Abstract. This study explored the association of matrix metalloproteinase-9 (MMP-9) serum concentration and gene polymorphism with childhood asthma. Serum levels of MMP-9 were determined by sandwich enzyme-linked immunosorbent assay (ELISA) in 65 children with asthma (cases) and 68 healthy children (controls), and the -1562C/T polymorphism in MMP-9 was detected by polymerase chain reaction and restriction fragment-length polymorphism (PCR-RFLP) analysis. The results showed that the mean serum levels of MMP-9 in the children with asthma (136.53±29.96 ng/ml) were significantly higher than that in the healthy controls (45.08±12.53 ng/ml; P<0.05). At MMP-9 base position -1562, the frequencies of the genotypes CC, CT and TT in cases were 67.7, 29.2 and 3.1% and in controls were 73.5, 25.0 and 1.5%. The allele frequencies of C and T in cases vs. controls were 82.3 and 17.7% vs. 86.0 and 14.0%, respectively. No statistically significant difference was detected in genotype or allele frequency between these groups. In addition, no significant difference in serum levels of MMP-9 was observed within groups among children with different genotypes (P>0.05). Therefore, whereas serum levels of MMP-9 are associated with the occurrence of childhood asthma, the MMP-9 -1562C/T gene polymorphism has no correlation with the pathogenesis of childhood asthma.

Introduction
Bronchial asthma (abbreviated asthma) has a complex etiology that remains to be fully understood. A common consequence of asthma is airway remodeling, resulting from irreversible airflow limitation and persistent airway hyper-responsiveness (1). Excess deposition of extracellular matrix (ECM) in the airway wall is a major cause of airway remodeling in asthma, and is closely related to airway wall fibrosis and airflow limitation (2). Matrix metalloproteinases (MMPs) are a zinc- and calcium-dependent endopeptidase superfamily containing proteases able to degrade most ECM components (3). MMP-9 is particularly able to degrade ECM, and has played a role in the pathogenesis of many inflammatory processes (4). Indeed, studies have shown that MMP-9 is closely correlated with airway inflammatory cell migration and airway hyper-responsiveness in an asthmatic rat model (5). Furthermore, polymorphisms in MMP-9 have been associated with several diseases, including chronic obstructive pulmonary disease (6). To determine the role of MMP-9 in the pathogenesis of asthma, we compared serum MMP-9 concentrations and frequencies of MMP-9 -1562C/T polymorphisms in 65 children with asthma and 68 healthy children.

Patients and methods
Patients. Sixty-five children with asthma, who had been diagnosed and hospitalized at the Pediatric Clinic of the Affiliated Hospital, Hainan Medical College Hospital between January 2010 and January 2011, participated in the present study (cases). This group included 32 males and 33 females, aged 2-13 years, with a mean age of 8.2±2.3. All cases met conventional diagnostic criteria for childhood asthma. The control group was comprised of 68 healthy children from the Physical Examination Center of our hospital, during the same period. This group included 34 males and 31 females, aged 4-14 years, with a mean age of 9.7±2.7. No significant differences were observed in age and gender between the two groups of children.

Determination of MMP-9 serum levels. Venous blood samples were collected from all participants. Five milliliters was centrifuged (4°C, 2000 rpm), sub-packaged, and cryopreserved at -20°C. Serum MMP-9 was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using various kits (Bio-Key International Inc., Wall, NJ, USA), according to the manufacturer's instructions. OD values (492 nm wavelength) were assessed on a microplate reader (BioRad-550).

Detection of the -1562C/T polymorphism of MMP-9. DNA was extracted from peripheral blood according to Lahiri et al (7). Primers for polymerase chain reaction (PCR) included the following: forward, 5'-GCCTGGCACATAGTAGGCCC-3' and reverse, 5'-CTTCCTAGCCAGCCGGCATC-3', which were used for polymorphism detection according to a previous report (8). The reaction mixture (total 50 µl) contained 1.0 µg template DNA, 0.2 mM dNTP, 4 U TaqDNA polymerase, 2.0 mM MgCl₂, 1X PCR buffer, 60 ng forward primer, and

Correspondence to: Dr Zhu Hong, Department of Clinical Laboratory, Affiliated Hospital of Hainan Medical College, Longhua Road 31, Haikou, Hainan 571102, P.R. China
E-mail: zhu_hong1970@126.com

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60 ng reverse primer. Cycling conditions were as follows: pre-denaturation at 95°C for 5 min; denaturation at 94°C for 1 min, annealing at 63°C for 1 min and extension at 74°C for 1 min, 30 cycles total; and final extension at 72°C for 10 min. Amplified products were resolved in 2% agarose gel. Restriction digestion was performed on 15 µl PCR with 5 U SphI (MBI product) at 37°C for 16 h. Restriction fragments were resolved in 2% agarose gel.

Statistical methods. SPSS 13.0 statistical software was used to analyze data. Single-factor analysis of variance was used to compare MMP-9 serum levels between the groups. The χ² and t-test were used to compare genotypes and gene frequencies. All analyses were two-sided tests, and α=0.05 was considered statistically significant.

Results

Serum MMP-9 levels in children with asthma. Mean MMP-9 serum levels were 136.53±29.96 ng/ml in the children with asthma and 45.08±12.53 ng/ml in the healthy children (Fig. 1). This difference in circulating MMP-9 was statistically significant (t=23.139, P<0.001), indicating that MMP-9 was highly expressed in children with asthma.

The -1562C/T polymorphism of MMP-9 in children with asthma. As expected, PCR-RFLP produced 2 bands (194 and 242 bp) for the TT genotype, 3 bands (436, 194, and 242 bp) for the heterozygous TC genotype, and 1 band (436 bp) for the CC genotype (Fig. 2). As shown in Table I, genotype frequencies for MMP-9 -1562C/T were 67.7 CC, 29.2 CT, and 3.1% TT for cases and 73.5 CC, 25.0 CT, and 1.5% TT for controls. These genotype distributions were not significantly different, suggesting that polymorphism at MMP-9 base position -1562 are not associated with asthma. Similarly, allele frequencies of C and T at MMP-9 -1562 were 82.3 and 17.7%, respectively, in the cases, 86.0 and 14.0%, respectively, in the controls. Again, these frequencies were not significantly different between the groups, with the relative risk odds ratio equal to 0.755 (95% confidence interval: 0.390-1.464).

MMP-9 serum levels by MMP-9 genotype. To determine whether the MMP-9 genotype was associated with higher levels of circulating MMP-9 in children with asthma, we assessed serum MMP-9 according to genotype within each group (cases and controls). In children with asthma, mean circulating MMP-9 levels were 133.59±29.50 ng/ml for the CC genotype, 140.67±31.52 ng/ml for the CT genotype, and 161.56±13.69 ng/ml for the TT genotype (Fig. 3); these
differences were not statistically significant. In the healthy children, mean circulating MMP-9 levels were 44.22±12.721, 46.08±11.90 and 58.46±7.94 ng/ml for the CC, CT, and TT genotypes, respectively; these differences were not statistically significant. These findings suggest that the -1562 polymorphism at MMP-9 does not affect circulating MMP-9 protein levels.

Discussion

MMP-9 identified in 1974 by Sopata and Dancewicz (9), has been associated with many disease processes, including those of respiratory diseases. MMPs, particularly MMP-9, break down most components of the extracellular matrix (ECM) (3) by degrading structural proteins such as collagen and elastin. MMP-9 is believed to play an important role in airway remodeling in chronic airway diseases, including asthma (10,11). Indeed, studies have shown that MMP-9 expression is closely related to the severity of asthma (12).

In the present study, we assessed expression levels of MMP-9 in 65 children with asthma and 68 healthy children. Notably, we observed significantly higher circulating levels of MMP-9 in children with asthma. To determine whether polymorphisms in the MMP-9 gene are also associated with the pathogenesis of asthma, we investigated MMP-9 genotypes for a previously identified SNP, -1562C/T, in our study population. However, we did not detect any significant differences in either the MMP-9 genotypes or allele frequencies for this polymorphic locus between children with asthma and healthy control individuals. Moreover, MMP-9 serum levels were not associated with particular genotypes. These findings suggest that, while circulating levels of MMP-9 are higher in children with asthma, polymorphisms at position -1562 in the gene are neither responsible for increased production of MMP-9 nor associated with the pathogenesis of asthma. Thus, the increased production of MMP-9 appears to be related to the inflammatory process of asthma, but is likely stimulated by other genes in the process.

In conclusion, while the etiology of asthma remains to be fully elucidated, MMP-9 plays a role in its pathogenesis (13). We found that children with asthma exhibit increased levels of MMP-9 in the serum. The lack of correlation between MMP-9 genotype and either asthma incidence or circulating MMP-9 levels indicates that further studies are required to identify the genetic components affecting MMP-9 expression.

References