



Contents lists available at SciVerse ScienceDirect



journal homepage: www.elsevier.com/locate/humimm



Polymorphisms in metalloproteinase-9 are associated with the risk for asthma in Mexican pediatric patients

Silvia Jiménez-Morales^a, Nora Martínez-Aguilar^b, Roberto Gamboa-Becerra^a, Juan Luis Jiménez-Ruiz^a, Diana López-Ley^a, Hong Lou^c, Yolanda Saldaña-Alvarez^a, Michael Dean^d, Lorena Orozco^{a,*}

^a Laboratorio de Inmunogenómica y Enfermedades Metabólicas, Instituto Nacional de Medicina Genómica, SS, Mexico City, Mexico

^b Hospital Pediátrico de Coyoacan, SS, Mexico City, Mexico

^c Human Genetics Section, Basic Research Program, SAIC-Frederick Inc., National Cancer Institute-Frederick, Frederick, MD 21702, USA

^d Cancer and Inflammation Program, Laboratory of Experimental Immunology, Center for Cancer Research, National Cancer Institute-Frederick, Frederick, MD 21702, USA

ARTICLE INFO

Article history:

Received 26 September 2012

Accepted 10 April 2013

Available online xxxx

ABSTRACT

Asthma is characterized by chronic airway inflammation, which induces airway remodelling of the extra-cellular matrix over time. Matrix metalloproteinases (MMPs) are involved in this process, and single-nucleotide polymorphisms (SNPs) in MMP genes may influence their mRNA expression levels or abilities to bind substrates and inhibitors, thereby contributing to asthma predisposition and severity. MMP-9 is highly expressed in airways and many studies support its involvement in asthma pathogenesis; however the contribution of *MMP-9* SNPs is controversial. To investigate whether *MMP-9* SNPs are associated with childhood-onset asthma in Mexican patients. We conducted a case-control study including 403 children with clinical asthma diagnoses and 426 healthy controls from Mexico. The cases and controls were matched by ethnicity and gender. We found that the SNPs rs2274755, rs17577, and rs3918249 were associated with asthma risk. The most significant associations were with rs2274755 (OR = 2.10, 95% CI 1.31–3.39, $P = 0.001$) and rs17577 (OR = 2.07, 95% CI 1.29–3.30, $P = 0.001$); which were in strong linkage disequilibrium. Both SNPs were also associated with atopic asthma (OR = 2.38, 95% CI 1.44–3.96, $P = 0.0005$). The SNP rs3918249 exhibited a female gender-dependent association with asthma (OR = 1.66, 95% CI 1.14–2.43, $P = 0.007$). Our results suggest that *MMP-9* polymorphisms could play a role in the susceptibility to childhood-onset asthma.

© 2013 Published by Elsevier Inc. on behalf of American Society for Histocompatibility and Immunogenetics.

1. Introduction

The matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases comprising over 26 proteins with homologous sequences, which can be divided into collagenases, stromelysins, matrilysins, and gelatinases [1–4]. The wide variability of MMPs explains their implication in a range of pathological conditions, including tissue destruction, matrix weakening, and fibrosis [1,5–8]. The available data support a major role for MMPs in lung development and architecture [4,6,8,9]. MMPs can be produced by a variety of inflammatory cells in the respiratory tract, where they mediate airway remodelling and cell trafficking [4,10,11]. MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 (or gelatinase B) are each overexpressed in asthmatic and allergic patients [6–8]; however, MMP-9 shows the highest activity in the airway and exhibits proteolytic activity against the major

component of the basement membrane (type IV collagen) [6,12–15]. In chronic obstructive pulmonary disease (COPD) and allergic inflammation, it has been observed that MMP-9 facilitates the recruitment of inflammatory cells through the airway, bronchial epithelial migrations, and alveolar destruction and permeability [1,8,14]. Furthermore, asthmatic patients exhibit high levels in serum and sputum, and has enhanced gelatinolytic activity of this endopeptidase [1,15–19]. Otherwise, functional studies of *MMP-9* polymorphisms have also contributed to establishing a correlation between this MMP and asthma pathogenesis; the –1562T (rs3918242) allele of *MMP-9* increases the transcription activity of the promoter [20].

Despite the abundance of evidence indicating the role of MMP-9 in asthma pathogenesis, few epidemiological studies have focused on the associations between *MMP-9* single nucleotide polymorphisms (SNPs) with asthma susceptibility, and the available data are still controversial [20–23]. Here we conducted a case-control study in pediatric patients from Mexico to determine whether *MMP-9* polymorphisms are associated with asthma in this population.

* Corresponding author. Address: Instituto Nacional de Medicina Genómica, Periférico Sur No. 4809, Col. Arenal Tepepan, Delegación Tlalpan, CP 14610, México, D.F. Fax: +52 55 5350 1968.

E-mail address: lorozco@inmegen.gob.mx (L. Orozco).

2. Materials and methods

2.1. Studied population

Asthma diagnoses were made based on the definition used by the American Thoracic Society and the Global Initiative for Asthma (GINA) criteria. We recruited 403 asthmatic patients of less than 17 years of age from four tertiary-level institutions located in Mexico City. As healthy controls, we included 426 unrelated healthy subjects, older than 18 years of age and without autoimmune or inflammatory history. Patients and controls were previously ethnically matched using 10 validated Ancestry Informative Markers (AIMs) [24]. We included 319 cases with allergy information available to investigate the association between MMP-9 polymorphisms and atopy.

The study was conducted with the approval of the ethics and research committees from all participating institutions. Parents provided written consent for their child's participation, and the children assented.

2.2. Genotyping analysis

Genomic DNA was extracted from blood samples using the QiaAmp Blood Kit (Qiagen, Valencia, CA).

The SNPs included were selected because of their potential function and previous association with lung pathologies (Table 1) [20,23]. Genotyping analysis of the polymorphisms rs3918242 C/T (−1562), rs3918245 T/G (−386), rs41427445 A/G (Asn38Ser), rs3918249 T/C, rs2274755 G/T, rs17576 A/G (Gln279Arg), rs2250889 C/G, rs17577 G/A (Arg668Gln), and rs9509 T/C) was performed by allelic discrimination assay using TaqMan probes (Applied Biosystems, Foster City, CA, USA). The overall genotype call rate was 99% and the genotyping reproducibility was 100% in 100 randomized samples genotyped in duplicate. The genotyping of rs3918242 was carried out by the PCR-restriction fragment length polymorphism (PCR-RFLP) method, using the procedures proposed by Sadeghi et al. [25]. Meanwhile, the polymorphism rs2250889 C/G (Arg574Pro) was detected by PCR-mediated site-directed mutagenesis (PSM). The oligonucleotide primer sequences were those described previously [26].

To confirm the genotypes, 10 samples, including random homozygote and heterozygote individuals, were sequenced directly with a DNA Sequencing Kit employing the Big Dye Terminator (ABI PRISM 3100 DNA sequencer; Applied Biosystems); primer sequences are available by request.

2.3. Functional prediction analysis

We predicted the potential effects of the MMP-9 SNPs that were associated with asthma in our population using FastSNP [27], SNP Function Prediction (<http://snpinform.niehs.nih.gov/snpfunc.htm>),

Table 1
Polymorphisms of MMP-9 included in the present study.

SNP	Region ^a	Location in MMP-9 gene	Allele
rs3918242	Chr20:44069383	promoter (−1562)	C/T
rs3918245	Chr20:44070317	promoter (−386)	T/G
rs41427445	Chr20:44071085	Exon 1	A/G
rs3918249	Chr20:44071543	Intron 1	T/C
rs2274755	Chr20:44073099	Intron 4	G/T
rs17576	Chr20:44073632	Exon 6	A/G
rs2250889	Chr20:44075813	Exon 10	C/G
rs17577	Chr20:44076518	Exon 12	G/A
rs9509	Chr20:44078560	3'UTR	T/C

^a Positions are from NCBI dbSNP Build 137 for Human.

Human-transcriptome DataBase for Alternative Splicing (<http://www.h-invitational.jp/h-dbas/>), SNPs3D (<http://www.snps3d.org/>), and SplicePort (<http://www.spliceport.cs.umd.edu/SplicingAnalyzer2.html>) software programs.

2.4. Cell culture and gene expression

To evaluate the functional effect of the rs2274755 on exon 5 splicing, human lung carcinoma cell lines with known genoNCI-H226, NCI-460, and HOP-92; ATCC, Manassas VA, USA) were grown in RPMI medium supplemented with foetal bovine serum (10% v/v), L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml). Cultures were maintained at 37 °C and 95% humidity in an atmosphere of 5% CO₂.

Total RNA was isolated using the NORGEN purification kit (Norgen Biotek, Thorold, On, Canada) and cDNA was synthesized from 5 mg of total RNA (PROMEGA Madison Wi, USA), following the manufacturer's instructions.

PCR amplification of MMP-9 was performed using 0.1 µl of cDNA, 100 ng of each primer, 20 nM of each dNTPs, and 1 U of Taq Gold Polymerase in a final volume of 50 µl. Amplification was performed with an initial denaturing step of 95 °C for 10 min; followed by 33 cycles of 30 s denaturation at 94 °C, 1 min annealing at 60 °C or 58 °C, and 1 min extension at 72 °C; and a final extension step at 72 °C for 7 min. For each cDNA sample, the beta-2 microglobulin gene was co-amplified as an internal cDNA quality control. All PCR reactions were carried out with a GeneAMP PCR system 9700 (Applied Biosystems, Foster City, CA).

2.5. Statistical analysis

To test for deviations from Hardy–Weinberg equilibrium (HWE), all genotypes were analyzed using FINETTI software (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using allele frequencies for the cases and controls. The χ^2 values were estimated from 2 × 2 contingency tables, and P values <0.05 were considered statistically significant. Stat-Cal software (Epic Info 2005 V-3-2; Centers of Disease Control and Prevention, Atlanta, GA, USA) was used for all statistical calculations. Haplotype frequencies and the r^2 measure of linkage disequilibrium (LD) (r^2) were calculated using the Haploview software [28]. We applied the Bonferroni method and 100,000 permutations to adjust for multiple comparisons. The statistical power for the study was estimated using QUANTO software (version 1.2; <http://hydra.usc.edu/GxE/>) and it reached >90% employing the additive model.

3. Results

A total of 403 pediatric patients fulfilled the GINA criteria for asthma. The patients' ages ranged from 5 to 17 years old and the age at the onset of asthma was 11.5 ± 3.3 years (mean ± s.d.). The male/female ratio was 246/157 (61/39%) (Table 2). The control group included 426 blood donors, 251 (59%) male and 175 (41%) female, all of whom were older than 18 years of age.

No study participants carried the rs41427445 or rs3918245 minor alleles. The genotype distributions of the remaining SNPs were in HWE for both cases and controls.

The SNPs rs2274755 and rs17577 showed associations with asthma; both SNPs were in high linkage disequilibrium (LD > 99%) and their minor allele frequencies (MAF) were 6.5% and 6.6 vs. 3.2% and 3.3% in cases and controls, respectively (OR = 2.10, 95% CI 1.31–3.39, P = 0.001 and OR = 2.07, 95% CI 1.29–3.30, P = 0.001, respectively). Similar asthma risk was observed in a dominant model to rs2274755 (GG vs [GT + TT]):

Table 2

Clinical characteristics of patients enrolled in the study.

Clinical features	Mean (sd) or n (%)
Age at the study	11.5 ± 3.3
Gender	
Male	246 (61)
Female	157 (39)
Atopy ^a N = 319	266 (83)
Asthma severity ^a N = 323	
Intermittent	175 (54)
Mild persistent	52 (16)
Moderate persistent	94 (29)
Severe persistent	2 (<1)

^a Data are missing from several cases, atopy: positive skin test to atleast one aeroallergen, sd: standard deviation.

OR = 2.13, 95% CI 1.29–3.49, $P = 0.002$) and rs17577 (GG vs [GA + AA]: OR = 2.09, 95% CI 1.28–3.41, $P = 0.002$) (Table 3). These associations remained statistically significant after Bonferroni correction.

In a further step, we stratified the asthma patients according to the presence of atopy. We observed increasing associations between the rs2274755 and rs17577 and atopic asthma (OR = 2.38, 95% CI 1.44–3.96, $P = 0.0005$).

We did not find statistically significant differences in the distribution of the rs3918249 alleles or genotypes between cases or controls. However, after gender stratification, MAF was higher in female cases than female controls (25.7% vs 17.1%, respectively; OR = 1.66, 95% CI 1.14–2.43, $P = 0.007$). The homozygous mutant allele genotype displayed a higher OR (TT vs CC = 3.01, 95% CI 1.10–8.23, $P = 0.025$) (Table 4).

We found no association between the rs3918242, rs17576, rs2250889, or rs9509 polymorphisms and asthma or atopy, even after gender stratification.

The LD structure between the seven SNPs showed two high LD blocks ($r^2 > 0.9$); the first one involving the rs3918249 and rs17576 and the other one including, both associated SNPs (rs2274755 and

rs17576). Combining the four SNPs, only the haplotypes harboring all minor alleles, CTGA, showed a statistically significant association (OR = 2.13, 95% CI 1.27–3.58, $P = 0.003$), which remained significant after a 100,000 permutation run (Table 5). Likewise, the haplotype analysis stratified by gender showed significant differences between cases and controls in both females (OR = 2.08, 95% CI 1.04–4.21, $P = 0.025$) and males (OR = 2.39, 95% CI 1.08–5.41, $P = 0.018$).

Functional effect evaluation of rs2274755 showed that this variant did not alter exon 5 splicing, as had been predicted. The rs3918249 SNP was predicted to be involved in a transcription factor-binding site (TFBS); however, our analyses did not show different scores between both alleles. The SNP rs17577 leads to the substitution of arginine (R) with a glutamine (Q) at position 648 (R648Q). Bioinformatic tools (Pmut pathogenicity and SNP3D) showed that the 648R amino acid substitution might alter the MMP-9 structure because of a loss of salt interaction between the 648R and the 544E (glutamic acid).

4. Discussion

The physiological function of MMP-9 is to cleave structural proteins (fibrin, fibrinogen, fibronectin, and collagen types IV, V, VII, and X) and to modulate the activities of other proteases, proinflammatory cytokines, and several chemokines [1,29,30]. MMP-9 is the principal MMP detected in cases of asthma, where it has been implicated in chronic airway inflammation and airway remodelling [2,10,18,31–33]. A correlation was described between MMP-9 levels in exhaled breath condensate, and lung function and levels of inflammatory molecules in children with asthma [34,35]. Moreover, *Mmp*-9 knockout mice exhibit less peribronchial inflammation following OVA exposure compared with wild-type mice [36]. A few association studies in humans have evaluated the roles of MMP-9 SNPs in asthma or its related phenotypes susceptibility; however, the results are controversial [20–23,37].

In the present study, we examined the contribution of MMP-9 polymorphisms to the asthma susceptibility in Mexican pediatric patients. Because there is no evidence that the MMP-9 polymor-

Table 3

Genotype and allele frequencies of the rs2274755 and rs1757 polymorphisms in cases and controls.

SNP id	Control (N = 426)	Asthma (N = 403)	OR [95% CI]	P
rs2274755				
Genotype, n(%)				
GG	400 (93.9)	354 (87.8)		0.001
GT	25 (6.0)	46 (11.5)		
TT	1 (0.1)	3 (0.7)		
GG vs. TT			3.39 [0.35–32.7]	0.262
GG vs. GT			2.07 [1.25–3.45]	0.004
GG vs. [GT + TT]			2.13 [1.29–3.49]	0.002
Allele, n(%)				
G	825 (96.8)	754 (93.5)	2.10 [1.31–3.39]	0.001*
T	27 (3.2)	52 (6.5)		
rs17577				
Genotype, n(%)				
GG	399 (93.9)	353 (87.8)		0.006
GA	26 (6.0)	47 (11.5)		
AA	1 (0.1)	3 (0.7)		
GG vs. AA			3.39 [0.35–32.7]	0.262
GG vs. GA			2.04 [1.25–3.36]	0.004
GG vs. [GA + AA]			2.09 [1.28–3.41]	0.002
Allele, n(%)				
G	824 (96.7)	753 (93.4)	2.07 [1.29–3.30]	0.001*
A	28 (3.3)	53 (6.6)		

Bold word denotes statistical significance. *P value remain after Bonferroni correction ($P = 0.009$).

Table 4
Genotype and allele frequencies of the rs3918249 polymorphism in cases and controls groups.

Population	Control	Asthma	OR [95% CI]	P
All	N = 421 ^a	N = 397 ^a		
Genotype, n(%)				
TT	280 (66.5)	239 (60.2)		
TC	122 (29.0)	138 (34.8)		
CC	19 (4.5)	20 (5.0)		0.169
TT vs. CC			1.23 [0.64–2.36]	0.527
TT vs. TC			1.32 [0.98–1.78]	0.064
TT vs. [TC + CC]			1.31 [0.98–1.74]	0.061
Allele, n(%)				
T	682 (81.0)	616 (77.6)	1.23 [0.96–1.56]	0.088
C	160 (19.0)	178 (22.4)		
Male	N = 246	N = 245		
Genotype, n(%)				
TT	159 (64.6)	152 (62.0)		
TC	74 (30.1)	86 (35.1)		
CC	13 (5.3)	7 (2.9)		0.097
TT vs. CC			0.56 [0.21–1.45]	0.228
TT vs. TC			1.21 [0.83–1.78]	0.316
TT vs. [TC + CC]			1.11 [0.77–1.61]	0.551
Allele, n(%)				
T	392 (79.7)	390 (79.6)	2.10 [1.31–3.39]	0.974
C	100 (20.3)	100 (20.4)		
Female	N = 175	N = 152		
Genotype, n(%)				
TT	121 (69.1)	87 (57.2)		0.010
TC	48 (27.5)	52 (34.4)		
CC	6 (3.4)	13 (8.6)		
TT vs. CC			3.01 [1.10–8.23]	0.025
TT vs. TC			1.50 [0.93–2.43]	0.093
TT vs. [TC + CC]			1.67 [1.06–2.63]	0.0256
Allele, n(%)				
T	290 (82.9)	226 (74.3)	1.66 [1.14–2.43]	0.007*
C	60 (17.1)	78 (25.7)		

*P value remain after Bonferroni correction.

^a Genotype data from five controls and six cases were no available; bold word denotes statistical significance.

Table 5
Distribution of the haplotypes in controls and cases.

Haplotype ^a	Control n (%)	Asthma n (%)	OR [95% CI]	P
rs3918249 T/C, rs2274755 G/T, rs17576 A/G, rs17577 G/A				
TGAG	671 (78.7)	626 (77.7)	0.83 [0.65–1.05]	0.114
CGGG	128 (15.0)	117 (14.5)	0.96 [0.73–1.27]	0.771
CTGA	25 (2.9)	50 (6.2)	2.13 [1.27–3.58]	0.003*
CGAG	7 (0.08)	13 (1.6)	1.98 [0.73–5.50]	0.211

*P value remain after 100,000 permutations (P = 0.016).

^a Haplotypes >1.0% are showing.

rs3918242 to be associated with asthma; other functional variant could be the causal to asthma in our population. The rs2274755 is located in a potential exon 5 splicing site, hence we analyzed its functional effect in cell lines (NCI-H226, NCI-460, and HOP-92) with known genotypes; the results did not show an error in exon 5 splicing. Thus, we hypothesize that rs17577 could have a role in causing predisposition to childhood-onset asthma in Mexican patients. Consistent with this hypothesis, our bioinformatic analyses revealed that the 648R amino acid substitution with 648Q at the hemopexin-like domain might alter the MMP-9 structure due to the loss of salt interaction between 648R and 544E. Because this domain is essential in MMP-9 activity regulation through its inhibitor (tissue inhibitor of metalloproteinase 1); the 648Q variant could increase the bioavailability of active MMP-9 [39–42]. In fact, increased MMP-9 activity has been reported in the bronchoalveolar fluids of severe asthmatic patients, and the proteolytic activity is increased after allergen treatment [17]. The variant rs17577 has also been associated with many other diseases, including COPD, allergies, and cancer [37,43–48]. Although there are convincing data suggesting a direct biological effect of rs17577, it remains possible that this SNP is a proxy for another variant within the MMP-9 gene or a neighbouring gene, which could be causal of the association.

Otherwise, we found a female-dependent association between the rs3918249 polymorphism and asthma in our patients; to our knowledge, this SNP has not been previously reported to be associated with other lung diseases. However, a recent study suggested that this SNP could be involved in extracellular matrix remodeling because of the proteolytic activity exerted by MMP-9 against collagen IV, and because this polymorphism is associated with primary angle closure glaucoma, which is characterized by increased sclera remodeling and increased MMP production and activation [26,48]. A gender-dependent association is frequently documented in immune pathologic, actually in a previous study, we documented an association between TNF α and FCERL3 SNPs and JRA and asthma in females and males respectively [24,49]. This phenomenon was also reported for several other immune entities, including multiple sclerosis, rheumatoid arthritis, asthma (IL4, IL13, and COX-2 SNPs), and inflammatory bowel disease (HLA SNPs) [50–53], suggesting that the interaction between the genotype and the sexual hormones during the development of the immune-mediated diseases should be investigated.

In conclusion, our results showed a significant association between MMP-9 polymorphisms and asthma in a Mexican pediatric population. Further studies are needed to confirm these findings and to increase our knowledge regarding the molecular mechanisms causing this lung disease.

5. Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

NMA, RGB, JLJR, DLL and YSA performed DNA biobank, SJM and RGB carried the research and analyzed the data; NMA contributed with patients; HL contributed the cell lines for the study; SJM, MD and LO designed the research study and wrote the paper. This study was supported by a grant from Consejo Nacional de Ciencia y Tecnología, Mexico (CONACyT): 2011-C01-161936. The authors also would like to acknowledge the patients and their families for their participation.

References

- [1] Amălinei C, Căruntu ID, Giuscă SE, Bălan RA. Matrix metalloproteinases involvement in pathologic conditions. *Rom J Morphol Embryol* 2010;51:215–28.
- [2] Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol* 2003;28:12–24.
- [3] Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol* 2004;16:558–64.
- [4] Shapiro SD, Senior RM. Matrix metalloproteinases. Matrix degradation and more. *Am J Respir Cell Mol Biol* 1999;20:1100–2.
- [5] Parks WC, Shapiro SD. Matrix metalloproteinases in lung biology. *Respir Res* 2001;2:10–9.
- [6] Demedts IK, Brusselle GG, Bracke KR, Vermaelen KY, Pauwels RA. Matrix metalloproteinases in asthma and COPD. *Curr Opin Pharmacol* 2005;5:257–63.
- [7] Mori S, Pawankar R, Ozu C, Nonaka M, Yagi T, Okubo K. Expression and Roles of MMP-2, MMP-9, MMP-13, TIMP-1, and TIMP-2 in Allergic Nasal Mucosa. *Allergy Asthma Immunol Res* 2012;4:231–9.
- [8] Winkler MK, Földes JK, Bunn RC, Fowlkes JL. Implications for matrix metalloproteinases as modulators of pediatric lung disease. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L65–L557.
- [9] Chen L, Nakai M, Belton Jr RJ, Nowak RA. Expression of extracellular matrix metalloproteinase inducer and matrix metalloproteinases during mouse embryonic development. *Reproduction* 2007;133:405–14.
- [10] Mehra D, Sternberg DI, Jia Y, Canfield S, Lemaitre V, Nkyimbeng T, et al. Altered lymphocyte trafficking and diminished airway reactivity in transgenic mice expressing human MMP-9 in a mouse model of asthma. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L149.
- [11] Bratcher PE, Weathington NM, Nick HJ, Jackson PL, Snelgrove RJ, Gaggar A. MMP-9 Cleaves SP-D and Abrogates Its Innate Immune Functions In Vitro. *PLoS One* 2012;7:e41881.
- [12] Izbicka E, Streeter RT, Michalek JE, Loudon CL, Diaz 3rd A, Campos DR. Plasma bio markers distinguish non-small cell lung cancer from asthma and differ in men and women. *Cancer Genomics Proteomics* 2012;9:27–35.
- [13] Wang LF, Chien CY, Chiang FY, Chai CY, Tai CF. Correlation between matrix metalloproteinase 2 and 9 expression and severity of chronic rhinosinusitis with nasal polyposis. *Am J Rhinol Allergy* 2012;26:e1–4.
- [14] Beeh KM, Beier J, Kornmann O, Buhl R. Sputum matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, and their molar ratio in patients with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and healthy subjects. *Respir Med* 2003;97:634–9.
- [15] Cundall M, Sun Y, Miranda C, Trudeau JB, Barnes S, Wenzel SE. Neutrophil-derived matrix metalloproteinase-9 is increased in severe asthma and poorly inhibited by glucocorticoids. *J Allergy Clin Immunol* 2003;112:1064–71.
- [16] Hong Z, Lin YM, Qin X, Peng JL. Serum MMP-9 is elevated in children with asthma. *Mol Med Report* 2012;5:462–4.
- [17] Kelly EA, Busse WW, Jarjour NN. Increased matrix metalloproteinase-9 in the airway following allergen challenge. *Am J Respir Crit Care Med* 2000;162:1157–61.
- [18] McMillan SJ, Kearley J, Campbell JD, Zhu XW, Larbi KY, Shipley JM, et al. Matrix metalloproteinase-9 deficiency results in enhanced allergen-induced airway inflammation. *J Immunol* 2004;172:2586–94.
- [19] Oshita Y, Koga Kamimura T, Matsuo K, Rikimaru T, Aizawa H. Increased circulating 92 kDa matrix metalloproteinase (MMP-9) activities in exacerbations of asthma. *Thorax* 2003;58:757–60.
- [20] Nakashima K, Hirota T, Obara K, Shimizu M, Doi S, Fujita K, et al. A functional polymorphism in MMP-9 is associated with childhood atopic asthma. *Biochem Biophys Res Commun* 2006;344:300–307.
- [21] Ganter K, Deichmann KA, Heinzmann A. Association study of polymorphisms within matrix metalloproteinase 9 with bronchial asthma. *Int J Immunogenet* 2005;32:233–6.
- [22] Lose F, Thompson PJ, Duffy D, Stewart GA, Kedda MA. A novel tissue inhibitor of metalloproteinase-1 (TIMP-1) polymorphism associated with asthma in Australian women. *Thorax* 2005;60:623–8.
- [23] Pinto LA, Depner M, Klopp N, Illig T, Vogelberg C, von Mutius E, et al. MMP-9 gene variants increase the risk for non-atopic asthma in children. *Respir Res* 2010;11:23.
- [24] Jiménez Morales S, Velázquez Cruz R, Ramírez-Bello J, Bonilla-González E, Romero-Hidalgo S, et al. Tumor necrosis factor- α is a common genetic risk factor for asthma, juvenile rheumatoid arthritis, and systemic lupus erythematosus in a Mexican pediatric population. *Hum Immunol* 2009;70:251–256.
- [25] Sadeghi M, Motovali-bashi M, Hojati Z. MMP-9 promoter polymorphism associated with tumor progression of breast cancer in Iranian population. *Int J Integ Biol* 2009;6:33–7.
- [26] Cong Y, Guo X, Liu X, Cao D, Jia X, Xiao X, et al. Association of the single nucleotide polymorphisms in the extracellular matrix metalloproteinase-9 gene PACG in Southern China. *Mol Vis* 2009;15:1412–7.
- [27] Yuan HY, Chiou JJ, Tseng WH, Liu CH, Liu CK, Lin YJ, et al. FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization. *Nucleic Acids Res* 2006;34(Web Server Issue):W635–W641.
- [28] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–5.
- [29] Van Lint P, Libert C. Chemokine and cytokine processing by matrix metalloproteinases and its effect on leukocyte migration and inflammation. *J Leukoc Biol* 2007;82:1375–81.
- [30] Wolf M, Albrecht S, Märki C. Proteolytic processing of chemokines: implications in physiological and pathological conditions. *Int J Biochem Cell Biol* 2008;40:1185–8.
- [31] Bosse M, Chakir J, Rouabhi M, Boulet LP, Audette M, Laviolette M. Serum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio correlates with steroid responsiveness in moderate to severe asthma. *Am J Respir Crit Care Med* 1999;159:502–96.
- [32] Bradley LM, Douglass MF, Chatterjee D. Matrix metalloproteinase 9 mediates neutrophil migration into the airways in response to influenza virus-induced toll-like receptor signaling. *PLoS Pathog* 2012;8:e1002641.
- [33] Zhang JY, Wenzel SE. Tissue and BAL based biomarkers in asthma. *Immunol Allergy Clin North Am* 2007;27:623–32.
- [34] Cataldo DD, Tournay KG, Vermaelen K, Munaut C, Foidart JM, Louis R, et al. Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation. *Am J Pathol* 2002;161:491–8.
- [35] Karakoc GB, Yukselen A, Yilmaz M, Altintas DU, Kendirli SG. Exhaled breath condensate MMP-9 level and its relationship with asthma severity and interleukin-4/10 levels in children. *Ann Allergy Asthma Immunol* 2012;108:300–4.
- [36] Vignola AM, Riccobono L, Mirabella A, Profita M, Chanez P, Bellia V, et al. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1998;158:1945–50.
- [37] Erbek SS, Yurtcu E, Erbek S, Sahin FI. Matrix metalloproteinase-9 promoter gene polymorphism (-1562C>T) in nasal polyposis. *Am J Rhinol Allergy* 2009;23:568–70.
- [38] Inoue H, Mashimo Y, Funamizu M, Yonekura S, Horiguchi S, Shimojo N, et al. Association of the MMP9 gene with childhood cedar pollen sensitization and pollinosis. *J Hum Genet* 2012;57:176–83.
- [39] Butler GS, Butler MJ, Atkinson SJ, Will H, Tamura T, Schade van Westrum S, et al. The TIMP2 membrane type 1 metalloproteinase “receptor” regulates the concentration and efficient activation of progelatinase A. A kinetic study. *J Biol Chem* 1998;273:871–80.
- [40] Cao J, Sato H, Takino T, Seiki M. The C-terminal region of membrane type matrix metalloproteinase is a functional transmembrane domain required for pro-gelatinase A activation. *J Biol Chem* 1995;270:801–5.
- [41] Gohlke U, Gomis-Ruth FX, Crabbe T, Murphy G, Docherty AJ, Bode W. The C-terminal (haemopexin-like) domain structure of human gelatinase A (MMP2): structural implications for its function. *FEBS Lett* 1996;378:126–30.
- [42] Murphy G, Knauper V. Relating matrix metalloproteinase structure to function: why the “hemopexin” domain? *Matrix Biol* 1997;15:511–8.
- [43] Mishra A, Srivastava A, Mittal T, Garg N, Mittal B. Association of matrix metalloproteinases (MMP2, MMP7 and MMP9) genetic variants with left ventricular dysfunction in coronary artery disease patients. *Clin Chim Acta* 2012;413:1668–74.
- [44] Nan H, Niu T, Hunter DJ, Han J. Missense polymorphisms in matrix metalloproteinase genes and skin cancer risk. *Cancer Epidemiol Biomarkers Prev* 2008;17:3357–551.
- [45] Sharma KL, Misra S, Kumar A, Mittal B. Higher risk of matrix metalloproteinase (MMP-2, 7, 9) and tissue inhibitor of metalloproteinase (TIMP-2) genetic variants to gallbladder cancer. *Liver Int* 2012. <http://dx.doi.org/10.1111/j.1478-3231.2012.02822>.
- [46] Shchankova P, Pettersson R, Nordenström K, Holm G, Ekman A. Personality traits and the R668Q polymorphism located in the MMP-9 gene. *Behav Brain Res* 2012;228:232–5.
- [47] Wu S, Hsu LA, Teng MS, Lin JF, Chang HH, Chang PY, et al. Association of matrix metalloproteinase 9 genotypes and cardiovascular disease risk factors with serum matrix metalloproteinase 9 concentrations in Taiwanese individuals. *Clin Chem Lab Med* 2010;48:543–9.
- [48] McBrien NA, Lawlor P, Gentle A. Scleral remodeling during the development of and recovery from axial myopia in the tree shrew. *Invest Ophthalmol Vis Sci* 2000;41:3713–9.
- [49] Ramírez-Bello J, Jiménez-Morales S, Espinosa-Rosales F, Gómez-Vera J, Gutiérrez R, Velázquez-Cruz R, et al. Juvenile rheumatoid arthritis and asthma, but not childhood-onset systemic lupus erythematosus are associated with FCRL3 polymorphisms in Mexicans. *Mol Immunol* 2012. <http://dx.doi.org/10.1016/j.molimm.2012.09.004>.
- [50] Yamada R, Tanaka T, Unoki M, Nagai T, Sawada T, Ohnishi Y, et al. Association between a single-nucleotide polymorphism in the promoter of human interleukin-3 gene and rheumatoid arthritis in Japanese patients, and maximum-likelihood estimation of combinatorial effect that two genetic loci have on susceptibility to the disease. *Am J Hum Genet* 2001;68:674–85.
- [51] Fisher SA, Hampe J, Macpherson AJ, Forbes A, Lennard-Jones JE, Schreiber S, et al. Sex stratification of an inflammatory bowel disease genome search shows male-specific linkage to the HLA region of chromosome 6. *Eur J Hum Genet* 2002;10:259–65.
- [52] Szczeklik W, Sanak M, Szczeklik A. Functional effects and gender association of COX-2 gene polymorphism G-765C in bronchial asthma. *J Allergy Clin Immunol* 2004;114:248–53.
- [53] Akkad DA, Arning L, Ibrahim SM, Epplen JT. Sex specifically associated promoter polymorphism in multiple sclerosis affects interleukin 4 expression levels. *Genes Immunol* 2007;8:703–6.