Association between peripheral blood mononuclear cell ORMDL3 expression and the asthma predictive index in preschool children

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Abstract

Objective: This study aimed to assess whether orosomucoid 1-like 3 (ORMDL3) expression and environmental and clinical factors are associated with wheezing episodes in preschool children.

Methods: Children diagnosed with wheezing episodes were classified according to their asthma predictive index (API) in the past year as follows: API+ (≥4 wheezing episodes), API− (1–3 wheezing episodes), and API0 (without wheezing). ORMDL3 expression was assessed by real-time polymerase chain reaction in peripheral blood mononuclear cells (PBMCs). Receiver operating characteristic curve analysis of ORMDL3 expression and the API was performed for diagnosing wheezing episodes. Correlations between ORMDL3 expression and asthma risk factors were examined using Spearman's correlation.

Results: PBMC ORMDL3 expression was higher in the API+ group compared with the API− and API0 groups. The area under the curve for ORMDL3 expression was 0.820 (95% confidence interval, 0.771–0.869). ORMDL3 expression was positively correlated with the API (r = 0.447), infantile eczema (r = 0.499), wheezing (r = 0.516), total immunoglobulin E (r = 0.208), and environmental factors, including Dermatophagoides pteronyssinus (r = 0.357), house dust mites (r = 0.112), dog fur (r = 0.226), and Aspergillus (r = 0.257). ORMDL3 expression was negatively correlated with amaranth (r = −0.122).

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Conclusions: ORMDL3 expression in PBMCs is positively associated with the API and some asthma-related clinical and environmental risk factors in preschoolers.

Keywords
ORMDL3, asthma, wheezing, child, biomarker, asthma predictive index, peripheral blood mononuclear cells

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Introduction
Asthma is the most common chronic disease in childhood. In Western countries, the prevalence of asthma is estimated at up to 10% in children aged ≤5 years. Although approximately 50% of children will experience wheezing, shortness of breath, and other asthma-like symptoms at least once before the age of 3 years, only approximately 30% of children will have recurrent symptoms by school age. The occurrence of wheezing episodes in children is related to multiple allergens, such as dust mites, pets, cockroaches, mice, mold, tobacco smoke, endotoxins, and air pollution. Additionally, a family history of asthma, age, family smoking habits, and total immunoglobulin E (IgE) levels are significantly associated with wheezing episodes in children. Unfortunately, for many reasons, detection of pulmonary function in infants is not reliable. Currently, there is no exact diagnostic method for wheezing episodes. Therefore, new factors for reliable wheezing episodes and prediction of asthma during infancy are required.

One important diagnostic tool for asthma in children is the asthma predictive index (API), but its clinical value remains controversial. In 2010, Castro-Rodriguez first proposed the API as a simple and convenient clinical indicator of asthma in infants and preschool children. The API is increasingly used in clinical practice and is approved by various international guidelines. However, the accuracy of the API for prediction is affected by genetic polymorphism, environmental and socioeconomic factors, sex, race, and family health beliefs. Moreover, a follow-up study of 1954 children with asthma (aged 7–10 years) showed that the ability of API to predict asthma was relatively weak and that it requires improvement. To be reliable, such improvements require a better understanding of the underlying pathophysiology.

The orosomucoid 1-like 3 (ORMDL3) gene, known as ORMDL sphingolipid biosynthesis regulator 3, was found to be a candidate gene for asthma in a genome-wide association study. Expression is stimulated by allergens and cytokines, and mainly occurs in airway epithelial cells. ORMDL3 expression is positively associated with recurrent wheezing in children. However, the diagnostic value of ORMDL3 in children requires further investigation.

Wheezing in children aged ≤5 years is not diagnostic of asthma. Therefore, a reliable test for determining the risk of developing asthma in this population is necessary. This study aimed to assess whether ORMDL3 expression and environmental and clinical factors are associated with
asthma in preschool wheezing children. We detected ORMDL3 expression levels in children (<5 years of age) with wheezing episodes who were grouped according to their API results. We also analyzed the predictive value of ORMDL3 expression on the API, and the correlations between ORMDL3 expression and sex, age, family history, environmental factors, dietary factors, and other risk factors associated with wheezing episodes. Our results could help determine whether ORMDL3 is a reliable clinical biomarker for early prediction of asthma in children.

Materials and methods

Patient information

This was a retrospective study of consecutive children aged <5 years who visited the Respiratory Health outpatient clinic and inpatient ward of Renji Hospital (Shanghai, China) between April 2013 and August 2014. The inclusion criteria were (1) wheezing symptoms and lung sounds of expiratory wheezing, and (2) age <5 years. The exclusion criteria were as follows: (1) other causes of breathing problems, such as foreign bodies in the bronchi, bronchopulmonary dysplasia, gastroesophageal reflux, trachea ring, and congenital heart disease; or (2) other diseases affecting ORMDL3 expression, such as infectious diseases, autoimmune diseases, hematological diseases, and cancer. The research design was approved by Renji Hospital ethics committee. Written informed consent was obtained for all children from their parents or guardians. Blood samples were collected from enrolled patients. Clinical and demographic data were collected from the medical charts, which contained a routine questionnaire that covered information, such as demographics, medical history, risk factors for asthma, and life habits. The child’s legal guardians had to fill out this questionnaire at the first visit to our center. Information, including the API, sex, age, family history, presence of infantile eczema, asthma duration, and rhinitis, was recorded.

API-based classification

We classified the patients based on their API according to classification criteria described in a previous study.17 The positive API (API+) group of patients had ≥four wheezing episodes in the past year and one of the following major risk factors or two of the minor risk factors. The major risk factors were (1) a parental history of asthma, (2) doctor-diagnosed eczema or atopic dermatitis, and (3) sensitization to inhaled allergens. The minor risk factors were (1) food allergen-induced sensitization (including milk, peanuts, and eggs), (2) non-cold wheezing; and (3) ≥4% peripheral blood eosinophils. The negative API (API−) group of patients had one to three wheezing episodes within the past year and no major risk factor or up to two of the minor risk factors. Children without wheezing were defined as the control group (API0). All of the children were routinely tested for IgE using ImmunoCAP analysis (Thermo Fisher Scientific, Waltham, MA, USA). Except for some exceptions, the prick test was not performed. Because the prick test is a commercial test, no other dust was detected and no Aspergillus was cultured.

Isolation of individual peripheral blood cells

Collection and detection of cells were performed on the day after blood collection. A total of 5 mL of venous blood (anti-coagulated with 1.5–2 mg/mL EDTA) was diluted (1:1) with phosphate-buffered saline. An equal volume of diluted blood was slowly added to lymphocyte separation liquid (Ficoll) in a centrifugal tube. Careful
attention was paid to maintain a clear interface. The solution was centrifuged (room temperature, 2500 rpm, 20 min), and the mononuclear cell layer (lymphocytes and monocytes) was gently extracted by capillary suction, added to tubes containing 5 mM of phosphate-buffered saline, and mixed fully and evenly. The number of live cells was counted (to ensure that it exceeded 95%). Finally, the cells were centrifuged again (1500 rpm for 10 minutes) and the supernatant was removed.

**Real-time quantitative polymerase chain reaction**

Total RNA from peripheral blood mononuclear cells (PBMCs) that were isolated from peripheral blood was extracted by the TRIzol method (TRIzol reagent, #15596-026; Life Technologies, Gaithersburg, MD, USA). Next, cDNA samples were obtained by reverse transcription using the RevertAid Fist Strand cDNA Synthesis Kit (#K1622; Thermo Fisher Scientific). ORMDL3 expression was detected by real-time polymerase chain reaction (PCR) and quantitative PCR (qPCR) amplification (Maxima SYBR Green qPCR Master Mix, #K0252, Thermo Fisher Scientific). β-actin was used as a reference gene. The primer sequences for ORMDL3 and β-actin are shown in Table 1.

**Statistical analysis**

SPSS Statistics for Windows, Version 19.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous data that fit a normal distribution are expressed as mean and standard deviation (SD) and were analyzed using one-way analysis of variance and the least significant difference post hoc test. Skewed continuous data are presented as median (range) and were analyzed using the Kruskal–Wallis test and compared pairwise using the S-N-K test. Categorical data are expressed as frequencies and were analyzed using the chi-squared test. To determine the accuracy of ORMDL3 gene expression as an API+ marker, receiver operating characteristic (ROC) curve analysis was performed. Correlation analysis of ORMDL3 gene expression with the personal history of patients, family history, and environmental and dietary factors was conducted using Spearman’s correlation analysis. P < 0.05 was considered statistically significant.

**Results**

**Patients’ baseline characteristics**

A total of 144 consecutive children aged <5 years from the Renji Hospital inpatient ward and outpatient clinic were included. There were 46 patients in the API+ group (30 months old), 47 in the API– group (months old), and 51 in the API0 group (28 months old). There were no significant differences in sex distribution and age among the three groups. However, the median API score was significantly higher in the API+ group than in the other two groups (P < 0.001), as expected (Table 2).

**Individual and family history of patients in the three API groups**

Because wheezing episodes in children are a multifactorial condition, we conducted comparative analysis of the personal allergy history and family allergy history of the

| Gene      | Primers                                      |
|-----------|----------------------------------------------|
| β-actin-F | 5′-ATGATGATATCGCCGCGCTC-3′                   |
| β-actin-R | 5′-CCACCATCACGCCCTGG-3′                     |
| ORMDL3-F  | 5′-CAGCCCGGGTTTGTTACAG-3′                   |
| ORMDL3-R  | 5′-CCCTCTCTGTGCTGTGGTGTT-3′                 |

F: forward; R: reverse.

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Table 1. Primers used in this study.
disease according to the API. We found significant differences in the individual history among the three API groups. The occurrence rate of wheezing, eczema, and rhinitis was significantly higher in the API+ groups than in the other two groups (all $P < 0.01$) (Table 3). However, a family allergy history was not significantly different among the three groups.

**Table 2.** Baseline data of the three API groups.

|       | API+ | API- | API0 | P value |
|-------|------|------|------|---------|
| n     | 46   | 47   | 51   |         |
| Sex ratio (M/F) | 2.07 (31/15) | 1.35 (27/20) | 0.96 (25/26) | 0.19 |
| Age (months), median (min, max) | 30 (19,49) | 25 (7,52) | 28 (8,54) | 0.834 |
| API, median (min, max) | 5 (4,10.5) | 1 (1,3) | 0 (0,0) | $<0.001$ |

API: asthma predictive index; API+: asthma predictive index ($\geq$4 wheezing episodes); API−: asthma predictive index (1–3 wheezing episodes); API0: asthma predictive index (no wheezing); M: male; F: female; min: minimum; max: maximum.

**Table 3.** Personal case history and family history of the different API groups.

|       | Total | API+ | API− | API0 | P value |
|-------|-------|------|------|------|---------|
| Individual history | | | | | |
| Wheezing | 93 (64.58%) | 46 (100%) | 47 (100%) | 0 | $<0.001$ |
| Eczema | 52 (36.11%) | 33 (71.74%) | 0 | 19 (37.25%) | $<0.001$ |
| Rhinitis | 21 (14.58%) | 13 (28.26%) | 5 (10.64%) | 3 (5.88%) | 0.007 |
| Family history | | | | | |
| Rhinitis | 15 (10.42%) | 6 (13.04%) | 5 (10.64%) | 4 (7.84%) | 0.708 |
| Wheezing | 9 (6.25%) | 8 (17.39%) | 1 (2.13%) | 0 | 0.001 |
| Dermatitis | 9 (6.25%) | 2 (4.35%) | 4 (8.51%) | 3 (5.88%) | 0.707 |

API: asthma predictive index; API+: asthma predictive index ($\geq$4 wheezing episodes); API−: asthma predictive index (1–3 wheezing episodes); API0: asthma predictive index (no wheezing).

ORMDL3 expression was observed between the API− and API0 groups (Figure 1).

**ROC curve analysis**

ROC analysis was performed to evaluate the diagnostic value and appropriate cutoff point of ORMDL3 expression for API+. Figure 2 shows that the area under the curve (AUC) for ORMDL3 expression was 0.820 (95% confidence interval, 0.771–0.869). These results strongly suggest that PBMC ORMDL3 expression can improve the sensitivity and specificity of diagnostic tests for API+.

**Correlation analysis between ORMDL3 expression and asthma-related variables in children**

To analyze the associations of ORMDL3 expression with the API and wheezing
episodes, we evaluated the correlations between ORMDL3 expression, the API, and other asthma-related variables. There were positive correlations between ORMDL3 expression and the API (r = 0.447, P < 0.001), the individual history of patients with infantile eczema (r = 0.499, P < 0.001), wheezing (r = 0.516, P < 0.001), and total IgE (r = 0.208, P = 0.002). ORMDL3 expression was also correlated with environmental factors, including exposure to Dermatophagoides pteronyssinus (r = 0.357, P < 0.001), house dust mites (r = 0.112, P = 0.039), dog fur (r = 0.226, P < 0.001), and Aspergillus (r = 0.257, P < 0.001). Moreover, ORMDL3 expression levels were negatively correlated with amaranth consumption (r = −0.122, P = 0.024). There were no correlations between ORMDL3 expression and a family history, age, exposure to cat fur and trees, and streptavidin, as well as dietary factors (milk, egg white, beef, shrimp, crab, cashews, and mango) (Table 4).

Figure 1. ORMDL3 expression was increased in PBMCs of children in the API+ groups (API = 4, API = 5, and API ≥ 6) compared with the API− or API0 group. The mRNA levels of ORMDL3 were analyzed by real-time polymerase chain reaction. The numbers of patients for the API0, API−, API = 4, API = 5, and API ≥ 6 groups were 28, 47, 12, 14, and five, respectively. There was no overlap among the groups. ***P < 0.001. PBMCs: peripheral blood mononuclear cells; API: asthma predictive index; API+: asthma predictive index (≥4 wheezing episodes); API−: asthma predictive index (1–3 wheezing episodes); API0: asthma predictive index (no wheezing).

Figure 2. Peripheral blood mononuclear cell ORMEL3 expression is a potential biomarker of API+. ROC curve analysis shows the diagnostic power in predicting peripheral blood mononuclear cell ORMLD3 expression as an API+ marker (AUC: 0.820 [0.771–0.869]). API: asthma predictive index; API+: asthma predictive index (≥4 wheezing episodes); ROC: receiver operating characteristic; AUC: area under the curve.
Although the API is an important predictive indicator of wheezing episodes in children, its use in clinical diagnosis remains controversial. In this study, we investigated whether PBMC *ORMDL3* expression levels are associated with wheezing episodes in children, especially at <5 years of age.

PBMC *ORMDL3* expression in children aged <5 years was positively associated with their API and was significantly correlated with their personal history of immune diseases (infantile eczema, wheezing, total IgE) and living environment (*D. pteronyssinus*, house dust mites, dog fur, and *Aspergillus*). Therefore, *ORMDL3* expression levels in PBMCs could be used as a clinical indicator of potential development of asthma in children.

Previous studies have shown that *ORMDL3* is closely related to wheezing episodes in children. In the present study, *ORMDL3* expression was significantly higher in children with API+ compared with those with API− or API0. Additionally, *ORMDL3* expression was positively correlated with an increased API, infantile eczema, wheezing, total IgE levels, *D. pteronyssinus*, house dust mites, dog fur, and *Aspergillus*. *ORMDL3* expression was not correlated with a family history, sex, age, rhinitis, cat fur, trees, and streptavidin, as well as other dietary factors (i.e., milk, egg white, beef, shrimp, crab, cashews, and mango). These results further suggest a close association of *ORMDL3* with recurrent wheezing in children, as well as with environmental (*D. pteronyssinus*, house dust mites, dog fur, and *Aspergillus*) and clinical (infantile eczema, wheezing, and total IgE levels) factors associated with recurrent wheezing. Additionally, *ORMDL3* expression was not associated with common allergies. Taken together, these results suggest that *ORMDL3* could be useful for diagnosing wheezing episodes. However, additional studies are necessary to validate this association, especially with allergens that can be found in households. That some allergens are associated with wheezing, while others are not, warrants more in-depth studies.

Numerous studies have shown associations between *ORMDL3* polymorphisms and asthma. However, the mechanism for involvement of *ORMDL3* in the inflammatory process of asthma in children is not completely understood. Studies in mice showed that *ORMDL3* expression was increased by up to 127 fold in wild-type mice that were exposed to antigens and cytokins. A study based on a house dust mite-induced mouse model of allergic asthma showed that *ORMDL3*

| Spearman's correlation coefficient (r) | P     |
|--------------------------------------|-------|
| API                                  | 0.447 | <0.001 |
| Sex                                  | 0.105 | 0.053  |
| Family history                       | −0.043| 0.432  |
| Infantile eczema                     | 0.499 | <0.001 |
| Wheezing                             | 0.516 | <0.001 |
| Rhinitis                             | −0.064| 0.240  |
| Age (months)                         | 0.024 | 0.661  |
| Total IgE                            | 0.208 | 0.002  |
| *Dermatophagoides pteronyssinus*     | 0.357 | <0.001 |
| House dust mites                     | 0.112 | 0.039  |
| Dog fur                              | 0.226 | <0.001 |
| Cat fur                              | 0.068 | 0.208  |
| Trees                                | 0.029 | 0.598  |
| *Aspergillus*, streptavidin          | 0.257 | <0.001 |
| Milk                                 | 0.040 | 0.459  |
| Egg white                            | −0.058| 0.288  |
| Beef                                 | −0.006| 0.911  |
| Cashews                              | −0.038| 0.480  |
| Amaranth                             | −0.122| 0.024  |
| Crab                                 | 0.016 | 0.774  |
| Mango                                | 0.103 | 0.056  |
| Shrimp                               | 0.010 | 0.850  |

API: asthma predictive index; IgE: immunoglobulin E.
overexpression increased production of ceramide, and promoted chronic inflammation and upper airway allergic reactions. A recent study showed that higher ORMDL3 expression induced the p-extracellular-regulated kinase/matrix metalloproteinase-9 pathway, which led to airway remodeling in asthma. Two single nucleotide polymorphisms in the promoter region of ORMDL3 (rs7216389 and rs7216558) are significantly associated with early-onset wheezing episodes in infants and young children. Furthermore, a correlation has been found between rs7216389 and asthma susceptibility in children. Such polymorphisms in the promoter region of ORMDL3 affect ORMDL3 transcription, which in turn, could increase recurrent wheezing in children. These studies support the role of ORMDL3 expression in asthma. However, further studies are still necessary to determine the exact relationship, which could help for diagnosis and management of asthma.

There are some limitations to our study. Because of the retrospective nature and the small sample size, the detailed phenotypes for each recruited subject are lacking. Additionally, the expected high degree of heterogeneity needs to be considered when interpreting the data. Because of the retrospective nature of the study, we were limited to the data available in the charts. The environmental data were from questionnaires that were filled in by the child’s legal guardians at the first visit to our center, and no formal inquiry was made. Furthermore, the exact initial reason for consulting the clinic was not consistently recorded in the charts. Because our study population was pediatric, pulmonary function tests cannot be performed in young children, adding ORMDL3 expression to the API could significantly improve our understanding of wheezing episodes in preschool children. This could also improve diagnosis and management of patients.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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References

1. Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; 368: 733–743.

2. Castro-Rodriguez JA. The Asthma Predictive Index: a very useful tool for predicting asthma in young children. *J Allergy Clin Immunol* 2010; 126: 212–216.

3. Zahran HS, Bailey CM, Damon SA, et al. Vital signs: asthma in children – United States, 2001-2016. *MMWR Morb Mortal Wkly Rep* 2018; 67: 149–155.

4. Rao D and Phipatanakul W. Impact of environmental controls on childhood asthma. *Curr Allergy Asthma Rep* 2011; 11: 414–420.

5. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2018, 2018: 99–122.

6. British Thoracic Society and Scottish Intercollegiate Guidelines Network (BTS/SIGN) national clinical guideline on management of asthma. 2016: 117–125.

7. Wu CC, Chen RF and Kuo HC. Different implications of paternal and maternal atopy for perinatal IgE production and asthma development. *Clin Dev Immunol* 2012; 2012: 132142.

8. Castro-Rodriguez JA, Cifuentes L and Rodriguez-Martinez CE. The Asthma Predictive Index remains a useful tool to predict asthma in young children with recurrent wheeze in clinical practice. *J Allergy Clin Immunol* 2011; 127: 1082–1083.

9. Leonardi NA, Spycher BD, Strippoli MP, et al. Validation of the Asthma Predictive Index and comparison with simpler clinical prediction rules. *J Allergy Clin Immunol* 2011; 127: 1466–1472.e6.

10. Huffaker MF and Phipatanakul W. Utility of the Asthma Predictive Index in predicting childhood asthma and identifying disease-modifying interventions. *Ann Allergy Asthma Immunol* 2014; 112: 188–190.

11. Chang TS, Lemanske RF Jr, Guilbert TW, et al. Evaluation of the modified Asthma Predictive Index in high-risk preschool children. *J Allergy Clin Immunol Pract* 2013; 1: 152–156.

12. Castro-Rodriguez JA. The necessity of having asthma predictive scores in children. *J Allergy Clin Immunol* 2013; 132: 1311–1313.

13. Luo G, Nkoy FL, Stone BL, et al. A systematic review of predictive models for asthma development in children. *BMC Med Inform Decis Mak* 2015; 15: 99.

14. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007; 448: 470–473.

15. Miller M, Tam AB, Cho JY, et al. ORMDL3 is an inducible lung epithelial gene regulating metalloproteases, chemokines, OAS, and ATF6. *Proc Natl Acad Sci U S A* 2012; 109: 16648–16653.

16. Jin R, Xu HG, Yuan WX, et al. Mechanisms elevating ORMDL3 expression in recurrent wheeze patients: role of Ets-1, p300 and CREB. *Int J Biochem Cell Biol* 2012; 44: 1174–1183.

17. Caudri D, Wijga A, A Schipper CM, et al. Predicting the long-term prognosis of children with symptoms suggestive of asthma at preschool age. *J Allergy Clin Immunol* 2009; 124: 903–910.

18. Schedel M, Michel S, Gaertner VD, et al. Polymorphisms related to ORMDL3 are associated with asthma susceptibility, alterations in transcriptional regulation of ORMDL3, and changes in TH2 cytokine levels. *J Allergy Clin Immunol* 2015; 136: 893–903.e814.

19. Zhao CN, Fan Y, Huang JJ, et al. The association of GSDMB and ORMDL3 gene polymorphisms with asthma: a meta-analysis. *Allergy Asthma Immunol Res* 2015; 7: 175–185.

20. Shi H, Cheng D, Yi L, et al. Association between ORMDL3 polymorphism and susceptibility to asthma: a meta-analysis. *Int J Clin Exp Med* 2015; 8: 3173–3183.
21. Shahid M, Sabar MF, Bano I, et al. Sequence variants on 17q21 are associated with the susceptibility of asthma in the population of Lahore, Pakistan. *J Asthma* 2015; 52: 777–784.

22. Ballardini N, Bergstrom A, Bohme M, et al. Infantile eczema: prognosis and risk of asthma and rhinitis in preadolescence. *J Allergy Clin Immunol* 2014; 133: 594–596.

23. Burgess JA, Dharmage SC, Byrnes GB, et al. Childhood eczema and asthma incidence and persistence: a cohort study from childhood to middle age. *J Allergy Clin Immunol* 2008; 122: 280–285.

24. Tenero L, Piazza M and Piacentini G. Recurrent wheezing in children. *Transl Pediatr* 2016; 5: 31–36.

25. Just J, Belfar S, Wanin S, et al. Impact of innate and environmental factors on wheezing persistence during childhood. *J Asthma* 2010; 47: 412–416.

26. Wu H, Romieu I, Sienra-Monge J-J, et al. Genetic variation in ORM1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. *Allergy* 2009; 64: 629–635.

27. Galanter J, Choudhry S, Eng C, et al. ORMDL3 gene is associated with asthma in three ethnically diverse populations. *Am J Respir Crit Care Med* 2008; 177: 1194–1200.

28. Jing Z, Wang JF and Li H. Children’s life style in Beijing, the correlation study of immune state, ORMDL3 gene SNPs and the occurrence of asthma. *J Med Res* 2010; 39: 21–24.

29. Tavendale R, Macgregor DF, Mukhopadhyay S, et al. A polymorphism controlling ORMDL3 expression is associated with asthma that is poorly controlled by current medications. *J Allergy Clin Immunol* 2008; 121: 860–863.

30. Hirota T, Harada M, Sakashita M, et al. Genetic polymorphism regulating ORM1-like 3 (Saccharomyces cerevisiae) expression is associated with childhood atopic asthma in a Japanese population. *J Allergy Clin Immunol* 2008; 121: 769–770.

31. Kim SJ, Kim CH, Ahn JH, et al. Time sequence of airway remodeling in a mouse model of chronic asthma: the relation with airway hyperresponsiveness. *J Korean Med Sci* 2007; 22: 183–191.

32. Oyeniran C, Sturgill JL, Hait NC, et al. Aberrant ORM (yeast)-like protein isoform 3 (ORMDL3) expression dysregulates ceramide homeostasis in cells and ceramide exacerbates allergic asthma in mice. *J Allergy Clin Immunol* 2015; 136: 1035–1046.

33. Yu F, Sun Y, Yu J, et al. ORMDL3 is associated with airway remodeling in asthma via the ERK/MMP-9 pathway. *Mol Med Rep* 2017; 15: 2969–2976.