Cross-Sectional and Longitudinal Replication Analyses of Genome-Wide Association Loci of Type 2 Diabetes in Han Chinese

Qi Zhao1, Jianzhong Xiao2, Jiang He1,2, Xuelian Zhang2, Jing Hong2, Xiaomu Kong2, Katherine T. Mills1, Jianping Weng4, Weiping Jia5, Wenying Yang2*

1 Department of Epidemiology, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana, United States of America, 2 Department of Endocrinology, China-Japan Friendship Hospital, Beijing, China, 3 Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana, United States of America, 4 Sun Yat-Sen University Third Affiliated Hospital, Guangzhou, Guangdong, China, 5 Shanghai Jiaotong University Affiliated Sixth People’s Hospital, Shanghai, China

Abstract

This study aimed to examine genomic loci of type 2 diabetes (T2D) initially identified by genome-wide association studies in populations of European ancestry for their associations with T2D and quantitative glycemic traits, as well as their effects on longitudinal change in fasting plasma glucose (FPG) and T2D development, in the Chinese population. Single nucleotide polymorphisms (SNP) from 25 loci were genotyped in a large case-control sample of 10,001 subjects (5,338 T2D cases and 4,663 controls) and a prospective cohort of 1,881 Chinese. In the case-control sample, 8 SNPs in or near WFS1, CDKAL1, CDKN2A/2B, CDC123, HHEX, TCF7L2, KCNQ1, and MTNR1B were significantly associated with T2D (P<0.05). Thirteen SNPs were associated with quantitative glycemic traits. For example, the most significant SNP, rs10811661 near CDKN2A/2B (P=1.11×10−8) for T2D, was also associated with 2-h glucose level of an oral glucose tolerance test (P=9.11×10−5) and insulinogenic index (P=2.71×10−2). In the cohort study, individuals carrying more risk alleles of the replicated SNPs had greater FPG increase and T2D incidence in a 7.5-year follow-up period, with each quartile increase in the number of risk alleles being associated with a 0.06 mmol/l greater increase in FPG (P=0.03) and 19% higher odds of developing T2D (P=0.058). Our study identified the associations of several established T2D-loci in Europeans with T2D and quantitative glycemic traits in the Chinese population. The prospective data also suggest their potential role in the risk prediction of T2D in the Chinese population.

Introduction

China has experienced an explosive increase in the prevalence of diabetes in the past two decades [1]. Although environmental and lifestyle factors undoubtedly contribute to the increase of type 2 diabetes (T2D) in China, genetic factors determine individual susceptibility to these risk factors. Multiple lines of evidence have indicated that T2D and its related glycemic traits have considerable heritability [2,3]. Recent genome-wide association studies (GWAS) identified more than 60 novel genomic loci associated with T2D, which greatly advanced the understanding of the genetic basis of T2D [4].

Because of the potential genetic heterogeneity of T2D across populations of different racial/ethnic backgrounds and because most novel GWAS-loci were initially identified in populations of European ancestry, it is necessary to replicate the association of these novel loci with T2D in independent populations with various ethnicities. Several genetic replication studies have been conducted in Chinese populations and reported inconsistent findings on these associations [5-8]. There is still lack of evidence for the associations between the T2D-related loci identified in populations of European ancestry and T2D in Han Chinese, which constitutes the majority of Chinese population. In addition, the associations of these novel loci with 2-h postload glucose level after an oral glucose tolerance test (OGTT) and insulin resistance measures have not been examined among the Chinese population.

In the current study, we tested the association of established T2D-loci in populations of European ancestry and T2D among a large case-control sample of Han Chinese and investigated their associations with quantitative glycemic traits. In addition, we studied the cumulative effects of significant loci on changes in fasting glucose and the incidence of T2D among study participants from north rural China of the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) over more than 7 years of follow-up.
informed consent was obtained from each participant of the two
Tulane University, respectively. Written Committee of China-Japan Friendship Hospital and the Institu-
tional Review Board of Tulane University, respectively. A total of 1,881 GenSalt study participants without
their siblings, spouses, and offspring. The participant recruitment
potentials and their families. Probands with prehyperten-
sion or stage-1 hypertension and no use of antihypertensive
medications were recruited for dietary interventions, along with
their siblings, spouses, and offspring. The participant recruitment
and baseline data collection were conducted from 2003 to 2005.
Two follow-up examinations were completed in 2008 and 2011,
respectively. A total of 1,881 GenSalt study participants without
T2D at baseline were included in the current study. Data on T2D
and baseline data collection were conducted from 2003 to 2005.

The GenSalt cohort study sample. The GenSalt study is a
family-based dietary feeding study designed to investigate genetic
factors associated with BP response to dietary sodium and
potassium interventions among a Han Chinese population [10].
A community-based BP screening was conducted among people
18–60 years of age who resided in the study villages to identify
potential probands and their families. Probands with prehyperten-
sion or stage-1 hypertension and no use of antihypertensive
medications were recruited for dietary interventions, along with
their siblings, spouses, and offspring. The participant recruitment
and baseline data collection were conducted from 2003 to 2005.

Quantitative glycemic trait measurements
In the DMS study, blood samples were obtained at baseline
after fasting and at 30 minutes and 2 hours after oral glucose
administration during the OGTT in all study participants. Plasma
glucose was measured with the use of a hexokinase enzymatic
method and serum insulin was measured by double-antibody
radioimmunoassay. Indices of beta-cell function (HOMA-B) and
insulin resistance (HOMA-IR) were derived from paired fasting
glucose and insulin measures using homeostasis model assessment
[11]. In addition, insulinogenic index was calculated using the
formula (insulin at 30 minutes – insulin at 0 minutes)/(glucose at
30 minutes – glucose at 0 minutes) to assess the early insulin
secretion phase in response to the oral glucose challenge [12]. In
the GenSalt study, FPG was measured during baseline and follow-
up examinations using the hexokinase enzymatic method.

SNP selection and genotyping
A total of 29 single nucleotide polymorphisms (SNPs) from 28
established T2D loci in populations of European ancestry were
genotyped among the DMS case-control sample using the
Illumina GoldenGate Indexing assay (Illumina Inc., San Diego,
CA). Twenty-five SNPs from 25 loci were successfully genotyped
with an average call rate of 98.4% (Table S1 in File S1). Two of
these SNPs, rs1801282 and rs7578597, are non-synonymous SNPs
and are predicted to have potential impacts on exonic splicing.
There are no predicted functions for the other SNPs based on the
SNPinfo database (http:// SNPinfo.niehs.nih.gov/ SNPinfo/ SNPfunc.
htm), a web tool for SNP function prediction [13]. The concordance rate was 100% for 229 duplicate samples. The
genotypes of the selected SNPs were extracted from the genotyped
(Allymetrix Genomewide Human SNP array 6.0 (Allymetrix, Inc.,
Santa Clara, CA) and imputed data of the GenSalt sample [14].

Statistical analyses
Each SNP was tested for deviation from the Hardy-Weinberg
equilibrium (HWE) within the DMS control group and the
GenSalt sample using an exact test implemented in Haploview
software [15]. In the DMS case-control sample, an additive
genetic model with age and sex as covariates was used to test for
the association of each SNP with T2D using logistic regression
models. Body mass index (BMI) was further adjusted for in these
tests to examine whether a SNP’s effect on T2D was
independent of BMI. Associations between SNPs and quantitative
glycemic traits under an additive genetic model were analyzed
among DMS controls using general linear models that included
age and sex. BMI was further adjusted in these models. Log-
transformed values for fasting insulin, HOMA-B, HOMA-IR, and
insulinogenic index were used as dependent variables.

During the conduction of this study, the association results of 22
genotyped SNPs in this study became available in the Asian
Genetic Epidemiology Network (AGEN) consortium, which
included 6,952 T2D cases and 11,865 controls of East Asian
descent in its GWAS meta-analysis discovery stage. To provide
more precise estimates of effect sizes for risk alleles of the tested
SNPs in East Asians, we conducted a meta-analysis to combine our
results with those from the Asian Genetic Epidemiology Network
(AGEN) consortium using a fixed effects model weighted by
inverse variance [14].

To test the effect of the replicated SNPs on long-term change in
FPG and the development of T2D, a genetic risk score was
calculated for each individual in the GenSalt sample. The sum
of the number of risk alleles at each SNP was weighted according to
the SNP’s relative effect size which was derived from the meta-
analysis of AGEN and this study. We rescaled the weighted score
to reflect the number of risk alleles each individual carried, and
each point of the genetic-predisposition score corresponded to one
risk allele [16]. Generalized estimating equations were used to test
the associations of genetic risk score with FPG change and T2D
incidence over follow-up accounting for non-independence of
GenSalt family members. Age, sex, and baseline BMI were
adjusted in these models. SAS statistical software (version 9.2; SAS
Institute Inc., Cary, NC) was used to conduct association analyses.

Results
The clinical characteristics of the DMS case-control sample and
the baseline characteristics of GenSalt participants are shown in
Table 1. None of the SNPs deviated significantly from HWE after
correcting for multiple testing among the control sample of the
DMS study (the smallest P-value = 0.02, Table S1 in File S1). In
addition, allele frequencies of the genotyped SNPs in this study
and the GenSalt sample using an exact test implemented in Haploview
software [15]. The DMS case-control sample, an additive
genetic model with age and sex as covariates was used to test for
association of each SNP with T2D using logistic regression
models. Body mass index (BMI) was further adjusted for in these
tests to examine whether a SNP’s effect on T2D was
independent of BMI. Associations between SNPs and quantitative
glycemic traits under an additive genetic model were analyzed
among DMS controls using general linear models that included
age and sex. BMI was further adjusted in these models. Log-
transformed values for fasting insulin, HOMA-B, HOMA-IR, and
insulinogenic index were used as dependent variables.

During the conduction of this study, the association results of 22
genotyped SNPs in this study became available in the Asian
Genetic Epidemiology Network (AGEN) consortium, which
included 6,952 T2D cases and 11,865 controls of East Asian
descent in its GWAS meta-analysis discovery stage. To provide
more precise estimates of effect sizes for risk alleles of the tested
SNPs in East Asians, we conducted a meta-analysis to combine our
results with those from the Asian Genetic Epidemiology Network
(AGEN) consortium using a fixed effects model weighted by
inverse variance [14].

To test the effect of the replicated SNPs on long-term change in
FPG and the development of T2D, a genetic risk score was
calculated for each individual in the GenSalt sample. The sum
of the number of risk alleles at each SNP was weighted according to
the SNP’s relative effect size which was derived from the meta-
analysis of AGEN and this study. We rescaled the weighted score
to reflect the number of risk alleles each individual carried, and
each point of the genetic-predisposition score corresponded to one
risk allele [16]. Generalized estimating equations were used to test
the associations of genetic risk score with FPG change and T2D
incidence over follow-up accounting for non-independence of
GenSalt family members. Age, sex, and baseline BMI were
adjusted in these models. SAS statistical software (version 9.2; SAS
Institute Inc., Cary, NC) was used to conduct association analyses.

Association analyses with T2D in the DMS study
In the DMS sample, 9 SNPs were significantly associated with
T2D (P<0.05) in logistic regression analysis without adjustment
for BMI (Table S2 in File S1). The associations of the SNP of the
Method (even after correcting for multiple testing using the Bonferroni correction) rs10811661 of the CDC123 gene was involved in insulin resistance.

CDC123 of the CDKN2A/2B pathway mediated through BCL11A and PPARG were involved in insulin resistance. In addition, T2D risk allele G of rs10811661 showed the most significant association with fasting glucose among GenSalt participants.

Discussion

In this study we replicated the association of several genomic loci, which were previously predominately reported in GWAS of European populations, with T2D among a large Han Chinese population. We also observed that most of the replicated variants had comparable effects between these two different populations. In addition, some of the variants were associated with quantitative glycemic traits, highlighting their potential effects on beta-cell dysfunction and insulin resistance. More notably, we observed that the cumulative effects of replicated variants predicted FPG increase and T2D development among the Chinese population over time.

Meta-analysis of DMS results with published AGEN data

Among the 8 replicated SNPs in the DMS sample, 6 SNPs were available for comparison with the published AGEN results. The associations of 4 SNPs (within or near CDEK2A1/2B, HHEX, TCF7L1, and KCNQ1) were further confirmed by the AGEN results (P < 0.05), showing consistent association directions (Table S3 in File S1). There was no significant heterogeneity for the effects of the 22 SNPs which were available for comparison between the DMS study and the AGEN study (data were not shown). The combined analysis of the DMS and AGEN results showed another 8 significant loci which includes SNPs within or near GCKR, BCL11A, PPARG, JAZF1, TP53INP1, ARAPI, PRC1, and FTO (P < 0.05, Table 2). Most of replicated SNPs showed similar effects with those observed in European populations (Table 2).

Association analyses with quantitative glycemic traits in DMS controls

In the DMS control sample, multiple SNPs showed significant associations with quantitative glycemic traits (P < 0.05, Table 3 and Table S3 in File S1). The risk allele T of the most significant SNP in the association with T2D, CDEK2A1/2Brs10811661, was associated with a higher glucose level during the OGTT (β (SE) = 0.06 (0.02), P = 9.11 × 10⁻⁶) and a lower insulinogenic index (β (SE) = −0.05 (0.02), P = 0.03), suggesting that the role of this locus in T2D may be mediated through beta-cell dysfunction. In addition, T2D risk allele G of CDC123-rs12779790 was associated with a higher fasting insulin level (β (SE) = 0.03 (0.01), P = 8.58 × 10⁻⁵) and a greater HOMA-IR (β (SE) = 0.04 (0.01), P = 9.39 × 10⁻⁵), suggesting that this locus may be involved in insulin resistance.

Cumulative effect of replicated SNPs on the progression to diabetes among GenSalt participants

The replicated SNPs showed cumulative effects on FPG change and T2D incidence over a follow-up of 7.5 years among the GenSalt participants. A total of 1,634 participants (86.9%) were examined in the follow-up studies and 126 participants developed T2D during the follow-up. Subjects with more risk alleles had a greater FPG increase and T2D incidence (Figure 1). On average, each quartile increase in the number of risk alleles was associated with a 0.06 mmol/l greater increase in FPG (P = 0.03 for trend across quartiles) and 19% higher odds of developing T2D (P = 0.058 for trend across quartiles) during the follow-up.

Table 1. Characteristics of study participants.

| Characteristic             | DMS Case (N = 5,338) | DMS Control (N = 4,663) | GenSalt N = 1,881 |
|----------------------------|----------------------|-------------------------|-------------------|
| Age, year                  | 55.0 (11.8)          | 50.7 (8.4)              | 38.7 (9.5)        |
| Male, %                    | 43.4                 | 32.2                    | 52.8              |
| Body mass index, kg/m²     | 25.9 (3.7)           | 23.0 (2.5)              | 23.3 (3.2)        |
| Waist circumference, cm    | 88.2 (10.0)          | 79.0 (8.5)              | 80.3 (9.8)        |
| Fasting glucose, mmol/l    | 8.0 (2.7)            | 5.0 (0.5)               | 4.8 (0.7)         |
| 2-Hour glucose in OGTT, mmol/l | 14.2 (5.2)        | 5.7 (1.1)               | -                 |
| Fasting insulin, pmol/l    | 60.7 (42.2–87.5)     | 43.7 (33.8–58.7)        | -                 |
| HOMA-B, %                  | 46.9 (27.9–77.0)     | 85.3 (60.6–125.3)       | -                 |
| HOMA-IR                    | 3.0 (1.9–4.6)        | 1.4 (1.1–1.9)           | -                 |
| Insulinogenic index        | 2.3 (0.7–5.5)        | 8.9 (4.5–16.7)          | -                 |
| Diabetes Treatment, %      | 37.5                 | 0                       | 0                 |

Continuous variables are presented as mean (standard deviation) or median (interquartile range). DMS, the China National Diabetes and Metabolic Disorders study; GenSalt, the Genetic Epidemiology Network of Salt Sensitivity; HOMA-B, homoeostasis model assessment of beta-cell function; HOMA-IR, homoeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test.

doi:10.1371/journal.pone.0091790.t001

Characteristics of study participants.

| Characteristic             | Case (N = 5,338) | Control (N = 4,663) | N = 1,881 |
|----------------------------|------------------|---------------------|-----------|
| Fasting glucose, mmol/l    | 8.0 (2.7)        | 5.0 (0.5)           | 4.8 (0.7) |
| 2-Hour glucose in OGTT, mmol/l | 14.2 (5.2)    | 5.7 (1.1)           | -         |
| Fasting insulin, pmol/l    | 60.7 (42.2–87.5) | 43.7 (33.8–58.7)    | -         |
| HOMA-B, %                  | 46.9 (27.9–77.0) | 85.3 (60.6–125.3)   | -         |
| HOMA-IR                    | 3.0 (1.9–4.6)    | 1.4 (1.1–1.9)       | -         |
| Insulinogenic index        | 2.3 (0.7–5.5)    | 8.9 (4.5–16.7)      | -         |
| Diabetes Treatment, %      | 37.5             | 0                   | 0         |
| SNP       | Chr | Physical Position | Nearby gene | Gene Region | Alleles | MAF     | OR (95% CI) | P-value | OR (95% CI) | P-value | Reported OR |
|-----------|-----|-------------------|-------------|-------------|---------|---------|-------------|---------|-------------|---------|-------------|
| rs780094  | 2   | 27741237          | GCKR        | Intronic    | A:G     | 0.476  | 1.06 (0.99–1.13) | 0.08    | 1.05 (1.02–1.09) | 4.52 × 10⁻³ | 1.06         |
| rs243021  | 2   | 65584819          | BCL11A     | Intergenic  | T:C     | 0.320  | 1.02 (0.96–1.10) | 0.49    | 1.04 (1.00–1.08) | 0.04    | 1.08         |
| rs1801282 | 3   | 12393125          | PPARG      | Exonic      | C:G     | 0.065  | 1.10 (0.96–1.25) | 0.17    | 1.12 (1.02–1.22) | 0.02    | 1.14         |
| rs10010131| 4   | 6592915           | WFS1       | Intergenic  | G:A     | 0.046  | 1.21 (1.04–1.41) | 0.02    | 1.05 (0.97–1.14) | 0.21    | 1.11         |
| rs7756992 | 6   | 20679709          | CDX2L1     | Intronic    | G:A     | 0.477  | 1.16 (1.08–1.23) | 1.02 × 10⁻⁵ | -           | -       | 1.20         |
| rs864745  | 7   | 28180556          | JAZF1      | Intronic    | A:G     | 0.239  | 1.04 (0.96–1.12) | 0.37    | 1.05 (1.00–1.10) | 0.03    | 1.10         |
| rs996854  | 8   | 95960511          | TP53N1     | Intronic    | G:A     | 0.341  | 1.02 (0.96–1.10) | 0.49    | 1.06 (1.02–1.10) | 6.30 × 10⁻³ | 1.06         |
| rs10811661| 9   | 22134094          | CDKN2A     | Intergenic  | T:C     | 0.476  | 1.21 (1.13–1.29) | 1.11 × 10⁻⁸ | 1.21 (1.16–1.26) | 6.87 × 10⁻³ | 1.19         |
| rs12779790| 10  | 12328010          | CDC123     | Intergenic  | A:G     | 0.165  | 1.14 (1.05–1.24) | 2.27 × 10⁻³ | 1.13 (1.06–1.20) | 1.16 × 10⁻⁴ | 1.11         |
| rs1111875 | 10  | 94462882          | HHEX       | Intergenic  | A:G     | 0.284  | 1.13 (1.05–1.21) | 8.05 × 10⁻⁴ | 1.12 (1.07–1.17) | 4.09 × 10⁻⁷ | 1.13         |
| rs7903146 | 10  | 11478349          | TCF7L2     | Intergenic  | C:T     | 0.039  | 1.34 (1.15–1.57) | 1.97 × 10⁻⁴ | 1.23 (1.11–1.36) | 3.41 × 10⁻⁵ | 1.40         |
| rs2237895 | 11  | 2857194           | KCNQ1      | Intronic    | A:C     | 0.320  | 1.22 (1.13–1.31) | 5.45 × 10⁻⁸ | -           | -       | 1.29         |
| rs1552224 | 11  | 72433098          | ARAPI      | Exonic      | T:G     | 0.090  | 1.06 (0.95–1.19) | 0.31    | 1.12 (1.04–1.20) | 1.69 × 10⁻³ | 1.14         |
| rs10830963| 11  | 92708710          | MTNR1B     | Intronic    | C:G     | 0.413  | 1.08 (1.01–1.15) | 0.03    | 1.03 (0.99–1.08) | 0.18    | 1.09         |
| rs8042680 | 15  | 91521337          | PRC1       | Intronic    | A:C     | 0.019  | 1.12 (0.89–1.42) | 0.33    | 1.27 (1.04–1.54) | 0.02    | 1.07         |
| rs9936090 | 16  | 53820527          | FTO        | Intronic    | T:A     | 0.113  | 1.09 (0.99–1.21) | 0.07    | 1.13 (1.08–1.19) | 1.91 × 10⁻⁶ | 1.34         |

P-values <0.05 are shown in bold in DMS and combined DMS+AGEN analyses.
*Major allele: minor allele; previously reported risk alleles (effect alleles) are shown in bold.
*Association results of the logistic regression analysis with adjustment for body mass index.
*Previously reported effects mainly among Europeans.
*The nearest gene is provided if a SNP is intergenic.

AGEN, the Asian Genetic Epidemiology Network; Chr, chromosome; DMS, the China National Diabetes and Metabolic Disorders study; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism.

doi:10.1371/journal.pone.0091790.t002
Table 3. Significant associations (P<0.05) of reported-T2D loci with quantitative glycemic traits in controls of the DMS case-control sample.

| SNP         | Nearby gene | Effect allele | Fasting glucose (mmol) | OGTT 2-h glucose (mmol/l) | Fasting insulin (pmol/l) | HOMA_IR | HOMA_B | Insulinogenic index |
|-------------|-------------|---------------|------------------------|---------------------------|-------------------------|---------|--------|---------------------|
| rs780094    | GCKR        | G             | 0.02 (0.01) 0.07        | 0.03 (0.02) 0.17          | 0.01 (0.01) 0.43        | 0.01 (0.01) 0.21 | −0.01 (0.01) 0.52 | 0.05 (0.02) 0.047  |
| rs243021    | BCL11Ac     | T             | 0.02 (0.01) 0.055       | −0.03 (0.02) 0.24         | 0.02 (0.01) 0.08        | 0.02 (0.01) 0.03  | 0 (0.01) 0.91    | 0.02 (0.03) 0.41   |
| rs4607103   | ADAMT9c     | C             | 0.01 (0.01) 0.25        | 0.02 (0.02) 0.45          | 0 (0.01) 0.67          | 0.01 (0.01) 0.56  | 0 (0.01) 0.95    | −0.07 (0.02) 5.33×10^{-3} |
| rs7756992   | CDKAL1      | G             | 0.02 (0.01) 0.09        | 0.04 (0.02) 0.55          | −0.01 (0.01) 0.21       | −0.01 (0.01) 0.37 | −0.02 (0.01) 0.12 | −0.07 (0.02) 6.21×10^{-3} |
| rs896854    | TPS3BP1     | A             | 0.01 (0.01) 0.20        | −0.01 (0.02) 0.65         | −0.03 (0.01) 0.01       | −0.02 (0.01) 0.03  | −0.03 (0.01) 0.04 | −0.03 (0.03) 0.32  |
| rs10811661  | CDKN2A/Bc   | T             | 0.01 (0.01) 0.49        | 0.06 (0.02) 9.11×10^{-3}  | 0 (0.01) 0.96          | 0 (0.01) 0.78     | −0.01 (0.01) 0.53 | −0.05 (0.02) 0.03   |
| rs13292136  | CHEK1       | C             | −0.02 (0.02) 0.42       | −0.08 (0.04) 0.03         | −0.02 (0.02) 0.32       | −0.02 (0.02) 0.28 | −0.01 (0.02) 0.78 | −0.04 (0.04) 0.33   |
| rs12779790  | CDC123c     | G             | 0.01 (0.01) 0.60        | 0.02 (0.03) 0.42          | 0.03 (0.01) 8.58×10^{-3} | 0.04 (0.01) 9.39×10^{-3} | 0.02 (0.02) 0.18 | 0 (0.03) 0.99      |
| rs1111875   | HHEXc       | G             | −0.02 (0.01) 0.12       | −0.01 (0.03) 0.79         | −0.01 (0.01) 0.29       | −0.02 (0.01) 0.17  | 0.01 (0.02) 0.71  | −0.09 (0.03) 6.73×10^{-4} |
| rs7903146   | TCF7L2      | T             | 0.03 (0.03) 0.34        | 0.17 (0.06) 4.90×10^{-3}  | 0.03 (0.03) 0.26        | 0.03 (0.03) 0.24  | 0.02 (0.04) 0.48  | −0.01 (0.06) 0.87   |
| rs10830963  | MTNR1B      | G             | 0.03 (0.01) 0.56        | 0 (0.02) 0.86             | 0 (0.01) 0.83          | 0 (0.01) 0.43     | −0.02 (0.01) 0.16 | −0.02 (0.02) 0.43   |
| rs11634397  | ZFAND6c     | G             | 0.03 (0.02) 0.12        | −0.05 (0.04) 0.21         | 0 (0.02) 0.78          | 0 (0.02) 0.97     | 0.03 (0.02) 0.15  | −0.11 (0.04) 4.67×10^{-3} |
| rs9939609   | FTO         | A             | −0.01 (0.02) 0.75       | 0 (0.04) 0.90             | −0.04 (0.02) 0.02       | −0.04 (0.02) 0.02  | −0.02 (0.02) 0.33 | 0.01 (0.04) 0.73    |

*P*-values <0.05 are shown in bold.

*Previously reported risk alleles.

*Log-transformed values were used in general linear regression models.

*The nearest gene is provided if a SNP is intergenic.

HOMA-B, homoeostasis model assessment of beta-cell function; HOMA-IR, homoeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; SE, standard error; SNP, single nucleotide polymorphism.

doi:10.1371/journal.pone.0091790.t003
either our study or a previous study of Han Chinese [5], we did observe that this SNP was associated with 2-h glucose during OGTT and the insulinogenic index. These findings suggest that this locus may be involved in insufficient insulin secretion of β-cells in response to glucose challenge.

A previous GWAS of Han Chinese failed to replicate the association of CDC123-rs12779790 with T2D, but identified an adjacent SNP rs10906115 (about 13 kb away from rs12779790, \( r^2 = 0.196 \) based on the HapMap CHB data) associated with T2D [21]. Our study replicated the association of CDC123-rs12779790 with T2D in Han Chinese for the first time (\( P = 0.002 \)), although the AGEN meta-analysis has replicated this locus among East Asians (\( P = 0.01 \)). These findings indicate that this locus may have at least two independent signals regarding the association with T2D. The associations with fasting insulin and HOMA-IR suggest that this locus may play a role in insulin resistance in the pathogenesis of T2D.

Our study not only replicated the associations of CDKAL1-rs7756992 and HHEX-rs1111875 with T2D in Han Chinese, but also confirmed their associations with β-cell function. The risk alleles of these two SNPs were significantly associated with a lower insulinogenic index measured through the OGTT. These findings were consistent with those observed in Europeans [22,23]. In addition, we observed that MTNR1B-rs10830963 was associated with T2D and FPG in the Han Chinese of the DMS study, which was also consistent with the finding in Europeans [20].

TCF7L2, the susceptibility gene with the largest effect on T2D discovered to date, was identified pre-GWAS in 2006 [24], with rapid replication by subsequent GWAS among European populations [17,18,20,25–30]. The TCF7L2 gene has been linked to β-cell function [31], and SNP TCF7L2-rs7903146 has allelic-specific enhancer effects on the TCF7L2 gene, which might explain its association with T2D [32]. Although it exhibited a strong effect on T2D among Europeans, the association of TCF7L2-rs7903146 with T2D in Han Chinese has not been well replicated previously. The most likely reason is the relatively low risk allele frequency of rs7903146 in Han Chinese compared to Europeans (2.6% vs. 27.9%).

In this large replication study, we did replicate the association of this SNP with T2D and also observed its association with 2-h glucose level during the OGTT.

Variants in KCNQ1 were first identified in Japanese and replicated in European and South Asian populations [33–35]. Our study further confirmed its association with T2D in the Han Chinese population. A meta-analysis of the FTO gene in East Asians (17,255 case and 19,703 control subjects) has shown variants of FTO associated with both obesity and T2D [36]. After adjusting for BMI, FTO-rs9939609 showed borderline significance (\( P = 0.07 \)) in the DMS sample, with the direction of association consistent with the AGEN meta-analysis. Although BMI was not adjusted in the AGEN analysis, the combined effect from DMS and AGEN (OR [95% CI] = 1.13 [1.08, 1.19]) was close to that from the aforementioned FTO meta-analysis (OR [95% CI] = 1.10 [1.03, 1.17]), in which BMI was adjusted in included studies.

Longitudinal replication is necessary to confirm the role of the GWAS-identified variants in the development of a disease and to assess the predictive value of the genetic markers on disease risk. Very few longitudinal studies have examined the effects of GWAS-T2D loci on the incidence of T2D among Han Chinese [7,37]. Our study provided further evidence for the cumulative effect of these replicated loci, most of which have small to moderate effects, on the increase in FPG and the risk of developing T2D.

To the best of our knowledge, this is the largest replication study of GWAS-T2D loci among Han Chinese. Genetic homogeneity of study participants further improved the study power. Both cross-sectional and longitudinal replication analyses were implemented in the study. In addition, a series of glucose metabolism measurements was conducted and analyzed to explore possible mechanisms of replicated genetic factors. However, our study has some limitations. First, only the reported lead SNP from each locus was tested. This approach may fail to replicate some loci in which the lead SNPs had different linkage disequilibrium with causal variants between Caucasians and Han Chinese. Second, many associations were only significant at \( a = 0.05 \) and could not tolerate correction for multiple testing. However, it should be noted that this study is a replication of previous GWAS findings with high prior probability, so a stringent threshold may not be necessary for statistical significance.

In summary, our large and comprehensive analyses replicated the associations of several GWAS-T2D loci, established in European populations, with T2D and a variety of quantitative glycemic traits in Han Chinese populations. The cross-ethnicity replication of these T2D-related loci further highlights their importance in the genetic basis of this disease. Future studies on fine mapping causal variants within these loci are necessary to understand the mechanism underlying these replicated associations.

Figure 1. The associations of risk scores with FPG change and accumulative T2D incidence over a 7.5-year follow-up period in the GenSalt study. Panel A is for the FPG change (95% CI) and Panel B is for the accumulative T2D incidence (95% CI) according to the quartiles of the number of risk alleles in the GenSalt participants. FPG, fasting plasma glucose; T2D, type 2 diabetes.
Supporting Information

File S1  Information of genotyped SNPs and associations of all genotyped SNPs with type-2 diabetes and quantitative glycemic traits.

References

1. Pan XR, Yang WY, Li GW, Lin J (1997) Prevalence of diabetes and its risk factors in China, 1994. National Diabetes Prevention and Control Cooperative Group. Diabetes Care 20: 1664–1669.

2. Das SK, Elbein SC (2006) The Genetic Basis of Type 2 Diabetes. Cell biology 2: 130–131.

3. Kota SK, Meher LK, Jammula S, Modi KD (2012) Genetics of type 2 diabetes mellitus and other specific types of diabetes; its role in treatment modalities. Diabetes Metab Syndr 6: 54–58.

4. Sanghera DK, Blackett PR (2012) Type 2 Diabetes Genetics: Beyond GWAS. J Diabetes Metab 3: 6948.

5. Wu Y, Li H, Loos RJ, Yu Z, Ye X, et al. (2008) Common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLCO1A1, and HHEX/IDE are associated with type 2 diabetes and impaired fasting glucose in a Chinese Han population. Diabetes 57: 2833–2842.

6. Liu C, Li H, Qi L, Loos RJ, Qi Q, et al. (2011) Variants in GLIS3 and CRY2 are associated with type 2 diabetes and impaired fasting glucose in Chinese Hans. PLoS One 6: e21464.

7. Xu M, Bi Y, Xu Y, Yu B, Huang Y, et al. (2010) Combined effects of 19 common variants on type 2 diabetes in China: results from two community-based studies. PLoS One 5: e10422.

8. Cui B, Zhu X, Xu M, Guo T, Zhu D, et al. (2011) A genome-wide association study confirms previously reported loci for type 2 diabetes in Han Chinese. PLoS One 6: e22353.

9. 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.

10. Group GCR GenSalt Collaborative Research Group (2007) GenSalt: rationale, design, methods and baseline characteristics of study participants. J Hum Hypertens 21: 639–646.

11. Matthews DR, Hockaday 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.

12. 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.

13. Xu Z, Taylor JA (2009) SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic Acids Res 37: W600–605.

14. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, et al. (2011) Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. Nat Genet 43: 531–538.

15. Barrett JC, Fry B, Mallory J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–265.

16. Qi Q, Chu AY, Kang JH, Jensen MK, Curhan GC, et al. (2012) Sugar-sweetened beverages and genetic risk of obesity. N Engl J Med 367: 1387–1396.

17. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, et al. (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316: 1331–1336.

18. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, et al. (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316: 1341–1345.

19. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, et al. (2007) Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 316: 1336–1341.

20. Manning AK, Hivert MF, Scott RA, Grinshvald J, Bonnycastle LL, et al. (2012) A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet 44: 659–669.

21. Shu XO, Long J, Cai Q, Qi L, Xiang YB, et al. (2010) Identification of new risk variants for type 2 diabetes. Proc Natl Acad Sci USA 107: 10011–22.

22. Pascoe I, Turz A, Patel SK, Ibrahim IM, Ferrannini E, et al. (2007) Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. Diabetes 56: 3101–3104.

23. Grapin N, Rose CS, Andersson EA, Andersen G, Nielsen AL, et al. (2007) Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. Diabetes 56: 3105–3111.

24. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, et al. (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 38: 320–323.

25. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, et al. (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445: 885–893.

26. Stefansson H, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, et al. (2007) A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. Nat Genet 39: 770–775.

27. Salonen JT, Uimarri P, Aalto JM, Pirkkanen M, Kaikkonen J, et al. (2007) Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. Am J Hum Genet 81: 338–345.

28. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, et al. (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40: 630–645.

29. Rung J, Cauchi S, Albrechtson A, Shen L, Rocheleau G, et al. (2009) Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet 41: 1110–1115.

30. Voight BF, Scott LJ, Steinthorisdottir V, Morris AP, Dina C, et al. (2010) Twelve type 2 diabetes susceptibility loci identified through large-scale association analyses. Nat Genet 42: 579–589.

31. Gloyn AL, Braum M, Rorsman P (2009) Type 2 diabetes susceptibility gene TCF7L2 and its role in beta-cell function. Diabetes 58: 800–802.

32. Savie D, Park SY, Bailey KA, Bell GI, Noreaga MA (2013) In vitro scan for enhancers at the TCF7L2 locus. Diabetologia 56: 121–125.

33. Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, et al. (2008) Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. Nat Genet 40: 1092–1097.

34. Ueoka H, Takahashi A, Kasaquchi T, Hara K, Horikoshi M, et al. (2008) SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet 40: 1098–1102.

35. Sun Q, Song K, Shen X, Cai Y (2012) The association between KCNQ1 gene polymorphism and type 2 diabetes risk: a meta-analysis. PLoS One 7: e41576.

36. Xi B, Wang C, Wang R, Huang Y (2011) FTO gene polymorphisms are associated with obesity and type 2 diabetes in East Asian populations: an update. Obesity 19: 236–237.

37. Chang YC, Chiu YF, Liu PH, Shih KC, Lin MW, et al. (2012) Replication of genome-wide association signals of type 2 diabetes in Han Chinese in a prospective cohort. Clin Endocrinol 76: 365–372.