Association of four gene polymorphisms in Chinese Guangxi population with diabetic retinopathy in type 2 diabetic patients

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Abstract

Background: Diabetic retinopathy (DR) is one of the most common chronic microvascular complications of diabetes. Many studies have suggested that genetic factors are important in the context of DR. This study evaluated the associations of GWAS-identified DR-associated SNPs in a Chinese population in Guangxi Province with type 2 diabetes mellitus (T2DM).

Methods: A total of 386 hospitalized T2DM patients without proliferative diabetic retinopathy (PDR) and 316 hospitalized T2DM patients with PDR were included in this case–control study. Four tag SNPs, rs1800896 in the IL-10 gene, rs2010963 in the VEGFA gene, rs2070600 in the RAGE gene and rs2910164 in the miR-146a gene, were examined using KASP (Kompetitive Allele Specific PCR) Genotyping Assays.

Results: There were no significant differences in the genotype or allele frequencies of the miR-146a polymorphism (rs2910164) between subjects with DR and without DR. For rs1800896, the TT allele was determined to be associated with a decreased risk of PDR ($P_{adjusted}$ = 0.022). For rs2010963, 2 alleles (CC and GG) were associated with an increased risk of PDR ($P_{adjusted\text{-}CC}$ = 0.048, $P_{adjusted\text{-}GG}$ = 0.028). For rs2070600, 2 alleles (TT and CT) were associated with a decreased risk of PDR ($P_{adjusted\ TT}$ = 0.033, $P_{adjusted\ CT}$ = 0.002).

Conclusions: The polymorphisms rs1800896 in the IL-10 gene, rs2010963 in the VEGFA gene and rs2070600 in the RAGE gene are associated with the risk of PDR in the Han Chinese population of Guangxi Province. Our findings provide suggestive evidence that these polymorphisms may be involved in the pathogenesis of PDR and should be investigated further.

1. Introduction

Diabetes is an endocrine disease that severely impacts human health, and its disability and fatality rates are second only to those of cardiovascular and cerebrovascular diseases and cancer [1]. It is estimated that the percentage of people with diabetes worldwide will reach 4.4% by 2030 [2]. It has recognized that this disease is a main source of morbidity and mortality because of its related acute and chronic side effects. Diabetic retinopathy (DR) is one of the most usual chronic microvascular side effects of diabetes [3]. With the incidence of diabetes increasing worldwide, the incidence of DR is expected to increase to alarming levels [4]. Furthermore, DR mainly brings blindness in diabetic patients [3].

Diabetes duration, poor glycaemic control and hypertension are known as the primary risk factors related to the growth of DR [4]. However, it has been shown through clinical observation that some patients with poorly controlled or long-lasting diabetes do not develop retinopathy, whereas others, even those with relatively good glycaemic control, eventually develop advanced retinopathy [5]. These clinical observations suggest that there are other factors involved in the development of DR. Many studies suggest that genetic factors are important in the context of DR, that DR exhibits a complicated inheritance mode and that genetic relationship researches are employed for the identification of the genetic elements impacting its growth[6]. According to these information, genetic elements exert an effect on the growth of DR [7]. It has been estimated that the heritability of DR is about 25% [8]. Through knowing the genetic foundation of DR, the hidden pathophysiological mechanisms governing its development can be identified. This genetic data may also bring the risk profiling of DR among patients suffering from diabetes, thereby promoting its early treatment and administration. Robust

relationships of DR-susceptibility variants may make them be genetic markers to enhance the prediction of DR via traditional clinical predictors and thus achieve more precise risk stratification.

Genome-wide relationship research(GWAS) represent a selective strategy for the detection of new genetic loci related to DR, and some GWAS conducted in different ethnic groups have been employed to identify novel genetic variants related to DR susceptibility in type 2 diabetes mellitus (T2DM) cohorts [9, 10]. Single nucleotide polymorphisms (SNPs) mean DNA sequence differences commonly occurring in populations [11]. These changes in DNA sequences may affect gene expression if they occur in putative regulatory regions [11]. In several previous reports, the serum concentrations of some GWAS-identified SNPs were determined to be highly associated with DR [6, 12–14]. Whether these DR-associated SNPs influence DR in the Han Chinese population of Guangxi Province has not been examined in a systematic way. Therefore, this study primarily aims to assess the relationships of GWAS-recognized DR-related SNPs in this population with T2DM.

2. Materials And Approaches

2.1 Research population

The research protocol was examined by the Research Ethics Committee of the Affiliated Hospital of Guilin Medical University and stuck to the principles of the Declaration of Helsinki. All participants obtained written informed consent before their enrolment. A total of 386 hospitalized T2DM patients without proliferative diabetic retinopathy (PDR) and 316 hospitalized T2DM patients with proliferative PDR were included in this case–control research. All patients lived in Guangxi Province and were Han Chinese. The diagnosis of Type 2 diabetes was made on basis of the American Diabetes Association criteria [15]. All patients received ophthalmic examination such as best-modified visual acuity, intraocular pressure, slit lamp, and dilated fundus examinations, in the Department of Ophthalmology, Affiliated Hospital of Guilin Medical University. PDR was defined as having eyes with definite neovascularization and/or vitreous/preretinal haemorrhages. Medical records about age, sex, age at diabetes diagnosis, presence of arterial hypertension, application of medication or insulin, and other comorbidities was collected by a questionnaire. People with peripheral vascular diseases, coronary artery diseases, acute infection, history of any thrombotic event, or any other ocular disorders were excluded.

2.2 Candidate gene and single nucleotide polymorphism selection

This research selected 4 SNPs in total. The selected SNPs are listed in Table 2. According to the report, these genes were related to DR in at least one population or are logical candidate genes according to the present understanding of the pathogenesis of DR; there are selected SNPs in the promoter areas, 5’ UTR regions, or coding areas of candidate genes.

2.3 Genotyping

Whole-blood specimens from all patients were gathered in EDTA tubes and kept at -20°C for less than two months. A TIANamp Genomic DNA Kit (TianGen, Beijing, China) was used to extract Genomic DNA from whole blood before analysis. Genotyping for SNP screening analyses was conducted with the KASP (Kompetitive Allele Specific PCR) assay. Equal amounts of genomic DNA (0.8 μl/patient) from DR and DNR patients were mixed with KASP MasterMix and KASP Assay mix. Next, the SNP-containing DNA fragments were amplified by PCR. The PCR program was below: 15-minute initial denaturation at 94°C, 20-second 10 cycles of denaturation

at 94°C, 1-minute annealing at 65°C (0.8°C decrease every cycle), and 27 cycles of final extension at 59°C for 1 min. Primers for the KASP SNP assays were designed using Primer Premier 5.0, and allele frequencies were analysed using IntelliQube software (LGC Genomics, UK).

2.4 Statistical analyses

Continuous data are shown as the mean ± SD. It is reported that categorical variables are numbers (percentages) or percentages. The normality of the distribution of quantitative variables was verified by Kolmogorov-Smirnov test. The comparison of continuous variables among groups of diabetic subjects was made by ANOVA for normally distributed variables. The χ^2 test was used to compare categorical variables. Gene counting was applied to determine allele frequencies, and the χ^2 test was used to verify departures from Hardy-Weinberg equilibrium. The comparison between allele and genotype frequencies was made among groups of subjects through the χ^2 test. *SPSS* (version 20.0; *SPSS*, Inc., Chicago, IL, USA) was employed to conduct statistical analyses. We considered P-values below 0.2 to be great for inclusion in multivariate analysis. Multivariate analysis, adjusting for the use of insulin treatment, systolic blood pressure and glomerular filtration rate (GFR), was performed with binary logistic regression analysis. According to the outcomes, hazard ratios and 95% confidence intervals (CIs) were shown. We considered *P*-values below 0.05 to be great.

3. Results

3.1 Clinical data of the research population

Table 1 summarizes the features of the subjects. The cases and controls were at the age of 58.5 and 58.95 on average, and the groups included 44.04% and 44.87% females, respectively, indicating well match between two groups with regard to age and sex (both $P > 0.05$). No great variation was found in the duration of DM, body mass index, HbA1c, HDL cholesterol, total cholesterol or LDL cholesterol between the groups. There was a great relationship in analyzing systolic blood pressure and using insulin treatment and glomerular filtration rate (GFR) between the groups.

3.2 Polymorphisms of 9 SNPs in Type 2 Diabetic Subjects Based on the Presence of PDR

The distribution of the genotype and allele frequencies of the 4 SNPs' polymorphisms in type 2 diabetic subjects based on the presence of PDR are shown in Table 2. No great variation was found in the genotype or allele frequencies between subjects with DR and without DR regarding the miR-146a polymorphism (rs2910164), indicating that this SNP might not be related to the presence of PDR. However, the data show that the other 3 SNP polymorphisms (rs1800896, rs2010963 and rs2070600) were significantly associated with the presence of PDR.

Multivariable logistic regression analysis showed that 3 SNPs (rs1800896, rs2010963 and rs2070600) were associated with the PDR phenotype after adjustment for insulin therapy, systolic blood pressure and GFR. For rs1800896, the frequency of the TC allele was higher among subjects with PDR than in those without DR (8.29% vs. 16.03%, $P = 0.002$). The frequency of the TT allele was lower in subjects with PDR (82.69% vs. 91.71%, $P = 0.001$). After multivariable analysis, the other TT allele was determined to be related to a decreased risk of PDR. The odds ratio (OR) was 0.429, with a 95% confidence interval (CI) ranging from 0.207 to 0.885 (*P*

$_{adjusted}$ = 0.022). For rs2010963, the frequency of the CG allele was lower in subjects with PDR than in those without DR (44.87% vs. 56.02%, P = 0.003). After multivariable analysis, the other 2 alleles (CC and GG) were related to an increased risk of PDR. The OR of the CC allele was 2.171, with a 95% CI ranging from 1.006 to 4.682 ($P_{adjusted}$ = 0.048). The OR of the GG allele was 1.700, with a 95% CI ranging from 1.058 to 2.733 ($P_{adjusted}$ = 0.028). For rs2070600, the frequency of the CC allele was higher among subjects with PDR than in those without DR (66.67% vs. 51.81%, P = 0.001). The frequency of the CT allele was lower in subjects with PDR (30.67% vs. 41.97%, P = 0.002). After multivariable analysis, the other 2 alleles (TT and CT) were related to a reduced risk of PDR. The OR of the TT allele was 0.180, with a 95% CI ranging from 0.037 to 0.872 ($P_{adjusted}$ = 0.033). The OR of the CT allele was 0.448, with a 95% CI ranging from 0.266 to 0.753 ($P_{adjusted}$ = 0.002).

4. Discussion

In this study, we analysed the association of 92 SNPs selected from 11 DR-associated factors in an independent cohort of patients in Guangxi Province with type 2 diabetes mellitus (T2DM). Besides, pyrosequencing KASP analysis of the allele frequency showed that 4 SNPs (rs1800896, rs2010963, rs2070600 and rs2910164) had allele frequency differences greater than 10%.

It is proposed that DR manifests persistent low-grade inflammation [16]. Interleukin-10 (IL-10) can stop the generation of proinflammatory cytokines and stimulate the proliferation, differentiation and survival of several immune cells [17]. Most cells of the adaptive and inborn immune systems such as dendritic cells, leukocytes, and macrophages express IL-10 [17]. The growth and progression of DR may include IL-10, an anti-inflammatory cytokine with strong deactivating nature [12]. IL-10 expression [18] was affected by IL-10 gene rs1800896 polymorphism (IL-10 -1082G/A polymorphism) in the promoter region, It is reported that the IL-10 gene polymorphism is related to the risk of DR among various populations [12, 19, 20]. This study displayed that the TT genotype was associated with a reduced risk for PDR. Various genetic backgrounds and living contexts of the study samples, sample sizes, exposure elements, and clinical phenotypes of PDR may occupy the conflicting outcomes obtained by the abovementioned researches. According to the multivariable analyses, there is significant risk of PDR confirmed by the IL-10 gene rs1800896 polymorphism. However, these outcomes should be explained carefully due to the limited sample sizes in the stratified analyses and the limited power. However, evidence for a possible effect between the rs1800896 polymorphism and several PDR risk elements is provided by our findings.

Vascular endothelial growth factor (VEGF) drives angiogenesis, brings the breakdown of the blood-retinal barrier, excites the growth of endothelial cells, induces neovascularization, and enhances vascular permeability in the ischaemic retina [21, 22]. Increased expression levels of VEGF and its receptors have been observed in DR patients [23]. The best-known and most widely used VEGF gene in clinical practice is VEGFA [13]. Our KASP assay analysed the SNP of VEGFA (rs2010963). Because the expression of VEGFA is important for the progression of DR, SNPs of VEGFA have been analysed in several DR-associated studies [24–26]. Our analysis demonstrated that carriers of the GG genotype had a 1.7-fold higher risk for DR than those with the CG genotype. Carriers of either the CC genotype or the GG genotype exhibited altered susceptibility to DR, suggesting that rs2010963 might be an important genetic marker for DR among patients in Guangxi Province with T2DM.

Receptor for advanced glycation end product (RAGE) gene polymorphisms impact DR due to pathophysiological information related to retinopathy and advanced glycation end products (AGEs) [6]. The RAGE gene is located on the short arm of chromosome 6: 6p21.3 [27]. AGEs outcome from the nonenzymatic glycation of proteins and lipids [28]. AGEs are observed at enhanced levels in individuals with diabetes and can result in enhanced oxidative stress and receptor-mediated activation and secretion of different cytokines [28]. The polymorphism in RAGE happens at a predicted N-linked glycosylation motif in the AGE binding site, impacting AGE-RAGE interactions [6]. This study analysed the RAGE SNP (rs2070600), and the results showed that TT genotype or T allele carriers were associated with a reduced risk for PDR. Similar great relationships between rs2070600 and diabetic retinopathy were also observed in Asian Indians and Asian Chinese people with type 2 diabetes and in an Indian study [6, 27]. Moreover, an allelic frequency of rs2070600 in different ethnic groups could cause various outcomes. The T allele frequency in this research was 18.00%, which is similar to the findings of an earlier report in the Chinese population (23.1%) [29] and another report in the Japanese population (17.3%) [30]. In past reports, the T allele happened with an incidence of 5% in Caucasians [31] and 2% in Indians [32]. Allelic variants of the RAGE gene may change protein roles and gene expression, which may deeply influence the disease result. The high proportion of variant alleles in the Chinese population may confer enhanced susceptibility to diabetic side effects in this population.

In Kaidonis' study [14], rs2910164 was found to potentially enhance susceptibility to retinal injury via a pathway taking part in both angiogenesis and breakdown of the blood retinal barrier. This SNP was determined to be significantly related to DN in patients suffering from type 1 diabetes mellitus (T1DM) after multivariate analysis [14]. In our study, we collected samples from T2DM patients to analyse the risk of DR, but T1DM and T2DM are unique diseases with various aetiologies. The growth of DR is impacted by environmental elements that may happen under the background of a given kind of diabetes mellitus. Furthermore, DR most commonly develops early in susceptible patients with T1DM [33]. After multivariable analysis, we did not observe that rs2910164 was a risk factor that was significantly associated with DR. Further researches with a larger cohort size are warranted to dig these given phenotypes more accurately in relation to miR-146a SNPs.

Hidden restrictions of the current research should be taken into account. First, the sample size was not great, which may have caused our work to be underpowered. Second, we cannot exclude confounding effects of unmeasured variables that may affect the stability of blood glucose levels, such as dietary and other lifestyle factors. Third, no detailed information regarding DR severity or treatment response were got, which limited our discussions. Fourth, we planned to avoid population substructures in our research. Nevertheless, it is possible that the positive and negative outcomes obtained in this study may be because of subtle population stratification, and the results should be used as suggestive instead of definitive. Moreover, our study requires a more direct assessment of the association between SNPs and related serum levels. The mechanisms underlying these SNPs in DR merit further study.

5. Conclusion

According to the outcomes of this research, the polymorphisms rs1800896 in the IL-10 gene, rs2010963 in the VEGFA gene and rs2070600 in the RAGE gene are related to the risk of PDR in the Han Chinese population of Guangxi Province. Our findings provide suggestive evidence that these polymorphisms may take part in the pathogenesis of PDR and should be examined further. Moreover, our study suggests that the rs2910164

polymorphism in the miR-146a gene may not be related to DR in the Guangxi Province population. Nevertheless, these findings should be examined by additional well-designed multicentre researches with larger sample sizes including gene–environment interaction assessments.

List Of Abbreviations

DR diabetic retinopathy

PDR proliferative diabetic retinopathy

T2DM type 2 diabetes mellitus

T1DM type 1 diabetes mellitus

KASP Kompetitive Allele Specific PCR

GWAS Genome-wide association studies

GFR glomerular filtration rate

IL-10 Interleukin-10

VEGF vascular endothelial growth factor

RAGE receptor for advanced glycation end product

Declarations

Ethics approval

No animals were used in this research. The study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their enrolment. All experiments were approved by the Ethics Committee of Affiliated Hospital of Guilin Medical University (Acceptance number: GLMU1A2019120).

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All the authors declare that they have no competing interests.

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Author contributions

All authors made substantial contributions to conception and design. Dongdong Jiang, Liu Zheng, Yu Xiong and Binbin Yang collected the data. Zhixiang Ding, Xinsheng Zeng and Miaoyun Liao made substantial contributions to analysis and interpretation of data. He Jin and Dongdong Jiang wrote the first draft of the manuscript. All authors were involved in revising the manuscript critically for important intellectual content. And He Jin has given final approval of the version to be published. All authors read and approved final manuscript.

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Tables

Table 1. Demographic and clinical characteristics of participants by type of diabetes

ical characteristics (Variable)	NDR group (n=386)	PDR group (n=316)	*P-value*
ıale [n (%)]	170 (44.04)	140 (44.87)	0.877
[years, mean ± SD]	58.50±11.44	58.95±10.16	0.699
ation of DM [years, mean ± SD]	9.53±7.07	10.10±7.24	0.472
y mass index (BMI) [kg/m^2, mean ± SD]	23.922±3.127	24.701±4.760	0.093
A1c [% (mmol/mol), mean ± SD]	8.668±2.432	8.677±2.520	0.976
ılin treatment [n (%)]	180 (46.63)	212 (67.52)	**0.001**
ertension [n (%)]	168 (43.52)	142 (45.22)	0.750
:olic blood pressure [mmHg, mean ± SD]	130.65±17.41	135.05±18.06	**0.025**
stolic blood pressure [mmHg, mean ± SD]	81.01±9.56	80.17±10.40	0.437
ıl cholesterol [mM, mean ± SD]	4.290±1.188	4.448±1.258	0.259
L cholesterol [mM, mean ± SD]	1.028±0.273	1.080±0.360	0.155
. cholesterol [mM, mean ± SD]	2.711±0.967	2.853±0.908	0.190
merular filtration rate (GFR) [ml/min, mean ±	84.970±33.218	75.951±28.971	**0.013**

Bold indicates statistically significant results.

Table 2. Analysis of 4 SNPs in NDR and PNR groups.

Gene	SNP	Allele	NDR [%]	PDR [%]	Geno-type	NDR [n (%)]	PDR [n (%)]	χ^2 test	Logistic regression analysis	
								P-value	odds ratio (95% CI)	P_{adjust}-*value*
IL-10	rs1800896	T	95.85	90.71	TC	32 (8.29)	50 (16.03)	**0.002**	Reference	
		C	4.15	9.29	CC	0 (0)	4 (1.28)	**0.026**	6.839 (0)	0.999
					TT	354 (91.71)	258 (82.69)	**0.001**	0.429 (0.207, 0.885)	**0.022**
VEGFA	rs2010963	C	39.00	34.62	CG	214 (56.02)	140 (44.87)	**0.003**	Reference	
		G	60.99	65.38	CC	42 (10.99)	38 (12.18)	0.627	2.171 (1.006, 4.682)	**0.048**
					GG	126 (32.98)	134 (42.95)	**0.007**	1.700 (1.058, 2.733)	**0.028**
RAGE	rs2070600	C	72.80	82.00	CC	200 (51.81)	200 (66.67)	**0.001**	Reference	
		T	27.20	18.00	TT	24 (6.22)	8 (2.67)	**0.029**	0.180 (0.037, 0.872)	**0.033**
					CT	162 (41.97)	92 (30.67)	**0.002**	0.448 (0.266, 0.753)	**0.002**
miR-146a	rs2910164	C	63.68	65.88	CC	158 (41.58)	132 (44.59)	0.432	-	
		G	36.32	34.12	GG	54 (14.21)	38 (12.84)	0.606	-	
					CG	168 (44.21)	126 (42.57)	0.669	-	

Bold indicates statistically significant results