

# Association of Combined *TCF7L2* and *KCNQ1* Gene Polymorphisms with Diabetic Micro- and Macrovascular Complications in Type 2 Diabetes Mellitus

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**Background:** Vascular complications are the major morbid consequences of type 2 diabetes mellitus (T2DM). The transcription factor 7-like 2 (*TCF7L2*), potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*), and inwardly-rectifying potassium channel, subfamily J, member 11 gene (*KCNJ11*) are common T2DM susceptibility genes in various populations. However, the associations between polymorphisms in these genes and diabetic complications are controversial. This study aimed to investigate the effects of combined gene-polymorphisms within *TCF7L2*, *KCNQ1*, and *KCNJ11* on vascular complications in Thai subjects with T2DM.

**Methods:** We conducted a case-control study comprising 960 T2DM patients and 740 non-diabetes controls. Single nucleotide polymorphisms in *TCF7L2*, *KCNQ1*, and *KCNJ11* were genotyped and evaluated for their association with diabetic vascular complications.

**Results:** The gene variants *TCF7L2* rs290487-T, *KCNQ1* rs2237892-C, and *KCNQ1* rs2237897-C were associated with increased risk of T2DM. *TCF7L2* rs7903146-C, *TCF7L2* rs290487-C, *KCNQ1* rs2237892-T, and *KCNQ1* rs2237897-T revealed an association with hypertension. The specific combination of risk-alleles that have effects on T2DM and hypertension, *TCF7L2* rs7903146-C, *KCNQ1* rs2237892-C, and *KCNQ1* rs2237897-T, as genetic risk score (GRS), pronounced significant association with coronary artery disease (CAD), cumulative nephropathy and CAD, and cumulative microvascular and macrovascular complications (respective odds ratios [ORs] with 95% confidence interval [95% CI], comparing between GRS 2–3 and GRS 5–6, were 7.31 [2.03 to 26.35], 3.92 [1.75 to 8.76], and 2.33 [1.13 to 4.79]).


**Conclusion:** This study demonstrated, for the first time, the effect conferred by specific combined genetic variants in *TCF7L2* and *KCNQ1* on diabetic vascular complications, predominantly with nephropathy and CAD. Such a specific pattern of gene variant combination may implicate in the progression of T2DM and life-threatening vascular complications.

**Keywords:** Diabetes mellitus, type 2; Diabetic angiopathies; Hypertension; *KCNQ1* potassium channel; Polymorphism, genetic; Transcription factor 7-like 2 protein

## INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a leading cause of morbid-

ity and mortality, arising substantial public health burden around the world. The predominant clinical outcomes of chronic T2DM are vascular complications that contribute to

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serious health problems, reducing the quality of life, and have a significant impact on healthcare budgets [1]. Diabetic vascular complications, partly arising from chronic hyperglycemia, can affect both microvascular and macrovascular system. Microvascular complications include retinopathy (leading to blindness and visual disability), nephropathy (leading to renal failure), and neuropathy (leading to incapability and amputation). Macrovascular complications comprise of coronary artery disease (CAD), peripheral arterial disease, and stroke [2]. Hypertension is the most common comorbidity in patients with T2DM, which is likely to aggravate the progression of both micro- and macrovascular complications [3]. Furthermore, several studies have revealed the common metabolic pathway shared by diabetes and hypertension during their development and progression, suggesting the involvement of common genetic and environmental factors in their etiology [4]. The global prevalence of diabetic vascular complications, in a recent observational study in 38 countries worldwide, was 18.8% and 12.7% for microvascular and macrovascular complications, respectively [5]. The prevalence of diabetic complications in Thai was 37% for diabetic nephropathy, 31.2% for diabetic retinopathy, 28.9% for cardiovascular disease (CVD), and 10.6% for the cerebrovascular disease [6].

The genetic susceptibility to T2DM has been widely investigated. Several single nucleotide polymorphisms (SNPs) in the transcription factor 7-like 2 gene (*TCF7L2*), the potassium voltage-gated channel subfamily Q member 1 gene (*KCNQ1*), and the inwardly-rectifying potassium channel, subfamily J, member 11 gene (*KCNJ11*) have reported to be associated with the development of T2DM and its vascular complications [7-18], with variable results among the different ethnic population. *TCF7L2* is the transcriptional effector in the canonical Wnt-signaling pathway involving in the regulation of incretin hormone production, pancreatic  $\beta$ -cell development, vascular development, and may involve in insulin signaling [19,20]. A common T2DM-associated variant, *TCF7L2* rs7903146, has also been associated with hypertension [21], and the development of diabetic retinopathy [7,8], nephropathy [9,10], and CAD [8]. *KCNQ1* gene encodes a pore-forming subunit of voltage-gated potassium (K<sup>+</sup>) channel, also known as Kv7.1, which plays a physiological role in several tissues including the cardiovascular system, the pancreas, and the kidney [22]. Genetic variations within *KCNQ1* are strongly associated with T2DM, insulin secretion, and impaired fasting blood glucose [23,24]. *KCNQ1* SNPs have also been associated with hyper-

tension [13], as well as an increased risk of diabetic nephropathy [12] and macrovascular disease [13]. The *KCNJ11* encodes a protein Kir6.2 that is a pore-forming subunit of ATP-sensitive potassium channel (KATP). KATP channel is highly expressed in the pancreas and plays an important role in the regulation of insulin secretion in pancreatic  $\beta$ -cells [25,26]. Polymorphisms in *KCNJ11* have been initially reported to be associated with T2DM [27] and subsequently with hypertension [17], CAD [16], and diabetic retinopathy [18].

Although the association of polymorphisms in *TCF7L2*, *KCNQ1*, and *KCNJ11* genes with T2DM have consistently replicated in multiple ethnic groups, their association with vascular complications remains inconsistent [7-18]. The influence of single SNPs on the risk of diabetic complications generally has a modest effect, therefore the cumulative effect of multiple risk alleles as genetic risk score (GRS) may provide a better tool for the evaluation of the risk for T2DM-related complications. Several studies have reported that GRSs comprising diabetes-risk SNPs were associated with T2DM [28], hyperglycemia [29], and insulin secretion or insulin resistance [30,31], but not with diabetic complications. There is also evidence that SNPs in these T2DM-related genes have been associated with hypertension [13,17,21]. We speculated that a combination of risk-associated alleles for both T2DM and hypertension might better confer susceptibility to diabetic vascular complications. However, there were no studies regarding the effect of such a risk-variant combination on diabetic complications. The present study therefore aimed to investigate the effects of individual gene variants and GRS comprising diabetes and hypertension risk SNPs on various diabetic vascular complications.

## METHODS

### Study population

The study protocol was reviewed and approved by Khon Kaen University Ethics Committee for Human Research (Expedited Review: HE591430 and HE601094), in accordance with the Declaration of Helsinki, before the study was conducted. Left-over blood samples were collected from (1) community-based Thai subjects attending annual health check-up at Health Center 15, Bangkok; (2) hospital-based Thai subjects who visited for clinical investigation at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University; and (3) Thai patients who attended for cardiovascular investigation at Queen Sirikit Heart Center of the Northeast, Faculty of Medicine, Khon

Kaen University. Exclusion criteria: subjects with type 1 diabetes mellitus, gestational diabetes, or maturity-onset diabetes of young adults were excluded; those who have chronic diseases that affect the metabolic or biochemical parameters, such as hypothyroidism, hyperthyroidism, liver disease, kidney disease, and cancer were also excluded from the study. Based on the retrospective medical record and laboratory data, 1,700 samples collected from Thai subjects age  $\geq 35$  years who were enrolled, comprising 960 T2DM patients and 740 non-diabetic subjects. T2DM was defined for subjects who (1) had fasting plasma glucose (FPG) of  $\geq 7.0$  mmol/L; (2) had glycated hemoglobin (HbA1c) of  $\geq 6.5\%$ ; and/or (3) were under anti-diabetic medication [32]. T2DM patients who had at least one complication were categorized as diabetes mellitus (DM) with complication subgroup. Definition and assessment for diabetic complications have detailed description in the Supplementary methods [33–36]. Among 960 DM patients, 383 were patients with diabetic complications. The non-diabetes (non-DM) group definition for individuals who had no history of T2DM medication, whose blood HbA1c content was less than 6.5%, and the FPG level was less than 7.0 mmol/L.

#### Biochemical parameters and demographic data

Hypertension was considered if the subject had systolic blood pressure (SBP)  $\geq 140$  mm Hg and diastolic blood pressure (DBP)  $\geq 90$  mm Hg or had a medical record and treatment of hypertension [37]. Obesity defined by body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup> [38]. The definition of metabolic syndrome (MetS), dyslipidemia (DL), hypertriglyceridemia (HTG), and low level of high-density lipoprotein cholesterol (HDL-C) followed the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guideline. The MetS was defined upon presenting with three or more of the following conditions: (1) waist circumference  $\geq 90$  cm (male) or  $\geq 80$  cm (female); (2) serum triglyceride (TG) levels  $\geq 1.7$  mmol/L (HTG); (3) HDL-C  $< 1.03$  mmol/L (male) or  $< 1.29$  mmol/L (female) (low HDL-C); (4) SBP  $> 130$  mm Hg or DBP  $> 85$  mm Hg; and (5) FPG  $\geq 6.1$  mmol/L [39]. DL was defined by having one or more of the abnormal lipid profile (total cholesterol [TC]  $\geq 5.17$  mmol/L, TG  $\geq 1.7$  mmol/L, low-density lipoprotein cholesterol [LDL-C]  $> 2.6$  mmol/L, and HDL-C  $< 1.03$  mmol/L for male or  $< 1.29$  mmol/L for female).

#### SNPs selection

Interested SNPs were selected for the association study with

T2DM, hypertension, and diabetic complications. According to the candidate gene studies, the original genome-wide association studies, and the replication studies in various ethnic groups, five common SNPs within three known functional genes, *TCF7L2*, *KCNQ1*, and *KCNJ11*, were selected for the present study. Except for *TCF7L2* rs7903146, the minor allele frequency (MAF) of SNPs *TCF7L2* rs290487, *KCNQ1* (rs2237892 and rs2237897), and *KCNJ11* rs5219, were greater than 5% in East Asian population, and these SNPs were also reported to be associated with T2DM in several populations. Although the MAF frequency of *TCF7L2* rs7903146 was low in the East Asian population (Chinese and Japanese with MAF 0.02 and 0.03, respectively), two studies in Indian population have indicated that the MAF of *TCF7L2* rs7903146 (T allele) was 14.2% [40] and 31% [41].

#### Genotyping

Genomic DNA extracted from peripheral blood leukocytes utilizing commercial DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For *TCF7L2* rs290487 and *KCNQ1* rs2237892, genotyping was performed using allele-specific polymerase chain reaction (AS-PCR) technique. Other SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). All primers were designed using Primer-BLAST program (National Center for Biotechnology Information) (Supplementary Table 1). The PCR reaction was performed in a total volume of 25  $\mu$ L mixture containing 100 ng of DNA template, 200 mM of each deoxynucleoside triphosphate (dNTP; Vivantis, Shah Alam, Malaysia), 1.0 mM of each primer (Integrated DNA Technologies, Coralville, IA, USA), and 0.5 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). For PCR-RFLP, the DNA amplicon was further digested with restriction endonuclease (New England Biolabs, Ipswich, MA, USA). The analysis of DNA products was performed by electrophoresis on 2.5% agarose gels and then visualized under a UV transilluminator. The genotyping results were validated by DNA sequencing (1st BASE, Seri Kembangan, Malaysia) of 10% randomly selected DNA samples. All eight SNPs' evaluations performed for the association with, T2DM, hypertension, and diabetic complications.

#### Statistical analysis

All of the statistical analysis was carried out using SPSS software version 17 (SPSS Inc., Chicago, IL, USA). The distribu-

tions of all SNP genotypes and alleles in the study population were analyzed for the concordance with the Hardy-Weinberg equilibrium (HWE) utilizing the HW calculator (<http://www.oege.org/software/>). The difference of continuous variables between two groups was analyzed using Student's *t*-test and among three or more groups was by one-way analysis of variance (ANOVA). Clinical and biochemical parameters were reported as the mean  $\pm$  standard deviation (for continuous variables) or percentages (for categorical parameters). The individual or combined effect of multiple SNPs on the risk of diabetic complication was evaluated by logistic regression model and expressed as odds ratio (OR) with 95% confidence interval (95% CI), adjusted for covariates including age, sex, hypertension, DL, HbA1c, and BMI. Haplotype analysis was carried out using software programs "Haploview ver.4.2 (<https://haploview.software.informer.com/4.2/>)" and "Arlequin ver.3.5 (<http://cmpg.unibe.ch/software/arlequin35/Arl35Downloads.html>)." For all analyses, a two-sided *P* value of  $<0.05$  considered as statistically significant. A permutation test was used to adjust for a significant threshold in multiple comparison testing. The statistical power for each SNP was estimated using Genetic Association Study (GAS) Power Calculator ([http://csg.sph.umich.edu/abecasis/gas\\_power\\_calculator/index.html](http://csg.sph.umich.edu/abecasis/gas_power_calculator/index.html)).

## RESULT

### Baseline demographic and clinical characteristics of the study population

A total number of 1,700 subjects enrolled, comprising 740 non-DM, 577 DM without complications, and 383 DM with complications. The baseline demographic and clinical characteristics of study subjects are presented in Table 1. The prevalence of male subjects was higher in DM (44.7%) than in the non-DM group (25.1%) ( $P<0.001$ ). The percentages of subjects with hypertension, obesity, MetS, HTG, and low HDL-C were significantly greater in the T2DM group than in the non-DM group. The means of BMI, SBP, HbA1c, FPG, TG, TG/HDL-C ratio, blood urea nitrogen (BUN), creatinine, and uric acid were also significantly higher in T2DM patient than in non-DM group. Other parameters such as DBP, TC, HDL-C, LDL-C, non-HDL-C, and estimated glomerular filtration rate (eGFR), revealed significantly lower in T2DM patients than in non-DM. For the medication in T2DM patients, the proportion of patients who received treatment with anti-diabetic drugs was not different between T2DM patients without and

with complications; with an exception for biguanides, the percentage of patients under treatment was higher in a group without complication than in those with complication. The proportion of patients using insulin was higher in patients with complications than in those without complications. The percentage of patients treated with antihypertensive drugs and anti-hyperlipidemic drugs were higher in patients with complications than in patients without complications (Supplementary Table 2).

### Analysis of the association between SNPs and T2DM

All SNPs were first investigated for their relationship with T2DM. The genotype distribution for each of these gene variants was consistent with the HWE ( $P>0.05$ ) in the study population. As shown in Table 2, the following SNPs were significantly associated with an increased risk of T2DM after adjusting for age, sex, hypertension, DL, and BMI. *TCF7L2* rs290487 presented with OR 1.33, 95% CI 1.05 to 1.68,  $P=0.017$  (recessive model, TT vs. CC+TC). *KCNQ1* rs2237892 revealed an association with OR (95% CI) as: 1.65 (1.13 to 2.43),  $P=0.010$  (CC vs. TT); 1.54 (1.06 to 2.23),  $P=0.024$  (dominant model, CT+CC vs. TT); and 1.25 (1.01 to 1.54),  $P=0.037$  (recessive model, CC vs. TT+CT). *KCNQ1* rs2237897 had an association with OR (95% CI) as: 1.88 (1.22 to 2.90),  $P=0.004$  (CC vs. TT); 1.76 (1.15 to 2.68),  $P=0.009$  (dominant model, CC+CT vs. TT); and 1.26 (1.02 to 1.55),  $P=0.031$  (recessive model, CC vs. TT+CT). There were no associations with T2DM for rs7903146 in *TCF7L2* and rs5219 in *KCNJ11*.

### Analysis of the association between SNPs and hypertension

Hypertension is the most common comorbidity of T2DM that may aggravate the development of micro- and macrovascular complications in T2DM patients [3]. The influence of hypertension on diabetic vascular complications firstly evaluated in all T2DM patients. In the present study, T2DM patient with hypertension had 4.32 times risk of progression with overall vascular complications (OR, 4.32; 95% CI, 2.75 to 6.79;  $P<0.001$ ), 4.06 time of developing CAD (OR, 4.06; 95% CI, 1.88 to 8.75;  $P<0.001$ ), and 7.43 time of developing nephropathy (OR, 7.43; 95% CI, 3.39 to 16.32;  $P<0.001$ ), as compared with those without hypertension (Supplementary Tables 3 and 4).

To investigate the relationship of all five SNPs with hypertension, regardless of diabetes, all study population was categorized into hypertension and non-hypertension groups (Supplementary Table 5). Individual SNPs then went through eval-

uation, by which only *TCF7L2* rs7903146 and rs290487 revealed an association with a risk of hypertension (Supplementary Table 6). The CC genotype of rs7903146, as compared with CT, revealed a significant association (OR, 1.56; 95% CI, 1.05 to 2.32;  $P=0.030$ ), whereas C allele of rs290487 showed a marginal effect (OR, 1.29; 95% CI, 1.00 to 1.66;  $P=0.050$ ; dominant model). Because the incidence of hypertension is likely to increase with age; thus, an analysis was conducted in an age

group of 60 to 94 (mean 68.44 years). In these old age subjects, *KCNQ1* rs2237892 and rs2237897 revealed a significant association with hypertension. Comparing with CC genotypes, TT genotypes of both rs2237892 and rs2237897 increased the risk of hypertension (OR, 1.75; 95% CI, 1.03 to 2.99;  $P=0.040$ ) and (OR, 1.99; 95% CI, 1.08 to 3.67;  $P=0.027$ ), respectively (Table 3). Association was also found for *TCF7L2* rs290487 C allele (OR, 1.41; 95% CI, 1.04 to 1.90;  $P=0.026$ ; dominant model).

**Table 1.** Demographic and clinical characteristics of the study population

Parameter	Non-T2DM (n=740)	T2DM without complication (n=577)	T2DM with complication (n=383)	P value <sup>a</sup>	P value <sup>b</sup>	P value <sup>c</sup>	P value <sup>d</sup>
Subjects, men/women	186/554	220/357	209/174	<0.001	<0.001	<0.001	<0.001
Hypertension	447/740 (60.4)	431/577 (74.7)	355/383 (92.7)	<0.001	<0.001	<0.001	<0.001
Obesity	325/728 (44.6)	303/568 (53.3)	206/366 (56.3)	0.002	<0.001	0.379	<0.001
Dyslipidemia	681/740 (92.0)	489/572 (85.5)	291/346 (84.1)	<0.001	<0.001	0.569	<0.001
MetS	143/740 (19.3)	327/577 (56.7)	280/383 (73.1)	<0.001	<0.001	<0.001	<0.001
Hypertriglyceridemia	193/740 (26.1)	250/570 (43.9)	144/337 (42.7)	<0.001	<0.001	0.740	<0.001
Low HDL-C	225/740 (30.4)	286/571 (50.1)	214/336 (63.7)	<0.001	<0.001	0.040	<0.001
Age, yr	64.19±9.53	63.60±9.31	64.38±9.91	0.267	0.754	0.220	0.396
BMI, kg/m <sup>2</sup>	24.81±3.98	26.02±4.87	26.01±4.25	<0.001	<0.001	0.957	<0.001
SBP, mm Hg	133.63±18.64	135.74±18.66	138.42±19.81	0.042	<0.001	0.035	<0.001
DBP, mm Hg	77.25±11.88	74.89±11.21	72.26±10.60	<0.001	<0.001	<0.001	<0.001
HbA1c, %	5.57±0.39	7.52±1.76	7.82±1.76	<0.001	<0.001	0.024	<0.001
FPG, mmol/L	5.40±0.58	8.29±3.10	8.55±3.55	<0.001	<0.001	0.246	<0.001
TC, mmol/L	5.48±1.10	4.90±1.28	4.49±1.27	<0.001	<0.001	<0.001	<0.001
TG, mmol/L	1.43±0.69	1.86±1.23	1.84±1.07	<0.001	<0.001	0.831	<0.001
HDL-C, mmol/L	1.52±0.41	1.33±0.38	1.20±0.36	<0.001	<0.001	<0.001	<0.001
LDL-C, mmol/L	3.52±1.01	3.00±1.13	2.75±1.08	<0.001	<0.001	0.001	<0.001
Non-HDL-C, mmol/L	3.96±1.09	3.58±1.27	3.30±1.26	<0.001	<0.001	0.002	<0.001
TC/HDL-C	3.81±1.12	3.92±1.37	4.02±1.52	0.093	0.010	0.308	0.033
TG/HDL-C	1.06±0.71	1.62±1.47	1.78±1.35	<0.001	<0.001	0.093	<0.001
LDL-C/HDL-C	2.47±0.93	2.40±1.03	2.47±1.14	0.202	0.914	0.299	0.390
Non-HDL-C/HDL-C	2.81±1.12	2.92±1.37	3.02±1.52	0.093	0.010	0.308	0.033
BUN, mmol/L	4.90±1.49	5.51±2.68	7.78±4.62	<0.001	<0.001	<0.001	<0.001
Creatinine, μmol/L	78.05±38.26	86.11±64.2	158.11±181.80	0.005	<0.001	<0.001	<0.001
eGFR, mL/s/m <sup>2</sup>	1.43±0.27	1.37±0.32	0.89±0.47	0.023	<0.001	<0.001	<0.001
Uric acid, μmol/L	315.75±88.64	332.73±89.17	348.89±99.16	0.004	<0.001	0.054	<0.001

Values are presented as number/total number (%) or mean ± standard deviation.  $P<0.05$  indicated significant associations. T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

<sup>a</sup> $P$  value, comparing between Non-T2DM and T2DM, <sup>b</sup> $P$  value, comparing between non-T2DM and T2DM with complication, <sup>c</sup> $P$  value, comparing between T2DM and T2DM with complication, <sup>d</sup> $P$  value, statistical comparison among groups.

**Table 2.** Association between SNPs and type 2 diabetes mellitus in all subjects

SNP	Non-DM (n=740)	DM (n=960)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>TCF7L2</i> rs7903146							
Additive							
CC	676 (91.4)	885 (92.2)	0.592	1		1	
CT	64 (8.6)	75 (7.8)		0.90 (0.63–1.27)	0.533	0.93 (0.64–1.36)	0.713
Allele							
C	1,416 (95.7)	1,845 (96.1)	0.600	1		1	
T	64 (4.3)	75 (3.9)		0.90 (0.64–1.26)	0.542	0.93 (0.64–1.35)	0.714
<i>TCF7L2</i> rs290487							
Additive							
CC	174 (44.8)	214 (55.2)	0.035	1		1	
TC	379 (45.8)	449 (54.2)		0.96 (0.76–1.23)	0.762	0.97 (0.74–1.26)	0.799
TT	187 (38.6)	297 (61.4)		1.29 (0.99–1.69)	0.065	1.30 (0.97–1.74)	0.084
Dominant							
CC	174 (44.8)	214 (55.2)	0.560	1		1	
TC+TT	566 (43.1)	746 (56.9)		1.07 (0.85–1.35)	0.552	1.07 (0.84–1.38)	0.571
Recessive							
CC+TC	553 (45.5)	663 (54.5)	0.011	1		1	
TT	187 (38.6)	297 (61.4)		1.33 (1.07–1.64)	0.010	1.33 (1.05–1.68)	0.017
Allele							
C	727 (45.3)	877 (54.7)	0.048	1		1	
T	753 (41.9)	1,043 (58.1)		1.15 (1.00–1.32)	0.046	1.07 (0.84–1.38)	0.571
<i>KCNQ1</i> rs2237892							
Additive							
TT	72 (9.7)	67 (7.0)	0.062	1		1	
CT	308 (41.6)	386 (40.2)		1.35 (0.94–1.94)	0.110	1.40 (0.95–2.07)	0.092
CC	360 (48.6)	507 (52.8)		1.51 (1.06–2.17)	0.024	1.65 (1.13–2.43)	0.010
Dominant							
TT	72 (9.7)	67 (7.0)	0.049	1		1	
CT+CC	668 (90.3)	893 (93.0)		1.44 (1.02–2.03)	0.041	1.54 (1.06–2.23)	0.024
Recessive							
TT+CT	380 (51.4)	453 (47.2)	0.096	1		1	
CC	360 (48.6)	507 (52.8)		1.18 (0.98–1.43)	0.089	1.25 (1.01–1.54)	0.037
Allele							
T	452 (30.5)	520 (27.1)	0.029	1		1	
C	1,028 (69.5)	1,400 (72.9)		1.18 (1.02–1.38)	0.027	1.50 (1.04–2.18)	0.031

(Continued to the next page)

Table 2. Continued

SNP	Non-DM (n=740)	DM (n=960)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>KCNQ1</i> rs2237897							
Additive							
TT	58 (7.8)	48 (5.0)	0.037	1		1	
CT	297 (40.1)	377 (39.3)		1.53 (1.02–2.32)	0.042	1.60 (1.03–2.48)	0.037
CC	385 (52.0)	535 (55.7)		1.68 (1.12–2.52)	0.012	1.88 (1.22–2.90)	0.004
Dominant							
TT	58 (7.8)	48 (5.0)	0.020	1		1	
CC+CT	682 (92.2)	912 (95.0)		1.62 (1.09–2.40)	0.017	1.76 (1.15–2.68)	0.009
Recessive							
TT+CT	355 (48.0)	425 (44.3)	0.141	1		1	
CC	385 (52.0)	535 (55.7)		1.16 (0.96–1.41)	0.129	1.26 (1.02–1.55)	0.031
Allele							
T	413 (27.9)	473 (24.6)	0.033	1		1	
C	1067 (72.1)	1,447 (75.4)		1.18 (1.02–1.38)	0.031	1.76 (1.15–2.68)	0.009
<i>KCNJ11</i> rs5219							
Additive							
GG	329 (45.8)	390 (54.2)	0.136	1		1	
GA	308 (40.8)	446 (59.2)		1.22 (0.99–1.50)	0.057	1.25 (1.00–1.56)	0.050
AA	103 (45.4)	124 (54.6)		1.02 (0.75–1.37)	0.919	0.99 (0.71–1.36)	0.931
Dominant							
GG	329 (45.8)	390 (54.2)	0.113	1		1	
GA+AA	411 (41.9)	570 (58.1)		1.17 (0.96–1.42)	0.113	1.18 (0.96–1.46)	0.117
Recessive							
GG+GA	637 (43.2)	836 (56.8)	0.565	1		1	
AA	103 (45.4)	124 (54.6)		0.92 (0.69–1.22)	0.547	0.88 (0.65–1.19)	0.408
Allele							
G	966 (44.1)	1,226 (55.9)	0.406	1		1	
A	514 (42.5)	694 (57.5)		1.06 (0.92–1.23)	0.392	0.88 (0.65–1.19)	0.408

Values are presented as number (%). OR adjusted for age, sex, hypertension, body mass index, and dyslipidemia.

SNP, single nucleotide polymorphism; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval.

<sup>a</sup>P value after 100,000 permutation.

**Analysis of the association between individual SNPs and diabetic complications**

Of all T2DM patients (n=960), 383 subjects had cumulative (micro- and macro-) vascular complications, of which 239 patients had microvascular complications (12.0% retinopathy, 18.9% nephropathy, and 3.6% neuropathy), 121 had macrovascular complications (11.7% CAD and 3.5% Stroke), and 23 had both micro- and macrovascular complications (Supplementa-

ry Table 7). Among 960 T2DM patients, of all five SNPs analyzed, there were no associations for individual SNPs with the complications (Supplementary Table 8).

**Combined effects of *TCF7L2* and *KCNQ1* risk alleles on diabetic complications**

To evaluate the combined effect of T2DM susceptibility gene polymorphisms on diabetic complications, a GRS was formu-

**Table 3.** Association between SNPs and hypertension in old-age subjects

SNP	Non-HT (n=325)	HT (n=907)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>TCF7L2</i> rs7903146							
Additive							
CT	37 (11.4)	72 (7.9)	0.068	1		1	
CC	288 (88.6)	835 (92.1)		1.49 (0.98–2.26)	0.062	1.54 (0.99–2.39)	0.057
Allele							
T	37 (5.7)	72 (4.0)	0.075	1		1	
C	613 (94.3)	1,742 (96.0)		1.46 (0.97–2.19)	0.068	1.50 (0.98–2.31)	0.064
<i>TCF7L2</i> rs290487							
Additive							
TT	95 (28.9)	234 (71.1)	0.492	1		1	
TC	155 (25.5)	453 (74.5)		1.19 (0.88–1.60)	0.264	1.40 (1.02–1.93)	0.039
CC	75 (25.4)	220 (74.6)		1.19 (0.84–1.70)	0.334	1.42 (0.98–2.07)	0.067
Dominant							
TT	95 (28.9)	234 (71.1)	0.243	1		1	
TC+CC	230 (25.5)	673 (74.5)		1.19 (0.90–1.57)	0.231	1.41 (1.04–1.90)	0.026
Recessive							
TT+TC	250 (26.7)	687 (73.3)	0.705	1		1	
CC	75 (25.4)	220 (74.6)		1.07 (0.79–1.44)	0.669	1.14 (0.83–1.57)	0.408
Allele							
T	345 (27.3)	921 (72.7)	0.315	1		1	
C	305 (25.5)	893 (74.5)		1.10 (0.92–1.31)	0.313	1.14 (0.83–1.57)	0.408
<i>KCNQ1</i> rs2237892							
Additive							
CC	174 (53.5)	453 (49.9)	0.299	1		1	
CT	130 (40.0)	373 (41.1)		1.10 (0.85–1.44)	0.473	1.15 (0.87–1.53)	0.319
TT	21 (6.5)	81 (8.9)		1.48 (0.89–2.47)	0.131	1.75 (1.03–2.99)	0.040
Dominant							
CC	174 (53.5)	453 (49.9)	0.272	1		1	
CT+TT	151 (46.5)	454 (50.1)		1.16 (0.90–1.49)	0.266	1.23 (0.94–1.61)	0.125
Recessive							
CC+CT	304 (93.5)	826 (91.1)	0.197	1		1	
TT	21 (6.5)	81 (8.9)		1.42 (0.86–2.34)	0.168	1.64 (0.98–2.76)	0.061
Allele							
C	478 (73.5)	1,279 (70.5)	0.143	1		1	
T	172 (26.5)	535 (29.5)		1.16 (0.95–1.42)	0.143	1.64 (0.98–2.76)	0.061

(Continued to the next page)

**Table 3.** Continued

SNP	Non-HT (n=325)	HT (n=907)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>KCNQ1</i> rs2237897							
Additive							
CC	185 (56.9)	479 (52.8)	0.207	1		1	
CT	125 (38.5)	364 (40.1)		1.13 (0.86–1.47)	0.384	1.15 (0.87–1.52)	0.328
TT	15 (4.6)	64 (7.1)		1.65 (0.92–2.97)	0.096	1.99 (1.08–3.67)	0.027
Dominant							
CC	185 (56.9)	479 (52.8)	0.218	1		1	
CT+TT	140 (43.1)	428 (47.2)		1.18 (0.92–1.52)	0.202	1.24 (0.94–1.62)	0.122
Recessive							
CC+CT	310 (95.4)	843 (92.9)	0.146	1		1	
TT	15 (4.6)	64 (7.1)		1.57 (0.88–2.79)	0.126	1.88 (1.03–3.41)	0.039
Allele							
C	495 (76.2)	1,322 (72.9)	0.107	1		1	
T	155 (23.8)	492 (27.1)		1.19 (0.97–1.46)	0.104	1.88 (1.03–3.41)	0.039
<i>KCNJ11</i> rs5219							
Additive							
GG	134 (25.5)	392 (74.5)	0.785	1		1	
GA	145 (26.8)	397 (73.2)		0.94 (0.71–1.23)	0.635	0.85 (0.64–1.14)	0.280
AA	46 (28.0)	118 (72.0)		0.88 (0.59–1.30)	0.512	0.88 (0.59–1.34)	0.556
Dominant							
GG	134 (25.5)	392 (74.5)	0.557	1		1	
AA+GA	191 (27.1)	515 (72.9)		0.92 (0.71–1.19)	0.534	0.86 (0.66–1.13)	0.276
Recessive							
GA+GG	279 (26.1)	789 (73.9)	0.634	1		1	
AA	46 (28.0)	118 (72.0)		0.91 (0.63–1.31)	0.603	0.96 (0.65–1.41)	0.826
Allele							
G	237 (27.2)	633 (72.8)	0.503	1		1	
A	413 (25.9)	1,181 (74.1)		0.93 (0.78–1.13)	0.473	0.96 (0.65–1.41)	0.826

Values are presented as number (%). Old-age subjects, age range 60 to 94 years (mean 68.44 years); OR adjusted for sex, type 2 diabetes mellitus, body mass index, and dyslipidemia.

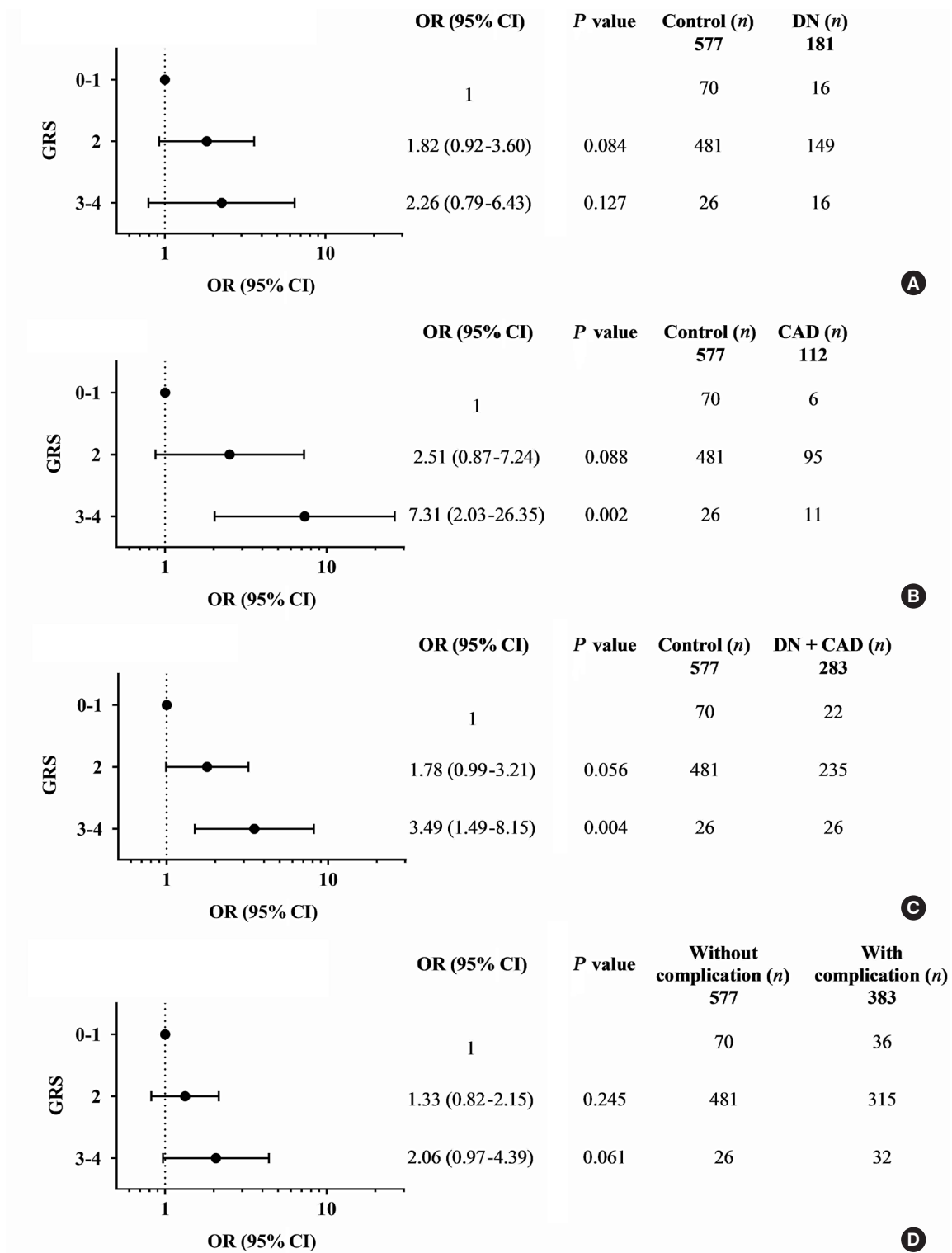
SNP, single nucleotide polymorphism; HT, hypertension; OR, odds ratio; CI, confidence interval.

<sup>a</sup>P value after 100,000 permutation.

lated based on the summation of risk-contributing alleles from the studied SNPs that were associated with T2DM and hypertension. Based on the previous analysis, four SNPs (*TCF7L2* rs7903146, *TCF7L2* rs290487, *KCNQ1* rs2237892, and *KCNQ1* rs2237897) were selected for the construction of GRS. For each biallelic SNP, a risk score of 0 was defined for homozygous of

non-risk alleles, score 1 for heterozygous of risk and non-risk alleles, and score 2 for homozygous of two risk alleles. For a combination of two or more SNPs, the GRS would be between 0 and 4 for combined two SNPs, and between 0 and 6 for combined three SNPs.

Diabetic nephropathy and CAD are two major complica-



**Fig. 1.** Adjusted odds ratios (ORs) and 95% confidence interval (CI) for the combined effect of *KCNQ1* rs2237892 and rs2237897 risk alleles, as genetic risk score (GRS), on vascular complications in type 2 diabetes mellitus patients: (A) diabetic nephropathy (DN), (B) coronary artery disease (CAD), (C) cumulative DN and CAD, and (D) cumulative micro- and macrovascular complications; GRS 0–1 as reference; ORs adjusted for age, sex, hypertension, dyslipidemia, glycosylated hemoglobin, and body mass index.

tions in our study T2DM population and known to cause mortality in T2DM patients. They were mainly considered for the association study. A combination of any two risk-alleles was first investigated; the overall analyses are presented in Supplementary Tables 9-16. However, only combined two risk-alleles in *KCNQ1* SNPs, rs2237892 (C) and rs2237897 (T), revealed the significant associations. The high-GRS group (GRS 3-4) was associated with increased risks of CAD (OR, 7.31; 95% CI, 2.03 to 26.35;  $P=0.002$ ), cumulative nephropathy and/or CAD (OR, 3.49; 95% CI, 1.49 to 8.15;  $P=0.004$ ), and borderline association with cumulative micro- and macrovascular complications (OR, 2.06; 95% CI, 0.97 to 4.39) as compared with a group of GRS 0-1 (Fig. 1). Haplotype analysis was performed for rs2237892 (C/T) and rs2237897 (C/T). In accordance with the combined effect of two risk-alleles, C-T haplotype was also significantly associated with CAD (OR, 5.64; 95% CI, 1.26 to 25.28;  $P=0.024$ ), as compared with T-C haplotype (Supplementary Tables 17-19).

A combination of multiple risk-alleles was subsequently investigated, from which a significant association was presented only for the combined three risk-alleles, *TCF7L2* rs7903146 (C), *KCNQ1* rs2237892 (C), and *KCNQ1* rs223797 (T). As compared with a group of GRS 2-3, the high-GRS group (GRS 5-6) revealed significant association with CAD (OR, 6.90; 95% CI, 2.30 to 20.71;  $P=0.001$ ), cumulative nephropathy and/or CAD (OR, 3.92; 95% CI, 1.75 to 8.76;  $P=0.001$ ), and cumulative micro- and/or macrovascular complications (OR, 2.33; 95% CI, 1.13 to 4.79;  $P=0.022$ ) (Fig. 2 and Supplementary Tables 9-16).

The association between the combined risk alleles (GRS) and the parameters related to the severity of T2DM (blood level of HbA1c and FPG) and the kidney function (BUN, creatinine, and the magnitude of eGFR) was explored, which may be related to the outcomes of diabetic complications. Although GRS did not directly show significant relationship with the level of HbA1c or FPG, the level appeared to increase according to the increase of GRS. For the kidney function-related parameters, the trend toward an increasing level of BUN and creatinine, with the decrease of eGFR, was associated with the increase of GRS, significantly for combined two risk alleles ( $P$  trend  $<0.05$ ) and significant only at creatinine level for combined three risk alleles (Supplementary Table 20).

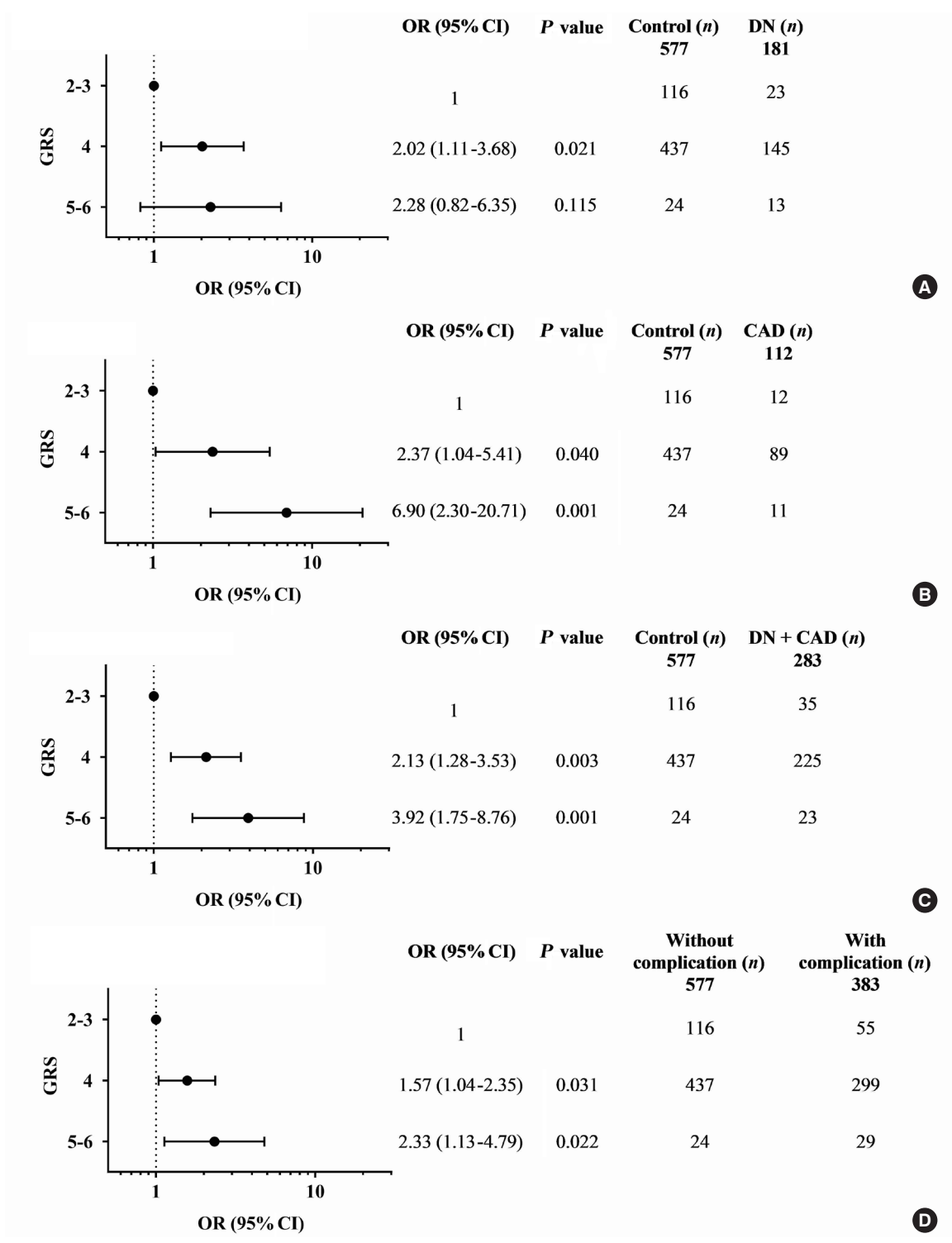
## DISCUSSION

The present study initially evaluated the individual effect of five

SNPs in *TCF7L2*, *KCNQ1*, and *KCNJ11* genes for their association with T2DM and its comorbidity, hypertension, in Thai subjects with T2DM and related complications. Of five SNPs, we found that only three SNPs (with effecting alleles): *TCF7L2* rs290487 (T), *KCNQ1* rs2237892 (C), and *KCNQ1* rs2237897 (C), were significantly associated with risk of T2DM. We subsequently demonstrated that *TCF7L2* rs7903146 (C) revealed an association with the risk of hypertension in all subjects; whereas *TCF7L2* rs290487 (C), *KCNQ1* rs2237892 (T), and *KCNQ1* rs2237897 (T) were associated with risk of hypertension only in old-age subjects. With the speculation that combined T2DM and hypertension associated gene variants might confer susceptibility to diabetic vascular complications; we further demonstrated, however, that only specific combination of *KCNQ1* rs2237892 (C) and rs2237897 (T) with *TCF7L2* rs7903146 (C) increased the risk of T2DM related CAD, cumulative diabetic nephropathy and/or CAD, and cumulative micro- and/or macrovascular complication.

Several studies have published the association of common SNPs in *TCF7L2* with T2DM. However, the results have been variable among different racial populations [8,42,43]. The previous studies in the Chinese [42] and the Japanese population [43] have reported a significant association between the T allele of *TCF7L2* rs7903146 and T2DM risk, whilst no association found for *TCF7L2* rs290487. In the present study, we found that only *TCF7L2* rs290487 (T) was associated with risk of T2DM, but such association was not found for rs7903146. For the rs7903146, although the T allele revealed no association with T2DM in our study Thai population, the C allele was associated with an increased risk of hypertension. The difference in the association results conferred by *TCF7L2* SNPs could be due to the difference in population characteristics as well as the difference in frequencies of the effecting allele among the various study population. For *KCNQ1*, in accordance with the previous study in both European [44] and Asian population [45], the present study also found that *KCNQ1* rs2237892 (C) and rs2237897 (C) had a significant association with T2DM. There is evidence suggesting that the risk gene variants in *KCNQ1* are gain-of-function polymorphisms that enhance T2DM risk. Such gain-of-function polymorphisms could result in increased *KCNQ1* expression, which by mechanistic function of *KCNQ1* (Kv7.1) could decrease insulin exocytosis and reduce insulin secretion [46].

Hypertension is the most common comorbidity of T2DM that plays a dominant role in the pathophysiology and progres-



**Fig. 2.** Adjusted odds ratios (ORs) and 95% confidence interval (CI) for the combined effect of *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892 and rs2237897) risk alleles, as genetic risk score (GRS), on vascular complications in type 2 diabetes mellitus patients: (A) diabetic nephropathy (DN), (B) coronary artery disease (CAD), (C) cumulative DN and CAD, and (D) cumulative micro- and macrovascular complications; GRS 2–3 as reference; ORs adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index.

sion of micro- and macrovascular complications [3]. Interestingly, the present study showed that the low-risk allele of the same SNP for T2DM appeared to act inversely as the high-risk allele for hypertension; as such *TCF7L2* rs7903146 (C), *TCF7L2* rs290487 (C), *KCNQ1* rs2237892 (T), and *KCNQ1* rs2237897 (T) revealed a significant association with risk for hypertension. The low-risk allele for T2DM in *KCNQ1* (high-risk for hypertension) could reduce the expression of *KCNQ1*, as opposed to the gain-of-function allele, and result in increased insulin secretion. Similarly, there is an in vitro report showing that inhibition of *KCNQ1* (Kv7.1) function in pancreatic  $\beta$ -cell line results in increased insulin secretion [47]. Reduced activity and expression of Kv7.1 have also resulted in an increase of vascular resistance and renin release with subsequent hypertension [48]. Insulin resistance and/or hyperinsulinemia may contribute to elevated blood pressure via several possible mechanisms; it may cause sodium retention, increased sympathetic activity, and alteration of tissue angiotensin II and aldosterone actions, which are the important causes of hypertension [49]. In addition, polymorphisms in the Wnt effector, *TCF7L2* gene, have been associated with the prevalence and severity of CAD [50]. There is evidence showing that impaired *TCF7L2* activity affects vascular smooth muscle cell differentiation and leads to vascular pathology-related atherosclerotic CAD [51].

T2DM and hypertension are common comorbidities that exacerbate the progression of diabetic complications in the context of progressive renal disease and accelerated CVD. In the present study, we investigated the effect of single SNP on diabetic complications and found no significant association between individual SNP and diabetic complications. However, a combination of risk-alleles that confer susceptibility to both T2DM and hypertension appeared to have a significant effect. As shown by a combination of two risk-alleles in *KCNQ1*, rs2237892 (C) and rs2237897 (T), which respectively related to T2DM and hypertension, revealed a significant association predominantly with CAD and with cumulative diabetic nephropathy and CAD. Additionally, the haplotype linking rs2237892 (C) with rs2237897 (T) also conferred a significant association with CAD. Such evidence of C-T haplotype association could support a specific combined effect of both alleles. In contrast, a combination of two risk-alleles in *KCNQ1*: rs2237892 (C) and rs2237897 (C), which both related only to T2DM, revealed no association with either microvascular or macrovascular complications. Furthermore, a combination of three risk-alleles, *TCF7L2* rs7903146 (C), *KCNQ1* rs2237892

(C), and *KCNQ1* rs2237897 (T), pronounced the association with greater effect compared to a combination of only two risk-alleles, *KCNQ1* rs2237892 (C) and *KCNQ1* rs2237897 (T). This finding indicated the cumulative effect of gene variants that confer risk for both T2DM and hypertension on the progression of diabetic micro- and macrovascular complications. The development and progression of T2DM and its complications are complex. Age may be a contributing factor. However, in the present study, there were no differences in the average age among three groups of subjects. Among individuals with T2DM, during the diabetic progression, such genetic variation might be a driving factor that differentiates those with from those without complications. Our finding for the association of a specific pattern of combined risk alleles might be a part of such driving factor. Because this study explored in the limited sampling population, further replicative study in another larger group with more representative subjects may be of interest to confirm this finding in the general population.

The limitation of this study was due to the restricted access of the patient medical history; so that we were unable to address the effect of others factors such as the onset and duration of T2DM, environmental factors (smoking and alcohol use), that may modify the outcome of genetic association.

In conclusion, to the best of our knowledge, this study demonstrated for the first time the combined effect of genetic variants in *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892 and rs2237897) on the risk of both micro- and macrovascular complications, predominantly with nephropathy and CAD, in subjects with T2DM. Such an effect was conferred by a specific pattern of a combination of risk-alleles for both T2DM and hypertension.

## SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at <https://doi.org/10.4093/dmj.2020.0101>.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

## AUTHOR CONTRIBUTIONS

Conception or design: R.R., N.S., C.S.

Acquisition, analysis, or interpretation of data: R.R., N.S., C.S.  
 Drafting or revising the work: R.R., N.S., N.K., U.K., K.S., P.I., V.S., C.S.

Final approval of the manuscript: R.R., N.S., N.K., U.K., K.S., P.I., V.S., C.S.

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## Supplementary methods

### Definition and assessment for diabetic complications

All patients who were diagnosed with diabetes have been followed-up for diabetes progression at the diabetes mellitus clinic every 3 months and were undergone screening for the presence of complications. Diabetic retinopathy was defined and diagnosed according to The International Council of Ophthalmology guidelines: the presence of microaneurysms, hemorrhages, intraretinal microvascular abnormalities, venous beading, hard exudates, cotton-wool spots, and retinal neovascularization. Screening for retinopathy was performed specifically at the eye clinic by an ophthalmologist who also examined the results of fundus photography. Diagnosis of diabetic neuropathy was by clinical neurological examination and electromyography according to the recent criteria [33,34]. The frequency of neuropathy in our study subjects was 35 per 960 diabetic cases (3.6%), somewhat lower than those reported in the Western countries (6% to 51%). However, they were all confirmed cases being undergone regular intervention. Besides, the prevalence of neuropathy in Thai subjects with type 2 diabetes mellitus (T2DM) has also been reported in two nationwide cross-sectional studies, as 2.7% (1,808 per 65,904 T2DM patients) [35] and 3.1% (951 per 30,423) [36]. The Kidney Disease Improving Global Outcomes (KDIGO) guideline was generally used for diagnosis and evaluation for the intervention of kidney diseases of either diabetic or non-diabetic causes. For the diabetic patient, the definition of diabetic nephropathy was usually based on persistent albuminuria (albumin/creatinine ratio  $>3$  mg/mmol for more than 3 months) and a decline in estimated glomerular filtration rate (less than 60 mL/min/1.73 m<sup>2</sup>). The investigation for electrolyte abnormalities was also included to evaluate the renal function. The presence of hematuria was used along with renal histopathology to rule out non-diabetic kidney disease, such as glomerulonephritis. Structural abnormalities (by imaging) may also be used to monitor the progression and severity of kidney disease. Cardiovascular disease was defined as one or a combination of the pathological states: ischemic heart disease, congestive heart failure, and ischemic cerebral stroke. Clinical appearances of cardiovascular disease were confirmed by relevant biochemical analysis, coronary angiography, electrocardiogram, echocardiogram, and vascular diagnostic criteria.

**Supplementary Table 1.** Primers for genotyping of SNPs in *TCF7L2*, *KCNQ1*, and *KCNJ11* genes

Gene and SNP	Method	Primer	Sequences (5'-3')	PCR amplicon size, bp	Restriction enzyme	Digested products, bp (and alleles)	Annealing temperature, °C	MgCl <sub>2</sub> , mM
<i>TCF7L2</i>								
rs7903146	PCR-RFLP	F	TGGTAATGCAGATGTGATG	170	HpyCH4 III	TT = 170 CC = 148, 22	55	2.5
		R	TCATACGGCAATTAAATTATACA					
rs290487	AS-PCR	F (outer)	TACTTTTGGGATTCTGCCCTTTC	283			52	2.0
		R (outer)	CTCCTCTCATGCTGCTCATT TTT					
		F (T allele)	CCAGTACAAATCATGGTGACACAAT	200				
		R (C allele)	CACCTTCTCATT TTTCAAT TTTTCG	133				
<i>KCNQ1</i>								
rs2237892	AS-PCR	F (outer)	CTGGCTTGAGCATGTACACAGG	330			5.8	1.5
		R (outer)	GCTGGTAGGGAACA ACTGGAGAC					
		F (C allele)	TGTCACAGGACTTTGCCAACC	149				
		R (T allele)	GTTTCTAGGCCCTCACCACA	223				
rs2237897	PCR-RFLP	F	GACACCGTCTTGAGCCAGCAGG	167	HpyCH4 IV	TT = 167 CC = 145, 22	65	1.5
		R	AGGGGAAGGATGAGGCCCCAC					
<i>KCNJ11</i>								
rs5219	AS-PCR	F (outer)	TGCTCCCGGATGTTCTTG TG	236			62	1.5
		R (outer)	AGGACTCTGCAGTGAGGCCCTA					
		F (T allele)	GGGCACGGTACCTGGGCTT	166				
		R (C allele)	CTGGCAGAGGACCCTGCCG	107				

SNP, single nucleotide polymorphism; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *KCNJ11*, inwardly-rectifying potassium channel, subfamily J, member 11 gene; PCR, polymerase chain reaction; bp, base pair; RFLP, restriction fragment length polymorphism; F, forward; R, reverse; AS, allele-specific.

**Supplementary Table 2.** Therapeutic drug treatment in patients with type 2 diabetes mellitus

Medication	DM without complication (n=356)	DM with complication (n=382)	P value
Treatment for diabetes			
Biguanides	237 (66.6)	189 (49.5)	<0.001
Sulfonylureas	127 (35.7)	120 (31.4)	0.220
Thiazolidinediones	43 (12.1)	63 (16.5)	0.088
Alpha-glucosidase inhibitor	4 (1.1)	1 (0.3)	0.154
DPP-4 inhibitors	19 (5.3)	32 (8.4)	0.104
SGLT2 inhibitors	3 (0.8)	3 (0.8)	0.931
Using insulin	21 (5.9)	62 (16.2)	<0.001
Treatment for hypertension			
Angiotensin II receptor blockers	63 (17.7)	70 (18.3)	0.824
ACE inhibitors	132 (37.1)	175 (45.8)	0.016
Alpha-blockers	23 (6.5)	54 (14.1)	0.001
Beta-blockers	42 (11.8)	119 (31.2)	<0.001
Calcium channel blockers	29 (8.1)	87 (22.8)	<0.001
Diuretic drugs	23 (6.5)	42 (11.0)	0.030
Vasodilators	8 (2.2)	52 (13.6)	<0.001
Treatment for dyslipidemia			
Statin	43 (12.1)	89 (23.3)	<0.001
Fibrate	211 (59.3)	237 (62.0)	0.441

Values are presented as number (%).  $P < 0.05$  indicated statistical significance.

DM, diabetes mellitus; DPP-4, dipeptidyl peptidase 4; SGLT2, sodium-glucose co-transporter type 2; ACE, angiotensin-converting enzyme.

**Supplementary Table 3.** Relationship between risk factors and diabetic vascular complications

Related risk factor	Diabetes		Hypertension		Nephropathy		CAD	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.00 (0.99–1.01)	0.558	1.03 (1.02–1.05)	<0.001	1.03 (1.01–1.04)	0.004	1.00 (0.98–1.02)	0.945
Sex	2.41 (1.95–2.97)	<0.001	1.73 (1.37–2.18)	<0.001	2.42 (1.94–3.03)	<0.001	3.22 (2.44–4.25)	<0.001
HbA1c	NA	NA	1.08 (1.00–1.18)	0.065	1.33 (1.22–1.45)	<0.001	1.26 (1.07–1.48)	0.006
Hypertension	2.96 (2.38–3.69)	<0.001	NA	NA	12.43 (7.24–21.34)	<0.001	5.72 (3.51–9.33)	<0.001
T2DM with HT <sup>a</sup>	NA	NA	NA	NA	7.43 (3.39–16.32)	<0.001	4.06 (1.88–8.75)	<0.001
BMI	1.07 (1.04–1.09)	<0.001	1.09 (1.06–1.12)	<0.001	1.05 (1.01–1.08)	0.009	1.01 (0.97–1.06)	0.557
Obese	1.49 (1.22–1.80)	<0.001	1.76 (1.41–2.19)	<0.001	1.34 (1.07–1.67)	0.011	1.24 (0.93–1.64)	0.137
TG/HDL ratio (<3 vs. ≥3)	2.88 (2.34–3.56)	<0.001	1.45 (1.16–1.81)	0.001	1.97 (1.54–2.51)	<0.001	4.44 (3.23–6.09)	<0.001
TG/HDL ratio	1.36 (1.28–1.44)	<0.001	1.09 (1.04–1.14)	0.001	1.08 (1.03–1.14)	0.002	1.19 (1.12–1.25)	<0.001
Low HDL level	2.81 (2.29–3.45)	<0.001	1.62 (1.30–2.03)	<0.001	1.85 (1.31–2.60)	<0.001	7.46 (4.37–12.73)	<0.001

Crude OR for related risk factors. Statistical analysis using binary regression model,  $P < 0.05$  indicated significant associations.

CAD, coronary artery disease; OR, odds ratio; CI, confidence interval; HbA1c, glycated hemoglobin; NA, no available data; T2DM, type 2 diabetes mellitus; HT, hypertension; BMI, body mass index; TG, triglyceride; HDL, high-density lipoprotein.

<sup>a</sup>OR adjusted for age, sex, BMI, and dyslipidemia.

**Supplementary Table 4.** Relationship between risk factors and vascular complications

Related risk factor	Microvascular		Macrovascular		Micro- and macrovascular <sup>a</sup>		Micro- and macrovascular <sup>b</sup>	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.01 (0.99–1.02)	0.261	1.01 (0.99–1.02)	0.562	1.00 (0.99–1.02)	0.753	1.01 (1.00–1.03)	0.220
Sex	2.42 (1.85–3.17)	<0.001	3.43 (2.67–4.40)	<0.001	3.58 (2.76–4.64)	<0.001	1.95 (1.50–2.53)	<0.001
HbA1c	1.40 (1.29–1.51)	<0.001	1.25 (1.11–1.41)	<0.001	57.14 (27.81–117.40)	<0.001	1.10 (1.01–1.20)	0.024
Hypertension	6.78 (4.14–11.09)	<0.001	5.50 (3.59–8.42)	<0.001	8.31 (5.50–12.55)	<0.001	4.30 (2.80–6.59)	<0.001
T2DM with HT <sup>c</sup>	4.49 (2.63–7.66)	<0.001	4.06 (2.03–8.15)	<0.001	NA	NA	4.32 (2.75–6.79)	<0.001
BMI	1.05 (1.02–1.08)	0.001	1.01 (0.97–1.05)	0.691	1.07 (1.04–1.11)	<0.001	1.00 (0.97–1.03)	0.957
Obese	1.42 (1.08–1.86)	0.012	1.16 (0.90–1.50)	0.239	1.60 (1.24–2.06)	<0.001	1.13 (0.87–1.47)	0.379
TG/HDL ratio (<3 vs. ≥3)	1.68 (1.26–2.23)	<0.001	3.69 (2.81–4.84)	<0.001	3.45 (2.63–4.52)	<0.001	1.33 (1.01–1.74)	0.040
TG/HDL ratio	1.07 (1.03–1.12)	0.002	1.17 (1.11–1.23)	<0.001	1.39 (1.30–1.49)	<0.001	1.04 (0.99–1.08)	0.096
Low HDL level	1.88 (1.41–2.49)	<0.001	5.86 (3.78–9.08)	<0.001	4.02 (3.06–5.27)	<0.001	1.75 (1.33–2.30)	<0.001

Crude OR for related risk factors; Microvascular complications: retinopathy, neuropathy, and nephropathy; Macrovascular complications: coronary artery disease and stroke. Statistical analysis using binary regression model,  $P < 0.05$  indicated significant associations.

OR, odds ratio; CI, confidence interval; HbA1c, glycated hemoglobin; T2DM, type 2 diabetes mellitus; HT, hypertension; NA, no available data; BMI, body mass index; TG, triglyceride; HDL, high-density lipoprotein.

<sup>a</sup>Non-diabetes mellitus (DM) vs. DM with complication, <sup>b</sup>DM without complication vs. DM with complication, <sup>c</sup>OR adjusted for age, sex, BMI, and dyslipidemia.

**Supplementary Table 5.** Clinical and biochemical characteristics of the study population (categorized by hypertensive status)

Parameter	Non-HT ( <i>n</i> =467)	HT ( <i>n</i> =1,233)	<i>P</i> value
Subjects, men/women	128/339	487/746	<0.001
Diabetes	174/467 (37.3)	786/1,233 (63.7)	<0.001
Obesity	183/457 (40.0)	651/1,205 (54.0)	<0.001
Dyslipidemia	422/465 (90.8)	1,039/1,193 (87.1)	0.038
Metabolic syndrome	106/467 (22.7)	644/1,233 (52.2)	<0.001
Hypertriglyceridemia	146/465 (31.4)	441/1,182 (37.3)	0.024
Low HDL-C level	166/466 (35.6)	559/1,181 (47.3)	<0.001
Age, yr	61.87±9.17	64.85±9.56	<0.001
BMI, kg/m <sup>2</sup>	24.42±3.79	25.89±4.54	<0.001
SBP, mm Hg	121.73±11.83	140.60±18.62	<0.001
DBP, mm Hg	71.72±8.71	76.70±12.16	<0.001
HbA1c, %	6.85±2.03	7.08±1.68	0.064
FPG, mmol/L	6.60±2.86	7.22±2.87	<0.001
TC, mmol/L	5.44±1.19	4.93±1.27	<0.001
TG, mmol/L	1.55±1.00	1.70±1.01	0.006
HDL-C, mmol/L	1.47±0.41	1.36±0.40	<0.001
LDL-C, mmol/L	3.48±1.09	3.07±1.10	<0.001
Non HDL-C, mmol/L	3.97±1.16	3.59±1.22	<0.001
TC/HDL-C	3.93±1.19	3.88±1.34	0.505
TG/HDL-C	1.23±1.08	1.46±1.24	<0.001
LDL-C/HDL-C	2.51±0.96	2.41±1.03	0.086
Non HDL-C/HDL-C	2.93±1.19	2.88±1.34	0.505
BUN, mmol/L	4.93±1.68	6.05±3.39	<0.001
Creatinine, μmol/L	81.07±61.89	105.44±113.26	<0.001
eGFR, mL/s/m <sup>2</sup>	1.45±0.30	1.18±0.45	<0.001
Uric acid, μmol/L	306.21±87.19	335.73±91.67	<0.001

Values are presented as number/total number (%) or mean ± standard deviation. *P*<0.05 indicated significant associations.

HT, hypertension; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

**Supplementary Table 6.** Association between SNPs and hypertension in all subjects

SNP	Non-HT (n=467)	HT (n=1,233)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>TCF7L2 rs7903146</i>							
Additive							
CT	47 (10.1)	92 (7.5)	0.092	1		1	
CC	420 (89.9)	1,141 (92.5)		1.39 (0.96–2.01)	0.082	1.56 (1.05–2.32)	0.030
Allele							
T	47 (5.0)	92 (3.7)	0.099	1		1	
C	887 (95.0)	2,374 (96.3)		1.37 (0.95–1.96)	0.088	1.52 (1.03–2.25)	0.034
<i>TCF7L2 rs290487</i>							
Additive							
TT	142 (29.3)	342 (70.7)	0.542	1		1	
TC	223 (26.9)	605 (73.1)		1.13 (0.88–1.45)	0.348	1.27 (0.97–1.67)	0.081
CC	102 (26.3)	286 (73.7)		1.16 (0.86–1.57)	0.319	1.33 (0.96–1.84)	0.087
Dominant							
TT	142 (29.3)	342 (70.7)	0.279	1		1	
TC+CC	325 (26.7)	891 (73.3)		1.14 (0.90–1.44)	0.276	1.29 (1.00–1.66)	0.050
Recessive							
TT+TC	365 (27.8)	947 (72.2)	0.561	1		1	
CC	102 (26.3)	286 (73.7)		1.08 (0.84–1.40)	0.553	1.14 (0.86–1.50)	0.354
Allele							
T	507 (28.2)	1289 (71.8)	0.299	1		1	
C	427 (26.6)	1,177 (73.4)		1.08 (0.93–1.26)	0.294	1.14 (0.86–1.50)	0.354
<i>KCNQ1 rs2237892</i>							
Additive							
CC	247 (52.9)	620 (50.3)	0.539	1		1	
CT	186 (39.8)	508 (41.2)		1.09 (0.87–1.36)	0.459	1.08 (0.85–1.38)	0.518
TT	34 (7.3)	105 (8.5)		1.23 (0.81–1.86)	0.326	1.30 (0.83–2.02)	0.252
Dominant							
CC	247 (52.9)	620 (50.3)	0.356	1		1	
CT+TT	220 (47.1)	613 (49.7)		1.11 (0.90–1.37)	0.337	1.12 (0.89–1.40)	0.353
Recessive							
CC+CT	433 (92.7)	1,128 (91.5)	0.429	1		1	
TT	34 (7.3)	105 (8.5)		1.19 (0.79–1.77)	0.407	1.25 (0.81–1.92)	0.309
Allele							
C	680 (72.8)	1,748 (70.9)	0.269	1		1	
T	254 (27.2)	718 (29.1)		1.10 (0.93–1.30)	0.269	1.25 (0.81–1.92)	0.309
<i>KCNQ1 rs2237897</i>							
Additive							
CC	261 (55.9)	659 (53.4)	0.527	1		1	
CT	181 (38.8)	493 (40.0)		1.08 (0.86–1.35)	0.505	1.10 (0.87–1.40)	0.436
TT	25 (5.4)	81 (6.1)		1.28 (0.80–2.06)	0.299	1.41 (0.85–2.33)	0.182

(Continued to the next page)

Supplementary Table 6. Continued

SNP	Non-HT (n=467)	HT (n=1,233)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Dominant							
CC	261 (55.9)	659 (53.4)	0.383	1		1	
CT+TT	206 (44.1)	574 (46.6)		1.10 (0.89–1.37)	0.367	1.14 (0.90–1.43)	0.276
Recessive							
CC+CT	442 (94.6)	1,152 (93.4)	0.372	1		1	
TT	25 (5.4)	81 (6.1)		1.24 (0.78–1.97)	0.355	1.35 (0.83–2.21)	0.229
Allele							
C	703 (75.3)	1,811 (73.4)	0.294	1		1	
T	231 (24.7)	655 (26.6)		1.10 (0.93–1.31)	0.278	1.35 (0.83–2.21)	0.229
<i>KCNJ11 rs5219</i>							
Additive							
GG	191 (26.6)	528 (73.4)	0.736	1		1	
GA	214 (28.4)	540 (71.6)		0.91 (0.73–1.15)	0.435	0.87 (0.68–1.11)	0.267
AA	62 (27.3)	165 (72.7)		0.96 (0.69–1.35)	0.824	1.06 (0.74–1.52)	0.768
Dominant							
GG	191 (26.6)	528 (73.4)	0.475	1		1	
AA+GA	276 (28.1)	705 (71.9)		0.92 (0.74–1.15)	0.474	0.91 (0.72–1.15)	0.425
Recessive							
GA+GG	405 (27.5)	1,068 (72.5)	1.000	1		1	
AA	62 (27.3)	165 (72.7)		1.01 (0.74–1.38)	0.954	1.14 (0.81–1.59)	0.463
Allele							
G	338 (28.0)	870 (72.0)	0.630	1		1	
A	596 (27.2)	1,596 (72.8)		0.96 (0.82–1.13)	0.621	1.14 (0.81–1.59)	0.463

Values are presented as number (%). OR adjusted for age, sex, BMI, type 2 diabetes mellitus, and dyslipidemia.

SNP, single nucleotide polymorphism; HT, hypertension; OR, odds ratio; CI, confidence interval; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *KCNJ11*, inwardly-rectifying potassium channel, subfamily J, member 11 gene.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 7.** Prevalence of diabetic complications in the study population

Diabetic complication	Case no./total no. (%)
0 Disease	577/960 (60.1)
1 Disease	305/960 (31.8)
Retinopathy	61/305 (20.0)
Nephropathy	112/305 (36.7)
Neuropathy	11/305 (3.6)
CAD	100/305 (32.8)
Stroke	21/305 (6.9)
2 Diseases	63/960 (6.6)
Retinopathy and nephropathy	32/63 (50.8)
Retinopathy and neuropathy	6/63 (9.5)
Nephropathy and neuropathy	11/63 (17.5)
Retinopathy and CAD	1/63 (1.6)
Retinopathy and stroke	1/63 (1.6)
Nephropathy and CAD	6/63 (9.5)
Nephropathy and stroke	6/63 (9.5)
3 Diseases	14/960 (1.5)
Retinopathy, nephropathy, and neuropathy	6/14 (42.9)
Retinopathy, nephropathy, and CAD	3/14 (21.4)
Retinopathy, nephropathy, and stroke	4/14 (28.6)
Neuropathy, CAD, and stroke	1/14 (7.1)
4 Diseases	1/960 (0.1)
Retinopathy, nephropathy, CAD, and stroke	1/1 (100.0)
All complications	383/960 (39.9)
Microvascular complication	239/960 (24.9)
Macrovascular complication	121/960 (12.6)
Micro- or macrovascular complication	360/960 (37.5)
Micro and macrovascular complication	23/960 (2.4)
Retinopathy	115/960 (12.0)
Nephropathy	181/960 (18.9)
Neuropathy	35/960 (3.6)
CAD	112/960 (11.7)
Stroke	34/960 (3.5)

Microvascular complications: retinopathy, neuropathy, and nephropathy;  
 macrovascular complications: CAD and stroke.  
 CAD, coronary artery disease.

**Supplementary Table 8.** Association between individual SNPs and micro- and macrovascular complications in type 2 diabetes mellitus patients

SNP	DM without complications (n=577)	DM with complication (n=383)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>TCF7L2 rs7903146</i>							
Additive							
CC	526 (59.4)	359 (40.6)	0.177	1		1	
CT	51 (68.0)	24 (32.0)		0.69 (0.42–1.14)	0.148	0.65 (0.32–1.33)	0.234
Allele							
C	1,103 (59.8)	742 (40.2)	0.186	1		1	
T	51 (68.0)	24 (32.0)		0.70 (0.43–1.15)	0.156	0.58 (0.31–1.07)	0.080
<i>TCF7L2 rs290487</i>							
Additive							
CC	124 (57.9)	90 (42.1)	0.347	1		1	
TC	281 (62.6)	168 (37.4)		0.82 (0.59–1.15)	0.252	0.81 (0.53–1.24)	0.334
TT	172 (57.9)	125 (42.1)		1.00 (0.70–1.43)	0.994	0.93 (0.59–1.46)	0.751
Dominant							
CC	124 (57.9)	90 (42.1)	0.477	1		1	
TC+TT	453 (60.7)	293 (39.3)		0.89 (0.66–1.21)	0.464	0.86 (0.58–1.27)	0.445
Recessive							
CC+TC	405 (61.1)	258 (38.9)	0.355	1		1	
TT	172 (57.9)	125 (42.1)		1.14 (0.86–1.51)	0.353	1.07 (0.75–1.52)	0.715
Allele							
C	529 (60.3)	348 (39.7)	0.888	1		1	
T	625 (59.9)	418 (40.1)		1.02 (0.85–1.22)	0.860	0.86 (0.58–1.27)	0.445
<i>KCNQ1 rs2237892</i>							
Additive							
TT	37 (55.2)	30 (44.8)	0.459	1		1	
CT	240 (62.2)	146 (37.8)		0.75 (0.44–1.27)	0.282	0.76 (0.40–1.42)	0.387
CC	300 (59.2)	207 (40.8)		0.85 (0.51–1.42)	0.538	0.81 (0.43–1.50)	0.495
Dominant							
TT	37 (55.2)	30 (44.8)	0.438	1		1	
CT+CC	540 (60.5)	353 (39.5)		0.81 (0.49–1.33)	0.398	0.78 (0.43–1.43)	0.426
Recessive							
TT+CT	277 (61.1)	176 (38.9)	0.553	1		1	
CC	300 (59.2)	207 (40.8)		1.09 (0.84–1.41)	0.532	1.02 (0.73–1.42)	0.921
Allele							
T	314 (60.4)	206 (39.6)	0.916	1		1	
C	840 (60.0)	560 (40.0)		1.02 (0.83–1.25)	0.878	0.78 (0.43–1.43)	0.426

(Continued to the next page)

Supplementary Table 8. Continued

SNP	DM without complications (n=577)	DM with complication (n=383)	P value <sup>a</sup>	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>KCNQ1 rs2237897</i>							
Additive							
TT	26 (54.2)	22 (45.8)	0.274	1		1	
CT	218 (57.8)	159 (42.2)		0.86 (0.47–1.58)	0.629	1.09 (0.51–2.34)	0.829
CC	333 (62.2)	202 (37.8)		0.72 (0.40–1.30)	0.272	0.94 (0.44–1.99)	0.863
Dominant							
TT	26 (54.2)	22 (45.8)	0.450	1		1	
CC+CT	551 (60.4)	361 (39.6)		0.77 (0.43–1.39)	0.390	1.00 (0.48–2.09)	0.994
Recessive							
TT+CT	244 (57.4)	181 (42.6)	0.144	1		1	
CC	333 (62.2)	202 (37.8)		0.82 (0.63–1.06)	0.129	0.87 (0.62–1.21)	0.405
Allele							
T	270 (57.1)	203 (42.9)	0.130	1		1	
C	884 (61.1)	563 (38.9)		0.85 (0.69–1.05)	0.122	1.00 (0.48–2.09)	0.994
<i>KCNJ11 rs5219</i>							
Additive							
GG	235 (60.3)	155 (39.7)	0.861	1		1	
GA	265 (59.4)	181 (40.6)		1.04 (0.79–1.37)	0.805	1.21 (0.85–1.74)	0.292
AA	77 (62.1)	47 (37.9)		0.93 (0.61–1.40)	0.715	1.12 (0.67–1.86)	0.665
Dominant							
GG	235 (60.3)	155 (39.7)	0.947	1		1	
GA+AA	342 (60.0)	228 (40.0)		1.01 (0.78–1.32)	0.936	1.19 (0.85–1.67)	0.316
Recessive							
GG+GA	500 (59.8)	336 (40.2)	0.694	1		1	
AA	77 (62.1)	47 (37.9)		0.91 (0.62–1.34)	0.627	1.01 (0.63–1.61)	0.974
Allele							
G	735 (60.0)	491 (40.0)	0.884	1		1	
A	419 (60.4)	275 (39.6)		0.98 (0.81–1.19)	0.856	1.01 (0.63–1.61)	0.974

Values are presented as number (%). OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index. SNP, single nucleotide polymorphism; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *KCNJ11*, inwardly-rectifying potassium channel, subfamily J, member 11 gene.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 9.** Combined effect of two risk alleles on diabetic nephropathy in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	Control (n=577)	DN (n=181)	P value <sup>a</sup>	OR (95% CI)	P value	
1	<i>KCNQ1</i> rs2237892 (C)	0–1	70 (81.4)	16 (18.6)	0.050	1	0.084	
		2	481 (76.3)	149 (23.7)		1.82 (0.92–3.60)		
		3–4	26 (61.9)	16 (38.1)		2.26 (0.79–6.43)		
2	<i>TCF7L2</i> rs7903146 (C)	1–2	153 (75.0)	51 (25.0)	0.532	1	0.238	
		3	263 (78.0)	74 (22.0)		0.75 (0.46–1.22)		
		4	161 (74.2)	56 (25.8)		0.82 (0.48–1.39)		
3	<i>TCF7L2</i> rs7903146 (C)	1–2	59 (77.6)	17 (22.4)	0.536	1	0.712	
		3	243 (77.9)	69 (22.1)		1.15 (0.55–2.39)		
			4	275 (74.3)		95 (25.7)		1.47 (0.72–2.99)
4	<i>TCF7L2</i> rs7903146 (C)	1	25 (86.2)	4 (13.8)	0.445	1	0.497	
		2	331 (77.0)	99 (23.0)		1.59 (0.42–6.08)		
			3	198 (74.2)		69 (25.8)		1.77 (0.46–6.86)
			4	23 (71.9)		9 (28.1)		1.68 (0.34–8.32)
5	<i>TCF7L2</i> rs290487 (T)	0–1	73 (74.5)	25 (25.5)	0.232	1	0.495	
		2	197 (75.8)	63 (24.2)		1.26 (0.65–2.42)		
			3	225 (79.5)		58 (20.5)		0.94 (0.49–1.80)
			4	82 (70.1)		35 (29.9)		1.18 (0.56–2.50)
6	<i>KCNQ1</i> rs2237892 (C)	0	74 (73.3)	27 (26.70)	0.519	1	0.070	
		1	210 (78.9)	56 (21.1)		0.57 (0.31–1.05)		
			2	201 (75.8)		64 (24.2)		0.63 (0.34–1.18)
			3–4	92 (73.0)		34 (27.0)		0.67 (0.34–1.33)

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. Control, T2DM without complication. OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index.

T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; DN, diabetic nephropathy; OR, odds ratio; CI, confidence interval; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *TCF7L2*, transcription factor 7-like 2.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 10.** Combined effect of three risk alleles on diabetic nephropathy in T2DM patients

Model no.	SNPs in model (effect allele)	No.of effect allele (GRS)	Control (n=577)	DN (n=181)	P value <sup>a</sup>	OR (95% CI)	P value
1	<i>TCF7L2</i> rs7903146 (C)	2-3	116 (83.5)	23 (16.5)	0.029	1	
	<i>KCNQ1</i> rs2237892 (C)	4	437 (75.1)	145 (24.9)		2.02 (1.11-3.68)	0.021
	<i>KCNQ1</i> rs2237897 (T)	5-6	24 (64.9)	13 (35.1)		2.28 (0.82-6.35)	0.115
2	<i>TCF7L2</i> rs7903146 (C)	1-3	93 (76.9)	28 (23.1)	0.521	1	
	<i>TCF7L2</i> rs290487 (T)	4	199 (75.7)	64 (24.3)		1.74 (0.92-3.29)	0.089
	<i>KCNQ1</i> rs2237892 (C)	5	206 (78.3)	57 (21.7)		1.20 (0.63-2.28)	0.578
		6	79 (71.2)	32 (28.8)		1.42 (0.68-3.00)	0.354
3	<i>TCF7L2</i> rs7903146 (C)	1-2	91 (75.8)	29 (24.2)	0.678	1	
	<i>TCF7L2</i> rs290487 (T)	3	208 (78.2)	58 (21.8)		0.57 (0.31-1.03)	0.062
	<i>KCNQ1</i> rs2237897 (T)	4	197 (75.8)	63 (24.2)		0.68 (0.37-1.24)	0.207
		5-6	81 (72.3)	31 (27.7)		0.72 (0.36-1.43)	0.344
4	<i>TCF7L2</i> rs290487 (T)	0-2	149 (74.9)	50 (25.1)	0.102	1	
	<i>KCNQ1</i> rs2237892 (C)	3	266 (79.6)	68 (20.4)		0.89 (0.54-1.45)	0.626
	<i>KCNQ1</i> rs2237897 (T)	4-6	162 (72.0)	63 (28.0)		0.99 (0.59-1.66)	0.961

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. Control, T2DM without complication. OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index.

T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; DN, diabetic nephropathy; OR, odds ratio; CI, confidence interval; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 11.** Combined effect of two risk alleles on CAD in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	Control (n=577)	CAD (n=112)	P value <sup>a</sup>	OR (95% CI)	P value	
1	<i>KCNQ1</i> rs2237892 (C) <i>KCNQ1</i> rs2237897 (T)	0–1	70 (92.1)	6 (7.9)	0.012	1	0.088	
		2	481 (83.5)	95 (16.5)		2.51 (0.87–7.24)		
		3–4	26 (70.3)	11 (29.7)		7.31 (2.03–26.35)		0.002
2	<i>TCF7L2</i> rs7903146 (C) <i>TCF7L2</i> rs290487 (T)	1–2	153 (84.5)	28 (15.5)	0.675	1	0.845	
		3	263 (84.6)	48 (15.4)		0.94 (0.52–1.72)		
		4	161 (81.7)	36 (18.3)		1.12 (0.59–2.12)		0.738
3	<i>TCF7L2</i> rs7903146 (C) <i>KCNQ1</i> rs2237892 (C)	1–2	59 (84.3)	11 (15.7)	0.113	1	0.399	
		3	243 (87.1)	36 (12.9)		0.69 (0.29–1.65)		
		4	275 (80.9)	65 (19.1)		1.25 (0.55–2.86)		0.596
4	<i>TCF7L2</i> rs7903146 (C) <i>KCNQ1</i> rs2237897 (T)	1	25 (86.2)	4 (13.8)	0.502	1	0.276	
		2	331 (84.4)	61 (15.6)		3.16 (0.40–25.11)		
		3	198 (83.5)	39 (16.5)		3.49 (0.43–28.04)		0.241
		4	23 (74.2)	8 (25.8)		7.35 (0.79–68.37)		0.080
5	<i>TCF7L2</i> rs290487 (T) <i>KCNQ1</i> rs2237892 (C)	0–1	73 (82.0)	16 (18.0)	0.273	1	0.368	
		2	197 (86.4)	31 (13.6)		0.70 (0.32–1.52)		
		3	225 (84.3)	42 (15.7)		0.72 (0.35–1.50)		0.381
		4	82 (78.1)	23 (21.9)		1.23 (0.54–2.84)		0.620
6	<i>TCF7L2</i> rs290487 (T) <i>KCNQ1</i> rs2237897 (T)	0	74 (84.1)	14 (15.9)	0.796	1	0.682	
		1	210 (85.4)	36 (14.6)		0.85 (0.38–1.89)		
		2	201 (82.0)	44 (18.0)		1.12 (0.51–2.49)		0.776
		3–4	92 (83.6)	18 (16.4)		0.96 (0.39–2.37)		0.925

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. Control, T2DM without complication. OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index.

CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; OR, odds ratio; CI, confidence interval; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *TCF7L2*, transcription factor 7-like 2.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 12.** Combined effect of three risk alleles on CAD in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	Control (n=577)	CAD (n=112)	P value <sup>a</sup>	OR (95% CI)	P value
1	<i>TCF7L2</i> rs7903146 (C)	2–3	116 (90.6)	12 (9.4)	0.006	1	
	<i>KCNQ1</i> rs2237892 (C)	4	437 (83.1)	89 (16.9)		2.37 (1.04–5.41)	0.040
	<i>KCNQ1</i> rs2237897 (T)	5–6	24 (68.6)	11 (31.4)		6.90 (2.30–20.71)	0.001
2	<i>TCF7L2</i> rs7903146 (C)	1–3	93 (83.0)	19 (17.0)	0.162	1	
	<i>TCF7L2</i> rs290487 (T)	4	199 (87.3)	29 (12.7)		0.74 (0.35–1.57)	0.436
	<i>KCNQ1</i> rs2237892 (C)	5	206 (83.4)	41 (16.6)		0.87 (0.43–1.77)	0.704
		6	79 (77.5)	23 (22.5)		1.48 (0.66–3.31)	0.345
3	<i>TCF7L2</i> rs7903146 (C)	1–2	91 (85.0)	16 (15.0)	0.678	1	
	<i>TCF7L2</i> rs290487 (T)	3	208 (85.6)	35 (14.4)		0.90 (0.41–1.95)	0.785
	<i>KCNQ1</i> rs2237897 (T)	4	197 (81.7)	44 (18.3)		1.32 (0.62–2.82)	0.475
		5–6	81 (82.7)	17 (17.3)		1.09 (0.45–2.68)	0.843
4	<i>TCF7L2</i> rs290487 (T)	0–2	149 (86.1)	24 (13.9)	0.321	1	
	<i>KCNQ1</i> rs2237892 (C)	3	266 (84.8)	49 (15.6)		0.96 (0.52–1.78)	0.895
	<i>KCNQ1</i> rs2237897 (T)	4–6	162 (80.6)	39 (19.4)		1.35 (0.71–2.56)	0.368

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. Control, T2DM without complication. OR adjusted for age, sex, hypertension, dyslipidemia, glycosylated hemoglobin, and body mass index.

CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; OR, odds ratio; CI, confidence interval; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 13.** Combined effect of two risk alleles on nephropathy and/or CAD in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	Control (n=577)	DN+CAD (n=283)	P value <sup>a</sup>	OR (95% CI)	P value	
1	<i>KCNQ1</i> rs2237892 (C)	0-1	70 (76.1)	22 (23.9)	0.006	1		
		2	481 (67.2)	235 (32.8)		1.78 (0.99–3.21)	0.056	
		3-4	26 (50.0)	26 (50.0)		3.49 (1.49–8.15)	0.004	
2	<i>TCF7L2</i> rs7903146 (C)	1-2	153 (66.5)	77 (33.5)	0.588	1		
		3	263 (68.8)	119 (31.2)		0.95 (0.63–1.42)	0.800	
		4	161 (64.9)	87 (35.1)		1.08 (0.70–1.69)	0.721	
3	<i>TCF7L2</i> rs7903146 (C)	1-2	59 (67.8)	28 (32.2)	0.271	1		
		3	243 (70.0)	104 (30.0)		0.94 (0.51–1.70)	0.825	
			4	275 (64.6)		151 (35.4)	1.32 (0.74–2.35)	0.350
4	<i>TCF7L2</i> rs7903146 (C)	1	25 (75.8)	8 (24.2)	0.290	1		
		2	331 (68.5)	152 (31.5)		2.63 (0.75–9.23)	0.132	
			3	198 (65.1)		106 (34.9)	3.04 (0.86–10.80)	0.086
			4	23 (57.5)		17 (42.5)	4.18 (1.01–17.34)	0.049
5	<i>TCF7L2</i> rs290487 (T)	0-1	73 (64.0)	41 (36.0)	0.248	1		
		2	197 (68.4)	91 (31.6)		0.98 (0.57–1.66)	0.931	
			3	225 (69.7)		98 (30.3)	0.85 (0.50–1.44)	0.546
			4	82 (60.7)		53 (39.3)	1.25 (0.68–2.29)	0.474
6	<i>TCF7L2</i> rs290487 (T)	0	74 (65.5)	39 (34.5)	0.614	1		
		1	210 (69.8)	91 (30.2)		0.76 (0.45–1.28)	0.297	
			2	201 (66.6)		101 (33.4)	0.87 (0.51–1.46)	0.592
			3-4	92 (63.9)		52 (36.1)	0.96 (0.53–1.72)	0.886

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. Control, T2DM without complication. OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index.

CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; DN, diabetic nephropathy; OR, odds ratio; CI, confidence interval; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *TCF7L2*, transcription factor 7-like 2.

<sup>a</sup>P value after 100,000 permutation.

**Supplementary Table 14.** Combined effect of three risk alleles on nephropathy and/or CAD in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	Control (n=577)	DN+CAD (n=283)	P value <sup>a</sup>	OR (95% CI)	P value
1	<i>TCF7L2</i> rs7903146 (C)	2–3	116 (76.8)	35 (23.2)	0.002	1	0.003
	<i>KCNQ1</i> rs2237892 (C)	4	437 (66.0)	225 (34.0)		2.13 (1.28–3.53)	
	<i>KCNQ1</i> rs2237897 (T)	5–6	24 (51.1)	23 (48.9)		3.92 (1.75–8.76)	
2	<i>TCF7L2</i> rs7903146 (C)	1–3	93 (66.4)	47 (33.6)	0.458	1	0.573
	<i>TCF7L2</i> rs290487 (T)	4	199 (68.9)	90 (31.1)		1.16 (0.70–1.93)	
	<i>KCNQ1</i> rs2237892 (C)	5	206 (68.2)	96 (31.8)		1.09 (0.66–1.81)	
		6	79 (61.2)	50 (38.8)		1.46 (0.80–2.64)	
3	<i>TCF7L2</i> rs7903146 (C)	1–2	91 (67.9)	43 (32.1)	0.600	1	0.604
	<i>TCF7L2</i> rs290487 (T)	3	208 (69.3)	92 (30.7)		0.88 (0.53–1.45)	
	<i>KCNQ1</i> rs2237897 (T)	4	197 (66.3)	100 (33.7)		1.04 (0.63–1.72)	
		5–6	81 (62.8)	48 (37.2)		1.16 (0.64–2.07)	
4	<i>TCF7L2</i> rs290487 (T)	0–2	149 (67.4)	72 (32.6)	0.181	1	0.691
	<i>KCNQ1</i> rs2237892 (C)	3	266 (69.8)	115 (30.2)		0.92 (0.61–1.39)	
	<i>KCNQ1</i> rs2237897 (T)	4–6	162 (62.8)	96 (37.2)		1.21 (0.78–1.87)	

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. Control, T2DM without complication. OR adjusted for age, sex, hypertension, dyslipidemia, glycosylated hemoglobin, and body mass index.

CAD, coronary artery disease; T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; DN, diabetic nephropathy; OR, odds ratio; CI, confidence interval; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1.

<sup>a</sup>P value after 100,000 permutation.

**Supplementary Table 15.** Combined effect of two risk alleles on micro- and macrovascular complications in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	DM without complication (n=577)	DM with complications (n=383)	P value <sup>a</sup>	OR (95% CI)	P value
1	<i>KCNQ1</i> rs2237892 (C) <i>KCNQ1</i> rs2237897 (T)	0–1	70 (66.0)	36 (34.0)	0.027	1	
		2	481 (60.4)	315 (39.6)		1.33 (0.82–2.15)	0.245
		3–4	26 (44.8)	32 (55.2)		2.06 (0.97–4.39)	0.061
2	<i>TCF7L2</i> rs7903146 (C) <i>TCF7L2</i> rs290487 (T)	1–2	153 (60.5)	100 (39.5)	0.549	1	
		3	263 (61.6)	164 (38.4)		1.01 (0.70–1.45)	0.969
		4	161 (57.5)	119 (42.5)		1.18 (0.79–1.75)	0.414
3	<i>TCF7L2</i> rs7903146 (C) <i>KCNQ1</i> rs2237892 (C)	1–2	59 (59.6)	40 (40.4)	0.603	1	
		3	243 (62.0)	149 (38.0)		0.93 (0.56–1.56)	0.794
		4	275 (58.6)	194 (41.4)		1.06 (0.64–1.76)	0.819
4	<i>TCF7L2</i> rs7903146 (C) <i>KCNQ1</i> rs2237897 (T)	1	25 (67.6)	12 (32.4)	0.229	1	
		2	331 (62.2)	201 (37.8)		1.80 (0.70–4.64)	0.227
		3	198 (57.1)	149 (42.9)		2.25 (0.86–5.88)	0.097
		4	23 (52.3)	21 (47.7)		2.83 (0.91–8.83)	0.072
5	<i>TCF7L2</i> rs290487 (T) <i>KCNQ1</i> rs2237892 (C)	0–1	73 (57.5)	54 (42.5)	0.467	1	
		2	197 (60.8)	127 (39.2)		0.98 (0.61–1.56)	0.920
		3	225 (62.3)	136 (37.7)		0.85 (0.54–1.36)	0.496
		4	82 (55.4)	66 (44.6)		1.09 (0.63–1.88)	0.766
6	<i>TCF7L2</i> rs290487 (T) <i>KCNQ1</i> rs2237897 (T)	0	74 (58.3)	53 (41.7)	0.354	1	
		1	210 (63.6)	120 (36.4)		0.77 (0.48–1.23)	0.274
		2	201 (59.5)	137 (40.5)		0.92 (0.58–1.46)	0.716
		3–4	92 (55.8)	73 (44.2)		1.07 (0.63–1.80)	0.807

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. DM with complications: micro- and/or macrovascular complications; microvascular complications: retinopathy, nephropathy, and neuropathy; macrovascular complications: coronary artery disease and stroke. OR adjusted for age, sex, hypertension, dyslipidemia, glycosylated hemoglobin, and body mass index.

T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; *TCF7L2*, transcription factor 7-like 2.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 16.** Combined effect of three risk alleles on micro- and macrovascular complications in T2DM patients

Model no.	SNPs in model (effect allele)	No. of effect allele (GRS)	DM without complication (n=577)	DM with complications (n=383)	P value <sup>a</sup>	OR (95% CI)	P value
1	<i>TCF7L2</i> rs7903146 (C)	2-3	116 (67.8)	55 (32.2)	0.010	1	0.031
	<i>KCNQ1</i> rs2237892 (C)	4	437 (59.4)	299 (40.6)		1.57 (1.04-2.35)	
	<i>KCNQ1</i> rs2237897 (T)	5-6	24 (45.3)	29 (54.7)		2.33 (1.13-4.79)	
2	<i>TCF7L2</i> rs7903146 (C)	1-3	93 (59.6)	63 (40.4)	0.678	1	0.633
	<i>TCF7L2</i> rs290487 (T)	4	199 (61.2)	126 (38.8)		1.11 (0.71-1.74)	
	<i>KCNQ1</i> rs2237892 (C)	5	206 (61.1)	131 (38.9)		1.04 (0.66-1.61)	
		6	79 (55.6)	63 (44.4)		1.24 (0.73-2.11)	
3	<i>TCF7L2</i> rs7903146 (C)	1-2	91 (60.7)	59 (39.3)	0.325	1	0.472
	<i>TCF7L2</i> rs290487 (T)	3	208 (63.2)	121 (36.8)		0.85 (0.54-1.33)	
	<i>KCNQ1</i> rs2237897 (T)	4	197 (59.3)	135 (40.7)		1.05 (0.67-1.64)	
		5-6	81 (54.4)	68 (45.6)		1.26 (0.75-2.12)	
4	<i>TCF7L2</i> rs290487 (T)	0-2	149 (60.1)	99 (39.9)	0.135	1	0.571
	<i>KCNQ1</i> rs2237892 (C)	3	266 (63.2)	155 (36.8)		0.90 (0.63-1.29)	
	<i>KCNQ1</i> rs2237897 (T)	4-6	162 (55.7)	129 (44.3)		1.19 (0.80-1.75)	

Values are presented as number (%).  $P \leq 0.05$  indicated statistical significance. DM with complications: micro- and/or macrovascular complications; microvascular complications: retinopathy, nephropathy, and neuropathy; macrovascular complications: coronary artery disease and stroke. OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index.

T2DM, type 2 diabetes mellitus; SNP, single nucleotide polymorphism; GRS, genetic risk score; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; *TCF7L2*, transcription factor 7-like 2; *KCNQ1*, potassium voltage-gated channel subfamily Q member 1.

<sup>a</sup>P value after 100,000 permutations.

**Supplementary Table 17.** Association of *KCNQ1* haplotype and diabetic nephropathy in type 2 diabetes mellitus patients

<i>KCNQ1</i>	rs2237892	rs2237897	DM without complication ( <i>n</i> =1,154)	DN ( <i>n</i> =362)	<i>P</i> value	OR (95% CI)	<i>P</i> value
Haplotype	C/T	C/T			0.147		
H1	T	C	72 (6.2)	16 (4.4)		1	
H2	T	T	242 (21.0)	77 (21.3)		0.97 (0.39–2.40)	0.940
H3	C	C	812 (70.4)	253 (69.9)		1.20 (0.52–2.77)	0.678
H4	C	T	28 (2.4)	16 (4.4)		1.08 (0.24–4.80)	0.923

Values are presented as number (%). OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index. *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; DM, diabetes mellitus; DN, diabetic nephropathy; OR, odds ratio; CI, confidence interval.

**Supplementary Table 18.** Association of *KCNQ1* haplotype and CAD in type 2 diabetes mellitus patients

<i>KCNQ1</i>	rs2237892	rs2237897	DM without complication (n=1,154)	CAD (n=224)	P value	OR (95% CI)	P value
Haplotype	C/T	C/T			0.020		
H1	T	C	72 (6.2)	6 (2.7)		1	
H2	T	T	242 (21.0)	46 (20.5)		1.61 (0.44–5.88)	0.471
H3	C	C	812 (70.4)	160 (71.4)		1.62 (0.48–5.53)	0.441
H4	C	T	28 (2.4)	12 (5.4)		5.64 (1.26–25.28)	0.024

Values are presented as number (%). OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index. *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; CAD, coronary artery disease; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval.

**Supplementary Table 19.** Association of *KCNQ1* haplotype and nephropathy and/or CAD in type 2 diabetes mellitus patients

<i>KCNQ1</i>	rs2237892	rs2237897	DM without complication ( <i>n</i> = 1,154)	Nep+CAD ( <i>n</i> = 566)	<i>P</i> value	OR (95% CI)	<i>P</i> value
Haplotype	C/T	C/T					
H1	T	C	72 (6.2)	22 (3.9)		1	
H2	T	T	242 (21.0)	122 (21.6)	0.015	1.15 (0.53–2.51)	0.719
H3	C	C	812 (70.4)	395 (69.8)		1.31 (0.63–2.71)	0.467
H4	C	T	28 (2.4)	27 (4.8)		2.40 (0.82–6.96)	0.109

Values are presented as number (%). OR adjusted for age, sex, hypertension, dyslipidemia, glycated hemoglobin, and body mass index. *KCNQ1*, potassium voltage-gated channel subfamily Q member 1; CAD, coronary artery disease; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval.

**Supplementary Table 20.** Combined effect of risk alleles on the parameters related to the severity of type 2 diabetes mellitus in patients

Variable	No. of effect allele (GRS)			P value	P for trend
	0–1	2	3–4		
Combined two risk alleles					
Number	106	796	58		
HbA1c, %	7.54±1.41	7.65±1.80	7.73±1.93	0.812	0.521
FPG, mmol/L	8.24±2.60	8.39±3.38	8.73±3.13	0.672	0.403
eGFR, mL/s/m <sup>2</sup>	1.21±0.42	1.13±0.46	1.03±0.59	0.131	0.044
BUN, mmol/L	5.98±2.32	6.39±3.73	7.54±5.52	0.046	0.024
Creatinine, µmol/L	93.66±40.76	113.02±120.23	179.30±273.98	<0.001	<0.001
Combined three risk alleles					
Number	171	736	53		
HbA1c, %	7.57±1.55	7.65±1.80	7.78±2.01	0.792	0.511
FPG, mmol/L	8.42±3.25	8.37±3.30	8.64±3.23	0.854	0.843
eGFR, mL/s/m <sup>2</sup>	1.19±0.43	1.13±0.47	1.09±0.57	0.356	0.163
BUN, mmol/L	6.11±2.51	6.43±3.87	7.14±5.11	0.243	0.113
Creatinine, µmol/L	96.01±44.50	116.22±130.90	157.42±246.26	0.010	0.004

Values are presented as mean±standard deviation. Two combined risk alleles, potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) rs2237892 (C) and *KCNQ1* rs2237897 (T); Three combined risk alleles, transcription factor 7-like 2 (*TCF7L2*) rs7903146 (C), *KCNQ1* rs2237892 (C) and *KCNQ1* rs2237897 (T).  $P < 0.05$  indicated statistical significance.

GRS, genetic risk score; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen.