Several Polymorphisms of \textit{KCNQ1} Gene Are Associated with Plasma Lipid Levels in General Chinese Populations

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Abstract

\textbf{Background:} Potassium voltage-gated channel, KQT-like subfamily, member 1 (\textit{KCNQ1}) is thought to be an important candidate gene of diabetes. Several single nucleotide polymorphisms (SNPs) in a 40-kb linkage disequilibrium (LD) block in its intron 15 have been identified to be associated with diabetes in East Asian populations in recent genome-wide association studies. The aim of this study was to investigate whether \textit{KCNQ1} polymorphisms influence the levels of the metabolic phenotypes in general Chinese populations.

\textbf{Methodology/Principal Findings:} We investigated the associations of two SNPs (rs2237892 and rs2237895) in the aforementioned 40-kb LD block, a missense variant rs12720449 (P448R) in exon 10, and a synonymous variant rs1057128 (S546S) in exon 13 with metabolic phenotypes in a Uyghur population (n = 478) and replicated these associations in a Han population (n = 2,485). We found that rs2237892-T allele was significantly associated with decreased triglyceride levels ($p_{\text{combined}} = 0.001$). The minor G allele of the rs12720449, with sharp difference of the allelic frequency between European and East Asian populations (0.2% versus 14%, respectively), was associated with a lower triglyceride levels than G allele in Uyghur subjects ($p = 0.004$), in Han subjects ($p = 0.052$), and in subjects of meta-analysis ($p_{\text{combined}} = 0.001$). Moreover, the minor A allele of the rs1057128 was also associated with decreased triglyceride levels in meta-analysis ($p_{\text{combined}} = 0.010$).

\textbf{Conclusions:} To the best of our knowledge, this is the first report associating a missense mutation of \textit{KCNQ1}, rs12720449, with triglyceride levels. Rs2237892, representing the 40-kb LD block, is also associated with triglyceride levels in Han population. Further studies are required to replicate these findings in other East Asian populations.

Introduction

The potassium voltage-gated channel, KQT-like subfamily, member 1 (\textit{KCNQ1}), which encodes the pore-forming voltage-gated K$^+$ channel subunit KvlQ1T1 and is widely expressed in myocardial tissue, plays a key role in the repolarization of the cardiac action potential and in the transport of water and salt in epithelial tissues [1–3]. Loss-of-function and gain-of-function mutations in this gene have been associated with cardiac long QT syndrome, Lange-Nielsen cardioauditory syndrome, atrial fibrillation, and congenital deafness [2–6].

\textit{KCNQ1} is expressed in the inner ear, stomach, intestine, liver, kidney, and pancreatic islets [5]. In the pancreas, \textit{KCNQ1} contributes to the regulation of insulin secretion, and a blockade of the KvlQ1T1 channel may lead to increased insulin secretion [7,8]. Recently, two independent genome-wide association studies (GWAS) revealed that several SNPs (including rs2237892, rs2237895, rs2263228, and rs2237897) within a 40-kb linkage disequilibrium (LD) block in intron 15 of \textit{KCNQ1} were consistently associated with type 2 diabetes and the impairment of insulin secretion in East Asian populations [9,10]. For these SNPs, the associations with type 2 diabetes have been replicated, predominantly not only in East Asian ethnic groups, including Chinese [11,12] and Singaporean populations [13], but also in Euro-Caucasian populations from Denmark [10,14] and Sweden [15]. Moreover, rs2237892 and rs2237895 have also been associated with metabolic phenotypes, such as glucose and body mass index (BMI) levels, in East Asian populations [13,16].

Because \textit{KCNQ1} is critical for the regulation of insulin secretion [5] and insulin is important for the regulation of metabolic phenotypes, we hypothesized that genetic polymorphisms in the \textit{KCNQ1} gene may underlie differences in metabolic phenotypes, such as triglyceride (TG) and total cholesterol (TC). In addition to SNPs located in the 40-kb LD region, the pathogenesis effects of another two important SNPs in the exon regions of the \textit{KCNQ1} gene, rs1057128 and rs12720449, need to be explored. Synony-
mous variant rs1057128 (S546S), located in exon 13 of the KCNQ1 gene, has been found to be associated with various cardiac arrhythmias, such as Long-QT syndrome, atrial flutter, and atrial fibrillation, in European and Chinese individuals [17,18]. Non-synonymous variant rs12720449 (P448R), located in exon 10 of KCNQ1, was also found to be associated with Long-QT syndrome [19,20]. The minor allele frequency (MAF) of this SNP is distinct between Europeans and East Asians (0.2% and 5.8%, respectively), according to the NCBI SNP databank [http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs = 12720449]. No prior studies have investigated the association between these two SNPs and metabolic phenotypes.

The aim of the present study was to investigate the effects of rs1057128, rs12720449, and two SNPs (rs2237892 and rs2237895) within the aforementioned 40-kb LD block on several metabolic phenotypes. We first observed the associations of these four SNPs with BMI, waist-to-hip ratio (WHR), systolic blood pressure (SBP), glucose (Glu), triglyceride (TG), and total cholesterol (TC) in a Uyghur population, an ethnic group with European and East Asian ancestry. Then we observed the associations in a Han population, the ethnic group that accounts for 94% of the Chinese populations, with a larger sample size.

**Results**

The genotypic and allelic frequencies of the studied SNPs

The genomic characteristics of rs1057128, rs2237892, rs2237895, and rs12720449 were summarized in Table 1. All SNPs were consistent with Hardy–Weinberg expectations in both populations (p>0.01). The MAFs of rs12720449 were different for the Han and Uyghur populations in the present study (0.14 and 0.05, respectively). Pair-wise linkage analysis with the four SNPs showed weak LDs (data not shown). To the best of our knowledge, this is the first study investigating SNPs in KCNQ1 in a Uyghur population. Because the sample size for the Uyghur population was relatively small, we compared the metabolic phenotypes of rare allele carriers (CG/GG for rs12720449) with those of non-carriers in the subsequent analysis. The analysis was performed as previously described in the original GWAS [21].

The association between the four SNPs in KCNQ1 and plasma TG levels

We presented the association results between the four SNPs in KCNQ1 and plasma TG levels in Table 2. For SNP rs12720449, significantly lower TG levels were observed in CG/GG carriers (0.94±0.35) than in CC carriers (1.08±0.35) (p=0.004) in the Uyghur population. A preliminary significant association was also observed in the Han population, and the mean TG levels of GG, CG, and CC carriers were 0.87±0.30, 1.03±0.92, and 1.08±0.96, respectively (p=0.052). The minor alleles of rs1057128 (A) and rs2237892 (T) were significantly associated with decreased TG levels in Han subjects (p=0.011 for both alleles).

To investigate allelic associations with TG levels, we performed a meta-analysis across Uyghur and Han datasets (Table 2). We found that the minor alleles of rs12720449 (G), rs1057128 (A), and rs2237892 (T) decreased TG levels at a multiple correction threshold level of p<0.0125 (p=0.001, 0.010 and 0.001, respectively). We also evaluated these associations by including all four SNPs in one model, and the associations between

### Table 1. Genomic characteristics of the four SNPs in studied subjects.

| SNPs         | Gene region | Major/Minor Allele | Population | Genotyped frequency | MAF   | p value* |
|--------------|-------------|---------------------|------------|---------------------|-------|---------|
| rs1057128    | Exon 13     | G/A                 | Han        | 1207/1053/211       | 0.30  | 0.381   |
|              |             |                     | Uyghur     | 252/181/34          | 0.27  | 0.848   |
| rs2237892    | Intron 15   | C/T                 | Han        | 1161/1048/254       | 0.32  | 0.967   |
|              |             |                     | Uyghur     | 310/130/16          | 0.18  | 0.605   |
| rs12720449   | Exon 10     | C/G                 | Han        | 1857/559/60         | 0.14  | 0.022   |
|              |             |                     | Uyghur     | 425/46/2            | 0.05  | 0.533   |
| rs2237895    | Intron 15   | A/C                 | Han        | 1186/1048/238       | 0.31  | 0.769   |
|              |             |                     | Uyghur     | 173/222/77          | 0.40  | 0.684   |

*p value* Deviation from Hardy–Weinberg expectation for the variants was tested using the chi-square statistic. ( doi:10.1371/journal.pone.0034229.t001)

### Table 2. Association between studied SNPs in KCNQ1 and plasma TG levels.

| SNPs     | Uyghur   | Chinese Han | p value |
|----------|----------|-------------|---------|
|          | Uyghur   | Chinese Han | Meta-analysis |
| rs1057128| GG       | 1.05±0.33   | 1.10±0.12 | 0.052 |
|          | GA       | 1.06±0.37   | 1.02±0.84 |       |
|          | AA       | 1.15±0.32   | 1.05±1.05 | 0.271 | 0.011 | 0.010 |
| rs2237892| CC       | 1.06±0.34   | 1.08±0.92 |       |
|          | CT       | 1.08±0.36   | 1.07±0.99 |       |
|          | TT       | 1.00±0.39   | 0.97±0.73 | 0.410 | 0.011 | 0.001 |
| rs12720449| CC      | 1.08±0.35   | 1.08±0.96 |       |
|          | CG       | 0.94±0.35   | 1.03±0.92 |       |
|          | GG       | 0.87±0.30   | 0.004    | 0.052 | 0.001 |
| rs2237895| AA       | 1.05±0.34   | 1.03±0.91 |       |
|          | AC       | 1.07±0.36   | 1.08±0.94 |       |
|          | CC       | 1.05±0.34   | 1.13±1.03 | 0.851 | 0.095 | 0.040 |

*p values were inferred from GLM adjusted for age gender smoking and alcohol drinking.

Bold fonts represent significant difference (p<0.0125).

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rs1057128, rs2237892, rs12720449 and TG levels remained significant \(p = 0.007, 0.001, \) and \(0.001\), respectively).

The association between the four SNPs and the principal components of the metabolic phenotypes

To further validate our results, we conducted a principal component analysis in the Han population using nine metabolic-related traits: BMI, WHR, SBP, DBP, Glu, HDL, LDL, TG, and TC. First, we identified two principal components (PC) of these traits to generate canonical variables (PC1 and PC2) (Table 3). Next, we performed a classic single-trait approach to test the associations between SNPs and the canonical variables. As shown in Table 3, PC2 is strongly correlated with TG (with an eigenvalue of 0.98). All four SNPs were significantly associated with PC2 at a significant level of 0.05 \(p = 0.017, 0.020, 0.043, \) and 0.050, but this association did not reach the multiple correction threshold level of \(p < 0.0125\).

The association between SNPs in KCNQ1 and other metabolic phenotypes excluding TG

We investigated the relationships of the four SNPs in \textit{KCNQ1} with other metabolic-related parameters, including BMI, WHR, SBP, DBP, Glu, HDL, LDL, and TC. We found a significant association between rs1057128 and SBP in the Han population at the significant level of 0.0125. In the Uyghur population, we found a significant association between rs2237892 and WHR at the significant level of 0.05 (Table 4). We also performed a meta-analysis across the two datasets for the Uyghur and Han populations, but we found that none of associations reached the multiple correction threshold level of \(p < 0.0125\).

Discussion

In this population-based association study, we replicated the association between rs2237892, a SNP in intron 15 of \textit{KCNQ1} gene, and TG levels. We also identified a novel association between rs1057128 (S546S), which is located in exon 13 of the \textit{KCNQ1} gene, and TG levels. Most importantly, we identified a missense mutation, rs12720449 (P448R), located in exon 10 of \textit{KCNQ1}, associated with TG levels.

Intron 15 of the \textit{KCNQ1} gene spans approximately 70 kb on chromosome 11p15.5. An extremely long LD block, covering approximately 40 kb, was observed in a Japanese population, and several SNPs (including rs2237892, rs2237895, rs2283228, and rs2237897) located in this LD block were found to be associated with diabetes in Japanese individuals [9,10]. However, the LD block in this 40-kb region was weak in other ethnically distinct East Asian groups, such as Singaporean and Han Chinese populations [11,13,16,22]. Moreover, the associations of the SNPs in this region with other metabolic phenotypes, such as lipid levels, assessed in East Asian populations were inconsistent [23]. In the present study, we did not observe an association between glucose levels and the two SNPs, rs2237892 and rs2237895, within this region. However, we detected associations between rs2237892 and TG levels, rs2237895 and LDL levels, and rs2237895 and TC levels. In the Japanese population, the diabetes causal variant may be in LD with this 40-kb region. In other East Asian ethnic groups, such as Han Chinese or Singaporean populations, different variants affecting lipid metabolic regulation may be in LD with rs2237892 or rs2237895 [13]. Further studies will need to be performed to identify these functional variants.

In the present study, rs1057128 was associated with TG levels in the Han population. This SNP was originally identified in association with various cardiac arrhythmias [17,18]. Although rs1057128 (S546S) is a synonymous variant, there is evidence indicating that synonymous mutations can affect the thermodynamic stability of mRNA secondary structures or affect splicing through phenomena such as exon skipping; therefore, synonymous mutations may not be neutral in evolution [24]. Additionally, a small domain between residues 589 and 620 in the \textit{KCNQ1} C terminus may function as an assembly domain for \textit{KCNQ1} subunits.

### Table 3. PCA results of parameters of MS in Chinese Han.

| variable | Prin1 | Prin2 |
|----------|-------|-------|
| BMI      | 0.37  | 0.05  |
| WHR     | -0.34 | -0.02 |
| LDL     | 0.23  | -0.06 |
| HDL     | 0.35  | 0.10  |
| GLU     | 0.37  | 0.06  |
| TG      | -0.14 | 0.98  |
| SBP     | 0.37  | 0.05  |
| DBP     | 0.37  | 0.05  |
| TC      | 0.37  | 0.08  |
| Eigenvalue | 7.13 | 0.88  |
| Proportion | 0.79 | 0.10  |

### Table 4. Association between SNPs in KCNQ1 and other metabolic phenotypes.

| SNP       | Common genotype | Rare genotype | \(p\) value |
|-----------|-----------------|---------------|-------------|
| rs1057128 |                 |               | 0.017       |
| rs2237892 |                 |               | 0.020       |
| rs12720449|                 |               | 0.043       |
| rs2237895 |                 |               | 0.050       |

The \(p\) values were inferred from GLM adjusted for age, gender, smoking, and alcohol drinking.

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was an open-ended prospective study with very broad research aims. The design and baseline characteristics of this study have been previously described [31].

Subjects who reported receiving medications for the treatment of metabolic-related diseases were excluded. Subjects who reported having tumors, autoimmune diseases, or hematological diseases were also excluded. Written informed consent was obtained from each participant, and all protocols were approved by the Human Ethics Committee of Fudan University.

Data collection
A standardized interview was conducted by trained personnel, and detailed information was collected concerning medical history and lifestyle characteristics, such as smoking and alcohol consumption. All participants received a physical examination and blood tests at a local hospital after overnight fasting. A standardized mercury sphygmomanometer was used to measure SBP and DBP, and these measurements were performed by two cardiologists. Body weight and height were measured with subjects wearing only light indoor clothing and without shoes. BMI was calculated by dividing weight (kg) by height squared (m²). The waist circumference was measured midway between the caudal point of the costal arch, as palpated laterally, and the iliac crest. The hip circumference was measured at the symphysis-trochanter femoris level. WHR was calculated by dividing the waist circumference by the hip circumference. The blood specimens were drawn after overnight fasting, immediately subjected to centrifugation, and analyzed within 8 h for Glu, TG, and TC. The distribution of the clinical parameters is listed in Table 5.

Genotyping
Blood samples were collected into EDTA-containing receptacles, and genomic DNA was extracted using a standard method. Sample DNA (10 ng) was amplified by PCR according to the manufacturer’s instructions. Genotyping of the four SNPs in KCNQ1, rs1057128(G>A), rs2237892(C>T), rs2237895(A>C), and rs12720449(C>G) was performed using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA). The
statistical analysis
Deviation from Hardy-Weinberg expectation for the variants was tested using the chi-square statistic. Haplotype inference was conducted using Haplovew software [32]. We treated the metabolic phenotypes [BMI, WHR, SBP, DBP, FBG, LDL, HDL, TG, and TC] as quantitative traits. Because these traits exhibited a skewed distribution, we normalized these traits using the Box-Cox method, which can be used to automatically identify a suitable power transformation for the data. To estimate the association of the variants with the metabolic parameters, we applied a general linear regression model under an additive model, adjusting for sex, age, smoking, and drinking, with the normalized BMI, WHR, SBP, DBP, FBG, LDL, HDL, TG, and TC. Each SNP was separately entered into the model as the explanatory variable. A pre-specified threshold of $p < 0.0125$ was used for multiple correction significance (corresponding to $p < 0.05$ after adjusting for four loci). We also performed a principal component analysis on the nine variables representing metabolic phenotypes in the Han population. The association of the variants in KCNQ1 with changes in the extracted principal components was estimated using a general linear regression model. All analyses were performed using SAS statistical software (release 8.2, SAS Institute Inc, Cary, NC, USA).

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Author Contributions
Conceived and designed the experiments: XIW LJ YJ. Performed the experiments: XIJC SYL LZ. Analyzed the data: XIJC QgP XIW. Contributed reagents/materials/analysis tools: YJY LJ XJW. Wrote the paper: XIJC XIW.

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