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Type 2 Diabetes Genetic Variants and Risk of Diabetic Retinopathy

Yong He Chong, MSc,[1,2] Qiao Fan, PhD,[2] Yih Chung Tham, PhD,[1] Alfred Gan, MSc,[1] Shu Pei Tan, BSc,[1] Gavin Tan, MMed,[1] Jie Jin Wang, PhD,[3] Paul Mitchell, MD, PhD,[3] Tien Yin Wong, FRCS, PhD,[1,2,4,] Ching-Yu Cheng, MD, PhD[1,2,4,*]*

Purpose: Genetic association studies to date have not identified any robust risk loci for diabetic retinopathy (DR). We hypothesized that individuals with more diabetes genetic risk alleles have a higher risk of developing DR.

Design: Case-control genetic association study.

Participants: We evaluated the aggregate effects of multiple type 2 diabetes–associated genetic variants on the risk of DR among 1528 participants with diabetes from the Singapore Epidemiology of Eye Diseases Study, of whom 547 (35.8%) had DR.

Methods: Participants underwent a comprehensive ocular examination, including dilated fundus photography. Retinal photographs were graded using the modified Airlie House classification system to assess the presence and severity of DR following a standardized protocol. We identified 76 previously discovered type 2 diabetes–associated single nucleotide polymorphisms (SNPs) and constructed multilocus genetic risk scores (GRSs) for each individual by summing the number of risk alleles for each SNP weighted by the respective effect estimates on DR. Two GRSs were generated: an overall GRS that included all 76 discovered type 2 diabetes–associated SNPs, and an Asian-specific GRS that included a subset of 55 SNPs previously found to be associated with type 2 diabetes in East and/or South Asian ancestry populations. Associations between the GRSs with DR were determined using logistic regression analyses. Discriminating ability of the GRSs was determined by the area under the receiver operating characteristic curve (AUC).

Main Outcome Measures: Odds ratios on DR.

Results: Participants in the top tertile of the overall GRS were 2.56-fold more likely to have DR compared with participants in the lowest tertile. Participants in the top tertile of the Asian-specific GRS were 2.00-fold more likely to have DR compared with participants in the bottom tertile. Both GRSs were associated with higher DR severity levels. However, addition of the GRSs to traditional risk factors improved the AUC only modestly by 3% to 4%.

Conclusions: Type 2 diabetes–associated genetic loci were significantly associated with higher risks of DR, independent of traditional risk factors. Our findings may provide new insights to further our understanding of the genetic pathogenesis of DR. *Ophthalmology 2016;■:1–7 © 2016 by the American Academy of Ophthalmology*

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Diabetic retinopathy (DR) is the most common microvascular complication of diabetes mellitus[1] and is a leading cause of preventable blindness in working-aged adults worldwide.[2] The global prevalence of DR, proliferative DR (PDR), and vision-threatening DR among individuals with diabetes is estimated to be 35%, 7%, and 12%, respectively.[3] DR has also been estimated to be the cause of 2.6% of cases of blindness worldwide.[4]

Our understanding of the pathophysiology of DR is incomplete and constantly evolving with research. Risk factors such as hyperglycemia, hypertension, and prolonged diabetes duration are well established but explain less than 50% of the risk of DR.[3,5–9] Genetic susceptibility to DR has also been suspected.[10] Previous studies have shown racial and ethnic differences[11] and familial aggregation in DR,[12,13] suggesting a role for genetic factors in DR development. Several candidate gene studies and genome-wide association studies (GWAS) have also identified potential genetic loci associated with DR.[14–22] However, few results have been consistently replicated across different populations.

Because DR is the most common microvascular complication of type 2 diabetes,[2] it is reasonable to postulate that both diseases share a common genetic background. Type 2 diabetes is a multifactorial disease influenced by many different genetic variants with heritability estimated to be between 40% and 80%.[23] Over the past decade, GWAS have identified over 70 susceptibility loci for type 2 diabetes.[24–29] Of note, 3 of the type 2 diabetes risk loci (near *TCF7L2*, *PPARG*, and *KCNJ11*) have previously been shown to be associated with DR,[30–32] further suggesting that type 2 diabetes susceptibility genes may have an influence on DR development.

In this study, we hypothesized that individuals with more risk alleles of type 2 diabetes are more likely to have DR. We aimed to evaluate the association between the aggregate

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effects of multiple type 2 diabetes genetic variants with DR, through the construct of multilocus genetic risk scores (GRSs), in a population-based, multiethnic study.

Methods

Study Population

The Singapore Epidemiology of Eye Diseases (SEED) Study is a population-based cross-sectional study of 3 major ethnic groups in Singapore: Malays (2004−2006), Indians (2007−2009), and Chinese (2009−2011). The detailed study methodology has been previously described.[33,34] In brief, 4168 Malays, 4497 Indians, and 4605 Chinese aged 40 to 80 years were selected using an age-stratified random sampling strategy and invited to participate in the study. From these, a total of 10 033 participated, including 3280 Malays (response rate of 78.7%), 3400 Indians (75.6%), and 3353 Chinese (72.8%), giving an overall response rate of 75.6%. For this analysis, we focused on all participants with diabetes, defined as persons having a random glucose of 11.1 mmol/L or higher, using diabetic medication, or having a self-reported history of diabetes. Ethics approval was obtained from the SingHealth Centralised Institutional Review Board. Written informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki.

Diabetic Retinopathy and Risk Factor Assessments

DR was assessed through standardized retinal photographs using a digital retinal camera (Canon CR-DGi with a 10-D SLR back; Canon, Tokyo, Japan) at the Singapore Eye Research Institute. After pupil dilation, 2 retinal photographs, centered at the optic disc and macula, were taken from both eyes. Photographs were sent to the University of Sydney and graded for retinopathy by masked, trained graders. DR was considered present if any characteristic lesion as defined by the Early Treatment Diabetic Retinopathy Study severity scale (i.e., microaneurysms, hemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading, and new vessels) was present in either eye.[11] DR severity was based on the worse eye and was graded according to the modified Airlie House classification system[35] as follows: level 10, DR absent; levels 14−15, questionable DR; level 20, minimal non-PDR (NPDR); level 35, mild NPDR; level 43, moderate NPDR; level 47, moderately severe NPDR; level 53, severe NPDR; level 61, mild PDR; level 65, moderate PDR; level 71, severe PDR; and levels 81 and 85, advanced PDR.

All participants underwent a standardized interview for collection of demographic data, lifestyle risk factors, and medical history (e.g., diabetes duration). Blood pressure was measured according to the protocol used in the Multi-Ethnic Study of Atherosclerosis[11] and was taken with the study participants seated and after 5 minutes of rest. Systolic and diastolic blood pressure were measured with a digital automatic blood pressure monitor. Blood pressure was measured on 2 occasions 5 minutes apart. If the blood pressures differed by more than 10 mmHg systolic and 5 mmHg diastolic, a third measurement was made. The blood pressure of the individual was then taken as the mean between the 2 closest readings. Hypertension was defined as systolic blood pressure of 140 mmHg or more, diastolic blood pressure of 90 mmHg or more, or use of antihypertensive medication. Nonfasting venous blood samples were collected to measure serum glycosylated hemoglobin (HbA1c) levels and for DNA extraction.

Genotyping and Genetic Ancestry Inference

Genome-wide genotyping was performed using Illumina Human 610 Quad BeadChips (Illumina Inc, San Diego, CA) based on the manufacturer's protocols in 7584 of the SEED participants. The detailed data quality control procedure has been previously described.[36] Genotype imputation was carried out using the Markov Chain Haplotyping software package,[37] using 1000 Genomes Project as reference panels.

Individual genetic ancestry was inferred using principal component (PC) analysis to account for spurious associations owing to ancestral differences of individual single nucleotide polymorphisms (SNPs). This was carried out using the smartPCA program (EIGENSTRAT software version 4.2).[38] The details of the PC analysis have been previously described.[39]

Statistical Analysis

A 2-stage approach was adopted for analysis. First, we adopted a candidate gene approach by identifying and selecting 76 type 2 diabetes−associated SNPs identified in the most recent and largest-to-date meta-analysis of type 2 diabetes GWAS by the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium.[24] To determine the association between DR and each type 2 diabetes susceptibility locus in our study population, we performed logistic regression analyses between the individual SNPs with DR under additive genetic models adjusting for age, gender, and the first 3 PCs in each ethnic group. We then obtained the combined effect estimates of individual SNPs by performing random-effect meta-analysis using individual-level data across the 3 ethnic groups. Next, we evaluated the aggregate effects of the type 2 diabetes susceptibility loci by constructing 2 GRSs: (1) an overall GRS that included all 76 SNPs identified by the DIAGRAM Consortium, and (2) an Asian-specific GRS that included a subset of 55 SNPs showing nominally significant association ($P < 0.05$) in East and/or South Asian ancestry groups from the DIAGRAM Consortium's aggregated meta-analysis. This was achieved by summing the number of risk alleles for each of the type 2 diabetes−associated SNPs for each GRS, weighted by the estimated individual SNP effect size on DR (logarithm of the odds ratio).[40] Multivariable logistic and ordinal logistic regression analyses were performed to determine the association between GRSs with DR and DR severity levels, respectively, adjusting for diabetes duration, HbA1c, and hypertension (collectively termed "traditional DR risk factors"). In the ordinal logistic regression analyses, the dependent outcome variable DR severity level was coded accordingly on an ordinal scale as 0 (DR absent), 1 (questionable DR), 2 (minimal NPDR), 3 (mild NPDR), 4 (moderate NPDR), 5 (moderately severe NPDR), 6 (severe NPDR), 7 (mild PDR), 8 (moderate PDR), 9 (severe PDR), or 10 (advanced PDR).

To evaluate the discriminating ability of the GRSs for DR compared with traditional risk factors, we calculated the area under the receiver operating characteristic curve (AUC) for 2 different models: the first model consisted of traditional DR risk factors and the second model consisted of both the traditional risk factors plus the GRSs. The difference in AUCs between models was compared using C-statistic. All statistical analyses were performed using Stata 14 (StataCorp LP, College Station, TX).

Results

Of the 7584 study participants in the SEED study with genome-wide genotype information, after excluding participants without diabetes (n = 5805) and participants with diabetes with missing