


Association between 1p13 polymorphisms and peripheral arterial disease in a Chinese population with diabetes

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ABSTRACT

Aims/Introduction: Variants on chromosome 1p13 have been associated with coronary artery disease and acute myocardial infarction risk in different ethnic groups. The present study aimed to investigate the association between 1p13 polymorphisms and the development of peripheral artery disease (PAD) in a Chinese population with type 2 diabetes mellitus.

Materials and Methods: 1p13 polymorphisms, rs599839, rs646776 and rs12740374, were assessed in a cohort of 882 type 2 diabetes mellitus patients including 440 type 2 diabetes mellitus patients with PAD (DM + PAD group) and 442 patients without PAD (DM group). Genotyping was carried out using TaqMan assay.

Results: Compared with the DM group, the frequencies of the minor G allele of both rs599839 and rs646776 and the minor T allele of rs12740374 decreased ($P = 0.013$, $P = 0.019$ and $P = 0.005$, respectively), and the frequencies of rs599839 AG + GG, rs646776 AG + GG and rs12740374 CT+TT genotypes were statistically significantly decreased as well ($P = 0.017$, $P = 0.011$ and $P = 0.007$, respectively) in the dominant model in the DM + PAD group than in the DM group. Multivariate unconditional logistic regression analyses adjusted for age, glycated hemoglobin, triglyceride, low-density lipoprotein cholesterol, smoking, hypertension, diabetes duration, coronary heart disease and cerebral infarction showed that the genotypic distribution of rs599839 AG + GG, rs646776 AG + GG and rs12740374 CT + TT remained statistically different between the DM and DM + PAD group ($P = 0.014$, $P = 0.003$ and $P = 0.004$, respectively). The frequencies of haplotype GGT were statistically significantly different between groups ($P = 0.08$).

Conclusions: The present study strongly supports that genotypes of rs599839, rs646776 and rs12740374 on 1p13 are protective factors for diabetic PAD in a Chinese population. Haplotype GGT generated by rs599839, rs646776 and rs12740374 might also decrease the risk of the disease.

INTRODUCTION

Peripheral arterial disease (PAD), characterized by reduced blood flow to the lower extremities, is a manifestation of systemic atherosclerosis. It commonly affects people with type 2 diabetes mellitus, which influences the development and progress of PAD. Each 1% increase in hemoglobin A1c, an indicator for the average level of

blood glucose over the past 2 or 3 months, is associated with a 28% increased risk of PAD (95% confidence interval [CI]: 12–46)¹. Compared with general population-based prevalence, a higher prevalence of PAD over the age of 50 years was observed in diabetes patients². The prevalence of PAD in diabetes patients ranged from 6.3% to 36.1% in different countries and races^{3–6}. In addition, there are several other traditional risk factors for PAD, such as smoking, hypertension and dyslipidemia.

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Genetic factors also play an important role in the development of PAD. Genome-wide association studies (GWAS) have discovered multiple loci associated with the risk of disease^{7–12}. It has been found that chromosome 1p31 locus is specific to peripheral arterial occlusive disease, which is diagnosed angiographically and/or surgically¹³. Rs1333049 on chromosome 9p21 was not only associated with coronary artery disease (CAD) susceptibility^{14–16}, but also associated with PAD^{17,18}, which indicated that CAD and PAD might have a common genetic basis.

It was found that 1p13 single-nucleotide polymorphisms (SNPs), rs599839, rs646776 and rs12740374, were associated with the incidence of CAD in Asia, Europe and America^{19–24}. Rs599839 and rs646776 SNPs and myocardial infarction (MI) risk are also significantly correlated^{21,25,26}, and they also showed nominal significance with vascular calcification^{27,28}. However, the association between 1p13 polymorphisms and PAD has not yet been fully investigated. In the present study, we investigated the association of rs599839, rs646776 and rs12740374 with diabetic PAD.

METHODS

This study was approved by the Institutional Ethics Review Board of Tangshan Gongren Hospital, and all patients provided written informed consent. The design of the study conforms to the provisions of the Declaration of Helsinki II.

Participants

A total of 442 patients with type 2 diabetes mellitus (DM group) and 440 PAD patients with type 2 diabetes mellitus (DM + PAD group) were recruited from Tangshan Gongren Hospital (Tangshan, China) from March 2012 to June 2015. All of them were from the Chinese Han population and unrelated. Diagnosis of type 2 diabetes mellitus was carried out in accordance with the criteria established by the World Health Organization (1999) diagnostic standard. Inclusion criteria included type 2 diabetes mellitus patients aged ≥ 45 years and patients in the DM + PAD group who had an ankle brachial index ≤ 0.9 or a history of PAD therapy (stent, atherectomy and other surgical treatment). Exclusion criteria included patients with type 1 diabetes mellitus; patients who refused to provide informed consent; patients with ankle brachial index ≥ 1.4 were excluded to eliminate the effects of incompressible and calcified arteries²⁹; serious heart failure, liver and kidney dysfunction; venous embolism; infection; malignant tumor and diseases of the immune system; and patients for whom genotyping of 12 samples failed.

Coronary artery disease was defined as a history of coronary artery revascularization or percutaneous coronary angiography (70% stenosis in a major coronary artery) or myocardial infarction according to the World Health Organization criteria³⁰. Cerebral infarction was defined as a history of cerebral infarction or was diagnosed according to brain computed tomography or magnetic resonance imaging.

Peripheral blood deoxyribonucleic acid extraction and genotyping

For peripheral blood deoxyribonucleic acid (DNA) extraction and genotyping, 3 mL of peripheral blood was collected into sterile tubes containing ethylenediaminetetraacetic acid from all the study populations. Genomic DNA was extracted from peripheral blood leukocyte using a QIAamp DNA mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's guidelines. Spectrophotometric DNA quantification was carried out using a TU-1800 UV spectrophotometer (Bio-Rad, Hercules, California, USA). Three SNPs (rs599839/rs646776/rs12740374) were selected from the Hapmap Chinese Han in Beijing, China, data (<http://hapmap.ncbi.nlm.nih.gov/>) using Haploview Software 4.2 (Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA). The distance between rs599839 and rs646776, rs646776 and rs12740374, and rs599839 and rs12740374 was 3,637, 941 and 4,577 bp, respectively. The minor allele frequency of the three SNPs among Chinese Han in Beijing, China, was as follows: rs599839 $M_G = 0.033$, rs646776 $M_G = 0.012$, rs12740374 $M_T = 0.011$. According to previously published literature, polymorphisms of these loci might also be associated with the susceptibility to CAD or MI.

The SNPs of rs599839, rs646776 and rs12740374 were detected by TaqMan technology. The TaqMan probes to the selected loci were found on the Applied Biosystems website, where each number of the probes was sent to synthesize probes and to synthesize primers of the loci. The target DNA sequences were amplified by polymerase chain reaction in the 7900 HT Real-Time PCR System using TaqMan[®] SNP Genotyping Assays (Applied Biosystems, Foster City, California, USA). The DNA sequence was amplified using 1 ng of genomic DNA, 0.12 μL of Assay Mix ($\times 40$), 2.5 μL of Master Mix ($\times 2$) and 20 pmol of each primer in a total reaction volume of 5 μL with the following procedure: initial DNA denaturation at 95°C for 10 min followed by 40 cycles of 15 s of denaturation at 95°C, 60 s of annealing and extension at 60°C to complete the reaction. Quality control was carried out with 5% of samples, to ensure reproducibility.

Statistical analysis

Statistical analysis was carried out by SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA). The Hardy–Weinberg equilibrium was assessed in the DM group and DM + PAD group, by the χ^2 -test. Continuous data were expressed as mean \pm standard deviation or median (interquartile range), and between-group differences were analyzed using independent-samples *t*-test or the Mann–Whitney *U*-test. Measurement data and the frequencies of genotypes and alleles in each group were compared by the χ^2 -test.

Linkage disequilibrium calculation and haplotype construction were carried out using the SheSIS software (Bio-X Center, Shanghai Jiao Tong University, Shanghai, China). The linkage disequilibrium coefficient *D'* and the value of r^2 were calculated.

The values of D' and r^2 ranged from 0 (linkage equilibrium) to 1 (complete linkage disequilibrium). The haplotype analysis was carried out to compare frequencies of haplotypes generated by rs599839, rs646776 and rs12740374 between the two groups. The association between genetic polymorphisms and PAD was assessed by multivariate unconditional logistic regression analyses in dominant genetic model of inheritance. Odds ratios (ORs) and 95% CIs were determined. A P -value <0.05 was considered to show statistical significance.

RESULTS

Demographic and clinical characteristics of each group

We studied 440 type 2 diabetes patients with PAD, and 442 sex-matched type 2 diabetes patients without PAD. The demographic and clinical characteristics of the study population are described in Table 1. There was no significant difference between the groups in terms of sex and triglyceride. Whereas age, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol (LDL-C), body mass index, smoking, glycated hemoglobin, diabetes duration and CAD, cerebral infarction, hypertension, and lipid-lowering therapy were significantly different in the DM + PAD group compared with the DM group.

Association study of Rs599839, Rs646776 and Rs12740374 polymorphisms with diabetic PAD

Rs599839, rs646776 and rs12740374 were in the Hardy–Weinberg genetic equilibrium in both groups. It was found that frequencies of the minor G allele of both rs599839 and rs646776, and the minor T allele of rs12740374 were significantly decreased in the DM + PAD group compared with the DM group. Genotype frequencies of three loci decreased significantly in the dominant model in the DM + PAD group compared

with the DM group. Genotype frequencies were not statistically different in the recessive model (Table 2).

Univariate unconditional logistic regression analyses showed that rs599839 AG + GG, rs646776 AG + GG and rs12740374 CT + TT genotypes were correlated with PAD in diabetes patients ($P = 0.018$, $P = 0.012$ and $P = 0.008$, respectively). To evaluate the independent genetic effect of each SNP on the susceptibility to diabetic PAD, multivariate unconditional logistic regression analyses were carried out (Table 3). Adjusted for age, glycated hemoglobin, triglyceride, LDL-C, smoking, hypertension and duration of diabetes, the genotypic distribution of rs599839 AG + GG, rs646776 AG + GG and rs12740374 CT + TT remained statistically different between the groups ($P = 0.014$, $P = 0.003$ and $P = 0.004$, respectively).

Linkage disequilibrium of the three loci was observed in each group and both. Linkage disequilibrium coefficients D' and r^2 of rs12740374 and rs599839, rs12740374 and rs646776, and rs599839 and rs646776 were 0.933, 0.977 and 0.930, and 0.689, 0.914 and 0.655, respectively in the DM group. In the DM + PAD group, Linkage disequilibrium coefficients D' and r^2 of rs12740374 and rs599839, rs12740374 and rs646776, and rs599839 and rs646776 were 0.915, 0.959 and 0.922, and 0.558, 0.849 and 0.613, respectively. We showed the haplotype of the three loci in Table 4. Frequencies of haplotype AAG generated from rs599839, rs646776 and rs12740374 between the DM group and DM + PAD group were statistically significantly different ($P = 0.011$), the odds ratio was 1.683 (95% CI: 1.118–2.535). Frequencies of haplotype GGT were statistically significantly different between the DM group and DM + PAD group ($P = 0.008$), the odds ratio was 0.502 (95% CI: 0.297–0.846).

Table 1 | Demographic and clinical characteristics of the study population

Variable	DM + PAD ($n = 440$)	DM ($n = 442$)	P
Male	223 (50.7)	246 (55.7)	0.139
Age (years)	66.70 \pm 7.57	60.43 \pm 7.95	$<0.001^{***}$
HbA1c (%)	9.4 (3.3)	8.9 (3.2)	0.024*
TC (mmol/L)	5.13 \pm 0.88	4.93 \pm 0.97	0.002**
TG (mmol/L)	2.55 (1.67)	2.43 (1.67)	0.119
HDL-C (mmol/L)	1.34 \pm 0.25	1.40 \pm 0.28	$<0.001^{***}$
LDL-C (mmol/L)	3.29 \pm 0.98	3.11 \pm 0.98	0.005**
BMI (kg/m ²)	25.55 \pm 3.09	25.06 \pm 3.17	0.020*
Smoking	200 (45.5)	159 (36)	0.004**
Hypertension	249 (56.6)	214 (48.4)	0.015*
Lipid-lowering therapy	151 (34.3)	122 (27.6)	0.031*
Duration of diabetes (year)	13.38 \pm 5.82	10.60 \pm 5.43	$<0.001^{***}$
Coronary heart disease	168 (38.2)	87 (19.7)	$<0.001^{***}$
Cerebral infarction	131 (29.8)	64 (14.5)	$<0.001^{***}$

Data are mean \pm standard deviation, medians (interquartile range) or percentages. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. BMI, body mass index; DM, patients with type 2 diabetes mellitus; DM + PAD, peripheral artery disease patients with type 2 diabetes mellitus; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

Table 2 | Rs599839, rs646776 and rs12740374 genotype and allele distribution in the peripheral artery disease patients with type 2 diabetes mellitus and patients with type 2 diabetes mellitus group

Genotypes	DM + PAD (n = 440)	DM (n = 442)	P
rs599839			
Allele			
G	37 (4.2)	61 (6.9)	0.013*
Dominant model			
AA	404 (91.8)	384 (86.9)	0.017*
AG + GG	36 (8.2)	58 (13.1)	
Recessive model			
AA + AG	439 (99.8)	439 (99.3)	0.624 [†]
GG	1 (0.2)	3 (0.7)	
rs646776			
Allele			
G	27 (3.1)	47 (5.3)	0.019*
Dominant model			
AA	414 (94.1)	395 (89.4)	0.011*
AG + GG	26 (5.9)	47 (10.6)	
Recessive model			
AA + AG	439 (99.8)	442 (100.0)	0.499 [†]
GG	1 (0.2)	0 (0.0)	
rs12740374			
Allele			
T	25 (2.8)	49 (5.5)	0.005**
Dominant model			
GG	415 (94.3)	395 (89.4)	0.007**
GT + TT	25 (5.7)	47 (10.6)	
Recessive model			
GG + GT	440 (100.0)	440 (99.5)	0.499 [†]
TT	0 (0.0)	2 (0.5)	

Data are expressed as n (%). * $P < 0.05$, ** $P < 0.01$. [†]Calculated by Fisher's exact test. DM, patients with type 2 diabetes mellitus; DM + PAD, peripheral artery disease patients with type 2 diabetes mellitus.

DISCUSSION

The current study showed an association between rs599839, rs646776 and rs12740374 genotypes and PAD in diabetes patients. Genotypic frequencies of rs599839, rs646776 and rs12740374 increased in the dominant model in diabetes

patients with PAD, which showed that rs599839 AG + GG, rs646776 AG + GG and rs12740374 GT + TT were associated with a decreased risk of PAD in diabetes patients. Haplotype GGT of rs599839, rs646776 and rs12740374 was also an independent protective factor for PAD in diabetes patients.

Rs599839, rs646776 and rs12740374 are located on chromosome 1p13 (Figure 1), in which SNPs are associated with cardiovascular diseases, as well as macrovascular disease. It has been found that the minor G allele of rs599839 and the minor G allele of rs646776 were independent protective factors for CAD and MI^{20,21,26}. Ellis *et al.*³¹ showed that an individual with cardiovascular disease or not carrying one or more rs599839 G allele had a lower prevalence of dyslipidemia or lower levels of LDL-C and total cholesterol and/or less history of MI compared with AA participants. Furthermore, AG/GG carriers with coronary disease and post-MI had better cardiac function, and fewer AG/GG carriers with coronary heart disease were readmitted for a non-ST-segment elevation MI³¹. One copy of the rs599839 G allele was associated with a decrease of serum LDL-C by 0.14 mmol/L and a 9% decrease in CAD risk²². The rs599839 has been reported to be associated with a reduced risk of coronary heart disease in the Chinese population, and its association with decreased LDL levels was also observed³². Rs646776 also showed a significant protective association with CAD (OR 0.422, 95% CI: 0.181–0.981)²⁰. Rs599839, rs646776 and rs12740374 are near the genes *SORT1*, *PSRC1* and *CELSR2*, which can interfere with lipid metabolism (Table 1). Rs12740374 T increased the expression of *SORT1* messenger ribonucleic acid and the level of SORT1 protein, which reduced the levels of very LDL-C and LDL-C in the blood, and then reduced the risk of CAD¹³. Similar to the present study, these studies suggest that the minor G allele of rs599839 and rs646776, and the minor T allele of rs12740374 are protective factors for atherosclerosis and cardiovascular disease risk.

Concordantly, some meta-analyses found that the A allele of rs599839 and T allele of rs646776 were associated with increased cardiovascular disease risk as well as total cholesterol and LDL-C, apolipoprotein B, and decreased high-density lipoprotein cholesterol^{33,34}. The similar association was also

Table 3 | Logistic regression analysis for the association of rs599839, rs646776 and rs12740374 polymorphisms with peripheral artery disease in diabetes

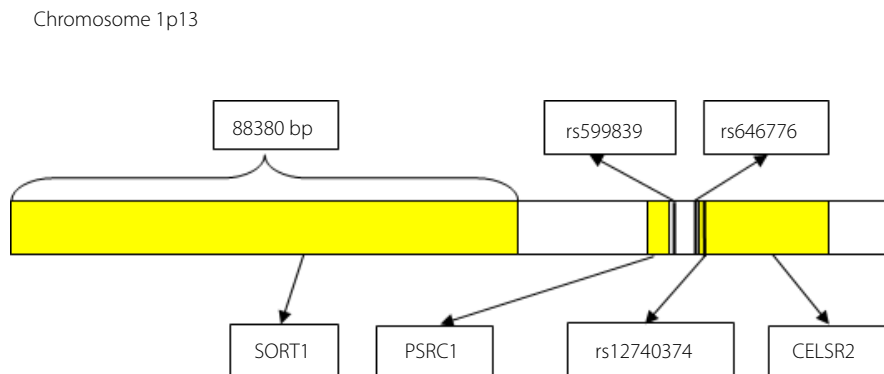
	Univariate regression		Multiple regression	
	Crude OR (95% CI)	Crude P	Adj OR (95% CI)	Adj P
rs599839 (dominant model)	0.590 (0.380–0.915)	0.018*	0.497 (0.285–0.866)	0.014*
rs646776 (dominant model)	0.528 (0.321–0.869)	0.012*	0.381 (0.200–0.728)	0.003**
rs12740374 (dominant model)	0.506 (0.306–0.838)	0.008**	0.385 (0.200–0.739)	0.004**

Multiple regression was adjusted by: sex, age, glycosylated hemoglobin, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, smoking, hypertension, lipid-lowering therapy, body mass index, duration of diabetes, coronary heart disease and cerebral infarction. * $P < 0.05$, ** $P < 0.01$. Adj, adjusted.

Table 4 | Haplotype analysis of rs599839, rs646776 and rs12740374 in the patients with type 2 diabetes mellitus group and peripheral artery disease patients with type 2 diabetes mellitus group

Haplotype	DM + PAD (freq)	DM (freq)	χ^2	<i>P</i>	OR	95% CI
AAG	840.97 (0.956)	819.95 (0.928)	6.333	0.011*	1.683	1.118–2.535
GAG	11.03 (0.013)	14.05 (0.016)	0.355	0.551	0.786	0.335–1.739
GGG	3.00 (0.003)	1.01 (0.001)	1.005	0.316	3.005	0.313–28.822
GAT	1.00 (0.001)	3.00 (0.003)	0.797	0.372	0.333	0.049–2.263
AGT	2.03 (0.002)	3.05 (0.003)	0.203	0.652	0.666	0.112–3.947
GGT	21.97 (0.025)	42.94 (0.049)	6.934	0.008**	0.502	0.297–0.846

Haplotypes were constructed for: rs12740374, rs646776 and rs599839. **P* < 0.05, ***P* < 0.01. CI, confidence interval; DM, patients with type 2 diabetes mellitus; DM + PAD, peripheral artery disease patients with type 2 diabetes mellitus; freq, frequency; OR, odds ratio.

**Figure 1** | Location of rs599839, rs646776 and rs12740374 on chromosome 1p13.

observed in a Korean population²⁴. However, the GWAS of PAD in East Asians had not found that 1p13 locus was significantly associated with PAD⁷. This is different from the present findings, which showed that rs599839 and rs646776 were protective factors in diabetic PAD patients. Considering the discordant reports from different ethnic groups, it is possible that the three SNPs are markers, but not causative variants, and the discrepancy in studies could also be related with different study population, ethnic differences, different genotypic frequency, gene–environment and gene–gene interactions. Sample bias and/or other confounding factors might also be responsible for such differences.

In addition, it was found that rs599839, rs646776 and coronary artery calcification were correlated^{27,28}. The occurrence of coronary artery calcification could increase the risk of cardiovascular disease, and it is also an important predictor of all-cause mortality and cardiovascular mortality.

There are fewer studies on the correlation between variant 1p13 and cerebrovascular disease. Rs646776 has been found to be associated with carotid artery disease³⁵, which is one of the important risk factors for stroke.

Haplotypes play key roles in the study of human disease and genetics³⁶. The haplotype GC generated by rs599839 and rs646776 was shown to be a protective factor of CAD²⁰. In the present study, diabetes patients with haplotype GGT had a

decreased PAD risk, whereas those with haplotype AAG had an increased risk. However, the association between haplotype AAG and risk of PAD was not supported by allelic analysis of rs599839 A, rs646776 A and rs12740374 G in the recessive model, which suggests that the susceptibility loci might exist in haplotype AAG or nearby.

In conclusion, the present study showed that genotypes of rs599839, rs646776 and rs12740374 on 1p13 are protective factors for PAD in a Chinese population with type 2 diabetic mellitus. Haplotype GGT generated by rs599839, rs646776 and rs12740374 might also decrease the risk of the disease. The mechanism of the association should be further confirmed. There were still some limitations to the present study, such as the ankle brachial index only being able to reflect local lesions, and it can be falsely high with poorly compressive vessels often seen in diabetes patients, so the diagnosis might need to be improved in future research.

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DISCLOSURE

The authors declare no conflict of interest.

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