

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*, rs1799884), glucokinase regulator protein (*GCKR*, rs780094), islet specific glucose-6-phosphatase (*G6PC2*, rs560887) and melatonin receptor 1B (*MTNR1B*, rs10830963) to be the variants that most influence FPG levels [3–5], with an effect size of >0.029 mmol/l per risk allele. Moreover, the significant associations between these variants and FPG were well replicated in other populations, including Asians and Africans [6,7].

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* rs560887 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]. Secondly, 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 I^2 test. I^2 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-variant (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 χ^2 based Q test [21]. Thirdly, 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 53 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 [3–7,11–15,38–65]. 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,

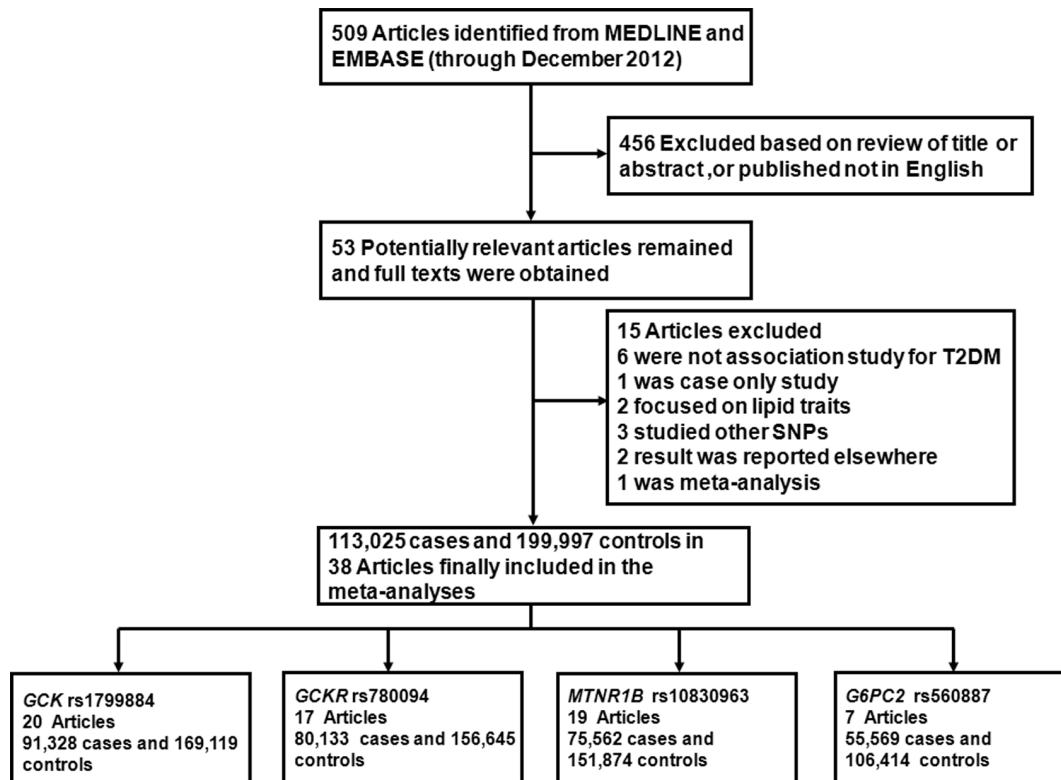


Figure 1. Flow diagram of study identification.
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rs4607517 and rs730497. Based on 1000 genome project, the SNP rs1799884 was in strong linkage disequilibrium (LD) with rs4607517 ($r^2 = 1.0$) and rs730497 ($r^2 = 1.0$) across different racial populations (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 *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 ($Q = 40.09$; $I^2 = 42.6\%$; $p = 0.015$).

After being stratified for ethnicity, significant difference between ethnic groups was detected (subgroup difference $\chi^2 = 8.79$; $p = 0.012$). The results indicated that the minor A-allele might be associated with an augmented T2DM risk (OR, 1.09; 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, 1.02; 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 ($Q = 46.49$; $I^2 = 59.1\%$; $p < 0.001$). After being stratified for ethnicity, significant associations were observed both in Caucasians (OR, 1.07; 95%CI, 1.03–1.10; $p = 1.3 \times 10^{-4}$) and Asians (OR, 1.09; 95%CI, 1.03–1.15; $p = 0.002$), with no difference in ORs observed (subgroup difference $\chi^2 = 1.34$; $p = 0.511$).

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 ($Q = 43.96$; $I^2 = 52.2\%$; $p = 0.002$), and an inconsistent effect was noted when studies were considered separately by ancestry (subgroup difference $\chi^2 = 21.71$; $p = 3.2 \times 10^{-6}$). 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, 1.08–1.13; $p = 6.7 \times 10^{-16}$), but it is not replicable in Asians (OR, 1.01; 95%CI, 0.98–1.04; $p = 0.547$).

Table 1. Characteristics of genetic association studies included in the current meta-analyses.

First author	Ethnicity	Year	Case			Control			<i>GCK</i>	<i>GCKR</i>	<i>G6PC2</i>	<i>MTNR1B</i>
			Number	Male%	Age	Number	Male%	Age	rs1799884	rs780094	rs560887	rs10830963
Rose et al. [12]	Danish	2005	1408	60.4	57	4441	46.54	45	✓			
Sparso et al. [13]	Danish	2007	3878	59.4	61.8	4891	46.5	46.6		✓		
Bouatia-Naji et al. [44]	French	2008	2972	62.2	50.4	4073	47.1	46.8			✓	
Cauchi et al. [45]	French+Swiss	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	513	50.3	60	✓			
Lyssenko et al. [11]	Swedish	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				✓
Ohshige 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			Control			<i>GCK</i>	<i>GCKR</i>	<i>G6PC2</i>	<i>MTNR1B</i>
			Number	Male%	Age	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

GCK rs1799884

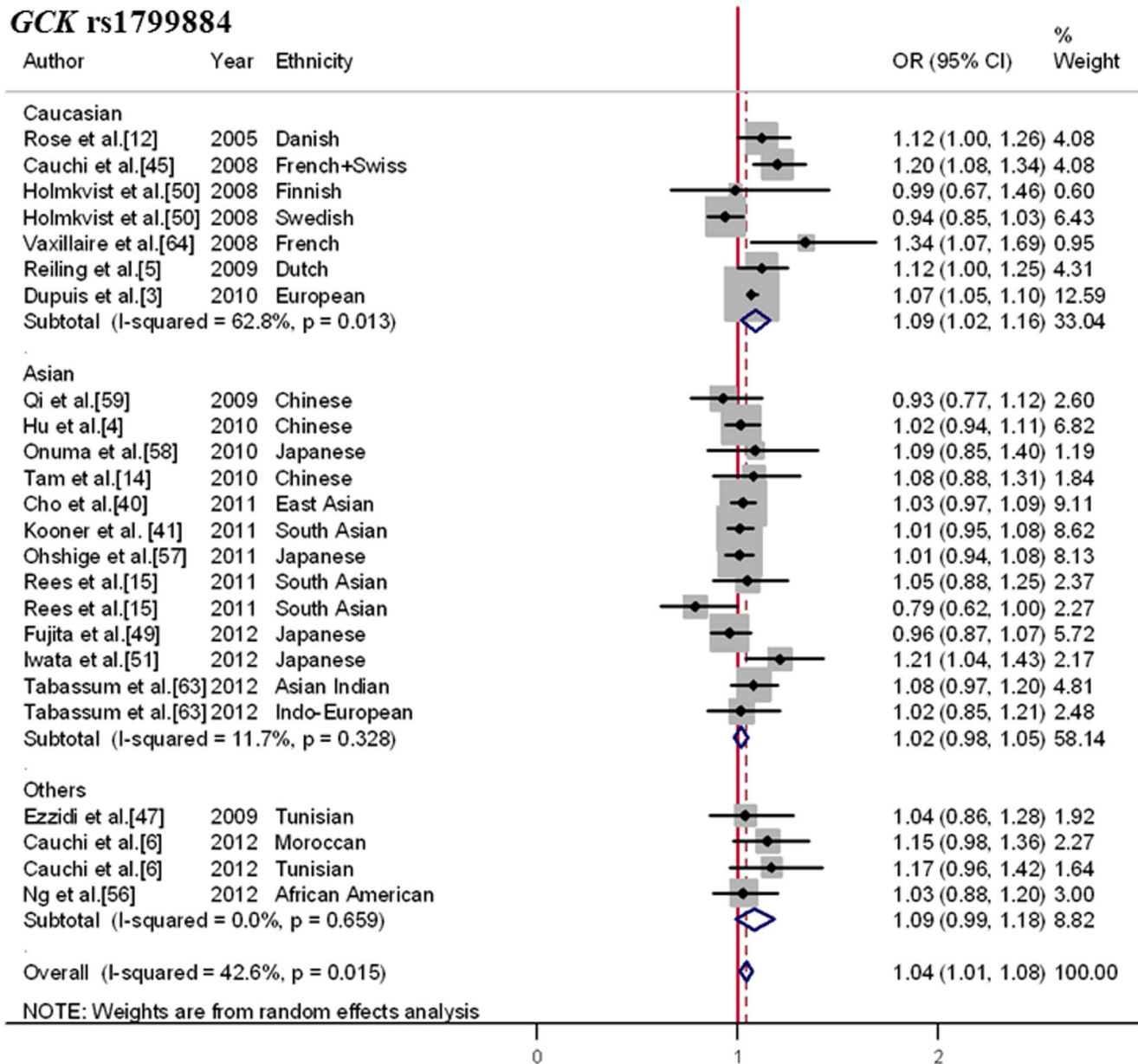


Figure 2. Forest plot for the association between GCK rs1799884 and T2DM. Pooled OR for the additive genetic model was shown under a random-effects model. Square sizes were proportional to weight of each study in the meta-analysis. Significant association was detected in Caucasians but not in Asians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

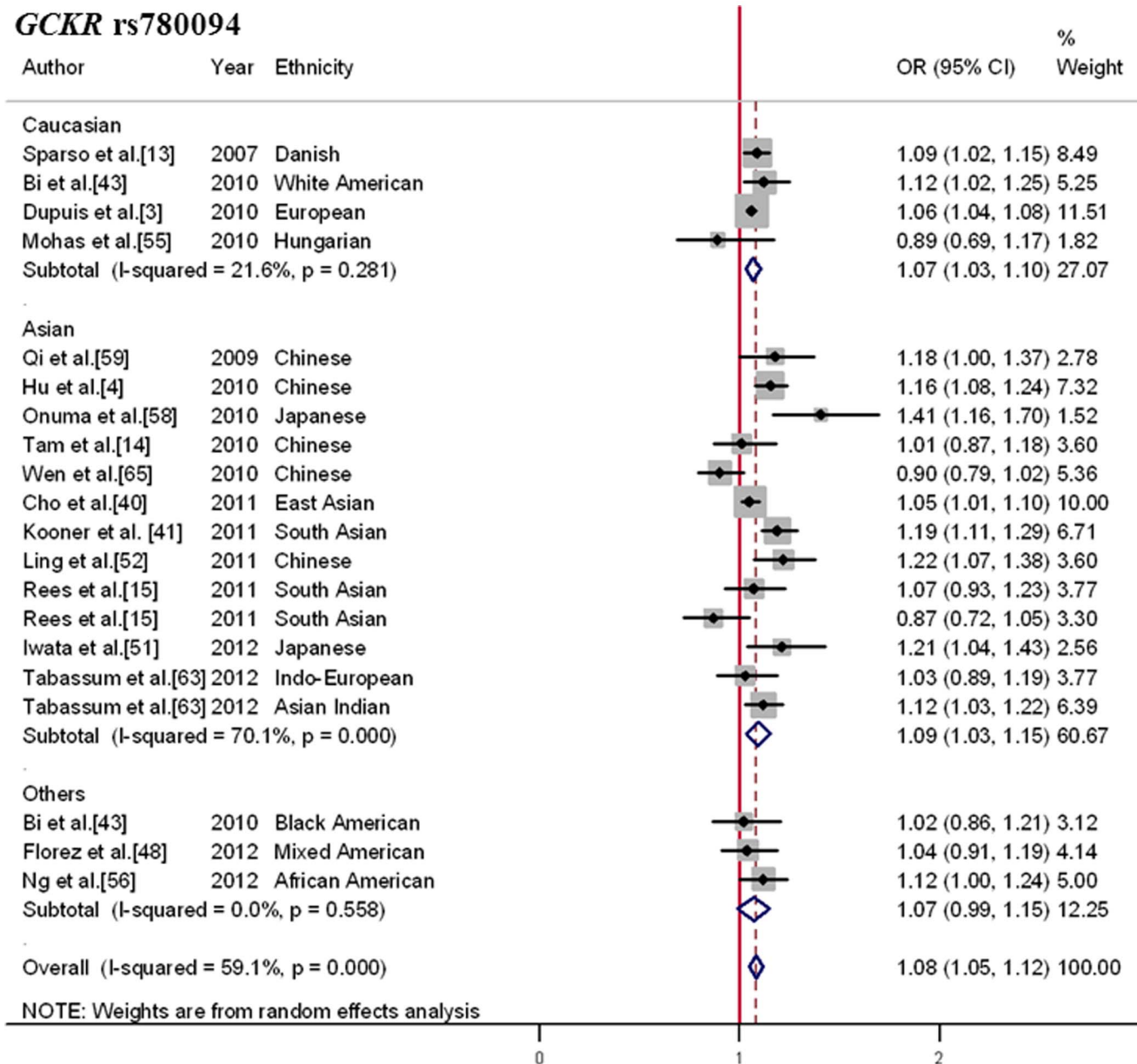
GCKR rs780094

Figure 3. Forest plot for the association between GCKR rs780094 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. Our meta-analyses demonstrated GCKR locus confers high cross-ethnicity risk for development of T2DM. doi:10.1371/journal.pone.0067665.g003

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 ($Q = 12.85$; $I^2 = 45.5\%$; $p = 0.002$). However, when considered separately by ethnicity, a contrasting effect of this variant on T2DM was observed (subgroup difference $\chi^2 = 2.94$; $p = 0.086$). 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 ($Q = 0.73$; $I^2 = 0.0\%$; $p = 0.867$). 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 $p > 0.05$).

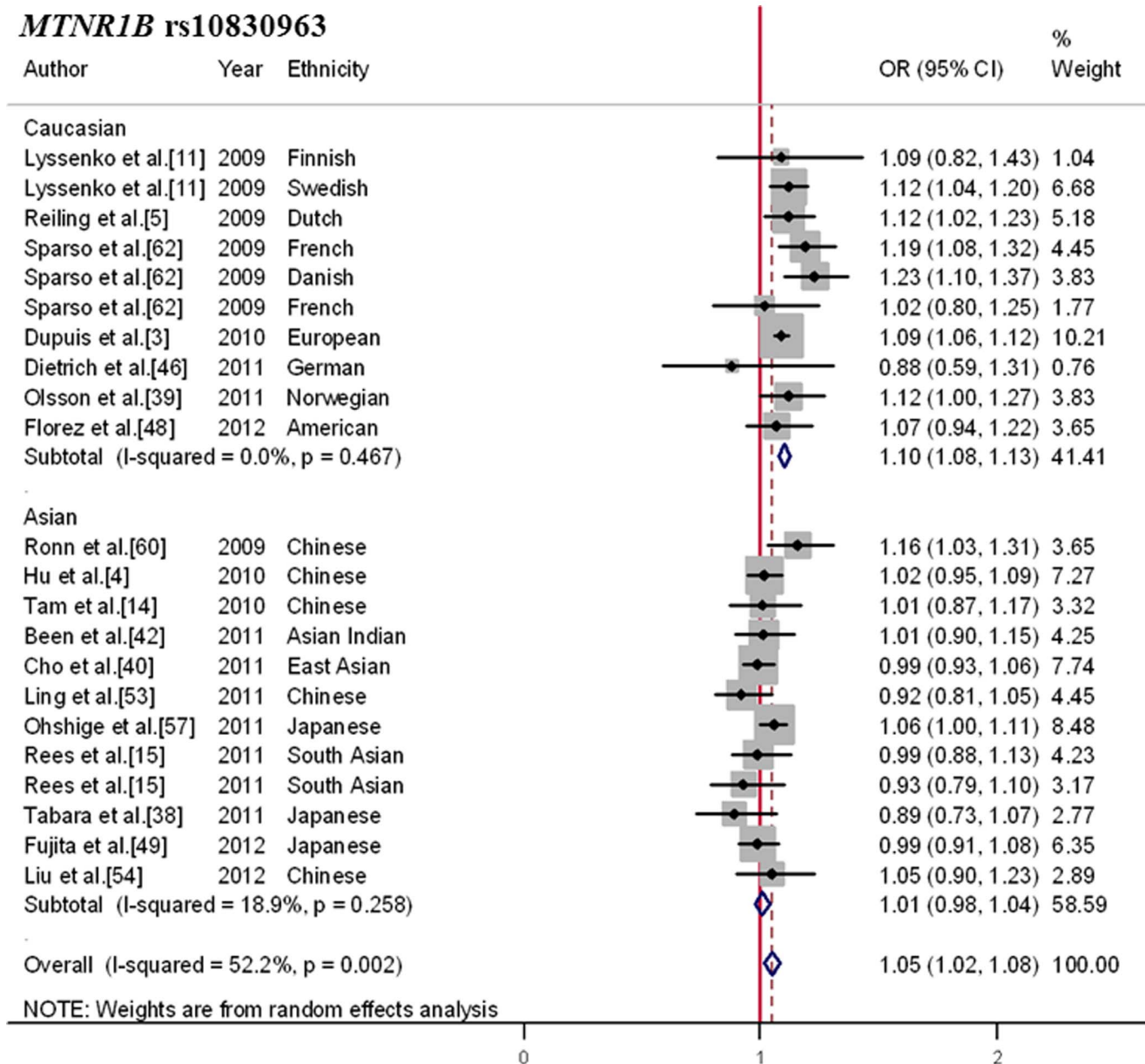
MTNR1B rs10830963

Figure 4. Forest plot for the association between *MTNR1B* rs10830963 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. The result indicated that significant association was limited to Caucasians. doi:10.1371/journal.pone.0067665.g004

Publication Bias, Sensitivity Test

Based on Egger'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

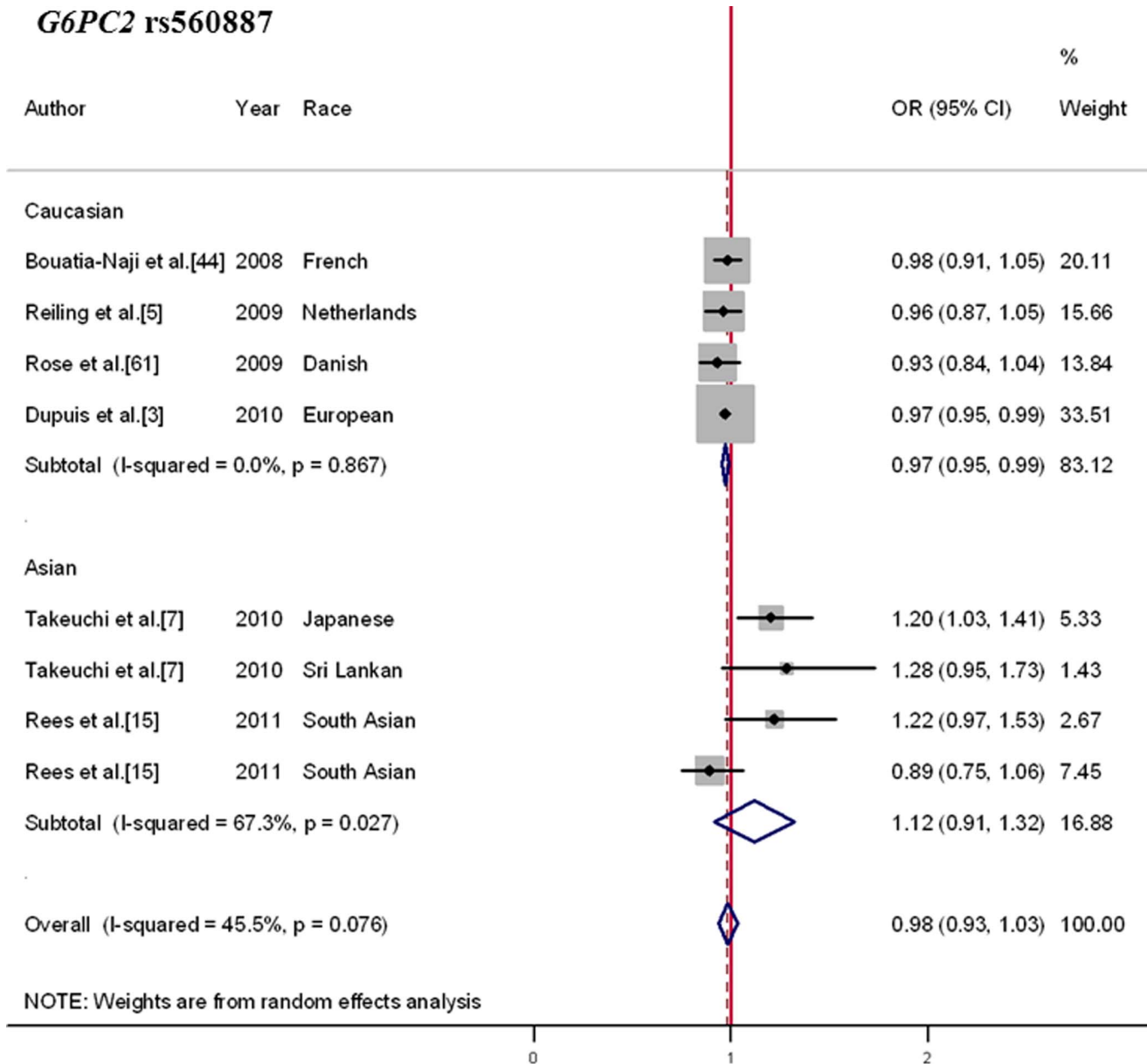
***G6PC2* rs560887**

Figure 5. Forest plot for the association between *G6PC2* rs560887 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. Contrasting results were detected between Caucasians and Asians.

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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 *GCKR* 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 *GCKR* 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 *GCKR* 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 G-allele of *MTNR1B* rs10830963.

(DOCX)

Table S5 Estimation of the pooled prevalence of the risk A-allele of *G6PC2* rs560887.

(DOCX)

Table S6 PRISMA Checklist for the current meta-analysis.

(DOC)

Table S7 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.

References

- Piche ME, Arcand-Bosse JF, Despres JP, Perusse L, Lemieux S, et al. (2004) What is a normal glucose value? Differences in indexes of plasma glucose homeostasis in subjects with normal fasting glucose. *Diabetes Care* 27: 2470–2477.
- Tirosh A, Shai I, Tekes-Manova D, Israeli E, Pereg D, et al. (2005) Normal fasting plasma glucose levels and type 2 diabetes in young men. *N Engl J Med* 353: 1454–1462.
- 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: 105–116.
- Hu C, Zhang R, Wang C, Yu W, Lu J, et al. (2010) Effects of *GCK*, *GCKR*, *G6PC2* and *MTNR1B* variants on glucose metabolism and insulin secretion. *PLoS One* 5: e11761.
- Reiling E, van 't Riet E, Groenewoud MJ, Welschen LM, van Hove EC, et al. (2009) Combined effects of single-nucleotide polymorphisms in *GCK*, *GCKR*, *G6PC2* and *MTNR1B* on fasting plasma glucose and type 2 diabetes risk. *Diabetologia* 52: 1866–1870.
- Cauchi S, Ezzidi I, El Achhab Y, Mtiraoui N, Chaieb L, et al. (2012) European genetic variants associated with type 2 diabetes in North African Arabs. *Diabetes Metab* 38: 316–323.
- Takeuchi F, Katsuya T, Chakrewarthy S, Yamamoto K, Fujioka A, et al. (2010) Common variants at the *GCK*, *GCKR*, *G6PC2-ABCB1* and *MTNR1B* loci are associated with fasting glucose in two Asian populations. *Diabetologia* 53: 299–308.
- Matsutani A, Janssen R, Donis-Keller H, Permutt MA (1992) A polymorphic (CA)_n repeat element maps the human glucokinase gene (*GCK*) to chromosome 7p. *Genomics* 12: 319–325.
- Warner JP, Leek JP, Intody S, Markham AF, Bonthon DT (1995) Human glucokinase regulatory protein (*GCKR*): cDNA and genomic cloning, complete primary structure, and chromosomal localization. *Mamm Genome* 6: 532–536.
- Martin CC, Bischof LJ, Bergman B, Hornbuckle LA, Hilliker C, et al. (2001) Cloning and characterization of the human and rat islet-specific glucose-6-phosphatase catalytic subunit-related protein (*IGRP*) genes. *J Biol Chem* 276: 25197–25207.
- Lyssenko V, Nagorny CL, Erdos MR, Wierup N, Jonsson A, et al. (2009) Common variant in *MTNR1B* associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat Genet* 41: 82–88.
- Rose CS, Ek J, Urhammer SA, Glumer C, Borch-Johnsen K, et al. (2005) A $-30G>A$ polymorphism of the beta-cell-specific glucokinase promoter associates with hyperglycemia in the general population of whites. *Diabetes* 54: 3026–3031.
- Sparso T, Andersen G, Nielsen T, Burgdorf KS, Gjesing AP, et al. (2008) The *GCKR* rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. *Diabetologia* 51: 70–75.
- Tam CH, Ho JS, Wang Y, Lee HM, Lam VK, et al. (2010) 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: e11428.

15. Rees SD, Hydrice MZ, O'Hare JP, Kumar S, Shera AS, et al. (2011) Effects of 16 genetic variants on fasting glucose and type 2 diabetes in South Asians: ADCY5 and GLIS3 variants may predispose to type 2 diabetes. *PLoS One* 6: e24710.
16. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. (2009) The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* 6: e1000100.
17. Attia J, Thakkinian A, D'Este C (2003) Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J Clin Epidemiol* 56: 297–303.
18. Thakkinian A, McEvoy M, Minelli C, Gibson P, Hancox B, et al. (2005) Systematic review and meta-analysis of the association between β 2-adrenoceptor polymorphisms and asthma: a HuGE review. *Am J Epidemiol* 162: 201–211.
19. Fleiss JL (1993) The statistical basis of meta-analysis. *Stat Methods Med Res* 2: 121–145.
20. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analyses. *BMJ* 327: 557–560.
21. Pettiti DB (2001) Approaches to heterogeneity in meta-analysis. *Stat Med* 20: 3625–3633.
22. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. *Biometrics* 50: 1088–1101.
23. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315: 629–634.
24. Brito EC, Lyssenko V, Renstrom F, Berglund G, Nilsson PM, et al. (2009) Previously associated type 2 diabetes variants may interact with physical activity to modify the risk of impaired glucose regulation and type 2 diabetes: a study of 16,003 Swedish adults. *Diabetes* 58: 1411–1418.
25. Chen G, Bentley A, Adeyemo A, Shriner D, Zhou J, et al. (2012) Genome-wide association study identifies novel loci association with fasting insulin and insulin resistance in African Americans. *Hum Mol Genet* 21: 4530–4536.
26. Ramos E, Chen G, Shriner D, Doumatey A, Gerry NP, et al. (2011) Replication of genome-wide association studies (GWAS) loci for fasting plasma glucose in African-Americans. *Diabetologia* 54: 783–788.
27. Renstrom F, Shungin D, Johansson I, Investigators M, Florez JC, et al. (2011) Genetic predisposition to long-term nondiabetic deteriorations in glucose homeostasis: Ten-year follow-up of the GLACIER study. *Diabetes* 60: 345–354.
28. 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.
29. Wagner R, Dudziak K, Herzberg-Schaefer SA, Machicao F, Stefan N, et al. (2011) Glucose-raising genetic variants in MADD and ADCY5 impair conversion of proinsulin to insulin. *PLoS One* 6: e23639.
30. Bonetti S, Trombetta M, Boselli ML, Turrini F, Malerba G, et al. (2011) Variants of GCKR affect both beta-cell and kidney function in patients with newly diagnosed type 2 diabetes: the Verona newly diagnosed type 2 diabetes study 2. *Diabetes Care* 34: 1205–1210.
31. Hishida A, Morita E, Naito M, Okada R, Wakai K, et al. (2012) Associations of apolipoprotein A5 (APOA5), glucokinase (GCK) and glucokinase regulatory protein (GCKR) polymorphisms and lifestyle factors with the risk of dyslipidemia and dysglycemia in Japanese - a cross-sectional data from the J-MICC Study. *Endocr J* 59: 589–599.
32. Chambers JC, Zhang W, Zabaneh D, Schmi J, Jain P, et al. (2009) Common genetic variation near melatonin receptor MTNR1B contributes to raised plasma glucose and increased risk of type 2 diabetes among Indian Asians and European Caucasians. *Diabetes* 58: 2703–2708.
33. Stancakova A, Paananen J, Soiminen P, Kangas AJ, Bonnycastle LL, et al. (2011) Effects of 34 risk loci for type 2 diabetes or hyperglycemia on lipoprotein subclasses and their composition in 6,580 nondiabetic Finnish men. *Diabetes* 60: 1608–1616.
34. Vaxillaire M, Cavalcanti-Proenca C, Dechaume A, Tichet J, Marre M, et al. (2008) The common P446L polymorphism in GCKR inversely modulates fasting glucose and triglyceride levels and reduces type 2 diabetes risk in the DESIR prospective general French population. *Diabetes* 57: 2253–2257.
35. 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.
36. Balkau B, Lange C, Fezeu L, Tichet J, de Lauzon-Guillain B, et al. (2008) Predicting diabetes: clinical, biological, and genetic approaches: data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care* 31: 2056–2061.
37. Xia Q, Chen ZX, Wang YC, Ma YS, Zhang F, et al. (2012) Association between the melatonin receptor 1B gene polymorphism on the risk of type 2 diabetes, impaired glucose regulation: a meta-analysis. *PLoS One* 7: e50107.
38. Tabara Y, Osawa H, Kawamoto R, Onuma H, Shimizu I, et al. (2011) Genotype risk score of common susceptible variants for prediction of type 2 diabetes mellitus in Japanese: the Shimanami Health Promoting Program (J-SHIP study). Development of type 2 diabetes mellitus and genotype risk score. *Metabolism* 60: 1634–1640.
39. Olsson L, Pettersen E, Ahlbom A, Carlsson S, Midthjell K, et al. (2011) No effect by the common gene variant rs10830963 of the melatonin receptor 1B on the association between sleep disturbances and type 2 diabetes: results from the Nord-Trøndelag Health Study. *Diabetologia* 54: 1375–1378.
40. Cho YS, Chen CH, Hu C, Long J, Ong RT, et al. (2012) Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. *Nat Genet* 44: 67–72.
41. Kooner JS, Saleheen D, Sim X, Schmi J, Zhang W, et al. (2011) Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. *Nat Genet* 43: 984–989.
42. Been LF, Hatfield JL, Shankar A, Aston CE, Ralhan S, et al. (2012) A low frequency variant within the GWAS locus of MTNR1B affects fasting glucose concentrations: genetic risk is modulated by obesity. *Nutr Metab Cardiovasc Dis* 22: 944–951.
43. Bi M, Kao WH, Boerwinkle E, Hoogveen RC, Rasmussen-Torvik LJ, et al. (2010) Association of rs780094 in GCKR with metabolic traits and incident diabetes and cardiovascular disease: the ARIC Study. *PLoS One* 5: e11690.
44. Bouatia-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: 1085–1088.
45. Cauchi S, Nead KT, Choquet H, Horber F, Potoczna N, et al. (2008) The genetic susceptibility to type 2 diabetes may be modulated by obesity status: implications for association studies. *BMC Med Genet* 9: 45.
46. Dietrich K, Birkmeier S, Schleinitz D, Breifeld J, Enigk B, et al. (2011) Association and evolutionary studies of the melatonin receptor 1B gene (MTNR1B) in the self-contained population of Sorbs from Germany. *Diabet Med* 28: 1373–1380.
47. Ezzidi I, Miraoui N, Cauchi S, Vaillant E, Dechaume A, et al. (2009) Contribution of type 2 diabetes associated loci in the Arabic population from Tunisia: a case-control study. *BMC Med Genet* 10: 33.
48. Florez JC, Jablonski KA, McAteer JB, Franks PW, Mason CC, et al. (2012) Effects of genetic variants previously associated with fasting glucose and insulin in the Diabetes Prevention Program. *PLoS One* 7: e44424.
49. Fujita H, Hara K, Shojima N, Horikoshi M, Iwata M, et al. (2012) Variations with modest effects have an important role in the genetic background of type 2 diabetes and diabetes-related traits. *J Hum Genet* 57: 776–779.
50. Holmkvist J, Almgren P, Lyssenko V, Lindgren CM, Eriksson KF, et al. (2008) Common variants in maturity-onset diabetes of the young genes and future risk of type 2 diabetes. *Diabetes* 57: 1738–1744.
51. Iwata M, Maeda S, Kamura Y, Takano A, Kato H, et al. (2012) Genetic risk score constructed using 14 susceptibility alleles for type 2 diabetes is associated with the early onset of diabetes and may predict the future requirement of insulin injections among Japanese individuals. *Diabetes Care* 35: 1763–1770.
52. Ling Y, Li X, Gu Q, Chen H, Lu D, et al. (2011) Associations of common polymorphisms in GCKR with type 2 diabetes and related traits in a Han Chinese population: a case-control study. *BMC Med Genet* 12: 66.
53. Ling Y, Li X, Gu Q, Chen H, Lu D, et al. (2011) A common polymorphism rs3781637 in MTNR1B is associated with type 2 diabetes and lipids levels in Han Chinese individuals. *Cardiovasc Diabetol* 10: 27.
54. Liu C, Wu Y, Li H, Qi Q, Langenberg C, et al. (2010) MTNR1B rs10830963 is associated with fasting plasma glucose, HbA1C and impaired beta-cell function in Chinese Hans from Shanghai. *BMC Med Genet* 11: 59.
55. Mohas M, Kisfali P, Jaromi L, Maasz A, Feher E, et al. (2010) GCKR gene functional variants in type 2 diabetes and metabolic syndrome: do the rare variants associate with increased carotid intima-media thickness? *Cardiovasc Diabetol* 9: 79.
56. Ng MC, Saxena R, Li J, Palmer ND, Dimitrov L, et al. (2013) Transferability and fine mapping of type 2 diabetes Loci in african americans: the candidate gene association resource plus study. *Diabetes* 62: 965–976.
57. Ohshige T, Iwata M, Omori S, Tanaka Y, Hirose H, et al. (2011) Association of new loci identified in European genome-wide association studies with susceptibility to type 2 diabetes in the Japanese. *PLoS One* 6: e26911.
58. Onuma H, Tabara Y, Kawamoto R, Shimizu I, Kawamura R, et al. (2010) The GCKR rs780094 polymorphism is associated with susceptibility of type 2 diabetes, reduced fasting plasma glucose levels, increased triglycerides levels and lower HOMA-IR in Japanese population. *J Hum Genet* 55: 600–604.
59. Qi Q, Wu Y, Li H, Loos RJ, Hu FB, et al. (2009) Association of GCKR rs780094, alone or in combination with GCK rs1799884, with type 2 diabetes and related traits in a Han Chinese population. *Diabetologia* 52: 834–843.
60. 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.
61. Rose CS, Garup N, Krarup NT, Poulsen P, Wegner L, et al. (2009) A variant in the G6PC2/ABCB11 locus is associated with increased fasting plasma glucose, increased basal hepatic glucose production and increased insulin release after oral and intravenous glucose loads. *Diabetologia* 52: 2122–2129.
62. Sparso T, Bonnefond A, Andersson E, Bouatia-Naji N, Holmkvist J, et al. (2009) G-allele of intronic rs10830963 in MTNR1B confers increased risk of impaired fasting glycaemia and type 2 diabetes through an impaired glucose-stimulated insulin release: studies involving 19,605 Europeans. *Diabetes* 58: 1450–1456.
63. Tabassum R, Chauhan G, Dwivedi OP, Mahajan A, Jaiswal A, et al. (2013) Genome-wide association study for type 2 diabetes in indians identifies a new susceptibility locus at 2q21. *Diabetes* 62: 977–986.
64. Vaxillaire M, Veslot J, Dina C, Proenca C, Cauchi S, et al. (2008) Impact of common type 2 diabetes risk polymorphisms in the DESIR prospective study. *Diabetes* 57: 244–254.

65. Wen J, Ronn T, Olsson A, Yang Z, Lu B, et al. (2010) Investigation of type 2 diabetes risk alleles support CDKN2A/B, CDKAL1, and TCF7L2 as susceptibility genes in a Han Chinese cohort. *PLoS One* 5: e9153.
66. Teo YY, Ong RT, Sim X, Tai ES, Chia KS (2010) Identifying candidate causal variants via trans-population fine-mapping. *Genet Epidemiol* 34: 653–664.
67. Hunter DJ (2005) Gene-environment interactions in human diseases. *Nat Rev Genet* 6: 287–298.
68. Yang Q, Khoury MJ, Sun F, Flanders WD (1999) Case-only design to measure gene-gene interaction. *Epidemiology* 10: 167–170.
69. Weber MB, Oza-Frank R, Stamez LR, Ali MK, Narayan KM (2012) Type 2 diabetes in Asians: prevalence, risk factors, and effectiveness of behavioral intervention at individual and population levels. *Annu Rev Nutr* 32: 417–439.