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Correlations of TIMP2 and TIMP3 gene polymorphisms with primary open-angle glaucoma

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Abstract. – OBJECTIVE: To explore the correlations of the tissue inhibitor of metalloproteinase-2 (TIMP2) and TIMP3 gene polymorphisms with primary open-angle glaucoma (POAG).

PATIENTS AND METHODS: 295 POAG patients were recruited as the observation group and 200 healthy people as the control group. The general clinical information, medical history (hypertension, diabetes and hyperlipemia) and family history were collected. The vascular endothelium and hemorheology indexes in each group were detected. Moreover, the polymorphisms of TIMP2 rs796391657 and TIMP3 rs8136803 were detected via TaqMan-MGB probe assay.

RESULTS: The observation group exhibited higher prevalence rates of diabetes, hyperlipemia and family history than those of the control group ($p<0.05$), and there were no differences in age, sex and hypertension prevalence rate between the two groups ($p>0.05$). The observation group had a lower nitric oxide (NO) level, but higher levels of endothelin-1 (ET-1), plasma viscosity (PV), hematocrit (HCT) and fibrinogen (FIB) than those of the control group ($p<0.05$). The distribution of the three genotypes and alleles of TIMP2 rs796391657 varied a lot between the two groups ($p<0.05$). The frequency of genotype CC was markedly higher than that of genotype TT; however, C allele had a higher frequency than T allele. Similarly, the three genotypes and alleles of TIMP3 rs8136803 were differentially distributed in the two groups ($p<0.05$). The frequency of genotype GG was higher than that of genotype TT, while the frequency of G allele was higher than that of T allele.

CONCLUSIONS: The TIMP2 and TIMP3 gene polymorphisms are correlated with POAG.

Key Words:

Tissue inhibitor of metalloproteinases, Primary open-angle glaucoma, Single nucleotide polymorphism.

Introduction

Clinically, primary open-angle glaucoma (POAG) is a common chronic progressive ocular disease and has no significant manifestations at its early stage. However, with the disease progressing, POAG will further develop into visual field defect, optic nerve damage, optic nerve cupping and atrophy. Glaucoma has become the second leading cause of blindness worldwide and POAG is a relatively common type of glaucoma[1]. According to the epidemiology survey, there were about 60.5 million glaucoma patients in 2010, and the total number is expected to increase to 79.6 million by 2020, 74% of which are POAG patients[2]. Glaucoma not only seriously affects the life quality of patients, but also causes a huge burden to the society and their families. Therefore, it is extremely urgent to study the related pathogenic factors for POAG. Currently, scholars believe that POAG features in significant familial aggregation[3-5] suggesting that this disease is notably associated with genetic heredity. Additionally, related studies have revealed that the disruption of the balance between tissue inhibitor of metalloproteinases (TIMPs) and their matrix metalloproteinases (MMPs) can lead to the occurrence and development of glaucoma[6,7]. Hence, with TIMP2 and TIMP3 genes from the TIMPs family as candidates, the work investigated the correlations of TIMP2 and TIMP3 gene polymorphisms with the onset of POAG. Moreover, we detected the polymorphisms of TIMP2 rs796391657 and TIMP3 rs8136803 using TaqMan-MGB probes, to provide certain theoretical support for PAOG genetic polymorphism.

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Patients and Methods

Patients

POAG patients treated in the Department of Ophthalmology in The Second Affiliated Hospital of Xi'an Medical University from January 2016 to January 2018 were enrolled. We diagnosed POAG according to the standard in the *Preliminary Recommendations on the Early Diagnosis of Primary Glaucoma* by Glaucoma Group, Ophthalmological Society, Chinese Medical Association in 1987. The standards were as follows: (1) The intraocular pressure of over 21 mmHg based on 24 h monitoring or multiple measurements, (2) Glaucomatous defect in retinal nerve fiber layer and (or) optic nerve head damage, (3) Glaucoma visual field defects, (4) Patients with open anterior chamber angles in 3 quadrants at least discovered *via* slit-lamp gonioscopy, (5) Those with favorable compliance and complete information.

Exclusion criteria: (1) Patients with other organic ocular diseases, (2) Dysfunctions of the heart, kidney, liver or other major organs, or (3) Mental diseases or other cognitive dysfunctions who could not cooperate. According to the above criteria, 295 POAG patients were included in this study, and they were aged (58.86±4.25) on average. Meanwhile, 200 healthy people without ocular diseases and undergoing eye surgeries in the same period were selected from the hospital as the control group, and they were aged (58.70±5.03) on average.

All study subjects were unrelated Chinese Han individuals and signed the informed consent. This study was approved by the Ethics Committee of The Second Affiliated Hospital of Xi'an Medical University.

Collection of General Clinical Information

Name, age, sex, medical history (hypertension, diabetes mellitus, and hyperlipemia) and family history of all subjects were collected. After 5 mL of venous blood was drawn from the elbows of patients and centrifuged for 5 min at 800 g and 4°C, the serum was sub-packaged into Eppendorf (EP) tubes (200 μL/tube) and stored at -80°C for standby use. The levels of vascular endothelial function indexes [nitric oxide (NO), endothelin-1 (ET-1)] and hemorheology indexes [plasma viscosity (PV), hematocrit (HCT) and fibrinogen (FIB)] were measured.

Extraction of Deoxyribonucleic Acid (DNA)

After 1 mL of venous blood was taken from the elbows of the subjects, DNA was extracted using a medium-amount whole blood genomic DNA extraction kit (BioTeke Corporation, Beijing, China) according to the instructions in the kit. Moreover, TaqMan® single nucleotide polymorphism (SNP) genotyping assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was employed to detect and analyze the genotypes of the samples (Table I).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Measurement data were expressed as ($\bar{x} \pm s$). The independent-samples *t*-test was employed for comparing the difference between the two groups. Chi-square (χ^2) test was adopted for the comparisons of count data. The likelihood-ratio χ^2-test was performed to analyze whether the genotype distribution met the Hardy-Weinberg equilibrium law. R×C χ^2-test was applied for comparing the frequency of genotypes and alleles in each group. $p<0.05$ was considered statistically significant.

Results

Comparisons of Basic Data

The prevalence rates of diabetes mellitus, hypertension, hyperlipidemia and family history

Table I. Measurement information of TIMP2 rs796391657 and TIMP3 rs8136803 via TaqMan®-MGB probe assay.

SNP reference	rs796391657	rs8136803
Assay ID	C_342702242_10	C__29344894_10
Protein ID	NP_003246.1	NP_001129246.1
SNP Type	Intron	Intron
Context sequence	TCCTTGGTTAAGCAGCAGCTGTTGG[C/T]GGGTGCTCTGTCCAGGCCTCCGCTA	TGGCACTTTATACAAGAAATCACAC[G/T]TAGCTTCCCATATTCAGCAAATACA

Table II. Comparisons of basic data between the two groups.

Group	No	Age (years old)	Male/ female	Hypertension (n)	Diabetes (n)	Hyperlipidemia (n)	Family history (n)
Observation group	295	58.86 ± 4.25	165/130	213 (72.20)	189 (64.07)	151 (51.19)	100 (33.90)
Control group	200	58.70 ± 5.03	113/87	130 (65.00)	53 (26.50)	73 (36.50)	27 (13.50)
t/χ^2		0.684	0.016	2.907	67.321	10.377	26.001
p		0.496	0.901	0.088	0.000	0.001	0.000

were higher in the observation group than those in the control group ($p<0.01$). In addition, there were no differences in name, sex and the prevalence rate of hypertension between the two groups ($p>0.05$) (Table II).

Comparisons of Vascular Endothelial Function and Hemorheology Indexes

The observation group exhibited a lower NO level but higher ET-1, PV, HCT and FIB levels than those of the control group ($p<0.05$) (Table III).

Genetic Equilibrium Test

The likelihood-ratio χ^2-test was conducted for the actual and theoretical frequency of TIMP2 rs796391657 and TIMP3 rs8136803 genotypes in the observation group and control group. The frequency distributions of TIMP2 rs796391657 and TIMP3 rs8136803 genotypes in both groups were consistent with the Hardy-Weinberg equilibrium law ($p>0.05$) and comparable (Table IV and V).

Comparison of Genotype Distribution Frequency

The distribution frequency of three genotypes of TIMP2 rs79639165 was different between the two groups. The frequency of genotype CC was significantly higher than that of genotype TT ($p<0.01$). The comparison also showed the difference in the distribution frequency of three genotypes of TIMP3 rs8136803 between the two groups, and genotype GG had a higher frequency than genotype TT ($p<0.05$) (Table VI).

Comparison of Allele Distribution Frequency

The distribution frequency of TIMP2 rs79639165 alleles was different between the two groups, and C allele had a substantially higher frequency than T allele ($p<0.01$). The comparison also revealed the difference in the distribution frequency of TIMP3 rs8136803 alleles between the two groups, and the frequency of G allele was a higher frequency than T allele ($p<0.01$) (Table VII).

Table III. Comparisons of vascular endothelial function and hemorheology indexes between the two groups.

Group	No	NO (μmol/L)	ET-1 (Pg/mL)	PV (mPa/s)	HCT (%)	FIB (g/L)
Observation group	295	44.45 ± 9.12	82.52 ± 9.69	1.73 ± 0.48	54.42 ± 4.37	3.87 ± 0.78
Control group	200	63.15 ± 9.82	54.18 ± 8.96	1.32 ± 0.49	40.59 ± 3.17	3.06 ± 0.88
t		5.672	6.876	3.572	3.876	4.985
p		0.032	0.021	0.042	0.037	0.035

Table IV. Genetic equilibrium test of TIMP2 rs796391657 genotype.

Group	No	CC		CT		TT		χ^2	p
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Observation group	295	250	247.12	40	45.76	5	2.12	4.68	0.10
Control group	200	117	118.58	74	70.84	9	10.58	0.40	0.82

Table V. Genetic equilibrium test of TIMP3 rs8136803 genotype.

Group	No	CC		CT		TT		χ^2	p
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Observation group	295	152	145.25	110	123.50	33	26.25	3.52	0.17
Control group	200	77	76.26	93	94.48	30	29.26	0.05	0.98

Table VI. Comparisons of the genotype distributions of TIMP2 rs79639165 and TIMP3 rs8136803 between the two groups.

Gene	SNP code	Genotype	Observation group	Control group	χ^2	p
TIMP2	rs796391657	CC/CT/TT	250 (84.75)/ 40 (13.56)/ 5 (1.69)	117 (58.50)/ 74 (37.00)/ 9 (4.50)	42.827	0.000
TIMP3	rs8136803	GG/GT/TT	152 (51.53)/ 110 (37.29)/ 33 (11.19)	77 (38.50)/ 93 (46.50)/ 30 (15.00)	8.200	0.017

Discussion

POAG is a common ocular disease that causes optic nerve head damage and visual field defects due to the pathological increase in intraocular pressure. Although pathologically elevated intraocular pressure is a crucial cause of POAG, its onset is also associated with the history of related diseases, family history, peripheral angiopathy and vasospasm[8-11]. In this work, the prevalence rates of diabetes, hyperlipidemia and family history in the observation group were higher than those of the control group. Those results indicated that POAG onset is correlated with diabetes, hyperlipidemia and familial heredity. POAG is a slowly progressive optic neuropathy, while diabetes is also a lifelong and chronic metabolic disease. The poor control of blood glucose levels in diabetes patients can cause autonomic and peripheral neuropathies. It also contributes to the changes in osmotic pressure of body fluids, which increases aqueous humor. It ultimately gives rise to the increase in intraocular pressure and induces POAG[12,13]. Hyperlipidemia can bring about raised PV to lower the ophthalmic artery perfusion pressure, thereby aggravating optic nerve injuries[14]. In recent years, other studies have pointed out that familial aggregation is discovered in POAG, a multi-factor hereditary disease[15]. The results of this work corroborated the correlation of POAG with familial heredity again. Therefore, more attention should be paid to the adjustment of blood glucose and lipid for patients with diabetes and hyperlipidemia, to reduce the risk of concurrent POAG. As to the high-risk groups with the POAG family history, measures are supposed to be actively taken for early prevention and treatment.

Some scholars have found that the occurrence of POAG is related to blood stasis and the pathological change of excessive accumulation of aqueous humor. The vascular endothelial function and hemorheology indexes can objectively reflect the blood stasis of body and have been widely applied in clinical practices. Other scholars have pointed out that ET-1 is correlated with

Table VII. Comparisons of the allele distributions of TIMP2 rs79639165 and TIMP3 rs8136803 between the two groups.

Gene	SNP code	Genotype	Observation group	Control group	χ^2	p
TIMP2	rs796391657	C/T	540 (91.53)/ 50 (8.47)	308 (77.00)/ 92 (23.00)	40.937	0.000
TIMP3	rs8136803	G/T	414 (70.17)/ 176 (29.83)	247 (61.75)/ 153 (38.25)	7.616	0.006

multiple pathological changes, such as those in intraocular pressure, choroid and retina regulation and believe that vascular endothelial function is involved in the occurrence of POAG[16]. According to the findings of this study, the observation group had a lower NO level than the control group, but the level of ET-1 was higher than that in the control group. These results indicated that vascular endothelial functions are crucial to the onset of POAG and visual nerve defects. Specifically, NO is the protective factor of reducing POAG incidence rate, while ET-1 is the risk factor of increasing it. Hemorheological abnormalities often induce systemic or local hemodynamic disorders, and then ischemia and hypoxia occur, finally causing necrosis, edema, inflammation and other pathological changes to tissues and organs. The increase of blood viscosity exerts a certain effect on the onset of POAG. Under normal physiological conditions, the vascular diameter and perfusion pressure are the main factors affecting microcirculation. The blood flow volume is negatively correlated with blood viscosity, but positively correlated with vascular diameter. Nevertheless, when the shear rate of retinitis declines and blood viscosity increases, the retinal blood perfusion remains decreased. As a result, local ischemia is induced, ultimately aggravating optic atrophy. The results of this work showed that the levels of PV, HCT and FIB were higher in the observation group than in the control group, suggesting the increased blood viscosity serves as the risk factor for POAG. Therefore, it is held that detecting vascular endothelial function and hemorheology indexes in patients with POAG are of great significance for effectively evaluating the disease and timely adjusting treatment regimens.

The recent studies have manifested that genetic factors play important roles in the onset of POAG. In the present work, the TIMP2 and TIMP3 genes were selected as candidates from the TIMPs family. And we explored whether the polymorphisms of these two genes are correlated with POAG onset. TIMPs (TIMP1, TIMP2, TIMP3, and TIMP4). TIMPs participate in the growth, apoptosis, proliferation and migration of cells and the angiogenesis as well as its inhibition. In the majority of reactions, TIMPs exert effects *via* suppressing MMPs. TIMP2, a soluble secretory protein, is the inhibitor of MMP-2, and TIMP3 is a non-soluble protein that can bind to ECM[17,18]. The over-expression of TIMP2 and TIMP3 can lead to the dynamic imbalance between the extracellular matrix synthesis and degradation, and influence the discharge of aqueous humor, further raising the intraocular pressure to worsen POAG damage[19,20]. In this study, the TIMP2 rs796391657 (C/T) and TIMP3 rs8136803 (G/T) were employed, and TaqMan-MGB probe assay was performed to analyze the frequency of genotypes and alleles in the observation group and control group. According to the results, there were significant differences in the composition of the mutation genotype distribution of TIMP2 rs796391657 (C/T) and TIMP3 rs8136803 (G/T) between the two groups. This indicated that the polymorphisms of TIMP2 rs796391657 (C/T) and TIMP3 rs8136803 (G/T) are correlated with the risk of POAG onset. Genotype CC at TIMP2 rs796391657 and genotype GG at TIMP3 rs8136803 were its risk genotypes. The alleles were further analyzed in the two groups. The alleles of TIMP2 rs796391657 (C/T) and TIMP3 rs8136803 (G/T) were notably differentially distributed in the two groups, suggesting that the C allele at TIMP2 rs796391657 and G allele at TIMP3 rs8136803 act as the risk alleles of POAG.

Conclusions

We showed that the TIMP2 and TIMP3 gene polymorphisms are correlated with POAG.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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References

1) Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90: 262-267.
2) Strom AR, Hassig M, Iburg TM, Spiess BM. Epidemiology of canine glaucoma presented to University of Zurich from 1995 to 2009. Part 2: secondary glaucoma (217 cases). Vet Ophthalmol 2011; 14: 127-132.
3) O'Brien JM, Salowe RJ, Fertig R, Salinas J, Pistilli M, Sankar PS, Miller-Ellis E, Lehman A, Murphy W, Homsher M, Gordon K, Ying GS. Family history in the primary open-angle African American glaucoma genetics study cohort. Am J Ophthalmol 2018; 192: 239-247.

4) Wang F, Li Y, Lan L, Li B, Lin L, Lu X, Li J. Ser-341Pro MYOC gene mutation in a family with primary open-angle glaucoma. Int J Mol Med 2015; 35: 1230-1236.

5) Pacella E, Pacella F, Cavallotti C, Librando A, Feher J, Pecori-Giraldi J. The combination latanoprost-timolol versus twice daily 0.50% timolol administration either associated or not with latanoprost: efficacy and tolerability in the primary open-angle glaucoma. Eur Rev Med Pharmacol Sci 2010; 14: 477-480.

6) Valimaki J, Uusitalo H. Matrix metalloproteinases (MMP-1, MMP-2, MMP-3 and MMP-9, and TIMP-1, TIMP-2 and TIMP-3) and markers for vascularization in functioning and non-functioning bleb capsules of glaucoma drainage implants. Acta Ophthalmol 2015; 93: 450-456.

7) Kaminska A, Banas-Lezanska P, Przybylowska K, Gacek M, Majsterek I, Szaflik J, Szaflik JP. The protective role of the -735C/T and the -1306C/T polymorphisms of the MMP-2 gene in the development of primary open-angle glaucoma. Ophthalmic Genet 2014; 35: 41-46.

8) Modrzejewska M, Grzesiak W, Zaborski D, Modrzejewska A. The role of lipid dysregulation and vascular risk factors in glaucomatous retrobulbar circulation. Bosn J Basic Med Sci 2015; 15: 50-56.

9) Liu CH, Su WW, Shie SS, Cheng ST, Su CW, Ho WJ. Association between peripheral vascular endothelial function and progression of open-angle glaucoma. Medicine (Baltimore) 2016; 95: e3055.

10) Choi J, Kook MS. Systemic and ocular hemodynamic risk factors in glaucoma. Biomed Res Int 2015; 2015: 141905.

11) Atas M, Arifoglu HB, Hashas AS, Sarli B, Demircan S, Ozkose A, Goktas A. Systemic endothelial function in primary open-angle glaucoma. J Ophthalmol 2014; 2014: 529082.

12) Srikanth K, Kumar MA, Selvasundari S, Prakash ML. Colour doppler imaging of ophthalmic artery and central retinal artery in glaucoma patients with and without diabetes mellitus. J Clin Diagn Res 2014; 8: C1-C2.

13) Khatri A, Shrestha JK, Thapa M, Khatri BK, Kharel M. Severity of primary open-angle glaucoma in patients with hypertension and diabetes. Diabetes Metab Syndr Obes 2018; 11: 209-215.

14) Chen HY, Hsu SY, Chang YC, Lin CC, Sung FC, Chen WC, Kao CH. Association between statin use and open-angle glaucoma in hyperlipidemia patients: a taiwanese population-based case-control study. Medicine (Baltimore) 2015; 94: e2018.

15) Kong X, Zhu W, Chen X, Chen Y, Sun X. Familial aggregation of primary open angle glaucoma in Shanghai, China. Mol Vis 2013; 19: 1859-1865.

16) Lopez-Riquelme N, Villalba C, Tormo C, Belmonte A, Fernandez C, Torralba G, Hernandez F. Endothelin-1 levels and biomarkers of oxidative stress in glaucoma patients. Int Ophthalmol 2015; 35: 527-532.

17) Cadete VJ, Arcand SA, Chaharyn BM, Doroszko A, Sawicka J, Mousseau DD, Sawicki G. Matrix metalloproteinase-2 is activated during ischemia/reperfusion in a model of myocardial infarction. Can J Cardiol 2013; 29: 1495-1503.

18) George SJ, Baker AH, Angelini GD, Newby AC. Gene transfer of tissue inhibitor of metalloproteinase-2 inhibits metalloproteinase activity and neointima formation in human saphenous veins. Gene Ther 1998; 5: 1552-1560.

19) Rasmussen CA, Kaufman PL. The trabecular meshwork in normal eyes and in exfoliation glaucoma. J Glaucoma 2014; 23: S15-S19.

20) Jayaram H, Lozano DC, Johnson EC, Choe TE, Cepurna WO, Saugstad JA, Morrison JC, Keller KE. Author response: comparison of microRNA expression in aqueous humour of normal and primary open-angle glaucoma patients using PCR arrays: a pilot study. Invest Ophthalmol Vis Sci 2017; 58: 4989.