SHORT COMMUNICATION

Variations in *KCNQ1* are associated with type 2 diabetes and beta cell function in a Chinese population

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Abstract

Aims/hypothesis Recent genome-wide association studies in East Asian populations reported that single nucleotide polymorphisms (SNPs) in *KCNQ1* are associated with type 2 diabetes. The aim of this study was to validate this finding in a Chinese population.

Methods We genotyped four SNPs, rs2074196, rs2237892, rs2237895 and rs2237897, in a group of 3,503 Shanghai Chinese individuals, comprising 1,769 type 2 diabetic patients and 1,734 normoglycaemic controls. Both the cases and the controls were extensively phenotyped for anthropometric and biochemical traits related to glucose metabolism. Arginine stimulation tests under fasting conditions were performed in a subgroup of 466 cases.

Results All four of the SNPs were associated with type 2 diabetes, with rs2237892 showing strongest evidence for association (OR 1.532, 95% CI 1.381–1.698, $p=5.0\times 10^{-16}$). The SNP rs2237897 was associated with both acute insulin and C-peptide response after arginine stimulation in a subgroup of cases ($p=0.0471$ and $p=0.0156$, respectively). The SNP rs2237895 was associated with both first- and second-phase insulin secretion in the controls ($p=0.0334$ and $p=0.0002$, respectively).

Conclusions/interpretation In this study we found that *KCNQ1* was associated with type 2 diabetes susceptibility in a Chinese population, possibly through its effect on beta cell function.

Keywords Association study · Genetics · KCNQ1 · Single nucleotide polymorphism · Type 2 diabetes

Abbreviations
ACPR	Acute C-peptide response
AIR	Acute insulin response
GWA	Genome-wide association
SNP	Single nucleotide polymorphism

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Introduction

Genome-wide association (GWA) analysis is a powerful tool for the identification of new disease susceptibility loci. The widespread application of GWA scans in populations of European descent has increased the number of confirmed type 2 diabetes susceptibility loci from three to almost 20 in the last 2 years [1]. Recently, two papers provided the first reports of type 2 diabetes GWA studies in East Asian populations and added *KCNQ1* to the list of type 2 diabetes susceptibility genes [2, 3]. Although the association was replicated in two Chinese case–control samples recruited from Hong Kong and Singapore, respectively [2, 3], it is still important to validate this novel finding and further establish the relationship between the variants and clinical

phenotypes of type 2 diabetes in the population resident on the mainland. We therefore genotyped the reported single nucleotide polymorphisms (SNPs) in the *KCNQ1* gene in our type 2 diabetes case–control cohort.

Methods

Participants The present study included a total of 3,503 individuals of Han Chinese ancestry who were resident in Shanghai, comprising 1,769 type 2 diabetic patients and 1,734 normoglycaemic controls. Detailed information on this study population has been described elsewhere [4]. Briefly, all cases were type 2 diabetic patients defined according to 1999 WHO criteria [5] and were treated with oral hypoglycaemic agents and/or insulin. The controls had normal glucose tolerance defined as a fasting plasma glucose level of <6.1 mmol/l and a 2 h 75 g OGTT plasma glucose level of <7.8 mmol/l. The clinical characteristics of the study population are shown in Electronic supplementary material (ESM) Table 1. The study was approved by our institutional review board, and all participants gave written informed consent.

Clinical measurements Both the cases and controls were extensively phenotyped for anthropometric and biochemical traits related to glucose metabolism. BMI was calculated as weight (kg)/height^2 (m^2). For the controls, blood samples were obtained at 0 and 120 min during a 75 g OGTT to measure plasma glucose and serum insulin levels. Insulin sensitivity and beta cell function were assessed by HOMA [6]. First- and second-phase insulin secretion were estimated using the glucose and insulin levels at 0 and 120 min during the OGTT and BMI measurements [7]. In a subgroup of cases, potential beta cell function was determined using intravenous arginine stimulation tests under fasting conditions. After taking a baseline blood sample, a 10% (wt/vol.) solution of arginine hydrochloride

(5 g) was injected intravenously for 30–45 s. The end of the injection period was designated time zero, after which samples were taken at 2, 4 and 6 min. The acute insulin response (AIR) to arginine was calculated as the mean of the insulin levels in the postinjection samples minus the insulin level in the prestimulus sample. The acute C-peptide response (ACPR) to arginine was calculated in the same way using sampled C-peptide levels.

SNP genotyping Four SNPs in the *KCNQ1* gene that have previously been reported to be associated with type 2 diabetes in Chinese individuals (rs2074196, rs2237892, rs2237895 and rs2237897) were genotyped in the present study. The genotyping was performed by primer extension of multiplex products with detection by matrix-assisted laser desorption/ionisation time-of-flight mass spectroscopy using a MassARRAY platform (MassARRAY Compact Analyzer; Sequenom, San Diego, CA, USA). The call rates of rs2074196, rs2237892, rs2237895 and rs2237897 were 98.7%, 98.2%, 93.4% and 92.5%, respectively. The concordant rates of all SNPs based on 100 duplicates were 100%.

Statistical analysis The Hardy–Weinberg equilibrium test was performed in the cases and controls separately for each variant before association analysis. The allelic frequencies between the diabetic patients and controls were compared using χ^2 tests, and ORs with 95% CIs are presented for each SNP. The genotype–disease association analyses were performed under the additive model. Quantitative traits with a skewed distribution were logarithmically transformed (log$_e$) to approximate univariate normality. Quantitative traits were analysed under the additive genetic model by linear regression. The statistical analyses were performed using SAS for Windows (version 8.0; SAS Institute, Cary, NC, USA). A two-tailed *p* value of <0.05 was considered statistically significant.

Based on the previous reported OR (1.3) and minor allele frequency (0.35) of the SNPs [2, 3], our case–control

Table 1 Associations of *KCNQ1* SNPs with type 2 diabetes

SNP	Major/ minor allele	Risk allele	Case		Control		OR (95% CI)	*p* value for risk allele	*p* value for genotype	Adjusted *p* value for genotype[b]
			Risk allele frequencies	Genotype count 11/12/22[a]	Risk allele frequencies	Genotype count 11/12/22[a]				
rs2074196	G/T	G	0.678	796/776/175	0.639	688/808/213	1.188 (1.076–1.313)	7.0×10^{-4}	6.1×10^{-4}	1.2×10^{-3}
rs2237892	C/T	C	0.738	947/643/129	0.648	706/816/198	1.532 (1.381–1.698)	5.0×10^{-16}	9.1×10^{-16}	2.4×10^{-16}
rs2237895	A/C	C	0.377	636/759/234	0.318	770/699/173	1.294 (1.169–1.434)	7.0×10^{-7}	5.6×10^{-6}	8.6×10^{-9}
rs2237897	C/T	C	0.717	832/654/131	0.635	660/740/223	1.457 (1.312–1.618)	1.7×10^{-12}	2.3×10^{-11}	7.0×10^{-13}

The OR with 95% CI shown is for the risk allele. The additive model was used in the association analyses between genotypes and type 2 diabetes

[a] 11, major allele homozygotes; 12, heterozygotes; 22, minor allele homozygotes

[b] *p* values were adjusted for age, sex and BMI as confounding factors

cohort had over 95% power to replicate the previous finding at a level of significance of 0.001.

Results

The genotype distributions of all SNPs were in Hardy–Weinberg equilibrium. The risk allele frequencies of these SNPs in our control group were similar to those reported recently in Hong Kong and Singapore Chinese controls (rs2074196 ~0.63, rs2237892 ~0.65, rs2237895 0.32–0.35, rs2237897 ~0.65). Linkage disequilibrium analysis revealed that these four SNPs were in modest linkage disequilibrium (ESM Fig. 1).

We found that all four SNPs were nominally associated with type 2 diabetes (Table 1). The SNP rs2237892 showed the strongest association with type 2 diabetes, with the C allele conferring a higher risk of the disease (OR 1.532, 95% CI 1.381–1.698, $p=5.0 \times 10^{-16}$). The significant associations remained after adjusting for age, sex and BMI as confounding factors (Table 1). Analysis of the association of all four SNPs with type 2 diabetes by logistic regression adjusted for age, sex and BMI showed that rs2237892 conferred the strongest independent risk ($p=0.0002$), while rs2074196 and rs2237897 tended to confer weaker independent risks ($p=0.0263$ and $p=0.0087$, respectively).

We next investigated the effect of these SNPs on clinical variables in a subgroup of 466 cases. The SNP rs2237897 was associated with both AIR and ACPR, with carriers of a greater number of type 2 diabetes risk alleles (C) exhibiting lower values for these indicators of beta cell function ($p=0.0471$ and $p=0.0156$, respectively; Fig. 1). Similarly, carriers of the risk allele (G) of rs2074196 also had lower values for AIR and ACPR compared with TT homozygotes (ESM Fig. 2). In the control group, rs2237895 was associated with both first- and second-phase insulin secretion ($p=0.0334$ and $p=0.0002$, respectively); beta cell function was lower in the type 2 diabetes risk allele (C) carriers (ESM Table 2). Although no significant association was detected for the other three investigated SNPs, beta cell function as indicated by first- and second-phase insulin secretion decreased (non-significantly) as the number of risk alleles increased (ESM Tables 3, 4 and 5). However, none of the SNPs showed an association with HOMA-B in our samples.

Discussion

KCNQ1 encodes a protein for a voltage-gated K$^+$ channel that is required for the repolarisation phase of the cardiac action potential. Previous studies reported that *KCNQ1* mutations were associated with long QT syndrome [8] and familial atrial fibrillation [9]. GWA scans have recently
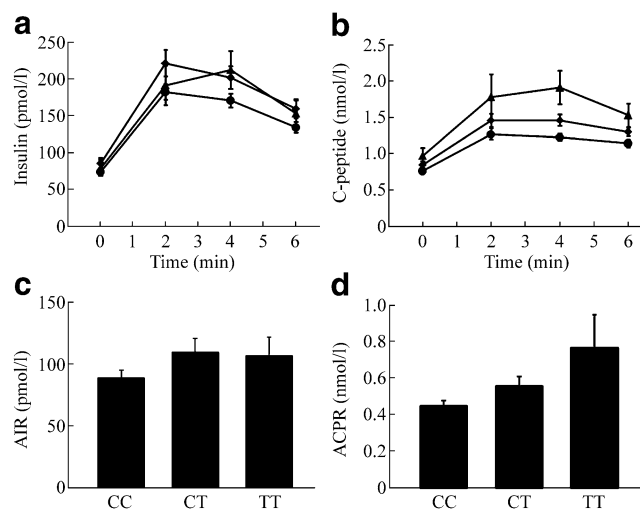
Fig. 1 Association of rs2237897 with AIR and ACPR during arginine stimulation tests in a subgroup of type 2 diabetic patients (CC $n=203$, CT $n=173$ and TT $n=26$; genotyping was unsuccessful for 64 cases). Insulin (**a**) and C-peptide (**b**) levels before and after arginine stimulation among CC (circles), CT (diamonds) and TT (triangles) genotypes. Arginine was injected up to time 0. **c** Association between rs2237897 and AIR (88.45±6.29 vs 109.15±11.43 vs 106.26± 15.27 pmol/l, respectively, $p=0.0471$). **d** Association between rs2237897 and ACPR (0.46±0.03 vs 0.56±0.05 vs 0.77±0.18 nmol/l, respectively, $p=0.0156$). Data are shown as means±SEM

linked *KCNQ1* to susceptibility to type 2 diabetes in a Japanese population [2, 3]. In the present study, we replicated the finding that four *KCNQ1* SNPs are associated with susceptibility to type 2 diabetes. The molecular mechanism responsible for the link between *KCNQ1* and the pathogenesis of type 2 diabetes has not been elucidated. However, functional investigations of *KCNQ1* revealed that it is produced in pancreatic islets and that selective blockade of this K$^+$ channel stimulates insulin secretion [10]. Furthermore, clinical trait association analysis showed that baseline insulin secretion is impaired in *KCNQ1* risk allele carriers [2]. In this study, we failed to replicate the association between HOMA-B and the *KCNQ1* SNPs, possibly as a result of the low power of our study to detect this quantitative trait. Based on the mean±SD value for HOMA-B (129.40±125.41) as well as the minor allele frequencies (~0.35) in our controls, we only have 45% power to replicate the reported effect size of *KCNQ1* SNPs on HOMA-B. Nevertheless, we found that the *KCNQ1* SNPs were associated with both the first and second phases of insulin secretion in the controls and with AIR and ACPR in the type 2 diabetic patients after arginine infusion. Although association signals were detected for different SNPs, together these findings suggest that *KCNQ1* SNPs probably contribute to diabetes susceptibility by impairing beta cell function. However, the causal variant(s) in this gene region is still unknown, and the reported associated SNPs may be just genetic markers in linkage disequilibrium

with the causal variant(s). This may also partly explain why association signals for clinical traits were observed for different SNPs. We also observed an association between rs2237895 and HOMA-IR in the controls, but we found no other evidence that *KCNQ1* was associated with insulin sensitivity. Whether this finding is a real signal rather than a false-positive remains unknown and needs to be replicated in other cohorts.

In summary, the present study reported that *KCNQ1* was associated with type 2 diabetes susceptibility in a Chinese population, possibly through its effect on beta cell function. Further effort on deep re-sequencing in *KCNQ1* gene is needed to identify the causal variant(s) and reveal the molecular mechanism under the association.

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