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Upregulated Thymic Stromal Lymphopoietin Receptor Expression in Children with Asthma

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Short title: TSLPR in asthmatic children

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Abstract

Background: Thymic stromal lymphopoietin (TSLP) plays an important role in pathogenesis in patients with asthma. However, the role of thymic stromal lymphopoietin receptor (TSLPR) and correlation with IL-7R α and clinical severity in asthmatic or non-asthmatic children remain unclear. We investigated TSLPR and IL-7R α mRNA levels in asthma and non-asthma and assessed TSLPR expression in children who were sensitive to mites.

Materials and Methods: We enrolled asthmatic and non-asthmatic children. In order to minimize the influence of allergy, we also divided participants into following 4 groups: non-allergic and non-asthmatic group (NN) (healthy children), allergic but non-asthmatic group (AN), non-allergic but asthmatic group (NA) and allergic asthmatic group (AA). We drew blood samples to check total IgE, allergen-specific IgE, TSLP, and measured expression of the TSLPR and IL-7R α genes using reverse-transcription polymeric chain reaction (RT-PCR) and real time PCR. Asthma symptom score was also recorded.

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Results: TSLP and TSLPR were significantly higher in asthmatic than non-asthmatic children. The levels of TSLP were significantly different between AA and NN group ($P < 0.05$). TSLPR in NA and AA group were significant higher than NN group ($P < 0.05$). TSLPR did not differ significantly between NA and AA. The TSLPR expression correlated strongly with IL-7R α and weakly with mite-specific IgE. Clinical asthmatic severity of children was found to exert no influence on TSLPR level.

Conclusion: TSLPR might be a significant disease biomarker for asthma. The levels of TSLPR were higher in asthmatic patients than in healthy children but not different between allergic and non-allergic asthmatic patients.

Keywords: allergy, asthma, IL-7R α , mite-sensitive, TSLPR

Introduction

Asthma manifests an inflammatory process in children. Although substantial progress has been made in understanding allergies, the precise cause remains unknown. The complex relationships among genetic, immunologic, pharmacologic, and environmental factors in these diseases require further investigation [1]. Recent studies on allergic and autoimmune diseases have focused on thymic stromal lymphopoietin (TSLP), which is a 140-amino acid IL-7-like 4-helix bundle cytokine [2]. It directly activates dendrite cells by upregulating

MHC Class I and II molecules and costimulatory molecules [3]. TSLP also directly costimulates mast cells to produce TH2 cytokines [3]. TSLP is known to be associated with atopic diseases [4, 5], and TSLP gene promoter polymorphisms have been shown to be associated with allergic inflammation including IgE levels, eosinophil levels, and bronchial asthma [6].

Several studies have shown that TSLP plays a potentially important role in the pathogenesis of allergic inflammation in patients with asthma [4, 6, 7], atopic dermatitis [8], and allergic rhinitis [9]. TSLP was first isolated from a murine thymic stromal cell line and has been shown to support B-cell development in the absence of IL-7 [2, 10]. In mice, TSLP has additional functions, including promoting B-cell and T-cell development and promoting the activation of T cells, mast cells, eosinophils, and macrophages [3]. TSLP has been shown to be a major switch factor in differentiating proinflammatory Th2 cells from naïve CD4⁺ T-cell precursors; these cells secrete high-level cytokines such as IL-4, IL-5, and IL-13 [11, 12, 13]. In humans, high expression of TSLP is a feature of keratinocytes in the skin lesions of patients with atopic dermatitis [11], and bronchial epithelial cells in asthmatic lungs [13]. In humans, the cytokine TSLP plays a key role in the pathogenesis of asthma [14]. TSLP has also been shown to induce asthmatic airway epithelial cell proliferation through an IL-13–dependent pathway [15]. Kato et al [16] reported that respiratory viral infection and the recruitment of TH2 cytokine-producing cells might amplify TH2 inflammation through the

induction of TSLP in the asthmatic airway. In the nasal mucosa of allergic rhinitis patients, TSLP expression increased and correlated strongly with the clinical severity of symptoms and the quantity of eosinophils [5, 9]. Matsuda et al [17] reported that TSLP plays a role in chronic allergic keratoconjunctivitis through the activation of dendritic cells and mast cells in synergy with other cytokines. In patients with autoimmune disease, TSLP may also contribute to tissue inflammation through the TH1 and TH17 cytokines [18]. TSLP and its receptor play a proinflammatory role in autoimmune arthritis by enhancing TH17 cells and causing tissue destruction [18].

The TSLPR is a heterodimeric Type I cytokine receptor that interacts with TSLP and shares the IL-7 receptor alpha (IL-7R α) subunit with the IL-7 receptor system [19]. Several studies have reported a relationship between the SNP in IL-7R α [20, 21] and TSLPR in patients with asthma [22]. Human TSLPR also known as CRLF2 (cytokine receptor like factor 2) has been identified in the Xp22.3 and Yp11.3 regions of T lymphocyte and dendritic cells in the cDNA cell library [22, 23]. Shi et al [24] reported that in asthmatics local blockade of TSLPR alleviated TH2-mediated airway inflammation by regulating the dendritic cells. A recent study showed that TSLPR expression induced upregulation of CD25 in activated T cells and persisted in the presence of ligand [12]. Furthermore, TSLPR was preferentially expressed on rare actively dividing TH2 cells and caused allergic inflammation [12]. A study targeting allergen to Fc γ RI revealed a novel TH2 regulatory

pathway linked to TSLPR [7]. TSLPR also mediated the expression of proinflammatory cytokine IL-6, chemokines CXCL8 and CCL11 in smooth muscle cells of the human airway [25]. However, the role of the mRNA expressions of the TSLPR and relationship between IL-7R α and clinical asthma severity among patients with asthma or non-asthma remains unclear. To investigate the hypothesis that TSLPR like TSLP might overexpress, influencing immune modulation in the course of asthma in children, we examined TSLPR and IL-7R α mRNA expression and TSLP secretion in children who were sensitive to mites.

Methods

Participants and data collection

Our study group included 84 children (45 girls and 39 boys). The asthmatic patient comprised 31 children, and the non-asthmatic group comprised 53 children. In the asthma group, the sex ratio was 0.82:1 (14 girls and 17 boys), and in the non-asthmatic group it was 1.41:1 (31 girls and 22 boys). All children were aged between 2 and 12 years at the time of the study, with a mean age of 8.44 years (mean $\pm$ SD; 8.4 ± 0.26 years). The average age of asthmatic children was 7.8 years (7.84 ± 0.43 years), and that of non-asthmatic children was 8.8 years (8.79 ± 0.32 years). The difference in age between the 2 groups was not statistically significant ($P = 0.09$). The demographic of participants was shown on Table 1. The study was approved by the institutional review board of Taipei Medical University and was conducted in accordance with the Declaration of Helsinki as revised in 1996. Inclusion criteria in our asthmatic group were based on the Global Initiative for Asthma (GINA) guidelines. The

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children were all acute asthma exacerbation and no steroid use within 1 week. In non-asthmatic group were children without asthma. Exclusion criteria were children with parasitic infections or hepatic disorders. All the children were compatible with criteria. After obtaining parental written informed consent, we recorded the child's age and sex, and collected a sample of 5 mL of peripheral blood in anti-coagulate tube. We analyzed total serum IgE, allergen-specific IgE levels (against *Dermatophagoides pteronyssinus*) using an ImmunoCAP250 analyzer (Phadia, Uppsala, Sweden) and TSLP expression by ELISA.

Asthma Symptom Score

Asthma Symptom Score was also recorded (Total points: 0 to 16) including night-time cough score (0 = absent, 1 = mild (present but not disturbing sleep), 2 = moderate (awake once due to cough), 3 = severe (awake more than once due to cough), 4 = extremely severe (insomnia in most night-time)); shortness of breath score early in the morning (0 = absent, 1 = mild (occasional and free of medications), 2 = moderate (occasional and medications needed), 3 = severe (frequent and medications needed), 4 = extremely severe (persistent and multiple doses of medications needed)); day-time wheeze or dyspnea score (0 = absent, 1 = mild (occasional wheeze), 2 = moderate (occasional wheeze and dyspnea but not disturbing), 3 = severe (persistent and hampering normal activities), 4 = extremely severe (totally unable)) and day-time cough score (0 = absent, 1 = mild (occasional but not disturbing), 2 = moderate (often but not disturbing), 3 = severe (frequent and disturbing), 4 = extremely severe

(persistent)) [26].

Subgroup classification

In order to classify the different phenotype of asthma, we divided the children into groups according to whether they were allergy or not. Allergy was defined as a total IgE above 250 KU/L with clinical signs. We divided the non-asthmatic and asthmatic group into 4 groups as follows: non-allergic and non-asthmatic (NN) (healthy children), allergic but non-asthmatic (AN), non-allergic but asthmatic (NA), and allergic asthmatic (AA). The classification of children into subgroups resulted in 36 children in the NN group, 17 children in the AN group, 10 children in the NA group, and 21 children in the AA group. The average age for NN was 8.6 years (8.56 ± 0.38 years), for AN it was 9.3 years (9.29 ± 0.62 years), for NA it was 7.4 years (7.4 ± 0.72 years), and for AA it was 8.1 years (8.05 ± 0.55 years). The differences in the mean ages among the groups were not significant ($P = 0.2$). The characteristics of the children are shown in Table 1.

Total IgE and Mite-specific IgE

We added 40 μ L of plasma to the Immuno-CAP (Phadia, Uppsala, Sweden) and incubated the mixture. The CAP tube was then washed with washing solution. Enzyme-labeled anti-IgE (conjugate against total IgE or specific conjugate against *Dermatophagoides pteronyssinus* IgE) was added to the CAP and was washed again. The bound total IgE and specific-IgE against *Dermatophagoides pteronyssinus* were quantified using a fluorescent substrate. The

CAP testing system is highly automated (ImmunoCAP 250, Phadia, Uppsala, Sweden), and the process required 1.5 h altogether.

Cytokine ELISA of TSLP

We checked cytokine levels of TSLP using sandwich ELISA method with commercial human cytokine kits (R&D, Minneapolis, MN, USA). To detect TSLP, we read plate at 450 nm and subtracted the values of 570 nm from those of 450 nm performed with SpectraMax M5 multidetection microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Molecular methods

PBMC isolation

Peripheral mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll-Hypaque (Pharmacia) density gradient centrifugation. In brief, plasma was first depleted by centrifugation ($400 \times g$ for 20 min), and cells were then added and mixed well with an equal volume of RPMI-1640 medium. Add Ficoll-Hypaque to the centrifuge tube bottom. The blood/RPMI-1640 mixture (the volume ratio was 2:1 (cells: Ficoll-Hypaque)) was slowly layered on Ficoll-Hypaque solution, then centrifuged for 30 min in a centrifuger (HERMLE, Germany) at $400 \times g$ at 20 °C. The mononuclear cell layer was transferred to a second centrifuge tube using a sterile pipet. Cells were washed by a 2-fold process of adding HBSS, and the cells were then collected for mRNA purification.

RNA extraction

RNA was extracted from the PBMC using a Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The RNA concentration was assessed using a spectrophotometer (Metertech, Taiwan), and then the amount of total RNA (μg) extracted was evaluated.

RNA product expression obtained from samples by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)

For each sample, 0.2 μg of RNA was reverse transcribed to complementary DNA (cDNA). The 0.2 μg of extracted RNA was mixed with RT reaction containing oligo (dT) primer and ddWater (nuclease-free), and was then incubated at 65 °C for 10 min. The following were added to the mixture: 5X reaction buffer, RiboLock RNase inhibitor (20 U/ μL), 10 mM dNTP mix, and RevertAid M-M μLV reverse transcriptase (200 U/ μL). (Fermentas is part of Thermo Fisher, Japan; RevertAid First Stand cDNA Synthesis Kit.) The mixture was incubated for 60 min at 42 °C. The reaction was terminated by heating to 70 °C for 5 min.

The cDNA was amplified by PCR. In brief, to each sample in ddWater the following were added: Taq polymerase (2 U/ μL) (Gene Maker, Taiwan), 10X Taq buffer (GenTaq), hTSLP-R primer (forward, 5'-GGA CCA ACC CTG ACT TTC CAC TAC A-3'; reverse, 5'-AAC ACT TCT CGG GCA TCC AAG-3'), hIL-7R α (forward, 5'-GCT ATG CTC AAA ATG GAG ACT TGG-3'; reverse, 5'-AGA CGA CAC TCA GGT CAA AAG GAG-3'),

GAPDH (forward, 5'- GAC ACC ATG GGG AAG GTG AAG GTC -3'; reverse, 5'- ACG TTT TTC TAG ACG GCA GGT CAG -3') , and 10 mM dNTP (Promega, Madison, USA). The final volume was 20 µL, and the mixture was gently agitated. For each sample, 40 cycles of PCR were performed in a DNA thermal cycler (BIO-RAD, Singapore) at the following temperatures: 94 °C for denaturing, 61 °C for annealing (30 s), and 72 °C (40 s) for polymerization. Following amplification, electrophoresis was performed (power supply: BIO RAD, Singapore; electrophoresis tank: MAJOR SCIENCE, Saratoga, California) on the DNA products on a 1.2% agarose gel for 30 min at 400 mA, 100 V (BIO RAD, Power Supplies). The bands were visualized by staining with ethidium bromide (AMRESCO, Canada). The gel was imaged using an AlphaImager EC Gel Documentation and Fluorescent Imaging System (Alpha Innotech, California, USA). IDV (integrated density value) was measured by the analysis tools with Alpha Imager software to confirm the mRNA levels (Alpha Innotech is now part of ProteinSimple, Santa Clara, CA).

Analysis of TSLPR and IL-7Rα gene expression by Real-Time Polymerase Chain Reaction (Q-PCR)

TSLPR and IL-7Rα gene expression were determined by reverse transcription, followed by Quantitative real-time polymerase chain reaction (Q-PCR) using appropriate primers. The primers were shown as below: TSLPR primer (forward, 5'- GAG CAG CGA GAC GAC ATT CTC -3'; reverse, 5'- ACA CGT CAC CGT CAC TGC AT -3'), IL-7Rα primer (forward,

5'- CAA CTT TTG GCA TGG TTT TTT CT -3'; reverse, 5'- CTG CGA TCC ATT CAC TTC CA -3'), GAPDH primer (forward, 5'- GCC AGA GAG CGC CAT CAG -3'; reverse, 5'- AGC TCG CTG CTG GGT GTT T -3'). Q-PCR amplification and detection were performed in MicroAmp optical 384-well reaction plates using the Applied Biosystems ViiA™ 7 Real-Time PCR System for 50 cycles (95 C for 15 s and 60 C for 1 min). Data were quantified using the comparative threshold cycle method with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference housekeeping gene.

Statistics

All data analyses were performed by STATA 8.2. Using the case-control method and a two-sample *t* test, we evaluated the levels of TSLPR, IL-7R α , IgE and TSLP among asthmatic children compared with non-asthmatic children. The levels of TSLPR, IL-7R α , IgE and TSLP across the subgroups were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's analysis. Linear regression was used to evaluate the correlations between TSLPR and IgE, between IL-7R α and IgE, between TSLPR and IL-7R α , between TSLPR and specific-IgE against *Dermatophagoides pteronyssinus* and between IL-7R α and specific-IgE against *Dermatophagoides*. Weak correlation were considered as $R < 0.4$. A *P* value of less than 0.05 was considered significant.

Results

Analysis of total serum IgE and serum TSLP levels

The mean IgE level in asthmatic children was higher than non-asthmatic children with a significant difference ($P < 0.05$) (Figure 1A). The IgE level for NN, AN, NA, and AA, one-way ANOVA showed that the difference between the subgroups was significant (F values=22.69, degrees of freedom= 83, $P < 0.0005$; Figure 1B). The mean TSLP level in asthmatic children was also higher than non-asthmatic children with a significant difference ($P < 0.05$) (Figure 1A). For the subgroups, the TSLP level for NN, AN, NA, and AA, One-way ANOVA also showed that the difference between the subgroups was significant (F values=2.84, degrees of freedom= 83, $P < 0.05$; Figure 1B).

RNA product expression obtained from samples

Total RNA was extracted from the PRMC, and RT-PCR was performed. The TSLPR and IL-7R α expression was detected for each sample using electrophoresis; the results between non-asthmatic group and asthmatic group are shown in Figure 2A. The results in the NN, AN, NA, and AA subgroups are shown in Figure 2B..

Analysis of TSLPR and IL-7R α mRNA levels

The TSPLR/GADPH in asthmatic children was higher than children with asthma ($P < 0.0005$; Figure 3A). The level of IL-7R α /GADPH in asthmatic children was higher than non-asthma ($P < 0.005$; Figure 3B). By subgroup with One-way ANOVA method, the level of

TSLPR/GADPH in the NN group, AN group, NA group and AA group showed that the difference was significant (F values=6.64, degrees of freedom= 83, $P < 0.0005$, Figure 3C). The level of IL-7R α /GADPH in the NN group, AN group, NA group and AA group also showed that the difference was significant by One-way ANOVA (F values=17.17, degrees of freedom= 83, $P < 0.0005$; Figure 3D). The Relative Quantitation (RQ) evaluated by Real-Time Polymerase Chain Reaction (Q-PCR) in asthmatic group and non-asthmatic group was shown in Figure 3. The data in NN, AN, NA and AA was also shown in Figure 3. Both TSLPR and IL-7 R α showed higher expression in asthmatic patients than non-asthmatic children. For the subgroups, the TSLPR level did not differ significantly between the NN and AN. We also found no significant difference in TSLPR level between the NA and AA subgroups. However, a significant difference in TSLPR level emerged for the following pairs of subgroups: NN versus AA, AN versus AA, NN versus NA, and AN versus NA ($P < 0.05$). The data analysis of IL-7R α showed that the AA subgroup exhibited a higher expression than the NN subgroup ($P < 0.05$). By contrast, the difference in the IL-7R α level between the NN and NA subgroups was not significant.

The clinical severity in asthmatic children

The Asthma Symptom Score in asthma group was 8.34 ± 0.52 , in NA was 8.3 ± 0.97 , in the AA was 8.43 ± 0.62 shown in Table 1. The statistical correlation between TSLPR and Asthma Symptom Score was 0.17 (R; $P = 0.35$). The R for the correlation between IL-7R α

and Asthma Symptom Score was 0.18 ($P=0.33$) shown in Table 2.

Strong correlation between TSLPR and IL-7R α and weak correlation between TSLPR and Total IgE and between IL-7R α and Total IgE

The statistical correlation between TSLPR and total IgE was 0.28 (R; $P<0.05$). The R for the correlation between IL-7R α and IgE was 0.22 ($P <0.05$). For TSLPR and IL-7R α , R was 0.77 with a highly significant P value (<0.0005) (Table 2).

Weak correlation between TSLPR and specific-IgE against *Dermatophagoides* and between IL-7R α and specific-IgE against *Dermatophagoides*

The statistical correlation between TSLPR and specific-IgE against *Dermatophagoides* was 0.37 (R; $P <0.005$). The R for the correlation between IL-7R α and specific-IgE against *Dermatophagoides* was 0.32 ($P <0.005$) (Table 2).

NO correlation between TSLPR and TSLP and between IL-7R α and TSLP

The statistical correlation between TSLPR and TSLP was 0.188 (R; $P = 0.11$). The R for the correlation between IL-7R α and TSLP was 0.1 ($P = 0.38$) (Table 2).

Discussion

Asthma is an airway inflammatory disease that is mediated by T-helper 2 (TH2) cells.

Recently, TSLP has been implicated as a key cytokine that initiates dendritic cell-mediated Th2 immune response [5]. In this study, our findings showed that asthma patients had higher

IgE, TSLP expression than non-asthmatic children. To minimize the influence of allergy, we divided the children into 4 groups according to IgE and clinical sign as previous described. The TSLP levels were significantly different between the subgroups NN (healthy children) and AA. We found no significant difference in TSLP level between the NN versus AN, AN versus AA, NN versus NA, AN versus NA and NA versus AA. That means only children with allergy and asthma had higher TSLP than children without allergy and asthma (healthy children); Non-allergic children with asthma had no difference in level of TSLP than healthy children. Liu et al [3] reported that TSLP may activate mast cells by directing the innate phase of allergic immune responses. This study was not evaluated using large number of population. Despite of the limitation, this study was able to state significant differences between asthma and non-asthmatic children.

For TSLPR and IL-7 $R\alpha$, the data analysis showed that asthmatic patients exhibited a higher expression than non-asthmatic children. For TSLPR in subgroup, both allergic and non-allergic asthmatic patients exhibited a higher expression of TSLPR than healthy children (non allergy and non asthma). The level of TSLPR is no different between allergic and non-allergic asthmatic patients. In other words, children with asthma had higher levels of TSLPR than healthy children whether they were allergic or not. For IL-7 $R\alpha$, only children with both allergy and asthma had higher levels of IL-7 $R\alpha$ than healthy children (non allergy and non asthma). Our analysis showed that TSLPR expression was strongly correlated with

IL-7R α . However, it was a little different expression of TSLPR and IL-7 R α among subgroup children, which means that the induction of TSLPR and IL-7 R α gene expression mechanism might be a little different.

A previous study reported that environmental factors such as airborne allergen exposure including house dust mite stimulate TSLP [27]. Dust mite lead to allergy by stimulating protease-dependent production of TSLP in airway epithelial cells [28]. In our finding, mite-sensitive allergy weakly influenced the level of TSLPR among children. Thus, mite-sensitive allergy appeared not only to be a factor influencing the level of TSLPR among children. One genetic study showed that single-nucleotide polymorphisms of gene IL-7R α chain (intron 2- rs969129 and exon 4- rs1494555) were associated with asthma [20]. The other study found that single-nucleotide polymorphisms of the TSLPR gene had a higher allele frequency of g.33G > C in asthma patients compared with non-asthmatic people [22]. The polymorphisms of TSLPR were not significantly associated with total IgE, FVC, or FEV1 in asthma patients [21]. The genetic risk factors seem to contribute an important role of TSLPR in the asthma onset. However, further studies are required to evaluate the possible interaction of mite-sensitive allergy between IL-7R α and TSLPR. In our analysis by clinical severity of asthma, the TSLPR and IL-7R α mRNA level did not correlated with asthma symptom score. In other words, TSLPR did not correlate with clinical severity of asthma.

TSLPR is an atypical Type I cytokine receptor that exhibits a remarkable 24 % match with

the common γ receptor chain (γ_c) [29]. The formation of a functional heteromeric TSLPR requires IL-7 R α without the γ_c chain [19]. TSLPR is more widely distributed; TSLPR mRNA has been detected on numerous peripheral blood mononuclear cell (PBMC) including DCs, T cells, B cells, mast cells, natural killer T (NKT) cells, and monocytes [30]. In the inflammatory process of asthma, the airway wall may infiltrate with lymphocytes and mast cells [31]; TSLP trigger DCs to attract chemokines, peripheral blood mononuclear cell (PBMC) also play a role in asthma [31]. We analyzed the TSLPR mRNA expression in PBMC. There are different types of cells including lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic cells. Further investigate the TSLPR complex (TSLPR and IL-7Ra) expression (mRNA and protein) by different cell types is needed. Although recent study reported that proinflammatory cytokines also played a role in TSLPR expression [32]. Cook et al [32] reported that TNF α and IL-3 increased TSLPR expression in eosinophils and enhanced degranulation. Reefer et al [12] reported that TSLPR were more detectable in TH2 cells in a proallergic milieu, and it seems to be induced by environmental factors such as bacterial superantigen. The induction of TSLPR expression by other stimulators still need more investigation. Furthermore, the regulation of functional TSLPR complex expression in allergic diseases will be more complicated.

In conclusion, this study showed that the TSLPR was overexpressed among children with allergic and non-allergic asthma than healthy children, and that TSLPR expression

correlated strongly with IL-7R α and weakly with mite-specific IgE. TSLPR was not different between allergic and non-allergic asthmatic patient. The present data suggest that anti-TSLPR therapeutics, should be further investigated as treatment alternatives in children with asthma.

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Conflict of interest

The authors do not have any conflict of interest or any financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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Figure legends

Figure 1. (A) Comparison between the IgE and TSLP levels of asthmatic children (n=31) and non-asthmatic children (n=53). (B) The total IgE and TSLP level among children in the non-allergic and non-asthmatic (NN) (healthy children), allergic but non-asthmatic (AN), non-allergic but asthmatic (NA), and allergic asthmatic (AA) subgroups. * indicates $p < 0.05$; ** indicates $p < 0.005$; *** indicates $p < 0.0005$.

Figure 2. The expression of TSLPR, IL-7R α , and GADPH was examined by electrophoresis. Results are expressed in (A) asthmatic children and non-asthmatic children. (B) non-allergic and non-asthmatic (NN) (healthy children), allergic but non-asthmatic (AN), non-allergic but asthmatic (NA), and allergic asthmatic (AA) subgroups.

Figure 3. (A) Percentage of TSLPR/GADPH and relative quantitation (RQ) by real time PCR of TSLPR level and between asthmatic and non-asthmatic children. (B) Percentage of IL-7R α /GADPH and relative quantitation (RQ) by real time PCR of IL-7R α between asthmatic and non-asthmatic children. (C) Percentage of TSLPR/GADPH and relative quantitation (RQ) by real time PCR of TSLPR among children in non-allergic and non-asthmatic (NN) (healthy children), allergic but non-asthmatic (AN), non-allergic but asthmatic (NA), and allergic asthmatic (AA) subgroups.

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asthmatic (NA), and allergic asthmatic (AA) subgroups (D) Percentage of IL-7R α /GADPH and relative quantitation (RQ) by real time PCR of IL-7R α among children in NN, AN, NA and AA subgroups.

Table1. Demographics of participants

Number of children (total)	84
Ratio of asthma: non-asthma	31:53
Ratio of NN:AN:NA:AA	36:17:10:21
Ratio of F: M (both groups)	45:39
Asthma (F:M)	14:17
Non-asthma (F: M)	31:22
NN: AN: NA: AA (F/M)	18/18:13/4:4/6:10/11
Mean age (total)	8.44±0.26
Age ratio of asthma: non-asthma	7.84±0.43 : 8.79±0.32
Age ratio of NN: AN: NA: AA	8.56±0.38:9.29±0.62:7.4±0.72:8.05±0.55
Mean asthma symptom score among asthmatic group	8.34±0.52
Asthma symptom score ratio of NA: AA	8.3±0.97 : 8.43±0.62
Percentage of mite sensitive children among asthmatic group (Positive Mite-specific IgE)	68%

NN: non-allergic and non-asthmatic children (healthy children); AN: allergic but non-asthmatic children ; NA: non-allergic but asthmatic children; AA: allergic asthmatic children ; F: Female; M: Male.

Table 2. The R for the correlation between groups

	IgE	ASS	TSLP	SID	IL-7R α	TSLPR
TSLPR	0.28 [*]	0.17	0.188	0.37 ^{**}	0.77 ^{***}	—
IL-7R α	0.22 [*]	0.18	0.1	0.32 ^{**}	—	0.77 ^{***}

ASS: Asthma Symptom Score, SID: Specific-IgE against *Dermatophagoide*

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$

Fig. 1

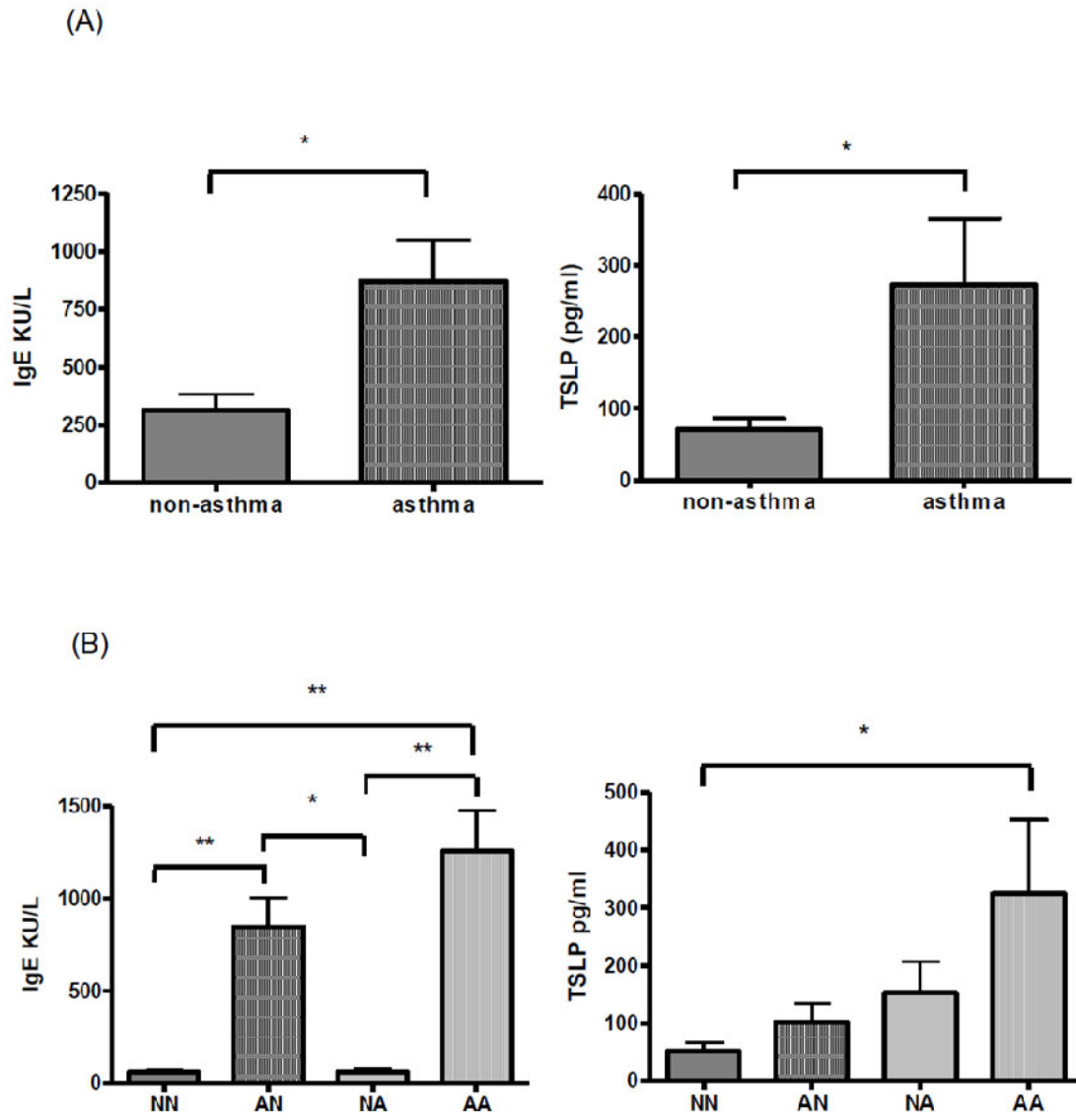
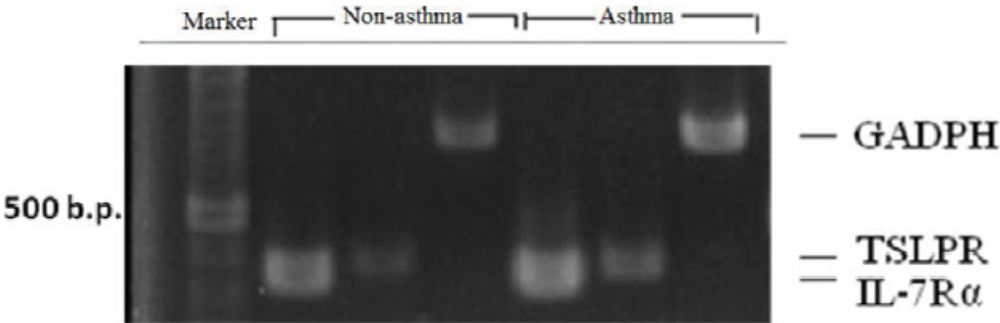


Figure 2.

(A)



(B)

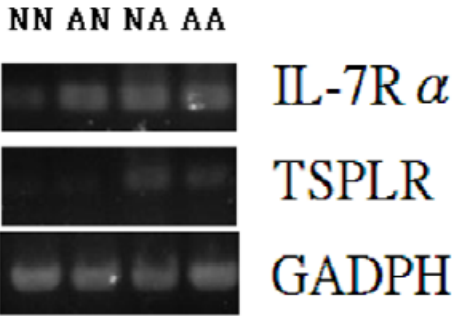


Fig.3

