. Besides, some studies included subjects with cerebral palsy and major neurological disabilities [31,46,49], which may amplify the likelihood that low SES can influence neurodevelopmental outcomes.

Although BSID-II is the gold standard in screening developmental delay of early childhood, it is most reliable when performed at or around 24 months corrected age [24,25]. Thus, DDST is applied as the supplemental tool to confirm the MNDs in our study. We found the results of BSID-II PDI<70 and failure in DDST motor subscale were highly corrected and the NMDs in our study are defined as at least two abnormal measures, which decrease the possibility of false positivity.

This study has some limitations. Not all premature neonates born during the study period were enrolled, because their parents declined to participate in the study. We do not know whether those refusing to participate were significantly different from our study group, and whether this potential difference could have influenced our results. This study is also limited by its inadequate sample size, which was contributed by certain proportion of drop out cases because of the longitudinal follow-up and repeated examinations.

In conclusion, considerable percentage of premature infants without major neurological deficits is found to have NMDs at 6 months corrected age, which is much earlier than we have thought, even in infants born at 33 to 36 weeks. ELBW and postnatal corticosteroid use are independently associated NMDs of premature infants. Given the high rate of NMDs and the significances in later school years, early intervention and rehabilitation programs are urgently needed in infancy period to optimize their neurodevelopmental outcomes.

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Table 4
Neonatal characteristics associated with psychomotor delay in 6-month-old neonates born preterm, with multivariate analysis comparing normal *vs* psychomotor developmental delay.

Characteristic	Neonates, no. (%)		Univariate analysis		Multivariate analysis	
	Normal (n = 131)	MND (n = 20)	Crude OR (95% CI)	*P* value	AOR (95% CI)	*P* value
GA, (weeks)						
≦28	29 (22.1)	8 (40.0)	3.1 (0.9–10.3)	0.066	10.0 (0.8–127)	0.075
29–32	46 (35.1)	7 (35.0)	1.7 (0.5–5.7)	0.389	17.4 (0.9–347)	0.062
33–36	56 (42.7)	5 (25.0)	1 (references)		1 (references)	
Birth body weight, (g)						
<1000	18 (13.7)	10 (50.0)	22.2 (2.6–186.9)	0.004	51.4 (1.9–1369)	0.019
1000–1499	42 (32.1)	4 (20.0)	3.8 (0.4–35.6)	0.241	5.8 (0.4–79.8)	0.190
1500–1999	31 (23.7)	5 (25.0)	6.5 (0.7–58.1)	0.096	10.2 (0.9–106)	0.052
≧2000	40 (30.5)	1 (5.0)	1 (references)		1 (references)	
SGA	12 (9.2)	1 (5.0)	0.5 (0.1–4.2)	0.543		
Male gender	68 (51.9)	14 (70.0)	2.2 (0.8–6.0)	0.137		
Caesarean section delivery	96 (73.3)	14 (70.0)	0.9 (0.3–2.4)	0.759		
Multiple births	35 (26.7)	4 (20.0)	0.6 (0.2–2.1)	0.722		
Antenatal steroids	63 (48.1)	13 (65.0)	1.8 (0.7–4.8)	0.242		
Apgar score≦7 at 5 min	33 (25.2)	11 (55.0)	3.4 (1.3–8.9)	0.014		
Acute fetal distress requiring resuscitation in the delivery room[a]	7 (5.3)	4 (20.0)	4.2 (1.1–15.9)	0.036		
Postnatal corticosteroid therapy	10 (7.6)	12 (60.0)	20.1 (5.3–77.0)	<0.001	11.2 (1.9–66.0)	0.008
Bronchopulmonary dysplasia	28 (21.4)	10 (50.0)	3.5 (1.3–9.2)	0.012		
length of mechanical ventilation (d) per additional 10 days increase[b]	–	–	1.3 (1.2–1.6)	<0.001		
Confirmed neonatal sepsis	19 (14.5)	8 (40.0)	3.7 (1.3–10.3)	0.012		
Retinopathy of prematurity[c]	16 (12.2)	9 (45.0)	5.6 (2.0–15.5)	0.001		
Cholestasis	9 (6.9)	8 (40.0)	8.6 (2.8–26.4)	<0.001	6.2 (1.16–33.1)	0.033
Intraventricular hemorrhage (Grade III or IV)	4 (3.1)	2 (10.0)	3.4 (0.6–19.7)	0.179		
Periventricular leukomalacia (PVL)	4 (3.1)	0 (0)	–	0.549		
Oxygen requirement at discharge[d]	10 (7.6)	4 (20.0)	2.9 (0.8–10.3)	0.104		
Breast feeding at discharge	113 (86.3)	18 (90.0)	1.3 (0.3–4.4)	0.578		

AOR: adjusted odds ratio; CI: confidence interval; GA: gestational age; MND: minor neurodevelopmental dysfunction; SGA: small for gestational age.
[a] Including those required cardiac massage or epinephrine through endotracheal tube in the delivery room.
[b] Including conventional ventilation and high frequency oscillatory ventilator use.
[c] Including those with stage II with plus disease or ≧stage III or those required laser therapy.
[d] Indicated neonates who required nasal-cannula and oxygen support at discharge.

Conflict of interest

The authors declared no conflict of interest and no financial support in this study.

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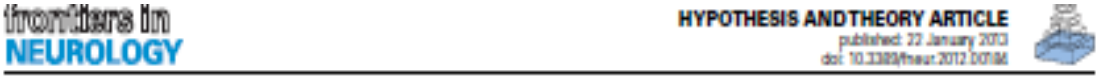

Pediatric obstructive sleep apnea and the critical role of oral-facial growth: evidences

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Pediatric Obstructive Sleep Apnea and the critical role of oral-facial growth (a review of our evidences placed in an historical context)

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Running title: Pediatric OSA and oral-facial growth

Abstract:

Aims: Review of evidence in support of an oral-facial growth impairment in the development of pediatric sleep apnea in non-obese children

Method: Review of experimental data from infant monkeys with experimentally induced nasal resistance. Review of early historical data in the orthodontic literature indicating the abnormal oral-facial development associated with mouth breathing and nasal resistance. Review of the progressive demonstration of sleep disordered breathing in children who underwent incomplete treatment of OSA with adenotonsillectomy, and demonstration of abnormal oral-facial anatomy that must often to be treated in order for the resolution of OSA. Review of long term recurrence data on OSA and indication of oral-facial myofunctional dysfunction in association with the recurrence of OSA.

Results: Presentation of prospective data on premature infants and sleep-disordered-breathing (SDB)-treated children, supporting the concept of oral-facial hypotonia. Presentation of evidence supporting hypotonia as a primary element in the development of oral-facial anatomic abnormalities leading to abnormal breathing during sleep. Continuous interaction between oral facial muscle tone, maxillary-mandibular growth and development of SDB. Role of myofunctional re-education with orthodontics and elimination of upper airway soft tissue in the treatment of non-obese SDB children.

Conclusion: Pediatric OSA in non-obese children is a disorder of oral facial growth.

Key-words: Pediatric sleep-disordered-breathing, Non-obese, Oral-facial anatomy, Hypotonia

Oral-facial growth, oral-facial myofunctional dysfunction

Introduction

Since the report of obstructive sleep apnea syndrome in children in 1976 (Guilleminault et al), recognition of abnormal breathing during sleep has progressed. There was the introduction of the nasal cannula-pressure transducer (Serebrisky et al., 2002), a more accurate technique than thermistors, the commonly previously used definitions to score abnormal breathing during sleep in association with esophageal manometry (Pes). It has allowed to recognized well the pattern of "flow limitation"; associated with an abnormal increase or decrease in respiratory effort and related to demonstrated sleep EEG changes indicative of sleep disturbances (Hosselet et al., 1998,Aittokallio et al., 2001). The sleep EEG changes were also shown to be better recognized using the "cyclic-alternating-pattern" (CAP) scoring system, a visual scoring system commonly used in Europe and Latin America (Terzano et al., 2002). This visual scoring system recognizes sleep disturbances, particularly arousals indicative of sleep disruption, better than the most commonly used atlas that require at least 3 seconds to score a disturbance. More accurate approaches have been used calling upon computerized analyses of the sleep EEG, based on specific algorithms (Chervin et al., 2004) or using well-known EEG analysis programs (fast-Fourier Transform, Wavelet or even better Hiller-Huang Transform programs). Usage of these recording techniques has allowed recognition of Sleep-Disordered-Breathing (SDB).

Follow- up of the first cases of children treated by tracheostomy, then by home nasal CPAP that was not well accepted in these teen-agers, led to usage of more specific surgery oriented toward treatment of the upper airway, performing maxilla-mandibular surgery (Powell et al., 1983) with follow-up for more than 25 years in one case indicating complete long term resolution of the OSAS.

Lessons from OSA treatment with adenotonsillectomy

Despite the widespread use of techniques with limited ability to identify the complete cessation of abnormal breathing and its effects during sleep, studies progressively reported an absence of complete elimination of sleep-disordered-breathing despite significant initial improvement in many tests. Early on two studies showed that prepubertal adolescents who were considered to be completely cured from OSA presented with OSA again as teenagers (Guilleminault et al., 1989). Interestingly, these subjects had a narrowing behind the base of tongue and orofacial anatomical abnormalities that either did not exist initially or had not been recognized at its earlier stage. Tasker et al (2002) also confirmed the presence of an abnormal upper airway and SDB twelve years after adenotonsillectomy. In more recent studies with larger number of patients, this point was made very clear: Despite a clear improvement of clinical symptoms, Guilleminault et al (2004) reported a complete resolution of OSA following adenotonsillectomy in only 51% of the non-obese prepubertal children they successively studied with systematic PSG at approximately 3 months post-surgery. Tauman et al (2006) and Guilleminault et al (2007) confirmed these findings. In a more recent multi-center study (Bhattacharjee et al., 2010), though 50% of the 500 recruited children had been obese, adenotonsillectomy again improved clinical symptoms and PSG results but did not cure the syndrome in about 70% of the enlisted children. Recently, Chen et al (2012) reported on a systematic prospective study of prepubertal children treated with adenotonsillectomy, who were then considered to have normal PSG according to AASM 2007

scoring criteria (Iber et al., 2007). All children whose parents signed informed consent were included in the prospective follow-up study, which spanned a 5-year period, and included not only systematic PSGs and clinical evaluations, but also attentional neurocognitive testing performed during the daytime that was interpreted blindly by the same trained psychologist and 3D-CT of the upper airway that was also interpreted blindly by the same cranial-facial specialist. The follow-up evaluations performed at 3- and 6- months showed normal PSG (AASM criteria) and neurocognitive scores. However, further follow-up showed once again the simultaneous presence of abnormal breathing during sleep (AASM criteria) and abnormal neurocognitive test results in 40% of the prepubertal children. Moreover, 3D-CT analysis showed abnormal oral-facial development in these children. Kim and Guilleminault (2011) also reported on a systematic investigation of the presentation of anatomical oral-facial abnormalities noted in 400 prepubertal children diagnosed with OSA and enlarged adenotonsillectomy before otolaryngological treatment. It came out that nearly all the children with OSA had at least one type of oral-pharyngeal abnormality from the list of pre-defined potential problems. As expected based on previous findings, clinical symptoms and abnormal PSG persisted in some children at 3-months follow-up after adenotonsillectomy. However, the study showed that many children had one of the many anatomical oral-facial abnormality investigated before surgery and scored based on systematic pre-established evaluation scales: this non specific scoring was insufficient to predict complete or incomplete results from adenotonsillectomy. Further analyses produced more specific clinical examination findings that better predicted persistence of abnormal PSG results post adenotonsillectomy. Similar to an analysis performed by Guilleminault and his coworkers (2007), the clinical findings included the presence of a Mallampati (or Friedman-Mallampati) scale score of 3 or 4 (Mallampati et al., 1985), the presence of a deviated septum, and the presence of a small mandible. The Mallampati scale does not identify one particular anatomical abnormality, but an abnormal score represent a combination of deficiencies involving both the nasomaxillary complex and the position of the mandible. The findings suggest the need to assess the upper airway on anatomic elements more related to oral facial growth and possibly more generalized oral-pharyngeal growth impairment rather than looking at many anatomic deviations from what is considered the norm.

Lessons from orthodontia and the experimental infant monkey model

European orthodontists showed that abnormal nasal resistance induced by enlarged adeno-tonsils in children were associated with mouth breathing and led to important cranio-facial changes (Haas, 1961;Linder-Aronson, 1969; 1970;Wertz, 1970;Timms, 1974;Gray, 1975;Hershey et al., 1976;McNamara, 1981;Timms, 1984). Ablation of the adeno-tonsils led to cessation of the mouth breathing and progressive return to normal facial development facilitated by orthodontia use in follow-up studies. Other orthodontists stricken by narrowness of the maxilla and its very negative impact on teeth position and facial growth during prepubertal development had performed “rapid maxillary expansion” (RME), and reported that such treatment had also made an impact on a sleep-related complaint. For example, treated children experienced elimination of their nocturnal enuresis. However, the most important findings were obtained on the infant monkeys, when the important role of abnormal nasal resistance during the developmental period was demonstrated.

Between 1970 and 1980, a number of very important experiments on newborn rhesus monkeyswere performed, whereby a small silicone head was placed within the nostrils of infant

monkeys and held by a thin thread to the nostrils in order to induce a clear nasal resistance for the first 6 months of life, and subsequent removal of the nasal obstruction thereafter (Harvold et al., 1981;Vargervik et al., 1984). As reported by Harvold and his team (1981), the blockade of the nasal passage led to narrowing of dental arches, decrease in maxillary arch length, anterior cross bite, maxillary over-jet and increase in anterior face height. These results were well illustrated, but the most significant findings in our view, was the report by Vargervik et al (1984) and Miller et al (1984). These researchers from the same team showed that experimentally induced abnormal nasal resistance led to systematic changes in the orofacial muscles. The changes were noted in the systematic recording of different muscles, more particularly the geniohyoid, the genioglossal muscles that constitute the tongue, and also, in the suprahyoid dorsal tongue fibers – the upper lip elevators, and the digastric muscles.

EMG changes were reported to show abrupt induction of rhythmic discharge patterns. This is contrary to normal firing, which is near continuous and desynchronized in any normal individual. Tonic EMG discharges changed back to the normal pattern when nasal breathing was restored at the end of the 6-month experiment. These alterations were entirely related to the abnormal nasal breathing accompanying the increase in nasal resistance. Increase in nasal resistance has a dramatic effect on the maxillomandibular skeleton, halting growth (Harvold et al., 1981) and bringing about adaptive changes in the soft tissues that are associated with deviation in jaw posture and tongue activity (Miller et al., 1984;Vargervik et al., 1984). That is, the nasal obstruction induces functional changes in the nasomaxillary complex and on the mandible.The consequences of the experiments were as follows: There was an absence of development, which impacted the maxilla and restricted the nose and upper jaw; there was a displacement of the mandible leading to mouth breathing and; oral breathing developed in association with increase in nasal resistance, leading to mouth opening and mouth breathing that occurred day and night. These led to the narrowing of the cranial skeleton (Harvold et al., 1981;Miller et al., 1984;Vargervik et al., 1984;Rubin, 1987;Vargervik and Harvold, 1987). Further follow-up showed that these changes were reversible if the experimental nasal resistance was withdrawn while the infant monkey was still in its developmental phase.

In summary: In growing animals in which the nasal airway is gradually occluded, there is an adverse effect on the morphology of the nasomaxillary complex, mandible, and pharyngeal airway space. The morphometric changes are induced by altered functioning of the muscles, with changes in muscle firing that are triggered by abnormal nasal resistance.

Obstructive sleep apnea syndrome was mostly unknown at the time of this investigation and no sleep recording was performed on the experimental animals.

Application of work in orthodontia in the field of SDB

More recently, findings that support the incomplete resolution of abnormal oral-pharyngeal growth by adenotonsillectomy have led to the usage of orthodontic techniques to help treat pediatric SDB. Several studies were performed over time, based on reports demonstrating the important role of the mesio-palatine suture in the nasomaxillary complex growth and examining its ossification process in depth. Cartilage is a connective tissue made of chondrocytes embedded in a matrix rich in collagen

(particularly type II), associated with proteoglycans in hyaline cartilage that strengthens it, and often elastin-(depending on the type of cartilage). Hyaline cartilage is the forerunner of skeletal bones in the fetus, and endochondral ossification is the process leading to formation of the nasomaxillary complex. Rapid Maxillary Expansion- RME-(Pirelli et al., 2004) is a procedure applying orthopaedic forces on the mid-palatal sutures using the first molars and permanent premolars as anchor teeth; while in deciduous dentition, the second primary molars are selected as long as they can provide the required firmness. The device is composed of a central expansion screw with four arms: 2 front arms and 2 back arms; the bone distraction at suture level enables an effective enlargement of the maxillary skeletal base. Enlargement is visually appreciable as the bone distraction leads to an interincisive space (a diastema), and with X-rays (as the gain appears as a radiotransparency corresponding to the visually seen space). The procedure usually last 3 to 4 weeks with daily turning of a mid-line screw that allows enlargement (distraction) of the space at the level of mid-line suture: The transpalatal force, that exceeds the orthodontic one, produces an orthopaedic force that opens the mid-palatal suture leading to maxillary movement without tipping teeth. Once the needed extension is obtained (end of the activation phase), the midline screw is blocked and the device is kept in place for at least 4 to 6 months more, to let the newly formed bone strengthen. Unfortunately, such important cartilage is missing from the mandible. Nevertheless, manipulation and verticalization of teeth can stimulate mandibular growth and such bimaxillary distraction is often needed in OSA children. In addition, maxillary widening also seems to independently impact mandibular growth. One negative element of RME is in its anteroposterior lengthening capabilities. That is, it is a limited approach when anteroposterior lengthening is needed. In the past, appliances such as the Herbs appliance or its equivalent were thought to be capable of producing anterior-posterior growth in prepubertal children. However, while such appliances may protrude the lower jaw forward, there is no evidence to date that more growth than expected with age is attained. Distraction osteogenesis may be performed in these cases, but while such approach is performed in children with clear malformations at birth, it has not been recommended in non-syndromic children with OSA till oral-facial growth is well advanced (Guilleminault and Li, 2004)Normally, 60% of the facial growth is obtained by 6 years of age and about 90% near 11 to 12 years. Thus, distraction osteogenesis is not usually performed before approximately 14 years of age in non-syndromic children with OSA. At that time, there is always a question of whether the anteroposterior advancement will be sufficient on its own or whether the teenager will need both the anteroposterior and the lateral extension. If the latter is need, as is most commonly the case, MMA (Holty and Guilleminault, 2010) is the best recommendation. On the other hand, distraction osteogenesis may be useful in certain cases, such as in the elimination of residual OSA.

In summary: Several studies have shown that RME or bimaxillary distraction have a clear impact on pediatric OSA and may resolve the residual problem that is seen post adenotonsillectomy. The combination of adenotonsillectomy and RME may potentially resolve OSA symptoms completely and a small prospective follow-up study indicates that results may be sustained 36 months post treatment (Villa et al., 2011).

Two investigations have looked at the effects of RME versus adenotonsillectomy either via random distribution of initial treatment or via initial selection based on the presence or absence of infectious tonsils (Guilleminault et al., 2011;Pirelli et al., 2012). In the initial investigation, there was presentation of both adenotonsil enlargement with the tonsils scored as level 3 + on the Friedman et al scale (Friedman et al., 1999) at entry and narrow jaws. Except for one child who only needed orthodontic

treatment, all other children needed both adenotonsillectomy and orthodontic treatment in order to see improvement. In the second study, a pre-selection was done with children with infectious tonsils sent first to adenotonsillectomy and others to orthodontic treatment; with plan to send children to the other arm of treatment if incomplete results were noted. More children were treated only with orthodontics, indicating that oral-facial factors may be dominant in at least a subgroup of OSA children. In both studies, several children were not completely cured with these approaches indicating that more aggressive treatment may be needed. The persistent oral-facial problems were always the prominent factor associated with the absence of a complete cure.

In summary, the studies presented here indicate that adenotonsillectomy in non-obese children does not "cure" OSA in many prepubertal children, and that oral-facial anatomical problems are clearly involved in the development of OSA in children. Moreover, these anatomical problems may be amenable to orthodontic treatment but not in all subjects.

Role of oral facial muscle hypotonia and usage of myofunctional reeducation

The investigation of the infant monkeys and the findings of the changes in EMG firing demonstrated that abnormal nasal resistance early in life leadsd to mouth breathing associated with abnormal muscle tone with oral-facial hypotonia and secondary changes in maxillary-mandibular growth (Harvold et al., 1981;Miller et al., 1984;Vargervik et al., 1984;Vargervik and Harvold, 1987).

In the 1970s, the concept that orofacial muscles were involved in many important functions including swallowing, breathing, phonation, mastication, facial mimic, and overall head posture was well demonstrated (Leech, 1958;Ricketts, 1958;Hawkins, 1965;Linder-Aronson, 1969; 1970;Solow et al., 1984;Rubin, 1987;Behlfelt et al., 1990). Orthodontists in different European countries concluded that myofunctional reeducation of the orofacial region was an important part of the treatment aimed at rehabilitation of abnormal local muscle activity when performing orthodontic treatment(Chauvois et al., 1991) aiming at correcting abnormal maxillary and mandibular growths and their impacts on teeth positions and normalcy of bite. Creation of oral-facial muscle reeducation programs meant training of specialized reeducators, and cumulated in specific university training with eventual deliverance of university diploma. Combine orthodontic and myofunctional reeducation was there after applied in children with narrow-jaws with investigation of long term outcome in orthodontia. The combination of both treatments was deemed more successful long term than one treatment alone. More recently, after demonstrating the involvement of maxillary-mandibular growth problems in SDB, children were treated with both myofunctional reeducation and orthodontia (Chauvois et al., 1991;Guilleminault et al, 2012in press). Brazil was one of the places at the forefront of such a treatment approach and these treatment were applied in children but also in adults, and a Brazilian team has published results of the combined treatment approach for adult OSA (Guimaraes et al., 2009). However, outcome reports of using myofonctional reeducation in SDB children continue to be rare. But the evaluation of children with abnormal oral-facial development seen for orthodontic treatment without sleep investigation, is well described in orthodontic monographs from the 1990s (Chauvois et al., 1991), including results obtained from appropriate reeducation regimen. Despite usage of combined approaches in specific geographic places for orthodontic problems, no prospective long-term study has been published where orthodontics and myofunctional reeducation were combined in the treatment of SDB children. Studies

have recently been initiated comparing results of adenotonsillectomy to that of orthodontic treatment without association to myofunctional treatment, and we performed one study investigating the role of myofunctional therapy in association with orthodontia in children with SDB. While our own investigation was limited due to its retrospective nature and the difficulty of retrieving original data from its multi–center setting, it produced evidence that the persistence of mouth breathing during sleep, which is related to myofacial hypotonia, led to the reoccurrence of SDB (Guilleminault et al., 2012b;a). The recurrence was documented along with the presentation of clinical symptoms, clinical signs and PSG findings in children appropriately treated with adenotonsillectomy and orthodontics. Myofunctional clinical evaluation revealed the presence of oral-facial hypotonia and of important mouth breathing during PSG in the children who experienced a recurrence of SDB. In this limited retrospective study (Guilleminault et al., 2012a) involving 24 early teen-agers with SDB previously diagnosed between 3 and ½ and 7 years of age and appropriately treated by adenotonsillectomy and orthodontia with recommendation of myofunctional reeducation, recurrence of OSA at teen-age in 13 of them, was associated with presence of oral-facial hypotonia, mouth breathing during sleep, and absence of myofunctional reeducation; while the other studied subjects with normal breathing at long term follow-up were with normal oral-facial tone and nasal breathing during sleep, and had completed myofunctional therapy.

In sum, this retrospective study indicated that myofunctional treatment may be an important tool in the treatment of SDB children and that having normal PSG findings may not be always sufficient to establish long-term control of abnormal breathing. Myofunctional reeducation is much less applied in early infancy. The premature cohort investigation indicates well that SDB is seen very early and that abnormal anatomic features of structures limiting the upper airway are also present very early. Applications of myofunctional reeducation techniques when the abnormalities of growth are recognized may be helpful. But if orthodontists are aware of these treatment approaches, their diffusion is rare in the pediatric arena, despite the fact that presence of generalized hypotonia is well known in premature infants and well investigated by neonatologist-neurologists.

Page (2003) has indicated the importance of dealing with oral-facial hypotonia and how to deal with it in infancy, as oral-facial hypotonia may be associated with negative facial anatomy problems at a later age. Today, there is data showing that the way an infant suck on a nipple (breast or bottle) is important for the development of normal oral-facial muscle tone and the prevention of local hypotonia (Davis and Bell, 1991;Paunio et al., 1993;Ogaard et al., 1994). Breastfeeding is a complex reflex for infants and considerable strength must be generated in order to succeed. Premature infants may experience significant apnea with severe drop in oxygen saturation during feeding. Thus, they often cannot breastfeed sufficiently well at their mothers' breasts and frequently end up being bottle-fed, an activity that requires less tongue and sucking efforts than breastfeeding. In our premature infant prospective study, more than 90% of women with premature infants bottle-fed their infants for many reasons, even though some fed their babies expressed breast milk. Several researchers have considered the oral-facial hypotonia presented by premature infants. As example Page (2003) has raised the issue of how to deal with such hypotonia in infancy: Bottle feeding may be performed with special nipples that require more effort from the orofacial muscles (e.g., NUK-Gerber nipples)(Ogaard et al., 1994) with different nipple adjustments possible and with usage of triggering early in life oral reflexes triggered by finger stimulation of lips and mouth. Progressive development of a normal palate can be attained using such approaches(In one case, there was documentation of sustain results up to 6 years of age -personal

communication Dr MJ Boileau, Dept of Orthodontics Bordeaux University Dental School France). In our own group, a non-randomized small study with 5 infants showed that when mothers follow feeding recommendations and use bottle nipples that require more effort from infants during feeding (but were otherwise similar to the less demanding regular nipple) and finger stimulation of oral reflexes, a progressive normalization of abnormal palatal anatomy associated with normal breathing during sleep was observed at 24-months follow-up, which was not the case in GA-matched infants with regular nipples. This was also demonstrated in our report above of the pair of premature twins with secondary development of normal oral-facial features and absence of SDB. Feeding was associated with usage of a special pacifier but reeducation of muscle hypotonia involved more participation from mothers, including stimulation of infants' lips by placing a parent's finger on the lips, and using chewing toys approved by the FDA after 6 months of age (e.g., ARK's Grabbers^{TM} chewing toys)(Bahr, 2010). These studies are very limited and are similar to case-reports but they add to the observations in older children and recurrence of SDB without myofunctional therapy associated with other SDB treatment when needed Guilleminault et al., 2012a).

In summary: Premature infants and some full-term infants may present with abnormal oral-facial presentation, particularly a high and narrow hard palate. Such presentation is associated with oral facial hypotonia. Systematic follow-up up to 36 months of post birth-age indicates persistence of abnormal tongue position and of abnormal breathing with presence of mouth breathing during PSG.

Information from orthodontists indicates that performing special oral-facial exercises during feeding and chewing in the first 2 years of life may eliminate the abnormal anatomy and result in a repositioning of the tongue and development of a normal nasomaxillary complex and mandible. A small non-randomized study indicates that premature infants may develop normal nasomaxillary complex and mandible when a strong effort is made to induce a normal oral-facial musculature. Years of experience in orthodontia also support the important role of myofunctional reeducation in the presence of abnormal oral-facial anatomy independent of sleep studies (Chauvois et al., 1991). In our investigations, absence of SDB is associated with normal nasal breathing during sleep, but recurrence of OSA at teen-age is associated mouth breathing during sleep and documentation of oral facial hypotonia.

Conclusion

The different data accumulated over time on SDB children and the experimental data obtained from the infant monkeys years ago all lean toward the same direction: There is a strong association between normal oral-facial muscle tone and normal development of the nasomaxillary complex and the mandible. Presence of abnormal muscle tone, either experimentally induced by creation of abnormal nasal resistance or due to premature birth, is quickly associated with mouth breathing particularly during sleep, abnormal placement of the tongue , and either development –as shown in the rhesus monkey and in some infants- or worsening of the oral-facial anatomy. In humans, SDB is noted in association with the pathological hypotonia of the tongue muscles: In a small group of infants seen at birth with a normal hard palate, development of a high and narrow hard palate and SDB was documented in children who also presented oral-facial hypotonia. When high and narrow hard palate was noted at birth, hypotonia was present and SDB was noted. In rare cases efforts to counteract oral-muscle hypotonia and high and narrow hard palate very early in life may bring normal development and absence of SDB at follow-up.

As suggested by Swedish investigators, enlargement of tonsils appears to be a secondary phenomenon, but it further impacts nasal resistance (no information on adenoids has been obtained in our infant studies, but it was obtained in the long-term follow-up of older children with 3D-CT scans). Adenotonsillectomy is often insufficient for the complete resolution of breathing problems.

Understanding the continuous interaction between muscle activity of the tongue and other oral-facial muscles as well as the development of normal anatomic structures supporting the upper airway may give potential avenues for more appropriate treatments involving myofunctional reeducation in our therapeutic arsenal. When does the interaction between oro-facial muscles and oro-facial other anatomic structures limiting the upper airway began is unknown.

Interruption of normal development with premature birth may explain the frequency of breathing problems during sleep in premature infants , but it may be seen in full term infants and may have very negative consequences (Rambaud and Guilleminault, 2012). Could the abnormality leading to oral-facial hypotonia begin in utero? It is possible: Investigation of facial expression and movements shows that beginning in early pregnancy, the fetus exhibits regular movements in the mouth and face; For example, the most frequent movement during the second trimester is sucking (Kurjak et al., 2005). Abnormal pregnancy and/or impairment of these movements may impede normal muscle activity at birth.

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Research Article: Open Access

Short Lingual Frenulum and Obstructive Sleep Apnea in Children

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Abstract

Background: Abnormal short lingual frenulum may lead to impairment of orofacial growth in early childhood. This may reduce the width of the upper airway—a pliable tube—increasing its risk of collapse, particularly during sleep.

Study: A retrospective study of prepubertal children referred for suspicion of obstructive sleep apnea, found 27 subjects with non-syndromic short lingual frenulum. The children had findings associated with enlarged adenotonsils and/or orofacial growth changes.

Results: Children with untreated short frenulum developed abnormal tongue function early in life with secondary impact on orofacial growth and sleep disordered breathing (SDB).

After presence of SDB, analysis of treatment results revealed the following: The apnea-hypopnea index (AHI) of children with adenotonsillectomy (T&A) performed without frenectomy improved, but surgery did not resolve fully the abnormal breathing. Similar results were noted when frenectomy was performed simultaneously with T&A. Finally, frenectomy on children two years or older without enlarged adeno tonsils also did not lead to normalization of AHI. The changes in orofacial growth related to factors including short lingual frenulum lead to SDB and mouth-breathing very early in life. Recognition and treatment of short frenulum early in life—at birth, if possible—would improve normal orofacial growth. Otherwise, myofunctional therapy combined with education of nasal breathing is necessary to obtain normal breathing during sleep in many children.

Conclusion: Short lingual frenulum may lead to abnormal orofacial growth early in life, a risk factor for development of SDB. Careful surveillance for abnormal breathing during sleep should occur in the presence of short lingual frenulum.

Keywords

Obstructive sleep apnea, Prepubertal, Short lingual Frenulum, Ankyloglossia, Frenectomy, Myofunctional therapy

Introduction

Abnormally short lingual frenulum may be seen in association with well-described syndromes such as Beckwitz-Weideman, orofacial digital syndrome, cleft palate, Optiz syndrome and others. Many of these syndromes are associated with abnormal breathing during sleep and obstructive sleep apnea (OSA) [1]. However, they are most commonly isolated, involving more boys than girls with a ratio of 3:1, and with a reported incidence of 5% at birth [1]. Studies of the impact of short frenulum have shown that such anatomic presentation impairs suction [2-5], chewing, swallowing, and frequently leads to speech disorders [6]. Recently, protocols have been published for evaluation of lingual frenulum both in infants and in children [2,7-9]. It was emphasized that a short lingual frenulum modifies the position of the tongue, particularly early in life, and impairs orofacial development. There is secondary association with anterior and posterior crossbite, disproportionate growth of the mandible, and abnormal growth of the maxilla [10,11]. Treatments for short lingual frenulum have been proposed and involve lingual frenectomy [12-14].

The anatomical changes seen with short lingual frenulum are related to the interdependence between function and form as indicated by Melvin Moss [15]: The observed anatomical orofacial changes lead to development of abnormal anatomic support of the upper airway. The upper airway is a collapsible tube submitted to negative intrathoracic pressure during inspiration; during sleep there is an increased risk of collapsibility due to the change of muscle tone related to sleep stages and state along with the recumbent position associated with sleep [16].

We performed a retrospective study of children and teenagers referred for suspicion of sleep-disordered breathing (SDB) during the last five years, and identified from our de-identified database, individuals with short lingual frenulum. We considered the associated orofacial anatomical changes (including the presence of enlarged adenotonsils and other orofacial changes), the recommended treatments, and the outcome of these treatments. This retrospective study of anonymous data was approved by the IRB.

Subjects and Testing

The de-identified charts of children, age 2 to 17 years, referred for suspicion of SDB were selected. Obese and syndromic children were eliminated from the review. To be included in the analysis, charts contained information on initial complaints leading to consult with usage of Pediatric Sleep Questionnaire [17], report of clinical

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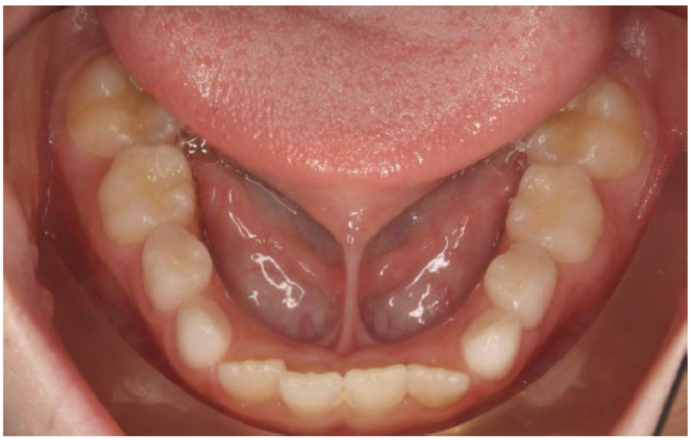

Figure 1: Example of a short lingual frenulum in a prepubertal child with obstructive sleep apnea

Table 1: Disease characteristics at entry and after first treatment

	At entry		After 1st treatment	
	n	(%)	n	(%)
Demographics (n=27)				
Boys	18	(63%)		
Mean Age (years) (SD)	11.4 ± 5.2			12.3 ± 4.6
Disease characteristics				
Overall symptoms	27	(100)	9	(90)
Fatigue	27	(100)	10	(37)
EDS	9	(35)	1	(4)
Poor sleep	18	(67)	9	(33)
Snoring	20	(74)	2	(7.5)
Speech problems+	13	(48)	2	(7.5)
Swallowing problems+	7	(26)	0	(0.0)
Chewing problems+	6	(22)	1	(3.7)
Tonsil scale				
0/1	8(30)			18(66.6)
2	9(33)			9(33)
3	5(18.5)			0 (0.0)
4	5(18.5)			0 (0.0)
Mouth breathing	27(100)			25 (92.5)
PSG findings				
AHI, mean ±SD	12 ± 4.6			3 ± 2
SaO2nadir, mean ±SD	89 ± 2.5			94 ± 1.6
Flow limitation, mean ±SD)	73 ± 11			31 ± 9
Mouth breathing (%TST)	78 ± 14			61 ± 16

+ indicates that symptom was reported during pre-pubertal period but not present necessarily at time of evaluation

evaluation of orofacial findings indicating investigation of tonsils using the Friedman scale [18], investigation of subjective upper-airway opening using the Mallampati-Friedman scale [18], evaluation of inferior nasal turbinates, dental crowding, presence of over jet and/or overbite [18], and determination of "facial harmony" with frontal measurements [18]. If nasal allergies were suspected, a consult with an allergist for treatment was obtained, and if orthodontic problems were suspected, results of evaluation by a specialist were available. Indication of short lingual frenulum and its potential association with speech, swallow, or mastication problems were outlined. Such impairment may have occurred during early childhood, and treatment of the secondary consequence (such as speech therapy) may have been implemented without addressing the primary cause (short lingual frenulum).

All patients had in-laboratory diagnostic polysomnography (PSG) that included the following: EEG (4 leads), EOG, chin and leg EMG, ECG (one lead), and body position. The respiration was monitored using nasal pressure transducer; mouth-breathing with thermos-couple; chest and abdominal movements with inductive plethysmography bands, diaphragmatic-intercostal, and rectus-oblique muscle EMG; pulse oximetry (Massimo ™) from which both oxygen saturation (SaO2) and finger-plethysmography were derived; and continuous video monitoring.

The conclusion of the testing was abnormal breathing during sleep with indication of the treatment plan.

Post treatment follow-up was available indicating:

a) Selected treatment

b) Changes compared to baseline, including questionnaire data

c) Post-treatment PSG findings

Based on the results obtained, a follow-up decision contained any further treatment recommendations and subsequent follow up information including PSG data.

Analysis

The data were collected and organized on spreadsheets. Sleep and respiratory scoring of PSGs followed the pediatric scoring guidelines, according to the American Academy of Sleep Medicine (AASM-2007) [19]. The presence of nasal flow limitation was determined using criteria published by Guilleminault et al. [20] and Palombini et al. [21]. The time spent mouth-breathing during sleep was also calculated as a percentage of total sleep time [22]. Data were analyzed using t-test for repeated measures.

Results

We identified 27 patients with association of short lingual frenulum and SDB who met entry criteria (Figure 1). The mean age was 11.4 years (range: 2 to 16 years). Children presented with symptoms of SDB (Table 1) such as snoring, poor sleep, and fatigue but also a history of symptoms associated with short lingual frenulum such as problems with speech, swallowing or suction, particularly early in life. Children with speech problems may have been sent to speech therapy, but none of the children had frenulum treatment. As can be seen (Table 1), 10 children had enlarged tonsils (Friedman scores of 3 and 4), five children had been recommended to consult an orthodontist due to evident crowding of dentition, 22 children presented with an abnormal Mallampati-Friedman score of 3 or 4 [18]. In all cases, there was presence of a high and narrow hard palate and distortion of the "harmonic face" [18] with either a longer lower third of the face or reduction of the middle third compared to the other thirds.

All children presented with abnormal orofacial anatomy and reduced oral cavity size. None of the children or their parents was aware of the presence of an abnormal short lingual frenulum, despite history of speech therapy early in life, feeding and/or swallowing

difficulties, or prior adenotonsillectomy (T&A) or orthodontic treatment.

All children had PSG s with an abnormal apnea-hypopnea-index (AHI) and abnormal nadir of oxygen saturation during sleep. Results are presented in table 1.

In sum: on initial presentation, children with a short lingual frenulum had associated anatomic problems linked to abnormal breathing during sleep, such as adenotonsillar hypertrophy, but all presented with orofacial features favoring collapse of the upper airway during sleep with presence of a high and narrow palatal vault [16,22].

Finally, detailed family history revealed that one parent had a short frenulum in 6 out of our 27 cases, and short frenulum was present in three siblings of the patients.

Following recognition of abnormal breathing during sleep, some children (n=10) had been referred to ear-nose-throat specialists (ENT) for T&A and frenectomy when large tonsils were present, and 8 children with normal sized tonsils (confirmed by naso-pharyngoscopy and/or lateral radiographs) were also referred to ENT for frenectomy. Nine children were referred to orthodontists for rapid maxillary expansion (RME) and need for frenectomy was also mentioned. Children were asked to have post-treatment follow-up by sleep medicine including investigation with PSG.

Follow-up post treatment#1

The sleep medicine follow-up occurred between three and four months post T&A with frenectomy or isolated frenectomy; and about one month after rapid maxillary expansion, with expander in place, for children sent to orthodontists. None of the children sent for orthodontia treatment had frenectomy and two children had T&A without frenectomy. In summary: 13 children had frenectomy either isolated (n=5) or in association with T&A (n=8), but frenectomy was not performed in 14 children despite recommendation to perform such treatment.

Table 1 presents the results obtained post treatment #1, independent of its type.

There was an overall improvement by clinical evaluation and PSG including children in which frenectomy had not been performed; parents reported, however, persistence of some symptoms particularly of "fatigue" and "poor sleep" in about one third of the cases. The AHI was significantly decreased, and oxygen saturation nadir was significantly improved (p=0.01 for each condition, paired t-test); flow limitation was also improved (p=0.05 paired t-test) but to a lesser degree.

There was persistence of mouth-breathing: only two children, treated by both T&A and frenectomy spent less than 10% of their sleep mouth breathing, a percentage considered as normal [28]. All others had abnormal mouth-breathing during sleep, including the five children that underwent isolated frenectomy.

The conclusion of this first follow-up was that children recognized with OSA were clearly improved after either T&A or orthodontics, performed with or without frenectomy. However, 92.5% of the treated children still had an abnormal amount of mouth-breathing during sleep.

After collection of data, children without frenectomy were referred back to ENT and recommended to undergo treatment (n=16). Considering the prior finding of persistence of mouth-breathing despite frenectomy, all children with residual AHI, flow limitation, and mouth-breathing were recommended to have myofunctional treatment with a specialist after surgery (n=25).

Follow-up post treatment #2

Eleven patients came back for further follow-up, six months after the first post treatment investigation. They had treatment with myofunctional therapy for 4 to 6 months [23-25].

These included those who underwent frenectomy with orthodontia (n=5), isolated frenectomy (n=4)), and children post T&A with new frenectomy subsequent to the first follow up visit (n=2).

None of these 11 patients or their parents had clinical complaints. At follow-up PSG, these cases had a mean AHI of 0.8 ± 0.9, a mean oxygen saturation nadir of 97.2 ± 1.0%, a mean flow limitation during sleep of 7.5 ± 6%, and time spent mouth breathing during sleep of 4 ± 4.1%.

Discussion

Diagnosing frenulum abnormalities can be difficult because the examiner has to be aware of the anatomy of the tongue, including different aspects of the frenulum and adjacent regions. In addition, the examiner must know what functions may be affected by the alterations of the lingual frenulum. Moreover, the cut-off point between normal and abnormal frenulum may be a challenge particularly early in life. In this study, all patients were non-syndromic and had a clearly recognizable short lingual frenulum at clinical evaluation. None of the children referred for suspicion of SDB had been recognized with such anatomical abnormality before referral. Short lingual frenulum is a known factor in altering orofacial growth particularly impacting development of the maxilla due to the low placement of the tongue. It leads to the abnormal development of a high and narrow hard palate, and secondarily, mouth-breathing during sleep. These changes occur early in life, as the orofacial growth is particularly fast during the first two years of life. The observed secondary anatomical changes occurring in the oral cavity are known to increase the degree of collapsibility of the upper airway during sleep, and recognition of a short lingual frenulum in a toddler and older children should lead to investigation of SDB.

It has also been hypothesized that mouth-breathing may be a factor in tonsillar enlargement: normally a child is an obligate nose-breather and the nose humidifies, heats, and regulates airflow before it reaches the distal portions of the upper airway. Mouth-breathing not only increases upper airway resistance [26] but also causes micro trauma to the back of the throat that may induce local inflammatory reactions in the tonsils, leading to their enlargement. Unrecognized short lingual frenulum at birth or very early in life may lead to suction, swallowing, masticatory, and speech acquisition problems. It negatively impacts orofacial growth leading to abnormal breathing during sleep, including development of mouth-breathing that will in turn worsen the abnormal orofacial growth. This can produce "nasal disuse," particularly during sleep, an often-missed condition [22] that will not spontaneously improve even after surgical elimination of the abnormal anatomic presentation; daytime reeducation and re-training of nasal usage will be needed.

Our study involves a relatively small number of children, and our longest follow-up investigation is with fewer children than at initial presentation due to loss to follow up, and such factors are limitations in the interpretation of our findings.

We have no indication why frenectomy was not performed earlier or even, in many cases, at time of T&A. Frenectomy does not significantly increase the surgical risks associated with T&A. Considering the negative impact of short lingual frenulum on orofacial development of children early in life, and the fact that the above children were already presenting with OSA, we suggest that lingual frenulum be systematically examined by otolaryngologists and pediatricians in the presence of early in life difficulties with function of the oral cavity and in the presence of SDB. Additionally, frenectomy should be integrated in surgical planning, independently of its timing [27].

Evaluation of breathing during sleep should be performed as early as possible in subjects recognized with short lingual frenulum. One fact is clear: frenectomy for short lingual frenulum in isolation or following T&A helps but is commonly insufficient to resolve all abnormal breathing patterns during sleep when SDB is present.

Myofunctional therapy, which has been previously demonstrated to allow return to normal nasal breathing [22-25] may be needed post surgery.

Finally, the existence of familial cases and the association with genetic syndrome suggest that presence of a short lingual frenulum may be part of a specific genetic predisposition, but no genetic study has been performed on familial cases of short lingual frenulum.

In conclusion: children with SDB should be evaluated for a short lingual frenulum, and conversely, children with an abnormally short frenulum should be investigated for the presence of SDB. Frenectomy should be performed as early as possible but it may not be sufficient to restore normal nasal breathing function during sleep [28], particularly if the frenulum-related problem has lingered over years, and nasal breathing reeducation may be needed in these cases.

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Inflammatory cytokines in pediatric obstructive sleep apnea

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Cognition and Inflammatory Cytokines in Pediatric Obstructive Sleep Apnea

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Abstract:

Backgrounds: Pediatric Obstructive-Sleep-Apnea (OSA) is associated with cognitive impairments and with chronic systemic inflammation. This study aimed to investigate cognition in pediatric OSA and the status of pro-inflammatory cytokines, particularly IL-17and IL 23..

Methods: 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 (WCST), questionnaires, analyses of plasma high-sensitivity C- reactive- protein (HS-CRP), tumor-necrosis-factor-alpha (TNF-α), interleukins 1(IL-1), 6 (IL-6), 17 (IL-17) and 23 (IL-23).

Results: Seventy-nine, 4-12-year-old subjects in 2 groups ended the study: 47 non-obese OSA children (mean age=7.84 $\pm$ 0.56 years, body mass index [BMI] = 16.95±0.47 kg/m2, BMI z score=0.15±0.21; mean AHI=9.13±1.67events/hour) and 32 healthy control children (mean age=7.02±0.65 years, with BMI =16.55±0.58 kg/m2, BMI z score=-0.12±0.27, mean AHI=0.41±0.07event/hour) were enrolled.

Serum cytokine analyses showed significantly higher levels of HS-CRP, IL-17 and IL-23 in OSA children ($p=0.002$, $p=0.024$, $p=0.047$).

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

Conclusion : OSA children have abnormal neurocognitive testing and abnormal levels of IL-17, an interleukin related toTh17, a T helper cell involved in development of auto-immunity and inflammation,and of IL-23 The high expression level of IL-17 may contribute to the brain complications of pediatric OSA as also would abnormal level of; IL-23 that show a significant influence on OSA abnormal neurocognitive testing.

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

Introduction

Symptoms of attention deficit/ hyperactivity disorder and neurocognitive functioning in children have been associated with obstructive sleep apnea (OSA) syndrome [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 associated with OSA are still incompletely chartered.

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 pro-inflammatory cytokines (C reactive protein (CRP), tumor-necrotic-factor (TNF-α), interleukin (IL)-6, and IL-10 in adult OSA patients and high-specific C reactive-protein(HS-CRP) in pediatric OSA patients) supports this hypothesis [13-15], with a possible association between the apnea-hypopnea-index(AHI) and inflammatory cytokine levels. The inflammatory responses may be reversed after OSA treatment [16-18]. 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 cerebro-spinal fluid with connection to the cervical lymphatic nodes, has been reported [19], and a direct impact of inflammatory cytokines and brain functioning may be occurring.

The pro-inflammatory cytokines IL-17 and IL-23 have been recently emphasized. IL-17 is a pro-inflammatory cytokine secreted predominantly by T helper 17 cells (TH 17) and various cells including innate immune cells and non-immune cells [15]. It is referred to as IL-17A as it is a member of the IL-17 family [20]. 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 (APC) in

response to antigen stimulation [20,21]. The interaction is as follow: IL-17A and IL-17F form homodimers or heretodimers 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 [23,24]. IL-17 also takes part in neutrophilic inflammation in the respiratory system [24,25], and leading to chronic inflammation of the airway [25]; as example there is 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 over-secreted pro-inflammatory cytokines contribute to the development of OSA and its co-morbidities [27]. In OSA children, cytokine profile obtained from tonsils shows high levels of IL-1b, 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, change in cerebral-blood-flow may be involved, Inflammatory cytokines may also play a role. We investigated cognition changes in OSA children and presence of interleukins 17 and 23. We hypothesized that chronic inflammation not only causes cardiovascular diseases in pediatric OSA patient, but also affect cognitive functions and wondered if a correlation between psychometric test and these cytokines could be shown [15]. A study found a relationship

between abnormal level of C-reactive protein and cognitive dysfunction in school age 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 neurocognitive functions investigated by psychometric tests and increase in inflammatory cytokines, [33] correlating the level of CRP, TNF-α, IL-1, IL-2, IL-6, Il-10, IL-17 and IL-23 with the sleep-polysomnogram-PSG- test results and neurocognitive test findings.

Methods

Inclusion/exclusion

Children aged 4-12 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 (ICSD)-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.(#103-0601C) All caregivers (parents) signed an informed consent. Two groups of participants were collected: Group A: normal control (n=32); 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 IQ<70 defined as mental retardation, were 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 presence of signs and symptoms evoking OSA for at least 3 months with confirmation by polysomnography-PSG- findings (apnea-hypopnea-index-AHI greater than 1 event/hour or respiratory-disturbance-index- RDI more than 5 events/hour) (OSA group) or absence of complaint, AHI<1 and presence of a non-inflammatory status (absence of asthma, allergies, eczema or other atopic/auto-immune diseases) (normal control).

Procedures

(a) All subjects underwent routine medical history and physical examination by otolaryngologist, craniofacial surgeon, pediatrician, and child psychiatrist assessing comorbidities.

(b) Demographic data, (age, sex, height, weight) and all systemic comorbidities, were collected on a standardized data-sheet.

(c) 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, Uppsala, Sweden), and duration and persistence of symptoms and co-morbidities according to the Allergic Rhinitis and its Impact on Asthma classification.

(d) Polysomnography (PSG)

The following variables were monitored: EEG (4 leads), eye movement chin and leg EMG, ECG (one lead), body-position. The respiration was recorded with nasal pressure transducer, mouth thermo-couple, chest and abdominal inductive plethysmography bands, neck microphone,

diaphragmatic-intercostal muscle EMGs, 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, Ontario, Canada], with continuous video monitoring. A family member was present during the nocturnal recording. Sleep and wake were scored using international criteria [33] with identification of stages 3 and 4. EEG arousal was defined according to the American Sleep Disorders Association [34]. 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 abnormal increase in respiratory effort leading to arousals as outlined by Lin and Guilleminault et al. [36] The AHI and the respiratory disturbance index (RDI: number of apneas, hypopneas, and respiratory effort-related arousals per hour of sleep) were calculated. PSG scoring was performed by a technician blind to the clinical status of the child.

(e) Inflammatory cytokine assessment

Blood samples were collected and allowed to clot for 30 min. The samples were then centrifuged and the serum was frozen at -70°C until assay. All samples were collected 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 ultra-sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN (see Appendix 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.) was employed in order to test for extreme outlying cytokine result.

Questionnaire evaluations and neurocognitive tests

Following PSG, four subjective questionnaires were filled out by caregivers to evaluating sleep quality and quality of life of children including the obstructive sleep disorder questionnaire (OSA-18), Children's Sleep Habits Questionnaire (CHSQ), and Child Behavior Checklist (CBCL).

Evaluation of neurocognitive function was carried out using the WPPSI-R intelligence test for 3-6 year-old children, the Wechsler-R intelligence (WPPSI-R) for 6-16 year-old children (WISC-Ⅲ) to assess IQ score; the Conners' Kiddie Continuous Performance Test(k-CPT) for 4-7 year old children (K-CPT) the Continuous Performance Test (CPT) that measures the subject's attention problem in three domains: inattention, impulsivity and vigilance and retention ability; and the Wisconsin Card Sorting Test for children assessing executive functioning ability.

The results of Continuous-performance-task (CPT) score are presented in T-scores. High T-scores indicate an attention problem, with any T-score >60 considered as abnormal. The high T- score of omissions, commissions, Hit reaction time (Hit RT), Hit RT std. Error, variability, detectability, Hit reaction-time inter-stimulus-interval Change (Hit RT ISI Change) and Hit-standard-error inter-stimulus-interval Change (Hit SE ISI Change) indicate inattention; while commission, Hit RT and Perseveration indicate impulsivity; and Hit reaction-time -block change (Hit RT block change) and Hit-standard- error (SE) block change indicate vigilance.

The Wisconsin Card Sorting Test (WCST) measures the subject's executive function. The total errors scores is an overall score of WCST, and higher score indicates worse performance. "Perseverative -response"and "error-T-score"are higher in subject 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.

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, BMI, gender, tonsil hypertrophy, adenoid hypertrophy, turbinate hypertrophy, and naso-septal deviation". All the reported *p*-values are two-tailed with statistical significance set at <0.05. Statistics were performed with SPSS version 18

Results

Eighty-two children, 3-12 years old, were enrolled; there were 3 drop-outs (3.6%). Demographic of the 78 children (mean age 7.43 $\pm$ 0.6 year) are in table 1. The OSA group was significantly different with symptoms of ADHD and enuresis (p=0.001 and p=0.036), presence of tonsil and adenoid hypertrophy (p<0.001), body-mass-index [BMI], BMI z score (p=0.001,and 0.036). The PSGs showed (table2) significantly higher AHI, AHI in REM, AI, Desaturation Index and snore index in OSA children (p<0.001, p<0.001, p=0.001, p<0.001 and p=0.002 respectively). In addition, (see table 3), the expression of inflammatory cytokines IL-17, IL-23 and HS-CRP was significantly elevated in children with OSA (p=0.002, p=0.024, p=0.047 respectively). Plasma levels of TNF-α, IL-1, IL-6 and IL-10 showed a non-significant elevation comparing with normal control. Results of CPT and WCST tests (see table 4) indicated significant difference between OSA and control in "Hit- RT-Std.- Error "(p=0.006) and "Hit-RT-ISI-Change"(p=0.004).

A Standardized Regression Test was performed to demonstrate the relationship of cytokines levels with PSG and neurocognitive outcomes after controlling the factors of "asthma, allergy, BMI, gender, tonsil hypertrophy, adenoid hypertrophy, turbinate hypertrophy, and naso-septal deviation" (see Appendix2). It revealed significant relationship between pro-inflammatory cytokines and PSG scores with higher AHI score and OSA severity, such as HS-CRP (β=0.390, P<0.05) and IL-17(β=0. 329, P<0.05 (see Appendix 2). Higher AI "influenced" serum levels of HS-CRP suggesting an impact of

inflammatory cytokines on soft tissues hypertrophy. Similarly, higher serum levels of IL-23 (β=0.403, P<0.05) was "influenced" by higher AI. Also lower mean SaO_2 "influenced" IL-10 level (β= -0.567, P<0.01), and 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 pro-inflammatory cytokines as shown in table 5. The Standardized-Regression-Test indicated significant findings between pro-inflammatory cytokines and neurocognitive-function tests. The elevated cytokines are related to domains of inattention, vigilance, such as "Hit-RT-ISI-Change T- score" and HS-CRP (β= -0.426, P<0.05); "Response-Style T-score" and TNF-α (β= -0.432, P<0.05); "Hit RT ISI Change T score" and "Hit SE ISI Change T score" with IL23 (β= -0.545, -0.526, P<0.01); and higher "Confidence-Index" with IL17 (β= 0.424, P<0.05). When looking at the influence between inflammatory cytokines and WCST, the results indicate that elevated cytokines, such as TNF-α and IL6 are related to decrease of executive functions: such as "non-Perseverative- Errors T-scores" (β= -0.553, P<0.05) ; "Learning-to-Learn" (β= -0.838, P<0.05) ; and "Percent-Conceptual-Level-Response- T-scores" (β= 0.476, P<0.05); especially IL-23 with significant poor performance of "non-Perseverative-Errors T- scores" (β= -0.729, P<0.01).

Our data shows the significant Spearman's correlation factors between pro-inflammatory cytokines and clinical findings such as asthma and IL-6 (ρ= 0.261, P=0.026); allergic rhinitis and HS-CRP(ρ= 0.280, P=0.022*), IL-6(ρ= 0.299, P=0.01*) and IL-10(ρ= -0.265, P=0.023*) ; Tonsil hypertrophy and HS-CRP(ρ= 0.244, P=0.046) ; Adenoid hypertrophy and IL-6 (ρ=0.232, P=0.048*)(Appendix 3).

Discussion

Plasma levels of pro-inflammatory 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 [17]. This last finding supported the concept that OSA induces a systemic inflammatory response activating the signal transduction pathway leading to up-regulation of inflammatory cytokines and down regulation 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 allows further understanding. We found a non- significant 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.

Also, although IL-6 did not appear to have a significantly high expression level in our OSA children, we cannot exclude a role for IL-6 in OSA-related inflammation, as IL-6 is a crucial cytokine signal in guiding the differentiation of naïve T cells into TH 17cells that release IL-17, and, in our subjects, we clearly found that IL-17 is elevated with pediatric OSA. But one may not necessarily have to call-upon IL-6 for stimulation, as IL-23 has been considered as a key-cytokine directing the differentiation of naïve T cells into IL-17-producing T_H17 cells. This issue however is still under debate as some studies support the concept that the production of IL-17 is dependent on IL-23[31]; while other studies may indicate that IL-17 could be produced without the presence of IL-23[37-39]. IL-17 may act synergistically with TNF-α to trigger the signaling pathway that upregulates the downstream cytokines, IL-6 and IL-8. IL-17 itself acted as a downstream of IL-1 in animal model of autoimmune arthritis [40,41]. IL-17, in turn, stimulates cell production of IL-1, TNF-α and ultimately leads to recruitment of inflammatory cells such as neutrophils and other leukocytes [42]. All of the studies above confirmed that IL-17, primarily secreted by Th17, a subset of T helper cells, is a pro-inflammatory cytokine that act, 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 auto-immunity. 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] and these co-morbidities are more common than cardiovascular dysfunction in non-obese pediatric OSA. But the mechanisms involved are still unknown. Evidence suggests that some peripheral pro-inflammatory cytokines such as IL-1 and IL-6 can pass blood-brain barrier [15,44] The new finding in rodent of a direct connection between cerebro-spinal- fluid and deep neck lymph nodes is also a very important clue [19]. These cytokines activate and regulate the vagus nerve system, and it affects the function of central nervous system (CNS) [44,45]. Moreover, chronically rising level of pro-inflammatory cytokines might also induce neuro-inflammation or neurodegeneration and cause impairment of neurocognitive functions[46,47].Other research shows reduction of cognitive function as related to increase level of peripheral IL-6 even in the normal aging Americans [48,49] .Our study shows that higher level of TNF-α and IL-23 are significantly related to some neurocognitive deficits in pediatric OSA. New studies should further address this issue.

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 that had an indication of abnormal levels of inflammatory cytokines. One important point our children were not all with "severe" OSA (only 17% had AHI>10), all however presented abnormal PSG findings: And our findings are in line with data showing that even children with low but abnormal AHI have often memory, attention problems and school difficulties [3-6]

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 net-work has been mentioned by many; our study indicates some interleukin abnormalities (IL 17 and IL 23) not reported before may be present very early with SDB, and should be investigated: these pro-inflammatory cytokines might be potential markers helping in diagnosis and post-treatment follow-up of pediatric OSA.

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Table 1 Demographic characteristics of OSA and healthy children

	Control (n=32)	OSA (n=47)	*p* value
Number of males (%)	21 (65.6%)	30 (63.8%)	0.428
Age (years)	7.02±0.65	7.84±0.56	0.366
BMI (kg/m^2)	16.55±0.58	16.95±0.47	0.601
BMI z score [a]	-0.12±0.27	0.15±0.21	0.442
AHI(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(%)[b]	0(0%)	1(2.1%)	0.461
ADHD(%)[b]	2(6.2%)	18(38.3%)	0.001**
Enuresis(%)[b]	4(12.5%)	15(31.9%)	0.036*
Other Physical Comorbidity History			
Asthma(%) [c]	4(12.5%)	6(12.8%)	0.561
Allergic rhinitis(%) [c]	4(12.5%)	23(48.9%)	<0.001***
Findings of ENT examination			
Tonsil hypertrophy (more than Gr. 2) (%) [c]	4(12.5%)	32(68.1%)	<0.001***
Adenoid hypertrophy (%)[c]	3(9.3%)	24(51.1%)	<0.001***
Turbinate hypertrophy(%) [c]	1(3.1%)	6(12.8%)	0.158
Nasoseptal deviation(%) [c]	0(0%)	1(2.1%)	0.461

†0.05≦$P<0.1$. *P<0.05. **P<0.01. ***P<0.001. BMI, body mass index; ENT, ear, nose and throat; ADHD, attention deficient hyperactivity disorder; PLM, periodic limb movement. [a]Corrected BMI z score based on the Center for Disease Control (CDC) growth charts. [b]Diagnosed according to the

criteria of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition-Text Revision.
[c]Diagnosed by pediatricians.

Table 2 Comparison of polysomonogram findings in OSA and healthy children

	Control (n=32)	OSA (n=47)	*p* value
BMI (kg/m^2)	16.55±0.58	16.95±0.47	0.601
BMI z score	-0.12±0.27	0.15±0.21	0.442
Polysomonograhic findings			
AHI(events/h)	0.37±0.06	9.13±1.67	<0.001***
AHI/REM (events/h)	0.65±0.18	16.25±3.68	<0.001***
AI(events/h)	0.18±0.05	2.12±0.51	0.001**
Desaturation Index(events/h)	0.41±0.06	7.27±1.59	<0.001***
Sleep efficiency (%)	89.70±1.32	83.65±2.28	0.131
Awake (%)	6.74±1.42	10.90±2.36	0.305
REM (%)	18.62±1.406	19.11±1.16	0.820
Stage N1 (%)	10.46±1.70	10.24±1.44	0.935
Stage N2 (%)	41.65±2.67	42.56±2.74	0.853
Stage N3 (%)	28.28±2.71	30.40±1.28	0.434
TST (mins)	405.26±10.96	383.34±11.15	0.274
Sleep latency(mins)	17.67±3.84	20.86±3.74	0.629
PLM Index(events/h)	0.13±0.10	0.93±0.41	0.067†
Snore Index(events/h)	30.41±15.28	156.02±35.62	0.002**

Mean SaO_2 (%)	95.97±1.12	90.12±3.61	0.332
Systolic pressure	100.80±17.20	106.76±19.40	0.387
Diastolic pressure	60.00±22.36	66.79±11.86	0.141

$^{\dagger}0.05 \leqq P < 0.1$. $^{*}P<0.05$. $^{**}P<0.01$. $^{***}P<0.001$. BMI, body mass index; AHI, apnea-hypopnea index; AHI/REM, AHI during REM; AI, apnea index; REM, rapid eye movement; TIB,Time in Bed; SPT, Sleep Period Time; TST, Total Sleep Time; WASO, Wake time after sleep onset; PLM, Periodic Limb Movement; Mean SaO_2, mean oxygen saturation; Lowest SaO_2, lowest oxygen saturation.

Table 3 Comparison of inflammatory cytokines in healthy and OSA children

	Control(n=32) mean±SD	OSA(n=47) mean±SD	*p* value
HS-CRP mg/l	0.41±0.48	1.90±0.44	0.002**
TNF-αug/dl	12.62± 0.94	12.58± 0.83	0.974
IL-1βpg/ml	0.42±0.27	0.36±0.16	0.857
IL-6 pg/ml	1.10±0.18	1.66±0.23	0.104
IL-10 pg/ ml	2.10±0.28	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*

$^{*}P<0.05$. $^{**}P<0.01$.

HS-CRP, high sensitivity-C reactive protein;TNF-α, tumor necrosis factor alpha; IL-1β, Interleukins 1 beta; IL-6, Interleukins 6; IL-10,Interleukins 10;IL-17, Interleukins 17; IL-23, Interleukins 23.

Table 4 Comparison of CPT and WCST findings in OSA children

	Control total(n=32)	OSA total(n=47)	*p* value
CPT			
Clinical, Confidence Index	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	46.43±7.31	51.42±10.57	0.099†
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
Perseverations 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	49.40±7.71	50.07±11.00	0.831
Hit RT ISI Change T score	47.88±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			
Total Errors Standard scores	107.20±20.97	99.67±24.26	0.392
Total Errors T scores	54.80±13.97	49.81±16.19	0.395
Perseverative Responses T scores	55.10±14.77	50.56±16.57	0.452
Perseverative Errors T scores	56.30±15.10	50.74±16.42	0.357

nonPerseverative Errors T scores	55.60±14.37	52.78±16.66	0.639
% Conceptual Level Response T scores	54.50±4.60	50.19±3.31	0.487
Learning to Learn	-3.49±11.66	-3.90±9.78	0.712

CPT, Continuous performance task. The result of CPT score is presented in T-scores. According to the Conners' CPT Computer Program User's Manual, high T-scores are designed to indicate an attention problem. Any T-score above 60 is considered abnormal. The Confidence Index presents the summary of the CPT. The Omissions reveals the number of targets which the person did not respond to. The Commission reveals the number of times when the person responds to a non-target. Hit RT, Hit reaction time; which reflects the mean response time. Hit RT std. Error, Hit reaction time standard error; which measures the speed consistency. The variability, also a measure of response time consistency, which calculates the standard deviation of the 18 standard error values calculated for each sub-block. The detectability is a measure of discriminative power. The higher response style T score indicates that the person act more cautiously to avoid commission error, and the lower score indicates that the person respond more freely to make sure they answer most of the target. The perseverations T score shows the frequency when responding time is lower than 100ms. Hit RT block change, Hit reaction time block change. The Hit RT block change shows the change in reaction time over the 6 time blocks; the higher Hit RT block change T scores indicates a slowing of reaction time as the test progress. Hit SE block change, Hit standard error block change, which indicates the consistency the person react to the targets as test progress. Hit RT ISI Change, Hit reaction time inter-stimulus interval change, reflects the change in reaction time over three inter-stimulus intervals (1,2 and 4 seconds.) Higher score reflects slowing of reaction time as the intervals between targets increased. Hit SE ISI Change, Hit reaction time inter-stimulus interval change. Higher score reflects the person became more erratic as the time between targets increased.

WCST, Wisconsin Card Sorting Test. The total errors scores is an overall score of WCST test, and the higher score indicates worse performance. The perseverative response and error T score are higher in the person with worse performance of mental flexibility and insight. The non-perseverative error reflects difficulty to forming concepts and insight even in flexible answer. The conceptual level response score indicates the insights in correct principle of the card combination. Learning to learn depicts the average tendency over successive categories for efficiency to change.

Table 5 Relationships between inflammatory cytokines and neurocognitive function tests

	HSCRP	TNF-α	IL-1β	IL-6	IL-10	IL-17	IL-23
CPT							
Clinical, Confidence Index [a]	-0.185	-0.177	-0.040	-0.027	0.181	0.424*	-0.317
Omissions T score [a]	-0.256	-0.154	-0.067	-0.109	0.002	0.112	-0.336†
Commissions T score [a]	0.037	0.075	0.170	0.030	0.250	-0.056	0.045
Hit RT T score [a]	-0.071	-0.127	0.006	-0.106	0.065	0.267	-0.249
Hit RT Std.Error T score [a]	-0.207	-0.216	0.083	-0.103	0.182	0.294†	-0.322†
Variability T score [a]	-0.247	-0.098	0.160	-0.009	0.354†	0.274	-0.291
Detectability T score [a]	-0.116	0.032	0.136	-0.004	-0.050	-0.186	0.034
Response Style T score [a]	-0.044	-0.432*	-0.101	-0.039	0.136	0.309†	-0.129
Perseverations T score [a]	-0.253	0.109	-0.013	-0.112	0.155	0.184	-0.203
Hit RT Block Change T score [a]	-0.146	-0.328†	-0.066	-0.017	0.105	-0.102	-0.051
Hit SE Block Change T score [a]	-0.100	-0.121	0.131	-0.029	0.201	-0.184	-0.051
Hit RT ISI Change T score [a]	-0.426*	-0.155	0.003	-0.168	-0.012	-0.036	-0.545**
Hit SE ISI Change T score [a]	-0.389†	-0.192	0.174	-0.159	0.049	0.114	-0.526**
WCST							

Total Errors Standard scores [a]	-0.123	-0.335	0.089	0.436[†]	0.065	-0.086	-0.443[†]
Total Errors T scores [a]	-0.124	-0.345	0.082	0.433[†]	0.068	-0.083	-0.446[†]
Perseverative Responses T scores [a]	-0.276	-0.093	0.186	0.324	-0.016	0.027	-0.179
Perseverative Errors T scores [a]	-0.262	-0.117	0.175	0.324	-0.021	0.012	-0.197
nonPerseverative Errors T scores [a]	0.047	-0.553[*]	-0.058	0.255	0.106	-0.250	-0.729[**]
% Conceptual Level Response T scores [a]	-0.131	-0.315	0.067	0.476*	0.081	-0.079	-0.404[†]
Learning to Learn [a]	0.336	-0.838[*]	0.019	0.221	0.119	0.330	0.295

[a]Standardized regression coefficient. Control factors: Asthma, allergy, BMI, gender, Tonsil hypertrophy, Adenoid hypertrophy, Turbinate hypertrophy, Nasoseptal deviation., [†]$0.05 \leqq P < 0.1$. [*]$P<0.05$. [**]$P<0.01$. WCST, Wisconsin Card Sorting Test; CPT, Continuous performance task

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FINAL CONCLUSION:

Our work is a work in progress. We are following our premature cohort, and despite the attrition that we expected, we see a persistence of abnormal breathing during sleep, with apnea and hypopnea in 77% of our group. Premature infants have more learning and attention problems but the children with sleep-disordered-breathing –SDB-have more dysfunctions than those without SDB.

Some of the premature as full term infants have short lingual frenulum [1], an anatomical presentation not checked in premature infants and often difficult to recognize in early premature, but that should be systematically checked with further growth. “Clipping” of the lingual frenulum may not be possible but dis-insertion of this vestigial abnormally placed fibrous tissue can be done.

Prematurity independently of short frenulum or not, is associated with abnormal oral facial functions such as sucking, swallowing, nasal breathing and sound emission (speech). Myofunctional therapy [MFT] [2-4] has received a large amount of attention in the recent past, despite the fact that it has been practiced for many years, particularly in Europe and Brazil. Usage of MFT should be much more applied to any child with regular mouth breathing during sleep as it leads to oral facial abnormal development and growth and SDB, with increasing the risk of collapsibility of the upper-airway during sleep. And such dysfunction will impact further sleep and cognition. But MFT is difficult to perform in young individuals and compliance is limited even with the most dedicated parents. The beginning investigations of the potential role of “passive reeducation” using orthodontic simple devices during sleep versus “active reeducation” with parental involvement and exercise during wakefulness have to be pursued, as these investigations are mostly in the stage of feasibility studies [5].

The involvement of inflammatory cytokines with SDB is also clear but much more work needs to be done in this area. Further work may help indicating if a treatment is successful performing a blood test and not having to perform systematic PSG. But the field of cytokines is getting always more complex and the selection of the cytokine(s) to systematically follow is still uncertain, and much more work is needed in this area [6]

Sleep is always affected in children with health problems, but sleep and investigation of vital variables during sleep is an important test to perform to understand the development and worsening of SDB, and despite its challenges, it should be a test used much more in clinical practice when daytime problems cannot be easily resolved

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