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HLA-DPA1 gene polymorphism in primary glaucoma

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Abstract. – OBJECTIVE: To explore the association between human leukocyte antigen (HLA)-DPA1 gene polymorphism and primary glaucoma.

PATIENTS AND METHODS: Six single nucleotide polymorphisms (SNPs) were genotyped in 51 patients and 51 healthy controls through Polymerase Chain Reaction (PCR). The possible association between HLA-DPA1 gene mutation and primary glaucoma was detected using the *t*-test and the Chi-square test.

RESULTS: Rs1676486 genotype had a significant genetic correlation. Rs3753841 and rs12138977 genotypes had a higher minor-allele frequency in control group. The CT + CC genotype frequency of rs12138977 showed a significant genetic correlation in both case group and control group. Moreover, the rs12138977 polymorphism and corneal thickness had little influence on the occurrence of primary angle-closure glaucoma (PACG). Also, the main risk factors for PACG were intraocular hypertension and short axial length.

CONCLUSIONS: The HLA-DPA1 gene polymorphism may be related to the severity of PACG.

Key Words:
HLA-DPA1, Gene polymorphism, Primary glaucoma.

Introduction

Glaucoma is the major cause of irreversible blindness in the world, as well as the second leading cause of blindness globally. It is estimated that 60 million people suffer from glaucomatous optic neuropathy worldwide, and glaucoma is the cause of blindness in 8.4 million people. There will be 79.6 million patients with primary glaucoma by 2020. Early diagnosis and early treatment are the main prevention measures for blindness in glaucoma. Primary angle-closure glaucoma (PACG) is a neurodegenerative disease characterized by the increased intraocular pressure (IOP) caused by mechanical obstruction due to the juxtaposition of the peripheral iris in trabecular meshwork and trabecula[1]. According to epidemiological studies[2], the morbidity rate of glaucoma was the highest in East Asia and Middle-South Asia in 2013, and it is expected that the number of glaucoma patients in Middle-South Asia will increase from 17.06 million to 32.9 million in 2013-2040 compared with other Asian regions. Glaucoma is characterized by the progressive degradation of retinal ganglion cells (RGC) and optic nerve axons, which can cause damage to the visual field. Currently, race, gender, and age have been identified as risk factors for PACG[3]. Although PACG is the leading cause of irreversible blindness, the visual ability can be retained if there is an early and appropriate treatment. According to the latest reports, the gene polymorphism is an important factor determining the individual disease susceptibility, phenotype, and therapeutic response. In addition, it is reported[4] that the gene polymorphism is closely related to susceptibility to glaucoma.

PACG is a complex heterogeneous disease, but its molecular mechanism is poorly understood, and its genetic susceptibility is still being studied. Several new PACG loci and genes, including PLEKHA7, HLA-DPA1, EPDR1, CHAT, GLIS3, FERMT2, and DPM2-FAM102A, have been recently found in the Genome-Wide Association Study (GWAS), which may reveal the molecular

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mechanism of PACG[5]. Among them, HLA-DPA1 may be an important influencing factor.

The major histocompatibility complex (MHC) region can be classified into class I (HLA-A, B, and C), class II (HLA-DR, DP, and DQ) and class III (C2, etc.) sub-regions. Increasing evidence[6] shows that HLA-DR, especially the HLA-DRB1 locus, is the most important known genetic factor causing a variety of diseases. According to the further data provided by Hosaka et al[7], there is an association between HLA-DP polymorphism and the decreased level of hepatitis B surface antigen. It is also reported[8] that HLA-DP gene polymorphism is associated with multiple sclerosis and systemic lupus erythematosus. However, the correlation between HLA-DP gene and PACG is rarely reported.

In the present work, the association between HLA-DPA1 and PACG was explored. Whether the HLA-DPA1 single nucleotide polymorphisms (SNPs) are associated with the different severity of PACG was also analyzed to provide new insights and a better understanding of the correlation of candidate gene SNPs with severity and specific clinical features of PACG.

Patients and Methods

Objects of Study

This investigation was approved by the Ethics Committee of the Affiliated Hospital of Weifang Medical University and all procedures were performed in accordance with the Declaration of Helsinki. Before enrollment, all subjects and normal controls signed the informed consent.

A total of 51 PACG patients and 51 matched healthy controls were recruited from the ophthalmology department of our hospital. They all received the standardized ophthalmologic examinations by glaucoma specialists, including the evaluation of refractive status, slit-lamp microscopic examination, and examinations of fundus, IOP, axial length (AL), anterior chamber depth (ACD), visual field, and anterior chamber angle. Mean defect (MD) and mean sensitivity (MS) were measured using the automatic instrument (Octopus, Haag-Streit AG, Koeniz, Switzerland), IOP was measured using the Goldmann applanation tonometer, while the retinal camera (TRC-NW200; Topcon Corp., Tokyo, Japan) was used for fundus photography, Finally, AL and ACD were detected through the A-scan ultrasound (A-Scan Pachymeter, Ultrasonic, Exton, PA, USA).

Diagnosis and Inclusion Criteria of Subjects

Inclusion criteria of PACG patients are as follows: (1) Hospitalized patients with PACG. (2) Patients diagnosed with glaucomatous optic neuropathy and corresponding visual field loss based on the narrow-angle. It is defined as follows: clusters of three or more points of discontinuity on the pattern deviation plot, without crossing the horizontal meridian, with the probability of less than 5% in the age-matched normal (one of them is less than 1%), abnormal pattern standard deviation ($p<0.05$) in the normal people, and meeting the test reliability criteria (namely the fixed loss <20%, false-positive <33%, and false-negative <33%). (3) PACG patients and controls without major systemic diseases (such as autoimmune diseases or cancer) that might confuse the research results. The subjects which had secondary angle-closure glaucoma due to the uveitis, trauma, formation of new blood vessels or any other optic nerve injuries affecting either eye were excluded.

SNP Screening

SNPs of HLA-DPA1 gene in the Chinese Han population were obtained from the HapMap database. Data were imported into Haploview Software (version: 4.2) to select the tag SNPs based on the following criteria: $r^2>0.8$ and minor allele frequency (MAF) >0.05. According to the confidence interval (CI) of linkage disequilibrium value (D' value), adjacent SNP of D ($t=0.70$ and 95% CI=0.98) were classified as the same haplotype block.

Polymerase Chain Reaction (PCR)

The genomic DNA was amplified *via* PCR (25 μL of reaction system) under the following conditions: denaturation at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 58°C for 45 s, extension for 35 cycles, extension at 72°C for 1 min, and extension at 72°C for 10 min using a thermal cycler (9700 model; PerkinElmer, Foster City, CA, USA). The IL-10-1082 polymorphism was amplified as follows: initial activation at 95°C for 15 min before the cycle program and annealing at 55°C during the cycle program. The amplified PCR products were digested with restriction enzymes and analyzed on the 3% agarose gel stained with ethidium bromide. Primer sequences were shown in Table I.

Table I. Primer sequences.

Gene	Primer sequence	
	Forward	Reverse
rs1676486	5'-TTGATGGGTCACAGAAGG-3'	5'-TGAGGTAGTCACAGGGAGGC-3'
rs3753841	5'-GAGGAATTGGAGCGTTAACTGT-3'	5'-GCTTATTCACCACGAAIAGCC-3'
rs12138977	5'-TCCCTGCGCCGCTGCAGTTTCT-3'	5'-TGGCGTGTGAGGCCTTACCTCC-3'
rs2126642	5'-ATAATACAATACAGATC-3'	5'-TTCTCCGACTACTACTTC-3'
rs2622848	5'-GGGTCACATCTGCTACTA-3'	5'-CGTTGGGTAGGAGGAGGT-3'
rs11024102	5'-CAGATCAGGCACAGGGGAGA-3'	5'-CGTCGGGTCGGAGCATCC-3'

Collection and Detection of Blood Samples

A total of 2 mL of whole blood samples were collected from each subject for DNA isolation and analysis of genetic polymorphisms. All samples were stored in the ethylenediaminetetraacetic acid (EDTA) tube (Wuhan Zhiyuan Medical Technology Co., Ltd., Wuhan, China) in a refrigerator at –80°C, followed by treatment.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis of data. The HWE and the difference in the allele frequency of each SNP between case group and control group were evaluated using the Chi-square test. The genotype frequency distribution was compared using the Logistic regression analysis and the odds ratio (OR) and 95% CI were calculated. The two-sided 95% CI was adopted in all tests and $p<0.05$ suggested the statistically significant difference.

Results

Genotyping of HLA-DPA1 Polymorphisms

The SNPs of HLA-DPA1 were analyzed using multiple PCR and the amplified fragments (digested by enzymes at four polymorphic sites) obtained for specific alleles on each primer were identified. It was found that the genotypes obtained by DNA sequencing were the same as those obtained by PCR-Sequence Specific Primer (SSP) (Figure 1).

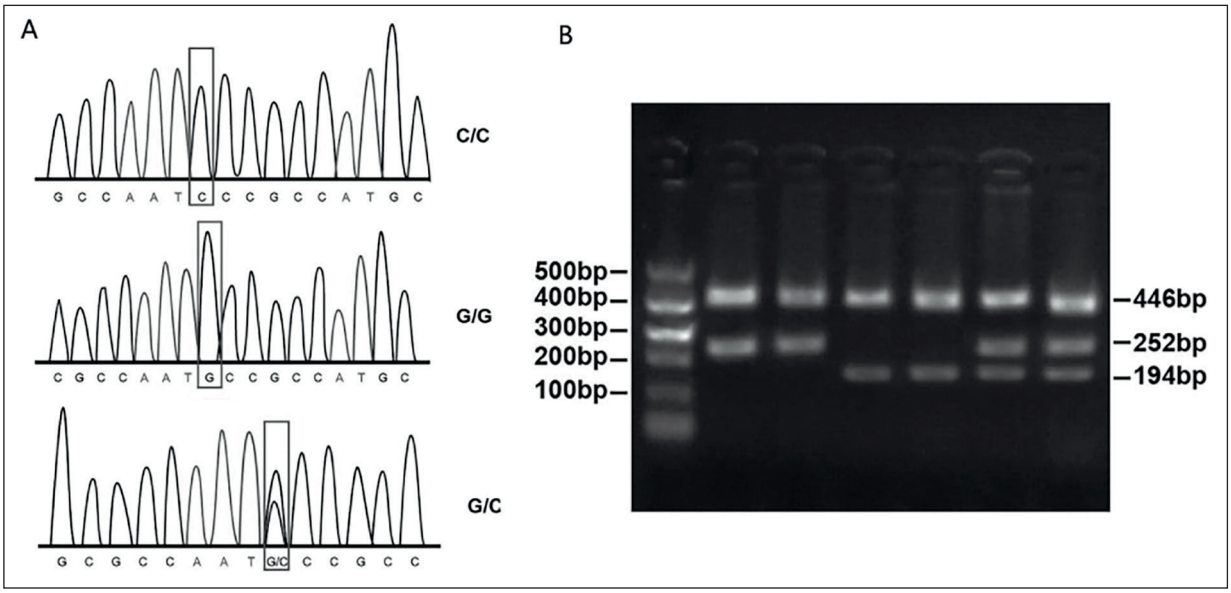

Figure 1. Agarose gel electrophoresis and PCR products of HLA-DPA1. After amplification, SNP Ser326 of HLA-DPA1 gene shows the 446 bp fragment, which produces three different fragments (194, 252, and 446 bp).

Comparison of Clinicopathological Features Between Case Group and Control Group

A total of 51 PACG patients, including 32 females and 19 males aged (59.29 ± 11.9) years old, and 51 normal controls, including 34 females and 17 males aged (58.24 ± 6.49) years old, were enrolled in the study. One eye was randomly selected as the object of research from patients with PACG in both eyes, and the average age, systemic blood pressure (SBP), diastolic blood pressure (DBP), and gender had no statistically significant differences between case group and control group (p>0.05) (Table II). All the 6 SNPs were successfully genotyped and the allele distribution met the HWE in both group (p>0.05) (Table III).

/Genotype Frequency and Correlation of 6 SNPs in Both Groups

Among the 6 SNPs, rs1676486 had a significant genetic correlation (p=0.026, OR=2.089, 95% CI=1.092-3.996). Rs3753841 (p=0.036, OR=1.886, 95% CI=1.038-3.426) and rs12138977 (p=0.024, OR=2.133, 95% CI=1.104-4.123) genotypes had higher MAF in control group (Table IV). The AG + GG genotype frequency of rs1676486 in case group was significantly higher than that in control group (p=0.031, OR=2.405, 95% CI=1.086-5.332). The GG genotype frequency of rs3753841 also had a significant correlation (p=0.040, OR=5.684, 95% CI=1.084-29.803). Besides, the CT + CC genotype frequency of rs12138977 (p=0.03, OR=2.417, 95% CI=1.088-5.368) showed a significant genetic correlation in both case group and control group (Table V).

Logistic Regression Analysis of Risk Factors for PACG

The binary Logistic regression analysis was performed with PACG as the dependent variable and rs11024102 genotype, rs3753841 genotype, rs12138977 genotype, corneal thickness, IOP, and AL as independent variables. As shown in Table VI, the rs3753841 and rs11024102 polymorphisms could increase the risk of PACG (p<0.05). It can be seen that the rs12138977 polymorphism and corneal thickness had little influence on the occurrence of PACG, and IOP, and short AL were the main risk factors for PACG (p<0.05).

Discussion

PACG is the main type of glaucoma in many Southeast Asian countries, and many PACG patients have similar anatomical features, such as shallow anterior chamber, increase in lens thickness, lens previa, narrow anterior chamber angle, and short AL[7]. It has been proved that genetic factors are related to the development of PACG. The genes involved in PACG susceptibility have been widely studied, and the correlation between individual gene polymorphism and PACG susceptibility has also attracted increasingly more attention[9]. However, the association of HLA-DPA1 gene polymorphism with PACG susceptibility and features has not been reported yet.

The correlation between HLA-DPA1 gene polymorphism and the risk of primary glaucoma was revealed in this study. The association between PACG susceptibility genes and risk factors for closure-angle glaucoma was explored. It was found that rs1676486, rs3753841, and rs12138977 in HLA-DPA1 had significant differences in MAF

Table II. The sequences of primers for RT-PCR.

Clinical feature	Case group	Control group	t/χ^2	p
Average age (years old)	59.29 ± 10.97	58.58 ± 10.14	0.558	0.887
Gender (male/female)	32/19	34/17	0.172	0.634
VCDR	0.61 ± 0.23	–		
IOP (mmHg)	22.89 ± 4.33	–		
ACD	1.8 ± 2.5	–		
AL	21.68 ± 3.08	–		
MD	12.86 ± 9.63	–		
MS	14.91 ± 9.59	–		
SBP	133.00 ± 13.36	129.31 ± 17.53	1.113	0.321
DBP	78.31 ± 7.42	79.90 ± 7.81	-0.234	0.311
HTN (Yes/No)	19/32	14/37	1.111	0.290
DM (Yes/No)	49/2	48/3	0.231	0.621

Table III. HWE.

Gene	SNP	Case group		Control group	
		t	*p*	*t*	*p*
HLA-DPA1	rs1676486	1.11	0.57	2.16	0.33
	rs3753841	0.06	0.97	0.5	0.80
	rs12138977	0.94	0.66	1.96	0.40
	rs2126642	0.05	0.98	1.02	1.00
	rs2622848	0.65	0.72	0.28	0.87
	rs11024102	0.37	0.83	0.01	1.00

Table IV Allele frequency of 6 SNPs and correlation in both groups.

SNP	BP	MA	Case group MAF	Control group MAF	*p*	OR (95% CI)
rs1676486	103354138	A	0.32	0.19	0.026	2.089 (1.092-3.996)
rs3753841	103379918	G	0.39	0.26	0.036	1.886 (1.038-3.426)
rs12138977	103393457	C	0.31	0.18	0.024	2.133 (1.104-4.123)
rs2126642	103405793	A	0.11	0.10	0.818	1.112 (0.450-2.746)
rs2622848	103421003	C	0.10	0.07	0.449	1.475 (0.539-4.040)
rs11024102	17008605	C	0.44	0.41	0.671	1.128 (0.647-1.965)

Note: Chi-square test and Logistic regression are adopted. BP: base pair position, MA: minor allele, MAF: minor allele frequency, OR: odds ratio, CI: confidence interval.

Table V. Genotype frequency of main SNPs and correlation in both groups.

SNP	Genotype	Case group		Control group		*p*	OR (95% CI)
	GG	21	41.20	32	62.70	1	
rs1676486	AG	27	52.90	19	37.3	0.060	2.165 (0.968-4.842)
	AA	3	5.88	0	0	–	–
	AG + AA	30	58.8	19	37.3	0.031	2.406 (1.086-5.332)
	AA	19	37.3	27	52.9	1	
rs3753841	AG	24	47.1	22	43.1	0.297	1.550 (0.680-3.534)
	GG	8	15.7	2	3.92	0.040	5.684 (1.084-29.803)
	AG + GG	32	72.4	24	47.1	0.11	0.528 (0.239-1.163)
	TT	22	43.1	33	64.7	1	
rs12138977	CT	26	51.0	18	35.3	0.061	2.167 (0.966-4.859)
	CC	3	5.9	0	0	–	-
	CT+CT	29	56.9	18	35.3	0.030	2.417 (1.088-5.368)
rs11024102	GG	40	78.4	42	82.4	1	
	AG	11	21.6	8	15.7	0.475	1.444 (0.527-3.958)
	AA	0	0	1	2.0	–	–
	AG + AA	11	21.6	9	17.6	0.6	1.283 (0.481-3.425)

Table VI. Logistic regression analysis of risk factors for PACG.

Independent variable	B	S.E.M.	*p*	OR	95% CI
rs12138977	-0.383	0.275	0.164	0.682	0.397-1.169
rs3753841	-1.059	0.341	0.002	2.251	1.958-3.261
rs11024102	-0.859	0.295	0.004	1.635	1.226-3.183
AL	-0.844	0.093	0	1.782	1.563-2.377
Corneal thickness	0.019	0.016	0.231	1.019	0.988-1.051
IOP	1.138	0.225	< 0.001	3.121	2.007-4.854

and genotype frequency between case group and control group, suggesting that the minor alleles of these SNPs in patients are about twice that in controls, and PACG may occur. In addition, none of the SNPs displayed a significant correlation with ACD or AL, demonstrating that the mechanism of disease is realized independently of the shallow anterior chamber and shorter eyeball length. Moreover, a significant advantage is that the correlation between HLA-DPA1 gene and severity of PACG was confirmed.

It was found in previous reports[10,11] that rs11024102 and rs3753841 in HLA-DPA1 are genetic risk factors for PACG. Studying their correlations in independent cohorts can help better understand the role of genetic risk factors in the pathogenesis of the disease. There are differences in genotyping techniques, but our research results are in agreement with previous works[12-14]. In the previous GWAS, rs3753841 is an encoding SNP with the amino acid variation from proline to leucine, which is the most closely-related SNP in HLA-DPA1 gene. However, rs12138977 showed the most significant correlation with PACG (p=0.024).

In addition, the correlation between susceptibility loci and severity of glaucoma was detected for the first time in this research. As is known to all, determining the risk factors at different stages of the disease may contribute to early diagnosis and prognosis in clinical practice. Notably, it was found that the allele frequency distribution of the above three SNPs (rs1676486, rs3753841, and rs12138977) was significantly different between moderate/severe PACG and controls, suggesting that they may be associated with severity of glaucoma. Therefore, the above three SNPs can be used clinically to predict the progression of PACG.

The extracellular matrix (ECM) in the outflow pathway of aqueous humor is thought to be necessary for the production of resistancetooutflow[15,16]. Both rs1676486 and rs3753841 are located in the coding region of HLA-DPA1, and each of them consists of two different amino acid coding modes. It is reported[17] that the HLA-DPA1 gene is up-regulated on the lamina cribrosa of glaucoma patients, further indicating the expression and regulatory effect of ECM gene in the pathogenesis of glaucoma. It is believed that the residues and combinations of these two loci may affect the configuration and function of collagen, and even ECM and traditional outflow pathways may be altered. Moreover, HLA-DPA1 is associated with congenital diseases type II Stickler and Marshall syndromes, including high myopia and blindness due to retinal detachment.

Conclusions

We demonstrated the findings of GWAS and the significant association between rs1676486, rs3753841, and rs12138977 polymorphisms and PACG was revealed. Therefore, they can serve as biological indexes of PACG. However, there are some limitations. For example, glaucoma is a disease involving multiple factors and genes, and the effects of various factors are easily offset by another factor, leading to misleading results. Besides, the distribution of HLA-DPA1 gene polymorphisms varies from region to region. Due to the limited sample size, it is necessary to conduct the case-control studies in different ethnic groups, expand the sample size, and perform the multivariate analysis to further confirm the results. Furthermore, although the correlation between the severity of PACG and HLA-DPA1 polymorphism was observed, its exact mechanism remains unknown and still needs further research.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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