Variants from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1, SLC30A8 and IGF1 Are Associated with Glucose Metabolism in the Chinese

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Abstract

Background: Recent meta-analysis of genome-wide association studies in European descent samples identified novel loci influencing glucose and insulin related traits. In the current study, we aimed to evaluate the association between these loci and traits related to glucose metabolism in the Chinese.

Methods/Principal Findings: We genotyped seventeen single nucleotide polymorphisms (SNPs) from fifteen loci including GIPR, ADCY5, TCF7L2, VPS13C, DGKB, MADD, ADRA2A, FADS1, CRY2, SLC2A2, GLIS3, PROX1, C2CD4B, SLC30A8 and IGF1 in 6,822 Shanghai Chinese Hans comprising 3,410 type 2 diabetic patients and 3,412 normal glucose regulation subjects. MADD rs7944584 showed strong association to type 2 diabetes (p = 3.5 x 10^{-16}, empirical p = 0.0002) which was not observed in the European descent populations. SNPs from GIPR, TCF7L2, CRY2, GLIS3 and SLC30A8 were also associated with type 2 diabetes (p = 0.0487−2.0 x 10^{-10}). Further adjusting age, gender and BMI as confounders found PROX1 rs340874 was associated with type 2 diabetes (p = 0.0391). SNPs from DGKB, MADD and SLC30A8 were associated with fasting glucose while PROX1 rs340874 was significantly associated with OGTT 2-h glucose (p = 0.0392−0.0014, adjusted for age, gender and BMI), the glucose-raising allele also showed association to lower insulin secretion. IGF1 rs535767 showed significant association to both fasting and 2-h insulin levels as well as insulin secretion and sensitivity indices (p = 0.0160−0.0035, adjusted for age, gender and BMI).

Conclusions/Significance: Our results indicated that SNPs from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1, SLC30A8 and IGF1 were associated with traits related to glucose metabolism in the Chinese population.

Introduction

Diabetes is one of the major health problems worldwide. According to the results of China National Diabetes and Metabolic Disorders Study, the prevalences of total diabetes and prediabetes in China were 9.7% and 15.5%, respectively [1]. Over 90% of the Chinese diabetes patients are type 2 diabetes. Type 2 diabetes is a metabolic disorder characterized by chronic hyperglycemia in the context of insulin resistance and relative insulin deficiency [2]. Although Western lifestyle contributes a lot to the type 2 diabetes epidemic, genetic determinants also influence type 2 diabetes susceptibility. Nowadays, multiple genes were identified to influence type 2 diabetes susceptibility, fasting and postprandial glucose levels [3,4,5,6]. Recent reports on meta-analysis of genome-wide association studies focusing on glucose and insulin related traits identified nine novel fasting glucose loci (ADCA5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1 and C2CD4B), five oral glucose tolerance tests (OGTTs) 2-h glucose loci (GIPR, ADCY5, VPS13C, GCKR and TCF7L2) and one locus (IGF1) associated with fasting insulin levels and insulin resistance [7,8]. Besides, the effects of previous reported type 2 diabetes and/or fasting glucose loci G6PC2, GCK, GCKR, MTNR1B, DGKB, SLC30A8 and TCF7L2 were also replicated in the meta-analysis [7,8]. However, as the initial studies were performed in the European descent adults, replication studies in other ethnic samples are important to fully understand their effects on disease susceptibility. Among these loci, only the effects of GCK, GCKR, G6PC2 and MTNR1B on fasting glucose levels and beta cell function had been well validated in multiple populations [9,10,11,12,13,14], while the effects of the other loci on fasting and 2-h glucose in non-European descent populations remained largely unknown. The effects of these novel loci on type 2 diabetes risk were also unclear. In the present study, we aimed to test for the association of SNPs from fifteen reported loci and type 2

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Participants
We recruited a total of 6,822 participants of Chinese Han ancestry residing in Shanghai, comprising 3,410 type 2 diabetes patients and 3,412 controls. All cases were unrelated type 2 diabetes patients recruited from the inpatient database of Shanghai Diabetes Institute. The controls were subjects with normal glucose tolerance as assessed by standard 75 g OGTTs, and with negative family history of diabetes that recruited from Shanghai Diabetes Study [15] and Shanghai Diabetes Study II [16]. The clinical characteristics of the cases and controls were shown in Table 1.

Clinical measurements
Phenotypes for anthropometric and biochemical traits related to glucose metabolism were extensively measured for both case and control subjects. OGTTs were performed in the controls in the morning after an overnight fast. Blood samples were obtained at the fasting and 2 h during OGTTs. Plasma glucose and serum insulin were measured. Basal insulin sensitivity and beta cell function were calculated from fasting plasma glucose and insulin using HOMA [17]. In addition, insulin secretion and sensitivity were also estimated according to the indices proposed by Stumvoll et al [18] and Gutt et al [19].

SNP selection, genotyping and quality control analysis
We selected seventeen SNPs from fifteen loci (GIPR rs10423928, ADCY5 rs2877716 and rs11708067, TC7FL2 rs1243326 and rs4506565, VPS13C rs17271305, DGKB rs2191349, MADH rs7944584, ADRA2A rs11605924, SLC2A2 rs11920090, GLIS3 rs7034200, PROX1 rs340874, C2CD4B rs11071657, SLC30A8 rs11558471, and IGF1 rs11071657).

**Methods**

Ethics statement
This study was approved by the institutional review board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital in accordance with the principle of the Helsinki Declaration II. Written informed consent was obtained from each participant.

**Table 1.** Clinical characteristics of the study samples.

| Samples (n) | 3,410 | 3,412 |
| Male/female (n) | 1,871/1,589 | 1,364/2,048 |
| Age (years) | 60.33 ± 12.49 | 50.10 ± 14.27 |
| BMI (kg/m²) | 24.38 ± 3.51 | 23.46 ± 3.25 |

**Table 2.** Effects of SNPs from fifteen glucose and insulin loci on type 2 diabetes susceptibility in the Chinese population.

| SNP | Gene | Risk/non-risk allele | Risk allele frequency | OR(95%CI) | P value | Empirical P value | OR(95%CI) | P value | Empirical P value |
|-----|------|----------------------|-----------------------|-----------|---------|------------------|-----------|---------|------------------|
| rs10423928 | GIPR | A*/T | 0.1981 | 0.1838 | 1.097(1.005–1.197) | 0.0378 | 0.4718 | 1.100(1.002–1.218) | 0.0462 | 0.5638 |
| rs2877716 | ADCY5 | C*/T | 0.9978 | 0.9976 | 1.060(0.524–2.145) | 0.8717 | 1.009 | 1.055(0.484–2.296) | 0.8932 | 1.000 |
| rs11708067 | ADCY5 | A*/G | 0.9891 | 0.9973 | 1.376(0.673–2.810) | 0.3940 | 0.9998 | 1.386(0.654–2.934) | 0.3940 | 0.9998 |
| rs12243326 | TC7FL2 | C*/T | 0.0048 | 0.0027 | 1.778(0.990–3.192) | 0.0564 | 0.6398 | 1.856(0.983–3.505) | 0.0564 | 0.6398 |
| rs4506565 | TC7FL2 | T*/A | 0.0467 | 0.0370 | 1.273(1.072–1.511) | 0.0057 | 0.8028 | 1.318(1.097–1.584) | 0.0057 | 0.8028 |
| rs17271305 | VPS13C | G/A | 0.1607 | 0.1556 | 1.084(1.000–1.174) | 0.0471 | 0.9998 | 1.086(0.983–1.205) | 0.0471 | 0.9998 |
| rs2191349 | DGKB | G/*A | 0.6643 | 0.6555 | 1.040(0.968–1.117) | 0.2863 | 0.9557 | 1.048(0.970–1.131) | 0.2863 | 0.9557 |
| rs7944584 | MADH | G/A | 0.9784 | 0.9651 | 1.637(1.327–2.020) | 3.5 x 10 –6 | 0.0002 | 1.624(1.294–2.038) | 2.9 x 10 –5 | 0.0006 |
| rs10885122 | ADRA2A | G/A | 0.5927 | 0.5795 | 1.057(0.913–1.198) | 0.3923 | 0.9998 | 1.073(0.936–1.229) | 0.3119 | 0.9998 |
| rs174550 | FADS1 | T/C | 0.5919 | 0.5787 | 1.056(0.985–1.131) | 0.1215 | 0.8887 | 1.039(0.965–1.119) | 0.3066 | 0.9988 |
| rs11605924 | CRY2 | C/*A | 0.2449 | 0.2303 | 1.084(1.000–1.174) | 0.0487 | 0.5821 | 1.078(0.989–1.176) | 0.0882 | 0.8028 |
| rs11920090 | SLC2A2 | A/*T | 0.0114 | 0.0092 | 1.249(0.889–1.754) | 0.1984 | 0.9741 | 1.407(0.975–2.031) | 0.0679 | 0.7074 |
| rs7034200 | GLIS3 | A/*C | 0.4489 | 0.4247 | 1.103(1.028–1.184) | 0.0062 | 0.9998 | 1.102(1.038–2.010) | 0.0037 | 0.0630 |
| rs340874 | PROX1 | C/T | 0.3918 | 0.3778 | 1.061(0.989–1.137) | 0.1082 | 0.9166 | 1.102(1.004–1.166) | 0.0391 | 0.5063 |
| rs11071657 | C2CD4B | G/A | 0.3703 | 0.3679 | 1.010(0.942–1.084) | 0.7710 | 1.000 | 1.036(0.962–1.116) | 0.3485 | 0.9994 |
| rs11558471 | SLC30A8 | A/G | 0.5969 | 0.5487 | 1.218(1.137–1.305) | 2.0 x 10 –4 | 0.0001 | 1.263(1.172–1.361) | 1.1 x 10 –4 | 0.0001 |
| rs35767 | IGF1 | A/G | 0.3420 | 0.3362 | 1.027(0.956–1.103) | 0.4724 | 0.9999 | 1.017(0.940–1.099) | 0.6813 | 1.000 |

* risk allele for type 2 diabetes in the European descent population.

* adjusted for age, gender and BMI.

P values < 0.05 were shown in bold.

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| SNP     | Gene | Effect allele*/other allele | Fasting glucose (n = 3,312) | 2-h glucose (n = 3,312) | Fasting insulin (n = 2,309) | 2-h insulin (n = 2,295) |
|---------|------|-----------------------------|-----------------------------|--------------------------|-----------------------------|--------------------------|
| rs10423928 | GIPR | A/T                        | Beta = -0.0118 (95%CI: -0.0428–0.0191) | -0.0185 (95%CI: -0.0866–0.0497) | 0.0074 (95%CI: -0.0115–0.0263) | -0.0061 (95%CI: -0.0318–0.0197) |
|         |      |                             |                             |                           |                             |                           |
| rs2877716 | ADCYS | C/T                        | Beta = -0.0190 (95%CI: -0.2637–0.2256) | -0.0528 (95%CI: -0.5908–0.4852) | 0.0657 (95%CI: -0.1071–0.2385) | -0.0457 (95%CI: -0.2814–0.1899) |
|         |      |                             |                             |                           |                             |                           |
| rs11708067 | ADCYS | A/G                        | Beta = 0.0736 (95%CI: -0.1452–0.2925) | 0.0421 (95%CI: -0.4400–0.5242) | -0.0323 (95%CI: -0.1679–0.1032) | -0.0126 (95%CI: -0.1978–0.1725) |
|         |      |                             |                             |                           |                             |                           |
| rs12243326 | TCF7L2 | C/T                        | Beta = -0.0780 (95%CI: -0.3081–0.1521) | 0.0306 (95%CI: -0.4764–0.5376) | 0.0642 (95%CI: -0.0642–0.2184) | -0.0274 (95%CI: -0.1082) |
|         |      |                             |                             |                           |                             |                           |
| rs4506565 | TCF7L2 | T/A                        | Beta = 0.0209 (95%CI: -0.0423–0.0842) | 0.0682 (95%CI: -0.0711–0.2074) | 0.0108 (95%CI: -0.0275–0.0491) | -0.0078 (95%CI: -0.0443–0.0599) |
|         |      |                             |                             |                           |                             |                           |
| rs17271305 | VPS13C | G/A                        | Beta = 0.0236 (95%CI: -0.0098–0.0571) | -0.0003 (95%CI: -0.0741–0.0735) | 0.0048 (95%CI: -0.0159–0.0255) | -0.0038 (95%CI: -0.0244–0.0319) |
|         |      |                             |                             |                           |                             |                           |
| rs2191349 | DGKB | T/G                        | Beta = 0.0335 (95%CI: -0.0985–0.0585) | 0.0099 (95%CI: -0.0452–0.0650) | 0.0025 (95%CI: -0.0177–0.0128) | -0.0168 (95%CI: -0.0375–0.0040) |
|         |      |                             |                             |                           |                             |                           |
| rs7944584 | MADD | A/T                        | Beta = 0.0673 (95%CI: 0.0034–0.1312) | 0.0483 (95%CI: -0.0926–0.1892) | 0.0045 (95%CI: -0.0440–0.0349) | -0.0006 (95%CI: -0.0545–0.0532) |
|         |      |                             |                             |                           |                             |                           |
| rs10885122 | ADRA2A | G/T                        | Beta = 0.0010 (95%CI: -0.0420–0.0440) | 0.0014 (95%CI: -0.0936–0.0965) | 0.0033 (95%CI: -0.0221–0.0288) | -0.0070 (95%CI: -0.0277–0.0416) |
|         |      |                             |                             |                           |                             |                           |
| rs174550 | FADS1 | T/C                        | Beta = 0.0149 (95%CI: -0.0093–0.0390) | -0.0364 (95%CI: -0.0896–0.0169) | 0.0095 (95%CI: -0.0240–0.0051) | -0.0017 (95%CI: -0.0182–0.0216) |
|         |      |                             |                             |                           |                             |                           |
| rs11605924 | CRY2 | A/C                        | Beta = 0.0057 (95%CI: -0.0338–0.0224) | 0.0596 (95%CI: -0.1214–0.0021) | 0.0061 (95%CI: -0.0234–0.0112) | -0.0304 (95%CI: -0.0541–0.0068) |
|         |      |                             |                             |                           |                             |                           |
| rs11920090 | SLC2A2 | T/A                        | Beta = 0.0193 (95%CI: -0.1068–0.1454) | 0.0239 (95%CI: -0.3016–0.2537) | 0.0174 (95%CI: -0.0944–0.0596) | -0.0529 (95%CI: -0.0518–0.1576) |
|         |      |                             |                             |                           |                             |                           |
| rs7034200 | GLS3 | A/C                        | Beta = 0.0090 (95%CI: -0.0153–0.0333) | 0.0157 (95%CI: -0.0380–0.0693) | 0.0099 (95%CI: -0.0247–0.0049) | 0.0012 (95%CI: -0.0189–0.0213) |
|         |      |                             |                             |                           |                             |                           |
| rs340874 | PROX1 | C/T                        | Beta = 0.0200 (95%CI: -0.0045–0.0444) | 0.0879 (95%CI: 0.0341–0.1416) | 0.0069 (95%CI: -0.0078–0.0217) | -0.0010 (95%CI: -0.0212–0.0192) |
|         |      |                             |                             |                           |                             |                           |
| rs11071657 | C2CD48 | A/G                        | Beta = 0.0039 (95%CI: -0.0204–0.0281) | 0.0002 (95%CI: -0.0533–0.0537) | -0.0029 (95%CI: -0.0177–0.0119) | -0.0040 (95%CI: -0.0242–0.0163) |
|         |      |                             |                             |                           |                             |                           |
| rs11558471 | SLC30A8 | A/G                        | Beta = 0.0376 (95%CI: 0.0139–0.0614) | -0.0199 (95%CI: -0.0722–0.0324) | 0.0018 (95%CI: -0.0181–0.0108) | -0.0338 (95%CI: -0.0328–0.0067) |
|         |      |                             |                             |                           |                             |                           |
rs35767) which were recently reported to be associated with fasting or OGTT 2-h glucose levels [7,8]. The SNPs were genotyped by using primer extension of multiplex products with detection by matrix-assisted laser desorption ionization – time of flight mass spectroscopy using a MassARRAY Compact Analyzer (Sequenom, San Diego, CA, USA). All seventeen SNPs passed genotype quality control analyses.

Statistical analysis

The Hardy-Weinberg equilibrium test was performed before the association analysis. SNPs failed Hardy-Weinberg equilibrium tests (p<0.01 in the controls) were excluded. The allelic frequencies between the diabetic patients and controls were compared using x^2 tests, and ORs with 95% CIs were presented. As age, gender and BMI differed in the cases and controls, we further adjusted them as confounding factors by logistic regression. Quantitative traits were analyzed by linear regression adjusted for age, gender and BMI under an additive genetic model. All skewly distributed quantitative traits, including fasting and 2-h insulin levels, HOMA-B, HOMA-IR, STUMVOLL and GUTT, were logarithmically transformed (log10) to approximate univariate normality (p<0.01 by Kolmogorov-Smirnov test). In order to adjust multiple comparison, 10,000 permutations were performed for each trait to assess empirical p values using PLINK [20]. The statistical analyses were performed using SAS for Windows (version 8.0; SAS Institute, Cary, NC, USA) unless specified otherwise. A two-tailed p value of <0.05 was considered statistically significant.

The statistic power was estimated under an additive genetic model based on the previously reported effect size and allele frequency observed in our samples. For SNPs with minor allele frequency over 0.2, our sample size had over 80% power to detect an effect size of 0.035 mmol/l for fasting glucose, 0.10 mmol/l for 2-h glucose and an OR of 1.13 for type 2 diabetes risk. For SNPs with minor allele frequency equal to 0.05, our sample size had over 80% power to detect an effect size of 0.08 mmol/l for fasting glucose, 0.17 mmol/l for 2-h glucose and an OR of 1.24 for type 2 diabetes risk.

Results

All SNPs were in accordance with Hardy-Weinberg equilibrium. Table 2 showed the analyses of associations between these SNPs and type 2 diabetes. GIPR rs10423928 (OR 1.097, 95%CI 1.003–1.197, p = 0.0378), TCF7L2 rs4506565 (OR 1.273, 95%CI 1.072–1.511, p = 0.0057), MADD rs7944504 (OR 1.637, 95%CI 1.327–2.020, p = 3.5 × 10^-5), PROX1 rs340874 (OR 1.082, 95%CI 1.004–1.166, p = 0.0391) showed a significant association to type 2 diabetes in our samples.

Discussion

In the current study, we tried to replicate the effects of recently reported loci influencing quantitative traits related to glucose metabolism in a Shanghai Chinese population. To our knowledge, this is the first replication study in Asian population focusing on these loci up to now. We confirmed the association between DKG8, MADD and SLC30A8 and fasting glucose. We also found PROX1 was associated with 2-h glucose in our samples. The effects of IGF1 rs35767 on fasting insulin and insulin sensitivity were also observed. However, the direction of effects was opposite to that observed in the European descent samples [7,21]. It should also be noted that the allele frequencies of rs35767 differed between European and Chinese populations (0.15 vs 0.35 for A allele). It suggests causal variant within this locus remained to be identified.
Table 4. Association between SNPs from fifteen loci and insulin secretion and sensitivity indices in the Chinese normal glucose regulation subjects.

| SNP      | Gene | Effect allele/* other allele | HOMA-B (n = 2,302) | STUMVOLL (n = 2,303) | HOMA-IR (n = 2,309) | GUTT (n = 2,291) |
|----------|------|-----------------------------|--------------------|----------------------|---------------------|------------------|
| rs10423928 | GIPR | A/T 0.0082 | 0.0048 | 0.0067 | 0.0333 |
|          |      | (95% CI) (-0.0135--0.0299) | (-0.0046--0.0142) | (-0.0129--0.0263) | (-0.0076--0.0141) |
| rs2877716 | ADCYS | C/T 0.0105 | -0.0375 | 0.0820 | -0.0033 |
|          |      | (95% CI) (-0.2088--0.1877) | (-0.1236--0.0486) | (-0.0972--0.2613) | (-0.1026--0.0960) |
| rs11708067 | ADCYS | A/G 0.1094 | -0.0402 | -0.0149 | -0.0065 |
|          |      | (95% CI) (-0.2648--0.0460) | (-0.1078--0.0274) | (-0.1556--0.1257) | (-0.0845--0.0716) |
| rs12243326 | TCF7L2 | C/T 0.0273 | -0.0127 | 0.0870 | 0.0146 |
|          |      | (95% CI) (-0.1346--0.1892) | (-0.0829--0.0576) | (-0.0594--0.2335) | (-0.0663--0.0955) |
| rs4505665 | TCF7L2 | T/A 0.1113 | 0.1652 | 0.8352 | 0.8713 |
|          |      | (95% CI) (-0.0053--0.0327) | (-0.0333--0.050) | (-0.0231--0.0564) | (-0.0296--0.0142) |
| rs17271305 | VPS13C | G/A 0.0001 | -0.0043 | 0.0061 | -0.0053 |
|          |      | (95% CI) (-0.0236--0.0238) | (-0.0145--0.0060) | (-0.0153--0.0275) | (-0.0172--0.0066) |
| rs2191349 | DGKB | T/G 0.0149 | 0.5678 | 0.3861 |
|          |      | (95% CI) (-0.0317--0.0034) | (-0.0163--0.0012) | (-0.0151--0.0165) | (-0.0062--0.0113) |
| rs5944584 | MADD | A/T 0.0410 | -0.0214 | 0.0053 | -0.0043 |
|          |      | (95% CI) (-0.0863--0.0043) | (-0.0145--0.0017) | (-0.0356--0.0462) | (-0.0270--0.0183) |
| rs10885122 | ADRA2A | G/T 0.0149 | 0.0008 | 0.0080 |
|          |      | (95% CI) (-0.0143--0.0442) | (-0.0071--0.0182) | (-0.0255--0.0271) | (-0.0139--0.0154) |
| rs174550 | FADS1 | T/C 0.0125 | -0.0007 | -0.0084 | 0.0020 |
|          |      | (95% CI) (-0.0292--0.0042) | (-0.0079--0.0066) | (-0.0235--0.0066) | (-0.0064--0.0104) |
| rs11659294 | CRY2 | A/C 0.0109 | -0.0024 | 0.0130 |
|          |      | (95% CI) (-0.0308--0.0091) | (-0.0111--0.0063) | (-0.0227--0.0133) | (0.0031--0.0230) |
| rs11920090 | SLC2A2 | T/A 0.0154 | -0.0005 | 0.0168 | -0.0151 |
|          |      | (95% CI) (-0.0103--0.0729) | (-0.0478--0.0287) | (-0.0967--0.0631) | (-0.0593--0.0291) |
| rs7034200 | GLIS3 | A/C 0.0072 | -0.0005 | -0.0098 | -0.0009 |
|          |      | (95% CI) (-0.0242--0.0097) | (-0.0099--0.0048) | (-0.0252--0.0056) | (-0.0094--0.0076) |
| rs340874 | PROX1 | C/T 0.0013 | -0.0080 | 0.0089 | -0.0045 |
|          |      | (95% CI) (-0.0183--0.0158) | (-0.0154--0.0006) | (-0.0064--0.0242) | (0.0130--0.0040) |
| rs11071657 | C2CD4B | A/G 0.0060 | -0.0008 | -0.0024 | 0.0018 |
|          |      | (95% CI) (-0.0231--0.0111) | (-0.0082--0.0066) | (-0.0177--0.0130) | (-0.0067--0.0104) |
| rs11558471 | SLC30A8 | A/G 0.0205 | 0.0006 | 0.0050 |
|          |      | (95% CI) (-0.0371--0.0038) | (-0.0145--0.0001) | (-0.0144--0.0156) | (-0.0034--0.0133) |
We showed SNPs from seven loci, including GIPR, TCF7L2, MADD, CRY2, GLIS3, PROX1 and SLC30A8, had an effect on type 2 diabetes in our samples. Most of these associations were in consistence with findings in the European descent samples, except that MADD rs7944584 showed an association to type 2 diabetes only in the Chinese samples. MADD encodes mitogen-activated protein kinase activating death domain, which interacted with tumor necrosis factor alpha receptor 1 to activate mitogen-activated protein kinase and propagate the apoptotic signal [22]. Previous study in European samples showed the glucose-raising allele of MADD rs7944584 was associated with elevated fasting proinsulin without altering insulin secretion [21], suggesting this locus was associated with insulin processing defects. In this study, we found the glucose-raising allele was associated with a higher risk for type 2 diabetes in the Chinese population. The underlying mechanism is not clear, but it is known the defects in insulin processing may lead to endothelium reticulum stress and finally beta cell dysfunction [23]. However, this SNP showed a negligible effect on type 2 diabetes in the European population. It is not clear whether ethnic difference played a role in the effect of this locus as poor insulin compensation ability was observed in the Asians compared with the European descent populations [24]. On the other hand, we cannot exclude the possibility that the effect of MADD rs7944584 on type2 diabetes was over estimated or even this association was just a positive finding by chance. Thus further replication studies in the Asian samples are needed.

In this study, we failed to replicate the associations of several variants with fasting or 2-h glucose levels. Some of these unreplicated SNPs including the ones from ADCY5, TCF7L2, SLC2A2 and ADRB2A were much rarer in the Asians than they were in the European descent populations (e.g., ADCY5, with minor allele frequency 0.002 in the Chinese vs 0.25 in the European descents). We may not have enough statistical power to replicate the effects of some loci because of the smaller minor allele frequencies as well as linkage disequilibrium structure between the loci. However, there are still loci that failed to be replicated in our samples even though we had enough statistical power, e.g. GIPR, which suggests heterogeneous effects of these loci may exist in the Chinese comparing with European descent populations.

Although we analyzed these loci in relatively large samples, there are several limitations of our study. First, as multiple traits and SNPs were analyzed in the current study, we could not exclude the possibility that our findings were false positive. But considering these SNPs were originally identified in large-scale genome-wide association studies and all the traits analyzed were highly related, the impact of multiple comparisons may be limited. Second, we only analyzed the effects of these loci on insulin sensitivity and secretion in the normal glucose regulation subjects as most of the type 2 diabetes patients were receiving glucose lowering therapy. However, Heni et al showed the impact of genetic variation on insulin secretion depends on glycaemia [25,26], what are the effects of these variants in the diabetic patients and prediabete subjects remained unknown and to be investigated. Third, only the reported SNP(s) from each locus was analyzed in the current study. As differences exist in allele frequencies as well as linkage disequilibrium structure between Asians and European populations, detailed analyzing of more SNPs from each locus in Asian samples may help identify the causal variant.

In conclusion, we analyzed the effects of SNPs from fifteen loci recently reported to be associated with fasting and/or 2-h glucose or fasting insulin in the Chinese samples, and showed SNPs from GIPR, TCF7L2, DGKB, MADD, CRY2, GLIS3, PROX1, SLC30A8 and IGF1 were associated with traits related to glucose metabolism in the Chinese population. Moreover, our data suggest heterogeneous effects of SNPs from MADD and GIPR may exist in the Chinese population comparing with European population.

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**Author Contributions**

Conceived and designed the experiments: WJ KX CH. Performed the experiments: CH RZ CW JL WY FJ. Analyzed the data: CH. Contributed reagents/materials/analysis tools: XM XY YB. Wrote the paper: CH JW.

### References

1. Yang W, Lu J, Weng J, Jia W, Ji L, et al. (2010) Prevalence of diabetes among men and women in China. N Engl J Med 362: 1090–1101.
2. Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. Lancet 365: 1333–1346.
3. Bousit-Naji N, Rocheleau G, Van Lommel L, Lemaire K, Schuit F, et al. (2008) A polymorphism within the G6PC2 gene is associated with fasting plasma glucose levels. Science 320: 1083–1088.
4. Florez JC (2008) Clinical review: the genetics of type 2 diabetes: a realistic appraisal in 2008. J Clin Endocrinol Metab 93: 4633–4642.
5. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, et al. (2009) Variants in MTNR1B influence fasting glucose levels. Nat Genet 41: 77–81.
6. Tsai FJ, Yang CF, Chen CC, Chuang LM, Lu CH, et al. A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese. PLoS Genet 6: e1000417.
7. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, et al. (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42: 103–116.
8. Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, et al. (2010) Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat Genet 42: 142–148.
9. Takeuchi F, Katsuya T, Chakravarthy S, Yamamoto K, Fujikawa A, et al. (2010) Common variants at the GCK, GCKR, G6PC2-ABCB11 and MTNR1B loci are associated with fasting glucose in two Asian populations. Diabetologia 53: 299–308.
10. Hu C, Zhang R, Wang C, Yu W, Lu J, et al. (2010) The effects of GCK, GCKR, G6PC2 and MTNR1B variants on glucose metabolism and insulin secretion. PLoS ONE 5: e11761.
11. Hu C, Zhang R, Wang C, Ma X, Wang C, et al. (2009) A genetic variant of G6PC2 is associated with type 2 diabetes and fasting plasma glucose level in the Chinese population. Diabetologia 52: 451–456.
12. Tam CH, Ho JS, Wang Y, Lee HM, Lam VK, et al. Common polymorphisms in MTNR1B, G6PC2 and GCK are associated with increased fasting plasma glucose and impaired beta-cell function in Chinese subjects. PLoS One 5: e11429.
13. Liu C, Wu Y, Li H, Qi Q, Langenberg C, et al. (2010) MTNR1B rs10130963 is associated with fasting plasma glucose, HbA1C and impaired beta-cell function in Chinese Hans from Shanghai. BMC Med Genet 11: 59.
14. Ronn T, Wen J, Yang Z, Lu B, Du Y, et al. (2009) A common variant in MTNR1B, encoding melatonin receptor 1B, is associated with type 2 diabetes and fasting plasma glucose in Han Chinese individuals. Diabetologia 52: 830–833.
15. Jia WP, Pang C, Chen L, Bao YQ, Lu JX, et al. (2007) Epidemiological characteristics of diabetes mellitus and impaired glucose regulation in a Chinese adult population: the Shanghai Diabetes Studies, a cross-sectional 3-year follow-up study in Shanghai urban communities. Diabetologia 50: 286–292.
16. Bao Y, Ma X, Li H, Zhou M, Hu C, et al. (2010) Glycated haemoglobin A1c for diagnosing diabetes in Chinese population: cross sectional epidemiological survey. BMJ 340: c2249.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419.
18. Stumvoll M, Van Haften T, Frische A, Gerich J (2001) Oral glucose tolerance test indexes for insulin sensitivity and secretion based on various availabilitys of sampling times. Diabetes Care 24: 796–797.
19. Gutt M, Davis CL, Spitzer SR, Llibre MM, Kumar M, et al. (2000) Validation of the insulin sensitivity index (ISI30) comparison with other measures. Diabetes Res Clin Pract 47: 177–184.
20. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.
21. Ingelsson E, Langenberg C, Hivert MF, Prokopenko I, Lyssenko V, et al. (2010) Detailed physiologic characterization reveals diverse mechanisms for novel genetic Loci regulating glucose and insulin metabolism in humans. Diabetes 59: 1266–1275.
22. Kurada BR, Li LC, Mulherkar N, Subramanian M, Peasad KV, et al. (2009) MADD, a splice variant of R20, is indispensable for MAPK activation and protection against apoptosis upon tumor necrosis factor-alpha treatment. J Biol Chem 284: 13533–13541.
23. Scheuner D, Kaufman RJ (2008) The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. Endor Rev 29: 317–333.
24. Torrens JI, Skurnick J, Davidow AL, Korenman SG, Santoro N, et al. (2004) Ethnic differences in insulin sensitivity and beta-cell function in premenopausal or early perimenopausal women without diabetes: the Study of Women’s Health Across the Nation (SWAN). Diabetes Care 27: 354–361.
25. Henri M, Keterrer C, t Hart LM, Ranta F, van Haften TW, et al. The Impact of Genetic Variation in the G6PC2 Gene on Insulin Secretion Depends on Glycemia. J Clin Endocrinol Metab.
26. Henri M, Keterrer C, Thamer C, Herzberg-Schafer SA, Guthoff M, et al. Glycemia determines the effect of type 2 diabetes risk genes on insulin secretion. Diabetes.