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Title: Genome-wide association study of diabetic retinopathy in a Taiwanese population

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Abstract: Purpose: Diabetic retinopathy (DR) is a microvascular complication of diabetes with a complex multifactorial pathogenesis. The aim of this study was to identify the susceptibility genes that increase the risk of DR in type 2 diabetes (T2D) and to further elucidate the underlying mechanism of DR pathogenesis.
Design: A case-control study.
Participants: 749 unrelated individuals with T2D (174 with DR and 575 without DR) and 100 non-diabetic controls.
Methods: We conducted a genome-wide association study using Illumina HumanHap550-Duo BeadChips.
Main outcome Measures: Compared with the genotypic distribution of single nucleotide polymorphism (SNPs) between subjects with DR and without DR.
Results: Using statistical models, we selected a total of 12 SNPs with p-values <1 ? 10-6 that were associated with DR. After controlling for diabetes duration and hemoglobin A1C, nine of the 12 SNPs located on five chromosomal regions were found to associated with DR. Five loci not previously associated with DR susceptibility were identified in and around the following genes: MYSM1 (Myb-like, SWIRM and MPN domains 1) located on chromosome 1p (odds ratio [OR] = 1.50, 95% confidence interval [CI] = 1.03-2.20); PLXDC2 (plexin domain-containing 2) located on the chromosome 10p (OR=1.67, 95% CI=1.06-2.65); ARHGAP22 (Rho GTPase-activating protein 22) located on chromosome 10q (OR = 1.65, 95% CI = 1.05-2.60) and HS6ST3 (heparan sulfate 6-O-sulfotransferase 3) located on chromosome 13q (OR = 2.33, 95% CI = 1.13-4.77). The SNPs rs13163610 and rs17376456 located in the unknown gene on chromosome 5q were also associated with DR (OR = 3.63, 95% CI = 1.38-9.58).
Conclusions: We identified a genetic association for susceptibility to DR in five novel chromosomal regions and PLXDC2 and ARHGAP22, the latter two of which are genes implicated in endothelial cell angiogenesis and increased capillary permeability. These findings suggest unsuspected pathways in the pathogenesis of DR.

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Genome-wide association study of diabetic retinopathy in a Taiwanese population

Ophthalmology

Dear Prof Tsai,

I am pleased to inform you that your Manuscript entitled, "Genome-wide association study of diabetic retinopathy in a Taiwanese population," has been accepted for publication in Ophthalmology, pending your addressing the following:

Change from:

Five loci not previously associated with DR susceptibility were identified in and around the following genes: MYSM1 (Myb-like, SWIRM and MPN domains 1) located on chromosome 1p [odds ratio (OR) = 1.50, 95% confidence interval (CI) = 1.03-2.20];

to:

Five loci not previously associated with DR susceptibility were identified in and around the following genes: MYSM1 (Myb-like, SWIRM and MPN domains 1) located on chromosome 1p (odds ratio [OR] = 1.50, 95% confidence interval [CI] = 1.03-2.20)…

Review other instances of use of brackets. Use brackets to represent parentheses within parentheses and not the other way around. Numerous errors to correct.

Response: We have made this correction in the revised manuscript.

Use commas and not semicolons for consecutive reference callouts (e.g., 6, 7 and 9,10).

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p. 18 - add date accessed for website.

Response: We have added this information in the text, as follows

Data from the SNP database (dbSNP BUILD 131; http://www.ncbi.nlm.gov/SNP/)

Submit each table as a separate file.

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Define SD, NPDR, SNP ID, MPN, DR (table 2) in tables. Review for others.

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With apologies, we currently have very limited space for both print and online pages. I need to ask authors to help by removing tables or figures since they take so much room to print. Please submit tables 3 and 4 in PDF format for online only publication. Submit each table as a separate file. The PDF files will not be typeset or reformatted in any way by the publisher. Thank you for your understanding and assistance.

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Genome-wide association study of diabetic retinopathy in a Taiwanese population

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Conflict of Interest: None of the authors have any financial interests to disclose.

Short title: Genome-wide association study of diabetic retinopathy

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This article contains online-only material. The following should appear online-only: Tables 3 and 4.

Abstract

Purpose: Diabetic retinopathy (DR) is a microvascular complication of diabetes with a complex multifactorial pathogenesis. The aim of this study was to identify the susceptibility genes that increase the risk of DR in type 2 diabetes (T2D) and to further elucidate the underlying mechanism of DR pathogenesis.

Design: A case-control study.

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located on the chromosome 10p (OR=1.67, 95% CI=1.06-2.65); *ARHGAP22* (Rho GTPase-activating protein 22) located on chromosome 10q (OR = 1.65, 95% CI = 1.05–2.60) and *HS6ST3* (heparan sulfate 6-*O*-sulfotransferase 3) located on chromosome 13q (OR = 2.33, 95% CI = 1.13–4.77). The SNPs rs13163610 and rs17376456 located in the unknown gene on chromosome 5q were also associated with DR (OR = 3.63, 95% CI = 1.38–9.58).

Conclusions: We identified a genetic association for susceptibility to DR in five novel chromosomal regions and *PLXDC2* and *ARHGAP22*, the latter two of which are genes implicated in endothelial cell angiogenesis and increased capillary permeability. These findings suggest unsuspected pathways in the pathogenesis of DR.

Key words: type 2 diabetes, diabetic retinopathy, polymorphism

Introduction

Diabetes mellitus, of which more than 95% cases worldwide are attributed to type 2 diabetes mellitus (T2D), has a complex multifactorial pathogenesis.[1] The devastating complications of diabetes are mostly macro- and microvascular diseases, which are the major causes of morbidity and early mortality in diabetes.[2]

Diabetic retinopathy (DR) is the commonest microvascular complication of diabetes and is a leading cause of new-onset blindness in the working-age population in the developed countries.[3-5] The pathogenesis of DR is believed to be complex and have multifactorial biochemical causes. Alteration of glucose metabolism is the primary cause of DR.[6,7] Poor glycemic control and prolonged duration of diabetes are other major risk factors in the development of DR.[7,8] In addition, there is increasing evidence to implicate genetic factors in the susceptibility to DR independent of glycemic control and the duration of diabetes.[9,10]

To date, genome-wide linkage studies have been performed to identify susceptibility loci for DR. In Pima Indians with T2D, these studies have not only identified the susceptibility loci for diabetes and obesity[11] but have also reported weak evidence for linkage of DR to regions of chromosomes 3 and 9,[12] and suggestive evidence for linkage of DR to chromosome 1p36.[13] Furthermore, in a study of Mexican Americans with T2D, intervals of chromosomes 3 and 12 were shown to be

most strongly associated with any severity of DR.[14] In addition, numerous candidate gene association studies have shown a significant association between genetic factors and the development of DR, including the involvement of the following: aldose reductase (AKR1B1),[15-20] which is involved in the polyol pathway and catalyzes the NADPH-dependent reduction of glucose to sorbitol, and is particularly active under hyperglycemic conditions;[21] vascular endothelial growth factor (VEGF),[22-29] which promotes angiogenesis and is a potent mediator of microvascular permeability;[30] transforming growth factor-beta (TGF-β1),[31-34] which plays an important role in stimulating angiogenesis and inhibiting endothelial function in the eye;[35] endothelial nitric oxide synthase (NOS3);[26,36-38] nitric oxide synthase 2A (NOS2A);[39] erythropoietin[40]; α2β1 integrin (ITGA2);[41,42] and intercellular cell adhesion molecule 1 (ICAM1)[43,44] [for a detailed review, see Abhary *et al.*[45]]. Identification of the specific genetic risk factors for DR susceptibility will contribute to developing new treatments and help to improve existing screening methods.

A considerable amount of work has focused on dissecting the genetics of diabetes itself; however, fewer studies have been conducted on the molecular mechanisms leading to its specific complications such as DR. In order to identify susceptibility loci that are associated with T2D retinopathy in Taiwanese population, we conducted a genome-wide association study involving 749 T2D cases (174 with

retinopathy and 575 without retinopathy) and 100 non-diabetic controls, and identified 12 previously unknown susceptibility loci related to DR.

Methods

Subjects

The study involved 749 unrelated individuals with T2D over the age of 20 years, who were recruited from the China Medical University Hospital (CMUH), Taichung, Taiwan. Subjects were diagnosed using the American Diabetic Association Criteria. Subjects with type 1 diabetes, gestational diabetes, or maturity-onset diabetes of the young were excluded from this study. Of this group, 174 T2D subjects were diagnosed with DR: 102 (58.6%) with non-proliferative diabetic retinopathy (NPDR) and 72 (41.4%) with proliferative diabetic retinopathy (PDR). All T2D subjects underwent a complete ophthalmologic examination, including corrected visual acuity, fundoscopic examination, and fundus photography. DR was graded by an expert ophthalmologists according to the American Academy of Ophthalmology proposed international scales for severity of clinical diabetic retinopathy.[46] A questionnaire was designed for collecting information regarding gender, age, age at diagnosis of diabetes, and ocular history. For each patient, systolic and diastolic blood pressure, waist and hip circumferences, body mass index (BMI), and hemoglobin A_{1C} (HbA_{1C}) levels were determined. In addition, 100 non-diabetic controls were randomly selected from the Taiwan Han Chinese Cell and Genome Bank.[47] The criteria for selecting controls for inclusion in the association study were (i) no diagnostic history of T2D, (ii) an

HbA_{1C} ranging between 3.4% and 6.0%, and (iii) a BMI <32 kg/m^2. This study was approved by CMUH institutional review boards, and informed consent was obtained from all study participants.

Genotyping

For the genome-wide association study, genomic DNA was extracted from peripheral blood mononuclear cells using a PUREGENE DNA isolation kit (Gentra Systems, Minneapolis, MN). Whole genome genotyping using Illumina HumanHap550-Duo BeadChips was performed by deCODE genetics, Inc., Reykjavík, Iceland. Genotype calling was performed using the standard procedure implemented in BeadStudio, with default parameters suggested by the platform manufacturer. Quality control of the genotype data was performed by examining several summary statistics. First, the ratio of loci with heterozygous calls on the X chromosome was calculated to double check the subject gender. Total successful call rate and the minor allele frequency of the cases were also calculated for each SNP. SNPs were excluded if they showed one of the following: (i) no polymorphism; (ii) a total call rate of <95%; (iii) a minor allele frequency of <5%, or a total call rate of <99%. Genotyping validation was performed using the Sequenom iPLEX assay (SEQUENOM MassARRAY system, Sequenom, San Diego, CA, USA).

Statistical analysis

T2D association analysis was carried out to compare allele frequency and genotype distribution between T2D subjects with and without DR using six single-point methods: genotype, allele, trend (Cochran-Armitage test), additive, dominant, and recessive models for each SNP. The most significant test statistic obtained from the six models was selected. First, we selected SNPs with p-values less than 10^{-5} in any of the abovementioned models for subsequent analysis. We then used a logistic regression model with stepwise selection to determine the significant SNPs. SNPs with p-values less than $\alpha = 10^{-7}$, a cut-off for the multiple comparison adjusted by Bonferroni correction, were considered to be significantly associated with the traits. SNPs with p-values between 10^{-7} and 10^{-5} were considered to have suggestive significant associations. Characteristics and clinical data of subjects with DR and without DR were compared by Student's t-test for continuous variables and chi-square test for categorical variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were performed by logistic regression and were adjusted with diabetes duration and HbA_{1C} level. Linkage disequilibrium analysis (D' and r^2) between any two loci were performed using the HAPLOVIEW program, v4.1.[48]

Results

We conducted a genome-wide association study to identify genetic variants for DR in T2D Han Chinese residing in Taiwan. We genotyped 749 T2D patients (174 with DR and 575 without DR) and 100 non-diabetic controls using Illumina Hap550duov3 chips. The demographic and clinical characteristics of the above groups are summarized in Table 1. Subjects without DR were of a significantly younger age at the time of study and diagnosis, and had a shorter disease duration, lower HbA_{1C} levels, and lower systolic blood pressure compared with the subjects with DR (Table 1).

DR-associated SNPs were selected from those showing $-\log_{10}(\text{p-value}) \geq 5$ under the most significant test statistic obtained from any of the six statistical models. We then used a logistic regression model with stepwise selection to determine the significant SNPs. As shown in Table 2, twelve SNPs representing seven regions of five chromosomes were selected from the regression model. The strongest SNP association with DR (rs17376456) occurred on chromosome 5 [$-\log_{10}(\text{p-value}) = 14.52$]. This SNP is located in an intergenic region and has tight linkage disequilibrium with rs13163610 ($D' = 1$, $r^2 = 0.98$). The SNP rs2038823 associated with DR is located on chromosome 13 in an intronic region of the *HS6ST3* (heparan

sulfate 6-*O*-sulfotransferase 3) gene. The remaining five regions, located on chromosomes 1, 4, and 10, are also associated with DR. The rs2811893 and rs12092121 SNPs were localized to chromosome 1 in an intronic region of the *MYSM1* (Myb-like, SWIRM and MPN domains 1) gene and are in complete linkage disequilibrium with each other (D' = 1, r^2 = 1). The rs4470583 SNP is located in an intergenic region on chromosome 4. In addition, six SNPs located on three regions of chromosome 10 are associated with DR. The rs12219125 SNP in an intergenic region is strongly associated with DR [$-\log_{10}$(p-value) = 8.03], and is in strong linkage disequilibrium with rs1571942 (D' = 0.99, r^2 = 0.96) localized to the *PLXDC2* (plexin domain containing 2) gene. The rs4462262 SNP in an intergenic region is also strongly associated with DR [$-\log_{10}$(p-value) = 7.04]. Three SNPs (rs4838605, rs11101355, and rs11101357) are in an intronic region of *ARHGAP22* (Rho GTPase-activating protein 22). Results from a pairwise linkage disequilibrium analysis revealed complete linkage disequilibrium between rs11101355 and rs11101357 (D' = 1, r^2 = 1).

Furthermore, the etiology of DR can vary according to duration of diabetes and the status of glycemic control. Table 3 (available at http://aaojournal.org) shows the results of multiple logistic regression analysis of DR susceptibility SNPs in subjects

with and without DR after controlling for the diabetes duration and HbA_{1C}. The results show that 9 SNPs representing five regions of four chromosomes remained significantly associated with DR in the dominant model after controlling for diabetes duration and HbA_{1C} in the multiple logistic regression analysis. The risk genotypes were defined subjects with homozygous risk allele (higher allele frequency in subjects with DR than in subjects without DR). The risk TT genotype of rs2811893 was associated with a 1.50-fold increase in DR risk under the dominant model (OR = 1.50, 95% CI = 1.03–2.20). Since rs2811893 is in complete linkage disequilibrium with rs12092121, the AA genotype of rs12092121 was associated with the same 1.50-fold increase in DR risk. The AA genotypes of rs13163610 and rs17376456 were associated with a 3.59-fold (95% CI = 1.36–9.47) and 3.63-fold (95% CI = 1.38–9.58) increase, respectively, in DR risk in the multiple logistic regression model after controlling for diabetes duration and HbA_{1C}. The SNPs rs4838605, rs11101355, and rs11101357 lie within the *ARHGAP22* gene of chromosome 10 and were associated with 1.58-, 1.65- and 1.65-fold increases in DR risk, respectively. The SNP rs1571942, located in the *PLXDC2* gene of chromosome 10 was associated with a 1.67-fold (95% CI = 1.06–2.65) higher risk of developing of DR. The SNP rs12219125 was in strong linkage disequilibrium with rs1571942 and was associated with a 1.62-fold increase in DR risk (95% CI = 1.02–2.58). Although the SNP rs1571942 was in strong linkage

disequilibrium with rs12219125, it remains unclear which gene it is located in. The CC genotype of rs2038823 was associated with a 2.33-fold increase in DR risk (95% CI = 1.13–4.77). After controlling for diabetes duration and HbA_{1C}, there was no significant relationship between DR and the SNPs rs4470583 and rs4462262 located on chromosomes 4 and 10, respectively. In addition, we compared the frequency of genotypes between T2D patients with/without DR and non-diabetic controls. As shown in Table 3 (available at http://aaojournal.org), there were significant differences between subjects with DR and non-diabetic controls in rs13163610 and rs17376456 on chromosome 5 as well as for rs2038823 in the *HS6ST3* gene on chromosome 13. There also were significant differences between subjects without DR and non-diabetic controls with respect to three SNPs in the *ARHGAP22* gene on chromosome 10. For the remaining SNPs, we observed no statistically significant difference between the patients with/without DR and the controls.

We subsequently stratified the T2D subjects with DR according to DR severity scales; however, due to the relatively small sample size of the DR subjects after stratification, we pooled all the NPDR subjects together. We analyzed the 10 SNPs exhibiting a significant difference (including rs4838605 in the *ARHGAP22* gene, which showed a borderline significant difference) between subjects with and without

DR after controlling for HbA_{1C} and diabetes duration. As shown in Table 4 (available at http://aaojournal.org), the risk genotypes located in the *MYSM1* gene on chromosome 1 (rs2811893 and rs12092121) were associated with a 1.72-fold increase in NPDR risk under the dominant model (OR = 1.72, 95% CI = 1.09–2.71) after controlling for HbA1c and diabetes duration. The AA genotypes of rs13163610 and rs17376456 located in an unknown gene on chromosome 5 were associated with a 3.48-fold (95% CI = 1.05–11.6) and 3.54-fold (95% CI = 1.07–11.7) increase, respectively, in NPDR risk. The SNPs rs1571942 and rs12219125, which were in strong linkage disequilibrium and located in the *PLXDC2* gene and an unknown gene, respectively, on chromosome 10, were associated with a 1.86-fold (95% CI = 1.09–3.18) higher risk of developing NPDR. Furthermore, the CC genotype of rs2038823 was associated with a 3.15-fold increase in NPDR risk (95% CI = 1.18–8.39). The SNPs rs4838605, rs11101355, and rs11101357 lie within the *ARHGAP22* gene of chromosome 10 and were associated with 2.18-, 2.22-, and 2.22-fold increases in PDR risk, respectively.

Discussion

Here, we describe the results of a genome-wide association study designed to identify loci associated with the risk of DR in subjects with T2D. Significant associations were identified in regions of chromosomes 1, 5, 10, and 13 after controlling for diabetes duration and HbA_{1C}. The results implicate *MYSM1*, *PLXDC2*, *ARHGAP22*, *HS6ST3*, and an unknown gene on chromosome 5q as being involved in the pathogenesis of DR, and particularly, with the exception *ARHGAP22*, in NPDR, although the biology underlying these associations remains to be elucidated. None of these loci have previously been reported to be associated with genes implicated in the development of DR.

The SNP rs2811893 associated with DR, particularly NPDR, is in complete linkage disequilibrium with rs12092121 and is located in the *MYSM1* gene of chromosome 1p. The *MYSM1* gene encodes a JAMM/MPN^+ (Jab1/MPN domain metalloenzyme) domain-containing zinc metalloprotease that acts a deubiquitinylating enzyme specific for mono-ubiquitinylated histone H2A.[49-51] MYSM1 acts by coordinating histone acetylation and deubiquitination, and destabilizing the association of linker histone H1 with nucleosomes.[49] *MYSM1* is located on chromosome 1p32, and a previous genome-wide linkage study has suggested that the interval 1p32-33 is associated with autosomal dominant nonsyndromic renal

hypodysplasia and associated urinary tract malformations.[52] However, the role of MYSM1 in DR pathogenesis awaits further characterization. In addition to *MYSM1* located in chromosome 1p32, a previous study has also reported suggestive evidence linking DR with a region of chromosome 1p36 in Pima Indians with T2D.[13] This suggests that the shorter arm of chromosome 1 may harbor a number of genes conferring susceptibility to DR.

In this study, six of the DR-associated SNPs were located in three regions of chromosome 10. On the basis of the haplotype analysis, we can divide these SNPs (with the exception of rs4462262) into two major haplotype blocks. The larger of the two blocks, which includes the SNPs rs4838605, rs11101355, and rs11101357, is located in the *ARHGAP22* gene of chromosome 10q. *ARHGAP22* encodes a Rho family GTPase protein, which is involved in the signal transduction pathway that regulates endothelial cell capillary tube formation during angiogenesis.[53] In addition, a recent study has suggested that the expression levels of ARHGAP22 play an important role in determining the mode of tumor cell movement.[54] A further recent study has also suggested that ARHGAP22 may be involved in a novel insulin-regulated pathway.[55] In the present study, *ARHGAP22* was the only gene found to be associated with PDR. Since ARHGAP22 is involved in endothelial cell angiogenesis, it seemed to be a logical candidate gene for PDR. The other block,

which is located on chromosome 10p, includes the SNPs rs1571942 and rs12219125. SNP rs1571942 is located in the *PLXDC2* gene and is in strong linkage disequilibrium with SNP rs12219125, the gene location of which is unknown. In previous studies, PLXDC2, also called TEM7R (tumor endothelial marker 7-related protein), was found to be expressed at high levels not only in tumor endothelium but also in the vessels of some normal tissues, and may thus play a role in tumor angiogenesis.[56] A recent study has also shown that PLXDC2 may be associated with fasting insulin levels and insulin resistance.[57] Furthermore, a recent genome-wide study has shown that PLXDC2 may be associated with overgrowth in patients with Sotos syndrome-like features.[58] SNP rs4462262 was not significantly associated with DR after controlling for diabetes duration and HbA_{1C}.

rs2038823, the other SNP associated with DR, particularly NPDR, is located on chromosome 13q in a gene that encodes HS6ST3. HS6ST3 is a heparan sulfate (HS) biosynthetic enzyme that modifies HS to generate structures required for the interactions between HS and a variety of proteins. These interactions are regulated by a wide variety of biological activities and are implicated in proliferation and differentiation, adhesion, migration, inflammation, blood coagulation, and other diverse processes.[59] The Steno hypothesis states that in diabetes mellitus, changes in vascular heparan sulfate proteoglycan expression are involved in systemic endothelial

dysfunction and increase capillary permeability.[60] Previous studies using different animal models, namely, *Drosophila* and mice, have shown that HS6ST3 is involved in both limb bud development and tracheal branching.[61,62]

Finally, an additional locus (rs13163610 and rs17376456) is strongly associated with DR, even after controlling for diabetes duration and HbA_{1C}. However, the locus is located in an intergenic region on chromosome 5q, and therefore it is uncertain whether this locus plays a causal role in DR or is merely in linkage disequilibrium with other functional loci.

According to a recent report by Abhary et al., VEGF and associated genes involved in the renin-angiotensin system, the polyol pathway, and NO pathways are the most appropriate candidate genes for studying the pathogenesis of DR.[45] Since both ARHGAP22 and PLXDC2 are involved in endothelial cell angiogenesis—similar to VEGF, a major mediator of vascular permeability and angiogenesis—they may play a pivotal role in mediating the development and progression of DR.[29,30] In addition, it is worth noting that the unknown gene on chromosome 5q had the strongest association with DR and accordingly warrants further investigation in the future.

The major limitation of this study is the small sample size of the DR patients. In the present study, the statistical power (assuming $\alpha = 0.05$) reached 94%, 82%, and

83% for subjects with or without DR for the unknown gene on chromosome 5q, *PLXDC2*, and *ARHGAP22*, respectively. *MYSM1* and *HS6ST3*, nevertheless, lacked adequate power for subjects with/without DR. However, we have compared the allele frequencies of these significant SNPs in healthy controls among different ethnic groups [Data from the SNP database (dbSNP BUILD 131; http://www.ncbi.nlm.gov/SNP/)]. It is interesting to note that the allele frequencies of three SNPs located in the *ARHGAP22* gene are very different among the different ethnic groups. Since these three SNPs were in strong linkage disequilibrium with each other, we selected only the SNP rs11101357 for description. The A and G allele frequencies are reported, respectively, to be 12.2% and 87.8% in Chinese populations, 4.7% and 95.3% in Japanese populations, 65.3% and 34.7% in European populations, and 22.9% and 77.1% in sub-Saharan African populations. The allele frequencies found in the Chinese populations were similar to those seen in our Taiwanese population (A allele, 13.5%; G allele, 86.5%) in the present study. The allele frequency is very different among Africans and the Japanese, and particularly in Europeans. Therefore, future studies with a larger number of subjects or subjects from different ethnic backgrounds will be necessary in order to determine whether these findings can be replicated. As yet, we have been unable to find any evidence to link a shorter mean diabetes duration and risk genotype of these SNPs in subjects without

DR. In order to determine whether subjects with the risk genotypes of SNPs are more susceptible to developing DR, it will be necessary to undertake a long-term follow-up of T2D subjects without DR who carry these risk genotypes and to evaluate the effects of these genes on the development of DR.

In conclusion, the findings of this study contribute to our understanding of the genetic susceptibility of DR in type 2 diabetes. The novel DR risk loci occur in *ARHGAP22* and *PLXDC2* genes that are implicated in endothelial cell angiogenesis and increased capillary permeability. Although these findings require further study for confirmation, including a different cohort or larger sample size, it is interesting to speculate that these results may indicate new pathways in the pathogenesis of DR.

References

1. Prokopenko I, McCarthy MI, Lindgren CM. Type 2 diabetes: new genes, new understanding. Trends Genet 2008;24:613-21.

2. He Z, King GL. Microvascular complications of diabetes. Endocrinol Metab Clin North Am 2004;33:215-38.

3. Caldwell RB, Bartoli M, Behzadian MA, et al. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Diabetes Metab Res Rev 2003;19:442-55.

4. Moss SE, Klein R, Klein BE. The 14-year incidence of visual loss in a diabetic population. Ophthalmology 1998;105:998-1003.

5. Taylor HR, Keeffe JE. World blindness: a 21st century perspective. Br J Ophthalmol 2001;85:261-6.

6. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.

7. Jerneld B, Algvere P. Relationship of duration and onset of diabetes to prevalence of diabetic retinopathy. Am J Ophthalmol 1986;102:431-7.

8. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with

macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405-12.

9. Hallman DM, Huber JC, Jr., Gonzalez VH, et al. Familial aggregation of severity of diabetic retinopathy in Mexican Americans from Starr County, Texas. Diabetes Care 2005;28:1163-8.

10. Rema M, Saravanan G, Deepa R, Mohan V. Familial clustering of diabetic retinopathy in South Indian Type 2 diabetic patients. Diabet Med 2002;19:910-6.

11. Hanson RL, Ehm MG, Pettitt DJ, et al. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. Am J Hum Genet 1998;63:1130-8.

12. Imperatore G, Hanson RL, Pettitt DJ, et al. Sib-pair linkage analysis for susceptibility genes for microvascular complications among Pima Indians with type 2 diabetes. Pima Diabetes Genes Group. Diabetes 1998;47:821-30.

13. Looker HC, Nelson RG, Chew E, et al. Genome-wide linkage analyses to identify Loci for diabetic retinopathy. Diabetes 2007;56:1160-6.

14. Hallman DM, Boerwinkle E, Gonzalez VH, et al. A genome-wide linkage scan for diabetic retinopathy susceptibility genes in Mexican Americans with type 2

diabetes from Starr County, Texas. Diabetes 2007;56:1167-73.

15. dos Santos KG, Canani LH, Gross JL, et al. The -106CC genotype of the aldose reductase gene is associated with an increased risk of proliferative diabetic retinopathy in Caucasian-Brazilians with type 2 diabetes. Mol Genet Metab 2006;88:280-4.

16. Santos KG, Tschiedel B, Schneider J, et al. Diabetic retinopathy in Euro-Brazilian type 2 diabetic patients: relationship with polymorphisms in the aldose reductase, the plasminogen activator inhibitor-1 and the methylenetetrahydrofolate reductase genes. Diabetes Res Clin Pract 2003;61:133-6.

17. Wang Y, Ng MC, Lee SC, et al. Phenotypic heterogeneity and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. Diabetes Care 2003;26:2410-5.

18. Fujisawa T, Ikegami H, Kawaguchi Y, et al. Length rather than a specific allele of dinucleotide repeat in the 5' upstream region of the aldose reductase gene is associated with diabetic retinopathy. Diabet Med 1999;16:1044-7.

19. Ichikawa F, Yamada K, Ishiyama-Shigemoto S, et al. Association of an (A-C)n dinucleotide repeat polymorphic marker at the 5'-region of the aldose reductase

gene with retinopathy but not with nephropathy or neuropathy in Japanese patients with Type 2 diabetes mellitus. Diabet Med 1999;16:744-8.

20. Ko BC, Lam KS, Wat NM, Chung SS. An (A-C)n dinucleotide repeat polymorphic marker at the 5' end of the aldose reductase gene is associated with early-onset diabetic retinopathy in NIDDM patients. Diabetes 1995;44:727-32.

21. Kador PF, Kinoshita JH. Role of aldose reductase in the development of diabetes-associated complications. Am J Med 1985;79:8-12.

22. Abhary S, Burdon KP, Gupta A, et al. Common sequence variation in the VEGFA gene predicts risk of diabetic retinopathy. Invest Ophthalmol Vis Sci 2009;50:5552-8.

23. Kangas-Kontio T, Vavuli S, Kakko SJ, et al. Polymorphism of the manganese superoxide dismutase gene but not of vascular endothelial growth factor gene is a risk factor for diabetic retinopathy. Br J Ophthalmol 2009;93:1401-6.

24. Churchill AJ, Carter JG, Ramsden C, et al. VEGF polymorphisms are associated with severity of diabetic retinopathy. Invest Ophthalmol Vis Sci 2008;49:3611-6.

25. Uthra S, Raman R, Mukesh BN, et al. Association of VEGF gene polymorphisms with diabetic retinopathy in a south Indian cohort. Ophthalmic

Genet 2008;29:11-5.

26. Suganthalakshmi B, Anand R, Kim R, et al. Association of VEGF and eNOS gene polymorphisms in type 2 diabetic retinopathy. Mol Vis 2006;12:336-41.:336-41.

27. Awata T, Kurihara S, Takata N, et al. Functional VEGF C-634G polymorphism is associated with development of diabetic macular edema and correlated with macular retinal thickness in type 2 diabetes. Biochem Biophys Res Commun 2005;333:679-85.

28. Ray D, Mishra M, Ralph S, et al. Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes 2004;53:861-4.

29. Awata T, Inoue K, Kurihara S, et al. A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes 2002;51:1635-9.

30. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994;331:1480-7.

31. Prasad P, Tiwari AK, Kumar KM, et al. Association of TGFbeta1, TNFalpha, CCR2 and CCR5 gene polymorphisms in type-2 diabetes and renal insufficiency among Asian Indians. BMC Med Genet 2007;8:20.:20.

32. Buraczynska M, Baranowicz-Gaszczyk I, Borowicz E, Ksiazek A. TGF-beta1 and TSC-22 gene polymorphisms and susceptibility to microvascular complications in type 2 diabetes. Nephron Physiol 2007;106:69-75.

33. Wong TY, Poon P, Chow KM, et al. Association of transforming growth factor-beta (TGF-beta) T869C (Leu 10Pro) gene polymorphisms with type 2 diabetic nephropathy in Chinese. Kidney Int 2003;63:1831-5.

34. Beranek M, Kankova K, Benes P, et al. Polymorphism R25P in the gene encoding transforming growth factor-beta (TGF-beta1) is a newly identified risk factor for proliferative diabetic retinopathy. Am J Med Genet 2002;109:278-83.

35. Pascal MM, Forrester JV, Knott RM. Glucose-mediated regulation of transforming growth factor-beta (TGF-beta) and TGF-beta receptors in human retinal endothelial cells. Curr Eye Res 1999;19:162-70.

36. Awata T, Neda T, Iizuka H, et al. Endothelial nitric oxide synthase gene is associated with diabetic macular edema in type 2 diabetes. Diabetes Care 2004;27:2184-90.

37. Ezzidi I, Mtiraoui N, Mohamed MB, et al. Endothelial nitric oxide synthase Glu298Asp, 4b/a, and T-786C polymorphisms in type 2 diabetic retinopathy. Clin Endocrinol (Oxf) 2008;68:542-6.

38. Chen Y, Huang H, Zhou J, et al. Polymorphism of the endothelial nitric oxide synthase gene is associated with diabetic retinopathy in a cohort of West Africans. Mol Vis 2007;13:2142-7.:2142-7.

39. Warpeha KM, Xu W, Liu L, et al. Genotyping and functional analysis of a polymorphic (CCTTT)(n) repeat of NOS2A in diabetic retinopathy. FASEB J 1999;13:1825-32.

40. Tong Z, Yang Z, Patel S, et al. Promoter polymorphism of the erythropoietin gene in severe diabetic eye and kidney complications. Proc Natl Acad Sci U S A 2008;105:6998-7003.

41. Petrovic MG, Hawlina M, Peterlin B, Petrovic D. BglII gene polymorphism of the alpha2beta1 integrin gene is a risk factor for diabetic retinopathy in Caucasians with type 2 diabetes. J Hum Genet 2003;48:457-60.

42. Matsubara Y, Murata M, Maruyama T, et al. Association between diabetic retinopathy and genetic variations in alpha2beta1 integrin, a platelet receptor for collagen. Blood 2000;95:1560-4.

43. Kamiuchi K, Hasegawa G, Obayashi H, et al. Intercellular adhesion molecule-1 (ICAM-1) polymorphism is associated with diabetic retinopathy in Type 2 diabetes mellitus. Diabet Med 2002;19:371-6.

44. Liu L, Yu Q, Wang H, et al. Association of intercellular adhesion molecule 1 polymorphisms with retinopathy in Chinese patients with Type 2 diabetes. Diabet Med 2006;23:643-8.

45. Abhary S, Hewitt AW, Burdon KP, Craig JE. A systematic meta-analysis of genetic association studies for diabetic retinopathy. Diabetes 2009;58:2137-47.

46. Wilkinson CP, Ferris FL, III, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003;110:1677-82.

47. Pan WH, Fann CS, Wu JY, et al. Han Chinese cell and genome bank in Taiwan: purpose, design and ethical considerations. Hum Hered 2006;61:27-30.

48. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.

49. Zhu P, Zhou W, Wang J, et al. A histone H2A deubiquitinase complex coordinating histone acetylation and H1 dissociation in transcriptional

regulation. Mol Cell 2007;27:609-21.

50. Cope GA, Suh GS, Aravind L, et al. Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. Science 2002;298:608-11.

51. Verma R, Aravind L, Oania R, et al. Role of Rpn11 metalloprotease in deubiquitination and degradation by the 26S proteasome. Science 2002;298:611-5.

52. Sanna-Cherchi S, Caridi G, Weng PL, et al. Localization of a gene for nonsyndromic renal hypodysplasia to chromosome 1p32-33. Am J Hum Genet 2007;80:539-49.

53. Katoh M, Katoh M. Identification and characterization of ARHGAP24 and ARHGAP25 genes in silico. Int J Mol Med 2004;14:333-8.

54. Sanz-Moreno V, Gadea G, Ahn J, et al. Rac activation and inactivation control plasticity of tumor cell movement. Cell 2008;135:510-23.

55. Larance M, Rowland AF, Hoehn KL, et al. Global phosphoproteomics identifies a major role for AKT and 14-3-3 in regulating EDC3. Mol Cell Proteomics 2010;9:682-94.

56. Carson-Walter EB, Watkins DN, Nanda A, et al. Cell surface tumor endothelial

markers are conserved in mice and humans. Cancer Res 2001;61:6649-55.

57. Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010;42:105-16.

58. Visser R, Gijsbers A, Ruivenkamp C, et al. Genome-wide SNP array analysis in patients with features of sotos syndrome. Horm Res Paediatr 2010;73:265-74.

59. Habuchi H, Tanaka M, Habuchi O, et al. The occurrence of three isoforms of heparan sulfate 6-O-sulfotransferase having different specificities for hexuronic acid adjacent to the targeted N-sulfoglucosamine. J Biol Chem 2000;275:2859-68.

60. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, et al. Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetologia 1989;32:219-26.

61. Kamimura K, Fujise M, Villa F, et al. Drosophila heparan sulfate 6-O-sulfotransferase (dHS6ST) gene. Structure, expression, and function in the formation of the tracheal system. J Biol Chem 2001;276:17014-21.

62. Nogami K, Suzuki H, Habuchi H, et al. Distinctive expression patterns of

heparan sulfate O-sulfotransferases and regional differences in heparan sulfate structure in chick limb buds. J Biol Chem 2004;279:8219-29.

***Precis**

Five previously unknown susceptibility regions related to diabetic retinopathy in a genome-wide association study were identified, which possibly indicate novel pathways in the pathogenesis of this disease.

Table 1. Characteristics and clinical profiles of the study subjects

	T2D subjects		Non-diabetic controls	p-value* (DR vs. without DR)
	with DR	without DR		
	n = 174	**n = 575**	**n = 100**	
Gender				
Male	50.6%	53.9%	59.0%	0.440†
Female	49.4%	46.1%	41.0%	
Age at study (mean, years)	58.0	61.9	47.0	<0.001
Age at diagnosis T2D (mean ± SD, years)	47.7 ± 9.3	50.2 ± 9.2		0.002
Duration of diabetes (mean ± SD, years)	14.8 ± 8.3	8.3 ± 6.5		<0.001
HbA_{1C} (%)	8.3 ± 1.4	7.7 ± 1.4		<0.001
BMI (kg/m^2)	25.2 ± 4.0	25.1 ± 3.8		0.612
DR severity scales‡				
Non-proliferative DR (NPDR)				
-mild NPDR	31.0%	--		
-moderate NPDR	25.9%	--		
-severe NPDR	1.7%	--		
Proliferative DR	41.4%	--		
Systolic blood pressure (mmHg)	135.3 ± 18.5	127.6 ± 16.2		<0.001
Diatolic blood pressure (mmHg)	74.7 ± 11.3	76.4 ± 10.8		0.067
Waist (cm)	89.9 ± 10.3	88.6 ± 10.1		0.147
Hip (cm)	97.0 ± 8.1	97.1 ± 7.6		0.892

T2D, type 2 diabetes; DR, diabetic retinopathy; SD, standard deviation; HbA_{1C}, hemoglobin A_{1C}; NPDR, non-proliferative DR

*Student's t-test; †Chi-square test; ‡According to the American Academy of Ophthalmology proposed international scales for severity of clinical diabetic retinopathy

Table 2. Summary of the SNPs associated with diabetic retinopathy in type 2 diabetes

dbSNP ID	Chr.	Position (Mb)	Nearest gene	Risk allele* (non-risk allele)	p-value† (best model)	-log(p-value)
rs2811893	1p	5.89	*MYSM1*	T (C)	3.09×10^{-7}	6.51
rs12092121	1p	5.89	*MYSM1*	A (G)	3.09×10^{-7}	6.51
rs4470583	4q	16.24	Unknown	A (G)	4.25×10^{-7}	6.37
rs13163610	5q	9.35	Unknown	A (C)	3.22×10^{-15}	14.49
rs17376456	5q	9.35	Unknown	A (G)	2.99×10^{-15}	14.52
rs1571942	10p	2.05	*PLXDC2*	C (T)	3.47×10^{-7}	6.46
rs12219125	10p	2.06	Unknown	T (G)	9.29×10^{-9}	8.03
rs4838605	10q	4.93	*ARHGAP22*	C (T)	1.87×10^{-9}	6.73
rs11101355	10q	4.93	*ARHGAP22*	T (C)	8.92×10^{-7}	6.05
rs11101357	10q	4.93	*ARHGAP22*	A (G)	8.92×10^{-7}	6.05
rs4462262	10q	5.88	Unknown	C (T)	9.21×10^{-8}	7.04
rs2038823	13q	9.57	*HS6ST3*	C (A)	4.68×10^{-11}	10.33

dbSNP ID, SNP database identification; Chr, chromosome; *MYSM1*, Myb-like, SWIRM and MPN domains 1; *PLXDC2*, plexin domain-containing 2; *ARHGAP22*, Rho GTPase-activating protein 22; *HS6ST3*, heparan sulfate 6-*O*-sulfotransferase 3

*Risk allele: the allele with higher frequency in subjects with diabetic retinopathy (DR) compared with subjects without DR

† *P*-value (best model): *p*-value of the most significant statistic obtained from six models: genotype, allele, trend, additive, dominant, and recessive mo

Table 3. Genotypic distribution among type 2 diabetics with and without retinopathy and non-diabetic controls, and adjusted odds ratios of the diabetic retinopathy susceptibility SNPs in type 2 diabetic with and without retinopathy

Chr. (nearest gene)	dbSNP ID	Risk allele	Genotype (dominant model)	T2D subjects		Non-diabetic controls n (%)	aOR (95% CI)* (DR vs. without DR)
				with DR n (%)	without DR n (%)		
1p (*MYSM1*)	**rs2811893**	T	CC+CT	88 (50.6)	334 (58.3)	61 (61.0)	1.00 (Ref)
			TT	86 (49.4)	239 (41.7)	39 (39.0)	**1.50 (1.03-2.20)**
1p (*MYSM1*)	**rs12092121**	A	GG+GA	88 (50.6)	334 (58.3)	61 (61.0)	1.00 (Ref)
			AA	86 (49.4)	239 (41.7)	39 (39.0)	**1.50 (1.03-2.20)**
4q (Unknown)	rs4470583	A	GG	145 (83.3)	484 (84.2)	84 (84.0)	1.00 (Ref)
			GA+AA	29 (16.7)	91 (15.8)	16 (16.0)	1.16 (0.70-1.92)
5q (Unknown)	**rs13163610**†	A	CC+CA	5 (2.9)	60 (10.5)	13 (13.0)	1.00 (Ref)
			AA	169 (97.1)	514 (89.5)	87 (87.0)	**3.59 (1.36-9.47)**
5q (Unknown)	**rs17376456**†	A	GG+GA	5 (2.9)	62 (10.8)	13 (13.0)	1.00 (Ref)
			AA	169 (97.1)	513 (89.2)	87 (87.0)	**3.63 (1.38-9.58)**
10p (*PLXDC2*)	**rs1571942**	C	TT	128 (73.6)	481 (83.7)	82 (82.0)	1.00 (Ref)
			CT +CC	46 (26.4)	94 (16.3)	18 (18.0)	**1.67 (1.06-2.65)**
10p (Unknown)	**rs12219125**	T	GG	129 (74.1)	481 (83.7)	82 (82.0)	1.00 (Ref)
			GT+TT	45 (25.9)	94 (16.3)	18 (18.0)	**1.62 (1.02-2.58)**
10q (*ARHGAP22*)	rs4838605‡	C	TT	130 (74.7)	479 (83.7)	73 (73.0)	1.00 (Ref)
			CT+CC	44 (25.3)	93 (16.3)	27 (27.0)	1.58 (1.00-2.52)
10q (*ARHGAP22*)	**rs11101355**‡	T	CC	128 (73.6)	480 (83.8)	74 (74.0)	1.00 (Ref)
			CT+TT	46 (26.4)	93 (16.2)	26 (26.0)	**1.65 (1.05-2.60)**
10q (*ARHGAP22*)	**rs11101357**‡	A	GG	128 (73.6)	480 (83.8)	74 (74.0)	1.00 (Ref)
			AG+AA	46 (26.4)	93 (16.2)	26 (26.0)	**1.65 (1.05-2.60)**
10q (Unknown)	rs4462262	C	TT+TC	14 (8.0)	75 (13.0)	11 (11.0)	1.00 (Ref)
			CC	160 (92.0)	500 (87.0)	89 (89.0)	1.54 (0.79-2.99)
13q (*HS6ST3*)	**rs2038823**†	C	AA+AC	13 (7.5)	76 (13.3)	17 (17.0)	1.00 (Ref)
			CC	161 (92.5)	497 (86.7)	83 (83.0)	**2.33 (1.13-4.77)**

Chr, chromosome; dbSNP ID, SNP database identification; T2D, type 2 diabetes; DR, diabetic retinopathy; aOR, adjusted odds ratio; CI, confidence interval

*Adjusted odds ratio after controlling diabetes duration and HbA_{1C}; †Significant differences between T2D subjects with DR and non-diabetic control; ‡Significant differences between T2D subjects without DR and non-diabetic control

Table 4. Genotypic distribution and adjusted odds ratios of the diabetic retinopathy (DR) susceptibility SNPs in non-proliferative DR and proliferative DR

Chr. (nearest gene)	dbSNP ID	Genotype (dominant model)	T2D subjects			aOR (95% CI)*	
			with NPDR n (%)	with PDR n (%)	without DR n (%)	NPDR vs. without DR	PDR vs. without DR
1p (*MYSM1*)	rs2811893	CC+CT	48 (47.1)	40 (55.6)	334 (58.3)	1.00 (Ref)	1.00 (Ref)
		TT	54 (52.9)	32 (44.4)	239 (41.7)	**1.72 (1.09-2.71)**	1.18 (0.68-2.05)
1p (*MYSM1*)	rs12092121	GG+GA	48 (47.1)	40 (55.6)	334 (58.3)	1.00 (Ref)	1.00 (Ref)
		AA	54 (52.9)	32 (44.4)	239 (41.7)	**1.72 (1.09-2.71)**	1.18 (0.68-2.05)
5q (Unknown)	rs13163610	CC+CA	3 (2.9)	2 (28)	60 (10.5)	1.00 (Ref)	1.00 (Ref)
		AA	99 (97.1)	70 (97.2)	514 (89.5)	**3.48 (1.05-11.6)**	3.52 (0.80-15.6)
5q (Unknown)	rs17376456	GG+GA	3 (2.9)	2 (28)	62 (10.8)	1.00 (Ref)	1.00 (Ref)
		AA	99 (97.1)	70 (97.2)	513 (89.2)	**3.54 (1.07-11.7)**	3.55(0.80-15.7)
10p (*PLXDC2*)	rs1571942	TT	75 (73.5)	53 (73.6)	481 (83.7)	1.00 (Ref)	1.00 (Ref)
		CT +CC	27 (26.5)	19 (26.4)	94 (16.3)	**1.86 (1.09-3.18)**	1.31 (0.67-2.58)
10p (Unknown)	rs12219125	GG	75 (73.5)	54 (75.0)	481 (83.7)	1.00 (Ref)	1.00 (Ref)
		GT+TT	27 (26.5)	18 (25.0)	94 (16.3)	**1.86 (1.09-3.18)**	1.19 (0.60-2.39)
10q (*ARHGAP22*)	rs4838605	TT	81 (79.4)	49 (68.1)	479 (83.7)	1.00 (Ref)	1.00 (Ref)
		CT+CC	21 (20.6)	23 (31.9)	93 (16.3)	1.28 (0.72-2.27)	**2.18 (1.17-4.07)**
10q (*ARHGAP22*)	rs11101355	CC	80 (78.4)	48 (66.7)	480 (83.8)	1.00 (Ref)	1.00 (Ref)
		CT+TT	22 (21.6)	24 (33.3)	93 (16.2)	1.32 (0.75-2.32)	**2.22 (1.20-4.09)**
10q (*ARHGAP22*)	rs11101357	GG	80 (78.4)	48 (66.7)	480 (83.8)	1.00 (Ref)	1.00 (Ref)
		AG+AA	22 (21.6)	24 (33.3)	93 (16.2)	1.32 (0.75-2.32)	**2.22 (1.20-4.09)**
13q (*HS6ST3*)	rs2038823	AA+AC	6 (5.9)	7 (9.7)	76 (13.3)	1.00 (Ref)	1.00 (Ref)
		CC	96 (94.1)	65 (90.3)	497 (86.7)	**3.15 (1.18-8.39)**	1.62 (0.63-4.17)

Chr, chromosome; dbSNP ID, SNP database identification; T2D, type 2 diabetes; DR, diabetic retinopathy; NPDR, non-proliferative DR; PDR, proliferative DR; aOR, adjusted odds ratio; CI, confidence interval; *MYSM1*, Myb-like, SWIRM and MPN domains 1; *PLXDC2*, plexin domain-containing 2; *ARHGAP22*, Rho GTPase-activating protein 22; *HS6ST3*, heparan sulfate 6-*O*-sulfotransferase 3

*Adjusted odds ratio after controlling diabetes duration and HbA_{1C}