

## BIOMARKERS

# Transient Receptor Potential Vanilloid 2 (TRPV2), a Potential Novel Biomarker in Childhood Asthma

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**Background.** The presence of transient receptor potential vanilloid 2 (TRPV2) in human peripheral blood cells may suggest a role under pathological conditions. The aim of this study was to explore the relationship between the expression profile of TRPV2 gene and childhood asthma in the north of China. The effects of allergens exposure on the expression of TRPV2 gene were also investigated. **Methods.** Sixty asthmatic children confirmed by physician diagnosis and 60 healthy children as a control group were recruited. Serum total IgE and specific IgE were measured. Using quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), TRPV2 was detected in total RNA extracted from peripheral blood lymphocytes. Student's *t*-test and chi-square test were used to analyze the relationship between TRPV2 transcript and different parameter variables on susceptibility of childhood asthma. Multiple logistic regression was used to analyze the associations between TRPV2 gene and allergens. **Results.** The expression level of TRPV2 gene was increased 2.6 times in asthmatic children compared with controls ( $p < .01$ ). The up-regulation of TRPV2 gene and sensitization to one of three the allergens—spring pollen, dust mite, and dog and cat hair—were correlated with childhood asthma. In addition, the hypersensitivity to spring pollen, cockroach, and dust mite and up-regulation of TRPV2 gene expression may be the risk factors for the childhood asthma in Beijing. **Conclusions.** The increased expression of TRPV2 gene in peripheral lymphocytes is closely correlated with childhood asthma in the north of China. This study provides a potential new biomarker of childhood asthma and lays the basis for further clarification of the pathogenesis underlying asthma.

**Keywords** biomarker, case-control cohort, childhood asthma, gene expression, transient receptor potential vanilloid 2 (TRPV2)

## INTRODUCTION

Asthma is the most common chronic disease of childhood and the leading cause of childhood morbidity. Its prevalence has increased significantly worldwide in recent decades (1). The pathophysiology of asthma is characterized by airway inflammation, aggravation of asthmatic symptoms, airway hyperresponsiveness, and airway remodeling (2, 3). A broad range of cell types such as lymphocytes, mast cells, macrophages, eosinophils, airway epithelial cells, and airway endothelial cells are involved in modulating airway inflammation, as well as abnormalities in intracellular  $\text{Ca}^{2+}$  homeostasis (3, 4). Asthma is triggered by numerous stimuli, for example, cold air, exercise, viral infection, allergens, and environmental irritants (5–7). However, the underlying molecular mechanisms are not fully understood.

Transient receptor potential vanilloid (TRPV) family consists of the six mammalian members TRPV1–TRPV6 (8). Among the six members, four (TRPV1–TRPV4) are known to be activated by various physicochemical stimuli including nociception, thermo, and osmosensing (9, 10),

whereas the other two (TRPV5 and TRPV6) are highly selective for  $\text{Ca}^{2+}$  and are less affected by physical stimuli (11, 12). Changes in ion channel expression might have a role in regulating plasma membrane cation inflow in airways smooth muscle (ASM) cells. It has been identified that TRPV1 channels are up-regulated in ASM of individuals with cough (13–16). Similar to TRPV1, TRPV2 is activated by capsaicin, heat, particulate matter, and various noxious chemicals and is up-regulated in ASM cells during capsaicin-induced asthma (17–19). Besides, TRPV2 transcript was detected highly expressed in human mast cell line (20). However, the physiological significance of TRPV2 remains to be addressed. Moreover, a few recent studies have revealed that TRPV2 is also the predominant transcripts expressed in rat deendothelialized intralobar pulmonary arteries (PAs) and aorta (21). Taken together, these evidences indicate that TRPV2 might play a role in asthma.

It has been reported that TRPV2 is expressed in human peripheral blood cells (22). While no data have ever concerned the expression styles of TRPV2 gene in healthy and asthmatic children, it is still unknown whether changes in TRPV2 expression could be associated with the childhood asthma. Lymphocytes are easier to be obtained and handled than bronchial epithelial cells, which are recognized as the sources of the cytokines related to allergic responses. Therefore, comparison of TRPV2 gene

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expression level in peripheral blood lymphocytes between asthmatic children and healthy controls may provide clues regarding the disease pathway.

In this study, we determined the expression level of TRPV2 gene in peripheral blood lymphocytes from healthy and asthmatic children. In addition, the relation between the expression level of TRPV2 mRNA and serum-specific IgE was investigated.

## METHODS

### *Subjects*

This case-control study consisted of 60 asthmatic and 60 healthy children. The asthmatic children were recruited from asthma clinic in Children's Hospital and Capital Institute of Pediatrics, Beijing, China. The children were diagnosed as asthmatics by pediatricians according to the standardized diagnostic criteria established by National Executive Committee of Asthma Management and Prevention in China. Briefly, for children younger than 3 years old with a history of recurrent wheeze (more than 3 times), and a continuous high-pitched sound emitting from the chest during expiration, and excluding wheeze caused by other diseases, a diagnosis of asthma can be made possibly. For children aged 3 years or older with a history of recurrent wheeze, and a continuous high-pitched sound emitting from the chest during expiration, and a trial of treatment with short-acting bronchodilators and inhaled glucocorticosteroids, and excluding wheeze caused by other diseases, the diagnosis of asthma can be made. The asthmatic group comprised 36 males and 24 females aged from 2 to 12 years ( $5.81 \pm 2.85$  years). The control group comprised 60 healthy children (30 males and 30 females) aged from 3 to 12 years ( $8.35 \pm 2.48$  years) who had no present or past medical history suggestive of asthma or other allergic diseases, no family history of asthma, and were free from pulmonary or any chronic diseases based on the parents-completed International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Chinese version). An informed consent was obtained from the parents or caregivers of each subject prior to enrollment in the study. This study was approved by the Committee of Ethics at the Beijing Institute of Basic Medical Sciences.

### *Separation of Lymphocytes from Peripheral Blood*

According to the blood volume to be separated, 15 ml size centrifuge tubes are filled with 7 ml lymphocyte separation medium. Peripheral blood (3 ml) was collected in sterile tubes from each of the 60 healthy donors and the 60 asthmatic children. Heparinized whole blood is then carefully poured over the lymphocyte separation solution (Biomed Biotechnology Co., China). The separation process is performed by centrifugation at  $1200 \times g$  for 20 min.

The lymphocytes (70–100% enrichment) concentrate in the interphase (white layer) between the plasma and the separation solution. They are subsequently extracted with

a sterile Pasteur pipette and washed twice in the culture medium.

### *RNA Isolation*

RNA was immediately isolated from lymphocytes using the RNA Extraction Kit (Biomed Biotechnology Co., Beijing, China) according to the manufacturer's instructions. The concentration and purity of all RNA samples, eluted in a final volume of 20  $\mu$ l RNase-Free water, were determined by DU® 640 Spectrophotometer (BECKMAN, Maryland, USA). Based on the absorbance ratio at 260/280 nm and at 230/260 nm, all the samples were pure and free from protein and organic pollutants derived from the RNA-isolation procedure. The overall sample integrity was confirmed by denaturing formaldehyde agarose gel (1.2%) electrophoresis with ethidium-bromide staining, showing sharp and intense 18S and 28S ribosomal RNA bands without smears. Samples were stored at  $-80^{\circ}\text{C}$  until use.

### *Reverse Transcription*

RNA (3  $\mu$ g) was subjected to reverse transcription in a 20- $\mu$ l reaction mixture containing 10 $\times$  RT buffer, dNTP-Mix containing 5 mM each dNTP, oligo (dT) primer, RNase Inhibitor, and AMV Reverse Transcriptase (Promega, Wisconsin, USA). The reaction mixture was incubated at  $42^{\circ}\text{C}$  for 1 h followed by 5 min inactivation of enzyme at  $85^{\circ}\text{C}$ . The resulted cDNA was used as template for PCR amplification and the products were kept at  $-20^{\circ}\text{C}$  until use.

### *Quantitative Real-Time RT-PCR*

qRT-PCR assay of TRPV2 mRNA transcript was performed with the SYBR Green Master Mix (Fermantas, Ontario, Canada). Human TRPV2 primers were designed according to the sequence submitted to GenBank database under Accession no. NM 016113, forward primer 5'-CTTCCTTTTCGGCTTCGCTGTAG-3', and reverse primer 5'-GCACTGACTCTGTGGCATTGG-3'; amplicon length is 95 bp. Human Glyceraldehyde phosphate dehydrogenase (GAPDH) primers were designed according to the sequence submitted to GenBank database under Accession no. NM 002046, forward primer 5'-TGCACCACCAACTGCTTAGC-3', and reverse primer 5'-GGCATGGACTGTGGTCATGAG-3'; amplicon length is 87 bp. The endogenous control GAPDH was used to normalize the sample with the  $\Delta\Delta\text{CT}$  method for relative quantification by Mx3000P software (Santa clara, Agilent).

### *Serum Total IgE and Specific IgE Assay*

The serum was separated by centrifugation at 1500 rpm for 15 min after 2 h of incubation at  $25^{\circ}\text{C}$ , and then collected and stored at  $-20^{\circ}\text{C}$  until being assayed. Serum total IgE and specific IgE against dust mite, spring and autumn pollen, mold, hair of dog or cat, and cockroach were measured by Mediwiss sensitive screening and detection system for allergens (Mediwiss Medical Diagnostics, Moers, German). Serum sample (250  $\mu$ l) and nitrocellulose membrane that can absorb specific allergen were together incubated at room temperature for 45 min in reaction groove. After being washed with washing buffer, the

membrane was incubated with corresponding antibody at room temperature for 45 min. Then the membrane was incubated for 20 min with alkaline phosphatase-conjugated streptavidin at room temperature after thoroughly washing. Finally, the membrane was developed by the substrate. Grade and concentration (IU/ml) of each allergen was calculated by AllergyScreen quantitative analysis (Mediawiss Medical Diagnostics).

### Statistical Methods

The results were statistically analyzed via a standard computer program (SAS 9.1, Carolina, SAS Institute Inc). The data were presented as ratio or mean  $\pm$  standard deviation (s.d.). Student's *t*-test was used for the comparison of parametric data, and chi-square test or Fischer's exact test was used for the comparison of non-parametric data. The associations between TRPV2 gene and allergens were analyzed using multivariate logistic regression. Stepwise analysis was performed to test the interactive actions or confounding factors, and slentry 0.2 and slstay 0.2 were set as selection significant level. For all tests, *p*-values less than .05 were considered significant.

## RESULTS

### TRPV2 Gene Expression in Healthy and Asthmatic Children

The TRPV2 gene was shown to be expressed in leukocyte samples from the 60 healthy and 60 asthmatic children, determined by qRT-PCR with human TRPV2 primer pairs. To evaluate possible influence of sex and age, TRPV2 gene expression of the males ( $n = 30$ ) and females ( $n = 30$ ) aged 3–12 years of the healthy subjects was compared. The result showed that sex or age had no significant effect on the expression of TRPV2. ( $p = .106$  and  $p = .819$ , respectively). The expression level of TRPV2

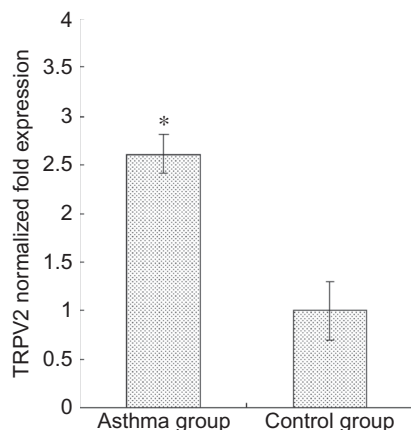


FIGURE 1.—Relative expression of the TRPV2 gene in human leukocytes from asthmatic children and controls ( $n = 120$ ). Relative expression measurements of the TRPV2 gene using qRT-PCR calculated with Mx3000P software (relative expression software tool). Fold change (y-axis) represents the relative expression of the TRPV2 in asthmatic children in comparison with control (less-expressed TRPV2 gene and was therefore considered equal to 1), normalized by GAPDH. \*means  $p < 0.01$ .

gene was elevated 2.6 times in the asthmatic children compared with the controls. The differences were statistically significant ( $t = 3.187$ ,  $p < 0.01$ ) (Figure 1).

### Analysis of Correlation between TRPV2 Gene Expression and Total Serum IgE

Correlation between TRPV2 gene expression and total serum IgE was analyzed. Total serum IgE level was considered as normal when lower than 100 IU/ml. As shown in Table 1, a significant difference of total IgE level existed between the case and the control group. However, the relative expression fold changes of TRPV2 gene was not significantly correlated with the IgE level within the asthmatic children. qRT-PCR analysis showed that the expression level of TRPV2 gene was comparable between asthmatic children with high levels of serum IgE and those with normal IgE (Table 2). The percentage of elevated expression of TRPV2 gene was significantly higher than that of serum total IgE in asthmatic children (Table 3).

TABLE 1.—General characteristics of subjects.

|                       | Mean $\pm$ SD   |                   | <i>P</i> -value     |
|-----------------------|-----------------|-------------------|---------------------|
|                       | Asthma          | Control           |                     |
| Age (year)            | 5.81 $\pm$ 2.85 | 8.35 $\pm$ 2.48   | .08*                |
| No. of males/females  | 36/24           | 30/30             | .106 <sup>†</sup>   |
| Total IgE (IU/ml)     | 227.9 $\pm$ 122 | 53.72 $\pm$ 16.24 | <.0001 <sup>‡</sup> |
| No. of normal IgE (%) | 14 (23.3)       | 60 (100)          | <.0001 <sup>‡</sup> |

\**p*-values were calculated by comparing age between asthma and control groups, <sup>†</sup>*p*-values were calculated by comparing sex difference between asthma and control groups, <sup>‡</sup>*p*-values were calculated by comparing total IgE or normal IgE between asthma and control groups.

TABLE 2.—Variation of TRPV2 gene in asthmatic children according to serum total IgE.

|                                          | Asthma            |                    | <i>P</i> -value     |
|------------------------------------------|-------------------|--------------------|---------------------|
|                                          | Normal IgE        | High IgE           |                     |
| TRPV2 gene ( $\Delta\Delta\text{Ct}^a$ ) | 1.43 $\pm$ 0.64   | 1.62 $\pm$ 0.78    | .478*               |
| Total IgE (IU/ml)                        | 58.62 $\pm$ 19.23 | 285.53 $\pm$ 80.06 | <.0001 <sup>†</sup> |

<sup>a</sup> $\Delta\Delta\text{Ct}$  was determined by calculating normalized  $\Delta\text{Ct}$  of TRPV2 from the PBMCs of the asthma group minus normalized  $\Delta\text{Ct}$  of TRPV2 from the PBMCs of the control group.

\**p*-values were calculated by comparing the expression of TRPV2 gene between normal and high IgE in the asthma group, <sup>†</sup>*p*-values were calculated by comparing total IgE between normal and high IgE in the asthma group.

TABLE 3.—Comparison of changes of TRPV2 gene expression and serum total IgE in asthmatic children.

|                | Asthma group     |                | <i>P</i> -value |
|----------------|------------------|----------------|-----------------|
|                | Number of normal | Number of high |                 |
| TRPV2 gene (%) | 0 (0%)           | 60 (100%)      | <.01*           |
| Total IgE (%)  | 14 (23.3%)       | 46 (76.7%)     |                 |

\**p*-values were calculated by chi-square analysis comparing the expression of TRPV2 gene and the level of total IgE between normal and high IgE in asthma group.

### Analysis of Correlation Between TRPV2 Gene Expression and Specific Serum IgE

Children from asthmatic and healthy group were subjected to the detection of total and specific serum IgE levels. A total of 50 cases out of 60 asthmatic children displayed positive allergic reaction (83.3%), among which the positive rate of mold (44%) is the highest, and then sequentially is dog and cat hair (42%), dust mite (36%), and autumn pollen (14%). The association between TRPV2 gene expression level in asthmatic group and each allergen was analyzed by logistic regression. The results showed that the expression of TRPV2 gene and sensitization to spring pollen, dust mite, and dog and cat hair were the correlation influence factors for asthma and the odds ratio (OR) of interaction between the TRPV2 gene expression and sensitization to spring pollen, dust mite, hair of dog and cat were 11.11 (2.444–50), 5.236 (1.114–27.16), and 3.497 (1.18–10.309), respectively (Table 4). Furthermore, multiple allergens as covariant were analyzed by multivariate logistic regression ( $\alpha = 0.2$  was taken when entering the levels;  $\alpha = 0.2$  was removed when rejecting the levels). Before adjusting the allergen, the expression of TRPV2 gene in asthmatic children was 1000 times as high as that of the control children and OR = 1000 (62.5–1000). After adjusting the allergen, the expression of TRPV2 gene was 1000 times higher than the control children, OR > 1000 (111–>1000). The results indicated that the spring pollen, cockroach, dust mite, and TRPV2 gene expression were correlated with childhood asthma by multiplying interaction. According to the standardized biased regression coefficient, the contribution rate of TRPV2 gene expression and allergen on childhood asthma in the order from large to small is as follows:

TRPV2, OR > 1000 (111–>1000); spring pollen, OR = 19.23 (2.88–125); dust mite, OR = 3.521 (0.585–21.27); and cockroach, OR = 0.059 (0.0026–1.366) (Table 5). Other multiple interactions between allergens and TRPV2 gene expression were not significant correlations with childhood asthma.

### DISCUSSION

In this study, we demonstrated that the expression level of TRPV2 gene was significantly increased in asthmatic children. In addition, the association of expression change of TRPV2 gene with allergens was also investigated. Our results indicate, for the first time in this study, that the expression of TRPV2 gene in peripheral blood lymphocytes might be associated with childhood asthma and may play important roles in the pathogenesis of childhood asthma.

Asthma is a heterogeneous disease characterized by intermittent inflammation of the airways, which may lead to irreversible airway remodeling and intractable airflow limitation. The pattern of IgE response (over time or to specific allergens) may reflect different atopic vulnerabilities, which are related to the presence of asthma in a fundamentally different way from current definition of atopy (23). This study showed that there was a statistically significant difference of total serum IgE level between the asthmatic and normal children group ( $p < .01$ ), and the IgE concentration of 46 cases in the asthmatic children group tremendously exceeded that of normal children, indicating the children of asthmatic group in this study mostly might suffer from atopic asthma. As IgE plays an important role in the pathogenesis of bronchial asthma, we firstly sought to determine whether a relationship between the expression of TRPV2 gene and serum total IgE levels exists in asthmatic children, but we could not prove such a link.

Although the expression change of TRPV2 gene was not significantly correlated with the differences of IgE level within the asthmatic children, the percentage of elevated TRPV2 gene expression was significantly higher than that of serum total IgE in asthmatic children. Approximately one half of patients with allergic disease have a total serum IgE level in the normal range (24–26); therefore, this parameter is not always informative of asthma activity, although closely related to the pathogenesis. Our results showed that asthmatic children with normal IgE level exhibited up-regulation of TRPV2 gene expression, suggesting that the expression change of TRPV2 gene might be more sensitive than that of total IgE and can potentially be used as a biomarker in diagnosing and determining the risk for asthma.

Allergen exposure is one of the environmental factors seemingly associated with the development of asthma. Therefore, we explored the relationship of TRPV2 gene expression with the childhood asthma-related allergens. As a result, the positive rate of allergens in the asthmatic children group was statistically significantly higher than that in the healthy control group ( $p < .05$ ), indicating that the increase of TRPV2 gene expression is closely

TABLE 4.—Correlation of TRPV2 gene expression and each allergens with childhood asthma.

| Variable            | OR    | 95% CI         | P      |
|---------------------|-------|----------------|--------|
| TRPV2               | 1000  | 62.5 – 1000    | <0.001 |
| Autumn pollen       | 2.671 | 0.385 – 18.18  | .332   |
| Cockroach           | 0.368 | 0.049 – 2.724  | .327   |
| Dust mite           | 5.236 | 1.114 – 27.16  | .036*  |
| Hair of dog and cat | 3.497 | 1.18 – 10.309  | .024*  |
| Mold                | 1.508 | 0.319 – 7.1428 | .604   |
| Spring pollen       | 11.11 | 2.444 – 50     | .0018* |

Notes: OR, odds ratio; CI, confidence interval.

\* $p < 0.05$ .

TABLE 5.—Association of TRPV2 gene expression with multiple allergens in asthma group.

| Variable      | STB     | OR    | 95% CI       | P     |
|---------------|---------|-------|--------------|-------|
| TRPV2         | –1.2065 | >1000 | 111–>1000    | <.001 |
| Spring pollen | –0.753  | 19.23 | 2.88–125     | .023  |
| Dust mite     | –0.515  | 3.521 | 0.585–21.27  | .169  |
| Cockroach     | 0.478   | 0.059 | 0.0026–1.366 | .077  |

Notes: STB, standardized estimate; OR, odds ratio; CI, confidence interval.

correlated with allergens. Though each specific IgE detected in the asthmatic groups was not correlated with the expression of TRPV2 gene, this research showed that the expression level of TRPV2 gene and sensitization to one of the three allergens—spring pollen, dust mite, and dog and cat hair—were correlated with childhood asthma. Furthermore, the multiple allergen-specific IgE as a covariant was analyzed, and the result showed that anaphylaxis to spring pollen, cockroach, and dust mite, as well as the up-regulation of TRPV2 gene expression, are the risk factors of childhood asthma. On the basis of standardized biased regression coefficient, it is evident that the order of contribution rate for the susceptibility to the childhood asthma from large to small is as follows: TRPV2 gene expression, spring pollen, dust mite, and cockroach.

Etiology and mechanisms of asthma are very complicated and the exact pathogenesis of asthma is still unclear. Lymphocyte plays an important role in bronchial asthma because of its involvement in immune, inflammation process, and distribution of circulating signals (cytokines and hormones). This research found that TRPV2 gene was expressed in peripheral blood lymphocytes of healthy and asthmatic children, and the expression was up-regulated in the latter, suggesting that TRPV2 gene not only plays a role at the normal physiological status, but might also be involved in the inflammatory reaction.

Additionally, there have some reports indicating that TRPV1 of TRPV family is closely related with the pathogenesis of asthma (27–30). The TRPV1 on the epithelial cells of human respiratory tract can be activated by negatively charged particulate matter (PM) of 2–10 µm diameter, resulting in apoptosis of the epithelial cells on airway (31). Considering the structural and function similarity to TRPV1 (32), further studies are required to explore whether TRPV2 acts with a similar mechanism as TRPV1 does.

In summary, our research demonstrated that the expression of TRPV2 gene was elevated in children suffering from asthma. In addition to the upregulated expression of the TRPV2 gene, spring pollen, cockroach and dust mite sensitization were found to be risk factors for childhood asthma. We conclude that TRPV2 might be a novel biomarker in diagnosis and determining the risk for childhood asthma. In addition, our research laid the basis for further exploration of the pathogenesis of TRPV2 in childhood asthma.

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#### DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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