Association of type 2 diabetes susceptibility loci with peripheral nerve function in a Chinese population with diabetes

Jingyi Lu1,2,3,4, Yi Luo1,2,3,4, Jie Wang1,2,3,4, Cheng Hu1,2,3,4, Rong Zhang1,2,3,4, Congrong Wang1,2,3,4*, Weiping Jia1,2,3,4

1Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, 2Shanghai Diabetes Institute, 3Shanghai Key Laboratory of Diabetes Mellitus, and 4Shanghai Clinical Center for Diabetes, Shanghai, China

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*Correspondence
Congrong Wang
Tel: +86-21-2405-8254
Fax: +86-21-6436-8031
E-mail address: crwang@sjtu.edu.cn

ABSTRACT
Aims/Introduction: Previous studies have suggested a possible relationship between type 2 diabetes mellitus susceptibility loci and diabetic complications. The present study aimed to investigate the associations between type 2 diabetes mellitus loci with peripheral nerve function in a Chinese population with type 2 diabetes mellitus.

Materials and Methods: A total of 1,900 type 2 diabetes mellitus patients were recruited in the study. We selected ten single nucleotide polymorphisms (SNPs) from ten type 2 diabetes mellitus susceptibility genes previously confirmed in Chinese patients. Genotyping was carried out by using a MassARRAY Compact Analyzer. Peripheral nerve function was evaluated by nerve conduction studies in all participants. The composite Z-scores for nerve conduction parameters including conduction velocity (CV), amplitude and latency were calculated, respectively.

Results: Rs5219 of KCNJ11 (E23K, G→A) was identified to be associated with all the parameters obtained from nerve conduction studies (Z-score of CV: \(β = 0.113, P = 0.01\); Z-score of amplitude: \(β = 0.133, P = 0.01\); Z-score of latency: \(β = -0.116, P = 0.01\)) after adjustment for covariates including age, duration and glycated hemoglobin. Specifically, each copy of the A allele was related to better outcomes.

CDKAL1 rs7756992 and TCF7L2 rs7903146 correlated with the composite Z-score of amplitude (\(P = 0.028\) and \(P = 0.016\), respectively), but not CV (\(P = 0.393\) and \(P = 0.281\), respectively) or latency (\(P = 0.286\) and \(P = 0.273\), respectively). There were no significant associations between the other seven SNPs and peripheral nerve function.

Conclusions: Rs5219 at KCNJ11 (E23K) was associated with peripheral nerve function in a Chinese population with type 2 diabetes mellitus, suggesting shared genetic factors for type 2 diabetes mellitus and diabetic polyneuropathy in this population.

INTRODUCTION
Diabetic polyneuropathy (DPN) is one of the most common forms of diabetic complications. The prevalence of DPN was estimated to be approximately 50\%\(^1\). Mechanisms underlying the development of DPN include activation of the polyol pathway, exaggerated oxidative stress, overactivity of protein kinase C and increased formation of advanced glycation end-products\(^2,3\). In addition, there is emerging evidence that genetic factors could contribute to the development of DPN. For instance, several studies reported that some patients with prediabetes might have neuropathic complications, whereas some show little evidence of neuropathy even after long-standing diabetes, suggesting genetic heterogeneity of DPN development\(^4\).

To date, approximately 80 type 2 diabetes mellitus susceptibility loci have been reported in different ethnic groups\(^5,6\). Of them, some have been confirmed in Chinese descents in our previous studies\(^7-10\). Recently, associations between type 2 diabetes mellitus susceptibility loci and diabetic nephropathy were reported including variants in JAZF1, FTO, CDKAL1, KCNJ11, KCNQ1 and HHEX/IDE genes\(^11-13\). In addition, TCF7L2 rs7903146 was identified to be associated with diabetic
retinopathy, nephropathy and neuropathy. These data suggest that type 2 diabetes mellitus and its complications might have shared genetic risk factors. By carrying out nerve conduction studies (NCS), the current study sought to evaluate the associations of type 2 diabetes mellitus susceptibility genes with peripheral nerve function in a Chinese population with type 2 diabetes mellitus.

**MATERIALS AND METHODS**

**Study population**

We recruited 1,900 patients with type 2 diabetes mellitus from the Shanghai Diabetes Institute Inpatient Database of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital, Shanghai, China. Diabetes was defined according to the 1999 World Health Organization criteria. Individuals were negative for glutamic acid decarboxylase and/or insulin-like 2 antibodies. Participants with known diseases that could possibly affect NCS parameters (such as Guillain–Barré syndrome and carpal tunnel syndrome) were excluded.

The study protocol was approved by the institutional review board of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital. The conduction of the study conforms to the provisions of the Declaration of Helsinki II. Written informed consent was obtained from each participant.

**Anthropometric and biochemical measurements**

Anthropometric parameters were height and weight. Body mass index was calculated as weight (kg)/height (m)\(^2\). Biochemical measurements including glycated hemoglobin (HbA1c), fasting plasma glucose and lipid profiles (total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol) were determined as previously described.

**Nerve conduction studies**

Electrophysiological examinations were carried out by using EMGMyto, EBNeuro (Esaote, Florence, Italy). Motor nerve studies were carried out in the median and tibial nerves, including the following parameters: conduction velocity (CV), distal latency and compound muscle action potential amplitude. CV, sensory nerve onset latency, and sensory nerve action potential amplitude were measured in the median and superficial peroneal nerves. Skin temperatures were kept at 32–35°C during testing.

Additionally, the composite Z-scores for nerve conduction (NC) parameters including CV, amplitude and latency were calculated, respectively. For each individual, every CV value was converted into a Z-score using the formula: 

\[
Z = \frac{X - \mu}{\sigma}
\]

where \(X\) is the individual value of control, \(\mu\) is the mean value of control group, and \(\sigma\) is the SD of control group. A composite Z-score of CV was then calculated as \(Z_{\text{CV}} = \frac{(Z_{\text{CV}} - \mu_{\text{CV}}) + (Z_{\text{CV}} \text{ motor median CV}) + (Z_{\text{CV}} \text{ superficial peroneal CV}) + (Z_{\text{CV}} \text{ tibial CV})}{4}\). The composite Z-scores for amplitude and latency were calculated similarly.

**Single nucleotide polymorphism selection and genotyping**

We selected ten single nucleotide polymorphisms (SNPs) from ten type 2 diabetes mellitus susceptibility genes previously confirmed in Chinese patients, including PPARG rs1801282, IGF2BP2 rs7651090, CDKAL1 rs7756992, CDKN2A/2B rs10811661, IDE-KIF11-HHEX rs1111875, TCF7L2 rs7903146, HNF1β rs4430796, KCNQ1 rs2237892, SLC30A8 rs13266634 and KCNJ11 rs251979. All the SNPs were genotyped using the primer extension of multiplex products with detecting by matrix-assisted laser desorption ionization-time of flight mass spectrometry on a MassARRAY Compact Analyzer (Sequenom, San Diego, CA, USA).

**Statistical analyses**

Allele frequencies for the SNP tested were calculated by gene counting. Genotype frequency distribution was tested for Hardy–Weinberg equilibrium with a chi-square test. All analyses were carried out under an additive genetic model, except that the associations of rs10811661 (PPARG) and rs7903146 (TCF7L2) with NC parameters were investigated under a dominant model because of the small number of minor allele homozygous. Student’s t-test and analysis of variance (ANOVA) were used to assess differences in continuous variables. The association of NC parameters with each SNP was analyzed by multiple linear regression analysis after adjusting for age, type 2 diabetes mellitus duration and HbA1c as confounding factors. Statistical analyses were carried out by using SPSS software version 11.0 (SPSS, Chicago, IL, USA). A two-tailed P-value of <0.05 was considered to be statistically significant.

**RESULTS**

Clinical characteristics of the participants are shown in Table 1. The mean age of the participants was 60.65 ± 12.16 years, with

| Table 1 | Clinical characteristics of the participants |
|------------------------------------------------|
| T2DM patients | 998/902 |
| Age (years) | 60.65 ± 12.16 |
| Duration of diabetes (years) | 7.44 ± 6.76 |
| BMI (kg/m\(^2\)) | 24.48 ± 3.46 |
| FPG (mmol/L) | 12.4 ± 4.95 |
| HbA1c (%) | 9.07 ± 2.31 |
| SBP (mmHg) | 133.66 ± 17.8 |
| DBP (mmHg) | 80.74 ± 9.46 |
| Total cholesterol (mmol/L) | 4.79 ± 1.15 |
| HDL cholesterol (mmol/L) | 1.16 ± 0.56 |
| LDL cholesterol (mmol/L) | 2.97 ± 0.87 |
| Triglycerides (mmol/L) | 1.88 ± 1.82 |

Continuous variables are presented as mean ± standard deviation; categorical variables are presented as numbers. BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.
the average diabetes duration of 7.44 ± 6.76 years and the mean HbA1c of 9.07 ± 2.31%.

All SNPs selected in the present study were in Hardy–Weinberg equilibrium. The minor allele frequencies of the SNPs in our study population are shown in Table 2. Of the ten SNPs, rs5219 of KCNJ11 (E23K: G→A) was identified to be associated with all the parameters obtained from NCS (Z-score of CV: $\beta = 0.113$, $P = 0.01$; Z-score of amplitude: $\beta = 0.133$, $P = 0.01$; Z-score of latency: $\beta = -0.116$, $P = 0.01$) after adjustment for age, duration and HbA1c, with each copy of the A allele relating to better NC parameters (Tables 2 and 3).

We also found that CDKAL1 rs7756992 and TCF7L2 rs7903146 were related to the composite Z-score of amplitude (CDKAL1 rs7756992: $\beta = -0.115$, $P = 0.028$; TCF7L2 rs7903146: $\beta = 0.123$, $P = 0.016$), but not CV ($P = 0.393$ and $P = 0.281$, respectively) or latency ($P = 0.286$ and $P = 0.273$, respectively). There was no significant association between the other seven SNPs and peripheral nerve functions (Table 2).

DISCUSSION

In the present study, we selected ten SNPs from ten type 2 diabetes mellitus susceptibility genes previously confirmed in Chinese people, and analyzed the association of these loci with peripheral nerve function in Chinese patients with type 2 diabetes mellitus. We found that rs5219 of KCNJ11 (E23K) was consistently associated with the parameters obtained from NCS, and the minor allele A seemed to have a protective effect on peripheral nerve function.

Although a large portion of diabetic patients are affected by DPN, our knowledge about its genetic contributors is limited. In previous studies, several gene variants were reported to be associated with DPN, and the minor allele A seemed to have a protective effect on peripheral nerve function.

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cardiovascular system, however, the activation of $K_{ATP}$ channels could upregulate the expression of vasorelaxant factors, and E23K was reported to exert an beneficial effect on coronary heart disease$^{34}$. More importantly, the presence of $K_{ATP}$ channels was also reported in both the central and peripheral nervous system$^{32,33}$. In peripheral sensory neurons, Kawano et al.$^{34}$ further showed that the $K_{ATP}$ channel plays an important role in the regulation of nerve excitability and neurotransmitter release, raising the possibility that variations/mutations in KCNJ11 might impact on nerve function through the modulation of $K_{ATP}$ channel activity. In support of this notion, a patient with neonatal diabetes caused by a KCNJ11 V59M mutation was observed to present with neurological symptoms including muscle weakness, and delayed motor function and mental development$^{35}$. In addition to sulfonylurea treatment, by closing the $K_{ATP}$ channel, motor development and glycemic control were remarkably improved for this patient. Based on these observations, it is plausible to speculate that KCNJ11 E23K might affect peripheral nerve function through its effect on the activity of the $K_{ATP}$ channel in the peripheral nervous system.

CDKAL1 rs7756992 and TCF7L2 rs7903146 were reported to be significantly associated with type 2 diabetes mellitus in multiple ethnic populations, although the exact mechanism remains unknown. We found these two SNPs correlated with the composite Z-score of amplitude, but not CV or latency. Established DPN is characterized by decreased amplitude, reduced CV and prolonged latency. Of them, amplitude is the most clinically relevant, as it reflects the density of functioning nerve fibers$^{36}$. Therefore, it is possible that CDKAL1 rs7756992 and TCF7L2 rs7903146 could be associated with the early onset and severity of DPN, although their effect might not be as strong as KCNJ11 E23K.

Two limitations of this the present should be pointed out. First, we did not carry out correction for multiple testing when analyzing the association of ten SNPs with NCS data. The positive findings we observed would be non-significant after Bonferroni correction. Therefore, our work should be regarded as a preliminary study. Further studies with larger sample sizes and in other populations are required to confirm the present findings. Second, we used the superficial peroneal nerve to assess sensory nerve function of the lower limbs, whereas most previous studies used the sural nerve in NCS. However, it was reported that the superficial peroneal nerve is more sensitive for detecting peripheral neuropathy as compared with the sural nerve$^{37}$. Nevertheless, caution should be taken to interpret the NCS data of the present study.

In conclusion, the current study shows that rs5219 at KCNJ11 (E23K) is significantly associated with peripheral nerve function as evaluated by NCS in a Chinese population with type 2 diabetes mellitus. The present findings suggest shared genetic factors for type 2 diabetes mellitus and DPN in this population.

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DISCLOSURE

The authors declare no conflict of interest.

Table 3 | Nerve conduction parameters of the participants according to KCNJ11 E23K genotypes

| Parameters | EE | EK | KK | P  |
|------------|----|----|----|----|
| Median (sensory) | CV (m/s) | 50.75 ± 9.13 | 51.47 ± 8.67 | 52.44 ± 8.56 | 0.0135 |
| Amp (mV) | 13.82 ± 9.54 | 13.97 ± 9.15 | 14.93 ± 9.08 | 0.0673 |
| Latency (ms) | 2.79 ± 0.80 | 2.73 ± 0.73 | 2.68 ± 0.69 | 0.255 |
| Median (motor) | CV (m/s) | 52.66 ± 7.41 | 52.59 ± 7.43 | 53.32 ± 7.10 | 0.063 |
| Amp (mV) | 8.79 ± 4.75 | 8.88 ± 5.31 | 9.02 ± 4.99 | 0.918 |
| Latency (ms) | 4.16 ± 1.57 | 4.12 ± 1.58 | 4.05 ± 1.47 | 0.875 |
| Superficial peroneal | CV (m/s) | 40.37 ± 7.99 | 40.05 ± 8.32 | 41.16 ± 9.15 | 0.359 |
| Amp (mV) | 15.07 ± 11.51 | 15.98 ± 12.71 | 16.78 ± 12.69 | 0.317 |
| Latency (ms) | 4.14 ± 3.84 | 4.20 ± 3.89 | 3.99 ± 3.71 | 0.936 |
| Tibial | CV (m/s) | 42.13 ± 7.99 | 42.21 ± 7.20 | 43.05 ± 6.12 | 0.867 |
| Amp (mV) | 6.34 ± 4.70 | 7.82 ± 5.08 | 8.54 ± 6.58 | 0.014 |
| Latency (ms) | 4.98 ± 1.75 | 4.74 ± 1.67 | 4.55 ± 1.35 | 0.276 |
| Composite | CV Z-score | -0.96 ± 0.98 | -0.86 ± 0.91 | -0.60 ± 0.80 | 0.010 |
| Amp Z-score | -0.53 ± 0.59 | -0.43 ± 0.60 | -0.22 ± 0.53 | 0.010 |
| Latency Z-score | 2.57 ± 3.16 | 2.28 ± 3.24 | 1.24 ± 2.31 | 0.010 |

P was adjusted for age, type 2 diabetes mellitus duration and glycated hemoglobin. Amp, amplitude; CV, conduction velocity; EE, GG genotype carriers of KCNJ11 rs5219; EK, GA genotype carriers of KCNJ11 rs5219; KK, AA genotype carriers of KCNJ11 rs5219.
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