Significant Association of MMP2 Promoter Genotypes to Asthma Susceptibility in Taiwan

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Abstract. Background/Aim: Matrix metalloproteinase 2 (MMP2) is reported to be overexpressed in asthma; however, its genotypic contribution to asthma is not well studied. Therefore, we examined the association of MMP2 genotypes with asthma risk among Taiwanese. Materials and Methods: One hundred and ninety-eight asthmatic patients and 453 non-asthmatic subjects were determined with respect to their MMP2 -1306 (rs243845) and -735 (rs2285053) genotypes. Results: CT and TT at MMP2 rs243845 are 17.7% and 1.5% among asthmatic cases, whereas their presence in healthy subjects is at 28.1% and 2.4%, respectively (p for trend=0.0118). In detail, the CT genotype in MMP2 rs243845 was associated with a decreased asthma risk [adjusted odds ratio (OR)=0.57, 95% confidence interval (CI)=0.37-0.78, p=0.0040], and the T allele conferred a significantly lower asthma risk compared to the wild-type C allele (adjusted OR=0.55, 95%CI=0.43-0.77, p=0.0042). No significance was found for MMP2 rs2285053. Conclusion: The genotype of CT in MMP2 rs243845 may serve as a novel biomarker in determining susceptibility to asthma in Taiwan.

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Asthma, typically characterized by airflow obstruction, airway inflammation and remodeling, is a complex disease with variable phenotypes in triggering coughing, making a wheezing sound and causing shortness of breath (1, 2). Over the past 30 years, the number of patients diagnosed with asthma has significantly increased (3), collectively comprising more than 300 million cases all over the world (4). In 2017, the annual global incidence of asthma was 43.12 million new cases (0.56%), while global prevalence and mortality accounted for 272.68 million cases (3.57%) and 0.49 million deaths (0.006%), respectively (5). Etiologically, asthma is determined by a complex interaction of genomic and environmental factors, while the contribution of heritability to the susceptibility to asthma has been estimated to vary between 36 to 79 percent (6, 7). Although the concept that the genetic inheritance plays a role in the pathogenesis of asthma has been recognized for more than one century (3), the exact mechanisms of asthma etiology are still poorly understood while asthma is believed to progresses with lots of unidentifiable and unpredictable causes. Following the code-breaking of human genome, more than 200 asthma candidate genes have been proposed (8, 9). Still, although it is intensely studied, lots of difficulties remain in figuring out the role of the specific causal genes and determining whether ethnic disparities should be attributed to the genetic control of certain factors leading to asthma.

Extracellular matrix (ECM) components play a critical role in providing support for surrounding cells and in regulating the remodeling of the cell micro-environment (10). Similarly, with the progression of asthma the airway undergoes structural and biochemical remodeling (11). Matrix metalloproteinases (MMPs), also known as matrixins, are a group of endopeptidases involved in ECM remodeling by degrading ECM components (10, 12). Many MMPs are
involved with the pathogenesis and modulation of the severity of asthma, among which, MMP-9 is the predominant one; however, MMP-2 is also gaining ground (13).

From the viewpoint of genetic contribution towards asthma risk, the MMP9 gene has been examined a few times (14-21), while MMP2 has not (13). The latter, composed of 12 introns and 13 exons, is located on chromosome 16q21 in the human genome (22). The MMP2 promoter C-1306T (rs243865) and C-735T (rs2285053) polymorphisms have been reported to affect the expression of MMP2 at both mRNA and protein levels, leading to variable susceptibilities to several types of cancer, including oral, nasopharyngeal, esophageal, breast, lung, and colon cancer (23-28). In addition, MMP2 has been found to be upregulated among patients with oral squamous cell carcinoma, especially those with lymph node metastasis (29). The only study investigating the contribution of MMP2 polymorphisms to asthma is by Toujani and his colleagues, who have reported that these are not associated with asthma risk in a population containing 150 asthma cases and 150 controls (13). Most importantly, MMP2 protein is in charge of the degradation of its various substrates, including MMP-13, and fibrillar collagen, elastin, endothelin, fibroblast growth factor, plasminogen, TGF-β, as well as MMP9 (30). Based on the evidence above showing that MMP2 genotypes may also involve in the susceptibility determination of asthma as other human diseases, the present case-control study aims at examining the contributions of MMP2 promoter C-1306T (rs243865) and C-735T (rs2285053) polymorphisms to the susceptibility of asthma in Taiwan.

Materials and Methods

Cohort. A total of 198 patients with asthma were recruited at the China Medical University Hospital from 2008 to 2010. Their medical histories were reviewed, and the data were collected into the hospital database. At the same period, 453 healthy individuals, matched with these patients by age (±5 years), were admitted to the exact same hospital for their health checkup (similar residential areas and same background, i.e., Taiwanese) with no previous diagnosis of neoplastic disease or any malignancy and were selectively enrolled into our matched control group. All the participants enrolled in this study were asked to provide their informed consent for publishing their tissue analysis results and other data. All the protocols of the current study were approved by the Human Research Committees of China Medical University Hospital (CMUH106-REC1-004). From each participant, 5 ml of venous blood sample was collected and used for DNA extraction and subsequent genotyping as described below. Demographics of the cohort and controls have also been used in our previous study (31) and are summarized in Table I.

**Table I. Distribution of age and gender among the 198 asthma patients and 453 controls (from 31).**

| Index | Controls (n=453) | Cases (n=198) | p-Value* |
|-------|-----------------|---------------|----------|
| Age (years) | | | |
| 25-40  | 285             | 133            | 0.2972   |
| >40    | 168             | 65             |          |
| Gender | | | |
| Male   | 190             | 83             |          |
| Female | 263             | 115            |          |

*Based on chi-square without Yate’s correction test.

MMP2 genotyping. Each participant’s genomic DNA was extracted from peripheral blood leukocytes within 24 h, carefully quantitated, diluted and stored at −80°C until further processing, as previously described (31-34). In the current study, the genotypes at MMP2 -1306 (rs243845) and -735 (rs2285053) were determined for all the investigated subjects via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using BioRad Mycycler (BioRad, Hercules, CA, USA). All PCR reactions were uniformly performed using the primers presented in Table II and the following thermal program: i) an initial cycle at 94°C for 5 min, ii) 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and iii) a final extension at 72°C for 10 min. The PCR DNA amplicons were digested by the corresponding restriction endonucleases Xsp I and Hinf I (New England Biolabs, Taipei, Taiwan) overnight, and their fragment were separated by 3% agarose gel electrophoresis under 100 Volt, stained with ethidium bromide, observed and taken pictured under UV-irradiation, and finally identified of their individual genotypes. All genotypic procedures were repeated by at least two researchers independently and blindly, and their results turned out to be 100% concordant with each other.

**Results**

The frequency distributions of selected demographic indexes, such as age and gender for the 198 asthma patients and 453 non-asthmatic controls have also been used in our previous study (31) and are presented in Table I.

The distribution of the MMP2 rs243865 and rs2285053 genotypes among the 198 asthma cases and the 453 non-asthmatic controls are presented and compared in Table III. First, the distributions of genotypes of both MMP2 rs243865 and rs2285053 in control and case groups fit well with Hardy-Weinberg Equilibrium (all p>0.05). Second, the
results show that the genotype of \textit{MMP2} rs2285053 among Taiwan citizens are not differently distributed between the asthma patients and the healthy control groups (Table III bottom part). Third, interestingly, the genotypes of \textit{MMP2} rs243845 are differentially distributed between the two groups ($p$ for trend=0.0118) (Table III top part). In detail, the \textit{MMP2} rs243845 heterozygous variant CT was associated with a decreased asthma risk, compared to the wild-type CC genotype (adjusted OR=0.57, 95%CI=0.37-0.78, $p$=0.0040; Table III top part).

Concerning the homozygous variant TT in \textit{MMP2} rs243845, this was not associated with a significantly altered asthma risk, compared to the wild-type CC genotype (adjusted OR=0.66, 95%CI=0.19-1.98, $p$=0.3377; Table III top part). To confirm these findings, we put the CT and TT groups into one. The results show that there is an association between the CT+TT genotype at \textit{MMP2} rs2285053 and decreased asthma risk, compared to CC wild-type genotype in the dominant model analysis, (adjusted OR=0.58, 95%CI=0.38-0.77, $p$=0.0118; Table III top part).

To confirm the novel findings presented in Table III, we also analyzed the allelic frequency distribution of \textit{MMP2} rs243845 and rs2285053 (Table IV). The results show that the variant T allele in \textit{MMP2} rs243845 was associated with a relatively lower asthma risk compared to the wild-type C allele. On the other hand, the T allele in \textit{MMP2} rs243845 was not a determinant of asthma risk for Taiwanese. In detail, the variant allele T was found to be at 10.4% in the asthma group, much lower compared to the 16.4% in the control group (adjusted OR=0.55, 95%CI=0.43-0.77, $p$=0.0042 (Table IV top part). On the contrary, there was no such significant difference in \textit{MMP2} rs2285053 between the two groups examined (Table IV bottom part).

**Discussion**

In the current study, the contribution of \textit{MMP2} to Taiwan asthma risk is firstly revealed among a representative population containing 358 asthma patients and 716 age- and gender-matched non-asthmatic subjects in Taiwan. Our results suggest that the heterozygous variant CT genotype of \textit{MMP2}...
rs243845, alone or together with TT, could serve as a protective biomarker for asthma in Taiwan. Although the finding is not consistent with that of the only one group having investigated the contribution of MMP2 genotype to asthma (13), and the detail mechanisms are still unrevealed, the findings are interesting and should be explored further. Two possible explanations behind the differences between the current study with the previous one are: i) the genetic background of the investigated populations (Taiwanese vs. Caucasian), and ii) differences in the sample size (our cohort involved 198 asthma patients and 453 non-asthmatic subjects while theirs involved 150 patients in each group). In the future, more investigations including different ethnic backgrounds and larger cohorts would be useful to validate the significance of the MMP2 rs243845 genotypes in asthma risk.

MMP2 protein is critically important in the regulation of extracellular components by metabolizing its various substracts (30). In the literature, overexpressed MMP2 has been reported in several tumor sites, and is correlated with poor prognosis in melanoma, colorectal, breast, ovarian, prostate and lung cancer (35). Now, we found that the MMP2 rs243845 is associated with a decreased risk of asthma. The genotype at MMP2 rs243845 has been reported to enhance the occurrence rates of bladder cancer (36) and sclerosing cholangitis (37).

Concerning molecular interactions, the promoter region of MMP2 bears specific binding cites for several transcription factors, including activator protein-1 (AP-1), specificity protein-1 (SP-1) and activator protein-2 (AP-2), which can regulate the transcriptional activity of MMP2 (38, 39). The most direct evidence strengthening the importance of MMP2 rs243845 genotypes comes from two studies. The first one found that substituting the C nucleotide with a T in MMP2 rs243845 inactivated the SP-1 binding region and led to down-regulation of the transcriptional and translational expression levels of MMP2 (40). The second study revealed that MMP2 is indeed overexpressed among people carrying the CC genotype in MMP2 rs243845, compared to those with the CT and TT genotypes (41). In addition, the MMP2 activity was enhanced in people carrying the MMP2 rs243845 CC genotype, compared to those carrying the variant CT and TT genotypes (41). The genotype-phenotype correlation in the MMP2 rs243845 SNPs and their contribution to asthma etiology should be revealed through further investigations.

We are interested in further investigating the genotypes of other MMPs, such as MMP9, which will also help understanding its role in ECM dysregulation and how this is involved in asthma etiology. In addition to MMP9, several other genes whose coded protein products closely interact with MMP2, such as the chemokine (C-C motif) ligand (CCL7) (42), tissue inhibitor of metalloproteinases 2 (TIMP 2) (43, 44), tissue inhibitor of metalloproteinases 4 (TIMP 4) (45, 46), thrombospordin1s 1 and 2 (THBS 1, THBS 2) (47), are also in our interest to examine in the future.

In conclusion, these results provide evidence that the variant CT genotypes at the MMP2 promoter rs243845 may play a role in determining susceptibility to asthma in the Taiwanese.

Conflicts of Interest

The Authors declare no conflicts of interest with regard to this study.

Authors’ Contributions

Research design was done by CLH, CKL and HTC; patient and questionnaire summaries by HTC and STC; experimental work by CWS and TCW; statistical analysis by LCH, SYC, and LYH; manuscript writing by CLH and BDT; manuscript review and revision by TCW and BDT.

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References

1. Holgate ST: Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol 104(6): 1139-1146, 1999. PMID: 10588993. DOI: 10.1016/S0091-6749(99)70005-9
2. Madore AM, Perron S, Turmel V, Laviolette M, Bissonnette EY and Laprise C: Alveolar macrophages in allergic asthma: an expression signature characterized by heat shock protein pathways. Hum Immunol 71(2): 144-150, 2010. PMID: 19913588. DOI: 10.1016/j.humimm.2009.11.005
3. Steinke JW, Rich SS and Borish L: 5. Genetics of allergic disease, J Allergy Clin Immunol 121(2 Suppl): S384-387; quiz S416, 2008. PMID: 18241687. DOI: 10.1016/j.jaci.2007.07.029
4. Himes BE, Hunninghake GM, Baurley JW, Raufman JS, Meulman B, Strachan DP, Wilk JB, Willis-Owen SA, Klanderer B, Lasky-Su J, Lazarus R, Murphy AJ, Soto-Quiros ME, Avila L, Beaty T, Mathias RA, Ruczinski I, Barnes KC, Celedon JC, Cookson WO, Gauderman WJ, Gilliland FD, Hakonarson H, Lange C, Moffatt MF, O’Connor GT, Raby BA, Silverman EK and Weiss ST: Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. Am J Hum Genet 84(5): 581-593, 2009. PMID: 19426955. DOI: 10.1016/j.ajhg.2009.04.006
5. Mattiuzzi C and Lippi G: Worldwide asthma epidemiology: insights from the Global Health Data Exchange database. Int Forum Allergy Rhinol 10(1): 75-80, 2020. PMID: 31645084. DOI: 10.1002/inf.25246
6. Koppelman GH: Gene by environment interaction in asthma, Curr Allergy Asthma Rep 6(2): 103-111, 2006. PMID: 16566859. DOI: 10.1007/s11882-006-0047-y
21 Jimenez-Morales S, Martinez-Aguilar N, Gamboa-Becerra R, Hong Z, Lin YM, Qin X and Peng JL: Serum MMP-9 is elevated.

22 Ye S: Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol 19(7): 623-629, 2000. PMID: 11102751. DOI: 10.1016/s0945-053X(00)00102-5

23 Tsai CW, Hsu HM, Wang YC, Chang WS, Shih LC, Sun KT, Hung YW, Yang YC, Gong CL and Bau DT: Contribution of MMP2 promoter genotypes to oral cancer susceptibility, recurrence and metastasis in Taiwan. Anticancer Res 38(12): 6923-6928, 2018. PMID: 30504396. DOI: 10.1186/1465-9921-9-4

24 Hsu SW, Lon CL, Hsu HM, Chao CC, Wang YC, Chang WS, Tsai YT, Shih LC, Tsai CW and Bau DT: Contribution of matrix metalloproteinase-2 promoter genotypes to nasopharyngeal cancer susceptibility and metastasis in Taiwan. Cancer Genomics Proteomics 16(4): 287-292, 2019. PMID: 31243109. DOI: 10.21873/cgg.20133

25 Waleh NeS, Murphy BJ and Zaveri NT: Increase in tissue inhibitor of metalloproteinase-2 (TIMP-2) levels and inhibition of MMP-2 activity in a metastatic breast cancer cell line by an anti-inflammatory small molecule SR13179. Cancer Lett 289(1): 111-118, 2010. PMID: 19751965. DOI: 10.1016/j.canlet.2009.08.006

26 Chen GL, Wang SC, Shen TC, Tsai CW, Chang WS, Li HT, Wu CN, Chao CY, Hsia TC and Bau DT: The association of metalloproteinase-2 promoter polymorphisms with lung cancer susceptibility in Taiwan. Chin J Physiol 62(5): 210-216, 2019. PMID: 31670285. DOI: 10.4103/cjphysiol.cjphysiol.4319

27 Gröbelska M, Mroczko B, Kozlowski M, Nikłinski J, Laudanski J and Szmitkowski M: Serum matrix metalloproteinase-2 and tissue inhibitor of matrix metalloproteinases-2 in esophageal cancer patients. Folia Histochem Cytobiol 50: 590-598, 2012. PMID: 23264224. DOI: 10.5603/20327

28 Kapral M, Wawszczyk J, Jurzak M, Dymitruk D and Weglarz L: Evaluation of the expression of metalloproteinases 2 and 9 and their tissue inhibitors in colon cancer cells treated with phytic acid. Acta Pol Pharm 67(6): 625-629, 2010. PMID: 21229878.

29 Patel BP, Shah PM, Rawal UM, Desai AA, Shah SV, Rawal RM and Patel PS: Activation of MMP-2 and MMP-9 in patients with oral squamous cell carcinoma. J Surg Oncol 90(2): 81-88, 2005. PMID: 15841488. DOI: 10.1002/jso.20240

30 Nagase H and Woessner Jr JF Jr.: Matrix metalloproteinases. J Biol Chem 274(31): 21491-21499, 1999. PMID: 10419448. DOI: 10.1074/jbc.274.31.21491

31 Hsiao, WY, Tsai CW, Chang WS, Wang S, Chao CY, Chen WC, Shen TC, Hsia TC and Bau DT: Association of polymorphisms in MMP2 promoters: implication in regulation of gene expression and susceptibility in asthma. Mol Immunol 74(8): 998-1002, 2013. PMID: 23695553. DOI: 10.1016/j.molimm.2013.04.036

32 Chen et al: MMP2 Genotypes in Asthma

33 Shih LC, Chang WS, Lee HT, Wang YC, Wang ZH, Chao CY, Yu CC, Lin HY, Shen TC, Kuo CC, Tsai CW and Bau DT: Interaction of interleukin-16 genotypes with betel quid chewing behavior on oral cancer in Taiwan. In Vivo 34(4): 1759-1764, 2020. PMID: 32606144. DOI: 10.21873/inivo.11969

34 Li MQ, Wang YC, Shen TC, Chang WS, Li HT, Liao CH, Gong CL, Wang ZH, Tsai CW, Hsia TC and Bau DT: Significant...
association of interleukin-16 genetic variations to Taiwanese lung cancer. In Vivo 34(3): 1117-1123, 2020. PMID: 32354900. DOI: 10.21873/inivo.11883

35 Björklund M and Koivunen E: Gelatinase-mediated migration and invasion of cancer cells. Biochim Biophys Acta 1755(1): 37-69, 2005. PMID: 15907591. DOI: 10.1016/j.bbcan.2005.03.001

36 Wieczorek E, Reszka E, Jablonowski Z, Jablonska E, Krol MB, Grzegorczyk A, Gromadzinska J, Sosnowski M and Wasowicz W: Genetic polymorphisms in matrix metalloproteinases (MMPs) and tissue inhibitors of MPs (TIMPs), and bladder cancer susceptibility. BJU Int 112(8): 1207-1214, 2013. PMID: 23819551. DOI: 10.1111/bju.12230

37 Korkmaz KS, de Rooij BJ, van Hoek B, Janse M, Coenraad MJ, van der Reijden JJ, Weersma RK, Porte RJ, Voorneveld PW, Baranski AG and Verspaget HW: MMP-2 is a disease-modifying gene in primary sclerosing cholangitis. Liver Int 34(2): 274-280, 2014. PMID: 23809662. DOI: 10.1111/liv.12237

38 Singh N, Hussain S, Sharma U, Suri V, Nijhawan R, Bharadwaj M and Sobti RC: The protective role of the -1306C>T functional polymorphism in matrix metalloproteinase-2 gene is associated with cervical cancer: implication of human papillomavirus infection. Tumour Biol 37(4): 5295-5303, 2016. PMID: 26564167. DOI: 10.1007/s13277-015-4378-y

39 Eftekhary H, Ziaee AA, Yazdanbod M, Shahpanah M, Setayeshgar A and Nassiri M: The influence of matrix metalloproteinase-2, -9, and -12 promoter polymorphisms on Iranian patients with oesophageal squamous cell carcinoma. Contemp Oncol (Poln) 19(4): 300-305, 2015. PMID: 26557778. DOI: 10.5114/wo.2015.48569

40 Price SJ, Greaves DR and Watkins H: Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 276(10): 7549-7558, 2001. PMID: 11114309. DOI: 10.1074/jbc.M010242200

41 Bchir S, Nasr HB, Anes AB, Benzarti A, Garrouch A, Tabka Z and Chahed K: MMP-2 (-1306 C/T) polymorphism affects serum matrix metalloproteinase (MMP)-2 levels and correlates with chronic obstructive pulmonary disease severity: A case-control study of MMP-1 and -2 in a Tunisian population. Mol Diagn Ther 20(6): 579-590, 2016. PMID: 27412345. DOI: 10.1007/s40291-016-0225-0

42 McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I and Overall CM: Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. Science 289(5482): 1202-1206, 2000. PMID: 10947989. DOI: 10.1126/science.289.5482.1202

43 Morgunova E, Tuuttila A, Bergmann U and Tryggvason K: Structural insight into the complex formation of latent matrix metalloproteinase 2 with tissue inhibitor of metalloproteinase 2. Proc Natl Acad Sci USA 99(11): 7414-7419, 2002. PMID: 12032297. DOI: 10.1073/pnas.102185399

44 Overall CM, Tam E, McQuibban GA, Morrison C, Wallon UM, Bigg HF, King AE and Roberts CR: Domain interactions in the gelatinase A,TIMP-2,MT1-MMP activation complex. The ectodomain of the 44-kDa form of membrane type-1 matrix metalloproteinase does not modulate gelatinase A activation. J Biol Chem 275(50): 39497-39506, 2000. PMID: 10991943. DOI: 10.1074/jbc.M005932200

45 Bigg HF, Shi YE, Liu YE, Steffensen B and Overall CM: Specific, high affinity binding of tissue inhibitor of metalloproteinases-4 (TIMP-4) to the COOH-terminal hemopexin-like domain of human gelatinase A. TIMP-4 binds progelatinase A and the COOH-terminal domain in a similar manner to TIMP-2. J Biol Chem 272(24): 15496-15500, 1997. PMID: 9182583. DOI: 10.1074/jbc.272.24.15496

46 Kai HS, Butler GS, Morrison CJ, King AE, Pelman GR and Overall CM: Utilization of a novel recombinant myoglobin fusion protein expression system to characterize the tissue inhibitor of metalloproteinase (TIMP)-4 and TIMP-2 C-terminal domain and tails by mutagenesis. The importance of acidic residues in binding the MMP-2 hemopexin C-domain. J Biol Chem 277(50): 48696-48707, 2002. PMID: 12374789. DOI: 10.1074/jbc.M209177200

47 Bein K and Simons M: Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. J Biol Chem 275(41): 32167-32173, 2000. PMID: 10900205. DOI: 10.1074/jbc.M003834200

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