Observational Study

Genetic association of ADAM33 polymorphisms with childhood asthma in Chinese Han population
A case-control study

Xuecong Ning, PhD, Yunxia Zhang, PhD, Hongzhi Wu, PhD, Linlin Bai, PhD, Cuike Gong, PhD, Zhihua Wang, PhD

Abstract
To explore the association of a disintegrin and metalloprotease 33 (ADAM33) polymorphisms with childhood asthma susceptibility, we conducted this case-control study.

In this case-control study, we selected 96 asthma children and 86 healthy children to conduct the genotyping of ADAM33 polymorphisms through polymerase chain reaction-direct sequencing (PCR-DS). Hardy-Weinberg equilibrium (HWE) status in the control group was detected adopting chi-square test. Frequency differences of genotypes, alleles, and haplotypes were compared by chi-square test between the case and control groups. Linkage disequilibrium (LD) between polymorphisms was checked using Haplovlew software. Association intensity of the polymorphisms with the disease risk was assessed by odds ratio (OR) and 95% confidence interval (95%CI). The frequency of rs678881 GA genotype was obviously higher in cases than in controls (P = .03) and the carriage of this genotype conferred higher risk of asthma among children than GG genotype (OR = 2.03, 95%CI = 1.05–3.91). However, neither rs2280089 nor rs2853209 polymorphism was significantly associated with the risk of childhood asthma. Strong LD was found among rs678881, rs2280089 and rs2853209, and haplotype GGT was distinctly associated with the risk of asthma in children (OR = 0.28, 95%CI = 0.13–0.57).

ADAM33 rs678881 polymorphism is significantly correlated with increased susceptibility to asthma in Chinese Han children. Besides, haplotype GGT among the 3 polymorphisms was obviously associated with decreased risk of childhood asthma.

Abbreviations: 95%CI = 95% confidence interval, ADAM33 = a disintegrin and metalloprotease 33, AGE = agaroase gel electrophoresis, COPD = chronic obstructive pulmonary disease, HWE = Hardy-Weinberg equilibrium, IPF = idiopathic pulmonary fibrosis, LD = Linkage disequilibrium, MDC = metalloprotease disintegrin cysteine-rich, OR = odds ratio, PCR-DS = polymerase chain reaction-direct sequencing, SNPs = single nucleotide polymorphisms.

Keywords: ADAM33, childhood asthma, haplotype, polymorphisms

1. Introduction
Asthma is one of the most common chronic inflammatory diseases among children, and characterized by recurrent seizures.[1,2] Its symptoms contain cough, wheezing, shortness of breath and chest tightness.[3] The occurrence of asthma could result in asthma, but only in a small part of children.[4] Environment factors, such as tobacco smoke, vitamin D insufficiency and high-lipid diet, are all risk factors for asthma in children.[5,6] Exposing to these risk factors could result in asthma, but only in a small part of children. Therefore, genetic factors may play a key role in asthma etiology. Scholars pay attentions to the disease heredity, especially genetic polymorphisms.[7–9] However, up to now, the pathology and etiology of asthma in children remain unclear.

A disintegrin and metalloprotease 33 (ADAM33) is a transmembrane metalloproteinase which belongs to the sub-group of zinc-dependent metalloproteinase super-family.[10,11] It is encoded by ADAM33 gene which is located on chromosome 20p13, and usually expressed in bronchial smooth muscle cells and lung fibroblasts.[12,13] ADAM33 has been proved to participate in cell adhesion, cell fusion, cell signaling, and cell-cell interaction, which suggests that it may play an important role in pulmonary defenses.[14] All the time, as a vital candidate gene, ADAM33 is widely studied in asthma among various populations.[15–17] ADAM33 gene covers many single nucleotide polymorphisms (SNPs). Until now, scholars have achieved inconsistent conclusions on the role of ADAM33 polymorphisms in childhood asthma in different races.

well as their growths and developments. The disease also generates heavy burden to family and society. Reportedly, asthma is a complex multifactorial disease, involving genetic and environmental triggers.[4] Environment factors, such as tobacco smoke, vitamin D insufficiency and high-lipid diet, are all risk factors for asthma in children. Exposing to these risk factors could result in asthma, but only in a small part of children. Therefore, genetic factors may play a key role in asthma etiology. Scholars pay attentions to the disease heredity, especially genetic polymorphisms. However, up to now, the pathology and etiology of asthma in children remain unclear.

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In this study, we selected 3 polymorphisms (rs678881, rs2280089, rs2853209) in ADAM33 to explore their associations with childhood asthma risk in Chinese Han population, which are studied rarely in asthma but widely in others diseases. Linkage disequilibrium among these polymorphisms was analyzed, and haplotype distributions were calculated to determine their association with asthma occurrence in children.

2. Materials and methods

2.1. The case and control groups

In this case-control study, 96 asthma children and 86 healthy children were selected. All subjects were Chinese Han people from the same region and had no blood relation between each other. This study was approved by the Research Ethics Committee of Xingtai People’s Hospital and all subjects’ guardians were informed of the whole study process. Written informed consents were signed by the participants’ parents before blood extraction.

Patients in the case group were diagnosed based on their clinical symptoms and through pathological examinations. Healthy controls were from the physical examination center of the hospital during the same period. The control group was frequency-matched with the case group in age and sex.

2.2. DNA extraction

2 ml peripheral venous blood from every participant was extracted into blood collection tube with EDTA anticoagulant, and then stored in −80°C freezer. Genomic DNA was extracted following the method of conventional chloroform/isooamyl alcohol extraction and then stored at −20°C for standby application.

2.3. Genotyping of ADAM33 polymorphisms

The genotyping of ADAM33 polymorphisms was conducted through polymerase chain reaction-direct sequencing (PCR-DS). PCR primers were acquired from published articles[18-20] and synthesized by Shanghai Sangon Biotech Co., Ltd. Primer sequences were shown in Table 1. PCR system was a mixture of 2.5 μl, including 1 μl DNA template, 12.5 μl PCR Master Mix, each 0.5 μl of forward and reverse primers and ddH2O added to 25 μl. PCR program was as follows: pre-denaturation at 95°C for 3 minutes, followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. The quality and concentration of PCR products were detected through 1.0% agarose gel electrophoresis (AGE) and NanoDrop 2000c instrument.

Eligible PCR products were sequenced in Shanghai Sangon Biotech Co., Ltd to determine the genotypes of ADAM33 polymorphisms in all subjects.

2.4. Statistical analysis

The genotype distributions of ADAM33 polymorphisms in the control group were tested for their conformity to Hardy-Weinberg equilibrium (HWE). Frequency differences in genotypes, alleles, and haplotypes were compared adopting chi-square test between the case and control groups. Odds ratio (OR) with 95% confidence interval (95% CI) was calculated to express the relative risk of childhood asthma. Data analyses were conducted utilizing PASW Statistics 18.0 software. P < .05 was considered as the significant threshold. Linkage disequilibrium (LD) and the role of haplotypes in childhood asthma were also analyzed.

3. Results

3.1. Detailed clinical information on all subjects

In the present study, a total of 182 subjects were selected, consisting of 96 cases and 86 controls. The case group included 57 boys and 39 girls between 3 and 12 years old, with a ratio of boys to girls at 1.46: 1. The control group consisted of 48 boys and 38 girls with age ranging between 4 and 14 years old, and the percentage of boys in the controls was near 56%. Therefore, difference in sex ratio between 2 groups was not significant (P > .05). Similarly, there was no significant difference in age between the case and control groups (P > .05).

3.2. Genotype and allele distributions of ADAM33 polymorphisms in the case and control groups

Genotype distributions of ADAM33 rs678881, rs2280089, and rs2853209 polymorphisms in the control group were all consistent with HWE (P > .05, Table 2), indicating that our study population came from a Mendelian population and possessed fine representativeness.

When compared with GG genotype, GC genotype of rs678881 polymorphism showed obviously higher frequency in cases than in controls (P = .03) and its carriers faced higher risk of childhood asthma than GG carriers (OR = 2.03, 95% CI = 1.05–3.91). However, all of the genotypes and alleles of ADAM33 rs2280089 and rs2853209 polymorphisms had no significantly different frequency between the case and control group, indicating they held no significant association with the susceptibility of asthma in children (Table 2).

3.3. Haplotype analysis of ADAM33 polymorphisms in childhood asthma

LD between ADAM33 rs678881, rs2280089, and rs2853209 polymorphisms was detected by Haplovie. As shown in Table 3, strong LD existed between them and 4 haplotypes were constructed, namely GGA, GGT, CGT, and GAT. Their percentages were 56.25%, 5.73%, 23.96%, and 14.06% in the case group and 55.23%, 20.35%, 16.28%, and 8.14% in the control group. Compared with GGA, GGT haplotype had significantly higher frequency in the control group than in the
Liang et al conducted a meta-analysis to explore the influence on asthma risk among people at any age, including children, according to relevant researches. But referring to rs678881, due to the scariness of related researches on Chinese population, its role in childhood asthma need to be further studied. Our study showed strong LD among these 3 polymorphisms, so their haplotypes were analyzed for the first time. Accordingly, haplotype GGT significantly associated with the decreased risk of childhood asthma, playing a protective role against childhood asthma.

ADAM, or metalloprotease disintegrin cysteine-rich (MDC), constitutes a glycoprotein family on membrane surface and covers multiple functional domains. It is consisted of approximately 800 amino acid and contains a number of conservative structural domains. In ADAM protein, there are signal peptide, regulatory region, metalloproteinase, disintegrin, cysteine-rich, endothelial growth factor and transmembrane domains, and intracellular glycoprotein domain from N to C terminal. The characteristics of ADAM domains include the interactions of cell-cell and cell-matrix mediated by a disintegrin domain, and ADAM participates in various signal transduction pathways through integrating a cysteine-rich domain into cell adhesion and an endothelial growth factor domain. Studies prove that the increased activity of ADAM proteolytic enzyme is involved in inflammatory airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD). ADAM33 is a member of ADAM family and expressed in airway smooth muscle cells and lung fibroblasts. Consequently, ADAM33 not only plays a role in lung defense but also participates in airway vascular remodeling, airway inflammation and obstruction, and the reduction of lung function. Chen et al concluded that ADAM33 polymorphisms might be involved in the development of mite-sensitized persistent allergic rhinitis in a Chinese population. Uh et al found that ADAM33 rs628977G>A polymorphism was correlated with idiopathic pulmonary fibrosis (IPF) risk and significantly decreased the disease susceptibility. Seven SNPs in ADAM33 have been associated with COPD risk in a Chinese Mongolian population. In addition, some inflammatory diseases are affected by ADAM33 polymorphisms as well, such as systemic lupus erythematosus and psoriasis. The role of ADAM33 in asthma has been explored in previous studies and several SNPs have been proposed as susceptible factors for asthma. But due to differences in allele distribution for ADAM33 polymorphisms in different races, their functional mechanism in childhood asthma is still not completely illustrated.

### Table 2
The genotype frequency comparison of ADAM33 polymorphisms between 2 study groups.

| SNP     | Case/control (n) | OR (95%CI) P | Allele       | Case/control (n) | OR (95%CI) P | P_HWE |
|---------|------------------|--------------|--------------|------------------|--------------|-------|
| rs678881|                   |              |              |                   |              |       |
| GG      | 55/82            | 1.00 (Ref.)  | G            | 146/144          | 1.00 (Ref.)  | .17   |
| GC      | 36/20            | 2.03 (1.05–3.91) | .03 | C                | 46/28        | 1.62 (0.96–2.73) | .07 |
| CC      | 5/4              | 1.41 (0.36–5.51) | .62 |                 |              |       |
| rs2280089|                  |              |              |                   |              |       |
| GG      | 71/72            | 1.00 (Ref.)  | G            | 165/158          | 1.00 (Ref.)  | .41   |
| GA      | 23/14            | 1.67 (0.79–3.50) | .17 | A                | 27/14        | 1.85 (0.93–3.65) | .07 |
| AA      | 2/0              | –            | –            |                  |              |       |
| rs2853209|                 |              |              |                   |              |       |
| AA      | 34/24            | 1.00 (Ref.)  | A            | 108/95           | 1.00 (Ref.)  | .33   |
| AT      | 40/47            | 0.60 (0.31–1.18) | .14 | T                | 84/77        | 0.96 (0.63–1.45) | .85 |
| TT      | 22/15            | 1.04 (0.45–2.40) | .94 |                 |              |       |

HWE = Hardy-Weinberg equilibrium.

### Table 3
The haplotype analysis of ADAM33 3 polymorphisms.

| Haplotype SNP1-SNP2-SNP3 | Case (%) | Control (%) | OR (95%CI) | P |
|--------------------------|----------|-------------|------------|---|
| GGA                      | 108 (56.25) | 95 (55.23) | 1.00 (Ref.) | – |
| GGT                      | 11 (5.73) | 35 (20.35) | 0.28 (0.13–0.57) | <.001 |
| GST                      | 46 (23.96) | 28 (16.28) | 1.45 (0.84–2.49) | .18 |
| GAT                      | 27 (14.06) | 14 (8.14) | 1.70 (0.84–3.43) | .14 |

SNP1: rs678881; SNP2: rs2280089; SNP3: rs2853209.
In conclusion, ADAM33 rs678881 polymorphism significantly increases the risk of childhood asthma in Chinese Han population, but not rs2280089 or rs2853209. Furthermore, strong LD exists among our focused three polymorphisms and GGT haplotype functions as a protective factor against childhood asthma. However, some disadvantages may affect the veracity of final results, such as small sample size, neglecting potential influences of environmental factors on the disease, and sole nationality of enrolled population. Therefore, well-designed further study with large enough sample size should be conducted to verify our findings and to explore the mechanism of ADAM33 polymorphisms affecting childhood asthma in the future.

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References
[1] Bateman ED, Hurud SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008;31:143–78.
[2] Afif AA, Korgonarik P. The association of childhood asthma with mental health and developmental comorbidities in low-income families. J Asthma 2015;52:1–9.
[3] Kew KM, Undela K, Kotsoriki I, et al. Macrolides for chronic asthma. Cochrane Database Syst Rev 2015;9:CD002997.
[4] Hollenbach JP, Cloutier MM. Childhood asthma management and environmental triggers. Pediatr Clin North Am 2015;62:1199–214.
[5] Wood LG, Garg ML, Gibson PG. A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. J Allergy Clin Immunol 2011;127:1133–40.
[6] von Mutius E, Hartter T. Update in asthma 2012. Am J Respir Crit Care Med 2013;188:150–6.
[7] Ramphul K, Hua L, Bao YX, et al. Identification of IL13 C1925T as a single nucleotide polymorphism for asthma in children from Mauritius. Pediatr Allergy Immunol Pulmonol 2015;28:92–5.
[8] Zhang YN, Li YJ, Li H, et al. Association of CD14 C159T polymorphism with atopic asthma susceptibility in children from Southeastern China: a case-control study. Genet Mol Res 2015;14:4311–7.
[9] Zhang H, Zhang Z, Li G, et al. Association of FCRL3 genetic polymorphisms with endometriosis-related infertility risk: an independent study in Han Chinese. Medicine 2015;94:e1168.
[10] Holgate ST, Davies DE, Rorke S, et al. ADAM 33 and its association with airway remodeling and hyperresponsiveness in asthma. Clin Rev Allergy Immunol 2004;27:23–34.
[11] Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. Mol Aspects Med 2008;29:258–89.
[12] Karimi MR, Faridhosseini R, Abbasszadegan MR, et al. Association of ADAM33 gene polymorphisms with allergic asthma. Iran J Basic Med Sci 2014;17:716–21.
[13] Holloway JW, Laxton RC, Rose-Zerilli MJ, et al. ADAM33 expression in atherosclerotic lesions and relationship of ADAM33 gene variation with atherosclerosis. Atherosclerosis 2010;211:224–30.
[14] Oth P, Reichert P, Wang W, et al. Crystal structure of the catalytic domain of human ADAM33. J Mol Biol 2004;335:129–37.
[15] Zhilif M, Zhilif N, Obeidad NM, et al. Association between ADAM33 polymorphisms and susceptibility with adult and childhood asthma among Jordanians. Genet Test Mol Markers 2014;18:767–74.
[16] Yildizhu N, Wushouer Q, Arkk, et al. Association of a disintegrin and metalloprotease 33 gene polymorphisms with asthma. Mol Clin Oncol 2014;2:1076–80.
[17] Zheng W, Wang L, Su X, et al. Association between V4 polymorphism in the ADAM33 gene and asthma risk: a meta-analysis. Genet Mol Res 2015;14:989–99.
[18] Xue W, Han W, Zhou ZS. ADAM33 polymorphisms are associated with asthma and a distinctive palm dermatoglyphic pattern. Mol Med Rep 2013;6:1795–800.
[19] Sabar MF, Gham MI, Shahid M, et al. Genetic variants of ADAM33 are associated with asthma susceptibility in the Punjabi population of Pakistan. J Asthma 2016;53:341–8.
[20] Matsusue A, Kiyohara C, Tanaka K, et al. ADAM33 genetic polymorphisms and risk of atopic dermatitis among Japanese children. Clin Biochem 2009;42:477–83.
[21] Miyake Y, Tanaka K, Arakawa M. ADAM33 polymorphisms, smoking and asthma in Japanese women: the Kyushu Okinawa Maternal and Child Health Study. Int J Tuberc Lung Dis 2012;16:974–9.
[22] Liang S, Wei X, Gong C, et al. A disintegrin and metalloprotease 33 (ADAM33) gene polymorphisms and the risk of asthma: a meta-analysis. Hum Immunol 2013;74:648–57.
[23] Bilodeau CP, Metalloprotease-disintegrins: links to cell adhesion and clevage of TNF alpha and Notch. Cell 1997;90:589–78.
[24] Orth P, Reichert P, Wang W, et al. Crystal structure of the catalytic domain of human ADAM33. J Mol Biol 2004;335:129–37.
[25] Miyake Y, Tanaka K, Arakawa M. ADAM33 polymorphisms, smoking and asthma in Japanese women: the Kyushu Okinawa Maternal and Child Health Study. Int J Tuberc Lung Dis 2012;16:974–9.
[26] Liang S, Wei X, Gong C, et al. A disintegrin and metalloprotease 33 (ADAM33) gene polymorphisms and the risk of asthma: a meta-analysis. Hum Immunol 2013;74:648–57.
[27] Blokz CL, Metalloprotease-disintegrins: links to cell adhesion and clevage of TNF alpha and Notch. Cell 1997;90:589–78.
[28] Paulissen G, Rocks N, Guerders MM, et al. Role of ADAM and ADAMTS metalloproteinases in airway diseases. Respir Res 2009;10:127.
[29] Paulissen G, Rocks N, Guerders MM, et al. ADAM-8, a metalloproteinase, drives acute allergic-induced airway inflammation. Eur J Immunol 2011;41:380–91.
[30] Oseo KM, Gibson PG, Simpson JL, et al. Sputum ADAM8 expression is increased in severe asthma and COPD. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology 2014;44:342–52.
[31] Puxeddu I, Peng YF, Harvey A, et al. The soluble form of a disintegrin and metalloprotease 33 promotes angiogenesis: implications for airway remodeling in asthma. J Allergy Clin Immunol 2008;121:1400–6. 1406.e1401–04.
[32] Chen RX, Lu WM, Zhu LP, et al. Association study on ADAM33 polymorphisms in mite-sensitized persistent allergic rhinitis in a Chinese population. PloS One 2014;9:e85033.
[33] Uh ST, Jang AS, Park SW, et al. ADAM33 gene polymorphisms are associated with the risk of idiopathic pulmonary fibrosis. Lung 2014;192:25–32.
[34] Tan J, Liu AP, Sun C, et al. Association of ADAM33 gene polymorphisms with COPD in the Mongolian population of China. Ann Hum Biol 2014;41:9–14.