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Original Article

The impact of genetic variants in matrix metalloproteinase-9 gene on lupus nephritis in Chinese Han population

Yan He, Xia Wang, Yuhuan Bu, Yan Song

Department of Nephrology, The People' Hospital of Rizhao, Rizhao, Shandong, China

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Abstract: Objectives: This study aimed to discuss the effect of matrix metalloproteinase-9 (*MMP9*) gene polymorphisms on lupus nephritis (LN) susceptibility in Chinese Han population, Shandong region. Methods: Firstly, the genotyping of *MMP9* rs3918242 (C-1562T), rs17576 (R279Q) polymorphisms was conducted by the way of polymerase chain reaction (PCR) in 105 LN patients and 117 healthy people. The genotype, allele and basic indexes distribution differences between two groups were calculated by χ^2 test which was also used to check Hardy-Weinberg equilibrium (HWE) in the control group. The association strength of *MMP9* two polymorphisms with LN susceptibility was revealed by odds ratio (OR) with corresponding 95% confidence interval (95% CI) calculated using χ^2 test. Results: In present study, the genotypes and alleles frequencies of *MMP9* rs17576 polymorphism were found that there was no significant difference between the case and control groups (P>0.05). But referring to rs3918242, TT genotype in LN patients was more common than that of in healthy people (P=0.026) and meanwhile, T allele showed a significant distribution difference between two group (P=0.028). Therefore, the carriage of TT genotype and T allele significantly increased the occurrence risk of LN (TT vs. CC: OR=2.272, 95% CI=1.103-6.745; T vs. C: OR=1.623, 95% CI=1.051-2.506). In LN patients, class IV was common and they were inclined to carrying genotype with T allele. Conclusion: *MMP9* rs3918242 polymorphism is correlated to the generation and development of LN in Chinese Han population, but not rs17576.

Keywords: Matrix metalloproteinase-9, polymorphism, lupus nephritis, classification

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a wide variety of clinical manifestations, which involves in multiple organs damage [1]. It severely affects the quality of life for patients and their family due to the high health-care costs and the loss of productivity [2]. Lupus nephritis (LN) is an immune complex nephritis caused by the involvement of kidney in SLE patients and LN is also a leading cause of death in patients with SLE [3, 4]. The clinical symptoms of LN are hematuresis, proteinuria, edema, active urinary sediment, and acute renal injury [5]. In clinical, LN patients can develop to end-stage renal disease (ESRD) and even renal failure from primary proteinuria, nephritic syndrome with different severity. The pathogenesis LN is proved to be influenced by multiple factors, including genetic and environmental factors [6-8]. Recently, increasing scholars focus on the effect of single nucleotide polymorphism (SNP) of gene on disease occurrence, LN is no exception [9, 10]. However, the ethology of LN still remains unclear.

Matrix metalloproteinases (MMPs) are the calcium-dependent zinc endopeptidases and involve in the remodeling of tissues and the degeneration of extracellular matrix [11, 12]. MMPs play a vital role in cell behaviors such as cell proliferation, differentiation, apoptosis, angiogenesis and host defense and regulate various biological processes and the occurrence of diseases, such as microglial activation, dopaminergic apoptosis, inflammation, and neurodegenerative disorders [13-15]. They are classified into four subgroups based on the domain structure, namely, stromelysins, collagenases, gelatinases, and film sort (MT)-MMPs [16]. MMP9 belongs to gelatinases in MMPs and is encoded by *MMP9* gene located on chromo-

some 20q11.2-13.1 [17]. In human body, MMP9 is secreted in the form of inactive precursor or zymogen and activated MMP9 involves in tumor cells and inflammatory cells. In previous study on NZB/NZW lupus mice model, the expression and activity of MMP-9 increased in kidney, so *MMP9* may be participate in the onset of LN, but so far, there is no report about the association of *MMP9* polymorphisms and LN.

In present study, two SNPs of *MMP9* gene were selected to perform the association study with LN in a total of 222 subjects from Chinese Han group. One SNP is located on promoter region and the other one is a missense mutation in exon 6 and they may modify the expression of *MMP9*.

Materials and methods

The selection of study subjects

This study was a population-based case-control design, enrolling 105 patients with LN and 117 healthy people as the case and control groups, respectively. All subjects were unrelated Chinese Han population from Shandong region (people have lived here for more than five years). This study project was assessed and supported by the Research Ethics Committee of The People' Hospital of Rizhao. The whole experiment flow was informed the patients and their family and we obtained written consents before beginning this study.

The LN patients in the case group were diagnosed by pathobiology in nephrology development of The People' Hospital of Rizhao during March, 2011 and June, 2013, according to the criteria of American Rheumatism Association in 1982 [18]. 79 of LN patients were females and only 26 were males with an age range of 13-47 years old. On the basis of renal biopsy for histopathological classification of the World Health Organization (WHO) classification system, the LN patients were divided into class I~class V [19]. The patients with others inflammatory disease and tumors were excluded in this study. The control group was all healthy persons experiencing the physical examination in the same hospital with the cases at the same period, including 84 females and 33 males. There age was from 12 to 48 years old. The frequency should be matched by age and gender between the case and control groups.

Samples collection and DNA extraction

All subjects were informed abrosia after supper of the day before and 2 ml venous blood was collected from every subject in the morning of the second day. The blood sample was put into blood collection tube with EDTA-2Na anticoagulation. Then genomic DNA was distilled using TIANGEN Blood Genomic DNA Extraction Kit purchased from TIANGEN BIOTECH CO., LTD (BEIJING), referring to the manufacturer's instruction. The DNA samples were stored at -20°C for standby application.

The genotypes determination of MMP-9 polymorphisms in all subjects

In present study, we selected two SNPs -rs-3918242 in *MMP9* promoter region and rs-17576 in exon6. The PCR primer sequences referred to the study of Wu et al. [20]. Rs-3918242: GCCTGGCACATAGTAGGCCC (forward), CTTCCTAGCCAGCCGGCATC (reverse); rs17-576: TCACCCTCCCGCACTCTGG (forward), CGG-TCGTAGTTGGCGGTGG (reverse). 20 μl PCR system were used to amplify SNP fragments, containing 1.0 μl DNA template with eligible concentration, each 0.5 μl of forward and reverse primers, 10.0 μl PCR Mix and ddH_2O added to 20 μl. The PCR reaction progress of rs3918242 was as follows: 94°C pre-degeneration for 5 min, followed by 35 cycles of 94°C degeneration for 35 s, 56°C annealing for 30 s, 72°C extension for 45 s and final extension at 72°C for 5 min. Rs17576 PCR program was: 94°C pre-degeneration for 5 min, followed by 30 cycles of 94°C degeneration for 30 s, 58°C annealing for 1 min, 72°C extension for 1 min, and final extension at 72°C for 10 min. The quality of PCR products were detected by 1.0% agarose gel electrophoresis (AGE).

The 10 μl eligible PCR products were sequenced to determine the genotype of every subject based on *MMP9* polymoprhisms in Shanghai Sangon Biotech Co., Ltd

Statistical analysis

The data analyses were conducted using PASW Statistics 18.0 software. All data were showed by $\bar{x} \pm s$ or %. The genotype distribution of *MMP9* polymorphisms in the control group were detected by Hardy-Weinberg equilibrium (HWE). The χ^2 test was used to compare the frequency differences of basic indexes, genotype,

Table 1. The basic information of all subjects in the case and control groups

Characteristic		Case n=105 (%)	Control n=117 (%)	P
Age	Mean age	27.53±9.56	26.78±8.33	>0.05
Gender	Female	79 (75.24)	84 (71.79)	>0.05
	Male	26 (24.76)	33 (28.21)	
Drinking	Yes	22 (20.95)	15 (12.82)	>0.05
	No	83 (79.05)	102 (87.18)	
Hormone	Yes	26 (24.76)	21 (17.95)	>0.05
	No	79 (75.24)	96 (82.05)	
LN classification	Class I	14 (13.33)		
	Class II	20 (19.05)		
	Class III	19 (18.10)		
	Class IV	50 (47.62)		
	Class V	2 (1.90)		

and allele between two groups. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to reveal the risk strength of gene polymorphisms for LN. $P<0.05$ was considered as a statistically significant association.

Results

The basic features of LN patients and the healthy people

In this study, a total of 222 subjects were enrolled, including 105 LN patients as the cases and 117 healthy people as the controls. The mean age of the LN patients and healthy people was 27.53±9.56 and 26.78±8.33, respectively. In LN patients, the females accounted for three quarters and nearly 72% of the controls were females. There was no significant distribution difference between two groups by age and gender ($P>0.05$). The alcohol and hormone consumption were also not associated with the onset of LN in our population ($P>0.05$). In LN histologic classification, about a half of LN patients belonged to class IV and the percentage of class I~class III was similar, but only 1.9% of the cases were in class V. The whole results were showed in **Table 1**.

The frequencies differences of genotype and allele in MMP9 polymorphisms between the case and control groups

The relative results were listed in **Table 2**. The genotype frequencies of *MMP9* rs17576 polymorphism in the case and control groups were 49.53%, 37.14%, 13.33% and 58.97%, 32.48%, 8.55% (GG, AG, AA), respectively. The frequency differences of genotypes in rs17576 didn't reach a significant level ($P>0.05$). Likely, the allele frequencies in the cases and controls (G: 68.10% & 31.90%, A: 75.21% & 24.79) were also not a statistical significant difference ($P>0.05$). So rs17576 may be not a direct risk factor for LN. Differently, the genotype frequencies of rs3918242 were 57.14%, 26.67%, 16.19% and 65.81%, 27.35%, 6.84%, respectively in cases and controls and had a significant difference in TT genotype ($P=0.026$), so was T allele frequency between two groups ($P=0.028$). The carriage of TT genotype and T allele may be the risk factors for LN development and procession (TT vs. CC: OR=2.272, 95% CI=1.103-6.745; T vs. C: OR=1.623, 95% CI=1.051-2.506).

The genotype distributions of MMP9 polymorphisms in IN patients with different types of histologic classification

The genotype distribution of *MMP9* polymorphisms were analyzed in the types of histologic classification of LN patients. We can see from **Table 3** that there was no significant distribution difference of genotype in class I~class III of LN patients based on not only rs3918242 but rs17576, compared with the controls ($P>0.05$). In class IV, the genotype carrying T allele of rs3918242 was common and the distribution difference reached a significant level of 0.01 and LN patients in class V were all TT genotype of rs3918242. But we didn't gain the similar result in re17576.

Discussion

In present study, we conducted a case-control design to reveal the association of *MMP9* polymorphisms with LN susceptibility for the first time in Chinese Han population. Two SNPs rs3918242, rs17576 of *MMP9* were analyzed and the former is located on the gene promoter region, the latter occurs in exon 6. In this study period, age and gender were checked to be frequency-matched between the case and control groups, meanwhile, alcohol and hormone

Table 2. The genotype and allele frequencies of *MMP9* polymorphisms between two study groups

Genotype/ allele	Case (%)	Control (%)	OR (95% CI)	P	PHWE
rs3918242					0.081
CC	60 (57.14)	77 (65.81)	1.000 [Ref.]	-	
CT	28 (26.67)	32 (27.35)	1.123 [0.611, 2.065]	0.709	
TT	17 (16.19)	8 (6.84)	2.272 [1.103, 6.745]	0.026	
C	148 (70.48)	186 (79.49)	1.000 [Ref.]	-	
T	62 (29.52)	48 (20.51)	1.623 [1.051, 2.506]	0.028	
rs17576					0.163
GG	52 (49.53)	69 (58.97)	1.000 [Ref.]	-	
AG	39 (37.14)	38 (32.48)	1.362 [0.767, 2.417]	0.291	
GG	14 (13.33)	10 (8.55)	1.858 [0.765, 4.514]	0.168	
A	143 (68.10)	176 (75.21)	1.000 [Ref.]	-	
G	67 (31.90)	58 (24.79)	1.422 [0.939, 2.153]	0.096	

Note: HWE: Hardy-Weinberg equilibrium.

Table 3. The genotype distributions of *MMP9* polymorphisms in LN patients with different types of histologic classification

Classification	rs3918242 genotypes (%)				rs17576 genotype (%)			
	CC	CT	TT	CT/TT	GG	AG	AA	AG/AA
Control	77	32	8	40	69	37	11	48
Class I	11	2	1	3	9	3	2	5
Class II	14	5	1	6	12	5	3	8
Class III	13	3	3	6	8	8	3	11
Class IV	22	18	10	28**	23	22	5	27
Class V	0	0	2	2*	0	1	1	2

Note: **represents that the difference reaches to the significant level of 0.01; *represents a significant difference of 0.05.

consumption were not the risk factors, either. However, a significantly increased risk of LN occurrence were found in people carrying TT genotype of rs3918242 and T allele was also associated with the development risk of LN in our study group. But in rs17576, we didn't gain the similar conclusion, that is, neither genotype nor allele of rs17576 had a direct association with LN occurrence.

SLE is a common chronic and systemic immune inflammatory disease and usually attacks the groups with the age of 20-40 years old, most are females [21]. The symptoms of SLE are inconspicuous in early stage so as not to perceive timely, which leads to the involvement of multiple organs, one of them is kidney. The first manifestation of some SLE patients is only the involvement of kidney and 90% of SLE was detected renal injury. The occurrence of SLE causes a quite burden for patients and their family in economy and life. As the nephritis caused by the kidney involvement of SLE, LN is a major contributor to mortality of SLE [22]. So far, the ethology of SLE has been not illuminated completely and involves in the genetic and environmental factors [23, 24]. So, in order to reveal the pathogenesis of LN, genetic variants of gene is a good way.

MMP9 belong to a kind of gelatinase in classification of MMPs according to substrates, namely gelatinase B. The function of gelatinase B is dependent on zinc ions and it is the most active in the presence of Ca (2+) ions. In 1994, Reponen and Sahlberg et al. detected the expression of MMP9 in osteoclasts of mouse embryos for the first time [25]. Its domain structure is complex, including a signal peptide, propeptide region, a catalytic domain with the Zn (2+) ion binding site, the insertion of three fibronectin type II, a proline-rich domian and a hemopexin domain from N terminal to C terminal in turn [26]. It can be expressed throughout the body and plays an important role in multiple physiological and pathological processes as a negative regulator [27, 28]. Increasing evidence shows that the imbalanced of MMPs activity has a vital influence on the onset of renal failure, especially MMP9 which involves in atherosclerosis in chronic kidney disease [29, 30]. In healthy people, MMP9 is in an inappreciable level in kidney glomeruli, however, it is induced when the renal is attacked by inflammatory diseases or others relative diseases. What's more, the data of Tveita et al. indicate that glomerular proteolytic activity increase in a model of LN based on (NZB×NZW) F1 mice when they suffer from proteinuria, MMP9 is a major cause [31]. In previous studies, *MMP9* polymorphisms, especially rs3918242, rs17576, can modify the expression of MMP9 in serum, therefore, *MMP9* polymorphisms are associated with LN

susceptibility, which is not guesswork unwarranted.

In this population, *MMP9* rs3918242 polymorphism may affect the expression of *MMP9* in transcription and then change the level of MMP9 in serum to involve in the onset of LN. However, as a missense mutation in exon6, rs17576 is also considered to change the MMP9 level in some diseases, such as end stage kidney disease [32]. Even so, we didn't detect the association between rs17576 and LN, which may be caused by others environmental factor and the interaction. In our population, patients with LN were mostly in class IV in histologic classification and they had a preference of TT genotype in rs3918242, so was LN patients in class V. From the above, our study obtains the positive results, in the meanwhile, it exists some limitations, too. For example, the environmental factors are ignored and the study population only includes a part of Han population in Shandong with small sample size.

In conclusion, *MMP9* rs3918242 polymorphism is directly associated with LN development in Chinese Han population based on our selection, but not rs17576. In order to verify the role of *MMP9* polymorphisms in LN occurrence and further interpret the ethology of LN, more studies about this topic should be conducted with enough large sample size and different aces, considering the environmental factors in the future.

Disclosure of conflict of interest

None.

Address correspondence to: Yan Song, Department of Nephrology, The People' Hospital of Rizhao, Rizhao 276800, Shandong, China. E-mail: songyanjier@sina.com

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