**Background and Objective:** Asthma and allergic rhinitis have been reported to be strongly associated with genetic factors. The aim of this study was to evaluate the accuracy of the TaqMan-MGB (minor groove binder) qPCR method for detecting CYSLTR1 rs320995 (T927C) and GSDMB rs7216389 (G1199A) gene polymorphisms as well as to explore the association of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms with genetic susceptibility of Chinese patients with asthma and allergic rhinitis.

**Methods:** In this study, 310 asthmatic patients and 60 healthy individuals were recruited in Peking Union Medical College Hospital. The CYSLTR1 rs320995 (T927C) and GSDMB rs7216389 (G1199A) gene polymorphisms in each group were analyzed by TaqMan-MGB qPCR and DNA sequencing which was regarded as the gold standard. After the validation of this method, additional 71 patients with allergic rhinitis and 72 patients with asthma combined with allergic rhinitis were selected and tested by using TaqMan-MGB qPCR.

**Results:** The TaqMan-MGB qPCR results were fully consistent with DNA sequencing results (Kappa = 1, P<0.001). In addition, the results of the TaqMan-MGB qPCR assay were not affected by bilirubin and lipids. We found differential distribution of CYSLTR1 rs320995 genotypes in female patients with asthma combined with allergic rhinitis ($\chi^2$=6.172, P=0.046, statistical power = 0.591). Specifically, the TT genotype is more frequent in women suffering from asthma with allergic rhinitis, whereas the TC genotype is more prevalent in healthy women. However, no such associations were observed in the GSDMB rs7216389 polymorphism.

**Conclusion:** We have established a reliable TaqMan-MGB qPCR method for the detection of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. Moreover, the CYSLTR1 rs320995 polymorphism may be associated with genetic susceptibility of Chinese female patients with asthma and allergic rhinitis. Multicenter studies with larger sample sizes are required in the future.

**Keywords:** asthma, allergy treatment, gene polymorphism, CYSLTR1, GSDMB

**Introduction**

Asthma is a common respiratory inflammatory disease that affects more than 300 million people worldwide. Despite there are a variety of medicines to control asthma progression, the incidence of asthma is still not well controlled. Asthma is a polygenic disease caused by the complex interplay of environmental and genetic factors.\(^1\) It may lead to different phenotypes, severity, and atopic responses. Allergic rhinitis is also a prevalent atopic disease that can affect the quality of life of a wide range of people in all age groups, especially teenagers. Although it is not fatal, in addition to the nasal and ocular damage caused by allergic reactions, it can also affect productivity at work by causing daytime sleepiness due to the interference of symptoms with sleep.\(^2\) At the same time, allergic rhinitis is also very common in asthma patients, and the pathogenesis of these two diseases are closely related. Genome-wide association studies (GWAS) have revealed that disease-related single nucleotide polymorphisms (SNPs) may significantly affect genetic susceptibility to asthma and allergic diseases.\(^3–6\) A large number of
SNPs of candidate genes, such as TH2LCRR, OPRM1, RORA and IL-33, have been identified as authentic risk genes associated with asthma or allergic rhinitis. Moreover, Levin et al reported a variant, rs3827907, that may affect responsiveness to inhaled corticosteroid (ICS) medication in diverse populations with asthma.

Therefore, it is important to detect SNPs which may influence the progression of the disease and predict patients’ responsiveness to drugs or vaccines. Among the pathophysiological mediators involved in bronchial asthma and rhinitis, cysteinyl leukotrienes (CYSLTs) have been reported to be some non-negligible components due to its ability to promote bronchoconstriction, vascular hyperpermeability, and mucous hypersecretion. Accordingly, cysteinyl leukotriene receptor 1 (CYSLTR1), as a receptor for CYSLTs has emerged as an important target for the treatment of asthma and rhinitis. Interaction of CYSLTs with CYSLTR1 expressed on immune/inflammatory cells, airway smooth muscle, and other types of structural cell is intimately involved in different aspects of the immunopathogenesis in bronchial asthma. The rs320995 C allele of CYSLTR1 upregulates CYSLTR1 protein and enhances the targeting of leukotriene modulators, thereby enhancing the targeting of leukotriene inhibitors and strengthening the therapeutic effect. Gasdermins are a family of structurally related proteins associated with pyroptosis, of which Gasdermin B (GSDMB) is the most characteristic one. Notably, the connection between GSDMB and genetic susceptibility to chronic mucosal inflammation has been proposed. In AA homozygous patients with GSDMB rs7216389, GSDMB expression was significantly increased, and lung function was significantly improved after treatment with glucocorticoids or β2 agonists compared to patients with GA heterozygous or GG homozygous individual with asthma. Regrettably, only a few reports have focused on the relationship between asthma or allergic rhinitis and SNPs in the GSDMB gene and the CYSLTR1 gene. Hence, it is a topic worth investigating and has the potential to be translated into clinical detection.

Several methods with various efficiency and cost of testing for genotyping SNPs have been developed over the last few years. At present, the common methods for SNPs detection in the clinic include DNA sequencing, restriction fragment length polymorphism (PCR-RELP), gene chip and fluorescence quantitative PCR. However, many current SNPs assays, including high-throughput probes or arrays, are limited by the high financial and time costs and cumbersome experimental steps. TaqMan-MGB (minor groove binder) qPCR technique combines TaqMan probe and MGB with classical quantitative PCR technique. The MGB is added to the 3’ end of the common TaqMan probe to improve the annealing temperature of the probe and template binding. Compared with TaqMan probe, TaqMan-MGB probe has the advantages of high specificity and sensitivity. It is suitable for detection of single base mutation of target gene. Currently, TaqMan-MGB qPCR is being used to detect SNPs. TaqMan MGB-probe qPCR has showed stable amplification efficiency in different reaction systems, even in multiple combination probe. In clinical study, TaqMan-MGB qPCR proved a more sensitivity method to detection DNA mutation, which compared with SYBR Green qPCR. There are usually substantial samples which need to be analyzed in clinical settings, so a detection method which has shorter cycle and lower cost would achieve a rapid road to adapt large flow rapid detection in outpatient and inpatient of hospital. Overall, TaqMan-MGB qPCR is an effective, rapid, simple, and inexpensive method to detect SNP genotypes. Remarkably, there are no human CYSLTR1 and GSDMB gene polymorphisms detection kits registered for marketing by the FDA or in China. It is believed that the use of the TaqMan-MGB qPCR method would be useful for clinical work in dealing with large numbers of samples.

This study is dedicated to verifying the feasibility of TaqMan-MGB qPCR in the SNPs detection of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. Besides, the association of CYSLTR1 rs320995 (T927C) and GSDMB rs7216389 (G1199A) gene polymorphisms with genetic susceptibility in Chinese patients with asthma and allergic rhinitis has also been investigated.

**Methods**

**Study Design**

From July 2019 to June 2020, a prospective study was conducted at Peking Union Medical College Hospital (PUMCH) in Beijing, China. Our trial was approved by Chinese Academy of Medical Sciences and Peking Union Medical College Hospital Drug Clinical Trial Ethics Committee, registration information: No.002062, ethics approval No. KS2019282. The Clinical Trial Ethics Committee agreed to waive the informed consent in this clinical trial. The main reasons for the waiver were that: (1) the patients have signed the terms of admission which consented to the use of their biological...
samples for scientific research purposes; and (2) the blood samples used in this study were residual from the clinical testing program, the relevant testing program has been completed, and no adverse or secondary harm to the patients. The study consisted of two phases: first, the efficacy of TaqMan-MGB qPCR method was assessed in 310 asthmatic patients and 60 healthy individuals by comparing with DNA sequencing which was regarded as the gold standard. After validation of this method, an additional 71 patients with allergic rhinitis and 72 patients with asthma combined with allergic rhinitis were selected and tested for SNPs using TaqMan-MGB qPCR.

Participants

Patients were evaluated with routine diagnostic workups according to their presentations and auxiliary test results. The criteria of asthma include: (1) obvious respiratory symptoms, (2) at least one FEV1 reduction, accompanied by a FEV1/FVC reduction, (3) positive bronchodilation (ΔFEV1 >12%) or bronchial stimulation test (ΔFEV1 decline >12%), (4) positive serum specific IgE. The criteria for allergic rhinitis patients include: (1) obvious symptoms of allergic rhinitis such as sneezing, watery nose, nasal congestion, and nasal itching, (2) positive serum specific IgE, (3) positive skin prick test or intradermal test. In addition, in order to verify whether the kit would be interfered by other samples, we also selected 10 jaundice and 10 lipid blood samples to evaluate the accuracy of the reagent.

TaqMan-MGB qPCR Method

In this study, the TaqMan-MGB qPCR kit (Wuhan Healthcare Biotechnology Co., Ltd) was used to detect human CYSLTR1 rs320995 (T927C) and GSDMB rs7216389 (G1199A) gene polymorphisms. β-Actin was used as the internal control (IC) gene, which was placed with target gene detection system. The probe of the target gene detection system was FAM and VIC fluorescence, and the probe of the internal control gene detection system was ROX fluorescence. The detailed sequences of probes are as follows:

- CYSLTR1 (927T) -P1: 5'-VIC-AGGCTGTCTACATTTAGA-MGB-NFQ-3';
- CYSLTR1 (927T) -P2: 5'-FAM-CTGTCTACATTCAGAAAG-MGB-NFQ-3';
- GSDMB (1199G)-P3: 5'-VIC-TCCATGCGTGTTTGT-MGB-NFQ-3';
- GSDMB (1199A)-P4: 5'-FAM-TCCATGCATGTTTGTG-MGB-NFQ-3';
- β-actin-P5: 5'-ROX-ACCACCACGGCCGAGCGG-BHQ-3'.

DNA was extracted from each blood sample by TIANamp Genomic DNA Kit (Tiangen Biotechnology (Beijing) Co., LTD.). Each DNA sample was divided into two groups: one was detected by TaqMan-MGB method; the other group was detected by DNA sequencing method. (Sangon Biotech (Shanghai) Co., Ltd.)

For the TaqMan-MGB method, the detection system was 20 μL of DNA mixed with 30 μL reaction solution, comprised of 25μL qPCR Mix and 5μL CYSLTR1 or GSDMB gene PCR reaction solution (include internal control gene) per well. PCR amplification was performed using the ABI 7500 fluorescent quantitative PCR instrument, to acquire the signal from the PCR instrument, wild-type gene was collected through VIC channel; mutation of target gene was collected by FAM channel; β-Actin as internal control gene was collected by ROX channel. The reaction conducted on the fluorescence quantitative PCR instrument under the following conditions: 95°C for 5s repeated 1 cycle; 95°C for 30s, 61°C for 32s repeated 15 cycles; 95°C for 5s, 61°C for 32s repeated 30 cycles. Positive results visible fluorescence quantitative curve is smooth S type, and the curve of Ct values was 17 or less, and Δ Rn value >100,000. For the negative results, FAM, VIC and ROX signals should be no curve rises, or Ct value >17, or Δ Rn value ≤100,000. The judgment criteria of genotype results are shown in the following table (Supplementary Table 1)

Data Statistics and Analysis

SPSS 19.0 (SPSS Inc., Chicago, IL, United States), R Project (version 4.2.0) and RStudio (Open-Source Edition) software was used for statistical analysis in this study. The Hardy-Weinberg equilibrium (HWE) test was used to assess whether the frequency distribution of polymorphisms across genomes was representative. Kappa test was used to analyze the consistency coefficient of TaqMan-MGB qPCR and DNA sequencing. Chi-square test was used to compare whether there was a significant difference in SNP between the disease group and the healthy control group. Statistical power analysis was performed using the “pwr” package in RStudio. P<0.05 was considered statistically significant.
Results

Demographic Characteristics of Participants

The demographic characteristics of the participants in this study are presented in Table 1. Among the 310 asthma patients, 123 were male and 187 were female, with a median age of 34 years. Of the 71 allergic rhinitis cases, 38 were male and 33 were female, and the median age was 20 years. The median age of the 72 patients with asthma combined with allergic rhinitis was 28.5 years, including 31 males and 41 females. In addition, 60 healthy individuals were included in the study as controls, consisting of 12 males and 48 females, with a median age of 34 years.

Methodological Assessment of Genotype Identification

A total of 310 asthma patients and 60 healthy individuals were used to evaluate the feasibility of TaqMan MGB qPCR for detecting CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. Venous blood samples were collected and detected by DNA sequencing and TaqMan-MGB qPCR method. Intriguingly, we noticed that CYSLTR1 gene is located on the X chromosome. Due to the differences between male (XY) and female (XX) sex chromosomes, CYSLTR1 gene polymorphism analysis must be grouped by gender. Thus, in the females, CYSLTR1 rs320995 (T927C) locus detection results included TT (homozygous wild type), TC (heterozygous mutant) and CC (homozygous mutant). In contrast, among males, CYSLTR1 rs320995 (T927C) locus detection results only included T (wild type) and C (mutant type). GSDMB rs7216389 (G1199A) locus detection results included GG (homozygous wild type), GA (heterozygous mutant) and AA (homozygous mutant). The amplification plots for genotypes of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms are shown in Figure 1.

The TaqMan-MGB qPCR was used to detect the CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. There were 179 participants that detected as TT/T genotype (48.38%), 126 participants as TC genotype (34.05%) and 65 participants as CC/C genotype (17.57%). As for the results of SNP in GSDMB rs7216389, GG genotype was detected in 32 participants (8.65%), GA genotype in 153 participants (41.35%), and AA genotype in 185 participants (50.00%). The results of DNA sequencing were perfectly consistent with the results of TaqMan-MGB qPCR (Table 2). Based on the concordance analysis, it was shown that the accuracy of TaqMan-MGB qPCR method is the same as that of the conventional method (Kappa = 1, P<0.001).

In addition, in order to evaluate whether the test results would be interfered by bilirubin or lipid, we selected 20 samples which were unrelated to rhinitis disease, including 10 cases of jaundice and 10 cases of lipid blood samples. The detection results of interference samples were 100% consistent with the detection results of DNA sequencing (Supplementary Table 2). It explained that the interference involved in the clinical trial did not affect the detection. Overall, the TaqMan-MGB qPCR method is accurate and reliable for detecting SNPs of CYSLTR1 rs320995 and GSDMB rs7216389.

Analysis of Association of CYSLTR1 rs320995 with Genetic Susceptibility to Asthma and Allergic Rhinitis

After validation of this method, an additional 71 patients with allergic rhinitis and 72 patients with asthma combined with allergic rhinitis were selected and tested for SNPs by using TaqMan-MGB qPCR. The distribution frequencies among

| Group                          | Number of Subjects | Age, Median (IQR) | Sex       |
|-------------------------------|--------------------|-------------------|-----------|
|                               |                    |                   | Male      | Female   |
| Asthma                        | 310                | 34 (23, 47)       | 123       | 187      |
| Allergic rhinitis             | 71                 | 20 (10, 39)       | 38        | 33       |
| Asthma with allergic rhinitis | 72                 | 28.5 (13, 42)     | 31        | 41       |
| Healthy individuals           | 60                 | 34 (31, 39)       | 12        | 48       |

Abbreviation: IQR, interquartile range.
asthmatic patients in CYSLTR1 rs320995 and GSDMB rs7216389 polymorphism with healthy individuals were conform to HWE equilibrium law ($P > 0.05$). We found differential distribution of CYSLTR1 rs320995 genotypes in female patients with asthma combined with allergic rhinitis ($\chi^2 = 6.172$, $P = 0.046$, statistical power $= 0.591$). Specifically, the TT genotype is more frequent in women suffering from asthma with allergic rhinitis (asthma with allergic rhinitis vs healthy individuals: 56.1% vs 31.3%), whereas the TC genotype is more prevalent in healthy women (asthma with allergic rhinitis vs healthy individuals: 36.6% vs 62.5%). (Table 3) However, in other subgroups, no associations of CYSLTR1 rs320995 with asthma and allergic rhinitis were observed.

**Table 2** Detection of the Genotypes of CYSLTR1 rs320995 and GSDMB rs7216389 by DNA Sequencing and TaqMan-MGB qPCR

| Gene Polymorphism | Genotype | DNA Sequencing | TaqMan-MGB qPCR | Kappa | $P$ value |
|-------------------|----------|----------------|----------------|-------|-----------|
| CYSLTR1 rs320995 (T927C) | TT/T    | 179 (48.38%) | 179 (48.38%) | I     | <0.001    |
|                   | TC       | 126 (34.05%) | 126 (34.05%) | I     | <0.001    |
|                   | CC/C     | 65 (17.57%) | 65 (17.57%) | I     | <0.001    |
| GSDMB rs7216389 (G1199A) | GG      | 32 (8.65%)  | 32 (8.65%)  | I     | <0.001    |
|                   | GA       | 153 (41.35%) | 153 (41.35%) | I     | <0.001    |
|                   | AA       | 185 (50.00%) | 185 (50.00%) | I     | <0.001    |
Similarly, we have also evaluated the association of GSDMB rs7216389 with asthma and allergic rhinitis. As shown in Table 4, no significant correlation was identified between GSDMB rs7216389 and asthma or allergic rhinitis in all subgroups.

Discussion

The addition of MGB to TaqMan probes can reduce the generation of non-specific products during amplification by increasing the Tm value of the probes. Due to its high specificity, the TaqMan-MGB qPCR technique has been continuously developed and utilized. Yang et al established a reliable multiplex real-time RT-PCR assay for type A and subtype H10 avian influenza viruses by using the TaqMan-MGB qPCR method. Xue et al developed a rapid, low-cost, easy to-handle, and high-throughput TaqMan-MGB probe qPCR procedure for the quantitative or qualitative detection of three primary Leber hereditary optic neuropathy (LHON) mitochondrial DNA mutations.

As there are currently no commercially available kits for the detection of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms, we have attempted to establish a stable method for the detection of these polymorphisms using TaqMan-MGB qPCR technology. Our data indicated that the TaqMan-MGB qPCR method showed a high degree of concordance with DNA sequencing in detecting CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. We also found that the TaqMan-MGB qPCR method was not interfered with by bilirubin and lipids in the sample thereby confirming its robustness. Apart from this, the TaqMan-MGB qPCR method also has numerous other advantages such as rapidity, high sensitivity, high throughput, cost and time savings. Consequently, we are convinced that the TaqMan-MGB qPCR method we have developed for the detection of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms is promising for clinical application.

Table 3 Analysis of Association of CYSLTR1 rs320995 with Genetic Susceptibility to Asthma and Allergic Rhinitis

| Diagnosis                                | Genotype | Group (%) | χ²   | P   |
|------------------------------------------|----------|-----------|------|-----|
|                                          |          | Cases     | Healthy Individuals |      |
|                                          |          |           |                  |      |
| Asthma (Female)                          | TT       | 72 (38.5) | 15 (31.3)        | 2.054| 0.358|
|                                          | TC       | 96 (51.3) | 30 (62.5)        |      |      |
|                                          | CC       | 19 (10.2) | 3 (6.25)         |      |      |
| Total                                    |          | 187       | 48               |      |      |
| Asthma (Male)                            | T        | 83 (67.5) | 9 (75.0)         | 0.044| 0.834|
|                                          | C        | 40 (32.5) | 3 (25.0)         |      |      |
| Total                                    |          | 123       | 12               |      |      |
| Allergic rhinitis (Female)               | TT       | 15 (45.5) | 15 (31.3)        | 1.883| 0.390|
|                                          | TC       | 17 (51.5) | 30 (62.5)        |      |      |
|                                          | CC       | 1 (3.0)   | 3 (6.3)          |      |      |
| Total                                    |          | 33        | 48               |      |      |
| Allergic rhinitis (Male)                 | T        | 29 (76.3) | 9 (75.0)         | 0.087| 0.768|
|                                          | C        | 9 (23.7)  | 3 (25.0)         |      |      |
| Total                                    |          | 38        | 12               |      |      |
| Asthma with Allergic rhinitis (Female)   | TT       | 23 (56.1) | 15 (31.3)        | 6.172| 0.046*|
|                                          | TC       | 15 (36.6) | 30 (62.5)        |      |      |
|                                          | CC       | 3 (7.3)   | 3 (6.3)          |      |      |
| Total                                    |          | 41        | 48               |      |      |
| Asthma with Allergic rhinitis (Male)     | T        | 21 (67.7) | 9 (75.0)         | 0.009| 0.925|
|                                          | C        | 10 (32.3) | 3 (25.0)         |      |      |
| Total                                    |          | 31        | 12               |      |      |

Note: *P<0.05.

Analysis of Association of GSDMB rs7216389 with Genetic Susceptibility to Asthma and Allergic Rhinitis

Similarly, we have also evaluated the association of GSDMB rs7216389 with asthma and allergic rhinitis. As shown in Table 4, no significant correlation was identified between GSDMB rs7216389 and asthma or allergic rhinitis in all subgroups.

Table 4 Analysis of Association of GSDMB rs7216389 with Genetic Susceptibility to Asthma and Allergic Rhinitis

| Diagnosis                                | Genotype | Group (%) | χ²   | P   |
|------------------------------------------|----------|-----------|------|-----|
|                                          |          | Cases     | Healthy Individuals |      |
|                                          |          |           |                  |      |
| Asthma (Female)                          | TT       | 72 (38.5) | 15 (31.3)        | 2.054| 0.358|
|                                          | TC       | 96 (51.3) | 30 (62.5)        |      |      |
|                                          | CC       | 19 (10.2) | 3 (6.25)         |      |      |
| Total                                    |          | 187       | 48               |      |      |
| Asthma (Male)                            | T        | 83 (67.5) | 9 (75.0)         | 0.044| 0.834|
|                                          | C        | 40 (32.5) | 3 (25.0)         |      |      |
| Total                                    |          | 123       | 12               |      |      |
| Allergic rhinitis (Female)               | TT       | 15 (45.5) | 15 (31.3)        | 1.883| 0.390|
|                                          | TC       | 17 (51.5) | 30 (62.5)        |      |      |
|                                          | CC       | 1 (3.0)   | 3 (6.3)          |      |      |
| Total                                    |          | 33        | 48               |      |      |
| Allergic rhinitis (Male)                 | T        | 29 (76.3) | 9 (75.0)         | 0.087| 0.768|
|                                          | C        | 9 (23.7)  | 3 (25.0)         |      |      |
| Total                                    |          | 38        | 12               |      |      |
| Asthma with Allergic rhinitis (Female)   | TT       | 23 (56.1) | 15 (31.3)        | 6.172| 0.046*|
|                                          | TC       | 15 (36.6) | 30 (62.5)        |      |      |
|                                          | CC       | 3 (7.3)   | 3 (6.3)          |      |      |
| Total                                    |          | 41        | 48               |      |      |
| Asthma with Allergic rhinitis (Male)     | T        | 21 (67.7) | 9 (75.0)         | 0.009| 0.925|
|                                          | C        | 10 (32.3) | 3 (25.0)         |      |      |
| Total                                    |          | 31        | 12               |      |      |

Note: *P<0.05.
Given the diverse etiology of asthma and allergic rhinitis, in which the environment, immunity and genetics may all be involved, the detection of polymorphisms in key genes may contribute to understand genetic susceptibility to the disease. The CYSLTR1 gene has received widespread attention as an influential target for the treatment of asthma. It is not to be neglected that since the CYSLTR1 gene is located on the X chromosome, the genotypes are not the identical for the different sexes: females with two copies of the X chromosome may have a genotype of TT, TC or CC, whereas males with only one copy of the X chromosome can only have a genotype of T or C. In this study, we observed the association of the CYSLTR1 rs320995 polymorphism with female patients suffering from asthma and allergic rhinitis. The TT genotype is more frequent in women suffering from asthma with allergic rhinitis, whereas the TC genotype is more prevalent in healthy women. The association of CYSLTR1 rs320995 polymorphisms with asthma is widely controversial. Hao et al analyzed SNPs of CYSLTR1 in 341 UK asthmatic families and determined that CYSLTR1 rs320995 927T/C is associated with atopy severity but not with asthma. Arriba-Méndez et al found a greater presence of CYSLTR1 rs320995 C allele in the Spanish male population with persistent asthma versus the control group. Likewise, they also revealed the CYSLTR1 rs320995 C allele was significantly more common among patients with asthma presented atopic dermatitis than in health controls. Sanz et al showed that the CYSLTR1 rs320995 C allele was more frequent among Spanish male patients with asthma than controls. On this basis, ethnic variation may be an essential factor in the diversity of conclusions. Moreover, differences in statistical power should be taken into account. Therefore, in the future, high-quality multicenter studies with large samples of various populations should be desired to clarify this issue.

GSDMB which located on chromosome 17q21.1 have also gained growing interest in recent years. A meta-analysis has reported that GSDMB rs7216389 variants is associated with asthma. Zack et al reported that GSDMB rs7216389 polymorphism is associated with chronic rhinosinusitis in a multi-institutional cohort. In our study, the relevance of GSDMB rs7216389 polymorphism with various conditions was not observed in the asthma, allergic rhinitis as well as asthma with allergic rhinitis groups. This finding would be verified by expanding sample sizes in the future.

Inevitably, the TaqMan-MGB qPCR method has its limitations. The kit we tested was only suitable for detecting CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. No other polymorphisms in the CYSLTR1 or GSDMB genes were identified in this clinical study. Clinicians should make a comprehensive judgement of test results based on the patients’ manifestations, drug indications the response to treatment and other laboratory tests.

In summary, we established a reliable TaqMan-MGB qPCR method for the detection of CYSLTR1 rs320995 and GSDMB rs7216389 polymorphisms. We found differential distribution of CYSLTR1 rs320995 genotypes in female patients with asthma combined with allergic rhinitis. Specifically, the TT genotypes are more frequent in women suffering from asthma with allergic rhinitis, whereas the TC genotypes are more prevalent in healthy women.

### Table 4 Analysis of Association of GSDMB rs7216389 with Genetic Susceptibility to Asthma and Allergic Rhinitis

| Diagnosis                        | Genotype | Cases Group (%) | Healthy Individuals Group (%) | \( \chi^2 \) | P   |
|----------------------------------|----------|-----------------|-------------------------------|-------------|-----|
|                                  |          | Cases           | Healthy Individuals           |             |     |
| **Asthma**                       | GG       | 23 (7.4)        | 9 (15.0)                      | 4.493       | 0.106|
|                                  | GA       | 133 (42.9)      | 20 (33.3)                     |             |     |
|                                  | AA       | 154 (49.7)      | 31 (51.7)                     |             |     |
|                                  | Total    | 310             | 60                            |             |     |
| **Allergic rhinitis**            | GG       | 6 (8.5)         | 9 (15.0)                      | 1.554       | 0.460|
|                                  | GA       | 23 (32.4)       | 20 (33.3)                     |             |     |
|                                  | AA       | 42 (59.2)       | 31 (51.7)                     |             |     |
|                                  | Total    | 71              | 60                            |             |     |
| **Asthma with Allergic rhinitis**| GG       | 6 (8.3)         | 9 (15.0)                      | 2.437       | 0.296|
|                                  | GA       | 32 (44.4)       | 20 (33.3)                     |             |     |
|                                  | AA       | 34 (47.2)       | 31 (51.7)                     |             |     |
|                                  | Total    | 72              | 60                            |             |     |

Table 4 Analysis of Association of GSDMB rs7216389 with Genetic Susceptibility to Asthma and Allergic Rhinitis
However, it is necessary to be cautious in interpreting our results due to the limited sample size and statistical power. Multicenter studies with larger sample sizes are required in the future.

**Author Contributions**

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

**Ethics Approval and Informed Consent**

This study has been performed in accordance with the principles stated in the Declaration of Helsinki, our trial was approved by Chinese Academy of Medical Sciences and Peking Union Medical College Hospital Drug Clinical Trial Ethics Committee, registration information: No.002062, ethics approval No. KS2019282. The Clinical Trial Ethics Committee agreed to waive the informed consent in this clinical trial.

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**Disclosure**

The authors report no competing interests.

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