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Correlations of MMP-9 and PPARγ gene polymorphisms with occurrence of preeclampsia

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Abstract. – OBJECTIVE: The aim of this study was to investigate the expressions of matrix metalloproteinase-9 (MMP-9) and peroxisome proliferator-activated receptor gamma (PPARγ) in the placenta of patients with preeclampsia and to elucidate the associations of their polymorphisms with the occurrence of preeclampsia.

PATIENTS AND METHODS: A total of 200 patients with preeclampsia (Preeclampsia group) and 100 pregnant women with normal delivery (Control group) were enrolled as research subjects. The expressions of MMP-9 and PPARγ in placentae of Preeclampsia group and Control group were measured by Western blotting. Conformation-difference gel electrophoresis was adopted for typing single nucleotide polymorphisms (SNPs) rs101201 and rs23102 in the promoter region of MMP-9 gene and rs201018 and rs102934 in the promoter region of PPARγ gene. Chi-square test was conducted to analyze whether the distribution frequency of MMP-9 and PPARγ genotypes was consistent with the law of genetic equilibrium. The correlations of the alleles and polymorphisms of MMP-9 and PPARγ with the occurrence of preeclampsia were analyzed. In addition, the associations of rs101201 genotype GG of MMP-9 gene and rs201018 genotype TT of PPARγ gene with the clinicopathological features of preeclampsia were analyzed.

RESULTS: Compared with Control group, the protein expression level of MMP-9 was significantly down-regulated ($p<0.05$), while the protein expression level of PPARγ was significantly up-regulated in placental tissues of Preeclampsia group ($p<0.05$). Based on Hardy-Weinberg equilibrium analysis, the two polymorphisms of both MMP-9 and PPARγ were consistent with the genetic equilibrium distribution ($p>0.05$). Gene correlation analysis showed that rs101201 polymorphism and its alleles in the promoter region of MMP-9 gene and rs201018 polymorphism and its alleles in the promoter region of PPARγ gene were correlated with the occurrence of preeclampsia ($p<0.05$). Besides, body mass index (BMI) value, gestational age, systolic blood pressure, and serum creatinine level in preeclampsia patients with the genotype GG of rs101201 in the promoter region of MMP-9 gene were not statistically significantly different from those in Control group ($p>0.05$). Furthermore, no statistically significant differences were observed in gravidity, parity, gestational age, systolic blood pressure, serum creatinine level, and plasma albumin level between preeclampsia patients with the genotype TT of rs201018 in the promoter region of PPARγ gene and those in Control group ($p>0.05$).

CONCLUSIONS: PPARγ and MMP-9 are abnormally expressed in the placenta of patients with preeclampsia. Moreover, rs201018 polymorphism in the promoter region of PPARγ gene and rs101201 polymorphism in the promoter region of MMP-9 gene are correlated with the occurrence of preeclampsia.

Key Words:
MMP-9, PPARγ, Preeclampsia, Polymorphism.

Introduction

Preeclampsia, a complication occurring after 20 weeks of pregnancy, is characterized by high blood pressure and proteinuria. It may induce multiple organ system dysfunction or failure or even death in severe cases[1,2]. The major pathophysiological changes of preeclampsia include systemic arteriole spasm, vascular endothelial cell damage, and visceral hemoperfusion reduction. About 34,000 women die from gestational hypertension worldwide annually, accounting for 14% of maternal deaths[3]. Previous evidence[4] has manifested that the occurrence of preeclampsia has a typical familial aggregation, implying that genetic factors play vital roles in its occurrence. For this reason, further elucidating the genetic pathogenesis of preeclampsia is of great significance for its targeted therapy in the future.

Single nucleotide polymorphisms (SNPs), the most common heritable variations in human genome sequences, refer to the insertion, replacement or deletion of single nucleotide in normal

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individuals. The frequency of alleles in a population is at least 1%[5]. With continuously in-depth studies of genetics and molecular biology, there is an increasing awareness that SNPs can lead to different susceptibility to preeclampsia[6]. Therefore, the distribution of different genotypes of genes in a population can be further understood by the exploration of human SNPs. All these findings may help to clarify the genetic mechanism and epidemiological characteristics of preeclampsia.

Previous studies have denoted that matrix metalloproteinase-9 (MMP-9) and peroxisome proliferator-activated receptor gamma (PPARγ) genes play important roles in hypertension and recurrent spontaneous abortion. However, their expressions in the placenta of patients with preeclampsia and the correlations of their polymorphisms with the occurrence and development of preeclampsia have not been fully elucidated. In this study, the correlations of SNPs rs101201 and rs23102 in the promoter region of MMP-9 gene and rs201018 and rs102934 in the promoter region of PPARγ gene with the occurrence of preeclampsia were analyzed. Our finding might aim to provide a certain basis for the further exploration of genetic pathogenesis of preeclampsia.

Patients and Methods

Subjects

A total of 200 patients with preeclampsia (Preeclampsia group) treated in our hospital from June 2016 to March 2019 were selected as research subjects, with an age of (32.19±2.48) years old. Venous blood (4 mL) was collected, anti-coagulated with sodium citrate and stored in a refrigerator at -20°C for use. Besides, 100 pregnant women with normal and term delivery in the corresponding period were enrolled in Control group, with an age of (28.02±5.82) years old. This study was approved by the Ethics Committee of Huadu District Maternal and Child Health Hospital. Informed consent was obtained from all participants before the study. All patients in Preeclampsia group met the diagnostic criteria in the *Guidelines for the Diagnosis and Treatment of Gestational Hypertension (2019)* and had no history of immunological diseases, such as chronic hypertension, nephropathy, placental abruption, gestational diabetes, diabetes and systemic lupus erythematosus.

Western Blotting

Freshly frozen placental tissues of patients in a refrigerator at -80°C were firstly taken out, cut into pieces using scissors, and ground thoroughly with a grinder. Subsequently, the tissues were subjected to ultrasonic lysis, and the resulting lysate was centrifuged. The supernatant was then aspirated and sub-packaged into Eppendorf (EP) tubes. Protein concentration was determined by the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA) and ultraviolet spectrophotometric assay. All the protein samples were maintained at the same constant concentration, sub-packaged and preserved in a refrigerator at -80°C. Total proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After incubation with primary antibodies at 4°C overnight, the membranes were incubated with goat anti-rabbit secondary antibodies in the dark for 1 h. Immuno-reactive bands were finally scanned and quantified using an Odyssey membrane scanner (Seattle, WA, USA).

Deoxyribonucleic Acid (DNA) Extraction and Polymerase Chain Reaction (PCR) Amplification

EDTA-anticoagulated blood (4 mL) was collected from patients in the two groups, from which genomic DNAs were extracted according to the instructions of a DNA extraction kit (Wuhan Servicebio Technology Co., Ltd., Wuhan, China). Extracted DNAs were taken for mass measurement with 1.5% agarose gel electrophoresis, and an ultraviolet spectrophotometer was used to detect the concentration of extracted DNAs at the same time. Primers of rs101201 and rs23102 polymorphisms in the promoter region of MMP-9 gene and rs201018 and rs102934 polymorphisms in the promoter region of PPARγ gene were designed for amplification (Table I). The PCR amplification was performed in a 20 μL system composed of 2.0 μL (0.2 ug) of DNA template, 10.0 μL of 2× MIX, 0.4 μL (10-100 pmol) of forward primers, 0.4 μL (10-100 pmol) of reverse primers, and 7.2 μL of ddH_2O. Specific procedures were as follows: 95°C for 120 s, 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s, for a total of 35 cycles, followed by extension at 72°C for 10 min. Finally, the amplification of gene fragments was determined by agarose gel electrophoresis.

Table I. Primer sequences and product sizes of different polymorphisms in the promoter region of MMP-9 and PPARγ genes.

Gene	Polymorphism	Primer sequence (5′-3′)	Product (bp)
MMP-9	rs101201	Forward: GTCGTAGATGATGTACCAAGG Reverse: ATGATGCTGTAGATGTCGTAGC	145
	rs23102	Forward: TGATGCTGATGCTGCTAAAA Reverse: ACGTAGTAGTAGCTGATGCTA	156
PPARγ	rs201018	Forward: AGCTGATGTCGATGTGCAAAAC Reverse: ACGTAGTCGTAGTCGTGATGTC	234
	rs102934	Forward: CCTGATGTCGTACCACCGTGAC Reverse: AGCTGATGTCGTAGTCGTAGTC	231

Ligase Detection Reaction

The forward and reverse probes were designed and synthesized by BGI. After modification by 5' terminal phosphorylation, all forward probes were prepared into a probe mixture with a concentration of 12.5 pmol/μL. The ligase detection reaction system (3.05 μL) consisted of 0.05 μL of ligase, 1 μL of buffer, 1 μL of PCR products, and 1 μL of probe mixture. PCR amplification conditions were as follows: 95°C for 120 s, 94°C for 15 s and 50°C for 25 s. After 30 cycles, the concentration was detected using an ultraviolet spectrophotometer. Next, BGI was commissioned to conduct the sequencing and fragment analysis of target genes. All data were analyzed using GeneMapper (Table II).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was utilized for all statistical analysis. Enumeration data were expressed as frequency and percentage, and measurement data were expressed as mean ± standard deviation. Hardy-Weinberg equation was used to test the calculation frequency of genotypes in samples. Enumeration data were subjected to chi-square test and multiple comparisons, and *t*-test and analysis of variance were employed for measurement data. $p<0.05$ was considered statistically significant.

Results

Demographic Data of Two Groups of Research Subjects

There were statistically significant differences in age, body mass index (BMI), gravidity, parity, gestational age, systolic blood pressure, diastolic blood pressure, serum creatinine, blood urea nitrogen, plasma albumin, neonatal birth weight, neonatal birth length and 24-h urinary protein quantity between Control group and Preeclampsia group ($p<0.05$) (Table III).

Protein Expressions of MMP-9 and PPARγ in the Placenta of Patients in the Two Groups

Western blotting results revealed that in comparison with Control group, Preeclampsia group

Table II. Probe sequences and product sizes of different polymorphisms of MMP-9 and PPARγ genes in ligase detection reaction.

Gene	Polymorphism	Probe	Probe sequence (5′-3′)	Product (bp)
MMP-9	rs101201	rs101201	P-CTGCTAGTCGTAAACTTTTTTTTTTTTTTTTTTT-FAM	190
		rs101201-C	TTTTTTTTTCAGTCGTAGTCGTGATGTCTTTTTTTTTAT	
		rs101201-G	TTTTTTTTTTTTTACGTAGTGATGCTAGTAGTTTTTTAAA	
	rs23102	rs23102	P-AGCACACGTGTCAGCTTTTTTTTTTTTTT-FAM	214
		rs23102-C	TTTTTTTTTTTTTTTTTTTTACACACGTAGCTAGTCG	
		rs23102-T	TTTTTTTTTTTTTTTTTTTTTCAGCTAGTAGATGCTA	
PPARγ	rs201018	rs201018	P-ACGTAGTCGTAGAAACTTTTTTTTTTTTTTTTTTT-FAM	183
		rs201018-C	TTTTTTTTTAGCTGATGTCCCCGTAGTCTTTTTTTTTAT	
		rs201018-T	TTTTTTTTTTTTTTCGATCGTAGTCGTAGTCGTTTTTTTAAA	
	rs102934	rs102934	P-TGTCGATGCTGTAGCTGTCCATTTTTTTTTTTTTT-FAM	177
		rs102934-A	TTTTTTTTTTTTTTTTTTTTCACGTGATGTCGATGCTAGCT	
		rs102934-G	TTTTTTTTTTTTTTTTTTTTCAGTGATGTAGTTTACGTTTTT	

Table III. Comparisons of demographic data between two groups of research subjects.

	Preeclampsia group (n = 200)	Control group (n = 100)	p
Age (years old)	32.19 ± 2.48	27.66 ± 3.82	0.001*
BMI (kg/m^2)	23.02 ± 0.19	20.18 ± 1.92	0.002*
Gravidity (times)	3.19 ± 0.54	1.45 ± 0.68	0.000*
Parity (times)	1.99 ± 0.83	0.29 ± 0.13	0.002*
Gestational age (weeks)	33.10 ± 3.21	38.68 ± 1.54	0.000*
Systolic blood pressure (mmHg)	158.45 ± 8.57	123.26 ± 6.23	0.000*
Diastolic blood pressure (mmHg)	103.58 ± 2.13	74.63 ±3.92	0.000*
Serum creatinine (μmol/L)	68.49 ± 2.45	50.99 ± 3.68	0.000*
Blood urea nitrogen (μmol/L)	6.42 ± 0.38	3.04 ± 0.48	0.001*
Plasma albumin (g/L)	27.78 ± 2.55	33.92 ± 1.02	0.001*
Neonatal birth weight (g)	2598.66 ± 1014.53	3611.05 ± 400.98	0.001*
Neonatal birth length (cm)	42.56 ± 2.06	54.04 ±1.55	0.000*
24-h urinary protein quantity (g)	3.94 ± 0.93	0	0.000*

exhibited significantly down-regulated protein expression level of MMP-9 (p<0.05) and up-regulated protein expression level of PPARγ in placental tissues (p<0.05) (Figure 1).

Results of Hardy-Weinberg Equilibrium Test

Hardy-Weinberg equation was adopted to detect the linkage disequilibrium (LD) between rs101201 and rs23102 polymorphisms in the promoter region of MMP-9 gene and that between rs201018 and rs102934 polymorphisms in the promoter region of PPARγ gene. According to the results (Tables IV and V), the r^2 between polymorphisms was less than 0.33, suggesting that polymorphisms in each group were in accordance with the law of genetic equilibrium and constant.

Correlations of MMP-9 and PPARγ Gene Polymorphisms with Preeclampsia

The distribution frequencies of genotypes of MMP-9 and PPARγ gene polymorphisms in the two groups were shown in Table VI. The rs101201 polymorphism in the promoter region of MMP-9 gene was significantly correlated with the development of preeclampsia (p<0.05). However,

Table IV. Results of Hardy-Weinberg equilibrium test of MMP-9 gene.

	r^2	
Polymorphism	rs101201	rs23102
rs101201	–	0.003
rs23102	0.003	–

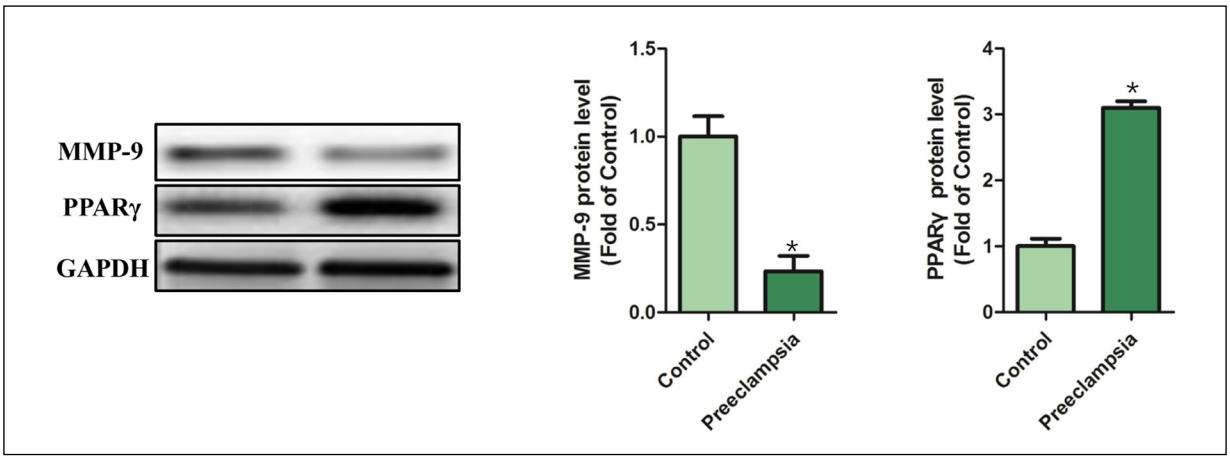

Figure 1. Protein expressions of MMP-9 and PPARγ in the placenta of patients in Preeclampsia group and Control group. *: a statistically significant difference *vs.* Control group.

Table V. Results of Hardy-Weinberg equilibrium test of PPARγ gene.

Polymorphism	r^2	
	rs201018	rs102934
rs201018	–	0.022
rs102934	0.022	–

there was no statistically significant correlation between the rs23102 polymorphism of MMP-9 gene and the development of preeclampsia (p>0.05). Similarly, the rs201018 polymorphism in the promoter region of PPARγ gene was significantly associated with the development of preeclampsia (p<0.05), whereas the rs102934 polymorphism of PPARγ gene showed no association with the development of preeclampsia (p>0.05).

Relations of Allelotypes of MMP-9 and PPARγ Genes to Preeclampsia

Based on Table VII, the occurrence of preeclampsia was significantly correlated with the allelotypes of MMP-9 gene polymorphism rs101201 (p<0.05) and PPARγ gene polymorphism rs201018 (p<0.05).

Associations of Genotype GG of MMP-9 Gene Polymorphism rs101201 and Genotype TT of PPARγ Gene Polymorphism rs201018 With Clinical Parameters of Preeclampsia

BMI value, gestational age, systolic blood pressure, and serum creatinine level in preeclampsia patients with genotype GG of the rs101201 polymorphism in the promoter region of MMP-9 gene were not statistically significantly different from those in Control group (p>0.05) (Table VIII). Meanwhile, no statistically significant differences were observed in gravidity, parity, gestational age, systolic blood pressure, serum creatinine level, and plasma albumin level between preeclampsia patients with genotype TT of the rs201018 polymorphism in the promoter region of PPARγ gene and subjects in Control group (p>0.05) (Table IX).

Table VI. Distribution of different genotypes of MMP-9 and PPARγ gene polymorphisms in Preeclampsia group.

Gene Group	MMP-9						PPARγ					
	rs101201			rs23102			rs201018			rs102934		
	CC	CG	GG	CC	CT	TT	CC	CT	TT	AA	AG	GG
Preeclampsia group (n = 200)	7%	23%	70%	29%	51%	20%	23%	12%	65%	23%	53%	24%
Control group (n = 100)	24%	52%	24%	27%	45%	28%	26%	49%	25%	25%	50%	25%
χ^2		5.231			0.883			4.022			0.778	
p		0.000*			0.492			0.001*			0.512	

Table VII. Distribution of allelotypes of MMP-9 and PPARγ gene polymorphisms in Preeclampsia group.

Gene Group	MMP-9				PPARγ			
	rs101201		rs23102		rs201018		rs102934	
	C	G	C	T	C	T	A	G
Preeclampsia group (n = 200)	18.5%	81.5%	54.5%	45.5%	29%	71%	49.5%	50.5%
Control group (n = 100)	50%	50%	49.5%	50.5%	50.5%	49.5%	50%	50%
χ^2	1.432		0.662		2.092		0.821	
p	0.000*		0.238		0.001*		0.892	

Table VIII. Associations of genotype GG of MMP-9 gene polymorphism rs101201 with clinical parameters of preeclampsia.

Indicator	Genotype GG		p
	Preeclampsia group (n = 46)	Control group (n = 52)	
Age (years old)	32.84 ± 2.04	28.04 ± 5.02	0.001*
BMI (kg/m^2)	20.82 ± 0.62	20.44 ± 1.92	0.942
Gravidity (times)	3.10 ± 0.03	0.63 ± 0.44	0.001*
Parity (times)	2.05 ± 0.59	0.46 ± 0.39	0.002*
Gestational age (weeks)	37.19 ± 3.02	38.08 ± 1.03	0.359
Systolic blood pressure (mmHg)	133.09 ± 1.45	131.09 ± 2.56	0.503
Diastolic blood pressure (mmHg)	95.62 ± 2.56	72.34 ± 1.93	0.000*
Serum creatinine (μmol/L)	58.56 ± 3.92	57.32 ± 3.98	0.172
Blood urea nitrogen (μmol/L)	5.04 ± 0.22	3.18 ± 0.67	0.038*
Plasma albumin (g/L)	31.19 ± 2.66	32.56 ± 2.93	0.084
Neonatal birth weight (g)	2422.56 ± 645.58	3656.99 ± 541.23	0.000*
Neonatal birth length (cm)	46.11 ± 2.34	50.39 ± 3.66	0.002*
24-h urinary protein quantity (g)	3.68 ± 0.54	0	0.000*

Discussion

Systemic arteriole spasm and visceral hemoperfusion reduction are the basic pathophysiological changes of preeclampsia, which will cause irreversible injuries in severe cases[7]. Up to date, the pathogenesis of preeclampsia remains unclear. The major theories about its pathogenesis include over-activation of inflammatory immune responses, dysfunction of uterine spiral arteriolar remodeling, vascular endothelial cell damage, genetic factors and insulin resistance[8]. Age, gravidity and parity, history of preeclampsia in a previous pregnancy, family history of hypertension, multiple pregnancy, poor socioeconomic status, malnutrition, assisted reproductive technique, and medical comorbidities (including diabetes, hypertension, nephropathy, thrombophilia, urinary tract infection, periodontitis and autoimmune diseases) are considered as high-risk factors for preeclampsia[9,10]. Therefore, the exploration of the etiology and pathogenesis of preeclampsia is of great significance for its early prevention and precise treatment in the future.

With the completion of the Human Genome Project and the development of molecular biological technology, many studies have been conducted to discover genes susceptible to preeclampsia. Human diseases are often caused by the joint action of several SNPs rather than a single SNP[11]. Some scholars have believed that the distance between different gene polymorphisms on a chromosome or in a certain region is able to affect the susceptibility of the gene itself. LD refers to the phenomenon that the combination frequency of alleles of different polymorphisms

Table IX. Associations of genotype TT of PPARγ gene polymorphism rs201018 with clinical parameters of preeclampsia.

Indicator	Genotype TT		p
	Preeclampsia group (n = 130)	Control group (n = 25)	
Age (years old)	31.24 ± 1.67	28.88 ± 3.26	0.001*
BMI (kg/m^2)	24.19 ± 0.46	18.57 ± 1.01	0.000*
Gravidity (times)	2.09 ± 0.23	2.09 ± 0.56	0.673
Parity (times)	1.02 ± 0.32	1.03 ± 0.81	0.582
Gestational age (weeks)	36.57 ± 3.56	37.83 ± 2.99	0.389
Systolic blood pressure (mmHg)	134.12 ± 2.82	133.67 ± 2.65	0.711
Diastolic blood pressure (mmHg)	96.24 ± 3.78	73.94 ± 3.29	0.000*
Serum creatinine (μmol/L)	58.56 ± 3.81	57.45 ± 2.87	0.431
Blood urea nitrogen (μmol/L)	4.78 ± 0.28	4.15 ± 0.68	0.038*
Plasma albumin (g/L)	30.92 ± 2.92	31.66 ± 1.82	0.078
Neonatal birth weight (g)	2647.45 ± 456.25	3827.83 ± 718.28	0.000*
Neonatal birth length (cm)	41.66 ± 2.58	52.02 ± 1.46	0.002*
24-h urinary protein quantity (g)	3.01 ± 0.64	0	0.000*

deviates significantly from that under equilibrium in a certain group[12]. Haplotypes are defined as the linear arrangement of many tightly linked alleles on a chromosome. SNP haplotypes refer to the linear arrangement of nucleotide bases of different SNPs, and each linear arrangement corresponds to one SNP haplotype. The genetic susceptibility of preeclampsia and other complex diseases of multifactorial inheritance mainly depends on the joint action of multiple polymorphisms. Meanwhile, the independent action of some micro-effective SNPs on preeclampsia may be covered by the effect of neighboring Ship. Therefore, the association between a SNP and preeclampsia is not obvious in SNP analysis. Moreover, the combination of different alleles of the same gene may have different effects on transcriptional regulation or protein function, so it is of crucial significance to analyze haplotypes[13,14]. However, preeclampsia-associated haplotypes constructed with multiple SNPs of MMP-9 and PPARγ genes have not been fully reported.

MMPs, a group of zinc ion-dependent endopeptidases, are widely distributed in plants, vertebrates, and invertebrates. Besides, they are the main enzymes for degradation of extracellular matrix and basement membrane. For example, they can degrade almost all components of extracellular matrix, such as collagen, elastin, and fibrin. MMPs act as important regulators of cell microenvironment and play crucial roles in cell proliferation, migration, differentiation, senescence and apoptosis and host defense. Furthermore, MMPs can destroy the basement membrane, change keratinocytes and epithelium of blood vessels or lymphatic ducts, and promote local invasion and metastasis of tumors[15]. MMP-9 gene is located on 20q13.12, with a total length of 7.7 kb. It consists of 13 exons and 12 introns[16]. A previous study[17] has shown that the activity of MMP-9, MMP-2 and serum elastase is visibly correlated with systolic hypertension and arteriosclerosis. In addition, MMP-9 gene polymorphism has been confirmed associated with the occurrence of hypertension[18]. However, the exact role of MMP-9 in gestational hypertension and preeclampsia has not been elucidated. In this study, it was found that MMP-9 was lowly expressed in placental tissues of patients with preeclampsia. Furthermore, the rs101201 polymorphism and its allelotypes in the promoter region of MMP-9 gene were closely correlated with the occurrence of preeclampsia.

PPARγ, a member of the PPAR family, is activated mainly by binding to ligands. It further participates in various biological functions of the body, such as controlling inflammation, affecting lipid metabolism, repressing cell differentiation and proliferation, and inducing cell apoptosis[19,20]. Activated PPARγ is capable of reducing the expression of MMP-9 in the serum of patients with type 2 diabetes and coronary heart disease[21]. In the present study, our results indicated that PPARγ was highly expressed in placental tissues of patients with preeclampsia. In addition, the rs201018 polymorphism and its allelotypes in the promoter region of PPARγ gene were associated with the occurrence of preeclampsia.

Conclusions

Briefly, our results revealed for the first time that MMP-9 and PPARγ were abnormally expressed in patients with preeclampsia, and their polymorphisms were associated with the occurrence of preeclampsia. All our findings suggested that the rs101201 polymorphism in the promoter region of MMP-9 gene and rs201018 polymorphism in the promoter region of PPARγ gene could be used as genetic markers for risk assessment for preeclampsia in women.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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