

Single nucleotide polymorphisms of metabolic syndrome-related genes in primary open angle glaucoma

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Abstract

• **AIM:** To analyze single nucleotide polymorphisms (SNP) of primary open angle glaucoma- and metabolic syndrome-related genes in primary open angle glaucoma (POAG), in order to elucidate the roles of metabolic syndrome as a risk factor in POAG progress.

• **METHODS:** SNP genotypes and alleles of interleukin-6 (IL-6), IL-6 receptor (IL-6R), dopamine D₂ receptor (DRD₂), beta-fibrinogen (FGB), peroxisome proliferator-activated receptor- γ 2 (PPARG), transforming growth factor- β 1 (TGF- β 1), E-selectin (E-Sel), apolipoprotein A-5 (APOA5), C-reactive protein (CRP), ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), hepatic lipase (LIPC), adiponectin (ADIPOQ), paraoxonase 1 (PON1) and serine protease inhibitor E (SERPINE1) genes in POAG ($n=37$) and normal control ($n=100$) groups were measured with ABI Prism 7900HT Fluorescence Quantitative PCR and TaqMan SNP Genotyping fluorescence probe kit.

• **RESULTS:** Genotypes and allele frequencies of IL-6R, IL-6, FGB, CRP, ENPP1, LIPC, ADIPOQ, PON1, and SERPINE1 in total POAG group were significantly different compared to the control group.

• **CONCLUSION:** Metabolic syndrome as a risk factor for POAG may be associated with genotypes and allele frequencies of the related genes. The corresponding gene expression and function can affect POAG progress, including roles of SERPINE1 in extracellular matrix, ENPP1 in insulin inhibition, IL-6 in endogenous neuroprotection, IL-6, IL-6R and E-Sel in autoimmune response, LIPC and FGB in blood hyperviscosity syndrome, ADIPOQ in NOS/NO production, PON1 in vascular endothelial protection.

• **KEYWORDS:** primary open angle glaucoma, metabolic syndrome, single nucleotide polymorphism

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INTRODUCTION

Metabolic syndrome, also known as insulin resistance syndrome, has a group of cardiovascular risk factors, including obesity, hypertension, diabetes, low/high density lipo proteinemia, high glycerin trinitrates lipemia, and fasting hyperlipidemia. Epidemiological survey found that the prevalence of primary open angle glaucoma (POAG) is higher in diabetes than that in the normal subjects as well as in the hypertension patients with the systolic blood pressure above 130mmHg. Based on optic disc and retinal nerve fiber layer photography and blood pressure measurement, blood pressure may be an independent risk factor of the early optic disc structural damage and glaucoma^[1]. Relative risk of open angle glaucoma would be raised by 0.91 when the systolic pressure reduces each 10mmHg^[2]. At the same time, a higher intraocular pressure is found in patients with metabolic syndrome than those without it^[1]. Atherosclerosis is also considered as risk factors of normal-tension glaucoma^[3]. Plasma lipids can be used as one of the POAG predictors^[4]. Therefore, there might be genetic susceptibility between the metabolic syndrome and POAG. To verify this inference, we examined the single nucleotide polymorphism (SNP) of the related metabolic syndrome genes in POAG and analyzed the role of metabolic syndrome as a risk factor in POAG.

MATERIALS AND METHODS

Subjects Thirty-seven POAG patients were examined by Goldmann tonometer, Humphrey 750 automatic perimeter, Heidelberg confocal scanning laser tomography of the optic nerve head (type 2.01), Zeiss-Humphrey optical coherence tomography (OCT Stratus), and gonioscopy in Zhongshan Ophthalmology Center, Sun Yat-sen University, Guangzhou, China from September 2005 to March 2009. POAG diagnosis criterion was based on the recommended national standards by Glaucoma Study Group in 1987 and revised draft 2001. The criterion included: (1) Intraocular pressure (IOP) >21mmHg (repeating measurement on Goldmann tonometer); (2) Glaucomatous optic disc damage; (3) Glaucomatous visual field damage (MD \geq 3dB, nasal step, arcuate scotoma, etc.); (4) Retinal nerve fiber layer defect; (5) Width of anterior chamber angle under high IOP. Among thirty-seven POAG cases, there were 25 male and 12 female, with a mean age of 40.9(14-69) years old, correction vision acuities from no light perception to 30/20, intraocular pressure 25-60mmHg. Their POAG were diagnosed at their age from 15 to 65 years, glaucomatous course from 1-19 years. In three cases, their eyes suffered from vision defective at the early stage, seven cases at the advanced stage, and three cases at the severe stage.

Table 1 Sequence numbers of dsSNP, position and missense expression

Relevant gene	dbSNP ID	Chrom. site	Chrom	mRNA site	Amino acid site	dbSNP allele	Missense expression
IL-6R	rs8192284	1q21.3e	1	1510	358	A → C	Asp [D] → Ala [A]
IL-6	rs1524107	7p15.3c	7				
DRD ₂	rs1800497	11q23.2a	11	2231	713	A → G	Lys [K] → Glu [E]
FGB	rs4220	4q31.3d	4	1548	478	A → G	Lys [K] → Arg [R]
PPARG	rs1797912	3p25.2b	3			Intron	
TGF-β1	rs 4803455	19q13.2c	19			Intron	
E-Sel	rs5368	1q24.2c	1	1518	468	C → T	His [H] → Tyr [Y]
APOA5	rs662799	11q23	11		1131	A → G	
CRP	rs1130864		1	1003	286	C → T	3'UTR
ENPP1	rs1409181		6			Intron	
LIPC	rs6083	15q21.3	15	701	215	A → G	Asn [N] → Ser [S]
ADIPOQ	rs2241766		3	129	15	G → T	
PON1	rs662	7q21.3-22.1	7	632	192	A → G	Gln [Q] → Arg [R]
Serpine1	rs7242	7q22-31.1	7	2006			3'UTR

Table 2 DNA sequences of probes in real-time quantitative PCR

Gene	ABI No.	DNA sequences
IL-6R	C_16170664_10	AATTTTTTTTTTAACCTAGTGCAAG [C/A] TTCTTCTTCAGTACCACTGCCACACA
IL-6	C_7449210_10	GTTGTAGCTTCATTTTTCTTAGAGA [C/T] TTTCTGGCTGTGGTTGAACAATGA
DRD ₂	C_7486676_10	CACAGCCATCCTCAAAGTGCTGGTC [A/G] AGGCAGGCGCCAGCTGGACGTCCA (reverse)
FGB	C_7429784_1	TGGAAGGGGTCATGGTACTCAATGA [A/G] GAAGATGAGTATGAAGATCAGGCC
PPARG	C_8756544_10	GCTAGGGAACAACCCTGGCAGATCC [A/C] TTTTGCCTTGACTAGAGCTTAAAG
TGF-β1	C_30031638_10	CCTGAATTCTCAGTAACTTAGAAGT [A/C] ATTTCTAATGATTCCGGCTGGGCAC
E-Sel	C_8919523_1	GTGCACTCAAGTTGAGTTGATCCAT [A/G] TAATTCAAATCCCTCCTCACAGCTG
APOA5	C_2310403_10	GAGCCCCAGGAACTGGAGCGAAAGT [A/G] AGATTTGCCCATGAGGAAAAGCTG
CRP	C_7479332_10	CCTCAAATTCTGATTCTTTGGACC [A/G] TTTCCAGCATAGTTAACCAGCTCC
ENPP1	C_1208017_10	TCTGGATTCCGAGTGGGATCGGAAA [C/G] TGATGTCCCGGATTAGAGGATGTG
LIPC	C_305149_10	AATCGTCTTTCTCCAGATGATGCCA [A/G] TTTTGTGGATGCCATTCATACCTTT
ADIPOQ	C_26426077_10	TTCTACTGCTATTAGCTCTGCCCGG [G/T] CATGACCAGGAAACCAGACTCAAG
PON1	C_2548962_20	TAAACCCAAATACATCTCCAGGAT [C/T] GTAAGTAGGGTCAAGAAAATAGTG
Serpine1	C_2620948_10	ATTTTTATAGGAATAGAGGAAGAAA [G/T] GTCAGATGCCGTGCCAGCTCTTCAC

Subjects in normal control group were healthy examinees, from the 12th People's Hospital of Guangzhou, with no history of metabolic syndrome, ocular diseases, particularly glaucoma. They all were Han nationality without close blood relationship. Their mean age was 58.3 (50-70) years old, the diopter ≤ -3.00D.

Methods Through the relevant published literatures from the Chinese National Knowledge Infrastructure and Foreign Medical Journal Full-Text Service (<http://192.168.2.10:8080/fmjs/index.jsp>), genes associated with POAG and metabolic syndrome were IL-6 (IL-6), IL-6 receptor (IL-6R), dopamine receptor-D₂ (DRD₂), β-fibrinogen (FGB), peroxidase peroxisome proliferator-activated receptor-γ2 (PPARG), transforming growth factor-β1 (TGF-β1), E-selectin (E-Sel), apolipoprotein A-5 (APOA5), C-reactive protein (CRP), outside the nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), hepatic lipase (LIPC), adiponectin (ADIPOQ), phospholipase of oxygen -1 (PON1) and serine protease inhibitor E (Serpine1) [5-11]. Their corresponding sequence numbers of single nucleotide polymorphisms (dsSNP ID) were listed in Table 1. Through GenBank's dbSNP database ([\[www.ncbi.nlm.nih.gov/SNP/\]\(http://www.ncbi.nlm.nih.gov/SNP/\)\), TSC \(The SNP Consortium\) database \(<http://snp.cshl.org/>\) and the ABI database \(<http://www.appliedbiosystems.com.cn/>\), data of the relevant SNP, the position, and missense translation were found \(Table 1\). Blood samples \(1.5mL\) were drawn from ulnar vein with 25g/L EDTA anticoagulation. Genomic DNA was extracted by a small amount of genomic DNA extraction kit \(Shanghai Songon Biological Engineering Technology & Services Co., Ltd\) with appropriate revision in experimental methods. DNA samples were preserved in -20°C. The SNP were detected by a quantitative PCR \(ABI Prism 7500HT\) with fluorescent TaqMan SNP Genotyping Kit and fluorescent probe technology. From ABI company database, the corresponding DNA sequences of the probes were listed in Table 2. Experimental results were showed as an allele distribution map and analyzed by the software. Real-time quantitative PCR was carried out in ABI Prism 7500HT fluorescent quantitative PCR with the reaction system 25μL, including 2 × Universal PCR Master mix 12.5μL; 40 × Probe/Primer mix 0.625μL; dATP, dCTP, dGTP, and dUTP 1μL; water 10.875μL. Primer/Probe Mix containing 6-FAM probe \(200nmol/L\),](http://</p>
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Table 3 Genotype and allele frequency of the SNP in Chinese and the other Asian

Gene	Chinese		Other Asian	
IL-6R	0.43 (C)	A/A (0.31-0.32)	A/C (0.51-0.52)	C/C (0.18-0.16)
IL-6	0.25 (C)	C/C (0.07-0.02)	C/T (0.36-0.23)	T/T (0.57-0.75)
DRD ₂	0.48 (T)	C/C (0.26-0.34)	C/T (0.48-0.48)	T/T (0.26-0.18)
FGB	0.18 (A)	A/A (0.04-0.02)	A/G (0.27-0.21)	G/G (0.69-0.77)
PPARG	0.41 (C)	A/A (0.35)	A/C (0.49)	C/C (0.16)
TGF-β1	0.46 (A)	A/A (0.22-0.16)	A/C (0.46-0.48)	C/C (0.31-0.36)
E-Sel	0.32 (A)	C/C (0.42-0.73)	C/T (0.51-0.27)	T/T (0.04-0.07)
APOA5	0.27 (G)	A/A (0.47-0.51)	A/G (0.44-0.49)	G/G (0.04-0.05)
CRP	0.31 (C)	C/C (0.89-0.93)	C/T (0.07-0.09)	T/T (0.03)
ENPP1	0.46 (C)	C/C (0.29-0.5)	C/G (0.29-0.47)	G/G (0.21-0.27)
LIPC	0.28 (A)	A/A (0.07-0.08)	A/G (0.11-0.42)	G/G (0.51-0.89)
PON1	0.42 (G)	A/A (0.07-0.20)	A/G (0.47-0.57)	G/G (0.30-0.46)
Serpine1	0.37 (T)	G/G (0.09-0.21)	G/T (0.46-0.68)	T/T (0.11-0.33)

VIC-probe (200nmol/L), forward primer (900nmol/L), and reverse primers (900nmol/L). FAM and VIC probes corresponded to wide type and mutant type genes. PCR cycle conditions: pre-denaturation 50°C 2 minutes → 95°C 10 minutes, 92°C 30 seconds → 60°C 60 seconds, 40 cycles. At least three blank controls were set at each test. The end point fluorescence signals of PCR were analyzed by SDS Data Analysis software (version 1.0). In the fluorescence distributions of examination results, each X represented a sample, X axis represented the VIC probe fluorescence, Y axis represented the FAM probe fluorescence. The zero point was the blank comparison. The diagonal line represented heterozygote. According to the map, correspondence allele category and genotyping were demonstrated (Figure 1).

Statistical Analysis SPSS13.5 software was used for the analysis. Genotype and allele frequencies in each SNP site were calculated by direct gene counting and frequency analysis. Allele frequencies were determined by the Hardy-Weinberg law of genetic equilibrium to evaluate the community representative degree of the samples. Differences between the genotype and gene frequency were compared with R x C contingency table of Chi-square test. The relative risks were represented by odds ratio (OR) and 95% confidence interval (CI). $P < 0.05$ means the statistically significant.

RESULTS

Through dbSNP, ABI and the TSC database, the genotypes and allele frequencies of SNP loci in POAG and metabolic syndrome-related genes in Asian population were shown in Table 3. Comparison of the genotypes and allele frequencies in normal control group and POAG group was in Table 4.

E-Sel, APOAS, LIPC, ADIPOQ, PON1, and Serpine1 allele frequencies were similar to those in Asian report and the control group as well as POAG group. IL-6 and DRD₂ allele frequencies were similar to those in Asian report and the control group, but not the POAG group. IL-6R allele frequency was similar to those in Asian report and the POAG group, but not the control group. PPARG, TGF-β1, ENPP1 allele frequencies were similar in both the control and POAG group, but not the Asian report. CRP and FGB allele frequencies

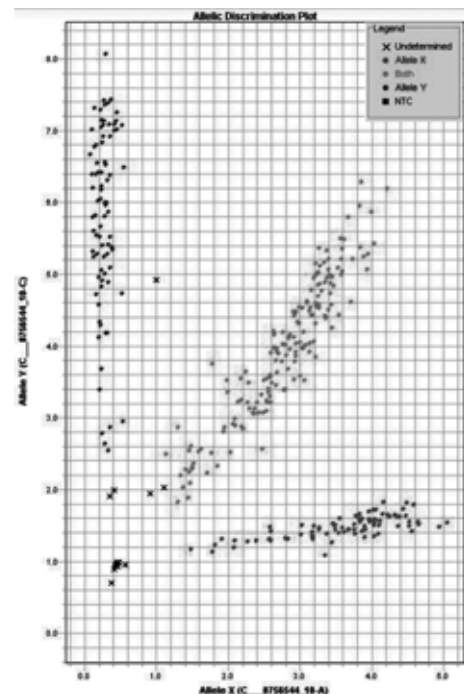


Figure 1 Results of PPARG detected by probe C__8756544_10

were not similar to Asian report and both groups. In addition to IL-6 in control and POAG group, CRP in POAG group, ADIPOQ and SERPINE1 in control group, the distribution of allele frequencies in both groups conformed to the Hardy-Weinberg balance ($\chi^2 = 3.84, P > 0.05$, Table 5).

Genotyped and allele frequencies of IL-6R, IL-6, FGB, CRP, ENPP1, LIPC, ADIPOQ, PON1, and the SERPINE1 were significantly different between POAG and control group ($\chi^2 = 11.33, P < 0.01$, Table 6). The OR of IL-6, FGB, CRP, ENPP1, LIPC and ADIPOQ were above 2.5 (Table 6). Gene expression of IL-6R, DRD₂, FGB, E-Sel, LIPC, and PON1 was the missense. In POAG group, the missense expressions in homozygous were from 0 to 5 with both eyes in the early stage (3 cases), the advanced stage (7 cases), the severe stage (3 cases), one eye in the early and the other in the advanced stage (5 cases), one eye in the advanced and the other in the severe stage (6 cases).

Table 4 Allele frequency of the Asian, control group and POAG group

Gene	Type	Origin	Asian			Control group			POAG group	
IL-6R	A/C	A	A(0.57-0.58)	C(0.43-0.42)	A(0.71)	C(0.29)	A(0.57)	C(0.43)		
IL-6	C/T	C	C(0.14-0.25)	T(0.86-0.75)	C(0.22)	T(0.78)	C(0.46)	T(0.54)		
DRD ₂	C/T	T	C(0.5-0.58)	T(0.42-0.5)	A(0.45)	G(0.55)	A(0.37)	G(0.63)		
FGB	A/G	A	A(0.13-0.18)	G(0.82-0.88)	A(0.36)	G(0.64)	A(0.26)	G(0.74)		
PPARG	A/C	C	A(0.59)	C(0.41)	A(0.46)	C(0.54)	A(0.49)	C(0.51)		
TGF-β1	A/C	C	A(0.4-0.46)	C(0.54-0.60)	A(0.35)	C(0.65)	A(0.36)	C(0.64)		
E-Sel	C/T	C	C(0.68-0.86)	T(0.32-0.14)	A(0.27)	G(0.73)	A(0.2)	G(0.8)		
APOA5	A/G	A	A(0.71-0.73)	G(0.27-0.29)	A(0.71)	G(0.29)	A(0.71)	G(0.29)		
CRP	C/T	C	C(0.93-0.97)	T(0.03-0.07)	A(0.95)	G(0.05)	A(0.35)	G(0.65)		
ENPP1	C/G	C	C(0.51-0.65)	G(0.35-0.49)	C(0.38)	G(0.62)	C(0.41)	G(0.59)		
LIPC	A/G	A	A(0.06-0.28)	G(0.72-0.94)	A(0.21)	G(0.79)	A(0.24)	G(0.76)		
ADIPOQ	G/T	T	G(0.27)	T(0.73)	G(0.27)	T(0.73)	G(0.32)	T(0.68)		
PONI	A/G	G	A(0.31-0.43)	G(0.57-0.69)	C(0.68)	T(0.32)	C(0.59)	T(0.41)		
Serpine1	G/T	G	G(0.37-0.55)	T(0.46-0.63)	G(0.42)	T(0.58)	G(0.43)	T(0.57)		

Table 5 Allele frequency comparison with Hardy-Weinberg law of genetic equilibrium (χ^2)

Group	IL-6R	IL-6	DRD ₂	FGB	PPARG	TGFβ1	E-Sel	APOA5	CRP	ENPP1	LIPC	ADIPOQ	PONI	Serpine1
POAG	0.38	4.46	0.00	0.23	0.67	0.00	0.28	0.73	10.22	0.39	0.03	0.69	0.54	0.00
Control	0.67	4.61	3.68	0.37	0.22	0.16	1.08	0.04	0.28	0.01	0.92	4.89	2.64	8.06

Table 6 Genotype and allele frequency comparison in POAG and control group

	IL-6R			IL-6			DRD ₂			FGB			PPARG												
	Genotype		Allele	Genotype		Allele	Genotype		Allele	Genotype		Allele	Genotype		Allele										
	AA	CC	AC	A	C	CC	TT	CT	C	T	AA	GG	AG	A	G	AA	CC	AC	A	C					
POAG	3	48	48	54	144	50	8	42	142	58	25	35	40	90	110	36	19	45	117	83	15	38	47	77	123
Control	48	7	45	141	59	1	58	41	43	157	25	35	40	90	110	14	43	43	71	129	20	28	52	92	108
χ^2	70.36		74.40		84.97		96.58		0.00		0.00		19.02		21.24		2.48		2.31						
OR	0.16		8.94		1		2.56		0.74																
(CI)	(0.10,0.24)		(5.67,14.09)		(0.67,1.48)		(1.71,3.84)		(0.49,1.09)																
P	0.00		0.00		0.00		0.00		1.00		0.00		0.00		0.00		0.29		0.16						
	TGF-β1			SELE			APOA5			CRP			ENPP1												
	Genotype		Allele	Genotype		Allele	Genotype		Allele	Genotype		Allele	Genotype		Allele										
	AA	CC	AC	A	C	AA	GG	AG	A	G	AA	GG	AG	A	G	CC	GG	CG	C	G					
POAG	14	43	43	71	129	1	58	41	43	157	48	7	45	141	59	3	60	37	43	157	90	10	0	190	10
Control	11	42	47	69	131	5	52	43	53	147	50	8	42	142	58	0	90	10	10	190	15	38	47	77	123
χ^2	0.55		0.04		3.04		1.37		0.21		0.01		24.51		23.69		116.91		143.83						
OR	1.04		0.76		0.98		5.20		30.35																
(CI)	(0.69,1.58)		(0.48,1.20)		(0.63,1.50)		(2.53,10.69)		(15.12,60.92)																
P	0.76		0.83		0.22		0.29		0.9		1.00		0.00		0.00		0.00		0.00						
	LIPC			ADIPOQ			PONI			Serpine1															
	Genotype		Allele	Genotype		Allele	Genotype		Allele	Genotype		Allele													
	AA	GG	AG	A	G	GG	TT	GT	T	T	CC	TT	CT	C	T	GG	TT	GT	G	T					
POAG	25	40	35	85	115	42	7	51	135	65	20	28	52	92	108	5	52	43	53	147					
Control	3	60	37	43	157	3	48	48	54	144	42	7	51	135	65	25	40	35	85	115					
χ^2	21.34		20.27		64.45		64.57		20.42		18.83		15.72		11.33										
OR	2.70		5.54		0.41		0.49																		
(CI)	(1.74,4.19)		(3.60,8.52)		(0.27,0.62)		(0.32,0.74)																		
P	0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00										

DISCUSSION

Like the metabolic syndrome, POAG is a complex diseases related to multi-genes. The analysis of single gene is insufficient to reveal the pathophysiology and pathogenesis of POAG. POAG is possibly caused by combined abnormal functions due to expression of SNPs in key genes together with

environmental factors. The interaction of these expressions of the SNPs could be combined, the synergistic, or the antagonistic. We have analyzed the genotype and the allele frequency of genes related with POAG and the metabolic syndrome, including IL-6, IL-6R, PPARG-γ2 (associated with the optic nerve damage and glaucomatous retinal

dysfunction); E-Sel, ENPP1, Serpine1, TGF- β 1 (associated with trabecular histology and function); DRD₂ (associated with intraocular pressure regulation and ocular length axial growth); β -FGB, APOA-5, LIPC (associated with high hemorrheologic viscosity and aggravation that increase glaucomatous retinal and optic nerve ischemic damage); CRP, PON1, ADIPOQ (associated with vascular endothelial damage and aggravation that also increase the ischemic damage). Among them, various genotypes of IL-6R, DRD₂, FGB, E-Sel, LIPC and PON1 are missense expression (Table 1). Except CRP in POAG group, ADIPOQ and Serpine1 in control group, IL-6 in both POAG and control groups, distributions of allele frequencies of the genes accord with Hardy-Weinberg law of genetic equilibrium (Table 5), which indicates that subjects in this research come from randomly stochastic marriage. Allele frequencies of this study are difficult to compare with the reports of Chinese that are reference scopes (Table 3). Referring to the results of the Asian report, we found that the allele frequency of IL-6 in control group and SERPINE1 in POAG group is similar to the Asian report, but not CRP in both POAG and control groups (Table 4). Therefore, although the number of subjects in this study is not enough, the results of genotypes and allele frequencies are comparable except that of CRP.

IL-6 and IL-6R are associated to the endogenous neural protection mechanism and autoimmune adjustment of POAG. Comparing to the normal, serum IL-6 concentration in POAG significantly decreased. The reduction of endogenous IL-6 activates tyrosinase and then IL-6-gp130-JAK-STAT3 signal pathway, which decreases the level of transcription factor 3 (STAT3) and then the expression of target gene Bcl-2 and Bcl-XL. As a result, apoptosis of retinal ganglion cell is increased. After vitreous cavity injection of exogenous IL-6, the apoptosis is significantly decreased. In addition, approximately 30% patients with normal tension glaucoma suffer from autoimmune diseases (such as dry syndrome, arthritis, thyroiditis) at the same time. Their serum concentrations of anti-heterogeneous protein antibody were increased. Decline of the generative levels of IL-6 and IL-6R is associated with these diseases and plays an important role in a variety of autoimmune pathogenesis. In autoimmune uveitis, stimulation from local IL-6 results in the increase of protein and cells in aqueous as well as the inflammatory cells in the trabecular meshwork. As a response to increase of intraocular pressure, expression of IL-6R in trabecular meshwork in POAG is increased obviously^[12]. The results of this study indicate that genotypes and allele frequencies of IL-6 and IL-6R were significantly different in POAG, comparing to the control group. The relative risk ratio of IL-6 was 8.94 (95% CI 5.67, 14.09) (Table 6). IL-6 and IL-6R also participate the transcription of FGB. IL-1 stimulated by a variety of injury activates the intranuclear factor that unifies IL-6 response element (STAT3 binding position), which inhibits

the combination of STAT3 to CTGGGAA and then the transcription of fibrinogen^[13]. Concentration of serum IL-6 decreases while fibrinogen is significantly increased in POAG. The increase of fibrinogen caused high hemorrheologic viscosity. In case of vasomotor reserve depletion, the slight increase in blood viscosity will become the key and limited factor in disposing the blood stream. When blood viscosity increases, small vessel will expand as compensation mechanism to maintain the normal circulation. At this time, if the intraocular pressure ascends, the vasodilatation will be hindered or cause the angiostenosis. As a result, decline of blood flow rate causes ischemia in optic nerve and retinal and glaucomatous damage. The result in this research indicates that genotypes and allele frequencies of FGP were significantly different in POAG, comparing to the control group. The relative risk is 2.56 (1.71, 3.84). Similarly, genotypes and allele frequencies of LIPC associated with high hemorrheologic viscosity also was significantly different in POAG comparison with control group. Missense expression of LIPC in mRNA 701 A/G (position of this study) might cause dysfunction of hepatic lipase and relate to hyperlipidemia type III. In this research, the relative risk of LIPC allele was 2.70 (1.74, 4.19) in POAG.

Not only genes associated with hyperviscosity, but also ADIPOQ related to vascular endothelial damage was found with various genotypes and the allele frequencies in POAG. Its relative risk is 5.54 (3.60, 8.52). Case-control study showed that POAG was related to endothelial dysfunction and peripheral vascular disorders. Adiponectin might indirectly inhibit expressions of IL-6 and CRP by restraining engendering TNF- α , which protects vascular endothelial against damage from peroxides and accelerates the synthesis of nitric oxide (NO) with nitric oxide synthase (NOS)^[7]. NOS and NO play a key role in apoptosis of glaucomatous ganglion cell. The massive release of NO might cause peroxidation of cellular membrane lipin, split of nuclear DNA, and damage or apoptosis of cells. Endogenous peroxynitrite anion at the same time causes toxic effects to retinal and optic nerve, which aggravates glaucomatous visual dysfunction.

Genotypes and allele frequencies of PON1, another gene related to vascular endothelial damaged, also were significantly different in POAG group. Its missense expression might affect vascular endothelial function. The 192Q/R genetic polymorphism of oxygen phospholipase coding region -1 (locus of this study) is the major molecular factor to determine its biological activity. The enzymatic activity in genotype 192RR is lower than that in 192QQ. The higher allele R frequency is, the lower enzymatic activity is^[14]. Allele frequencies of Q/R in normal are 0.45 and 0.55 in the Hans, 0.35 and 0.65 in Taiwanese, 0.39 and 0.61 in Shanghai, 0.46 and 0.54 in Shandong province, 0.32 and 0.68 this study^[15]. Allele Q and R frequencies were 0.41, 0.59 in POAG of this study (Table 4) as the same as patients with carotid plaque.

Results of this study show that allele frequency Q and R of PON1 in control group is close to that in Taiwan. There was no significant difference between normal control and POAG in Japan, which is not the same as this study result. However, among their POAG patients, intraocular pressure in PON1 genotype 192RR was obviously higher than that in genotype 192QQ^[16]. POAG might associate with vascular endothelial dysfunction caused by activity decrease of oxygen phosphorus esterase-1.

The main characteristic of POAG is abnormal deposition and decrease amount of trabecular meshwork cells, which impedes aqueous outflow through trabecular reticulum and raises intraocular pressure. Fibrinolytic enzyme is one of the main enzymes that degrades extracellular matrix. SERPINE1 inhibits fibrinolytic enzyme by restraining the plasminogen activator, which reduces degradation of extracellular matrix and increases the abnormal deposition. The results of this study indicate that genotypes and allele frequencies of Serpine1 were significantly different in POAG, comparing to the control group. In addition, there is also a significant difference between the genotype and allele frequencies of ENPP1 in POAG group and those in control group. The relative risk is 30.35 (15.12, 60.92). ENPP1 induces insulin resistance by combining with the insulin acceptor. When insulin binds to the (subunit of the acceptor, a combining division (CD) including 485-599 residue amino acids will transmit the signals to change the conformation of the acceptor, which activates tyrosine kinase region of (subunit and acidifies tyrosine phosphate. When ENPP1 combines with CD region, the change of (subunit will be hindered, which inhibits the activity of insulin^[6]. Insulin type growth factor-I(IGF-I) is multi-peptides which is similar to insulin in structure and function. Its acceptor also is very similar to the insulin acceptor in structure. The (subunit of IGF-I acceptor presents a binding site for IGF-I and insulin. IGF-I in trabecular meshwork cells coordinates mitosis activity of the ciliary cell growth factor, the epidermis growth factor and so on to promote the proliferation of trabecular meshwork cells and the cell regeneration after histological structure damage. At the same time, IGF-I also promotes the activation of fibrinolytic enzyme and degrades abnormal deposition of extracellular matrix in trabecular meshwork. Both effects improve outflow of aqueous and reduce intraocular pressure^[17]. ENPP1 decreases the protection effect of IGF-I and inhibits cellular proliferation of trabecular meshwork by combining with (subunit of IGF-I acceptor, which aggravates the occurrence and development of POAG. ENPP1 genotype is possibly associated with the insulin resistance of metabolic syndrome. It is reported that aqueous concentration of SERPINE1 in glaucoma was 3.3 times as that in the normal. It might infer that intraocular SERPINE1 was associated with pathogenic mechanisms of POAG.

In summary, metabolic synthesis, as a risk factor of POAG, is

possibly associated with genotypes and allele frequencies of its related genes. Its corresponding gene expression and function can affect POAG on the occurrence and the development, including Serpine1's effect on extracellular matrix of trabecular meshwork, activity of ENPP1 in the growth of trabecular meshwork cell by blocking IGF-I, neuroprotective effect of IL-6, autoimmune response involved with IL-6, IL-6R, E-Sel, effects of FGB and LIPC on hyperviscosity, ADIPOQ in NOS/NO generation, and the protective effect on vascular endothelium of PON1. Furthermore, according to clinical researches, concentrations of CRP in POAG were higher^[18] or the same^[19]. In this study, genotypes and allele frequencies of CRP in POAG were obviously different from those in control group ($\chi^2 = 23.69, P = 0.00$). The relative risk of CRP was 5.20 (2.53, 10.69). This is possibly related to inflammation responses and autoimmune diseases that CRP participates. To verify this inference, further research with more amounts of samples is needed because of the insufficient comparability.

In this study, no evidence was found to prove that the missense expression of homozygous genotypes is associated with initial age, duration, and severity degree of POAG. The possible interpretation for it might be that metabolic syndrome is not a major risk factor for POAG. The genes that we were studying might be not pathogenic genes of POAG. To prove the interpretation, researches with more amounts of samples and combination with proteid analyses are needed.

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原发性开角型青光眼的代谢综合征相关基因单核苷酸多态性

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摘要

目的:检测原发性开角型青光眼(POAG)和代谢综合征相关基因的单核苷酸多态性(SNP),分析代谢综合征作为危险因素在POAG发生发展中所起的可能作用。

方法:应用ABI Prism 7500HT型荧光定量PCR仪结合TaqMan SNP Genotyping试剂盒荧光探针技术检测POAG37和正常对照100例白介素6(IL-6)、白介素6受体(IL-6R)、多巴胺受体-D₂(DRD₂)、 β -纤维蛋白原(FGB)、过氧化物酶体增殖物激活受体- γ 2(PPARG)、转化生长因子- β 1(TGF- β 1)、E-选择素(E-Selectin)、脂蛋白A-5(APOA5)、C反应蛋白(CRP)、外核苷酸焦磷酸酶/磷酸二酯酶1(ENPP1)、肝脂肪酶(LIPC)、脂联素(ADIPOQ)、对氧磷酸酶-1(PON1)和丝氨酸蛋白酶抑制剂E(SERPINE1)基因的基因型,计算并比较其等位基因频率。

结果:POAG组IL-6R, IL-6, FGB, CRP, ENPP1, LIPC, ADIPOQ, PON1和SERPINE1的基因型和等位基因频率与正常对照组差异有显著性统计学意义。其中OR值>2.5的有IL-6, FGB, CRP, ENPP1, LIPC和ADIPOQ。

结论:代谢综合征作为POAG的危险因素,可能与其相关基因的基因型和等位基因频率有关。其相应的基因表达及其功能可影响POAG的发生和发展,包括SERPINE1作用于小梁网细胞外基质;ENPP1抑制胰岛素样因子活性,影响小梁网细胞生长;IL-6的内源性视神经保护作用;IL-6, IL-6R, E-Selectin参与的自身免疫反应;FGB和LIPC在高粘滞血症中作用;ADIPOQ促进NOS/NO生成;PON1的血管内皮保护作用。

关键词:原发性开角型青光眼;代谢综合征;单核苷酸多态性