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ARTICLE

A genetic variant of *G6PC2* is associated with type 2 diabetes and fasting plasma glucose level in the Chinese population

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Abstract
Aims/hypothesis Single nucleotide polymorphisms (SNPs) in *G6PC2* have been reported to be associated with fasting plasma glucose level in several populations of European descent. However, whether *G6PC2* variants have a similar effect in other ethnic groups is unknown. The aim of this study was to investigate the effect of common variants of *G6PC2* on type 2 diabetes and related clinical features in a Chinese population.
Methods We selected four SNPs, rs13387347, rs2232316, rs492594 and rs16856187, tagging all the common variants spanning the *G6PC2* gene ($r^2 \geq 0.8$) based on HapMap Chinese data, and genotyped them in a group of 3,676 Shanghai Chinese individuals, comprising 1,876 cases and 1,800 controls.
Results Three SNPs were nominally associated with type 2 diabetes, with rs16856187 showing the strongest evidence for association ($p=0.0009$, empirical $p=0.0047$). Further conditional analysis revealed that the association signal arose from an individual SNP, rs16856187. This SNP was also associated with fasting plasma glucose level in participants with normal glucose regulation ($p=0.0002$), with the fasting plasma glucose level observed to increase by 0.067 mmol/l with each copy of the rare C allele.
Conclusions/interpretation In this study we identified a novel risk-conferring *G6PC2* SNP for type 2 diabetes in a Chinese population and confirmed the previous finding that *G6PC2* variants are associated with fasting plasma glucose concentration.

Keywords Association study · Fasting glucose · G6PC2 · Genetics · Single nucleotide polymorphism · Type 2 diabetes

Abbreviations

G6PC2	Glucose-6-phosphatase catalytic subunit 2
MAF	Minor allele frequency
SNP	Single nucleotide polymorphism

Glucose-6-phosphatase catalytic subunit 2 (G6PC2), also known as islet-specific glucose-6-phosphatase-related protein (IGRP), belongs to the G6PC family of proteins, which are involved in catalysing the hydrolysis of glucose 6-phosphate [1]. Global knockout of the *G6pc2* gene in mice led to a mild metabolic phenotype characterised by a decrease of ~15% in fasting plasma glucose level [2]. Recent genome-wide association studies reported that the *G6PC2* single nucleotide polymorphisms (SNPs) rs560887 and rs563694 were associated with fasting plasma glucose level in people of European descent, and this association has been replicated in several independent Europid populations [3, 4]. However, the effects of this gene on fasting glucose in other ethnic populations have not been estab-

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lished. Moreover, although *G6PC2* variants were not linked to type 2 diabetes in people of European descent, given the different genetic backgrounds of Europid and Asian populations and the tight link between fasting glucose and type 2 diabetes, whether *G6PC2* variants have an effect on susceptibility of type 2 diabetes in the other ethnic groups is also worthy of study. As the HapMap database suggested that both rs560887 and rs563694 are rare variants in Asian and African populations [5, 6], we selected tagging SNPs that captured all common variants of *G6PC2* and genotyped them in a Chinese type 2 diabetes case–control cohort.

Methods

Participants We studied 1876 unrelated type 2 diabetic patients whose details were included in the Shanghai Diabetes Institute inpatient database and 1,800 unrelated controls who participated in the Shanghai Diabetes Studies [7]. All participants were of eastern Han Chinese ancestry and resided in Shanghai or nearby regions. Diabetes was defined according to the 1999 WHO criteria (fasting plasma glucose ≥7.0 mmol/l and/or 2 h plasma glucose ≥11.1 mmol/l) [8] and were treated with oral hypoglycaemic agents and/or insulin. Type 1 diabetes and mitochondrial diabetes were excluded by clinical, immunological (individuals with GAD and/or protein tyrosine phosphatase IA-2 antibodies were excluded) and genetic methods (mitochondrial $tRNA^{Leu(UUR)}$ A3243G mutation carriers were excluded). 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. All the controls were over 40 years old and had no family history of diabetes according to self-report questionnaire data. The clinical characteristics of the study groups are shown in Electronic supplementary material (ESM) Table 1. This study was approved by the institutional review board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Written informed consent was obtained from each participant.

Clinical studies All participants underwent a detailed clinical investigation as described previously [7, 9]. Briefly, anthropometric variables such as height, weight, waist and hip circumference and blood pressure were measured. For the cases, fasting and postprandial plasma glucose levels were measured. For the controls, blood samples were obtained at 0 and 120 min during a 75g OGTT to measure plasma glucose and serum insulin levels. Lipid profiles including total cholesterol, triacylglycerol, LDL-cholesterol and HDL-cholesterol were also obtained. HOMA was used to assess insulin resistance (HOMA-IR) and beta cell function (HOMA-B) [10].

SNP selection and genotyping We selected four tagging SNPs (rs13387347, rs2232316, rs492594 and rs16856187) (Fig. 1) from the HapMap Phase 3 Chinese population using the Tagger software incorporated in Haploview (version 4.1) [11, 12]. These four SNPs could tag all SNPs with a minor allele frequency (MAF) of >0.1 from 3 kb upstream to 10 kb downstream of *G6PC2* based on an r^2 of ≥0.8. 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

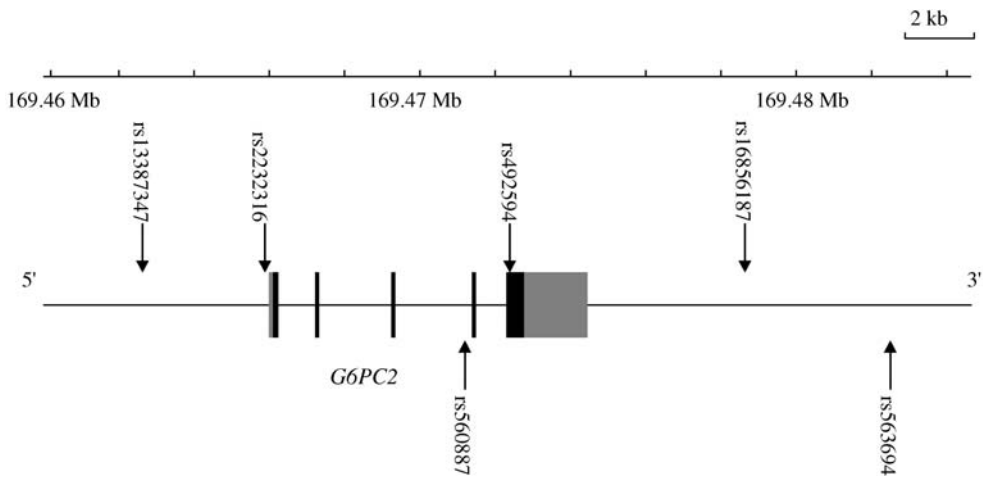

Fig. 1 Schematic map of the human *G6PC2* gene and genetic variants. Exons are represented by boxes, and introns and flanking sequence are shown as lines. Coding sequences are represented by black boxes, and untranslated regions as grey boxes. The SNPs genotyped in our study (rs13387347, rs2232316, rs492594 and rs16856187) are shown above the map and those reported to be associated with fasting plasma glucose in populations of European descent (rs560887 and rs563694) are shown below the map

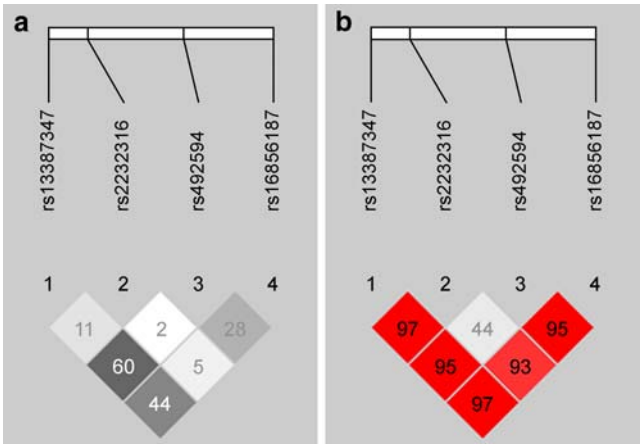

Fig. 2 Linkage disequilibrium analyses for SNPs genotyped in *G6PC2* region. **a** Shades of grey indicate the strength of pairwise linkage disequilibrium based on r^2, and numbers represent r^2 expressed as a percentage. **b** Shades of red indicate the strength of the pairwise linkage disequilibrium based on $|D'|$ and numbers represent $|D'|$ expressed as a percentage

for rs13387347, rs2232316, rs492594 and rs16856187 were 94.2%, 93.6%, 99.2% and 96.1%, respectively. The concordance rates based on 100 duplicates were over 99% for all these SNPs.

Statistical analysis A test for Hardy–Weinberg equilibrium was performed in case and control groups separately for each variant before association analyses. Pairwise linkage disequilibrium measures were calculated for all DNA samples using Haploview [12]. Haplotype frequencies were estimated using an expectation-maximization-based algorithm [13]. The allelic and haplotype frequencies between diabetic and control participants were compared using χ^2 tests, and the results are presented as ORs with 95% CIs. Empirical p values were assessed by 10,000 permutations by Haploview. Quantitative traits with skewed distribution (fasting insulin, 2 h insulin, HOMA-IR and HOMA-B) were logarithmically transformed ($\log_e$) to approximate univariate normality. Quantitative traits were analysed under an additive genetic model by linear regression adjusted for age, sex and BMI. A two-tailed p value of <0.05 was considered statistically significant. The statistical analyses were performed using SAS for Windows (version 8.0; SAS Institute, Cary, NC, USA) unless specified otherwise.

For SNPs with a MAF >0.2 under an additive genetic model, our control cohort had over 80% power to detect a previously reported effect size (0.06 mmol/l per allele) [3, 4], and our case–control cohorts had over 80% power to detect a minimal OR of 1.15 at a level of significance of 0.05.

Results

All SNPs were in Hardy–Weinberg equilibrium. The four SNPs rs13387347, rs2232316, rs492594 and rs16856187 were in moderate linkage disequilibrium in our population, consistent with the HapMap Chinese population (Fig. 2).

We found nominal significant evidence for an association with type 2 diabetes for three SNPs (rs13387347, rs492594 and rs16856187) in this region. The SNP rs16856187 showed the strongest association, with the A allele conferring a significant risk for type 2 diabetes (OR 1.191, 95% CI 1.074–1.321, p=0.0009, empirical p=0.0047) (Table 1). The SNPs rs13387347 and rs492594 were no longer significantly associated with type 2 diabetes after stratification of the groups according to rs16856187 genotype (ESM Table 2), indicating that the associations originally observed for these two SNPs reflected the association signal for rs16856187. In the haplotype association analyses, the haplotype CGCC showed a protective effect against type 2 diabetes (OR 0.8258, 95% CI 0.7452–0.9152, p=0.0003), while the haplotype TGGA conferred an increased risk (OR 1.1332, 95% CI 1.0318–1.2446, p=0.0089) (Table 2). The haplotype ORs were statistically indistinguishable from the individual SNP associations and merely recapitulated the single association signal at rs16856187.

We further investigated the effect of *G6PC2* genetic variants on clinical features related to glucose metabolism in individuals with normal glucose regulation. We found that rs16856187 was associated with fasting plasma glucose concentration after adjusting for age, sex and BMI (β=

Table 1 Associations of *G6PC2* SNPs with type 2 diabetes

SNP	Position	Major/minor allele	Risk allele	Risk allele frequencies		OR (95% CI)	p value
				Case	Control		
rs13387347	−2995	T/C	T	0.560	0.522	1.165 (1.059–1.280)	0.0016
rs2232316	−279	G/A	A	0.123	0.122	1.011 (0.875–1.167)	0.8854
rs492594	6335	C/G	G	0.458	0.426	1.138 (1.037–1.248)	0.0062
rs16856187	12545	A/C	A	0.733	0.697	1.191 (1.074–1.321)	0.0009

The ORs and 95% CIs shown are for the risk allele

Table 2 Associations of haplotypes consisting of four SNPs in the *G6PC2* region with type 2 diabetes

rs13387347	rs2232316	rs492594	rs16856187	Frequency		OR (95% CI)	*p* value
				Case	Control		
T	G	G	A	0.418	0.388	1.1332 (1.0318–1.2446)	0.0089
C	G	C	C	0.258	0.297	0.8258 (0.7452–0.9152)	0.0003
C	G	C	A	0.172	0.169	1.0179 (0.9009–1.1502)	0.7754
T	A	C	A	0.095	0.095	1.0062 (0.8604–1.1768)	0.9386
T	A	G	A	0.026	0.025	1.0379 (0.7763–1.3876)	0.8045
T	G	C	A	0.017	0.014	1.2432 (0.8515–1.8151)	0.2588

0.067 mmol/l per C allele, p=0.0002). However, no statistically significant difference was observed between rs16856187 and other clinical features, including 2 h plasma glucose, fasting and 2 h insulin levels, HOMA-IR and HOMA-B (Table 3). The SNPs rs13387347 and rs492594 were found to be more weakly associated with fasting plasma glucose (β=0.047 mmol/l per C allele, p=0.0045, and β=−0.036 mmol/l per G allele, p=0.0301, respectively) (ESM Tables 3 and 4).

Discussion

In the present study we analysed four SNPs in the *G6PC2* gene in a type 2 diabetes case–control cohort comprising 3,676 Shanghai Chinese individuals. We identified a novel risk-conferring SNP for type 2 diabetes, rs16856187, in our population. We also found that this SNP was associated with fasting plasma glucose level in participants with normal glucose regulation.

G6PC2 belongs to the G6PC family of proteins, which convert glucose 6-phosphate into glucose and phosphate. Overexpression of full-length *G6PC2* could increase glucose-6-phosphatase activity [14]; thus, it may functionally antagonise glucokinase activity, which is critical for glucose utilisation and glucose-stimulated insulin secretion. Moreover, increased G6PC2 activity has been observed in islets from several animal models of diabetes, including the streptozotocin-induced diabetic rat, Goto–Kakizaki rat and *ob/ob* mice [15–17].

Previous studies in populations of European descent suggested that *G6PC2* variants regulated fasting plasma glucose but made no significant contribution to type 2 diabetes susceptibility [3, 4]. A meta-analysis of genome-wide association studies by DIAGRAM did not find a strong association signal between *G6PC2* variants and type 2 diabetes in Europids (p=0.03 for rs560887, p=0.08 for rs492594) either [18]. However, we found that rs16856187 was associated with both fasting plasma glucose and susceptibility to type 2 diabetes in our Chinese population. Among the controls, carriers of the A allele displayed a higher risk of type 2 diabetes and a lower fasting plasma glucose level. Studies have shown that hypersensitivity to glucose and a decreased threshold for insulin secretion is an early step towards beta cell apoptosis [19]. Based on the suggestion that G6PC2 regulates the fasting plasma glucose level by altering the glucose cut-off level for beta cell insulin secretion [3], together with the evidence we found in this study, we hypothesise that A allele carriers may have a lower glucose threshold for insulin secretion, and thus, a higher probability of beta cell apoptosis. Recent reports in individuals of European descent also demonstrated a strong association between *G6PC2* variants and beta cell function [20, 21]. The allele that decreased fasting plasma glucose was also found to lower beta cell function, further strengthening our hypothesis. However, an association

Table 3 Associations of the rs16856187 genotype with clinical features related to glucose metabolism in the participants with normal glucose regulation

	AA (n=866)	AC (n=708)	CC (n=176)	*p* value
Fasting plasma glucose (mmol/l)	4.96±0.49	4.99±0.50	5.13±0.49	0.0002
2 h plasma glucose (mmol/l)	5.46±1.22	5.46±1.20	5.43±1.10	0.6929
Fasting insulin (pmol/l)	40.86 (27.66–59.64)	41.58 (28.14–59.34)	40.98 (28.44–61.62)	0.4021
2 h insulin (pmol/l)	217.86 (135.42–324.42)	210.12 (132.18–329.82)	229.74 (140.94–351.00)	0.2324
HOMA-IR	1.47 (0.98–2.22)	1.51 (0.99–2.19)	1.58 (1.01–2.23)	0.1884
HOMA-B	5.06 (3.55–7.77)	5.09 (3.48–7.39)	4.92 (3.23–7.14)	0.3727

Data are shown as mean±SD or median (interquartile range)
p values were adjusted for age, sex and BMI in an additive genetic model

between *G6PC2* SNPs and type 2 diabetes was not observed in Europids. It is unclear whether ethnic differences in beta cell function, such as those suggested between Asian populations and populations of European descent in several studies [22, 23], played a role in the effect of *G6PC2* variants.

As the SNP rs16856187 is located 3′ downstream of the gene, it is unlikely to play a functional role. It is possible that rs16856187 was only a genetic marker in linkage disequilibrium with the causal variant, as the extent of linkage disequilibrium in the *G6PC2* gene region was high. Also, we cannot exclude the possibility that the neighbouring gene, *ABCB11*, played a functional role, as several variants in the *ABCB11* region (e.g. rs503931, rs497692 and rs3755158) were in linkage disequilibrium (r^2>0.8) with rs16856187. *ABCB11* encodes the major canalicular bile salt export pump in man, and bile acid metabolism is essential for cholesterol homeostasis. Clinical trials have shown that bile acid sequestrants are able to lower the glucose level, probably by reducing the triacylglycerol level [24]. Therefore, rs16856187 would be expected to be associated with lipid profiles if the causal variant is harboured in *ABCB11*. However, we found no evidence for an association of rs16856187 with any variable related to lipid metabolism in the controls (p>0.05). Furthermore, the association between rs16856187 and fasting plasma glucose level remained significant after adjusting for triacylglycerol as a confounder (p=0.0003). Thus, our data do not support the existence of a causal variant harboured in *ABCB11*.

Although we found a novel risk-conferring SNP in *G6PC2*, our results do not indicate whether, as in populations of European descent, rs560887 and rs563694 were associated with glucose metabolism in the Chinese population, as we decided not to genotype these SNPs in our samples, on the basis of the statistical power calculation. As both of the SNPs only have a MAF of around 0.01 in our population, we would not have had enough power to detect any association between these variants and type 2 diabetes had these SNPs been genotyped. As we did not genotype any other SNP with a MAF of <0.1 in the present study, we are unable to conclude whether there is any other rare SNP with a strong effect in the Chinese population. However, this is unlikely given that the available evidence suggests that type 2 diabetes is a complex disease influenced by multiple genes that have a modest or small effect on disease susceptibility and no single variant seems to play a major role. It should be also noted that rs16856187, which is the SNP associated with type 2 diabetes susceptibility in our Chinese population, appears to be monomorphic in Europids according to the HapMap database. Moreover, differences in genetic architecture were observed between Chinese populations and populations of European descent. In our population the SNPs in the *G6PC2* region were in moderate linkage disequilibrium, whereas a greater extent of linkage disequilibrium was observed in European descendants. The substantial differences in MAFs and linkage disequilibrium structure between Europids and Chinese individuals suggest an evolutionary divergence. However, it is highly possible that the causal variant was tagged by different SNPs in the European and Chinese populations.

In summary, we found evidence for association of *G6PC2* SNPs with type 2 diabetes and fasting plasma glucose level in a Chinese population. Although different associated variant was reported, our results strengthen the fact that *G6PC2* variant had an effect on fasting plasma glucose level in the normal glucose regulation population. Further investigations in Asian populations, especially using prospective data, are needed to replicate the observed association with fasting glucose and especially with type 2 diabetes. Fine mapping and functional studies are also warranted to find the causal variant and define the mechanism underlying our observation.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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