Low expression and hypermethylation of FOXP3 in regulatory T cells are associated with asthma in children

XIAOHUA ZHU[1], QIANG CHEN[1], ZHIQIANG LIU[2], DAYA LUO[3], LAN LI[1] and YING ZHONG[4]

[1]Respiratory Department, Jiangxi Provincial Children's Hospital; [2]Clinical Laboratory, Jiangxi Children's Hospital; [3]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Nanchang University; [4]Graduate School of Medicine, Nanchang University, Nanchang, Jiangxi 330006, P.R. China

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Abstract. The aim of the present study was to determine the expression and methylation levels of forkhead transcription factor P3 (FOXP3) in peripheral blood $CD4^+CD25^+$ regulatory T cells (Tregs) harvested from children with asthma, and to explore the pathogenesis of asthma. The percentages of $CD4^+CD25^+FOXP3^+$ Tregs in $CD4^+$ T lymphocytes from 15 children with asthma and 15 healthy controls were measured by flow cytometry, and FOXP3 mRNA expression in the $CD4^+CD25^+$ Tregs was measured by reverse transcriptase-quantitative PCR. In addition, the forced expiratory volume in one second (FEV1) was measured to determine lung function. The methylation statuses of 16 CpG sites in two regions of the FOXP3 gene's exon and intron were analysed with bisulfite-specific PCR and pyrophosphate sequencing. The differences in methylation levels between the asthma and control groups were compared. The percentage of $CD4^+CD25^+FOXP3^+$ Tregs in $CD4^+$ T lymphocytes and FOXP3 mRNA expression were significantly lower in children with asthma than in control children ($P<0.05$). The FOXP3 mRNA levels in children with asthma were positively correlated with FEV1 ($P<0.001$; $r=0.895$). The methylation levels in 12 of the 16 studied CpG loci of the FOXP3 gene, and of the 6th CpG locus in the exon regions, were significantly higher in the asthma group compared with the control group ($P<0.05$). In summary, low expression and hypermethylation of the FOXP3 gene in the peripheral blood were associated with the pathogenesis of asthma in children. Thus, the FOXP3 mRNA expression level can be used to predict the severity of asthma in children.

Correspondence to: Dr Xiaohua Zhu, Respiratory Department, Jiangxi Provincial Children's Hospital, 122 Yangming Road, Nanchang, Jiangxi 330006, P.R. China
E-mail: nczxh2017@163.com

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Introduction

Asthma is a heterogeneous syndrome that is characterized by inflammation and hyper-responsiveness of the airway. Although all age groups are affected, the prevalence of asthma is increasing in many countries, especially among children (1). The aetiology and pathogenesis of asthma, which may be associated with genetic, immune and environmental factors, are incompletely understood and remain under investigation (2).

The helper T cell family include Th1, Th2, regulatory T (Treg) and Th17 cells. Recent developments in immunology and molecular biology have revealed that asthma is not only associated with the imbalance of Th1/Th2 function (3) but also with Tregs, since imbalances in forkhead transcription factor P3 $(FOXP3)^+$ Treg/Th17 and Th2/$FOXP3^+$ Treg cells lead to asthma (4,5). T cells play a central role in regulating airway inflammation in asthma. Tregs are a subset of $CD4^+$ T cells that play an essential role in maintaining peripheral immune tolerance and controlling allergic diseases, such as asthma. Tregs, together with effector T cells (Teffs), cytokines, immune antibodies and other cellular components, play an important role in maintaining immune balance (6). As important immunosuppressive cells, $CD4^+CD25^+$ Tregs act in cell-cell contact-dependent inhibition patterns and ultimately inhibit immune diseases by inhibiting helper T cell activation and differentiation, and directly inhibiting B cell activation to produce antibodies (7).

FOXP3 is the most reliable specific molecular marker of natural Tregs (nTregs) and is associated with the immunosuppressive function of $CD4^+CD25^+$ Tregs (8). The development and function of $CD4^+CD25^+$ Tregs depend on the expression of FOXP3 (9). Tregs, specifically $CD4^+CD25^+FOXP3^+$ Tregs, tightly control autoreactive B and T cell responses in the periphery (10). $FOXP3^+$ Tregs are the most widely-known type of immune cells and have the strongest inhibitory function and most extensive inhibitory targets. $FOXP3^+$ Tregs prevent autoreactive T cell activation, inhibit autoimmune and allergic disease occurrence, exert anti-inflammatory functions and maintain autoimmune tolerance (11,12). In addition, the downregulation of FOXP3 expression potentially results in the inability of Tregs to inhibit infection and tumours (13).

FOXP3 is an important transcription factor in the activation of Tregs, but its expression alone may not be sufficient to explain all Tregs functions. An additional mechanism is needed to explain the genes expressed by Tregs and their functional stability and cell lineage maintenance. One possible mechanism underlying this phenomenon is epigenetic regulation, which also provides a new understanding of the interaction between genes and the environment (14). Epigenetic inheritance can initiate and maintain FOXP3 expression in nTregs (15). FOXP3 expression is regulated by DNA methylation, histone modifications and posttranscriptional modifications (16). The epigenetic regulation and methylation of FOXP3 play an important role in its stable expression (17). Changes in the methylation level of the FOXP3 gene may affect Treg differentiation and regulate the occurrence of an immune response. Thus, detecting the methylation statuses of upstream enhancers of FOXP3 may help in the diagnosis and subtype classifications of diseases (18). A comprehensive study of epigenetic variation will promote our understanding of complex diseases, especially those in which genetic and environmental factors interact, such as asthma. Therefore, in order to further investigate the association between FOXP3 expression and methylation levels in Tregs and its pathogenesis in childhood asthma, the percentages of $CD4^+CD25^+$ FOXP3 Tregs in $CD4^+$ T lymphocytes in the peripheral blood mononuclear cells (PBMCs) from children with asthma and healthy controls were detected by flow cytometry. Furthermore, the mRNA expression of transcription factor FOXP3 in PBMCs was detected by RT-qPCR. The mRNA expression of FOXP3 in $CD4^+CD25^+$ FOXP3 Tregs was compared between asthmatic and healthy control groups and the correlation between FOXP3 mRNA expression and pulmonary function (FEV1) was analysed in the asthma group. Additionally, the methylation statuses of 16 CpG loci (including 7 sites in the exon and 9 sites in the intron areas) in the FOXP3 gene were compared by bisulfite transformation, PCR and pyrosequencing.

Patients and methods

Clinical samples. A total of 15 children with asthma, who were hospitalized in the respiratory department of Jiangxi Children's Hospital (Jianxi, China) between July 2016 and June 2017, were included in the asthma group. The group included 10 males and 5 females, aged 5-14 years, and all of these subjects met the diagnostic criteria for asthma (19). The healthy control group consisted of 15 children, including 9 males and 6 females, aged 5-14 years. These subjects simultaneously underwent physical examination at the children's health clinic. The subjects had no family history of atopic diseases or personal allergy history, no history of other diseases, and no respiratory tract infections in the past month. Subjects who failed to complete the study were excluded from both groups. Independent-Samples t-test confirmed that there were no significant differences in sex (P=0.702) or age (P=0.338) between the two groups. All human materials were obtained with informed consent, and the protocols were approved by the Ethics Review Committee of Jiangxi Children's Hospital.

Ficoll density gradient separation of PBMCs. Peripheral venous blood (5 ml) was collected for anticoagulation and mixed with 5 ml of phosphate-buffered saline (PBS). The liquid was added slowly against the wall of the tube and centrifuged at 4˚C, 500 x g for 20 min. Following centrifugation, the liquid in the tube was divided into four layers: Plasma and PBS; lymphocytes; red blood cells; and granulocytes. A white and cloudy layer, mainly composed of monocytes, including lymphocytes and monocytes, appeared at the junction of the upper and middle layers. A capillary pipette was inserted into the white and cloudy layer, and the PBMC layer was collected and placed in another centrifuge tube. Subsequently, 5 ml PBS solution was added, and the resulting mixture was centrifuged at 4˚C, 500 x g for 10 min. After discarding the supernatant, the cells were washed twice, and the cell concentration was adjusted to $1x10^6$ cells/ml with PBS.

Flow cytometry (FCM) analysis. For Treg analysis, cell suspensions were transferred into tubes and washed with PBS. The cell suspension (100 μl) was stained with fluorescein isothiocyanate (FITC) anti-human CD4 (20 μl; cat. no. 561005; BD Biosciences), and allophycocyanin (APC) anti-human CD25 (20 μl; cat. no. 560987; BD Biosciences). IgG1-FITC (20 μl; cat. no. 556649; BD Biosciences) and IgG1-APC (20 μl; BD Biosciences; cat. no. 550854) were used as homologous controls. After shaking and mixing, the solution was incubated for 15 min in the dark at room temperature (20-25˚C), following which 100 μl Reagent A (cat. no. 641776; BD Biosciences) was added to the tube and vortexed thoroughly. After a further incubation for 5 min in the dark at room temperature (20-25˚C), the mixture was washed with 1 ml PBS solution and centrifuged under 800-850 x g at 4˚C for 5 min. The supernatant was discarded, and 50 μl Reagent B (BD Biosciences, cat. no. 641776) and 20 μl phycoerythrin (PE) anti-human FOXP3 (BD Biosciences; cat. no. 560082) were added, IgG1-PE (20 μl; BD Biosciences; cat. no. 556650) was used as a homologous control. After shaking and mixing, the cells were incubated at room temperature (20-25˚C) for 15 min, washed with 1 ml of PBS solution and centrifuged under 800-850 x g at 4˚C, for 5 min. The supernatant was discarded, and the cells were suspended in 250 μl of PBS solution. All steps were performed using the BD Instrasure™ Kit according to manufacturer's protocols (Becton, Dickinson and Company).

FCM was performed on a BD FACSCanto™ II flow cytometer (BD, New Jersey, USA) using BD FACSDiVa software v6.1.2 (Becton, Dickinson and Company). $CD4^+$ cells, $CD4^+CD25^+$T cells and $CD4^+CD25^+FOXP3^+$ Tregs in the lymphocyte group were determined by an FSC-SSC scatter plot. The ratio of $CD4^+CD25^+FOXP3^+$ Tregs to $CD4^+$ T cells in children with asthma and healthy children was compared.

Pulmonary function test. Pulmonary function was measured using a MasterScreen IOS pulmonary function instrument (Jaeger), and the temperature, pressure and humidity were corrected before measurement. The pulmonary function was measured 3 times, and the best value was taken as the final value. The change in FEV1 was measured as a percentage of the normal predicted FEV1 (%).

Reverse transcription-quantitative (RT-q)PCR analysis of FOXP3 mRNA expression. The mRNA sequence of the FOXP3 gene was obtained from the GenBank database. β-actin

Table I. Primer sequences for the FOXP3 and β-actin genes.

Name	Direction	Sequence, 5'-3'	Fragment length, bp
FOXP3	F	CAAGTTCCACAACATGCGAC	91
	R	ATTGAGTGTCCGCTGCTTCT	
β-actin	F	ATCGTCCACCGCAAATGCTTCTA	105
	R	AGCCATGCCAATCTCATCTTGTT	

F, forward; R, reverse; FOXP3, forkhead transcription factor P3.

(cat. no. ab179467; Abcam) was used as an internal control. The primers were designed and synthesized by Shanghai Bioengineering Co., Ltd. The sequences of the primers are listed in Table I.

Total RNA was extracted using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) and then reverse-transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (cat. no. K1622; Thermo Fisher Scientific, Inc.) under the following incubation conditions: 65˚C for 5 min, 42˚C for 60 min and then 70˚C for 5 min according to the manufacturer's protocol. PCR amplification was carried out using a PCR kit (Beijing TransGen Biotech Co., Ltd.) under the following reaction conditions: 35 cycles of denaturing at 94˚C for 40 sec; annealing at 60˚C for 40 sec; and extension at 72˚C for 60 sec. The PCR products were subjected to a melting curve analysis to ensure that a single amplification product was produced. The cycle threshold (Ct) value of the quantitative results was calculated automatically and reported by a computer. The relative expression level of FOXP3 was calculated using the quantification cycle ($2^{-\Delta\Delta Cq}$) method (20).

DNA methylation assays were performed by bisulfate modification followed by PCR amplification and pyrosequencing. DNA was from PBMCs using TIANamp Genomic DNA kit (cat. no. DP304; Tiangen Biotech Co., Ltd.) before hydrogen sulfite conversion was performed. Subsequently, template DNA (2 μl) was added to each PCR tube for hydrogen sulfite conversion using a sodium bisulfite modification kit (EpiTect Fast DNA Bisulfite kit; Qiagen GmbH), according to the manufacturer's protocols. Each component of the reaction mixture, along with their respective volumes, are listed in Table II. PCR amplification at 20 μl volume per reaction was subsequently performed using Pyromark PCR Kit (cat. no. 978703; QIAGEN China Co., Ltd) according to the reaction programme shown in Table III. The primer sequences were designed and synthesized by KaiJie Transforming Medical Research Co. Ltd (Table IV). For methylation analysis, the microspheres were immobilized by the PCR products and prepared for sequencing on a pyrosequencing apparatus (Pyromark Q24; QIAGEN China Co., Ltd) that recorded the experimental results. 16 CpG sites from exons -6,210 to -6,334 and introns -2,262 to -2,376 of the FOXP3 gene were selected in asthma and control children, respectively to detect the degree of DNA methylation, which was expressed as the average methylation (Fig. 1).

Statistical analysis. The SPSS 19.0 software (IBM Corp.) was used for the statistically analysis of the data between the asthma and control group. The measurement data are expressed as the mean ± standard deviation. If the two group variances were homogeneous, Student's t-test was used for comparisons. If the variance was uneven, the Welch's t-test was used. The correlation between FOXP3 mRNA levels and FEV1 was analysed by Pearson's linear correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Table II. PCR system.

Components	Volume/reaction (μl)
PyroMark PCR Master Mix, 2X	12.5
CoralLoad Concentrate, 10X	2.5
Forward primer	0.5
Reverse primer	0.5
RNase-free water	7
Total	23

Table III. PCR programme.

Step	Process	Temperature, duration
1	Predenaturation	95˚C, 15 min
2	Denaturation	94˚C, 30 sec
3	Anneal	63˚C, 30 sec, -0.5˚C/cycle
4	Prolongation	72˚C, 30 sec
	Cycle number (steps 2-4)	10
5	Denaturation	94˚C, 30 sec
6	Anneal	58˚C, 30 sec
7	Prolongation	72˚C, 30 sec
	Cycle number (steps 5-7)	40
8	Final extension	72˚C, 5 min

Results

Percentages of peripheral blood $CD4^+CD25^+FOXP3^+$ Tregs in $CD4^+$ T cells in asthma and healthy control groups. The percentage of $CD4^+CD25^+FOXP3^+$ Tregs was significantly lower in the asthma group compared with the healthy control group (3.75±2.99 vs. 14.01±9.89, respectively; P<0.001; Fig. 2).

Table IV. PCR primer sequences and pyrosequencing primer sequences.

Name	Sequence, 5'-3'
FOXP3 F1-2	ATTTTTGTGGTGAGGGGAAGAAATTA (Biotin)
FOXP3 R1	AACCCCAAACCTCTCTCTTCTAATAATCCA
FOXP3 Seq1	CTCTCTCTTCTAATAATCCAA
FOXP3 F2	AAATTTGGATTATTAGAAGAGAGAGG
FOXP3 R2	AACTAACAAAAAAAAATCAACCTAACTTAT (Biotin)
FOXP3 Seq2	AGAAGAGAGAGGTTTG
FOXP3 F3	GGATGTTTTTGGGATATAGATTATGTTT (Biotin)
FOXP3 R3	ACCTATAAAATAAAATATCTACCCTCTTCT
FOXP3 Seq3	CCTCTTCTCTTCCTC
FOXP3 F4	GTTTGTTGTAGGATAGGGTAGT (Biotin)
FOXP3 Seq4	CCTATTATCACAACCCC

F, forward; R, reverse; FOXP3, forkhead transcription factor P3.

Lung function (FEV1%) is lower in children with asthma. The percentage change in FEV1 was significantly lower in the asthma group compared with the healthy control group (80.32±9.12 vs. 96.40±4.63%, respectively; P<0.001; Table V).

Table V. Change in FEV1.

Group	n	FEV1, %	t	P-value
Asthma	15	80.32±9.12	-6.091	P<0.001
Control	15	96.40±4.63		

Data are presented as the mean ± standard deviation. FEV1, forced expiratory volume in one second.

FOXP3 mRNA expression level is lower in the peripheral blood of children with asthma. The FOXP3 mRNA expression level in the peripheral blood was 6.09±3.04 in the asthma group compared with 9.38±4.54 in the healthy control group. The difference between the two groups was statistically significant (P<0.05; Fig. 3).

Correlation between peripheral blood FOXP3 mRNA levels and FEV1 in children with asthma. A correlation analysis demonstrated that FOXP3 mRNA level in the peripheral blood of children with asthma was positively correlated with FEV1, (P<0.001; r=0.895; Fig. 4).

Detection of FOXP3 gene sequence methylation levels. The average percentage methylation levels of each FOXP3 CpG loci were compared between the asthma and control groups. Overall, the average percentage methylation levels of 12 of the 16 FOXP3 CpG loci studied had the tendency to be higher in the asthma group compared with the control group. However, the methylation level at only CpG site 6 in exon 1 of sequence 1 was significantly higher in the asthma group compared with the control group (P<0.05; Fig. 5).

Discussion

The human immune system maintains a complex balance of self-defence and tolerance. Tregs differentiate in the human thymus and express CD4, CD25 and FOXP3, of which $CD4^+CD25^+$ Tregs are a specific subgroup of T cells that exist mainly in the peripheral blood and spleen of normal individuals. $CD4^+CD25^+$ Tregs have unique immunomodulatory functions, and FOXP3 is highly expressed in the cytoplasm. $CD4^+CD25^+$ Tregs can be divided into nTregs and induced $CD4^+CD25^+$ Tregs (iTregs), according to their origin and mechanism of action. Commonly, $CD4^+CD25^+FOXP3^+$ Tregs are referred to as nTregs (21).

Tregs have anti-inflammatory functions and maintain autoimmune tolerance. The transcription factor FOXP3 can regulate Treg differentiation, and FOXP3 serves as a key regulator of their formation and function. FOXP3 expression can be regulated by multiple epidermal susceptibility enhancers and promoters (22) and is associated with the occurrence of various immune diseases. Patients with autoimmune hepatitis have significantly decreased number of $CD3^+CD4^+CD25^+FOXP3^+$ Tregs and FOXP3 mRNA expression (23). Lower proportions of $CD4^+CD25^+FOXP3^+$ Tregs have also been found in $CD4^+$ T cells harvested from mice with asthma (24). The percentage of $CD4^+CD25^+FOXP3^+$ Tregs in the peripheral blood was significantly lower in children with allergic asthma compared with healthy controls (4). Furthermore, the expression of $CD4^+CD25^+$ Tregs and FOXP3 mRNA was lower in the acute asthma attack group compared with the asthma remission and normal control groups, whereas the increase in $CD4^+CD25^+$ Tregs and FOXP3 mRNA expression in the asthma remission group was similar to the control group (25). Thus, low FOXP3 expression and insufficient Treg cell function in target cells can lead to the occurrence of asthma.

Asthma can be both inherited and affected by environmental factors. Environmental exposure can induce DNA methylations (26), and hereditary epigenetic markers may play an important role in the pathogenesis of asthma (27,28).

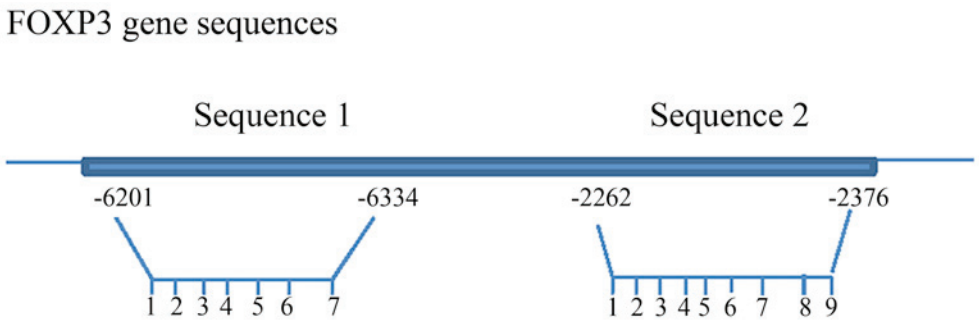

Figure 1. Schematic representation of the regions in the FOXP3 gene locus studied. Pyrosequencing data for the 16 CpG pair positions in the FOXP3 gene. FOXP3, forkhead transcription factor P3.

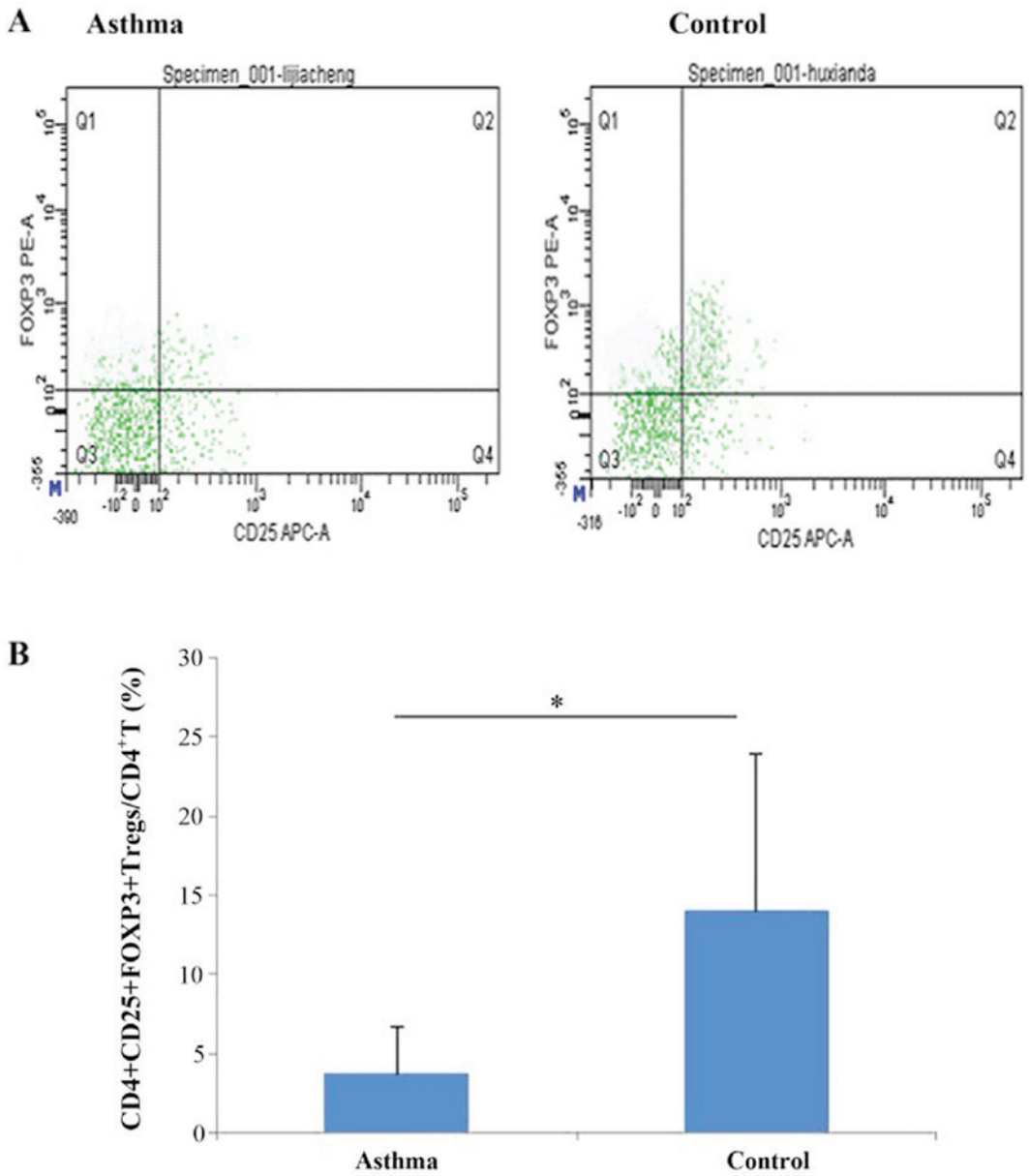

Figure 2. Flow cytometry analysis of Treg frequency in asthma and healthy groups. (A) Frequencies of $CD4^+CD25^+FOXP3^+$ Tregs. (B) Percentages of $CD4^+CD25^+FOXP3^+$ Tregs in $CD4^+$ T cells. Data are presented as the mean ± standard deviation, analysed by Welch's t-test due to uneven variance. $^*P=0.001$.

In order to explore the molecular mechanism of asthma in the present study, children with asthma and healthy controls were selected to compare the expression and methylation status of FOXP3 and for correlation analysis between

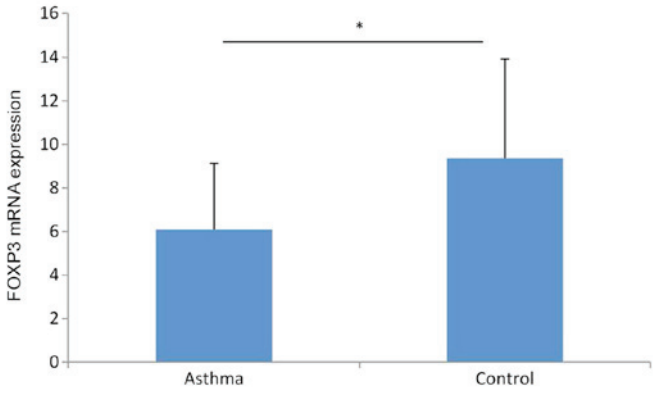

Figure 3. Expression of FOXP3 mRNA. The FOXP3 mRNA expression was lower in the asthma group compared with the control group. The data are presented as the mean ± standard deviation. *P<0.05. FOXP3, forkhead transcription factor P3.

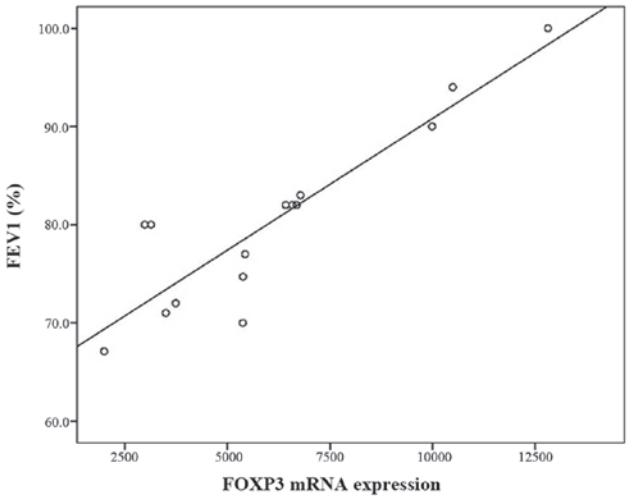

Figure 4. Pearson linear correlation analysis to compare peripheral blood FOXP3 mRNA levels and FEV1 in children with asthma. The y-axis refers to the change in FEV1, measured as a percentage of the normal predicted FEV1. The FOXP3 mRNA levels in the peripheral blood of children with asthma is positively correlated with FEV1. P<0.001; r=0.895. FOXP3, forkhead transcription factor P3; FEV1, forced expiratory volume in one second.

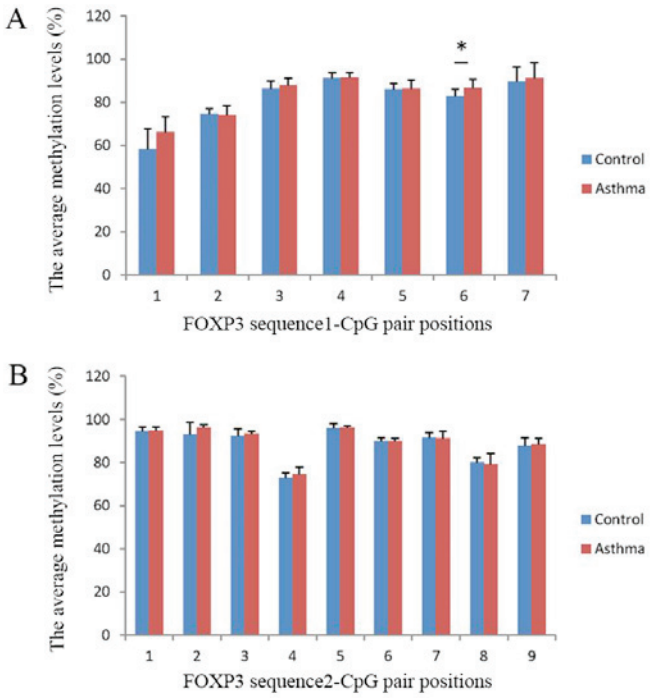

Figure 5. Methylation statuses of two FOXP3 sequences in the asthma and control groups. (A) Average methylation levels in the 7 FOXP3 sequence 1-CpG pair positions between Control and Asthma groups. (B) Average methylation levels in the 7 FOXP3 sequence 2-CpG pair positions between Control and Asthma groups. Comparison of the methylation statuses of the FOXP3 gene CpG loci reveals higher percentages FOXP3 methylation in the asthma group compared with the control group. The data are presented as the mean ± standard deviation, only the variance at CpG site 10 was uneven, the Welch's t-test was used. The methylation level at CpG site 6 in exon 1 was significantly higher in the asthma group than in the control group. *P<0.05. FOXP3, forkhead transcription factor P3.

FOXP3 mRNA expression and asthma severity. FCM and RT-qPCR were used to detect the percentage of $CD4^+$ T cells and FOXP3 mRNA expression in PBMCs of children with asthma. The number of $CD4^+CD25^+FOXP3^+$ Tregs and the percentage of $CD4^+CD25^+FOXP3^+$ Tregs were significantly lower in the asthma group compared with the healthy control group; these results were consistent with those of previous studies (4,24).

Children over five years of age were selected for the present study. The results showed that lung function (FEV1) was significantly lower in children with asthma than in healthy controls. FEV1 is the volume of maximal exhalation in the first second after a maximal deep inhale. The clinical measurement of FEV1 is often used to judge the severity of asthma. Since expiratory dyspnoea is most common in patients with asthma, FEV1 levels may be decreased. Some data showed that the Tregs and FOXP3 mRNA levels were decreased in patients with asthma, and that the FOXP3 mRNA expression levels were positively correlated with FEV1 (29); these results were consistent with the findings of the present study. The FOXP3 mRNA levels and FEV1 were positively correlated in the asthma group. Lower FOXP3 mRNA expression was correlated with a more significant decrease in FEV1 in the same period, which suggested that the FOXP3 gene had an antagonistic protective effect on lung function injury (30).

The CpG methylation statuses of the FOXP3 gene in Tregs were compared between children with asthma and healthy children, which revealed a tendency of hypermethylation in children with asthma. The methylation degree at the sixth CpG site of exon 1 was significantly higher in children with asthma than that of healthy children. Epigenetics, including DNA methylation, histone acetylation, chromatin recombination and nucleosome remodelling, is a popular topic in modern life sciences. Epigenetic mechanisms have been shown to regulate many genes, including those involved in inflammation and immune responses, and to ensure the stability of phenotypic inheritance and cell differentiation (31). DNA methylation has attracted increasing attention in the field of epigenetics, and it is of interest that a previous study has reported that DNA methylation serves an important role in the development of asthma (32). Changes in DNA methylation can inhibit gene expression and lead to the differentiation and reactivity of

T cells, where the hypermethylation of DNA CpG frequently leads to gene silencing and the overall reduction in gene expression (33). CpG methylation in specific DNA regions controls the expression of various key transcription factors, thus contributing to the differentiation of helper T cells (34). Moreover, DNA CpG hypermethylation usually results in gene silencing and overall decrease in gene expression (33), which can cause Tregs to differentiate into more Th2-type cells (35). Studies have found that the interleukin (IL)-4, IL-13 and runt-related transcription factor 3 genes were hypomethylated in patients with asthma (36), whereas the FOXP3 and IL-10 genes were hypermethylated (26). The methylation levels of the FOXP3 gene in children predisposed to risk factors of asthma and/or early, short wheezing were significantly increased (37). The methylation of CpG island in the FOXP3 gene could inhibit the decrease in mRNA expression, affecting DNA binding to transcription factors and the transcription of proteins, thus weakening the immunosuppressive effects of $CD4^+CD25^+$Tregs (38). The epigenetic mechanisms responsible for regulating FOXP3 expression were the key components of Treg suppressive activity. FOXP3 hypermethylation could impair the differentiation and function of Tregs, thus increasing the incidence of asthma and the severity of the disease (38).

The present study had a number of limitations, and further improvements are required. In the future, larger sample size for the correlation analysis between the expression and methylation levels of FOXP3 is required.

In conclusion, the present study demonstrated decreased proportion of $CD4^+CD25^+FOXP3^+$ Tregs and decreased expression of FOXP3 mRNA, accompanied by increased methylation level of FOXP3 in children with asthma. Moreover, a positive correlation was observed between FOXP3 mRNA expression level and the change in FEV1 in children with asthma. Thus, the epigenetic modification of FOXP3 could regulate the distribution of $CD4^+CD25^+FOXP3^+$ Tregs and affect the expression of FOXP3. The decreased number of $CD4^+CD25^+FOXP3^+$ Tregs and the low expression and hypermethylation of FOXP3 in the peripheral blood may be associated with the risk of developing asthma, and impact the pathogenesis and severity of asthma in children. Therefore, data from the present study suggest that the upregulation of FOXP3 expression, by suppressing its methylation, can potentially have immunosuppressive effect in asthma. Further epigenetic studies can provide new scientific evidence to aid and improve the clinical diagnosis and treatment of childhood asthma.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

XHZ was responsible for project design, clinical data collection, RT-qPCR, statistical analyses and wrote the article. QC was involved in the design of the study. ZQL, LL and YZ performed FCM analysis and pulmonary function test. WH, ZL and DL performed RT-qPCR and DNA methylation assays. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Jiangxi Children's Hospital (approval no. 2015006). All human materials were obtained with informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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