Role and mechanism of IL - 17 and its gene polymorphisms in dyslipidemia caused by obstructive sleep apnea syndrome in children

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ABSTRACT

The current research was carried out to explore the role and mechanism of IL - 17 and its gene polymorphisms in dyslipidemia caused by obstructive sleep apnea syndrome in children. For this aim, a total of 86 children with obstructive sleep apnea syndrome admitted to our hospital from January 2018 to January 2020 were selected as the study subjects, and the lipid-related indexes of the children were detected by a fully automatic biochemical analyzer, and they were divided into OSAHS group (54 cases), combined dyslipidemia group (32 cases), and another 40 healthy children who underwent physical examination in our hospital during the same period were selected as the healthy group. Levels of IL-7 and AHI, FEV1 / FVC were analyzed in each group, and levels of TC, TG, and LDL-C were compared to observe different sites of IL-17, namely IL-17A (rs3748067; The loci of IL-17F (rs763780, rs2397084) were genotyped in different groups to analyze the association between IL-17 and dyslipidemia in children with OSAHS. Results showed that higher IL-7 and AHI levels and lower FEV1 / FVC levels were found in the combined dyslipidemia and OSAHS groups compared with the healthy group, and higher IL-7 and AHI levels and lower FEV1 / FVC levels were found in the combined dyslipidemia group compared with the OSAHS group (P < 0.05); TC, TG and LDL-C level expression were higher in the combined dyslipidemia and OSAHS groups compared with the healthy group, and TC, TG and LDL-C level expression were higher in the combined dyslipidemia group compared with the OSAHS group (P < 0.05). IL-17 was positively correlated with TC, TG and LDL-C. It was concluded that in the development of OSAHS, IL-7 levels are significantly expressed, at the same time OSAHS children progress dyslipidemia, which has some correlation with IL-17, and IL-17 gene polymorphism, IL-17A (rs3748067); All were significantly expressed in the IL-17F (rs763780, rs2397084) locus.

Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAHS) is a common group of diseases in otorhinolaryngology, where the main clinical symptom is respiratory dysfunction during sleep (1). Studies have shown that the main cause of OSAHS is tonsillar and adenoid hypertrophy, which will cause a series of systemic pathophysiological changes in the body, leading to cardiovascular, renal and other related diseases and, in severe cases, death (2). Tonsils and adenoids, as lymphoid tissues, have a close association with the immune system. Therefore, OSAHS has a more profound and non-negligible impact on patients in childhood (3). OSAHS can lead to the development of multi-organ functional impairment. Intermittent hypoxia induces oxidative stress as well as an inflammatory response that affects insulin signaling, leading to the appearance of dyslipidemia (4). Disturbances in lipid metabolism often occur in adult OSAHS patients, while it has been less studied in pediatric OSAHS patients (5). The role of cytokines in the pathological process of OSAHS has received wide attention with the rise of medical technology (6). Therefore, this paper investigates the role and mechanism of IL-17 and its gene polymorphisms in dyslipidemia caused by obstructive sleep apnea syndrome in children.

Materials and methods

Study subjects: Eighty-six children with OSAHS admitted to our hospital from January 2018 to January 2021 were selected for the study, aged 3 to 11 years, with a mean of (8.1 ± 1.3) years. The children were divided into the OSAHS group and the combined dyslipidemia group after the detection of lipid-related indexes by the automatic biochemical analyzer. In the

OSAHS group, there were 54 cases, including 36 males and 18 females, aged from 3 to 11 years old, with a mean of (8.1±1.3) years. In the combined dyslipidemia group, there were 32 cases, including 25 males and 17 females, aged 4 to 12 years, with a mean of (8.2±1.5) years. Another 40 healthy children who underwent a physical examination at our hospital during the same period were selected as the healthy group. There were 22 males and 18 females in the healthy group, aged 4-11 years old, with a mean of (8.4±1.8) years. There was no statistical difference in the comparison of age and gender among the three groups. All parents signed an informed consent form.

Inclusion criteria: patients met the diagnostic criteria related to OSAHS (7); patients were less than 12 years of age; all had symptoms such as snoring and open-mouth breathing in sleep. Exclusion criteria: patients with combined cerebrovascular diseases, systemic diseases; those with congenital diseases; those with combined other related sleep disorder disease types; those with recurrent long-term tonsillar inflammation.

Methods

1 5 ml of venous blood was drawn from children in each group in the early morning on an empty stomach. After centrifugation of the venous blood, the IL-7 level was measured by an enzyme marker in strict accordance with the kit. The FEV1/FVC levels of each group were measured using a medical spirometer, and the measurement was carried out in strict accordance with the instrument instructions to avoid errors.

IL17A (rs3748067); IL-17F (rs763780, rs2397084) locus detection

The template DNA of the IL-17 gene was extracted by the NaI method, and the three loci of IL-17A (rs3748067), IL-17F (rs763780, rs2397084) (Table 1) were genotyped by RT-PCR. It is calculated by the $2^{-\triangle\triangle Ct}$ method with internal reference U6. The reverse transcription reaction conditions were set as follows: 25°C for 10min, 40°C for 60min, and 85°C for 5min. The amplification conditions were set as follows: 57°C for 20 s, 83°C for 30 s, and 58°C for 30 s and a total of 35 cycles were performed. The loci of IL-17A (rs3748067), and IL-17F (rs763780, rs2397084) were calculated by the 2-ΔΔCt method.

TC, TG, HDL-C, LDL-C level detection

Total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels were measured. 5 ml of venous blood was drawn from the children before and after treatment, respectively, in the early morning on an empty stomach, placed at room temperature for 15 min, and centrifuged at 3000 r for 10 min to obtain the serum layer. The assay was performed by Hitachi's fully automated biochemical assay.

Statistical analysis

SPSS 25.0 was used for relevant data analysis. The measurements were expressed as mean ± standard deviation ($\bar{x} \pm s$). The variance chi-squared was tested by the levene method and the normal distribution was tested by the Shapiro-Wilk test. The analysis was performed by rereading the measurement variance and comparing independent sample t-tests between groups at the same time point. The count data were expressed as a rate (%) using the χ^2 test, and $P<0.05$ indicated that the difference was statistically significant.

Table 1. Sequence list of IL-17 loci

Position	Enzyme	Upstream primer	Downstream primer	Product length (bp)	Annealing temperature (°C)
1249 (A/G,rs3748067)	EcoR I	GGGCTGAACTTTTCTCATACTTAGA	GAGACATTGTCTTCAGACTACAATG	212	56
7488 (T/C,rs763780)	Nla III	AGTACTGGGGCTCTCATT	GTTTCCATCCGTGCAGGTCT	412	52
7383 (T/C,rs2397084)	Ava II	GTGTAGGAACTTGGGCTGCATCAAT	AGCTGGGAATGCAAACAAAC	470	58

Results and discussion

Comparison of IL-7 and AHI, FEV1/FVC levels

As shown in Table 2, IL-7 and AHI levels were expressed higher and FEV1/FVC levels were lower in the combined dyslipidemia group and OSAHS group compared to the healthy group. In addition, IL-7 and AHI levels were higher and FEV1/FVC levels were lower in the combined dyslipidemia group compared with the OSAHS group, with statistical differences between the groups (P < 0.05).

Comparison of TC, TG and LDL-C levels

As shown in Table 3, the expression of TC, TG, and LDL-C levels was higher in the combined dyslipidemia group and OSAHS group compared with the healthy group. The expression of TC, TG, and LDL-C levels was higher in the combined dyslipidemia group compared with the OSAHS group, with statistical differences between the groups (P < 0.05).

IL-17A (rs3748067); IL-17F (rs763780, rs2397084) locus analysis

(i) The wild-type allele at the IL-17A (rs3748067) locus was G and the mutant type was A. The specimen electrophoresis results were seen for GG, GA and AA types. See Figure 1.

(ii) The size of the enzymatic cleavage product of IL-17F (rs2397084, rs763780) at these two loci differed greatly, and the results could be observed with ordinary agarose gels. The wild-type allele of rs2397084 was T and the mutant type was C. The specimen electrophoresis results can be seen in TT and CT types. The CC type of rs2397084 mutant pure heterozygote was not detected in this experiment, see Figure 2. The wild-type allele of rs763780 was T and the mutant type was C. The results of electrophoresis of the specimens could be seen as TT, CT and CC types, see Figure 3.

Table 2. Comparison of IL-7 and AHI and FEV1/FVC levels among study groups ($\overline{x} \pm s$)

Group	Number of cases	IL-7 (pg/ml)	AHI (times /h)	FEV1/FVC (%)
OSAHS	54	23.41±3.28ab	22.74±3.47	69.25±5.76
Combined dyslipidemia	32	34.22±5.16a	27.52±2.56	60.88±6.42
Healthy	40	17.65±1.09	2.46±0.28	83.59±12.36

Note: ^{a}P < 0.05 compared with the healthy group and ^{b}P < 0.05 compared with the combined dyslipidemia group.

Correlation analysis between IL-17 and dyslipidemia in children with OSAHS

IL-17 was positively correlated with TC, TG, and LDL-C, with IL-17 and TC (r=0.253, p=0.028), IL-17 and TG (r=0.343, p=0.002), and IL-17 and LDL-C (r=0.295, p=0.001).

Table 3. Comparison of TC, TG, and LDL-C levels among study groups ($\overline{x} \pm s$), mmol/L

Group	Number of cases	TC	TG	LDL-C
OSAHS	54	5.19±0.91ab	1.71±0.6ab	4.22±0.5ab
Combined dyslipidemia	32	7.26±0.92a	3.12±0.9a	4.81±0.75a
Healthy	40	4.48±0.50	1.22±0.3	2.63±0.44

Note: ^{a}P < 0.05 compared with the healthy group and ^{b}P < 0.05 compared with the combined dyslipidemia group.

Figure 1. Electrophoresis of IL-17A (rs3748067) locus PCR and digested product

Figure 2. Electropherogram of IL-17F (rs2397084) locus PCR and digested product
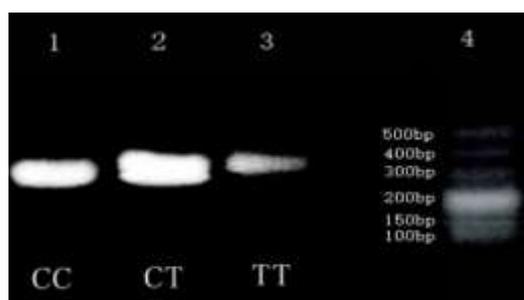

Figure 3. Electrophoresis of the IL-17F (rs763780) locus digestion product

OSAHS is a significant risk factor for diseases such as hypertension, coronary heart disease, and diabetes. OSAHS often affects the quality of sleep at night, which in turn causes daytime sleepiness, reduced daytime activity, impairment of normal food intake, and increased obesity. The relevant studies have shown that patients with OSAHS are prone to develop metabolic syndrome disorders due to their reduced sleep quality (8). Patients with OSAHS are prone to recurrent intermittent hypoxia and hypercapnia due to respiratory distress. The formation of lipid peroxides damages the vascular endothelium, which in turn causes patients to experience sympathetic excitation, increasing catecholamine concentrations and constricting the peripheral vasculature (9). Most scholars believe that this is the main cause of post-sleep drowsiness, pronounced daytime sleepiness, weakness and numbness in the extremities, and chest tightness in patients with OSAHS (10). OSAHS disease involves a variety of cells and cellular molecules acting in concert. In contrast, IL-7 has a strong activating and pro-inflammatory effect that accelerates the release of adhesion factors, which in turn allows the activation of inflammatory cells, while it can synergize with factors such as IL-6 and TNF-α to continue to increase the degree of inflammation (11).

The relevant studies have shown that OSAHS and hypertension are both risk factors for cardiovascular disease, and coexisting with hypertension in the same patient can aggravate the damage to their target organs (12). In recent years as the mechanism of action of multiple Th cells in asthma has been studied in depth, it has been found that Th1/Th2 type asthma already has its limitations and does not apply to all asthma. Other Th cells may also play a role in asthma, and Th17 cells have received extensive attention as new cells (13). Th17 cells are derived from naive D4+ T cells through differentiation. IL-17 is involved as a characteristic factor of Th17 cells in the eosinophil inflammatory response and plays an important role in the development of asthma (14). The results of this paper showed that IL-7 levels were significantly expressed in the development of OSAHS, which indicates the involvement of IL-7 in the development of disease in children with OSAHS.

The mutations in certain genes on IL-17A, and IL-17F may have an effect on their normal expression and thus act on the disease (15). IL-17A, as well as IL-17F, can increase the inflammatory response caused by the concentration of neutrophils in the bronchial airways, causing mucus cells to deform and lose their original function while promoting airway remodeling (16). It was found that the expression levels of IL-17A and IL-17F were significantly expressed in sputum, bronchial biopsies and alveolar lavage (BAL) fluid in patients with asthma disease, and the level of expression was significantly correlated with the severity of the disease in asthma patients (17). The results of this paper showed that among the IL-17 gene polymorphisms, IL-17A (rs3748067) and IL-17F (rs763780, rs2397084) loci were all associated with progression to dyslipidemia in children with OSAHS.

The related studies showed that the presence of disturbed lipid metabolism in OSAHS was directly associated with apnea and reduced blood oxygen in OSAHS patients (18). TC, LDL-C is now considered to be an important indicator of dyslipidemia and also one of the independent predictors of coronary heart disease (19). Lipid metabolism disorders are significantly associated with the degree of obesity in OSAHS patients. As OSAHS disease worsens, the immune system is severely affected and dyslipidemia ensues (20). It was demonstrated that plasma TG levels were negatively correlated with minimum oxygen saturation, which indicated that clinically patients with OSAHS often presented with both obesity and hyperlipidemia (21). The results of this paper showed that IL-17 was positively correlated with various indicators of lipids in children with OSAHS, suggesting a correlation with IL-17 in the progression to dyslipidemia in children with OSAHS.

In conclusion, IL-7 levels were significantly expressed in the development of OSAHS and also correlated with progressive dyslipidemia in children with OSAHS. Moreover, IL-17A (rs3748067), and IL-17F (rs763780, rs2397084) loci were significantly expressed among IL-17 gene polymorphisms.

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