Large Scale Meta-Analyses of Fasting Plasma Glucose Raising Variants in GCK, GCKR, MTNR1B and G6PC2 and Their Impacts on Type 2 Diabetes Mellitus Risk

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Abstract

Background: The evidence that the variants GCK rs1799884, GCKR rs780094, MTNR1B rs10830963 and G6PC2 rs560887, which are related to fasting plasma glucose levels, increase the risk of type 2 diabetes mellitus (T2DM) is contradictory. We therefore performed a meta-analysis to derive a more precise estimation of the association between these polymorphisms and T2DM.

Methods: All the publications examining the associations of these variants with risk of T2DM were retrieved from the MEDLINE and EMBASE databases. Using the data from the retrieved articles, we computed summary estimates of the associations of the four variants with T2DM risk. We also examined the studies for heterogeneity, as well as for bias of the publications.

Results: A total of 113,025 T2DM patients and 199,997 controls from 38 articles were included in the meta-analysis. Overall, the pooled results indicated that GCK (rs1799884), GCKR (rs780094) and MTNR1B (rs10830963) were significantly associated with T2DM susceptibility (OR, 1.04; 95%CI, 1.01–1.08; OR, 1.08; 95%CI, 1.05–1.12 and OR, 1.05; 95%CI, 1.02–1.08, respectively). After stratification by ethnicity, significant associations for the GCK, MTNR1B and G6PC2 variants were detected only in Caucasians (OR, 1.09; 95%CI, 1.02–1.16; OR, 1.10; 95%CI, 1.08–1.13 and OR, 0.97; 95%CI, 0.95–0.99, respectively), but not in Asians (OR, 1.02, 95% CI 0.98–1.05; OR, 1.01; 95%CI, 0.98–1.04 and OR, 1.12; 95%CI, 0.91–1.32, respectively).

Conclusions: Our meta-analyses demonstrated that GCKR rs780094 variant confers high cross-ethnicity risk for the development of T2DM, while significant associations between GCK, MTNR1B and G6PC2 variants and T2DM risk are limited to Caucasians.

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Introduction

Previous epidemiological studies have provided compelling evidence that fasting plasma glucose (FPG) levels that are on the high side of the normoglycemic range are associated with increased risk of type 2 diabetes mellitus (T2DM) [1,2]. Recently, multiple genome wide association studies (GWASs) performed in populations of European descent have identified common sequence variants in the promoter region of glucokinase (GCK), glucokinase regulator protein (GCKR), islet specific glucose-6-phosphatase (G6PC2), melatonin receptor 1B (MTNR1B) and melatonin receptor 1B (MTNR1B). These authors contributed equally to this work.

GCK encodes the key enzyme for the first step of glycolysis and is expressed only in liver and pancreatic islet beta cells [8]. Its activity is subject to inhibition by a regulatory protein, GCKR [9]. G6PC2 is also known as the encoding gene for islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), which is expressed in a highly pancreatic beta-cell-specific manner. But its catalytic activity has not been clearly described so far [10]. MTNR1B encodes a melatonin receptor that is found mainly in the brain. However, the presence of this receptor in islets suggests a possible association between its function and insulin secretion [11]. Given their biological relevance to glucose metabolism, it is no surprise that variants in these genes have been associated with FPG levels and T2DM.

Because of the significant impact of these variants on FPG, numerous studies have investigated further the association between these variants and T2DM risk. Rose et al. found the
GCK rs1799884 polymorphism was associated with impaired glucose regulation [12]. Sparso et al. reported that the G-allele of GCKR rs780094 polymorphism was associated with a modest increased risk of T2DM [13]. In two large prospective studies, Lyssenko et al. provided evidence that the risk genotype of the MTNR1B rs10830963 variant could predict future T2DM [11]. Dupuis et al. reported a significant association between the G6PC2 rs660887 variant and T2DM risk [3]. Furthermore, Reiling et al. demonstrated that there were combined effects of these four single nucleotide polymorphisms (SNPs) on FPG levels and T2DM risk [5]. However, in many other association studies, negative results were reported for these four SNPs, especially in studies performed in Asian populations. For example, Tam et al. failed to validate the association between genetic variants in GCK, GCKR, MTNR1B, G6PC2 and T2DM in a Chinese population [14], and this was consistent with the result of a study by Rees et al. in a south Asian population [15]. Given the discrepancies between the results of these studies and the low power of some of the small-scale association studies to detect small effect size results, we performed a comprehensive meta-analysis to give a more precise estimate of the associations between genetic variations in these four genes and T2DM risk.

Methods

Search Strategy
We conducted a systematic literature search (up to December 2012) of the MEDLINE and EMBASE databases in accordance with the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement [16]. For the search terms, we used gene name (GCK, GCKR, MTNR1B and G6PC2) and disease name (type 2 diabetes mellitus, T2DM or diabetes) to retrieve the association studies between genetic variants in GCK, GCKR, MTNR1B and G6PC2 and risk of T2DM. The computer-aided search was supplemented by including additional studies retrieved from the references and citations of the originally identified articles and from the PubMed option ‘Related Articles’.

Selection
Although several SNPs in the four studied genes have previously been linked to FPG levels and T2DM, only those variants that were studied in a total of >50,000 cases were analyzed. As a result, four SNPs (namely rs1799884 in GCK, rs780094 in GCKR, rs10830963 in MTNR1B and rs560887 in G6PC2) were finally included. Studies that met all the following criteria were included: (1) published in English; (2) with primary outcomes of T2DM; (3) described ethnicity and numbers of the study population; (4) provided the odds ratio (OR) with 95% confidence intervals (CIs) or enough genotype distribution data to calculate the ORs and 95% CIs. The exclusion criteria included: (1) not an association study for T2DM; (2) case-only study; (3) studied other SNPs; (4) meta-analysis. For duplicate publications, the study with the smaller data set was excluded.

Data Extraction
The characteristics extracted from each study included ethnicity, year of publication, study design, number and male percentages of cases/controls, estimated OR and 95% confidence interval, genotype distribution or allele frequency. Two authors (H.W. and L.L.) extracted data independently and in duplicate. All disagreements and uncertainties were discussed and resolved by consensus, with the involvement of another author (H.D.) if necessary.

Study Quality Assessment
The same two authors assessed the quality of included studies independently according to a quality assessment scores which was developed based on traditional epidemiologic and genetic considerations [17,18]. And total scores ranged from 0 (worst) to 12 (best). Details of the criteria that were used to develop the scoring system are available in the Table S1. Any discrepancies were adjudicated by another author (H.D.).

Meta-analyses
Data analyses were performed as follows. Firstly, we calculated the pooled prevalence of each risk allele in various ethnic groups using the inverse variance method described previously [18]. Second, the influence of these variants on T2DM risk was assessed by pooling together the per-allele ORs weighted by their inverse variance from each independent study. And a random-effects model was used by default to summarize the data as it properly takes into account the inter-study heterogeneity [19]. Heterogeneity was qualitatively assessed using the Q test and quantitatively evaluated with the I² test. I² test values of 25%, 50% and 75% were considered low, moderate and high, respectively [20]. In the presence of significant heterogeneity (Q test, p<0.05), the source of heterogeneity was explored by fitting a co-varient (quality score, case sample size, mean age and gender distribution of cases and controls) in a meta-regression model. Furthermore, considering the possible impact of ethnic variations on the results, we divided the study populations into three ethnic subgroups, including Caucasians, Asians and others. And differences between the subgroups were compared using the χ² based Q test [21]. Third, to evaluate the reliability and stability of our results, publication bias was evaluated with Egger’s linear regression and Begger’s funnel plot [22,23], and the influence of each study on the pooled-OR was investigated in a sensitivity test by excluding one study each time. All probability values were 2-sided, values of p<0.05 were considered to be statistically significant and values of p<10⁻⁸ were considered to have reached a genome-wide significance level. All analyses were performed using the STATA software version 10.0 (Stata Corporation, College Station, TX, USA).

Results

Literature Search Results
A total of 509 articles from MEDLINE and EMBASE were identified through the preliminary literature search up to December 2012. As shown in Figure 1, a total of 35 potentially relevant articles were retained on the basis of titles and abstracts, and full texts of these articles were obtained for detailed review. Fifteen articles were excluded for the following reasons: six were not association study for T2DM [24–29], one was case-only study [30], two focused on lipid traits [31], three were studies of other SNPs [32–34], two of the results were reported elsewhere [35,36], and the remaining one was a meta-analysis [37]. Totally, 38 articles consisting of 113,025 cases and 199,997 controls were finally included [3–7,11–15,38–63]. Of all the studies included, 15 were studies of populations of European descent, 19 were studies of populations of Asian descent and 4 were studies of mixed/other ethnicities. The detailed characters of the included association studies are listed in Table 1.

Heterogeneous Association of the GCK rs1799884 Polymorphism with T2DM Risk
Not all researchers used the same SNPs. The most widely used was rs1799884. The remaining 5 articles used 2 additional SNPs,
rs4607517 and rs730497. Based on 1000 genome project, the SNP rs1799884 was in strong linkage disequilibrium (LD) with rs4607517 ($r^2 = 1.0$) and rs730497 ($r^2 = 1.0$) across different racial populations (CEU, CHB, YRI), respectively. Therefore, the SNP rs1799884, which tags rs4607517 and rs730497, is probably the best proxy to evaluate the effect of this gene. Totally, 20 articles involving 91,328 cases and 169,119 controls were included to evaluate the effect of rs1799884 (or as proxy) for T2DM risk. As shown in Table S2, the pooled frequency of the minor A-allele was identical among Asians and Caucasians (minor allele frequency (MAF) = 0.16), while lower in others (MAF = 0.12). In the overall estimate (Figure 2), the minor A-allele of \textit{GCK} rs1799884 was significantly associated with increased risk of diabetes (OR, 1.04; 95%CI, 1.01–1.08; $p = 0.006$), with moderate heterogeneity ($Q = 40.09; \hat{I}^2 = 59.1\%$; $p < 0.001$). After being stratified for ethnicity, significant difference between ethnic groups was detected (subgroup difference $\chi^2 = 8.79; \hat{p} = 0.012$). The results indicated that the minor A-allele might be associated with an augmented T2DM risk (OR, 1.08; 95%CI, 1.02–1.16; $p = 0.015$) in Caucasians. However, no clear evidence for such an association was observed in either Asians (OR, 1.09; 95%CI, 0.98–1.05; $p = 0.329$) or others (OR, 1.09; 95%CI, 0.99–1.18; $p = 0.075$).

After being stratified for ethnicity, significant difference between ethnic groups was detected (subgroup difference $\chi^2 = 8.79; \hat{p} = 0.012$). The results indicated that the minor A-allele might be associated with an augmented T2DM risk (OR, 1.08; 95%CI, 1.02–1.16; $p = 0.015$) in Caucasians. However, no clear evidence for such an association was observed in either Asians (OR, 1.09; 95%CI, 0.98–1.05; $p = 0.329$) or others (OR, 1.09; 95%CI, 0.99–1.18; $p = 0.075$).

Homogeneous Association of the GCKR rs780094 Polymorphism with T2DM Risk

In total, 20 studies from 17 independent publications investigating the influence of the rs780094 on the risk of T2DM were combined, yielding a meta-analysis of data from 236,778 individuals (80,133 cases and 156,645 controls). As presented in Table S3, the pooled C-allele frequency was slightly lower in Caucasians (MAF = 0.62) than in Asians (MAF = 0.67), while much higher in African Americans (MAF = 0.82). In the overall estimate (Figure 3), a significant association was observed between the C-allele and elevated risk of T2DM (OR, 1.08; 95%CI, 1.05–1.12; $p = 3.8 \times 10^{-6}$) with high heterogeneity among studies ($Q = 46.49; \hat{I}^2 = 59.1\%$; $p < 0.001$). After being stratified for ethnicity, significant associations were observed both in Caucasians (OR, 1.07; 95%CI, 1.03–1.10; $p = 1.3 \times 10^{-6}$) and Asians (OR, 1.09; 95%CI, 1.03–1.15; $p = 0.002$), with no difference in ORs observed (subgroup difference $\chi^2 = 1.34; \hat{p} = 0.511$).

Heterogeneous Association of the MTNR1B rs10830963 Polymorphism with T2DM Risk

Meta-analysis on the relationship between rs10830963 and T2DM risk included 18 independent articles containing data from 227,436 subjects (75,562 cases and 151,874 controls). As shown in Table S4, the risk G-allele frequency was higher in Asians (MAF = 0.42) than in Caucasians (MAF = 0.30). In the overall estimate (Figure 4), the G-allele was significantly associated with increased risk of T2DM (OR, 1.05; 95%CI, 1.02–1.08; $p = 0.002$). A high level of heterogeneity was observed between the included studies ($Q = 43.96; \hat{I}^2 = 52.2\%$; $p = 0.002$), and an inconsistent effect was noted when studies were considered separately by ancestry (subgroup difference $\chi^2 = 21.71; \hat{p} = 3.2 \times 10^{-6}$). Indeed, the association between the minor G-allele and T2DM risk was well replicated and reached a genome wide significance level in populations of Caucasians (OR, 1.10; 95%CI, 1.00–1.13; $p = 6.7 \times 10^{-6}$), but it is not replicable in Asians (OR, 1.01; 95%CI, 0.90–1.04; $p = 0.547$).
Table 1. Characteristics of genetic association studies included in the current meta-analyses.

| First author       | Ethnicity | Year | Case Number | Male% | Age | Control Number | Male% | Age | GCK rs1799884 | GCKR rs780094 | G6PC2 rs560887 | MTNR1B rs10830963 |
|--------------------|-----------|------|-------------|-------|-----|----------------|-------|-----|---------------|---------------|----------------|-------------------|
| Rose et al. [12]   | Danish    | 2005 | 1408        | 60.4  | 57  | 4441           | 46.5  | 45  |               |               |                |                   |
| Sparso et al. [13] | Danish    | 2007 | 3878        | 59.4  | 61.8| 4891           | 46.5  | 46.6|               |               |                |                   |
| Bouatia-Naji et al. [44] | French+Swiss | 2008 | 2972 | 62.2 | 50.4 | 4073 | 47.1 | 46.8 |               |               |                |                   |
| Cauchi et al. [45]  | French    | 2008 | 2825        | 53.7  | 56.6| 4472           | 37.3  | 45.6 |               |               |                |                   |
| Holmkvist et al. [50] | Finnish | 2008 | 132 | 50.8 | 51.7 | 2293 | 45.8 | 44.9 |               |               |                |                   |
| Holmkvist et al. [50] | Swedish  | 2008 | 1872       | 78.3  | NA  | 13666         | 63.7  | 45.5 |               |               |                |                   |
| Vaxillaire et al. [64] | French  | 2008 | 2215 | NA | NA | 2251 | 50 | 47.7 |               |               |                |                   |
| Ezzidi et al. [47]  | Tunisian  | 2009 | 884        | 45.9  | 59.4| 1908           | 44.3  | 58.8 |               |               |                |                   |
| Lyssenko et al. [11] | French    | 2009 | 2063 | 64.9 | 45.5 | 13998 | 64.9 | 45.5 |               |               |                |                   |
| Lyssenko et al. [11] | Finnish   | 2009 | 138 | 50.8 | 44.9 | 2632 | NA | NA |               |               |                |                   |
| Qi et al. [59] | Chinese | 2009 | 424 | 44.3 | 58.6 | 1908 | 44.3 | 58.8 |               |               |                |                   |
| Reiling et al. [5]  | Netherlands | 2009 | 2628 | 55 | 64 | 2041 | 46 | 53 |               |               |                |                   |
| Ronn et al. [60] | Chinese | 2009 | 1165 | 39.1 | 60.3 | 1105 | 31.6 | 59.4 |               |               |                |                   |
| Rose et al. [61] | Danish | 2009 | 1408 | 60.4 | 57 | 4773 | 46.6 | 46.2 |               |               |                |                   |
| Sparso et al. [62] | Danish | 2009 | 1948 | 61.6 | 60.2 | 4905 | 46.4 | 46.2 |               |               |                |                   |
| Sparso et al. [62] | French | 2009 | 183 | 48.9 | 47 | 2894 | 48.9 | 47 |               |               |                |                   |
| Sparso et al. [62] | French | 2009 | 2622 | NA | NA | 4343 | NA | NA |               |               |                |                   |
| Bi et al. [43] | White American | 2010 | 992 | 47 | 54.3 | 9937 | 47 | 54.3 |               |               |                |                   |
| Bi et al. [43] | Black American | 2010 | 772 | 38.3 | 53.5 | 3188 | 38.3 | 53.5 |               |               |                |                   |
| Dupuis et al. [3] | Mixed | 2010 | 40655 | NA | NA | 87022 | NA | NA |               |               |                |                   |
| Hu et al. [4] | Chinese | 2010 | 3410 | 54.9 | 60.3 | 3412 | 40 | 50.1 |               |               |                |                   |
| Mohas et al. [55] | Hungarian | 2010 | 321 | 53.6 | 61.3 | 172 | 28.5 | 56.5 |               |               |                |                   |
| Onuma et al. [58] | Japanese | 2010 | 506 | 55.3 | 60 | 402 | 53.2 | 59 |               |               |                |                   |
| Takeuchi et al. [7] | Japanese | 2010 | 5629 | NA | NA | 6406 | NA | NA |               |               |                |                   |
| Takeuchi et al. [7] | Sri Lankan | 2010 | 599 | NA | NA | 515 | NA | NA |               |               |                |                   |
| Tam et al. [14] | Chinese | 2010 | 1342 | 40.5 | 44.5 | 1644 | 45.4 | 24.6 |               |               |                |                   |
| Wen et al. [65] | Chinese | 2010 | 1165 | 39.1 | 60.3 | 1136 | 31.1 | 59.1 |               |               |                |                   |
| Been et al. [42] | Asian Indian | 2011 | 1201 | 52.3 | 53.9 | 1021 | 52.4 | 50.7 |               |               |                |                   |
| Cho et al. [40] | East Asian | 2011 | 6952 | NA | NA | 11865 | NA | NA |               |               |                |                   |
| Dietrich et al. [46] | German | 2011 | 103 | NA | 48 | 547 | NA | 48 |               |               |                |                   |
| Kooner et al. [41] | South Asian | 2011 | 5561 | NA | NA | 14512 | NA | NA |               |               |                |                   |
| Ling et al. [52] | Chinese | 2011 | 1118 | 44.6 | 60.2 | 1161 | 42.7 | 56.5 |               |               |                |                   |
| Ling et al. [53] | Chinese | 2011 | 1118 | 44.6 | 60.2 | 1161 | 42.7 | 56.5 |               |               |                |                   |
| Ohtsuge et al. [57] | Japanese | 2011 | 2839 | 60.5 | 62.8 | 2125 | 47.6 | 51.6 |               |               |                |                   |
| Olsson et al. [39] | Norwegian | 2011 | 1322 | 48.9 | 68.4 | 1447 | 50.2 | 65.2 |               |               |                |                   |
| Rees et al. [15] | South Asian | 2011 | 821 | 52.4 | 54.6 | 1167 | 52.9 | 56.3 |               |               |                |                   |
| Rees et al. [15] | South Asian | 2011 | 857 | 45.3 | 56.9 | 417 | 52 | 54.9 |               |               |                |                   |
| Tabara et al. [38] | Japanese | 2011 | 506 | 55.3 | 60 | 402 | 53.2 | 59 |               |               |                |                   |
| Cauchi et al. [6] | Moroccan | 2012 | 1193 | 34.3 | 54 | 1055 | 30.3 | 58 |               |               |                |                   |
| Cauchi et al. [6] | Tunisian | 2012 | 1446 | 44.3 | 61 | 942 | 45.8 | 61 |               |               |                |                   |
| Florez et al. [48] | American | 2012 | 633 | 32.3 | 50.6 | 2890 | 32.3 | 50.6 |               |               |                |                   |
| Fujita et al. [49] | Japanese | 2012 | 2632 | NA | 64.1 | 2050 | NA | 69.7 |               |               |                |                   |
| Iwata et al. [51] | Japanese | 2012 | 1182 | 59.6 | 65.3 | 859 | 44.4 | 69.5 |               |               |                |                   |
| Liu et al. [54] | Chinese | 2012 | 424 | 44.3 | 58.6 | 2786 | 44.3 | 58.6 |               |               |                |                   |
| Ng et al. [56] | African American | 2012 | 2806 | 38.1 | 47.3 | 4265 | 39.4 | 51.1 |               |               |                |                   |
| Tabassum et al. [63] | Asian Indian | 2012 | 5482 | 42 | 50.1 | 4588 | 43.9 | 48.2 |               |               |                |                   |
Table 1. Cont.

| First author       | Ethnicity     | Year   | Case Number | Male% | Age | Control Number | Male% | Age | rs1799884 | rs780094 | rs560887 | rs10830963 |
|--------------------|---------------|--------|-------------|-------|-----|----------------|-------|-----|------------|----------|----------|------------|
| Tabassum et al. [63]| Indo-European| 2012   | 1256        | 57.8  | 45  | 1209          | 56.6  | 50  | /          | /        | ?        | ?          |

NA: not available; ! represents this SNP was studied.
doi:10.1371/journal.pone.0067665.t001

Figure 2. Forest plot for the association between GCK rs1799884 and T2DM. Pooled OR for the additive genetic model was shown under a random-effects model. Square sizes were proportional to weight of each study in the meta-analysis. Significant association was detected in Caucasians but not in Asians and others.
doi:10.1371/journal.pone.0067665.g002
Contrasting Effects of the G6PC2 rs560887 Polymorphism on Risk of T2DM between Caucasians and Asians

We pooled data from 6 articles containing a total of 55,569 cases and 106,414 controls. As indicated in Table S5, the risk A-allele frequency was much lower in Asians (MAF = 0.04) than in Caucasians (MAF = 0.30). In the overall estimate (Figure 5), the association between the rs560887-G allele and T2DM risk was non-significant (OR, 0.98; 95%CI, 0.93–1.03; \( p = 0.458 \)), with moderate heterogeneity (Q = 12.85; I² = 45.5%; \( p = 0.002 \)). However, when considered separately by ethnicity, a contrasting effect of this variant on T2DM was observed (subgroup difference \( x^2 = 2.94; p = 0.086 \)). Results from Caucasian studies indicated the FPG-raising G-allele might be associated with a decreased risk of T2DM (OR, 0.97; 95%CI, 0.95–0.99; \( p = 0.001 \)), with no heterogeneity observed (Q = 0.73; I² = 0.0%; \( p = 0.867 \)). Conversely, in Asians, the G-allele was associated with increased risk of T2DM, although statistically not significant (OR, 1.12; 95%CI, 0.91–1.32; \( p = 0.257 \)). Given the low frequency and limited sample size of Asian studies, the current meta-analysis may be still be under-powered to provide conclusive insights into this issue.

Meta-regression

In the meta-regression analyses, neither sample size, study quality, mean age of cases and controls nor sex distribution in cases and controls were significantly correlated with the magnitude of the genetic effect (all \( p > 0.05 \)).
Publication Bias, Sensitivity Test

Based on Begger’s funnel plots (Figures S1–S4) and Egger’s linear regression, we didn’t detect any publication bias for all the pooled analyses (Egger’s test, all \( p > 0.05 \)). Besides, in the sensitivity test (Figures S5–S8), the leave-one-out influential analyses showed that no individual study would significantly modify the estimates, and this further confirmed the stability and reliability of the pooled results.

Discussion

The present meta-analyses provided the most comprehensive evaluation of the associations between FPG-raising variants and T2DM risk. In the overall estimates comprising individuals from different ethnicities, significant associations with increased risk of T2DM were detected for the \( GCK \), \( GCKR \) and \( MTNR1B \) variants, but not for the \( G6PC2 \) variant. However, the results should be interpreted with caution when heterogeneity between Caucasians and Asians was detected. In particular, significant associations with T2DM risk were found in Caucasians for all four SNPs, whereas in Asians, no significant associations were detected for the \( GCK \), \( MTNR1B \) and \( G6PC2 \) variants.

Several possibilities may explain the divergence across diverse ethnic groups. First, the distributions of the SNPs were different between various ethnic populations. For instance, the allele A frequencies of rs560887 differ from 1.7% in Asians to 30.8% in Caucasians. Given that the low frequency of rs560887 in Asians, it may have limited statistical power to detect positive association.
with a small effect. Second, the genetic variant of interest might be in LD with other causal variants, and the extent of LD was reported to differ in some of study populations that were examined [66]. Third, there may be population-specific genetic effects as a result of gene-gene and gene-environment interactions [67,68]. Asians have been reported to have unique risk factor profiles for developing diabetes that differ from those in Caucasians [69]. All the above-mentioned factors might have contributed to the heterogeneous association results across ethnic groups.

The power of genetic association studies is always limited by sample size especially when the effect of a genetic variant is small, as was the case for the above-mentioned variants. Combining data from many studies to form a large sample size allows small effects to be detected and more precise estimates to be obtained. This was the main strength of the current meta-analysis. However, there are several limitations that should be noted. First, most of the study subjects were of European ancestry, the Asian subgroup only contained about 15,000 cases. And further Asian studies are required to give more precise estimate of the genetic effects. Second, although an exhaustive literature search was done, some publications (especially those published not in English) and unpublished work would have been missed, and publication bias may potentially exist. Third, because no original individual data were available, we were not able to further investigate the cumulative effect of the included variants and the gene-environment interactions could not be investigated.

In conclusion, our meta-analysis has provided robust evidence that the \(GCKR\) rs780094 polymorphism is an important variant that confers high cross-ethnicity risk for development of T2DM. Conversely, significant associations between the \(GCK\), \(MTNR1B\)
and G6PC2 variants and T2DM risk are limited to Caucasians, and the meta-analysis results of associations of those variants with T2DM are required for further evaluation in larger sample size in Asian population.

Supporting Information

Figure S1 Begg’s funnel plot of studies of the GCK rs1799884 variant and T2DM. Each point represents a separate study for the indicated association. Egger’s test, t = -0.42, p = 0.678.
(TIF)

Figure S2 Begg’s funnel plot of studies of the GCKR rs780094 variant and T2DM. Each point represents a separate study for the indicated association. Egger’s test, t = 0.86, p = 0.401.
(TIF)

Figure S3 Begg’s funnel plot of studies of the MTNR1B rs10830963 variant and T2DM. Each point represents a separate study for the indicated association. Egger’s test, t = -1.31, p = 0.205.
(TIF)

Figure S4 Begg’s funnel plot of studies of the G6PC2 rs560887 variant and T2DM. Each point represents a separate study for the indicated association. Egger’s test, t = 1.35, p = 0.225.
(TIF)

Figure S5 Sensitivity analyses of the GCK rs1799884 variant in an additive model by omitting one study at a time. The summary OR (95% CI) was indicated by each horizontal line when the labeled study was omitted and the reminders were reanalyzed.
(TIF)

Figure S6 Sensitivity analyses of the GCKR rs780094 variant in an additive model by omitting one study at a time. The summary OR (95% CI) was indicated by each horizontal line when the labeled study was omitted and the reminders were reanalyzed.
(TIF)

Figure S7 Sensitivity analyses of the MTNR1B rs10830963 variant in an additive model by omitting one study at a time. The summary OR (95% CI) was indicated by each horizontal line when the labeled study was omitted and the reminders were reanalyzed.
(TIF)

Figure S8 Sensitivity analyses of the G6PC2 variant in an additive model by omitting one study at a time. The summary OR (95% CI) was indicated by each horizontal line when the labeled study was omitted and the reminders were reanalyzed.
(TIF)

Table S1 Quality score assessment criteria.
(DOCX)

Table S2 Estimation of the pooled prevalence of the risk A-allele of GCK rs1799884.
(DOCX)

Table S3 Estimation of the pooled prevalence of the risk C-allele of GCKR rs780094.
(DOCX)

Table S4 Estimation of the pooled prevalence of the risk A-allele of G6PC2 rs560887.
(DOCX)

Table S5 PRISMA Checklist for the current meta-analysis.
(DOC)

Table S6 PRISMA Flow Diagram for the current meta-analysis.
(DOC)

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Author Contributions

Conceived and designed the experiments: HD DWW. Performed the experiments: HW LL JZ GC CC. Analyzed the data: HW LL JZ. Contributed reagents/materials/analysis tools: HD DWW. Wrote the paper: HW LL JZ.

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