·Basic Research·

MMP-2 gene polymorphisms in type 2 diabetes mellitus diabetic retinopathy

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Received:2010-04-12 Accepted:2010-05-26

Abstract

• AIM: To study the association between polymorphisms of the *MMP-2* gene and diabetic retinopathy (DR).

• METHODS: *MMP-2* C-1306T and C-735T SNPs was genotyped by polymerase chain reaction-restrictive fragment length polymorphism (PCR-RFLP) analysis in 151 DR patients and 150 healthy individuals served as control.

• RESULTS: There is no significant difference between the patient and control groups in allele or genotype distributions of *MMP-2* C-735T (P=0.263 and P=0.248). Also, there is no significant difference between the patient and control in allele of *MMP-2* C-1306T (P =0.03). However the result has significant deviation of C/C, C/T, T/T genotypic frequencies between the patient and control groups in *MMP-2* C-1306T (P=0.008). We found that subjects with the *MMP-2* C-1306T genotype had an overall 2-fold increase in the risk of developing DR [adjusted odds ratio (OR)=2.446; 95% confidence interval (CI)=1.239-4.829] compared with those with the T-1306T or C-1306T genotype. Stratification analysis showed that the *MMP-2* -1306C/T and -735C/T SNPs are not associated with the development of NPDR to PDR of DR in North Chinese Han population.

• CONCLUSION: *MMP-2* C-1306T genotypes may be associated with DR development in the Chinese population. However, there is no relationship between the *MMP-2* C-735T genotypes with the development of DR.

• KEYWORDS: MMP-2; diabetic retinopathy; gene; single nucleotide polymorphism
DOI:10.3980/j.issn.2222-3959.2010.02.10

Yang J, Fan XH, Guan YQ, Li Y, Sun W, Yang XZ, Liu R. *MMP-2* gene polymorphisms in type 2 diabetes mellitus diabetic retinopathy. *Int J Ophthalmol* 2010;3(2):137-140

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized primarily by hyperglycemia. It can lead to multiple complications. Diabetic retinopathy (DR) is a common and serious microvascular complication of diabetic mellitus that has become a leading cause of blindness worldwide. In fact, DR is one of the four main causes of blindness in the USA, as well as other developed countries[1]. In China, with economic development and improved living standards, the prevalence of diabetes has reached 3.2%. And accordingly, DR has become a major disease and the incidence of DR has increased rapidly in the last decade[2]. The exact mechanism of diabetic retinopathy is still unclear. Studies have demonstrated that DR is associated with the metabolic abnormalities promoted by long-term hyperglycemia. However, clinical studies on human subjects have revealed substantial variations in the onset and severity of retinopathy that are not fully explained with known risk factors such as the duration of diabetes, the level of glycemic control, or concomitant vascular disease. Some diabetic patients can tolerate a poorly controlled blood glucose level well. Furthermore, there are differences in the prevalence of DR in different ethnic populations. Also, diabetic retinopathy tends to cluster in families. Therefore, current data provides supporting evidence for the idea that genetic factors are responsible for an individual's susceptibility to DR.

Matrix metalloproteinases (MMPs) are an important family of proteolytic enzymes that are required for the degradation and turnover of extracellular matrix (ECM) components[3]. MMPs can degrade all types of ECM, except polyglycan, and can aid in cell proliferation, neovasculogenesis and tissue remodeling. Therefore, MMPs are important for a variety of pathopathysiological processes in the human body. Matrix metalloproteinase-2 (MMP-2) is especially interesting because of its ubiquitous expression and multiple functions [4]. For endothelial cells to migrate, proliferate, and to form capillaries, the extracellular matrix (ECM) proteins of the basement membrane have to be degraded. Quiescent endothelial cells are activated by soluble endothelial mitogens and ECM molecules [5] and produce proteases that degrade matrix proteins. Matrix metalloproteinase-2, also known as gelatinase A, is a 72kDa type IV collagenase and can degrade type IV collagen. It is a main component of the basement membrane. Increased expression of *MMP-2* may precipitate the degradation of type IV collagen and the gap

Table 1 PCR conditions for MMP-2 restriction fragment length polymorphisms

Polymorphism	Primers	Product length (bp)	Restriction enzyme	Fragment length (bp)
C-1306T	Forward:5'-CTTCCTAGGCTGGTCCTTACTGA-3'	193	XspI	188+5 (C)
	Reverse: 5'-CTGAGACCTGAAGA GCTAAAGAGCT-3'			162+26+5 (T)
C-735T	Forward: 5'-GGATTCTTGGCTTGGCGCAGGA-3'	391	HinfI	391 (C)
	Reverse: 5'-GGGGGCTGGGTAAAATGAGGCTG-3'			338+53 (T)

junction protein, expediting the vascular complications of diabetes. All previous studies have suggested that MMP-2 plays an important role in the development of DR. Transcriptional regulation is likely the most important factor among several regulating mechanisms for the overexpression of *MMP-2* [6]. Several functional single-nucleotide polymorphisms (SNPs) have been described in the *MMP-2* promoter. Among them, two SNPs, C-1306T and C-735T, are particularly interesting, because the C/T polymorphisms located at these loci disrupt a Sp1 regulatory element and the T allele. This results in strikingly lower activity of the *MMP-2* promoter compared with the C allele [7,8]. Additionally, an endogenous protein, tissue inhibitor of metalloproteinase-2 (TIMP-2)[9], regulates *MMP-2* activity. Several studies elsewhere have shown that these two polymorphisms of *MMP-2* are associated with some diseases [10-13]. On the basis of those findings, we sought to analyze the association of two polymorphisms in the promoter region of *MMP-2* with the risk of developing diabetic retinopathy in a case-control study. We examined the association between polymorphisms in matrix metalloproteinase-2 (*MMP-2*C-1306T and C-735T) and the development of Type 2 diabetic retinopathy in a North Chinese Han population.

MATERIALS AND METHODS

Participants All of the patients were recruited from the Department of Ophthalmology and Endocrinology in the Fourth Affiliated Hospital of Hebei Medical University and from a DM screening in the city of Shijiazhuang and surrounding counties from March 2006 to December 2007. These included 151 cases of diabetic retinopathy (DR), 118 cases of non-proliferative diabetic retinopathy (NPDR) and 33 cases of proliferative diabetic retinopathy (PDR). All patients were definitively diagnosed by standard international diabetic retinopathy typing. Additionally, 150 cases of healthy volunteers with no clinical evidence of diabetes mellitus, or any other disease, were randomly selected from Chinese blood donors as control subjects. All study subjects were from the North Chinese Han population. The study was approved by the ethics committee of the Heibei Provincial Health Bureau and carried out in accordance with the tenets of the Helsinki Declaration (revised in 2000). All participants provided informed consent, and all examination and treatment were provided free of charge. Participants were diagnosed with DR based on the criteria established by the International Diabetic Retinopathy Typing Standard.

Methods Genomic DNA was extracted by using proteinase K digestion, followed by a sorting-out procedure [14]. Genotypes were determined by using the PCR-restriction fragment-length polymorphism (PCR-RFLP) method. PCR was performed using 100ng of the DNA template, 2.4μL of a 10× PCR buffer, 1U of Taq DNA polymerase, 0.4μL of 10mmol/L deoxyribonucleotide triphosphates,and 200nmol/L of each primer, all in a 20μL volume. The PCR cycling conditions were as follows: 5 minutes at 94℃, 35 cycles of 45s at 94 ℃, 45s at 58℃ for C-1306T and 63.5℃ for C-735T, and 45 seconds at 72℃, with a final step at 72℃ for 10 minutes to allow for the complete extension of all PCR fragments. An 8μL aliquot of each PCR product was subjected to digestion at 37℃ overnight in a 10μL reaction containing 10IU of the respective restriction enzyme. After digestion, the products were separated on a 40g/L agarose gel containing ethidium bromide. The primers, length of PCR product, restriction enzymes, and fragment lengths are summarized below in Table 1. Distilled water was used as a negative control instead of DNA in the reaction system for each panel of PCR. The PCR reactions of 15% of the samples were run in duplicate for quality control, with 100% reproducibility.

Statistical Analysis Statistical analysis was performed using the Statistical Package for Social Sciences software package (version 11.5, SPSS, Chicago, IL). Hardy-Weinberg analysis was performed to compare the observed and expected genotype frequencies by using the Chi-square test. Comparison of the distribution of the *MMP-2*C-1306T and C-735T genotype in the study groups was performed by using two-sided contingency tables and the Chi-square test. Odds ratios and 95% confidence intervals were calculated using an unconditional logistic regression model. $P<0.05$ was considered significant.

RESULTS

The allelotype and genotype frequencies of *MMP-2* in the control group were found to be in equilibrium based on Hardy-Weinberg analyses. There was no significant difference in the C and T allelic frequencies between the

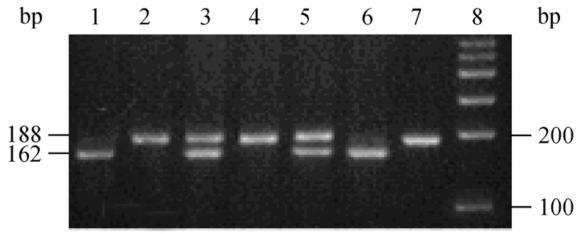

Figure 1 MMP -2 C -1306T genotyping by PCR -XspI digestion Lanes 1,6: T/T; 2,4: C/C; 3,5: C/T; 7: PCR product; 8: 100bp ladder

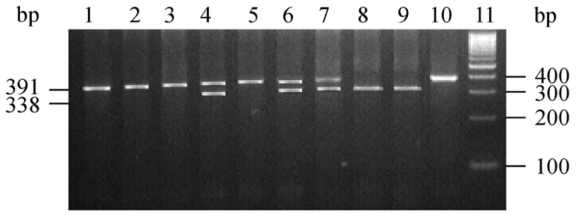

Figure 2 MMP -2 C -735T genotyping by PCR -HinfI digestion Lanes 1,2,3,5: C/C; 4,6,7: C/T; 8,9: T/T; 10: PCR product; 11: 100bp ladder

control group and DR group (χ^2=4.686, P=0.096). The C/C, C/T, T/T genotypic frequencies among the control subjects and DR patients were 80%, 20% and 0%, respectively, in controls and, 90.7%, 7.9% and 1.3%, respectively, in the DR group. There was significant difference in the C/C, C/T, and T/T genotypic frequency between the control group and DR group (χ^2=7.130, P= 0.028, Figure 1). Compared to the C/T and T/T genotypes, the C/C genotype may increase one's risk of diabetic retinopathy. The odds ratio (OR) between the control group and DR group was 2.446 (95% CI=1.239-4.829). The association between *MMP-2* C-735T SNP and diabetic retinopathy demonstrates that there was no significant difference with regard to the expression of the C and T alleles between the control group and the DR patients (χ^2= 2.671, P= 0.263). The frequencies of the three genotypes, C/C, C/T, T/T, among the control and DR group are 70.7%, 26.7%, and 2.7% (control) and 63.6%, 33.1%, and 3.3% (DR), respectively. The C and T allelic frequencies in the control and the DR groups were 84% and 16% (control) and 80.1% and 19.9% (DR), respectively and there was no significant difference between the genotypes(χ^2 =2.789, P= 0.248, Figure 2). Compared to the C/T and T/T genotypes, the C/C genotype is not associated with an increase in the risk of diabetic retinopathy. The odds ratio (OR) between the control group and DR group was 0.725 (95% CI= 0.447-1.175).

After stratification according to the approved international diabetic retinopathy typing, the frequency of C/C, C/T, T/T at the MMP-2 C-1306T locus in the NPDR group was 90.7% , 7.6% , and 1.7% , respectively. There was no significant difference when compared with the genotypic frequencies in the PDR group (90.9%, 9.1%, and 0%; χ^2 = 0.002, P =0.968). Compared to the C/T+T/T genotype, the C/C genotype did not increase the risk of the onset of PDR (OR=1.028, 95% CI=0.269-3.923). The C and T allelic frequencies in the two groups were not significantly different (χ^2=0.095, P =0.758). The C/C, C/T, T/T genotypic frequencies at the *MMP-2* C735T site in the NPDR group were 62.7%, 33.9%, and 3.4%, respectively, and these were not significantly different from the genotypic frequencies in the PDR group (66.7%, 30.3%, and 13.0%; χ^2=0.174, P= 0.676). Compared to the C/T+T/T genotype, the C/C genotype did not increase the risk of PDR (OR=1.189, 95% CI=0.527-2.685). The C and T allelic frequencies in the two groups were not significantly different between the NPDR and PDR groups (χ^2=0.151, P=0.698).

DISCUSSION

In the present study, we have examined the relationship between the C-1306T and C-735T polymorphisms in the *MMP-2* promoter region. *MMP-2* is a gene that plays a role in several diseases, and influences the risk of DR in a Chinese population. The results demonstrate that there is a significant association of the C-1306T polymorphism in the *MMP-2* promoter with an increased risk of developing diabetic retinopathy. However, no significant association was identified between the expression of the C-735T polymorphism and one's risk of developing diabetic retinopathy. Subjects carrying the *MMP-2* C-1306C genotype had a two-fold increase in their risk of developing DR. To the best of our knowledge, this is the first study to investigate the impact of these *MMP-2* polymorphisms on a individual's susceptibility to developing diabetic retinopathy. The C-1306T and C-735T are two common SNPs upstream from the transcriptional start site of the *MMP-2* gene that have a significant effect on *MMP-2* transcriptional activity. Because the human *MMP-2* promoter contains a number of cis-acting regulatory elements, the constitutive and inducible expression of *MMP-2* is likely to be regulated by transcription factors[15]. Among these regulatory elements, the Sp1 binding site is particularly interesting. Price *et al*[7] demonstrated that the C-1306T transition in the *MMP-2* promoter region could cause strikingly reduced promoter activity, which was due to disruption of the Sp1 binding site (CCACC box). Another C →T transition in the promoter region of *MMP-2* (C-735T) was also shown to disrupt this binding site and significantly diminish promoter activity[8]. Deletion or site-directed mutagenesis analysis of the *MMP-2* promoter has further demonstrated that the Sp1 site is critical for the constitutive activity of this gene [15]. In addition, Pan and Hung[16] reported that reduced Sp1 DNA-binding activity or phosphorylation by non-steroidal anti-inflammatory drugs could decrease *MMP-2* expression. Accumulating evidence

has shown that the C →T transition of these two polymorphisms could lead to the absence of the Sp1 consensus sequence and decrease transcriptional activity, which would further produce lower levels of MMP-2 protein in individuals carrying the T/T genotype compared to those with the C/C or C/T genotype. Considering the role of *MMP-2*, one would expect that individuals who carry the C/C genotype may be more susceptible to some diseases as a result of overexpression of this enzyme over one's lifetime. Hence, these two polymorphisms may be a latent genetic marker that can help identify individuals who are at higher risk for many diseases.

The association between the *MMP-2* C-1306T polymorphism and the risk of developing a number of diseases has been examined in several studies, but the results have been inconsistent. A number of reports have shown that the -1306C allele with high transcriptional activity was associated with increased risk of common cancers, including lung cancer, gastric cancer, cardiac cancer, and colorectal cancer [12,13,17,18]. Additionally, the *MMP-2* C-735T polymorphism is associated with the risk of developing some common diseases, and C-735C homozygosity may increase an individual's susceptibility to those conditions[8,19,20].

The results of this study demonstrate that *MMP-2*C-1306T SNPs may play a role in the genetic susceptibility to the development of diabetic retinopathy. At the same time, *MMP-2*C-735T is not associated with susceptibility to DR. What's more, considering the important functions of *MMP-2*, including the degradation of ECM components, the overexpression of *MMP-2* may play critical roles in the development of DR. Finally, our study may have certain limitations because our study subjects were recruited from one hospital in the community and may not be representative of the general population. However, the selection bias may be very low, because the genotype distributions of these two polymorphisms in the study groups did fit with the Hardy-Weinberg equilibrium, and we randomly recruited a large number of subjects.

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