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Correlation between MMP-2 gene polymorphism and cataract susceptibility

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Abstract. – OBJECTIVE: To investigate the correlation between matrix metalloproteinase-2 (MMP-2) gene polymorphism and cataract.

PATIENTS AND METHODS: 104 cataract patients and 100 healthy subjects were enrolled and assigned to the observation group and control group, respectively. General clinical data of the enrolled subjects were collected. The inflammatory factors were detected, and the rs243865 polymorphism of MMP-2 gene was detected using the TaqMan-MGB probe.

RESULTS: The levels of interleukin-6 (IL-6), IL-1β, C-reactive protein (CRP), and tumor necrosis factor-1α (TNF-1α) in the observation group were higher than those in the control group ($p<0.05$). There were significant differences in the genotype and allele distribution frequency between the two groups ($p<0.05$). In the genetic model analysis, the additive model was remarkably different between the two groups ($p<0.05$). However, the recessive model and dominant model were not different between the two groups ($p>0.05$).

CONCLUSIONS: Cataract is correlated with inflammatory factors, and the rs243865 polymorphism of MMP-2 gene has a correlation with the incidence of cataract.

Key Words:
Cataract, MMP-2, Single nucleotide polymorphism.

Introduction

Cataract is a kind of malignant ophthalmic disease, in which the long-term metabolic disorders lead to the protein denaturation in the lens, ultimately resulting in opacity. Cataract is believed as an important cause of blindness. The incidence rate of cataract increases with aging. According to the epidemiological survey report, the number of cataract-induced blind people in China will be up to 5.0625 million by 2020. Cataract not only brings an enormous influence on affected patients and families, but also directly affects the development of the whole society. The huge economic burden of cataract to the society makes it a serious social and public health problem. Therefore, it is of great urgency to study the relevant pathogenic factors of cataract. Currently, cataract is considered as the result of the combined effect of congenital genetic factors and acquired environmental factors. However, there is still a lack of reliable experimental data to prove this theory. Some reports have pointed out that the expression level of matrix metalloproteinase-2 (MMP-2) increases after cataract surgery. MMP-2 affects the cell migration, shuttle, proliferation and growth on the posterior capsule of the lens, which is closely related to the occurrence and development of cataract[1-4]. Therefore, cataract patients in our department were collected for detecting rs243865 polymorphism of the MMP-2 gene using the TaqMan-MGB probe. We further analyzed the correlation between the MMP-2 gene polymorphism and cataract, to provide theoretical support for genetic polymorphism of cataract.

Patients and Methods

Patients

This study was approved by the Ethics Committee of Tengzhou Central People's Hospital Cataract patients treated in Tengzhou Central People's Hospital from January 2016 to January 2018 were selected. Cataract was diagnosed based on the diagnostic criteria for cataract in Ophthalmology: 1) Emergence of phacoscotasmus in both eyes, gradual decline of visual acuity, fixed black spots before eyes or monodiplopia. 2) The diascope and slit-lamp microscope examinations of dilated pupils show the opacity of lens cortex. 3) All

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Table I. TaqMan®-MGB probe information of rs243865 site of MMP-2.

SNP Reference	rs243865
Assay ID	C_3225943_10
Protein ID	NP_001121363.1
SNP Type	Intron
Context Sequence	TCCCCATATTCCCCACCCAGCACTC[C/T]ACCTCTTTAGCTCTTCAGGTCTCAG

subjects signed the informed consent. 4) Patients have good compliance and complete data.

Exclusion criteria: 1) patients with other organic diseases in the eyes, 2) patients with dysfunction in the heart, kidney, liver or other important organs, or 3) patients who could not cooperate due to the mental diseases or other cognitive disorders. According to the above criteria, 104 cataract patients with an average age of 66.68±4.81 years were enrolled as the observation group, while 100 healthy people with an average age of 67.51±5.21 years who had no eye diseases and did not receive eye surgery in our hospital during the same period were enrolled as the control group. All objects of this work were Chinese Han people without kinship.

Collection of General Clinical Data

Name, age, gender and history of diseases (hypertension, diabetes mellitus and hyperlipidemia) of the enrolled subjects were collected.

Enzyme-Linked Immunosorbent Assay (ELISA)

5 mL elbow venous blood was drawn from patients and centrifuged at 800 g and 4°C for 5 min. Then, the serum was taken, subpackaged into the 0.5 mL EP tube (200 μL/tube) and stored at -80°C for standby application. The serum levels of interleukin-6 (IL-6), IL-1β, C-reactive protein (CRP) and tumor necrosis factor-1α (TNF-1α) were detected *via* enzyme-linked immunosorbent assay (ELISA).

Extraction of Deoxyribonucleic Acid (DNA)

After 1 mL elbow venous blood was drawn from patients, DNA was extracted using the whole blood genomic DNA extraction kit (Beijing BioTeke Corporation, Beijing, China) according to the instructions. The genotype of samples was detected and analyzed using the TaqMan®SNP Genotyping Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The specific probe information of gene sites was shown in Table I.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for the statistical analysis. Measurement data were expressed as ($\overline{\chi}$±s). The *t*-test was adopted for the comparison of measurement data between the two groups, and the chi-square test was adopted for the comparison of enumeration data. The genotype distribution in accordance with the Hardy-Weinberg equilibrium law was analyzed using likelihood-ratio chi-square test, and R×C chi-square test was performed for the comparison of genotype and allele frequency in each group. $p<0.05$ suggested that the difference was statistically significant.

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

Group	No.	Age (years old)	Male/ female	Hypertension (no.)	Diabetes mellitus (no.)	Hyperlipidemia (no.)
Observation group	104	66.68±4.81	54/50	30 (28.85)	30 (28.85)	16 (15.38)
Control group	100	67.51±5.21	48/52	33 (22.00)	24 (24.00)	12 (12.00)
t/χ^2		1.556	0.716	0.412	0.615	0.493
p		0.092	0.398	0.521	0.433	0.483

Table III. Comparisons of inflammatory factors levels between the two groups.

Group	No.	IL-6 (ng/L)	IL-1β (ng/L)	CRP (mg/L)	TNF-1α (ng/L)
Observation group	104	6.17±2.51	42.53±4.14	9.27±0.80	25.22±3.28
Control group	100	4.41±1.74	30.47±4.83	2.23±0.29	15.57±3.08
t		3.672	5.242	3.471	3.731
p		0.042	0.013	0.044	0.037

Table IV. Hardy-Weinberg equilibrium test of rs243865 genotype of MMP-2 gene.

		CC		CT		TT			
Group	No.	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	χ^2	*p*
Observation group	104	62	57.01	30	39.98	12	7.01	3.24	0.20
Control group	100	72	70.04	25	26.20	3	2.40	1.29	0.53

Table V. Comparison of rs243865 genotype distribution of MMP-2 gene between the two groups.

		Genotype [n (%)]				
Group	No.	CC	CT	TT	χ^2	*p*
Observation group	104	62 (57.01)	30 (28.85)	12 (11.54)	6.525	0.038
Control group	100	72 (72.00)	25 (25.00)	3 (3.00)		

Results

Comparisons of General Data

There were no differences in the general data between the two groups ($p>0.05$) (Table II).

Comparisons of Inflammatory Factors Levels

The serum levels of IL-6, IL-1β, CRP and TNF-1α in the observation group were higher than those in the control group ($p<0.05$) (Table III).

Hardy-Weinberg Equilibrium Test

The likelihood-ratio chi-square test was performed for the actual frequency and theoretical frequency of three genotypes in the observation and control group. It was found that the rs243865 genotype frequency of the MMP-2 gene was in accordance with the Hardy-Weinberg equilibrium law in both groups ($p>0.05$), and was comparable (Table IV).

Comparison of Genotype Distribution Frequency

The CC, CT and TT genotype distribution frequency in the observation group was 57.01%, 28.85% and 11.54%, respectively. In comparison, CC, CT and TT genotype distribution frequency in the control group was 72.00%, 25.00% and 3.00%, respectively, displaying differences between the two groups ($p<0.05$) (Table V).

Comparison of Allele Distribution Frequency

The C and T allele distribution frequency in the observation group was 74.04% and 25.96%, respectively, which was 84.50% and 15.50%, respectively, in the control group, showing differences between the two groups ($p<0.01$) (Table VI).

Rs243865 Genetic Model Analysis

In the genetic model analysis, the additive model was different between the two groups

Table VI. Comparison of C/T allele distribution of MMP-2 gene between the two groups [n (%)].

Group	No.	Allele [no. (%)]		χ^2	*p*
		CT	TT		
Observation group	104	154 (74.04)	54 (25.96)		
Control group	100	169 (84.50)	31 (15.50)	6.766	0.009

Table VII. MMP-2 rs243865 genetic model analysis between the two groups [n (%)].

Item	Observation group	Control group		χ^2	*p*
Recessive model	CC vs. CT+TT	62 (59.62)/42 (40.38)	72 (72.00)/28 (28.00)	3.469	0.063
Dominant model	CC+CT vs. TT	92 (88.46)/12 (11.54)	97 (97.00)/3(3.00)	0.004	0.949
Additive model	CC vs. CT vs. TT	62 (57.01)/30 (28.85)/12 (11.54)	72 (72.00)/25 (25.00)/3 (3.00)	6.525	0.038

($p<0.05$). However, the recessive model and dominant model were not different between the two groups ($p>0.05$), indicating that the additive model was suitable for describing the genetic model of MMP-2 rs243865 in cataract (Table VII).

Discussion

The lens is a transparent, refractive and avascular organ composed of lens fiber cells and anterior subcapsular monolayer epithelial cells, whose main function is the refraction, convergence of rays and ultimately imaging on the retina. Pathological changes in cellular metabolism and structural components in the lens lead to lens opacity, thus resulting in cataract. Cataract is a common disease of the lens, which is the main cause of blindness[5-7]. With the aging of the population, the incidence rate of cataract has been increasing year by year[8,9]. With the rapid development of molecular biological cytology in recent years, the pathogenesis of cataract has been deeply studied at the molecular and genetic levels, but its exact mechanism remains unclear. Studies have found that changes in inflammatory factor levels can retard cell proliferation, thereby prolonging the wound healing time. IL-6, IL-1β, CRP, and TNF-1α are important inflammatory factors mainly produced by vascular endothelial cells and mononuclear macrophages in the body. These inflammatory factors can activate the neutrophils, monocytes and natural killer cells, promote the local leukocyte aggregation and increase the vascular permeability, leading to the inflammatory response. Besides, TNF-1α can also trigger and initiate the inflammatory response[10-13]. In this work, the inflammatory factors between the observation group and the control group were compared. It was found that the levels of IL-6, IL-1β, CRP, and TNF-1α in the observation group were higher than those in the control group. Therefore, it is speculated that the inflammatory response may be involved in the occurrence of cataract, and they may be potential targets for clinical treatment of cataract patients.

Some studies[14-17] have demonstrated that the abnormal gene expression is closely related to the occurrence of cataract, which can lead to changes in the transcription and translation of various proteins in the lens, thus impairing lens structure and function, and ultimately resulting in cataract. Therefore, searching the genes specifically expressed in the lens is of great importance in exploring the pathogenesis of cataract. MMP is a kind of calcium-zinc ion-dependent proteolytic enzyme, which plays an important role in embryonic development, wound repair, cell migration, etc. MMP-2, an important member of the MMP family, is a proteolytic enzyme with the type IV collagen and laminin as the substrate, which can specifically degrade the matrix component of the normal lens capsule. The increased concentration of MMP-2 can enhance the degradation of the extracellular matrix, promote the cell proliferation and migration, and transform mesothelial cells into myofibroblasts. Finally, it leads to the matrix contracture, cell aggregation and collagen deposition,

ultimately resulting in the posterior capsular fold and opacity[18-20]. Therefore, it is speculated that the MMP-2 protein formed after transcriptional translation of MMP-2 gene may be related to the occurrence and development of cataract. In this study, the MMP-2 polymorphism site rs243865 (C/T) was selected, and the genotype and allele frequency in the observation group and control group was analyzed using the TaqMan-MGB probe. The results revealed that there were differences in the genotype and allele distribution frequency of MMP-2 rs243865 (C/T) between the two groups, suggesting that the MMP-2 rs243865 (C/T) polymorphism was correlated with the risk of cataract. Then the MMP-2 rs243865 (C/T) genetic model was further analyzed. It was found that the additive model was different between the two groups ($p<0.05$), indicating that the additive model is suitable for describing the genetic model of MMP-2 rs243865 in cataract.

Conclusions

We showed that MMP-2 rs243865 polymorphism is correlated with the risk of cataract, and the risk degree of cataract increases due to the mutation of MMP-2 rs243865.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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