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Polymorphisms in Sorbitol-Aldose Reductase (Polyol) Pathway Genes and Their Influence on Risk of Diabetic Retinopathy Among Han Chinese

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Sorbitol-aldose reductase (polyol) pathway genes have been strongly linked to diabetic retinopathy. Polymorphisms in these genes may affect their functions and influence the risk of retinopathy. In this work, we investigated the influence of the rs759853 polymorphism of *ALR2* gene and rs2055858 and rs3759890 polymorphisms of *SDH* gene on risk of diabetic retinopathy among Han Chinese.

Material/Methods: We included 3,000 subjects in our study, of which 1,500 were patients with diabetic retinopathy and 1,500 were controls without the said condition. Among the cases, 750 had the non-proliferative diabetic retinopathy (NPDR) and 750 had proliferative diabetic retinopathy (PDR). The polymorphisms were genotyped using established methods and logistic regression analysis was used to determine whether the polymorphisms were associated with risk of diabetic retinopathy.

Results: We found that variants of *ALR2* rs759853 polymorphism were significantly associated with an increased risk of diabetic retinopathy, whereas variants of *SDH* rs2055858 polymorphism were significantly associated with a lower risk. For the former, an odds ratio (OR) of 1.46 were noted for the heterozygous genotype (95% CI=1.25–1.70, P<0.01) and the homozygous variant genotype (OR=1.90, 95% CI=1.40–2.60, P<0.01). For *SDH* rs2055858 polymorphism, an OR of 0.51 (95% CI=0.43–0.61, P<0.01) and 0.34 (95% CI=0.28–0.42, P<0.01) was observed for heterozygous and homozygous variant genotype respectively. Subgroup analysis based on NPDR and PDR showed a similar finding as the combined results.

Conclusions: *ALR2* rs759853 and *SDH* rs2055858 polymorphisms were respectively associated with a higher and lower risk of diabetic retinopathy.

MeSH Keywords: Aldehyde Reductase • Diabetic Retinopathy • Polymorphism, Genetic • Sorbitol

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Background

One of the most common complications of diabetes mellitus is diabetic retinopathy, which is the damage to the retina caused by the prolonged exposure to altered metabolism associated with diabetes. This condition may lead to severe vision impairment and even blindness among the patients. Known risk factors for diabetic retinopathy include long disease duration and poor glycemic control [1]. However, not all patients with a long disease duration and poor glycemic control develop retinopathy. Similarly, not all patients who develop retinopathy have a long disease duration and poor glycemic control. Previous works have suggested that there is also a strong genetic influence in the development and progression of diabetic retinopathy [2].

Sorbitol-aldose reductase pathway, also known as the polyol pathway, is one of the major pathways that link hyperglycemia and diabetic retinopathy [3]. There are 2 main enzymes involved in this pathway, namely aldose reductase and sorbitol dehydrogenase. When excessive amount of glucose is available in the cell, aldose reductase converts glucose to sorbitol. Accumulation of sorbitol can led to osmotic stress, which can have deleterious effect to the retinal cells, leading to retinopathy [4]. Fortunately, sorbitol can be oxidized and converted into fructose by sorbitol dehydrogenase. Despite the important role of sorbitol dehydrogenase, a previous work has reported that overexpression of sorbitol dehydrogenase can cause glucose toxicity in retinal pericytes, which can lead to retinopathy [5]. Thus, the activities of both aldose reductase and sorbitol dehydrogenase need to be tightly regulated at an optimal level. Any disruption to the activities of either enzyme is likely to predispose a diabetic patient to diabetic retinopathy.

The activities of the enzymes are regulated, at least partially, by genetic polymorphisms in the genes that encode them. Aldose reductase is encoded by the *ALR2* gene, whereas sorbitol dehydrogenase is encoded by *SDH*. Several previous works have demonstrated a potential association between diabetic retinopathy and several polymorphisms in *ALR2* and *SDH* genes [6]. These include rs759853 polymorphism of *ALR2* and rs2055858 and rs3759890 polymorphisms of *SDH*. However, inconsistent findings have been obtained in different studies – while some studies demonstrated that a specific allele of the polymorphisms were associated with increased risk of diabetic retinopathy, some other studies showed the opposite [7–9]. One of the possible reasons for the inconsistent finding is the sample size. Most of the previous works employed low sample size, which might have led to false-positive or false-negative findings. In this work, we investigated the association between rs759853, rs2055858 and rs3759890 polymorphisms and risk of diabetic retinopathy among Han Chinese in a large sample size.

Material and Methods

This case-control study involving 3,000 subjects was conducted with ethical approval from Tongji Medical College, Huazhong University of Science and Technology. The subjects were pre-identified from the hospital's medical database prior to their recruitment into the study to ensure that the subjects were represented equally and that they were closely matched to one another. All subjects were type 2 diabetes patients [diagnosed based on the American Diabetes Association (2005) criteria] with a minimum duration of 5 years. Among these 3,000 subjects, 1,500 were cases with diabetic retinopathy and 1,500 were controls without diabetic retinopathy. Diagnosis of diabetic retinopathy was performed by direct ophthalmoscopic examination through dilated pupils. A positive diagnosis was defined by the presence of typical retinopathic manifestations, including but not limited to microvascular abnormalities, exudates and hemorrhages. The patients were classified into the less severe non-proliferative diabetic retinopathy (NPDR) and the severe proliferative diabetic retinopathy (PDR) based on fundus fluorescein angiography. We included 750 cases with NPDR and 750 cases with PDR. The classification of NPDR and PDR was confirmed by fundus fluorescein angiography. Written informed consent was obtained from all subjects before participating in the study and the study was accepted by the Ethics Board of the Central Hospital of Wuhan (approval SY/20160711).

Blood specimens were collected from all subjects. Genotyping was performed on DNA isolated from the blood specimens. For *ALR2* rs759853 polymorphism, genotyping was performed through direct bidirectional sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Massachusetts, USA), by using the primers 5'-CCT TTC TGC CAC GCG GGG CGC GGG-3' (forward) and 5'-CAT GGC TGC TGC GCT CCC CAG-3' (reverse) [10]. For *SDH* rs2055858 and rs3759890 polymorphisms, genotyping was performed with PCR-based method as described [11]. PCR products were visualized on 1.5% agarose gel and the genotypes were confirmed by sequencing 10% of the samples. The genotype and allele frequencies were determined by counting.

Statistical analysis was performed by using SPSS version 20.0. Difference between cases and controls were evaluated by using chi-squared test (categorical data) or t-test (continuous data). Goodness of fit test was used to calculate deviation from the Hardy-Weinberg equilibrium. Logistic regression analysis was used to determine whether the polymorphisms were associated with risk of diabetic retinopathy. P value of <0.05 was considered significant.

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Table 1. Clinical and biochemical parameters of cases and controls.

Characteristics	Cases	Controls	P
Number of subjects	1500	1500	–
Age (years)	57.5±5.6	57.1±6.1	0.097
Sex (% male)	49.1	49.5	0.855
Duration of diabetes (years)	8.2±3.5	7.9±3.7	0.104
BMI (kg/m^2)	26.4±2.7	25.9±3.0	0.148
Presence of hypertension (%)	68.9	67.9	0.556
HbA1c (%)	7.3±1.7	7.3±1.6	0.109
Total cholesterol (mmol/l)	6.0±1.6	5.8±1.2	0.343
LDL (mmol/l)	3.24±1.72	3.18±1.86	0.619
HDL (mmol/l)	1.28±0.33	1.21±0.29	0.283
Triglyceride (mg/dl)	150 (110–217)	147 (104–218)	0.450
Serum creatinine (mg/dl)	1.5 (1.1–2.6)	1.0 (0.8–1.3)	0.091
Smoking (%)	45.3	44.1	0.509

Table 2. Association of the polymorphisms with diabetic retinopathy.

	Cases (N=1500)	Controls (N=1500)	OR (95% CI)	P
ALR2 rs759853				
CC	812 (54.1%)	963 (64.2%)	Ref	
CT	574 (38.3%)	466 (31.1%)	1.46 (1.25–1.70)	<0.01
TT	114 (7.6%)	71 (4.7%)	1.90 (1.40–2.60)	<0.01
SDH rs2055858				
CC	540 (36.0%)	304 (20.3%)	Ref	
CG	701 (46.7%)	771 (51.4%)	0.51 (0.43–0.61)	<0.01
GG	259 (17.3%)	425 (28.3%)	0.34 (0.28–0.42)	<0.01
SDH rs3759890				
CC	968 (64.5%)	996 (66.4%)	Ref	
CG	461 (30.7%)	443 (29.5%)	1.07 (0.91–1.25)	0.40
GG	71 (4.7%)	61 (4.1%)	1.20 (0.84–1.71)	0.32

Results

Clinical and biochemical parameters of the subjects is shown in Table 1. As the controls were deliberately selected to match to the cases, significant difference between cases and controls was not observed for age, sex, duration of diabetes, BMI, presence of hypertension, HbA_{1c}, total cholesterol, LDL, HDL, triglyceride, serum creatinine and smoking status. It should be noted that due to the difficulty in identifying a large number of subjects who were similar in all aspects, the cases and controls were not *strictly* matched to each other (which is why the P value was not 1.00). Rather, we tried to match them as close as possible to ensure that they were not significantly different from one another (P>0.05).

Table 2 shows the association of the polymorphisms with diabetic retinopathy. It was observed that the CC and TT genotypes of *ALR2* rs759853 polymorphism were associated with increased diabetic retinopathy risk. The CC genotype was present at 38.3% of the cases, compared to 31.3% of the controls (OR=1.46, 95% CI=1.25–1.70, P<0.01), whereas the TT genotype was present in 7.6% of the cases, compared to 4.7% of

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Table 3. Deviation from Hardy-Weinberg equilibrium.

	P value in cases	P value in controls
ALR2 rs759853	0.370	0.136
SDH rs2055858	0.225	0.179
SDH rs3759890	0.095	0.188

the controls (OR=1.90, 95% CI=1.40–2.60, P<0.01). On the contrary, the CG and GG genotypes of *SDH* rs2055858 were associated with a lower risk of diabetic retinopathy. Among the cases, 46.7% had the CG genotype. In comparison, 51.4% of the controls had the same genotype (OR=0.51, 95% CI=0.43–0.61, P<0.01). Besides, only 17.3% of the cases had GG genotype compared to 28.3% in the controls (OR=0.34, 95% CI=0.28–0.42, P<0.01). For *SDH* rs3759890, there was no significant difference in the frequencies of CG and GG genotypes between cases and controls. The CG genotype was present in 30.7% of the cases and 29.5% of the controls (P=0.40), whereas the GG

Table 4. Association of the polymorphisms with non-proliferative diabetic retinopathy (NPDR).

	Cases (N=1500)	Controls (N=1500)	OR (95% CI)	P
ALR2 rs759853				
CC	812 (54.1%)	469 (62.5%)	Ref	
CT	574 (38.3%)	242 (32.3%)	1.37 (1.13–1.65)	<0.01
TT	114 (7.6%)	39 (5.2%)	1.69 (1.15–2.47)	0.01
SDH rs2055858				
CC	540 (36.0%)	148 (19.7%)	Ref	
CG	701 (46.7%)	381 (50.8%)	0.50 (0.40–0.63)	<0.01
GG	259 (17.3%)	221 (29.5%)	0.32 (0.25–0.41)	<0.01
SDH rs3759890				
CC	968 (64.5%)	507 (67.6%)	Ref	
CG	461 (30.7%)	210 (28.0%)	1.15 (0.95–1.40)	0.16
GG	71 (4.7%)	33 (4.4%)	1.13 (0.74–1.73)	0.58

Table 5. Association of the polymorphisms with proliferative diabetic retinopathy (PDR).

	Cases (N=1500)	Controls (N=1500)	OR (95% CI)	P
ALR2 rs759853				
CC	812 (54.1%)	494 (65.9%)	Ref	
CT	574 (38.3%)	224 (29.9%)	1.56 (1.29–1.89)	<0.01
TT	114 (7.6%)	32 (4.3%)	2.17 (1.44–3.26)	<0.01
SDH rs2055858				
CC	540 (36.0%)	156 (20.8%)	Ref	
CG	701 (46.7%)	390 (52.0%)	0.52 (0.42–0.65)	<0.01
GG	259 (17.3%)	204 (27.2%)	0.37 (0.28–0.47)	<0.01
SDH rs3759890				
CC	968 (64.5%)	489 (65.2%)	Ref	
CG	461 (30.7%)	233 (31.1%)	0.99 (0.83–1.21)	0.99
GG	71 (4.7%)	28 (3.7%)	1.28 (0.82–2.01)	0.28

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genotype was present in 4.7% of the cases and 4.1% of the controls (P=0.32). The frequencies of all the polymorphisms conforms to the Hardy-Weinberg equilibrium (Table 3).

Diabetic retinopathy can be divided into 2 types based on their severity. The less severe type is the non-proliferative diabetic retinopathy (NPDR) and the more severe one is proliferative diabetic retinopathy (PDR). In order to determine whether the polymorphisms influence the risk differently in the 2 types of diabetic retinopathy, we included both NPDR and PDR cases into our study and analyzed the results separately. The association of the polymorphisms with NPDR and PDR is shown in Tables 4 and 5 respectively. We found that when analyzed separately, the results in each type of diabetic retinopathy were similar to the combined results, that the CT and TT genotypes of *ALR2* rs759853 polymorphism were associated with an increased risk, the CG and GG genotypes of *SDH* rs2055858 polymorphism were associated with a lower risk, and the *SDH* rs3759890 polymorphism was not significantly associated with risk of diabetic retinopathy.

Among NPDR, the CT and TT genotypes of *ALR2* rs759853 polymorphism conferred an OR of 1.37 (95% CI=1.13–1.65, P<0.01) and 1.69 (95% CI=1.15–2.47, P=0.01) respectively. The magnitude of association was larger among PDR, with an OR of 1.56 (95% CI=1.29–1.89, P<0.01) and 2.17 (95% CI=1.44–3.26, P<0.01) respectively. For *SDH* rs2055858, the CG and GG genotypes respectively conferred an OR of 0.50 (95% CI=0.40–0.63, P<0.01) and 0.32 (95% CI=0.25–0.41, P<0.01) for NPDR, and an OR of 0.52 (95% CI=0.42–0.65, P<0.01) and 0.37 (95% CI=0.28–0.47, P<0.01) for PDR.

Discussion

Diabetic retinopathy is a serious complication of diabetes mellitus. Asians are inherently more susceptible to diabetic retinopathy compared to Caucasians [12]. Identification of genetic markers that can categorize diabetic patients into high risk or low risk group is especially needed for Asian populations. In this study, we screened the genotypes of 3 polymorphisms in sorbitol-aldose reductase (polyol) pathway genes among Han Chinese. We found that variants of *ALR2* rs759853 polymorphism were associated with an increased risk of diabetic retinopathy, whereas variants of *SDH* rs2055858 polymorphism were associated with a lower risk.

The *ALR2* rs759853 polymorphism is located close to the CCAAT box in the promoter region of the gene. The 2 different alleles of the polymorphism were found to result in distinct binding affinity of the gene to the transcription factor [13]. In fact, the C allele was shown to have twice as high mRNA expression level compared to the T allele [14]. This means that individuals with the C allele may have a higher level of aldose reductase, which leads to a more efficient conversion of glucose into sorbitol. Accumulation of sorbitol in the retina is known to cause osmotic stress and eventually retinopathy. This is the reason why a few studies had demonstrated that the C allele was associated with an increased risk of diabetic retinopathy [7,8,15], which is in contrast to our finding. The reason and mechanism underlying our observation is not understood. Nevertheless, one study has reported the same finding as ours and coincidentally, this study was conducted in the Chinese population [9]. This suggests that there could be unique mechanisms by which Chinese individuals regulate glucose conversion or handle sorbitol accumulation, although this warrants further research.

Sorbitol dehydrogenase is another enzyme involved in the sorbitol-aldose reductase (polyol) pathway. However, not many studies have investigated the association between its genetic polymorphisms with risk of diabetic retinopathy. For rs2055858, only one previous report is available [11]. In contrast to our finding, this report did not find any significant association between rs2055858 polymorphism and diabetic retinopathy, but noted that the effect of another polymorphism might be strengthened by rs2055858. It should be noted that the sample size used in this previous report was small. A study with a small sample size may not be adequately powered to detect a significant association. In comparison, our unprecedented large sample size was adequate to detect an association. However, how variants of *SDH* rs2055858 polymorphism contributed to a lower risk of diabetic retinopathy is not known, as no transcription factor binding site has been empirically demonstrated thus far.

Conclusions

There are a few major limitations of our study. First, although our sample size was largest to date in this area of investigation, it was still not sufficient to give a strong clinical impact. Second, we did not perform functional analysis to investigate the mechanism underlying the genetic association. Third, we did not consider gene-environment interactions in our work. Thus, further research is warranted to replicate our finding and clarify the mechanisms underlying the genetic association, by considering environmental effects as well. Nonetheless, our present work found that *ALR2* rs759853 and *SDH* rs2055858 polymorphisms were associated with a higher and lower risk of diabetic retinopathy respectively.

Acknowledgement

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Conflict of interest

None.

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