ORIGINAL ARTICLE

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

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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: $\beta = 0.113$, $P = 0.01$; Z-score of amplitude: $\beta = 0.133$, $P = 0.01$; Z-score of latency: $\beta = -0.116$, $P = 0.01$) after adjustment for 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[14–16]. 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[8]. Diabetes was defined according to the 1999 World Health Organization criteria[17]. Individuals were negative for glutamic acid decarboxylase and/or protein tyrosine phosphatase IA-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[18].

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-score = (individual value of patient − mean value of control group)/SD of control group. A composite Z-score of CV was then calculated as ([Z-score sensory median CV] + [Z-score motor median CV] + [Z-score superficial peroneal CV] + [Z-score 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* rs5219[7,9,10,19]. All the SNPs were genotyped using the primer extension of multiplex products with detecting by matrix-assisted laser desorption ionization-time of flight mass spectroscopy 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 rs1801282 (*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 patients 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
Male/female (*n*)	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 $9.07 \pm 2.31\%$.

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[20], our knowledge about its genetic contributors is limited. In previous studies, several gene variants were reported to be associated with DPN[21–26]. For example, Yigit *et al.*[25], Papanas *et al.*[22] and Monastiriotis *et al.*[26] identified that the methylenetetrahydrofolate reductase (*MTHFR*) gene C677T mutation, alpha2B adrenoceptor gene insertion/deletion polymorphism and ε4 allele of the apolipoprotein E gene were associated with DPN, respectively.

Of note, certain type 2 diabetes mellitus susceptibility loci have been observed to be associated with diabetic complications[11–16]. Franceschini *et al.*[11] reported that *FTO* rs8050136, *TCF7L2* rs7901695 and *KCNJ11* rs5219 were associated with a lower estimated glomerular filtration rate in patients with type 2 diabetes mellitus. In the study by Chen *et al.*[12], variants in *JAZF1*, *FTO* and *HHEX/IDE* loci were significantly associated with reduced estimated glomerular filtration in a She Chinese population with type 2 diabetes mellitus. Furthermore, Ciccacci *et al.*[15] found that rs7903146 of *TCF7L2* was related to the presence of cardiovascular autonomic neuropathy, with T allele carriers having an eightfold increased risk of cardiovascular autonomic neuropathy.

To the best of our knowledge, this is the first study to analyze the association of type 2 diabetes mellitus susceptibility loci with peripheral nerve function in a Chinese population. In the present study, *KCNJ11* (E23K) was observed to be associated with all the NC parameters including amplitude, latency and CV. Furthermore, the direction of the association was consistent, with A allele carriers relating to better electrophysiological outcomes (i.e., higher amplitude, faster CV and shorter latency). Therefore, the results of the present study are unlikely to be merely false positives.

The *KCNJ11* gene encodes the adenosine triphosphate-sensitive K$^+$ (K$_{ATP}$) channel subunit, Kir6.2, which belongs to the inward rectifying K$^+$ channel family. To date, K$_{ATP}$ channels have been reported to exist in multiple tissues, such as skeletal muscle, vascular smooth muscle and heart[27], suggesting pleiotropic effects. In pancreatic β-cells, E23K causes overactivity of Kir6.2, leading to the hyperpolarization of cell membrane, which inhibits calcium inflow and subsequent release of insulin[28]. In accord, E23K was identified to be associated with type 2 diabetes mellitus in several populations[9,29,30]. In the

Table 2 | Association between ten single nucleotide polymorphisms and nerve conduction parameters

Gene	SNP	MAF, %	Z-score					
			CV		Amplitude		Latency	
			β	P	β	P	β	P
PPARG	rs1801282	4.70	0.003	0.944	−0.029	0.576	−0.001	0.985
IGF2BP2	rs7651090	27.50	0.056	0.209	−0.026	0.614	−0.04	0.382
CDKAL1	rs7756992	44.30	0.039	0.393	−0.115	0.028*	−0.049	0.286
CDKN2A/2B	rs10811661	38.60	−0.024	0.589	−0.048	0.353	−0.003	0.939
IDE-KIF11-HHEX	rs1111875	30.90	0.014	0.752	0.102	0.052	−0.036	0.435
TCF7L2	rs7903146	4.20	0.048	0.281	0.123	0.016*	−0.05	0.273
HNF1β	rs4430796	31.20	−0.019	0.67	−0.055	0.291	−0.007	0.881
KCNQ1	rs2237892	25.70	−0.034	0.457	0.054	0.302	0.02	0.656
SLC30A8	rs13266634	38.60	−0.011	0.808	0.006	0.905	0.047	0.295
KCNJ11	rs5219	42.80	0.113	0.01*	0.133	0.01*	−0.116	0.010*

P-values were adjusted for age, type 2 diabetes mellitus duration and glycated hemoglobin. **P*-values <0.05. CV, conduction velocity; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

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	0.135
	Amp (mV)	13.82 ± 9.54	13.97 ± 9.15	14.93 ± 9.08	0.673
	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	0.643
	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.

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[31]. 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.

DISCLOSURE

The authors declare no conflict of interest.

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