Supplementary Appendix

Genetic Variation in *TCF7L2* rs7903146 Correlating with Peripheral Arterial Disease in Longstanding Type 2 Diabetes

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Supplementary Methods

Clinical and laboratory data

The clinical information of the subjects was recorded by their physicians. From each patient, their medical history, familial history of diabetes, age of diabetes onset, and smoking habit. , Body weight and height was measured, and their body mass index (BMI) was calculated as body weight kilograms divided by the square of height in meters. Blood pressure was measured using a standard methods. Venous blood samples were collected after an overnight fasting period of at least 12 h. HbA1c was measured by affinity chromatography (Bio-Rad Laboratories, Hercules, CA, USA) in a National Glycated Hemoglobin Standardization Program level II-certified laboratory. Fasting plasma glucose, aspartate transaminase (AST), alanine transaminase (ALT), and lipid profile were measured using an automated chemistry analyzer (Hitachi 7600, Tokyo, Japan). Fasting insulin and C-peptide were measured by chemiluminescence enzyme immunoassays (Abbott, Lake Forest, IL, USA). The estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease equation.

Assessment of peripheral arterial disease

PAD was defined as ankle-brachial index (ABI) ≤0.9 or previous history of lower extremity bypass and/or endovascular therapy. ABI was performed in the supine position after at least 5 minutes of rest, a trained technicians used a pulse wave Doppler and ABI device (VP-1000, Colin Co., Komaki, Japan) in both dorsalis pedis arteries and brachial arteries. Technicians followed a standard protocol, systolic BP (SBP) of the brachial artery was used for the upper extremities, whereas duplicate SBPs of the dorsalis pedis artery were used for the two lower

extremities. The ABI was calculated separately for each leg by taking the higher SBP in each lower extremity and dividing by the highest upper-extremity SBP. The lowest of these two leg-specific ABIs was used to classify the ABI score for each participant.

TCF7L2 genotyping

Genomic DNA was isolated (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and stored at −20℃. Genotyping master mix (ThermoFisher, *TCF7L2* TaqMan® single nucleotide polymorphism [SNP] Genotyping Assays, Cat #4351379, Waltham, MA, USA) and fluorescent probes (ThermoFisher, Waltham, MA, USA) was used for variant detection. The PCR conditions were as follows: (1) initial denaturation for 15 seconds at 94℃, (2) annealing, and (3) extension at for 60 seconds at 60℃. The fluorescent signal intensities were analyzed using the BioRad CFX96 Real-Time PCR system (BioRad, Hercules, CA, USA).

Statistical analysis

All data are expressed as the mean ± standard deviation or frequencies with percentage. The Kolmogorov-Smirnov test for normality was performed for the appropriate statistical analyses for continuous variables. Fasting insulin, fasting C-peptide, triglyceride, high-density lipoprotein cholesterol, AST, ALT were analyzed after logarithmic transformation. To assess Hardy-Weinberg equilibrium, a likelihood ratio test was performed. The baseline characteristics were examined using chi-square test for the categorical variables and one-way analysis of variance for the continuous variables. Due to the prevalence of PAD was different according to the duration of diabetes, the analysis was divided into two groups (long duration of diabetes, ≥10 years; short duration of diabetes, <10 years). We analyzed the association

between *TCF7L2* rs7903146 variant and PAD using following multivariate logistic regression models: model 1 unadjusted; model 2 adjusted for age, sex, and BMI; model 3 further adjusted for familial history of diabetes, smoking, laboratory measurements including HbA1c, and lipid profiles. In this analysis, the presence of T/T and C/T *TCF7L2* rs7903146 genotypes were combined into a single category. Covariates were selected as potential confounding factors based on their significance in univariable regression analyses or based on their probable cardiovascular risk. Adjusted associations were shown as odds ratios (ORs) with 95% confidence intervals (CI). Two-sided P value of <0.05 were considered statistically significant. Statistical analyses were performed with SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

Table S1. Clinical and laboratory characteristics of patients with type 2 diabetes according to the transcription factor 7-like 2 (*TCF7L2*) gene variant rs7903146.

	TCF7L2 rs7903146			
	C/C	C/T	T/T	P value
Number	1679	137	2	
Age, years	64.4 ± 11.9	64.8 ± 11.9	66.0 ± 0.0	0.930
Male, n (%)	917 (54.6)	75 (54.7)	2 (100.0)	0.436
BMI, kg/m^2	25.2 ± 3.3	24.9 ± 3.0	22.8 ± 0.8	0.308
Diabetes duration, years	14.5 ± 8.5	14.7 ± 8.4	9.0 ± 0.0	0.628
Family history of diabetes, n (%)	718 (42.8)	62 (45.3)	0 (0.0)	0.384
Current smoker, n (%)	396 (23.6)	35 (25.5)	1 (50.0)	0.598
Hypertension, n (%)	390 (23.2)	33 (24.1)	1 (50.0)	0.654
HbA1c (%)	7.5 ± 1.5	7.6 ± 1.6	7.3 ± 0.4	0.640
Fasting plasma glucose, mg/dL	141.5 ± 60.3	153.2 ± 57.2	137.5 ± 10.6	0.111
Fasting C-peptide, ng/mL	2.3 ± 1.8	2.5 ± 2.1	2.7 ± 0.4	0.346
Fasting insulin, μIU/mL	14.4 ± 25.9	14.1 ± 30.0	11.4 ± 1.1	0.979

MDRD eGFR	82.7 ± 30.1	81.9 ± 31.9	70.6 ± 7.6	0.814
Total cholesterol, mg/dL	166.6 ± 36.7	171.6 ± 39.8	197.0 ± 24.0	0.165
Triglycerides, mg/dL	154.8 ± 92.5	170.6 ± 148.7	115.5 ± 17.7	0.194
HDL cholesterol, mg/dL	48.5 ± 14.0	48.0 ± 13.3	49.5 ± 10.6	0.930
LDL cholesterol, mg/dL	97.7 ± 30.9	102.8 ± 34.9	134.0 ± 7.1	0.060
AST, IU/L	25.8 ± 13.8	25.5 ± 16.4	24.5 ± 3.5	0.952
ALT, IU/L	28.5 ± 20.5	26.7 ± 25.1	26.0 ± 0.0	0.613

Data are presented as mean ± standard deviation or number (%). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HbA1c, glycated hemoglobin; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; MDRD eGFR, estimated glomerular filtration rate using the Modification of Diet in Renal Disease Study equation.

75 **Table S2.** Univariate regression analysis for peripheral arterial disease and selected risk factors

76 (n = 1818)

	OR	95% CI	P value
Age (years)	1.047	1.032 – 1.062	<0.001
Male (yes)	1.670	1.223 – 2.280	0.001
BMI (≥25 kg/m^2)	1.064	0.769 – 1.471	0.708
Familial history of diabetes (yes)	1.616	1.180 – 2.213	0.003
Duration of diabetes (≥ 10 years)	1.493	1.109 – 2.011	0.008
Current smoker (yes)	2.138	1.344 – 3.401	0.001
Hypertension (yes)	2.536	1.801 – 3.572	<0.001
HbA1c (≥7.0%)	0.788	0.490 – 1.267	0.326
Fasting plasma glucose (≥ 126 mg/dl)	0.813	0.587 – 1.125	0.212
Total cholesterol (≥200 mg/dl)	1.866	1.166 – 2.986	0.009
Triglyceride (≥250 mg/dl)	1.975	1.101 – 3.541	0.022
HDL cholesterol (male <40 mg/dl, female <50 mg/dl)	0.986	0.974 – 1.001	0.300
LDL cholesterol (≥100 mg/dl)	2.272	1.294 – 3.990	0.004

77 BMI, body mass index; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high

78 density lipoprotein; LDL, low density lipoprotein; OR, odds ratio.

Table S3. Association of the *TCF7L2* rs7903146 (C/T) genotypes with the risk of peripheral arterial disease according to the duration of type 2 diabetes

	PAD (+)	PAD (-)	OR (95% CI)	P value
Total	195	1623		
C/C	175	1504	1.000	
C/T + T/T	20	119	1.444 (0.877-2.379)	0.149
Duration of type 2 diabetes ≥10years	100	671		
C/C	86	625	1.000	
C/T + T/T	14	46	2.212 (1.167-4.192)	0.015
Duration of type 2 diabetes <10years	95	952		
C/C	89	879	1.000	
C/T + T/T	6	73	0.812 (0.343-1.919)	0.635

Data are number (%). CI, confidence interval; OR, odds ratio; PAD, peripheral arterial disease; *TCF7L2*, transcription factor 7-like 2 gene.