An Exploratory Study of the Association between KCNB1 rs1051295 and Type 2 Diabetes and Its Related Traits in Chinese Han Population

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Abstract

Since the KCNB1 encoding Kv2.1 channel accounts for the majority of Kv currents modulating insulin secretion by pancreatic islet beta-cells, we postulated that KCNB1 is a plausible candidate gene for genetic variation contributing to the variable compensatory secretory function of beta-cells in type-2 diabetes (T2D). We conducted two studies, a case-control study and a cross-sectional study, to investigate the association of common single-nucleotide polymorphisms (SNPs) in KCNB1 with T2D and its linking traits. In the case-control study, we first examined the association of 20 tag SNPs of KCNB1 with T2D in a population with 226 T2D patients and non-diabetic subjects (screening study). We then identified the association in an enlarged population of 412 T2D patients and non-diabetic subjects (replication study). In the cross-sectional study, we investigated the linkage between the candidate SNP rs1051295 and T2D by comparing beta-cell function and insulin sensitivity among rs1051295 genotypes in a general population of 1051 subjects at fasting and after glucose loading (oral glucose tolerance tests, OGTT) in 84 fasting glucose impaired subjects, and several T2D-related traits. We found that among the 19 available tag SNPs, only the KCNB1 rs1051295 was associated with T2D (P = 0.027), with the rs1051295 TT genotype associated with an increased risk of T2D compared with genotypes CC (P = 0.009). At fasting, rs1051295 genotype TT was associated with a 9.8% reduction in insulin sensitivity compared to CC (P = 0.008); along with increased plasma triglycerides (TG) levels (TT/CC: P = 0.046) and increased waist/hip (W/H) ratio (TT/CC: P = 0.013; TT/TC: P = 0.002). OGTT confirmed that genotype TT exhibited reduced insulin sensitivity by 16.3% (P = 0.030) compared with genotype TC+CC in a fasting glucose impaired population. The KCNB1 rs1051295 genotype TT in the Chinese Han population is associated with decreased insulin sensitivity and increased plasma TG and W/H ratio, which together contribute to an increased risk for T2D.

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Introduction

Although the precise mechanisms underlying the development and progression of T2D remain unclear, a consensus is that pathophysiologic defects underlying T2D include insulin resistance of peripheral tissues and defects in pancreatic islet insulin secretory capacity [1], each influenced by environmental and genetic factors [2]. With respect to the latter, numerous genome-wide association studies (GWAS) have been conducted leading to identification of susceptibility loci for T2D. SNPs of ion channel genes contributing to beta-cell secretory defects in T2D include ATP-sensitive K+ (KATP) channels (KCNJ11) [3] and Ca2+ channels (CACNA1E) [4]. KATP channel closure causes cell depolarization which opens
Ca$^{2+}$ channels to enable Ca$^{2+}$ influx that evokes insulin exocytosis, and thus defects in these ion channels would result in insulin secretory insufficiency [5]. Most recently, studies revealed SNPs in KCNQ1, encoding Kv7.1, associated with T2D in the Japanese population [6], and confirmed to be present in Chinese [7,8], Koreans [6], and also Swedes [6] and Danes [9]. Furthermore, common variants of KCNQ1 have been shown to be associated with reduced insulin granule docking and depolarization-evoked insulin exocytosis [10], and impairment in insulin secretion during glucose loading [11].

Voltage-gated K$^+$ (Kv) channels regulate cell membrane repolarization that controls duration of Ca$^{2+}$ channel opening [12], which in beta-cells influences duration of insulin secretion [13,14]. Kv7.1 is however not the major Kv channel in pancreatic islets, but rather in the heart, where genetic defects account for [13,14]. Kv7.1 is however not the major Kv channel in pancreatic islets, but rather in the heart, where genetic defects account for [13,14].

Kv2.1 is the dominant Kv channel accounting for ~70% of membrane repolarisation in beta-cells is Kv2.1 [13,14], encoded by KCNB1 located in chromosome 20q13.2 [17]. The Kv2.1 channel is also expressed in brain, atria, ventricle, skeletal muscle and other tissues [17]. Based on our previous work on the beta-cell Kv2.1 channel identifying its important role in modulating of insulin secretion [18,19], we pursued the possibility and hypothesis that there may be a genetic variation in KCNB1 associated with T2D that could influence disease progression and/or compensatory capacity. This prompted us to search for candidate KCNB1 SNPs associated with T2D, employing a case-control study, followed by a cross-section study to examine underlying type 2 diabetic-related traits linking this association in the Chinese Han population.

**Materials and Methods**

This study was approved by the Ethics Committees of Capital Medical University (Beijing, China) and also the Beijing Geriatric Hospital and Beijing Xuanwu Hospital, and was conducted in accordance with the principles of the Helsinki Declaration II. Written consent was obtained from all participants.

**Study Participants and Study Design**

In the case-control study, the participants were composed of 412 Chinese Han participants. This included 176 unrelated individuals with T2D designated as Cases, identified by 1999 WHO criteria [20]. These subjects were recruited consecutively from the Department of Endocrinology of the two Beijing hospitals, Xuanwu Hospital Capital Medical University and Beijing Geriatric Hospital. 236 unrelated non-diabetic individuals were recruited consecutively, designated as Controls, from the Departments of Otolaryngology and Ophthalmology of the above two hospitals.

**Genotyping, PCR and Quality Control**

For the replication study and the cross section study, rs1051295 was genotyped using PCR-based pyro-sequencing technology and DNA sequencing with an ABI 3730 automated sequencer (Applied Biosystems, Foster city, CA). The primers are F: biotin-GGCCAAXAACCCTTACTCA AAT and R: GCCAGGGGG-CATTAGAAT for PCR amplification and 5'- TGGTATCT-TGGTATCT-TGGTATCT-CAA AATTTAATGT-3' for sequencing. The primers were designed according to the published sequence of KCNB1 (http://www.ncbi.nlm.nih.gov/pubmed/), the GenBank accession number is NT_002370.

PCR was conducted in a 50-μl reaction mixture containing 100 ng genomic DNA, 0.5 pmol primer, 2× master mix (mixture of reaction buffer, MgCl$_2$ and DNA polymerase) (Toyobo, Japan). PCR products were denatured for 2 min at 95°C and then thermal-cycled for 30 s at 95°C, 30 s at 59°C, and 60 s at 72°C, repeating the cycle 40 times. A final extension step at 72°C for 10 min completed the program. Pyrosequencing analysis was performed on Streptavidin Sepharose™ HP (Amersham, Sweden). sDNA prepared from 50 ul of biotinylated PCR product

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was then subjected to pyrosequencing. The call rates was 96.02% (169/176) and 94.92% (224/236) for cases and control, respectively, for the replication study, and 97.91% (1029/1051) and 100% (84/84), respectively, for the first and second stages of the cross-section study.

For both the case-control and the cross-section studies, genotyping was repeated in 5% of random samples for verification and quality control, which all revealed the genotype data had an error rate of <1%.

Assessment of Islet Beta-cell Secretory Function and Insulin Sensitivity

We employed the homeostatic model to calculate beta-cell function (HOMA-B%) and insulin sensitivity (HOMA-S%) at basal condition (the software HOMA calculator downloaded from www.dtu.ox.ac.uk/homa). In the glucose loading condition (OGTT), insulin sensitivity was assessed by the Matsuda index = 10,000/√(Ins0 * Glu0 + mean Glu * mean Ins). Ins0 is fasting insulin, Glu0 is fasting glucose. The islet beta-cell secretory function was assessed by the Insulin Secretion-Sensitivity Index-2 (ISSI-2) = AUC (insulin curve)/AUC (glucose curve) * Matsuda index. The Matsuda index is a validated OGTT-based measure of insulin sensitivity that is analogous to the disposition index obtained from iv glucose tolerance tests [21]. Area under curve (AUC) for glucose and insulin were calculated by the trapezoidal rule.

Statistical Analyses

In the case-control study, the χ² test was used to examine the differences in gender between case and control groups. The differences between T2D-related traits in T2D case and control groups were compared by an independent-sample t test. The distribution of SNPs in Cases and Controls were compared using the χ² test and an unconditional Logistic regression analysis, in which the association of a SNP with T2D was adjusted for age, gender and BMI. T2D-related traits among the three genotypes of rs1051295 were compared by one-way ANOVA test.

The program Haploview (http://www. broad. mit.edu/mpg/ haploview/) was used to calculate pair-wise linkage disequilibrium statistics and to test allelic and haplotype associations with T2D.

In the cross-section study, we used independent-samples t test to compare T2D-related traits between genotypes (TT vs TC+CC) in OGTT, or one-way ANOVA test among 3 genotypes at fasting. Association of rs1051295 genotypes with HOMA-B%, HOMA-S%, ISSI-2, Matsuda index and other T2D-related traits were examined by multiple linear regression analysis, in which association was adjusted for age, gender, and BMI.

Hardy–Weinberg equilibrium was determined for each SNP distribution. All analysis was done on SPSS software, version 18.0 (purchased by Capital Medical University, China). All tests were two-tailed, with a significance level of 0.05. Data were expressed as means ± SD, unless the data did not conform to a normal distribution, in which case the data were expressed as median and quartiles and natural Log-transformed for analysis.

Results

Candidate-gene Association Study Identified KCNB1 3’-UTR rs1051295 is Likely to be Associated with T2D

The demographic and clinical characteristics of the participants for the case-control study are summarized in Table 2. Among the 19 available tag SNPs, the distribution of rs742759 (P = 0.16) and rs1051295 (P = 0.18) showed a possible association with T2D. The other 17 tag SNPs did not show a trend of association with T2D.

Table 1. KCNB1 gene tag SNPs information.

| SNP ID   | Genomic position (bp) | Genic position | Alleles (major/minor) | MAF | HWE (P) |
|----------|-----------------------|----------------|-----------------------|-----|---------|
| rs1051295| 47988905              | 3’-UTR         | T/C                   | 0.45| 1.00    |
| rs186942 | 48045742              | Intron         | G/C                   | 0.24| 0.85    |
| rs1961192| 48063070              | Intron         | T/C                   | 0.47| 0.41    |
| rs237451 | 48024332              | Intron         | C/T                   | 0.33| 0.46    |
| rs237458 | 48031549              | Intron         | G/T                   | 0.24| 0.17    |
| rs237476 | 48051382              | Intron         | T/C                   | 0.28| 0.34    |
| rs237477 | 48057448              | Intron         | T/C                   | 0.38| 0.90    |
| rs3787318| 48058067              | Intron         | T/C                   | 0.35| 1.00    |
| rs4810952| 48006175              | Intron         | T/C                   | 0.20| 1.00    |
| rs533213 | 48097564              | Intron         | T/C                   | 0.24| 0.91    |
| rs562954 | 48092076              | Intron         | G/A                   | 0.24| 0.34    |
| rs572845 | 48076614              | Intron         | T/C                   | 0.11| 0.74    |
| rs579113 | 48075912              | Intron         | A/G                   | 0.23| 0.27    |
| rs610142 | 48078202              | Intron         | A/C                   | 0.24| 0.91    |
| rs6125647| 48027463              | Intron         | T/C                   | 0.11| 0.87    |
| rs653070 | 48087408              | Intron         | C/T                   | 0.13| 1.00    |
| rs7269864| 48096608              | Intron         | T/C                   | 0.13| 1.00    |
| rs742759 | 48061145              | Intron         | G/A                   | 0.19| 0.05    |
| rs802952 | 48086269              | Intron         | T/G                   | 0.19| 0.05    |

MAF: minor allele frequency. HWE: Hardy-Weinberg equilibrium.
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(P<0.20, data not shown). However, only rs1051295 had a trend towards association with T2D (P=0.08) when adjusted for age, gender and BMI (Table 3).

To determine whether these SNPs demonstrated any additional evidence of association with T2D when examined together, haplotypes for 19 SNPs were constructed using the Haploview program. We identified four haplotypes for the KCNB1 gene, and found no association between any of them and T2D (data not shown). It is noteworthy that rs1051295 was not in a haplotype block generated in this study due to weak linkage disequilibrium shown). It is noteworthy that rs1051295 was not in a haplotype block generated in this study due to weak linkage disequilibrium with other SNPs.

In order to identify this association, a replication study was performed in the source population of the above screening study. This population consisted of 176 cases and 236 controls. Hardy–Weinberg equilibrium testing showed P=0.06 and 0.14 for rs1051295 genotypes in cases and controls, respectively. TT genotype of rs1051295 demonstrated a significantly increased risk for T2D compared with the CC (P=0.009, OR =2.58, 95% CI = (1.27, 5.23)), but not with TC (P=0.226, OR = 1.36, 95% CI = (0.83, 2.25)) genotypes. In the dominant model, allele C (genotype CC and TC) did not show decreased risk for T2D compared with genotype TT (P=0.071, OR =0.64, 95% CI = (0.40, 1.04)); but in the recessive model, allele T (genotype TT+TC) showed increased risk for T2D compared with genotype CC (P=0.02, OR = 2.10, 95% CI = 1.13,3.90) (Table 4).

We further compared a number of T2D-related traits among the three variants of rs1051295 including BMI, W/H ratio, TG, fasting plasma glucose, SBP, DBP as well as gender and age, but found no association of any genotype with these traits (Table S1 and Table S2).

**Table 2.** Characteristics of the participants in the case-control study.

| Variables          | Screen study | Replication study |
|--------------------|--------------|-------------------|
|                    | Case (n = 112) | Control (n = 114) | P  | Case (n = 176) | Control (n = 236) | P  |
| Age (years)        | 66.96±13.32   | 67.32±15.25       | 0.85 | 65.10±14.06   | 63.92±15.01       | 0.42 |
| M/F                | 56/56         | 68/46             | 0.15 | 88/88         | 124/112           | 0.61 |
| BMI (kg/m²)        | 25.21±4.33    | 26.39±4.20        | 0.01 | 25.31±4.29    | 23.53±3.99        | <0.001 |
| W/H ratio          | 0.90±0.07     | 0.89±0.08         | 0.37 | 0.89±0.07     | 0.88±0.09         | 0.27 |
| TG (mmol/L)        | 2.15±1.78     | 2.17±0.62         | <0.001 | 2.02±1.57    | 1.41±0.75         | <0.001 |
| SBP (mmHg)         | 129.74±16.23  | 126.35±17.99      | 0.11 | 129.91±15.61  | 126.37±17.73      | 0.04 |
| DBP (mmHg)         | 74.55±9.41    | 76.06±12.00       | 0.30 | 75.87±9.50    | 76.05±12.00       | 0.87 |
| FPG (mmol/L)       | 9.29±3.06     | 9.29±2.75         | <0.001 | 9.16±3.68    | 5.50±0.79         | <0.001 |

M/F: male/female. W/H ratio: waist/hip circumference. TG: triglycerides. SBP: systolic blood pressure. DBP: diastolic blood pressure. FPG: Fasting plasma glucose. Data are presented as mean ±SD. P from independent-sample t test except P for M/F from x² test. doi:10.1371/journal.pone.0056365.t002

**Table 3.** Distribution of two tag SNPs of KCNB1 in the screening study.

| SNP ID | Cases | Controls | χ² | P₁ | P² | OR (95% CI) |
|--------|-------|----------|----|----|----|-------------|
| rs1051295 | n = 110 | n = 111 | 3.41 | 0.18 |    |              |
| CC     | 13    | 23       |    |    |    |              |
| TC     | 62    | 59       |    |    |    |              |
| TT     | 35    | 29       |    |    |    |              |
| rs742759 | n = 103 | n = 110 | 4.63 | 0.16 |    |              |
| TT vs CC | 0.08 | 2.18(0.89–5.31) |    |    |    |              |
| TT vs TC | 0.15 | 1.81(0.89–4.09) |    |    |    |              |
| GG     | 69    | 86       |    |    |    |              |
| GA     | 23    | 18       |    |    |    |              |
| AA     | 11    | 6        |    |    |    |              |
| AA vs GG | 0.19 | 1.44(0.84–2.56) |    |    |    |              |
| AA vs GA | 0.56 | 1.45(0.42–4.96) |    |    |    |              |

P₁: from χ² test; P²: from Logistic regression analysis and adjusted for age, gender and BMI. OR: odds ratio. CI: confidence interval. doi:10.1371/journal.pone.0056365.t003

**Table 4.** Distribution of KCNB1 rs1051295 SNPs in the replication study.

| SNP ID | Cases | Controls | χ² | P₁ | P² | OR (95% CI) |
|--------|-------|----------|----|----|----|-------------|
| rs1051295 | n = 169 | n = 224 | 7.21 | 0.027 |    |              |
| CC     | 21    | 48       |    |    |    |              |
| TC     | 93    | 123      |    |    |    |              |
| TT     | 55    | 53       |    |    |    |              |
| rs742759 | n = 103 | n = 110 | 4.63 | 0.16 |    |              |
| TT vs CC | 0.009 | 2.58(1.27,5.23) |    |    |    |              |
| TT vs TC | 0.226 | 1.36(0.83,2.25) |    |    |    |              |
| Dominant model | 0.051 | 0.071 | 0.64(0.40,1.04) |    |    |              |
| Recessive model | 0.020 | 0.020 | 2.10(1.13,3.90) |    |    |              |

P₁: from χ² test; P²: from Logistic regression analysis and adjusted for age, gender and BMI. Dominant Model: CC+TC compared with TT; Recessive Model: TT+TC compared with CC. OR: odds ratio. CI: confidence interval. doi:10.1371/journal.pone.0056365.t004
statistical significance (\(TT\) decreased insulin sensitivity (HOMA-S%) with that of genotype TT in the dominant model, the result showed genotype TT decreased insulin sensitivity \(P = 0.05, b = -5.22, 95\% CI = (-10.49, -0.00)\), whereas allele T (genotype TC and TT) did not show decreased insulin sensitivity compared with genotype CC in the recessive model \(P = 0.08, b = -5.67, 95\% CI = (-12.53, 0.79)\). Importantly, the rs1051295 genotype TT was associated with an unfavorable W/H ratio and higher plasma TG levels compared to genotype CC \((W/H\) ratio: C/T: 8/0.01, b = 0.91, 95\% CI (0.02, 1.84); CC: 8/0.01, b = 0.08, 95\% CI (0.01, 0.13)) compared with genotype CC at basal condition. To investigate whether the insulin secretory function of beta-cell in the carrier of genotype TT is superior to genotype CC, we performed OGTT to assess the compensatory secretory function and insulin sensitivity of beta-cells after a glucose challenge. Because of the small size of the groups, and genotype CC and TC demonstrated the similar phenotype in insulin sensitivity in the dominant model at basal glucose, we combined CC and TC as one to compare its insulin sensitivity with genotype TT.

OGTT also demonstrated genotype TT displayed decreased insulin sensitivity (Matsuda index: 5.05) by 16.3\% compared to CC+TC (Matsuda index: 6.03) \(P = 0.03, b = -1.34, 95\% CI = (-2.56, -0.12)\). This is most dramatically seen at 120min where insulin secretion from genotype TT (76.45 mmol/L) was 22.8\% higher than genotype CC+TC (54.04 mmol/L), whereas blood glucose levels at this time point were still 14.4\% higher in genotype TT (9.9 mmol/L) than CC+TC (8.5 mmol/L) [Fig. 1]. These results indicate that subjects with rs1051295 genotype TT exhibited greater insulin sensitivity than genotype TT during a glucose load and confirmed the finding at basal condition.

**Discussion**

In summary, we unexpectedly found that KCNB1 rs1051295 genotype TT was associated with decreased insulin sensitivity at basal conditions in a general population. Of the three variants in KCNB1 rs1051295, CC (99.9\%) and to a less extent of TT (95.2\%) exhibited normal insulin sensitivity at basal condition, whereas TT genotype (90.1\%), exhibited reduced insulin sensitivity at the fasting state compared to CC \((P = 0.008, b = -0.09, 95\% CI = (-0.16, -0.02)) and TC \((P = 0.096, b = -0.04, 95\% CI = (-0.09, 0.01))\), genotype TT also decreased insulin sensitivity compared with genotype (CC+TC) \((b = 0.05, b = -5.22, 95\% CI = (-10.49, -0.00))\). This was also confirmed in a fasting glucose impaired population at glucose loading condition. In the OGTT, the TT genotype also exhibited lower insulin sensitivity compared with genotype (CC+TC) \((P = 0.03, b = -1.34, 95\% CI = (-2.56, -0.12))\). Collectively, these results indicate that KCNB1 rs1051295 TT confers on its carriers the phenotype of decreased insulin sensitivity that is likely to increase the risk of T2D.

Kv2.1, encoded by KCNB1, is the major beta-cell Kv channel in humans [13,22] and rodents [13,14] accounting for >70\% of outward K+ currents. Kv2.1 deletion in mice caused severe perturbation in insulin release and blood glucose [14]. It is reasonable to expect the rs1051295 genotype to be associated with an increased the risk of T2D by impairing islet beta-cell secretory

| Table 5. Comparison of T2D-related traits among rs1051295 genotypes in a general population at fasting. |

| Variable | TT | TC | CC | TT vs TC | TT vs CC |
|----------|----|----|----|---------|---------|
| N (%)    | 346 (33.62) | 514 (49.95) | 169 (16.42) |
| M/F      | 153/193 | 226/288 | 74/95 |
| Age (years) | 40.03±10.64 | 40.30±10.16 | 38.72±9.66 |
| W/H ratio | 0.81±0.07 | 0.80±0.07 | 0.79±0.07 |
| TG (mmol/L)* | 1.04 (0.74,1.46) | 0.98 (0.68,1.38) | 0.93 (0.67,1.31) |
| HDL-C (mmol/L) | 1.65 ± 0.35 | 1.65 ± 0.35 | 1.65 ± 0.35 |
| LDL-C (mmol/L) | 2.72 ± 0.78 | 2.64 ± 0.66 | 2.63 ± 0.68 |
| TCH (mmol/L) | 4.69 ± 0.88 | 4.57 ± 0.83 | 4.55 ± 0.76 |
| FPG (mmol/L) | 4.98 ± 0.51 | 4.99 ± 0.46 | 4.99 ± 0.50 |
| FINS (mU/L) | 9.08 ± 3.51 | 8.60 ± 3.36 | 8.14 ± 3.07 |
| HOMA-%S* | 90.16 (85.27,110.18) | 91.18 (86.20,126.40) | 90.35 (85.40,126.98) |
| HOMA-β%* | 105.30 (86.85,125.75) | 102.30 (80.90,121.00) | 99.15 (79.40,118.50) |

M/F: male/female. W/H ratio: Waist/hip circumference. TG: Triglycerides. HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. TCH: total cholesterol. FPG: Fasting plasma glucose. FINS: Fasting insulin: b: unstandardized coefficients; CI: confidence interval. \(P^2\): from one-way ANOVA test except for P for M/F from \(\chi^2\) test; \(b\), and 95% CIs: from multiple linear regression analysis adjusted for age, gender and BMI. *presented as median and quartiles and natural Log-transferred for analysis.

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function. We compared the beta-cell function among rs1051295 genotypes at basal condition, whereby HOMA-B% (in vivo assessment of beta-cell function) paradoxically showed a higher value in genotype TT than CC ($P$ = 0.004, $b = 0.08$, 95% CI (0.03, 0.13)). This should not be interpreted to represent a superior beta cell secretory function of the TT genotype, but rather, the higher HOMA-B% in the TT genotype indicates a compensatory action of the pancreatic islet in response to the reduced insulin sensitivity.

To confirm this thinking, we compared the beta-cell function under glucose loading condition, and employed the ISSI-2 assessment as a more accurate measurement of beta-cell compensatory insulin secretory function. ISSI-2 assessment showed the KCNB1 rs1051295 CC and TC genotypes was 16.31% higher than TT (Table 6) ($P$ = 0.13, $b = 2.09$, 95% CI (2.03, 0.09)), suggesting that the TT genotype might have actually reduced insulin secretory capacity, at least with increased glycemic demand during OGTT, and presumably after a meal.

In this study we unexpectedly found that insulin sensitivity was significantly reduced in the KCNB1 rs1051295 genotype TT compared with genotypes CC and TC. Major insulin sensitive

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**Figure 1. Relationship between plasma insulin and glucose levels during OGTT (0, 30th, 60th and 120th min).** $n=23$ for rs1051295 genotype TT and 61 for rs1051295 genotypes CC+TC. doi:10.1371/journal.pone.0056365.g001

**Table 6.** Comparison of T2D-related traits between rs1051295 genotype CC+TC and genotype TT in a type 2 diabetic-suspected population undergoing an OGTT.

| Variable          | TT     | TC+CC | $p^1$ | $p^2$ | $b$ (95% CIs) |
|-------------------|--------|-------|------|------|----------------|
| N                 | 23     | 61    |      |      |                |
| M/F               | 9/14   | 25/36 | 0.82 |      |                |
| Age (years)       | 54.70±9.90 | 51.92±10.64 | 0.29 |      |                |
| BMI (kg/m²)       | 24.07±3.06 | 25.01±2.66 | 0.18 |      |                |
| Glucose0 (mmol/L) | 6.64±1.82 | 6.27±1.28 | 0.30 |      |                |
| Glucose30 (mmol/L)| 10.97±2.98 | 10.54±2.55 | 0.54 |      |                |
| Glucose60 (mmol/L)| 11.81±3.90 | 11.60±3.67 | 0.85 |      |                |
| Glucose120 (mmol/L)| 9.89±4.44 | 8.47±3.45 | 0.13 |      |                |
| Insulin0 (mmol/L)*| 12.84(9.94,16.06) | 12.53(9.83,15.25) | 0.61 |      |                |
| Insulin30 (mmol/L)*| 44.87(33.41,75.59) | 42.83(27.25,71.73) | 0.36 |      |                |
| Insulin60 (mmol/L)*| 66.53(49.38,107.76) | 65.64(43.42,98.72) | 0.74 |      |                |
| Insulin120 (mmol/L)*| 76.45(33.74,106.15) | 54.84(35.32,74.54) | 0.10 |      |                |
| ISSI-2*           | 32.23(26.67,37.71) | 37.54(21.45,50.33) | 0.30 | 0.13 | −0.29 (−0.67,0.09) |
| Matsuda index     | 5.05±1.94 | 6.03±2.38 | 0.11 | **0.03** | −1.34 (−2.56, −0.12) |

M/F: male/female. W/H ratio: waist/hip circumference. b: unstandardized coefficients. CI: confidence interval. $p^1$: from one-way ANOVA test except for $P$ for M/F from $\chi^2$ test; $p^2$, b and 95% CIs were from multiple linear regression analysis and adjusted for age, gender, and BMI. *presented as median and quartiles and natural Log-transferred for analysis. doi:10.1371/journal.pone.0056365.t006
tissues are skeletal muscle, adipose tissue and liver. While Kv2.1 is known to be present in skeletal muscle to regulate membrane excitability [23], it is not known to regulate GLUT4 vesicle transport and exocytosis. Recent work demonstrating that Kv2.1 directly interacts with exocytotic SNARE proteins to modulate exocytosis [19-24] raises the possibility of non-channel exocytotic function(s) of Kv2.1 in skeletal muscle regulating GLUT4 transport or function. Kv2.1 is abundant in neuronal tissue [12] which might influence insulin-sensitive tissues.

We also showed that rs1051295 genotype TT is associated with higher TG levels and affected fat distribution towards an unfavorable waist/hip ratio and abdominal obesity. It is not clear how Kv2.1 could affect adipose tissue metabolism and distribution. We demonstrated the waist mean HDL-C (TT: 1.65 versus CC: 1.67, \(P = 0.47\)) was lower and LDL-C (TT: 2.72 versus CC: 2.63, \(P = 0.46\)) was higher in the population with a TT genotype than those with a CC genotype, which may provide some clues for future investigation on how rs1051295 genotype TT might influence fat metabolism. In support of a role of Kv channels in adipocyte metabolism, Kv channel activity has been recorded in human adipocytes [26], which responded to insulin by increasing channel density [27]. However, the specific Kv isoform or its downstream actions in adipocytes have not been critically assessed, thus requiring much further study. Rs1051295 is located in the 3’-UTR region of KCNB1, and has been found implicated in rheumatoid arthritis [28]. Since this SNP is not located in the coding regions, we speculate that it might not influence channel pore kinetics per se, but rather it may influence the post-translational processing of the Kv2.1 channel. Further study should be aimed at elucidating at identifying the target tissues that rs1051295 exert its effect on insulin sensitivity.

This work has thus identified KCNB1 rs1051295 genotype TT to be associated with reduced insulin sensitivity and increased plasma TG and W/H ratio, these together likely leading to increase the risk of T2D. Further confirmation will be needed for this study to be performed in a larger population and also to determine if these results could be replicated in other (non-Han) populations. Nonetheless, this initial study opens a new avenue to explore a possible role of Kv2.1 and other Kv channel in influencing fat metabolism and insulin sensitivity.

Supporting Information

Table S1 T2D-related quantitative traits in different genotypes of KCNB1 rs1051295 in 226 case-control study.

Table S2 T2D-related quantitative traits in different genotypes of KCNB1 rs1051295 in 412 validation study.

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Author Contributions

Designed the experiments: YH FL. Performed the experiments: Y-XZ YL S-JH X-XP Z-XQ Q-XL NQ Y-XW FL G-QZ Y-HK Y-YX Q-QG. Analyzed the data: Y-XZ FL Y-XL LZ XH G-XL X-HY WW. Contributed reagents/materials/analysis tools: YL JD JW HW Y-QI K-BJ LW LZ S-FW HZ X-QZ S-HZ JZ H-HW. Wrote the paper: YH HG.

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