CPVL/CHN2 Genetic Variant Is Associated With Diabetic Retinopathy in Chinese Type 2 Diabetic Patients

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OBJECTIVE—Diabetic nephropathy and retinopathy are two important microvascular diabetes complications with a high concordance rate in diabetic patients. A recent genome-wide association study in type 1 diabetic patients of European descent identified four loci to be associated with diabetic nephropathy. The aim of this study was to test the effects of single nucleotide polymorphisms (SNPs) from these four loci on diabetic nephropathy and retinopathy in Chinese type 2 diabetic patients.

RESEARCH DESIGN AND METHODS—In stage 1, we recruited 1,276 type 2 diabetic patients, including 378 patients with diabetic nephropathy but no retinopathy, 374 patients with diabetic retinopathy but no nephropathy, 244 patients with both diabetic retinopathy and nephropathy, and 280 control subjects with diabetes for >10 years and no diabetic retinopathy or nephropathy. Fifty-five SNPs from four loci (*CPVL/CHN2*, *FRMD3*, *CARS*, and *IRS2*) were genotyped. The SNPs that showed associations to diabetic retinopathy or nephropathy were genotyped in stage 2 samples for replication.

RESULTS—SNPs from *CPVL/CHN2* and *FRMD3* were associated with diabetic retinopathy with rs39059 and rs10868025 as the top SNPs (odds ratio [OR] 1.292, 95% CI 1.097–1.523, $P = 0.0022$, for rs39059; 1.201, 1.014–1.422, $P = 0.0343$, for rs10868025) in stage 1 samples. In stage 2 analysis, only rs39059 showed similar effect to diabetic retinopathy (OR 1.269, 0.989–1.628, $P = 0.0689$), and meta-analysis showed a significant association between rs39059 and diabetic retinopathy, with an OR of 1.285 (1.120–1.474, $P = 0.0003$). *CPVL/CHN2* rs39059 was also associated with levels of diabetic retinopathy ($P = 0.0007$ for trend). However, no association was detected between these SNPs and diabetic nephropathy.

CONCLUSIONS—In this study, we found *CPVL/CHN2* rs39059 was associated with diabetic retinopathy in the Chinese type 2 diabetic patients. ***Diabetes*** **60:3085–3089, 2011**

Diabetic nephropathy and retinopathy, two important microvascular complications of diabetes, are the main causes of morbidity and mortality among diabetic patients (1). Diabetic nephropathy is the most common cause of chronic kidney failure and end-stage renal disease, whereas diabetic retinopathy is the leading cause of blindness in the adults (2,3). With the significant rise in the prevalence of diabetes, the increase of patients suffering from diabetic microvascular complications will be inevitable worldwide. Although diabetic nephropathy and retinopathy are clearly associated with the duration of diabetes and glycemic control (4), some patients develop severe complications despite well-controlled blood glucose. Conversely, not all diabetic patients with poor glycemic control develop advanced renal or retinal complications. The underlying mechanism of how these diabetic microvascular complications occur remains largely unknown, but family studies in Pima Indian and European descent populations suggest genetic factors participate in the development of these complications (5–7). However, the advance of susceptible gene identification in diabetic microvascular complications was much more limited than it was in type 1 and type 2 diabetes. Recently, a genome-wide association study using Genetics of Kidneys in Diabetes (GoKinD) samples identified four loci associated with diabetic nephropathy in the type 1 diabetic patients of European descent (8). Maeda et al. (9) further replicated the effect of one of them (rs1411766 near *IRS2*) in the Japanese type 2 diabetic patients. However, although diabetic retinopathy and nephropathy are two diseases with a high concordance rate in diabetic patients and might share common pathogenesis, no study reported if these single nucleotide polymorphisms (SNPs) had effects on diabetic retinopathy after stratification of the status of diabetic nephropathy. In this study, we aimed to test the effects of SNPs from these four loci on the diabetic nephropathy and retinopathy in Chinese type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

We used a two-stage approach for this study. In stage 1, we recruited 1,276 type 2 diabetic patients from the Shanghai Diabetes Institute Inpatient Database of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (10,11) and examined them for diabetic retinopathy and nephropathy. These patients included 378 individuals with diabetic nephropathy but no retinopathy, 374 with diabetic retinopathy but no nephropathy, 244 with both diabetic retinopathy and nephropathy, and 280 control subjects. The control subjects were defined as having normoalbuminuria, no retinopathy, and having diabetes for >10 years. In stage 2, we recruited 590 type 2 diabetic patients from the Shanghai Diabetic Complications Study (12) and Shanghai Diabetes Institute Inpatient Database, including 209 patients with diabetic retinopathy and 381 patients with diabetes for >5 years and without retinopathy. The basic characteristics of the study population were shown in Table 1.

This study was approved by the institutional review board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital and in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from each participant.

Nephropathy measurement. The 24-h albumin excretion rates (AERs) were measured in 3 consecutive days, and the mean value was recorded for each patient. Patients with AER <30 mg/24 h, 30 mg/24 h ≤ AER <300 mg/24 h, or AER ≥300 mg/24 h were classified as having normoalbuminuria, microalbuminuria, or proteinuria, respectively. Estimated glomerular filtration rate was calculated by using a formula developed by the Modification of Diet in Renal Disease study group with adjustment for Chinese ethnicity: $186 \times (\text{serum creatinine in } \mu\text{mol/L} \times 0.011)^{-1.154} \times (\text{age in years})^{-0.203} \times (0.742 \text{ if female}) \times (1.233 \text{ if Chinese})$ (13).

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TABLE 1
Clinical characteristic of the participants

	Stage 1				Stage 2	
	Control subjects	Diabetic nephropathy only	Diabetic retinopathy only	Diabetic nephropathy and retinopathy	Control subjects	Diabetic retinopathy
Male/female	102/178	213/165	157/217	135/109	170/211	99/110
Age (years)	67.04 ± 9.49	61.21 ± 13.74	61.24 ± 10.74	64.36 ± 10.56	64.60 ± 10.35	62.06 ± 11.81
BMI (kg/m^2)	23.85 ± 3.16	25.28 ± 3.77	23.97 ± 3.45	24.43 ± 3.95	24.72 ± 3.49	25.03 ± 3.39
Age at diagnosis of diabetes (years)	52.88 ± 9.77	54.79 ± 12.67	51.76 ± 10.17	51.70 ± 11.38	53.97 ± 10.50	51.57 ± 11.93
Duration of diabetes (years)	12.00 (10.00–16.00)	6.00 (0.80–10.00)	10.00 (5.00–14.00)	12.00 (8.00–18.00)	9.00 (6.90–13.00)	10.00 (5.00–15.00)
Hemoglobin A_{1c} (%)	8.47 ± 1.99	9.26 ± 2.34	8.98 ± 2.11	9.49 ± 2.21	7.88 ± 1.72	8.96 ± 2.53
Systolic blood pressure (mmHg)	133.90 ± 17.04	137.62 ± 18.58	135.27 ± 17.92	143.79 ± 19.73	133.42 ± 16.69	134.25 ± 20.98
Diastolic blood pressure (mmHg)	78.45 ± 8.68	82.23 ± 10.07	80.20 ± 9.37	82.98 ± 9.62	81.12 ± 9.22	80.66 ± 11.56
AERs (mg/24 h)	8.81 (6.00–13.44)	79.90 (44.03–203.60)	10.85 (7.17–16.28)	163.62 (54.31–607.54)	10.16 (5.87–29.77)	11.67 (6.18–43.53)
eGFR*	116.82 (102.05–137.85)	112.52 (87.24–138.57)	124.08 (105.21–149.32)	104.72 (76.85–134.74)	124.52 (106.74–145.06)	122.41 (97.73–146.77)

Data are *n*, means ± SD, or medians (interquartile range). eGFR, estimated glomerular filtration rate. *eGFR was calculated by using the formula developed by the Modification of Diet in Renal Disease study group with adjustment for the Chinese ethnicity.

Retinal assessment. Fundus photography was performed following a standardized protocol at the Department of Ophthalmology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Both eyes of each participant were photographed with a 45-degree 6.3-megapixel digital nonmydriatic camera (Canon CR6-45NM, Lake Success, NY). Level of retinopathy was defined according to the International Classification of Diabetic Retinopathy (14): mild nonproliferative diabetic retinopathy, moderate nonproliferative diabetic retinopathy, severe nonproliferative diabetic retinopathy, or proliferative diabetic retinopathy. The worse eye was recorded for each patient.

SNP selection and genotyping. In stage 1, we genotyped 55 tagging SNPs that capture 95% of the common variants, including one reported SNP from each locus (*CPVL/CHN2* rs39059, *FRMD3* rs10868025, *CARS* rs451041, and *IRS2* rs1411766). The SNPs showed associations to diabetic nephropathy or retinopathy and were genotyped in stage 2 samples. The genotyping was performed by using primer extension of multiplex products with detection by matrix-assisted laser desorption ionization – time of flight mass spectroscopy using a MassARRAY Compact Analyzer (Sequenom, San Diego, CA).

Statistical analysis. The Hardy-Weinberg equilibrium test was performed before the association analysis. The allelic frequencies between the diabetic patients with or without complications were compared by χ^2 tests using PLINK (v1.07) (15), and odd ratios (ORs) with 95% CIs were presented. Combined ORs from different studies were calculated by using a Comprehensive Meta Analysis (v2.2.057) with a fixed- or random-effect model after testing for heterogeneity. The test for homogeneity was assessed by the Cochran *Q* test. The genotype-disease association analyses were performed under the additive model by logistic regression with adjustment of confounding factors. The effects of SNPs on the levels of retinopathy severity were analyzed by trend analysis. Skewly distributed quantitative traits (estimated glomerular filtration rate and AER) were logarithmically transformed to approximate univariate normality before analysis. Quantitative traits were analyzed by linear regression with adjustment of confounding factors under an additive genetic model. The statistical analyses were performed using SAS for Windows (version 8.0; SAS Institute, Cary, NC) unless specified otherwise. A two-tailed *P* value of <0.05 was considered statistically significant.

On the basis of the previously reported effect size of these loci (~1.40) (8), our stage 1 samples (~600 case subjects vs. 600 control subjects) had >90% power to replicate the reported effects of SNPs with minor allele frequencies >0.2 and 75% power to replicate the effect SNP with minor allele frequency of 0.1 at a level of significance of 0.05.

RESULTS

We firstly analyzed the effects of these SNPs on diabetic retinopathy and nephropathy in stage 1 samples (Supplementary Tables 1 and 2). As shown in Table 2, four *CPVL/CHN2* SNPs (rs39059, rs17756941, rs245955, and rs245962) and *FRDM3* rs10868025 were nominally associated with diabetic retinopathy ($P < 0.05$), with rs39059 and rs10868025 showing the strongest association within each locus (OR 0.774, 95% CI 0.657–0.912, $P = 0.002$, for rs39059 G allele; 0.833, 0.703–0.987, $P = 0.034$, for rs10868025 G allele). However, none of the genotyped SNPs showed a significant association to diabetic nephropathy.

To further validate the effects of rs39059 and rs10868025 on diabetic retinopathy, we genotyped both SNPs in stage 2 samples. We found only *CPVL/CHN2* rs39059 showed similar effects to diabetic retinopathy, as identified in the first stage (OR 1.269, 95% CI 0.989–1.628, $P = 0.061$, for rs39059; 1.014, 0.789–1.302, $P = 0.9147$, for rs10868025). We then performed a meta-analysis with the fixed-effect model and found rs39059 was associated with diabetic retinopathy, with an OR of 1.285 (1.120–1.474, $P = 0.0003$) (Table 3). This association remained significant after adjusting confounding factors, including hemoglobin A_{1c} levels, duration of diabetes, systolic and diastolic blood pressure, and BMI (OR 1.242, 1.074–1.437, $P = 0.0034$).

We then analyzed the effects of *CPVL/CHN2* rs39059 on the disease severity of diabetic retinopathy in all the samples. As shown in Fig. 1, *CPVL/CHN2* rs39059 showed an association to the levels of diabetic retinopathy, with the risk allele more frequent in the more severe retinopathy patients ($P = 0.0007$ for trend analysis).

TABLE 2
Effects of the SNPs on diabetic retinopathy and nephropathy in stage 1 samples

Chromosome	SNP	Position	Minor/major allele	Diabetic retinopathy		Diabetic nephropathy	
				OR (95% CI)	*P*	OR (95% CI)	*P*
7	rs3812398	29237994	C,T	0.970 (0.816–1.153)	0.728	1.087 (0.915–1.292)	0.341
7	rs3812389	29244759	A,G	0.839 (0.679–1.036)	0.103	0.960 (0.778–1.185)	0.704
7	rs2269903	29247409	C,A	1.202 (0.976–1.481)	0.084	1.026 (0.832–1.264)	0.813
7	rs3812388	29249198	G,C	1.190 (0.967–1.464)	0.100	1.055 (0.858–1.298)	0.611
7	rs17756941	29250007	G,A	0.747 (0.598–0.933)	**0.010**	0.941 (0.755–1.174)	0.593
7	rs11981737	29250335	A,G	1.064 (0.736–1.536)	0.743	0.834 (0.578–1.204)	0.334
7	rs39059	29255470	G,A	0.774 (0.657–0.912)	**0.002**	0.959 (0.814–1.131)	0.621
7	rs39065	29262601	A,G	0.973 (0.711–1.331)	0.862	1.180 (0.862–1.614)	0.300
7	rs17157658	29274254	C,G	0.858 (0.719–1.024)	0.090	1.051 (0.881–1.254)	0.577
7	rs245955	29276307	C,T	0.784 (0.668–0.921)	**0.003**	0.989 (0.843–1.161)	0.896
7	rs245962	29290153	A,G	0.800 (0.681–0.939)	**0.006**	0.989 (0.842–1.161)	0.893
7	rs39099	29293095	A,G	0.910 (0.762–1.085)	0.293	1.042 (0.874–1.243)	0.646
7	rs39101	29294462	A,G	1.049 (0.846–1.301)	0.664	0.884 (0.712–1.096)	0.261
9	rs11140139	86145409	A,G	1.018 (0.848–1.221)	0.854	1.010 (0.841–1.211)	0.918
9	rs4877788	86146950	C,T	1.029 (0.866–1.221)	0.750	1.044 (0.880–1.240)	0.620
9	rs7849075	86149610	C,T	0.972 (0.787–1.199)	0.787	0.971 (0.787–1.198)	0.783
9	rs1888746	86155392	T,C	0.815 (0.538–1.234)	0.334	0.928 (0.602–1.380)	0.661
9	rs11140156	86163694	G,C	0.922 (0.717–1.186)	0.528	0.919 (0.714–1.183)	0.511
9	rs10868025	86164176	G,A	0.833 (0.703–0.987)	**0.034**	0.905 (0.764–1.072)	0.249
9	rs11535575	86165034	T,C	0.671 (0.439–1.025)	0.065	0.762 (0.501–1.160)	0.205
9	rs6559732	86168692	C,T	1.152 (0.948–1.399)	0.154	1.080 (0.889–1.311)	0.440
9	rs7470287	86172665	C,G	0.840 (0.687–1.027)	0.090	0.963 (0.788–1.177)	0.712
9	rs3934902	86177401	A,G	1.103 (0.938–1.298)	0.237	1.018 (0.865–1.197)	0.835
9	rs4451390	86179563	T,C	1.000 (0.848–1.180)	0.998	1.042 (0.883–1.229)	0.628
9	rs11793821	86184504	G,A	1.116 (0.861–1.447)	0.407	0.915 (0.706–1.187)	0.503
11	rs3764895	2945945	T,C	0.994 (0.802–1.231)	0.954	0.897 (0.724–1.112)	0.321
11	rs2583442	2956166	A,G	1.021 (0.860–1.211)	0.812	0.993 (0.837–1.179)	0.940
11	rs4758576	2973880	G,A	0.956 (0.726–1.259)	0.751	0.912 (0.692–1.201)	0.512
11	rs11024758	2981782	T,C	1.180 (0.640–2.174)	0.596	1.590 (0.853–2.962)	0.144
11	rs4758504	3000179	A,G	0.963 (0.724–1.279)	0.794	0.901 (0.678–1.198)	0.473
11	rs4758621	3009640	A,G	1.008 (0.851–1.194)	0.925	0.990 (0.836–1.173)	0.911
11	rs12363575	3030104	G,A	1.007 (0.783–1.294)	0.958	1.104 (0.859–1.420)	0.438
11	rs12421922	3035070	T,C	1.024 (0.868–1.209)	0.778	0.996 (0.844–1.175)	0.961
11	rs2071101	3050137	A,G	1.042 (0.885–1.225)	0.624	0.939 (0.798–1.105)	0.451
11	rs572373	3055361	C,T	1.061 (0.878–1.281)	0.541	1.032 (0.854–1.247)	0.745
11	rs451041	3060725	A,G	1.046 (0.881–1.243)	0.605	1.010 (0.851–1.200)	0.906
11	rs7111857	3068106	A,G	1.069 (0.876–1.306)	0.511	1.044 (0.855–1.274)	0.676
11	rs6578318	3072442	T,C	1.009 (0.792–1.284)	0.945	0.981 (0.770–1.248)	0.873
11	rs2290000	3073838	T,A	1.019 (0.801–1.296)	0.878	0.969 (0.762–1.233)	0.799
11	rs406598	3076285	C,T	1.016 (0.859–1.203)	0.850	1.002 (0.847–1.185)	0.980
11	rs10833173	3094505	A,T	1.033 (0.837–1.275)	0.760	0.950 (0.770–1.173)	0.635
11	rs2084239	3106659	G,A	0.983 (0.831–1.163)	0.845	0.958 (0.810–1.133)	0.617
13	rs914270	110243017	C,G	0.995 (0.847–1.169)	0.954	0.955 (0.812–1.122)	0.573
13	rs2391776	110243425	C,T	0.937 (0.735–1.194)	0.599	1.006 (0.790–1.281)	0.963
13	rs1041466	110244322	C,T	1.264 (0.945–1.691)	0.115	0.782 (0.584–1.048)	0.100
13	rs11069790	110244401	A,G	0.937 (0.784–1.121)	0.477	1.102 (0.921–1.317)	0.288
13	rs4462453	110251328	G,A	1.246 (0.902–1.719)	0.182	0.869 (0.629–1.199)	0.392
13	rs1411766	110252160	T,C	1.094 (0.828–1.445)	0.530	0.991 (0.750–1.310)	0.951
13	rs12184748	110253930	C,T	1.001 (0.845–1.186)	0.990	1.028 (0.868–1.217)	0.754
13	rs2150481	110256550	G,C	1.079 (0.917–1.269)	0.362	1.030 (0.876–1.212)	0.717
13	rs2391778	110258553	T,A	1.048 (0.888–1.236)	0.582	1.037 (0.879–1.223)	0.666
13	rs1547241	110273605	G,C	1.108 (0.824–1.490)	0.497	0.858 (0.637–1.155)	0.311
13	rs10161791	110281789	G,A	0.938 (0.786–1.121)	0.483	1.106 (0.926–1.321)	0.264
13	rs9587939	110284951	A,C	0.965 (0.810–1.150)	0.693	1.055 (0.885–1.257)	0.549
13	rs4773068	110288967	A,G	0.924 (0.771–1.107)	0.390	1.160 (0.968–1.390)	0.108

P values <0.05 are shown in boldface. ORs with 95% CIs were calculated for the minor allele.

DISCUSSION

In the current study, we analyzed the effects of SNPs from four loci on diabetic retinopathy and nephropathy in Chinese type 2 diabetic patients. We first reported that *CPVL/CHN2* rs39059 was associated with diabetic retinopathy. Although the association we observed was solid by replication in independent samples and the *P* value remained significant after Bonferroni correction of analysis on multiple SNPs (corrected $P = 0.033$, on the basis of the association analysis between 55 SNPs and two traits), we still cannot

TABLE 3
Association of rs39059 in *CPVL/CHN2* with diabetic retinopathy in Chinese type 2 diabetic patients

		Risk allele frequencies			
	n (case/control)	Case subjects	Control subjects	OR (95% CI)*	*P*
Diabetic retinopathy vs. control subjects	365/273	0.619	0.564	1.256 (1.003–1.575)	0.047
Diabetic nephropathy and retinopathy vs. diabetic nephropathy	231/363	0.636	0.567	1.334 (1.049–1.695)	0.018
Stage 2 validation	209/381	0.660	0.605	1.269 (0.989–1.628)	0.061
Meta-analysis	805/1,017	0.633	0.581	1.285 (1.120–1.474)	0.0003†

*ORs with 95% CIs were shown for risk allele. †*P* values of meta-analysis were calculated using the fixed-effect model; homogeneity test $P = 0.932$.

fully exclude the possibility that the association detected was false positive because of the relatively small sample size of this study. But considering we also found an association between rs39059 and disease severity in our samples, which supported the role of this locus in diabetic retinopathy from another aspect, the chance of a false-positive finding was limited. Although this locus was originally identified to be associated with diabetic nephropathy in a previous genome-wide association study (since the concordance rate of retinopathy and nephropathy was high in diabetic patients [16] and the genome-wide association study in the GoKinD samples (8) did not exclude the patients with retinopathy), *CPVL/CHN2* may be a susceptible locus of diabetic retinopathy other than nephropathy. However, whether this effect is restricted to type 2 diabetes is still unknown and needs to be investigated in studies with type 1 diabetic patients.

The SNP rs39059 locates in the intron of *CHN2*, which encodes β-2 chimerin that have been shown to regulate cell growth, proliferation, and migration (17). Previous studies showed decreased expression of *CHN2* is associated with high-grade malignant gliomas, breast cancer, and duodenal adenocarcinoma, whereas increased expression of *CHN2* is reported to be associated with lymphomas (17,18). One other gene within the same haplotype block of rs39059 is *CPVL*. It encodes a carboxypeptidase that cleaves a single amino acid from the COOH termini of peptides (19). However, the function of this gene is still largely unknown. Although hemoglobin A_{1c} levels and systolic blood pressure were also associated with diabetic retinopathy in our samples ($P = 0.0065$ for systolic blood pressure and $P = 7.0 \times 10^{-9}$ for hemoglobin A_{1c}), since rs39059 showed association to none of these traits ($P = 0.5031$ for systolic blood pressure and $P = 0.1561$ for hemoglobin A_{1c}), our data suggest this locus did not participate in the pathogenesis of diabetic retinopathy through the effects on blood pressure and glucose levels. Thus, the mechanism how this locus affected diabetic retinopathy susceptibility remains to be investigated. And the causal variant also remains to be identified by fine mapping studies.

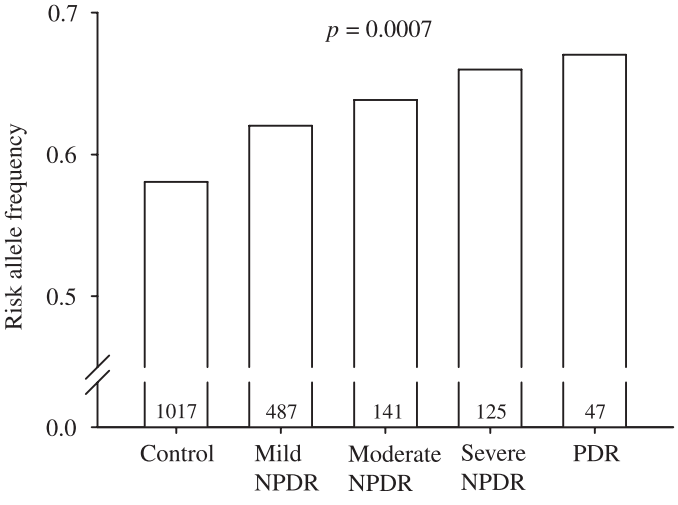

FIG. 1. The effects of *CPVL/CHN2* rs39059 on the disease severity of diabetic retinopathy. Numbers within the bar represent the number of participants of each group. NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

In this study, we failed to replicate the associations between these four loci and diabetic nephropathy in Chinese type 2 diabetic patients, although we had enough statistical power. Because we used a tagging SNP approach and captured most of the common variants within these loci, it was unlikely that causal variants were captured by different SNPs in different populations. One possible explanation may be the complicated phenotypes in type 2 diabetic patients. In our study, >60% of the diabetic nephropathy patients also suffered from hypertension; although we statistically adjusted blood pressure as a confounding factor, the impact of hypertension cannot be ignored. In a previous replication study in Japanese type 2 diabetic patients, Maeda et al. (9) also failed to replicate the effects of most of these loci in four independent samples; thus, it is highly possible that the effects of these loci on diabetic nephropathy are restricted in the European descent population or type 1 diabetic patients. Further studies with type 1 diabetic patients are needed to confirm the effects of these loci.

In summary, we found *CPVL/CHN2* rs39059 was associated with diabetic retinopathy in Chinese type 2 diabetic patients. Further studies are needed to replicate this finding.

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C.H. researched data, contributed to discussion, and wrote the manuscript. R.Z. and W.Y. researched data. J.W. contributed to discussion and wrote the manuscript. C.W. researched

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