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Gene Expression in Children with Obstructive Sleep Apnea: A Pilot Study

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Abbreviations: AHI: Apnea/Hypopnea Index; CLC4: Chemokine (C-C motif) Ligand 4; IL1Rl: Interleukin 1 Receptor-Like 1; OSA: Obstructive Sleep Apnoea Syndrome; PCA: Principal Component Analysis; PDK4: Pyruvate Dehydrogenase Lipoamide Kinase Isozyme 4; PSG: Polysomnography; TST: Total Sleep Time; SDB: Sleep Disordered Breathing

ABSTRACT

Background: Obstructive sleep disordered breathing is a syndrome presented with snoring and/or increased respiratory effort. Since its relatively high prevalence associated to the increased risk for end-organ effects OSA should be considered as a public health problem, in which both genetic and environmental factors could be involved. The recent advanced techniques allow to describe biological processes. The main objectives of this study were to analyze changing in gene expression in children with OSA, to highlight genes with changed expression and to understand activation of specific biological pathways.

Methods: We enrolled 5 pre-pubertal non-obese patients children with OSA; diagnosed with a positive polysomnography. A same number of non-obese control subjects were screened for symptoms of OSA using a validated questionnaire, and in absence of sleep disordered related symptoms they were invited to participate to the study; performing polysomnography.

Results: We recognize OSA patients and healthy children in two distinct clusters that suggest the real variability of expression pattern across the two individual groups. The main source of differences was represented by the genes differentially expressed, that were selected according to the stringent criteria previously described. We highlighted 118 and 205 genes in the OSA patients that are dowregulated and overexpressed respectively. Chemotaxis processes and G-protein receptors signaling were significantly enriched in the OSA children.

Conclusion: The study demonstrates that children with OSA presented changing in gene expression. We believe that the current study provides interesting informations on the underlying mechanism in OSA comorbidities.

Introduction

Obstructive Sleep Disordered Breathing (SDB) is reasonably considered as a syndrome of upper airway dysfunction during sleep presented with snoring and/or increased respiratory effort secondary to increased upper airway resistance and pharyngeal collapsibility [1]. The prevalence of habitual snoring was 7.45%; the prevalence of Obstructive Sleep Apnoea Syndrome (OSA) ranged from 0.1 to 13%, but most studies reported a frequency between 1 and 4% [2]. On the basis of its relatively high prevalence associated to the increased risk for cognitive and behavioral deficits, cardiovascular and metabolic end-organ effects [3-5] OSA

should be considered as a major public health problem [6], in which both genetic and environmental factors may be involved. Gold-standard exam for diagnosis of OSA is overnight Polysomnographic Evaluation (PSG).

Pathophysiology, genetic roles and comorbities of pediatric OSA in the last years have been valuated and understood, but specific genes connected with the condition were not still totally clarified. The recent advanced techniques, for example microarray technique, allow to describe biological processes. RNA microarrays perform a measure of gene expression entity for lots of genes or for the entire transcriptome, in order to permit the comprehension of different gene expression patterns [7]. Microarrays were utilized in many conditions, but not so often in sleep-related issues [8-9].

Material and Methods

The main objectives of this study were to analyze changing in gene expression in children with OSA followed at the Pediatric Pulmonology Service of the S. Orsola-Malpighi University Hospital of Bologna, to highlight genes with changed expression and to understand activation of specific biological pathways. The Ethical Comitee of our hospital approved the study (PED-OSAS; 21.04.2014) and all parents or legal tutors signed an informed consent to the treatment protocol. We recruited to the study 5 pre-pubertal non-obese patients' children with OSA; diagnosed with a positive polysomnography in association with no alterations in major inflammatory markers (C-reactive protein, white blood cell, velocity of Erythrocyte Sedimentation). A same number of non-obese control subjects matched for age, gender and ethnicity were screened for OSA utilizing a validated questionnaire [10], and in absence of sleep disordered related symptoms they were invited to participate to the study; performing polysomnography. Exclusion criteria were presence of any chronic medical condition (e.g asthma or allergies) or any genetic or craniofacial syndrome, presence of a recognized episode of infection in the eight weeks preceding the sleep study, and presence of a therapy during the period in study. A standard overnight multichannel polysomnographic exam was performed in our pediatric pneumologic unit [11]. The proportion of time spent in each sleep stage was counted as percentage of total sleep time (% TST). Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for duration of at least two breaths [11,12]. Hypopneas were defined as a decrease in oronasal flow of ≥50% with a corresponding decrease in SpO2 of ≥4% and/or arousal. The obstructive Apnea/Hypopnea Index (AHI) was defined as the number of apneas and hypopneas per hour [11].

Total RNA was extracted from peripheral blood leukocytes by RNeasy spin column method (Qiagen). Gene expression profile was assessed using GeneChip Human Transcriptome Array 2.0 (Affymetrix, Santa Clara, CA, USA). Microarray target sample processing was performed with WT Plus Reagent Kit (Affymetrix), while target hybridization, washing, staining and scanning steps were completed according to manufacturer's instructions (Affymetrix). Data normalization and summarization were performed using the RMA (Robust Multi-Array Average) method. Before proceeding with the expression profile analysis, the dimensions of the dataset were reduced by filtering out genes whose IQR is smaller than the 10th percentile of global IQR and whose expression level is below 5 in more than two samples. Then, the unsupervised analysis was performed using Principal Component Analysis (PCA) in order to estimate how the two group of children were similar in terms of gene expression.

To score the differences of expression between the two conditions, we used the moderated t-statistic for paired samples (implemented in limma package) with a significance level α =0,05. Differentially expressed genes were then classified as upregulated if logFC>0.5 and downregulated (logFC< -0.5). WEB-based GEne SeT AnaLysis Toolkit http://www.webgestalt.org/option.php was adopted to perform the Gene Ontology terms enrichment and the Gene Set Enrichment Analysis (http://software.broadinstitute.org/gsea/index.jsp) was implemented adopting the MsigDB curated gene sets C2 (http://software.broadinstitute.org/gsea/msigdb/collections.jsp).

Results

Demographic, clinical characteristics and principal polysomnographic index of the study population were summarized in Table 1; all OSA patients showed AHI >5 episodes·h−1 and all control children presented AHI < 1 episodes·h−1. Observing the PCA results (Figure 1), we were able to recognize OSA patients and healthy children in two distinct clusters that suggest the real variability of expression pattern across the two individual groups. The main source of differences was represented by the genes differentially expressed, that were selected according to the stringent criteria previously described. We highlighted 118 and 205 genes in the OSA patients that are dowregulated and overexpressed respectively (Figure 1B). Notably, the biological pathway of immune response, chemotaxis processes and G-protein receptors signaling were significantly enriched by the set of upregulated genes in the OSA children (Figure 1C).

Table 1: Summary of Demographic, Clinical Characteristics and Principal Polysomnographic Index of the Study Population.

	OSA (n=5)	Controls (n=5)
Male (n)	5	5
Age (Mean ± SD)	5 ± 3.2	5 ± 3.0
Caucasian (n)	5	5
BMI (z score)	0.41 ± 3.2	0.32 ± 4.1
AHI	8± 1.5	0,5± 0.8

Note: Data expressed as mean ± SD.

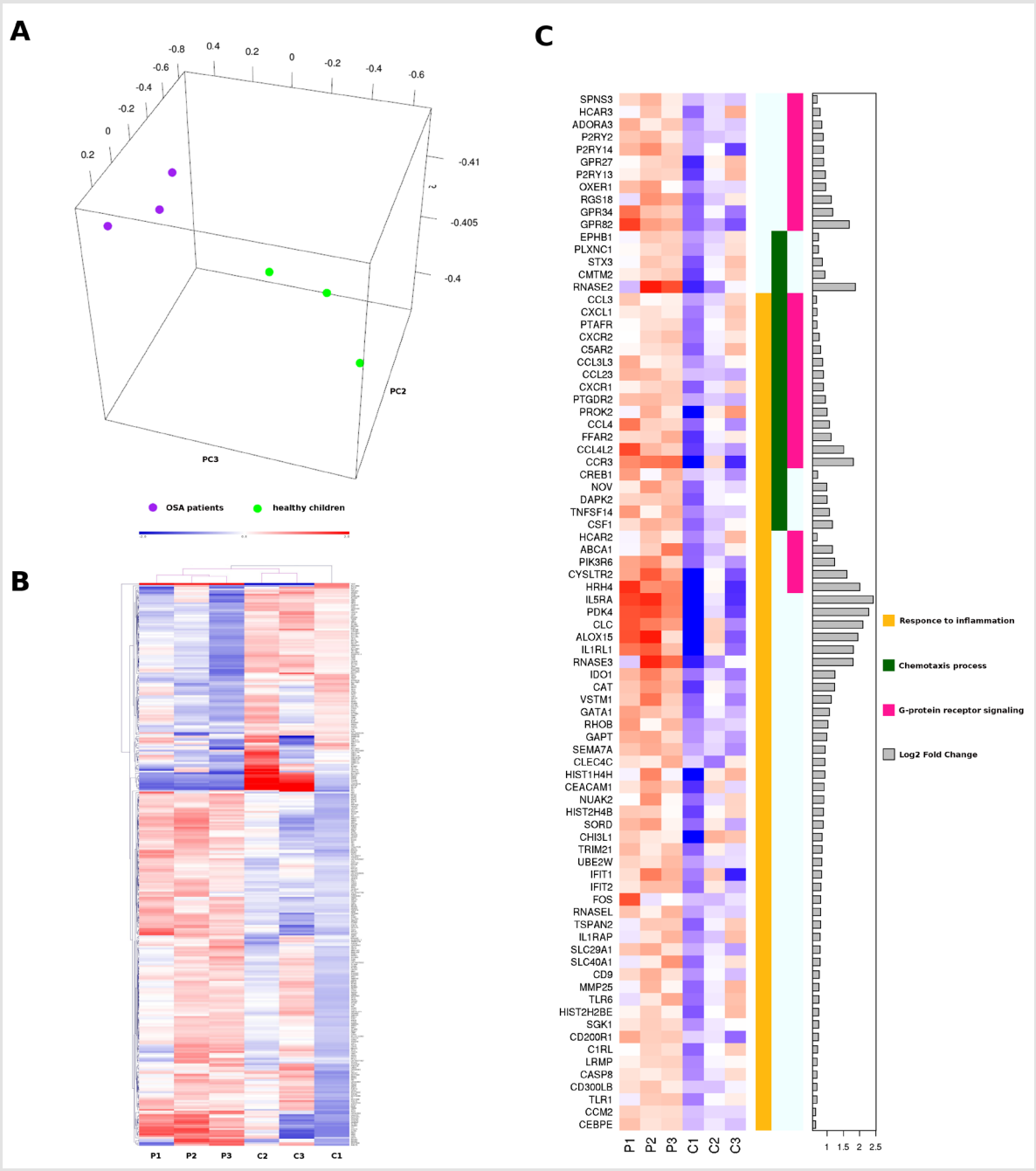

Figure 1: Summary of Principal Component Analysis (PCA) Results.

Discussion

Among the mainly differential overexpressed genes we found Pyruvate Dehydrogenase Lipoamide Kinase Isozyme 4 (PDK4), Chemokine (C-C motif) Ligand 4 (CLC4), Interleukin 1 Receptor-Like 1 (IL1Rl) were genes, whose up-regulation could be important in determining a specific comorbidity. PDK4 protein is found in the matrix of the mitochondria and inhibits the pyruvate dehydrogenase complex, reducing the conversion of pyruvate, which is produced from the oxidation of glucose and amino acids, to acetyl-CoA and contributing to the regulation of glucose metabolism. PDK4 is overexpressed in skeletal muscle in diabetes, resulting in impaired glucose utilization. In post-obese patients, there is a significant decrease in PDK4 mRNA expression, in conjunction with increased glucose uptake [13].

CCL4 is a CC chemokine with specificity for CCR5 receptors. It is a chemoattractant for natural killer cells, monocytes and a variety of other immune cells The chemokine has a crucial role in immune responses during infection and inflammation, in particular it activates human granulocytes which can lead to acute neutrophilic inflammation and also induce the synthesis and release of other pro-inflammatory cytokines such as Interleukin 1 (IL-1) and Interleukin 6 (IL-6) from fibroblasts and macrophages

[14]. IL1RL1 protein is directly implicated in the progression of cardiac disease: in case of scretching of the myocardium, the gene is upregulated, increasing the concentration of circulating soluble The ligand is the cytokine Interleukin-33 (IL-33); binding of IL-33 to the receptor, in response to cardiac disease or injury determinate a cardioprotective effect resulting in preserved cardiac function. In the presence of high levels of soluble protein, the heart is subjected to greater stress [15].

Biological pathway analysis confirmed the activation of specific inflammatory pathways, both in the individual parts that constitute inflammatory cascade (chemotaxis, response signal mediated by defense response, immune system process), and as inflammatory cascade. The main limit of our current findings is the low number of cases and controls, and our future efforts will be focused in confirming our data in larger cohorts.

Conclusion

The study demonstrates that children with OSA presented changing in gene expression. We believe that the current study provides interesting information on the underlying mechanism in OSA comorbidities. We believe that the current study provides interesting information on the underlying mechanism in OSA comorbidities and permits future researches about soluble markers of the disease.

Disclosure

The authors declare no conflicts of interest.

Conflicts of Interests and Source of Funding

All the authors declare no conflicts of interest. Sponsors were not involved in:

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