Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(8) 3727–3736

DOI: 10.1177/0300060519862674
journals.sagepub.com/home/imr
SAGE

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

Yaqin Li[1], Lanfang Cao[2], Qing Yu[1], Haiyan Xue[2] and Yanming Lu[1]

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 ($r = -0.122$).

[1]Department of Pediatrics, South Campus, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[2]Department of Pediatrics, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Corresponding author:
Yanming Lu, Department of Pediatric, South Campus, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 201112, China.
Email: luyanming@renji.com

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

Date received: 29 December 2018; accepted: 19 June 2019

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.[1] 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.[2,3] 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.[4–6] Additionally, a family history of asthma, age, family smoking habits, and total immunoglobulin E (IgE) levels are significantly associated with wheezing episodes in children.[5–7] 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.[2] The API is increasingly used in clinical practice and is approved by various international guidelines.[5,6] However, the accuracy of the API for prediction is affected by genetic polymorphism, environmental and socioeconomic factors, sex, race, and family health beliefs.[8–11] 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[9] and that it requires improvement.[12,13] 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.[14] *ORMDL3* expression is stimulated by allergens and cytokines, and mainly occurs in airway epithelial cells.[15] *ORMDL3* expression is positively associated with recurrent wheezing in children.[16] 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 (anticoagulated 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 mL 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.

Table 1. Primers used in this study.

Gene	Primers
β-actin-F	5′-ATGATGATATCGCCGCGCTC-3′
β-actin-R	5′-CCACCATCACGCCCTGG-3′
ORMDL3-F	5′-CAGCCGCGGGTTGTTACAG-3′
ORMDL3-R	5′-CCTCTCTGCTGTTCGTGTGG-3′

F: forward; R: reverse.

Statistical analysis

SPSS Statistics for Windows, Wersion 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

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 (≥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 (≥4 wheezing episodes); API−: asthma predictive index (1–3 wheezing episodes); API0: asthma predictive index (no wheezing).

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.

ORMDL3 *expression in the different API groups*

ORMDL3 expression in PBMCs was analyzed. When we examined relative expression of the *ORMDL3* gene in the API = 4, API = 5, API ≥6, API−, and API0 groups, all three API+ groups (API = 4, API = 5, and API ≥ 6) had significantly higher *ORMDL3* expression than did the API0 (all $P < 0.001$) and API− groups (all $P < 0.001$). However, no difference in *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

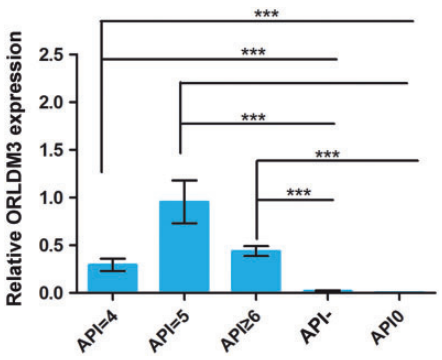

Figure 1. *ORMDL3* expression was increased in PBMCs of children in the API+ groups (API = 4, API = 5, and API ≥6) compared with the API− or API0 group. The mRNA levels of *ORMDL3* were analyzed by real-time polymerase chain reaction. The numbers of patients for the API0, API−, API = 4, API = 5, and 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).

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).

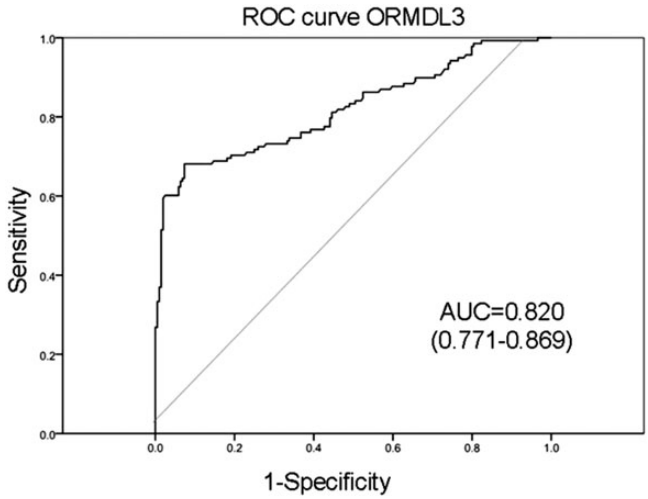

Figure 2. Peripheral blood mononuclear cell *ORMDL3* expression is a potential biomarker of API+. ROC curve analysis shows the diagnostic power in predicting peripheral blood mononuclear cell *ORMDL3* expression as an API+ marker (AUC: 0.820 [0.771–0.869]). API: asthma predictive index; API+: asthma predictive index (≥4 wheezing episodes); ROC: receiver operating characteristic; AUC: area under the curve.

Table 4. Correlations between *ORMDL3* expression and asthma-related parameters.

	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.

Discussion

Although the API is an important predictive indicator of wheezing episodes in children,[2,9] its use in clinical diagnosis remains controversial.[12,13] 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.[18–21] 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.[4,22–25] 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.[18–20,26–29] 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.[15,30,31] A study based on a house dust mite-induced mouse model of allergic asthma showed that *ORMDL3*

overexpression increased production of ceramide, and promoted chronic inflammation and upper airway allergic reactions.[32] 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.[33] 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.[19–21] Furthermore, a correlation has been found between rs7216389 and asthma susceptibility in children.[26] Such polymorphisms in the promoter region of *ORMDL3* affect *ORMDL3* transcription, which in turn, could increase recurrent wheezing in children.[18] 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 were not performed. IgE levels were qualitatively, not quantitatively, determined. Only *ORMDL3* expression was measured and other genes known to be associated with asthma were not examined. Because of the low number of patients in the API+ group, the correlation between the API and sex, as well as those between *ORMDL3* expression and a family history, age, rhinitis, dietary factors, API score, and other factors, should be further verified. Moreover, this was a retrospective study and we used the API, an imperfect measurement,[12,13] which probably introduced bias. Finally, isolating PBMCs represents an additional step, but it is a routine procedure in many hospitals. Therefore, use of more direct methods for measuring *ORMDL3* expression could be useful. Finally, no follow-up is yet available to determine the association of the API and *OMRDL3* expression on the eventual development of asthma. Therefore, to further confirm the relationships among *ORMDL3* expression, the API, and the incidence of wheezing in children, a prospective cohort study with a large sample size is necessary.

In conclusion, *ORMDL3* expression levels are associated with higher API values and other asthma-related factors. Because the reliability of the API is low and 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.

Funding

This work was supported by grants from the risk factors of recurrent infant wheeze and early intervention study in Pujiang Town (Project No. SHDC12014905) and the establishment of a new linkage model between tertiary hospitals and community hospitals for a study of prevention and cure of childhood asthma (Project No. 10DZ1951000).

ORCID iD

Yanming Lu https://orcid.org/0000-0001-5559-2574

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.