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CDKAL1 gene rs7756992 A/G polymorphism and type 2 diabetes mellitus: a meta-analysis of 62,567 subjects

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The *Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like (CDKAL1)* gene rs7756992 A/G polymorphism has been suggested to be associated with type 2 diabetes mellitus (T2DM), but the individual studies results are still controversial. To explore the association of *CDKAL1* gene rs7756992 A/G polymorphism with T2DM, a meta-analysis involving 62,567 subjects from 21 separate studies was conducted. In the whole population, a significant association was found between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM under allelic (OR: 1.180, 95% CI: 1.130–1.230, $P = 1.60 \times 10^{-14}$), recessive (OR: 1.510, 95% CI: 1.380–1.660, $P = 8.41 \times 10^{-18}$), dominant (OR: 1.175, 95% CI: 1.109–1.246, $P = 6.30 \times 10^{-8}$), homozygous (OR: 1.400, 95% CI: 1.282–1.530, $P = 8.02 \times 10^{-14}$), and heterozygous genetic models (OR: 1.101, 95% CI: 1.040–1.166, $P = 0.001$). *CDKAL1* gene rs7756992 A/G polymorphism was significantly associated with T2DM. The person with G allele of *CDKAL1* gene rs7756992 A/G polymorphism might be predisposed to T2DM.

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disturbance syndrome led by the combined actions of genetic gene and environmental factors. The data from International Diabetes Federation showed that in 2011, there were approximately 366 million diabetes mellitus (DM) patients in the globe and it has been speculated that the DM patients quantity will continually increase and be up to 552 million by 2030, of which the type 2 DM (T2DM) accounts for 90–95%[1]. The large scale investigations performed in the Chinese adults in June 2007 and May 2008 have shown that T2DM and impaired glucose tolerance (IGT) patients were about 92.4 million and 148 million respectively which are predominantly the young and middle-aged people and the number is in the first place of the world. Therefore, it is no time to delay to prevent T2DM[2].

The interaction of genetic and environmental factors is universally acknowledged as the primary underlying T2DM mechanism. It is now generally considered that T2DM is not a sole disorder, but a multi-gene disorder with extensive heredity heterogeneity which results from the insulin resistance and β cell dysfunction of pancreatic island. The T2DM risk in the first degree relatives of T2DM patients is 3.62 times that in the common population[3], so the researchers of various countries make great efforts to explore the T2DM susceptible genes. Once the T2DM susceptible genes are sought out, it means that the T2DM prevention clues have been found. It is an effective measure to screen the T2DM susceptible population and prevent T2DM progress.

It has been reported that more than 50 genes are closely associated with T2DM by genome wide association study (GWAS) technology[4,5]. *Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like* (*CDKAL1*) is one of the novel T2DM associated genes identified recently[6]. *CDKAL1* gene, located in 6p22.3, spans 37 kb which encodes 579 amino acids. *CDKAL1* gene encode tRNA decoration enzyme, namely methyl transfer enzyme which is responsible for the 2-methylthio-N6-threonylcarbamoyladenosine synthesis of the 37th base of tRNA $^{Lys(UUU)}$ [7]. Wei et al found the mitochondria adenosine triphosphate (ATP) generation obstacle and the first stage insulin secretion impairment in the *CDKAL1* gene knock-out mice[8]. In 2007, the GWAS study in Iceland population first reported that the *CDKAL1* gene rs7756992 A/G polymorphism was associated with T2DM risk which was repeatedly verified in Caucasian populations[6].

Although many studies on the relationship between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM have been performed so far, the researches results were still controversial. In 2011, Chistiakov et al found that the allele G of rs7756992 with higher diabetes risk thereby replicating the predisposing role of *CDKAL1* gene in etiology of T2DM in a Russian population (OR = 1.21, 95% CI: 1.04–1.42, P = 0.017)[9]. In 2013, Li W et al found the similar result in a Chinese population (OR = 1.50, 95% CI: 1.11–2.04, P = 0.009)[10]. In contrast, in 2007, Horikoshi et al observed that in a Japan population with *CDKAL1* gene rs7756992 GG genotypes, the T2DM risk was significantly decreased (OR:0.78, 95% CI: 0.61–0.98, P = 0.04)[11]. However, in 2010, Xu et al reported that *CDKAL1* gene rs7756992 A/G polymorphism was not significantly associated with T2DM susceptibility in another Chinese population (OR: 1.68, 95% CI: 0.91–3.09, P = 0.10)[12].

In the present study, a meta-analysis involving 26,120 T2DM patients and 36,447 controls from 21 separate studies was performed to estimate the relationship of *CDKAL1* gene rs7756992 A/G polymorphism and T2DM.

Results

Studies and populations. Twenty-eight publications were obtained through the retrieval process, among which fourteen manuscripts including twenty one studies met the inclusion criteria. Among the fourteen discharged papers, three papers were published repeatedly, four papers were of review character, and five papers were not involved with *CDKAL1* gene rs7756992 A/G polymorphism or T2DM. Two studies deviating from the Hardy-Weinberg equilibrium (HWE) were rejected. All of information was extracted from 26,120 T2DM cases and 36,447 controls (Table 1)[6,9–21]. One publication generally included one study. Additionally, some publications included multiple studies. For example, the manuscript published by Steinthorsdottir et al included five individual studies[6]. In addition, four individual studies were involved in the publication authored by Cauchi et al[14]. Thirteen countries were included in the present meta-analysis as China, Korea, Denmark, Iceland, Netherland, West Africa, Japan, France, Austria, United States, Morocco, Israel, and Russia. These populations belong to Caucasian, Asian and African subgroups respectively. The Caucasian subgroup includes 7 individual studies, the Asian subgroup consists of 12 individual studies, and the African subgroup comprises 2 individual studies.

Pooled analyses. In the whole population, a significant association was found between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM under allelic (OR: 1.180, 95% CI: 1.130–1.230, P = 1.60×10^{-14}), recessive (OR: 1.510, 95% CI: 1.380–1.660, P = 8.41×10^{-18}), dominant (OR: 1.175, 95% CI: 1.109–1.246, P = 6.30×10^{-8}), homozygous (OR: 1.400, 95% CI: 1.282–1.530, P = 8.02×10^{-14}), and heterozygous genetic models (OR: 1.101, 95% CI: 1.040–1.166, P = 0.001).

In the subgroup analysis, there was a significant association between them in Caucasian population under allelic (OR: 1.220, 95% CI: 1.170–1.270, $P = 5.85 \times 10^{-22}$), recessive (OR: 1.470, 95% CI: 1.340–1.610, $P = 4.22 \times 10^{-16}$), dominant (OR: 1.239, 95% CI: 1.176–1.305, $P = 7.85 \times 10^{-15}$), homozygous (OR: 1.530, 95% CI: 1.391–1.684, $P = 5.81 \times 10^{-18}$), and heterozygous genetic models (OR: 1.185, 95% CI: 1.122–1.252, $P = 1.54 \times 10^{-9}$).

In Asian subgroup analysis, a significant association between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM was also detected under allelic (OR: 1.190, 95% CI: 1.110–1.270, $P = 3.58 \times 10^{-7}$), recessive (OR: 1.570, 95% CI: 1.360–1.800, $P = 3.85 \times 10^{-10}$), dominant (OR: 1.173, 95% CI: 1.068–1.289, $P = 8.68 \times 10^{-4}$), and homozygous genetic models (OR: 1.404, 95% CI: 1.237–1.594, $P = 1.52 \times 10^{-7}$). No significant association was found under heterozygous genetic model (OR: 1.078, 95% CI: 0.990–1.174, $P = 0.083$).

In the African subgroup, there was no significant association between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM under allelic (OR: 1.070, 95% CI: 0.960–1.190, $P = 0.23$), dominant (OR: 1.008, 95% CI: 0.843–1.206, $P = 0.927$), homozygous (OR: 1.058, 95% CI: 0.840–1.332, $P = 0.631$), or heterozygous genetic models (OR: 0.954, 95% CI: 0.788–1.154, $P = 0.626$). Only

Table 1 | Characteristics of the investigated studies of the association between the *CDKAL1* gene rs7756992 A/G polymorphism and type 2 diabetes mellitus (T2DM)

				T2DM			Control				
Author	Year	Country	Subgroup	AA	AG	GG	AA	AG	GG	Matching criteria	sample size (T2DM/control)
Steinthorsdottir [6]a	2007	Iceland	Caucasian	751	539	108	3107	1887	277	Sex, ethnicity	**1398/5271**
Steinthorsdottir [6]b	2007	Denmark	Caucasian	735	663	174	2795	2139	444	Sex, ethnicity	**1572/5378**
Steinthorsdottir [6]c	2007	US	Caucasian	216	174	40	492	331	68	Age, sex, ethnicity	**430/891**
Steinthorsdottir [6]d	2007	Netherland	Caucasian	186	138	30	475	359	63	Ethnicity	**354/897**
Steinthorsdottir [6]e	2007	West Africa	African	137	349	344	160	499	397	Sex, ethnicity	**830/1056**
Horikoshi[11]	2007	Japan	Asian	238	426	188	191	450	216	Ethnicity	**852/857**
Omori[13]	2008	Japan	Asian	398	782	430	293	508	238	Sex, ethnicity	**1610/1039**
Cauchi[14]a	2008	France	Caucasian	1855	1751	430	2357	1783	336	Ethnicity	**4036/4476**
Cauchi[14]b	2008	Austria	Caucasian	233	174	43	368	269	56	Sex, ethnicity	**450/693**
Cauchi[14]c	2008	Morocco	African	238	225	57	204	172	41	Age, sex, ethnicity	**520/417**
Cauchi[14]d	2008	Israel	Asian	208	236	69	229	201	45	Ethnicity	**513/475**
Liu Y[15]	2008	China	Asian	394	800	506	471	956	457	Ethnicity	**1700/1884**
Ng [16]	2008	China,Korea	Asian	486	1054	742	698	1338	701	Continent	**2282/2737**
Horikawa[17]	2008	Japan	Asian	442	876	537	438	818	330	Ethnicity	**1855/1856**
Rong[18]	2009	India	Asian	610	547	178	820	744	184	Ethnicity	**1335/1748**
Takeuchi[19]	2009	Japan	Asian	387	773	463	467	729	310	Sex, ethnicity	**1104/1004**
Tabara[20]	2009	Japan	Asian	119	217	155	102	217	78	Age, sex, ethnicity	**491/397**
Xu M [12]	2010	China	Asian	16	30	21	154	338	164	Sex, ethnicity	**67/656**
Chistiakov [9]	2011	Russia	Caucasian	361	322	82	398	311	57	Age, sex, BMI,ethnicity	**765/766**
Lu F [21]	2012	China	Asian	570	1423	910	802	1615	847	Sex, ethnicity	**2903/3264**
Li W [10]	2013	China	Asian	114	260	160	115	225	113	Sex, ethnicity	**534/453**

Abbreviations: T2DM: type 2 diabetes mellitus; BMI: body mass index; Polymerase chain reaction-restriction fragment length polymorphism genotyping method and Case-control study design were adopted in the above studies.

under recessive genetic model, a significant association was found between them (OR: 1.420, 95% CI: 1.040–1.940, $P = 0.03$). (Table 2, Figure 1–5).

There was significant heterogeneity in the Asian subgroup under all of the genetic models ($P < 0.05$), while the heterogeneity did not exist under all of the genetic models in the Caucasian or African subgroup ($P > 0.05$). In order to explore the heterogeneity source, subsequent meta-regression was performed in the Asian population. Under the allelic, recessive, and homozygous genetic models, the GG genotype number of T2DM group (GG1) was verified to be the main confounding factor to explain the heterogeneity source ($P < 0.05$).

According to the GG genotype of T2DM group, the Asian population was separated into two subgroups. The studies with GG1 > 200 were grouped to subgroup 1 and the residual studies with GG1 < 200 belonged to subgroup 2. In the following subgroup analysis stratified by GG1, significant increased T2DM risk was only detected in the subgroup 1 (allelic: OR: 1.240, 95% CI: 1.180–1.290, $P = 2.85 \times 10^{-22}$; recessive: OR: 1.670, 95% CI: 1.510–1.840, $P = 4.52 \times 10^{-24}$; homozygous: OR: 1.511, 95% CI: 1.407–1.624, $P = 7.42 \times 10^{-27}$). Under the recessive genetic model, a significant association was detected in subgroup 2 (OR: 1.470, 95% CI: 1.030–2.100, P = 0.03). Nevertheless, no significant association was detected in subgroup 2 under the allelic or homozygous genetic model (allelic: OR: 1.120, 95% CI: 0.970–1.300, P = 0.13; homozygous: OR: 1.272, 95% CI: 0.938–1.725, P = 0.121). Moreover, the heterogeneity was distinctly lower in subgroup 1 than that in the whole population (allelic: $P_{heterogeneity} = 0.23$, $I^2 = 27.4\%$; recessive: $P_{heterogeneity} = 0.03$, $I^2 = 58.7\%$; homozygous: $P_{heterogeneity} = 0.234$, $I^2 = 26.7\%$), while in subgroup 2, the heterogeneity still existed (allelic: $P_{heterogeneity} = 0.0004$, $I^2 = 78.0\%$; recessive: $P_{heterogeneity} < 0.00001$, $I^2 = 87.0\%$; homozygous: $P_{heterogeneity} < 0.00001$, $I^2 = 77.6\%$).

Under the dominant and heterozygous genetic models, AA genotype number of control group (AA0) was suggested to be the main heterogeneity source ($P < 0.05$). According to AA0, the Asian population was divided into two subgroups. Subgroup 1 was defined as AA0 > 300 and subgroup 2 was denoted as AA0 < 300. In the following subgroup analysis stratified by AA0, there was a significant association between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM in subgroup 1 (dominant: OR: 1.229, 95% CI: 1.123–1.346, $P = 8.20 \times 10^{-6}$; heterozygous: OR: 1.114, 95% CI: 1.022–1.215, P = 0.014), but no significant association between them was found in subgroup 2 (dominant: OR: 1.088, 95% CI: 0.886–1.335, P = 0.421; heterozygous: OR: 1.010, 95% CI: 0.839–1.217, P = 0.914). Although the heterogeneity still existed in subgroup 2 under the two genetic models (dominant: $P_{heterogeneity} = 0.004$, $I^2 = 70.7\%$; heterozygous: $P_{heterogeneity} = 0.028$, $I^2 = 60.2\%$), it was reduced and even did not exist any longer in subgroup 1 (dominant: $P_{heterogeneity} = 0.033$, $I^2 = 58.8\%$; heterozygous: $P_{heterogeneity} = 0.086$, $I^2 = 48.2\%$). (Table 3).

Bias diagnostics. The publication bias among the individual studies was evaluated by funnel plot and Egger's test. There was no visual publication bias in the Begg's funnel plot (Figure 6). There was no significant difference in the Egger's test yet, which suggested that no publication bias was detected in the current meta-analysis by using recessive genetic model ($T = -0.29$, $P = 0.777$). As no duplicate publications were included in the meta-analysis and every included individual study was a case-control study, there was no sample overlap in the cases or controls. In addition, as the controls data in each individual study were originally collected by the authors themselves and not cited from other studies, the samples in each of the studies were entirely independent and could not cause the results to be biased.

Discussion

In the current meta-analysis, a significant association was detected in the whole population between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM under allelic (OR: 1.18), recessive (OR: 1.51), dominant (OR: 1.175), homozygous (OR: 1.40), and heterozygous genetic models (OR: 1.101). In the subgroup analysis, there was a significant association in Caucasian and Asian subgroups ($P < 0.05$), while no significant association was detected in African subgroup (P

Table 2 | Summary of meta-analysis of association of *CDKAL1* gene rs7756992 A/G gene polymorphism and type 2 diabetes mellitus (T2DM)

Genetic model	Pooled OR (95% CI)	Z value	P value	Study number	T2DM size	control size	$P_{heterogeneity(I2\%)}$
Allelic genetic model	1.180(1.130–1.230)	7.68	1.60×10^{-14}*	21	26120	36447	0.0001*(61.3%)
Caucasian subgroup	1.220(1.170–1.270)	9.51	5.85×10^{-22}*	7	9005	18372	0.47(0%)
Asian subgroup	1.190(1.110–1.270)	5.09	3.58×10^{-7}*	12	16765	16602	<0.0001* (73.3%)
African subgroup	1.070(0.960–1.190)	1.20	0.23	2	1350	1473	0.70(0%)
Recessive genetic model	1.510(1.380–1.660)	8.70	8.41×10^{-18}*	21	26120	36447	<0.00001* (70.3%)
Caucasian subgroup	1.470(1.340–1.610)	8.15	4.22×10^{-16}*	7	9005	18372	0.88(0%)
Asian subgroup	1.570(1.360–1.800)	6.26	3.85×10^{-10}*	12	16765	16602	<0.00001* (81.9%)
African subgroup	1.420(1.040–1.940)	2.20	0.03*	2	1350	1473	0.19(42.2%)
Dominant genetic model	1.175(1.109–1.246)	5.41	6.30×10^{-8}*	21	26120	36447	0.001* (56.4%)
Caucasian subgroup	1.239(1.176–1.305)	8.02	7.85×10^{-15}*	7	9005	18372	0.439(0%)
Asian subgroup	1.173(1.068–1.289)	3.33	8.68×10^{-4}*	12	16765	16602	<0.00001* (67.2%)
African subgroup	1.008(0.843–1.206)	0.09	0.927	2	1350	1473	0.212(35.8%)
Homozygous genetic model	1.400(1.282–1.530)	7.47	8.02×10^{-14}*	21	26120	36447	<0.00001* (59.9%)
Caucasian subgroup	1.530(1.391–1.684)	8.72	5.81×10^{-18}*	7	9005	18372	0.767(0%)
Asian subgroup	1.404(1.237–1.594)	5.25	1.52×10^{-7}*	12	16765	16602	<0.00001* (70.9%)
African subgroup	1.058(0.840–1.332)	0.48	0.631	2	1350	1473	0.537(0%)
Heterzygous genetic model	1.101(1.040–1.166)	3.31	0.001*	21	26120	36447	0.007* (48.6%)
Caucasian subgroup	1.185(1.122–1.252)	6.04	1.54×10^{-9}*	7	9005	18372	0.574(0%)
Asian subgroup	1.078(0.990–1.174)	1.74	0.083	12	16765	16602	0.012* (54.6%)
African subgroup	0.954(0.788–1.154)	0.49	0.626	2	1350	1473	0.103(62.4%)

*P < 0.05.

Abbreviations: T2DM: type 2 diabetes mellitus; CI: confidence interval; OR: odds ratio; T2DM size: the total number of T2DM cases; control size: the total number of control group; Allelic genetic model: G allele distribution frequency; recessive genetic model: GG vs. AA + AG; Dominant genetic model: AG + GG vs. AA; Homozygous genetic mode: GG vs. AA; Heterzygous genetic model: AG vs. AA; Additive genetic model: total G allele vs. total A allele.

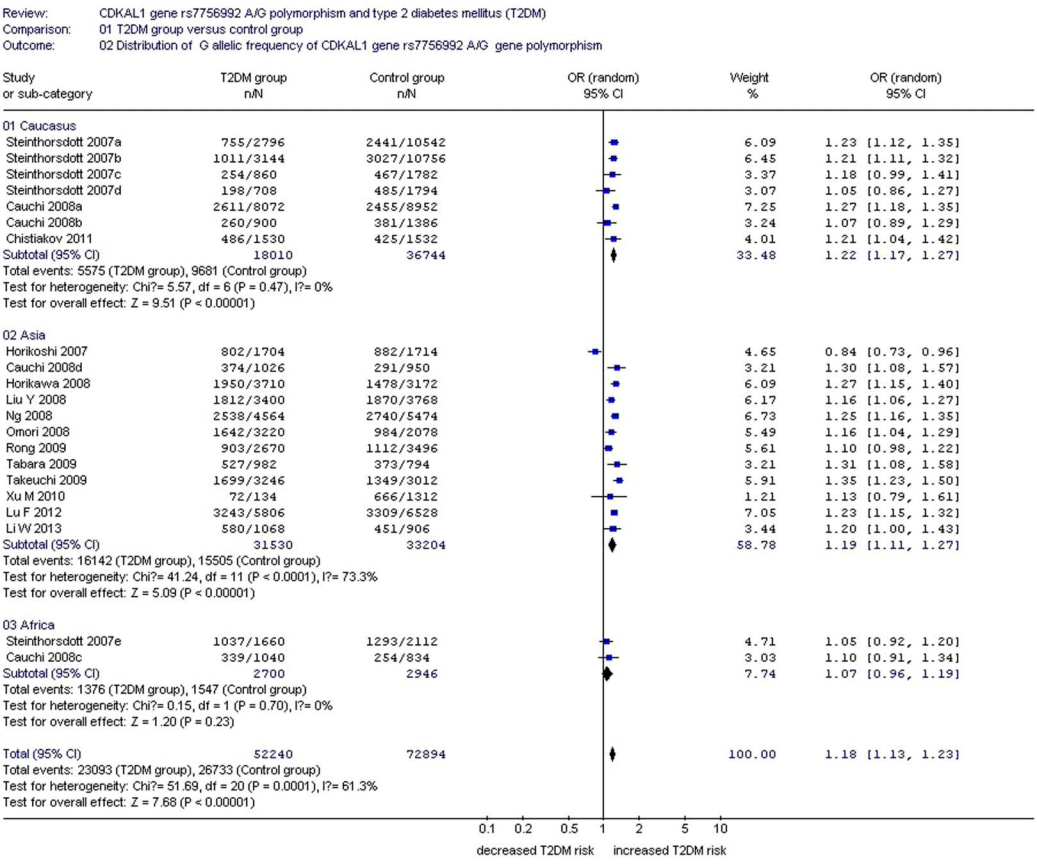

Figure 1 | Forest plot of T2DM associated with *CDKAL1* gene rs7756992 A/G polymorphism under an allelic genetic model (distribution of G allelic frequency of *CDKAL1* rs7756992 gene).

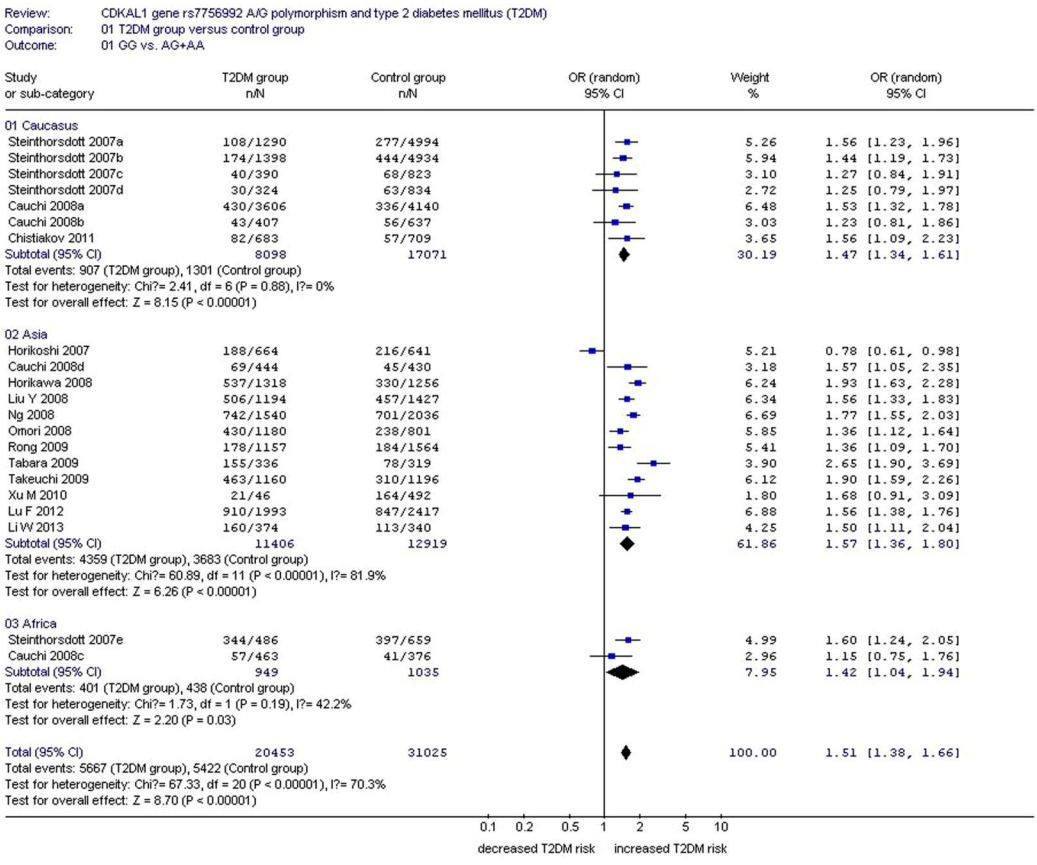

Figure 2 | Forest plot of T2DM associated with *CDKAL1* gene rs7756992 A/G polymorphism under a recessive genetic model (GG vs. AA + AG).

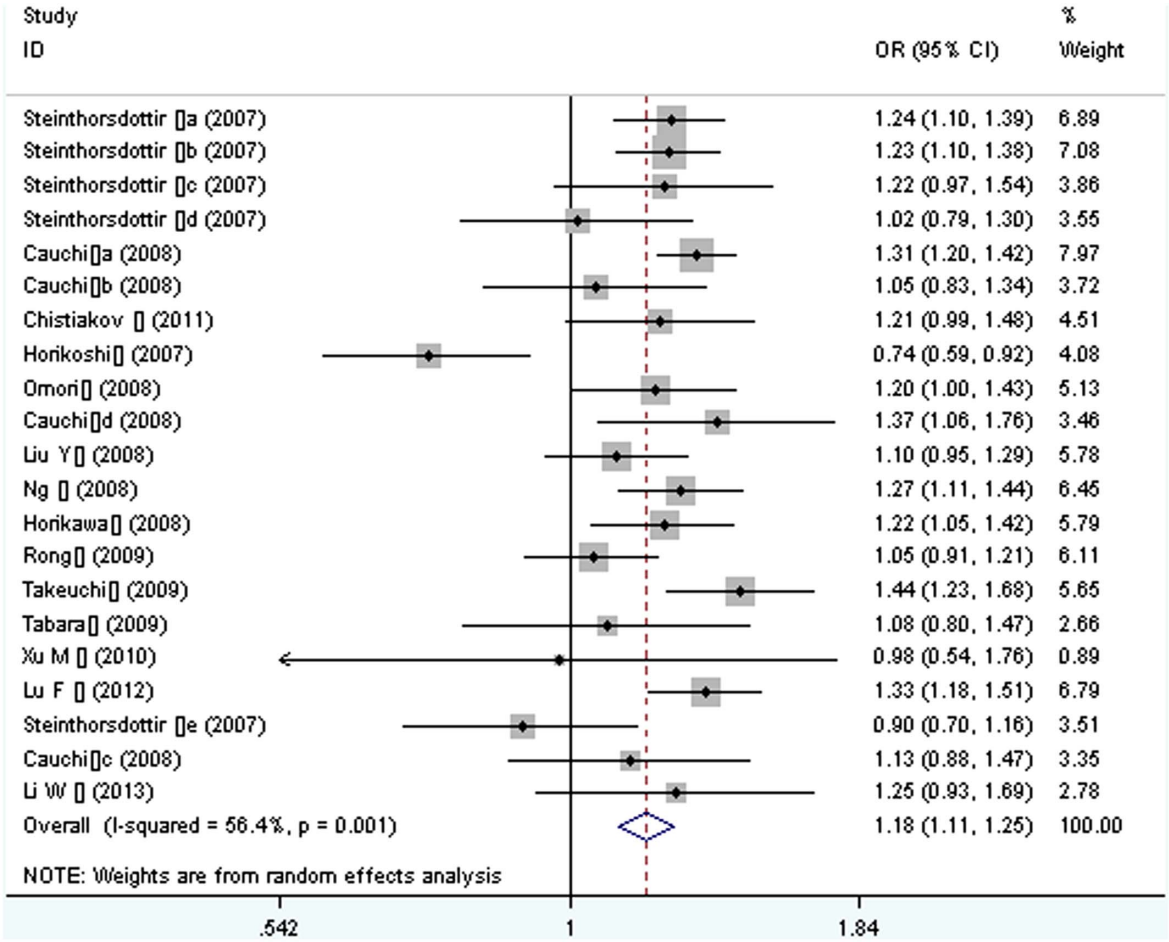

Figure 3 | Forest plot of T2DM associated with *CDKAL1* gene rs7756992 A/G polymorphism under a dominant genetic model (AG + GG vs. AA).

> 0.05). In conclusion, it was indicated that the G allele of *CDKAL1* gene rs7756992 A/G polymorphism might increase the T2DM risk, except in the African population. Moreover, the results in the whole population and Caucasian subgroup were genome-wide significant under most of the genetic models ($P < 8.0 \times 10^{-7}$). In Asian subgroup, the results reached the genome-wide significant threshold under allelic, recessive and homozygous genetic models. The negative results in the African population was perhaps not only associated with the different ethnicity, but also associated with the small sample size, because only two researches with 1350 T2DM subjects were included in this subgroup. In comparison to 9005 and 16,765 for the Caucasian and Asian studies respectively, the sample size1350 for the African studies was too small. Hence, the conclusion needs to be further verified by more and more studies with larger sample size in the African subgroup in the future.

In consideration of the significant heterogeneity in the Asian populations, the meta-regression has been performed. The confounding factor GG1 was confirmed to be the main heterogeneity source under the allelic, recessive, and homozygous genetic models. AA0 was considered as the main heterogeneity source under the dominant and heterogeneity genetic models. In the subgroup analysis stratified by the two confounding factors under the five genetic models, the larger the confounding factors number (the subgroup in GG1 > 200, AA0 > 300), the smaller the heterogeneity (allelic: $I^2 = 27.4\%$; recessive: $I^2 = 58.7\%$; dominant: $I^2 = 58.8\%$; homozygous: $I^2 = 26.7\%$; and heterozygous: $I^2 = 48.2\%$), the stronger the association between *CDKAL1* gene rs7756992 A/G polymorphism and T2DM risk (allelic: OR 1.240; recessive: OR 1.670; dominant: OR 1.229; homozygous: OR 1.511; heterozygous: OR 1.114). By contrast, the smaller the confounding factors number (the subgroup in GG1 < 200, AA0 < 300), the larger the heterogeneity (allelic: $I^2 = 78.0\%$; recessive: $I^2 = 86.9\%$; dominant: $I^2 = 70.7\%$; homozygous: $I^2 = 77.6\%$; and heterozygous: $I^2 = 60.2\%$), the weaker the association between them (allelic: OR 1.120; recessive: OR 1.470; dominant: OR 1.088; homozygous: OR 1.272; heterozygous: OR 1.010). It suggested that the larger sample size could reduce the heterogeneity between the individual studies and the research sample size needs to be further expanded in the future.

Cyclin-dependent kinase 5 (CDK5) is a serine/threonine protein kinase. The CDK5 is activated by producing CDK5/p35 compounds in the pancreatic tissue, thus the β cells are degenerated and the insulin secretion is inhibited, especially in the high glucose condition[22]. In 2006, Ubeda et al found that in the high glucose internal environment, the CDK5 overactivity could decrease the insulin release rate and reduce the insulin production and restrain the insulin gene expression. They found that inhibition of CDK5 activity could protect pancreatic cells from glucotoxity[23].

CDKAL1 is highly expressed in the human pancreas, skeletal muscle, and brain tissue and can specially inhibit CDK5 activity[24]. In 2010, Ohara-Imaizumi et al found that CDKAL1 controls first-phase insulin exocytosis in β cells by facilitating ATP generation, K (ATP) channel responsiveness and the subsequent activity of Ca (2+) channels through pathways other than CDK5-mediated regulation.*CDKAL1* gene rs7756992 A/G mutation probably leads to the inhibition effect loss on CDK5, thus the T2DM risk is increased[25]. However, the exact underlying mechanism of *CDKAL1* gene mutation changing the insulin secretion pattern are still unclear and need to be clarified in the further researches.

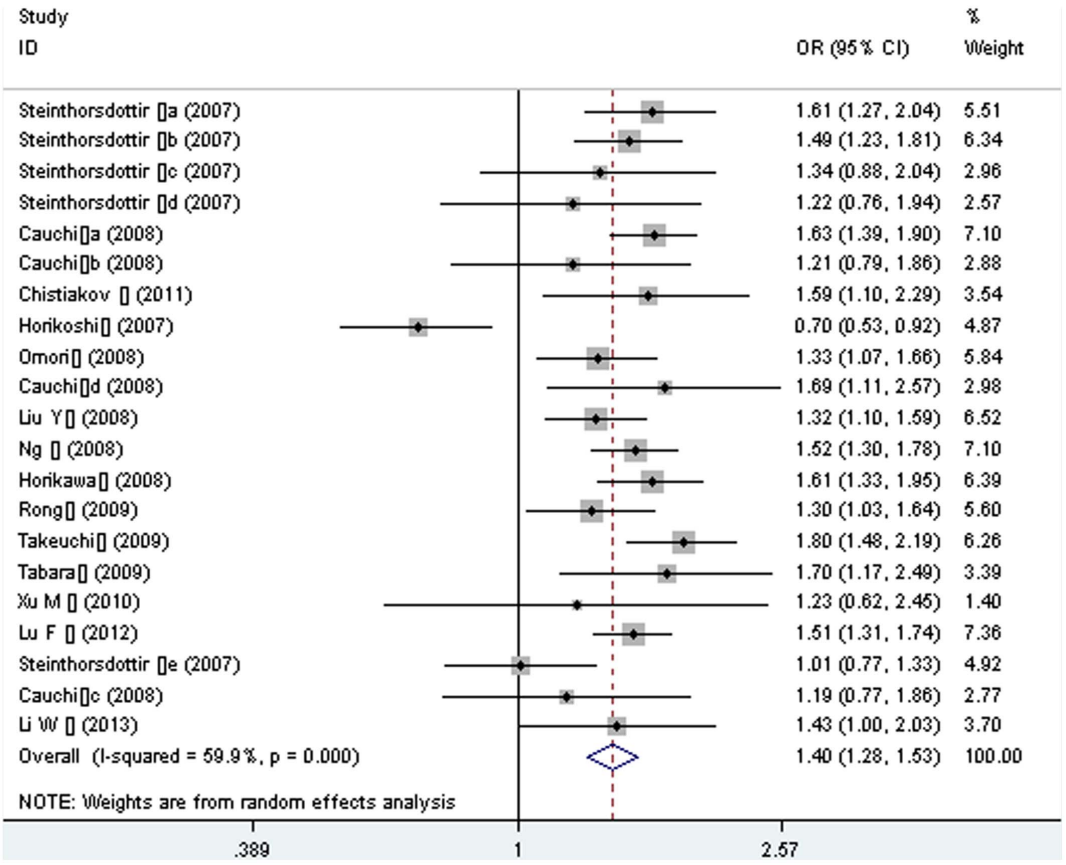

Figure 4 | Forest plot of T2DM associated with *CDKAL1* gene rs7756992 A/G polymorphism under a homozygous genetic model (GG vs. AA).

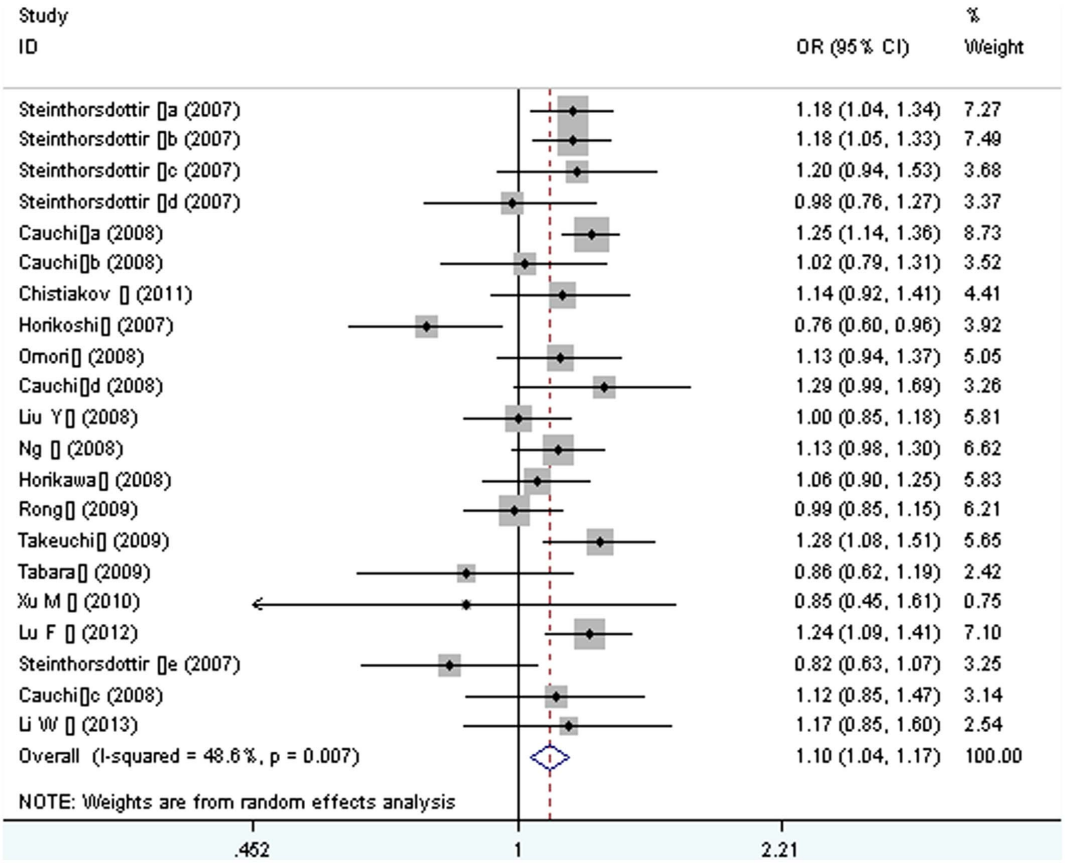

Figure 5 | Forest plot of T2DM associated with *CDKAL1* gene rs7756992 A/G polymorphism under a heterozygous genetic model (AG vs. AA).

Table 3 | Summary of meta-analysis of association of *CDKAL1* gene rs7756992 A/G gene polymorphism and type 2 diabetes mellitus (T2DM) in the Asian subgroup

Genetic model	Pooled OR (95% CI)	Z value	P value	Study number	T2DM size	control size	$P_{heterogeneity(I2\%)}$
Allelic genetic model	1.190(1.110–1.270)	5.09	3.58×10^{-7}*	12	16765	16602	<0.0001* (73.3%)
Subgroup 1:GG1 > 200	1.240(1.180–1.290)	9.70	2.85×10^{-22}*	6	11973	12016	0.23(27.4%)
Subgroup 2:GG1 < 200	1.120(0.970–1.300)	1.53	0.13	6	3792	4586	0.0004* (78.0%)
Recessive genetic model	1.570(1.360–1.800)	6.26	3.85×10^{-10}*	12	16765	16602	<0.00001* (81.9%)
Subgroup 1:GG1 > 200	1.670(1.510–1.840)	10.16	4.52×10^{-24}*	6	11973	12016	0.03* (58.7%)
Subgroup 2:GG1 < 200	1.470(1.030–2.100)	2.12	0.03*	6	3792	4586	<0.00001* (86.9%)
Dominant genetic model	1.173(1.068–1.289)	3.33	8.68×10^{-4}*	12	16765	16602	<0.00001* (67.2%)
Subgroup 1:AA0 > 300	1.229(1.123–1.346)	4.46	8.20×10^{-6}*	6	12698	12725	0.033* (58.8%)
Subgroup 2::AA0 < 300	1.088(0.886–1.335)	0.81	0.421	6	4067	3877	0.004* (70.7%)
Homozygous genetic model	1.404(1.237–1.594)	5.25	1.52×10^{-7}*	12	16765	16602	<0.00001* (70.9%)
Subgroup 1:GG1 > 200	1.511(1.407–1.624)	11.30	7.42×10^{-27}*	6	11973	12016	0.234(26.7%)
Subgroup 2:GG1 < 200	1.272(0.938–1.725)	1.55	0.121	6	3792	4586	<0.00001* (77.6%)
Heterzygous genetic model	1.078(0.990–1.174)	1.74	0.083	12	16765	16602	0.012* (54.6%)
Subgroup 1:AA0 > 300	1.114(1.022–1.215)	2.46	0.014*	6	12698	12725	0.086(48.2%)
Subgroup 2::AA0 < 300	1.010(0.839–1.217)	0.11	0.914	6	4067	3877	0.028* (60.2%)

*P < 0.05.
Abbreviations: T2DM: type 2 diabetes mellitus; CI: confidence interval; OR: odds ratio; T2DM size: the total number of T2DM cases; control size: the total number of control group; Allelic genetic model: G allele distribution frequency; recessive genetic model: GG vs. AA + AG; Dominant genetic model: AG + GG vs. AA; Homozygous genetic mode: GG vs. AA; Heterzygous genetic model: AG vs. AA; Additive genetic model. total G allele vs. total A allele.

No similar meta-analysis on the association of T2DM with *CDKAL1* gene rs7756992 A/G polymorphism has been found internationally so far. Some limitations still existed in the present meta-analysis. Large-scale researches on the association of T2DM with *CDKAL1* gene rs7756992 A/G polymorphism are still inadequate. The serum CDKAL1 level was influenced not only by the *CDKAL1* gene rs7756992 A/G polymorphism, but also by other gene polymorphism as rs7754840 G/C polymorphism, and unscientific dietary habits[20]. As the heredity model of T2DM is multiple gene inheritance which means that many micro-effect genes produce a general effect and leads to T2DM, other genes polymorphisms might be predisposed to T2DM risk[26].

Finally, *CDKAL1* gene rs7756992 A/G polymorphism was significantly associated with T2DM susceptibility, particularly in the Caucasian and Asian population. The persons with the G allele of *CDKAL1* gene rs7756992 A/G polymorphism might be predisposed to T2DM. This conclusion might guide us to formulate new T2DM therapy strategy. Taken account the above limitations, more studies on the association of *CDKAL1* gene rs7756992 A/G polymorphism and T2DM are needed to be carried out to further clarify the conclusion in the future.

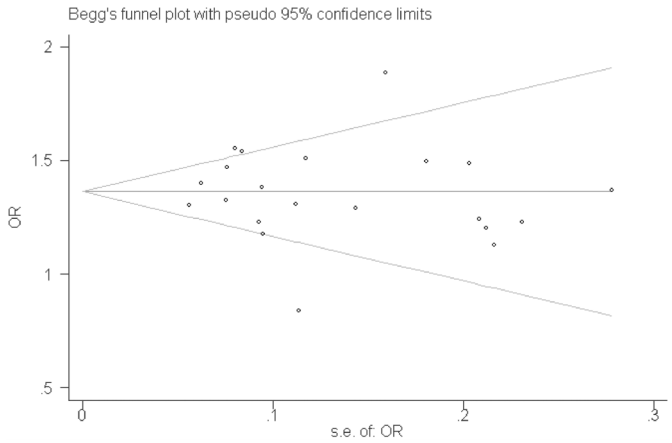

Figure 6 | Begg's funnel plot for studies of the association of T2DM associated with *CDKAL1* gene rs7756992 A/G polymorphism under a recessive genetic model (GG vs. AA + AG). The horizontal and vertical axis correspond to the OR and confidence limits. OR: odds ratio; SE: standard error.

Methods

Publication search and inclusion criteria. The electronic databases including PubMed, Web of Science, Embase, China Biological Medicine Database, and China National Knowledge Infrastructure were searched by using the words as "*CDKAL1*", "rs7756992", "polymorphism" and "type 2 diabetes mellitus". The last research was updated on October 15, 2013 with the publication years ranging from 2007 to 2013.

The following major criteria should be met by the included studies. The *CDKAL1* gene rs7756992 A/G polymorphism and T2DM.were evaluated. T2DM was diagnosed by the American Diabetes Association fasting plasma criteria (2005). The fasting plasma glucose level was no less than 7.0 mmol/L or the 2 h plasma glucose of oral glucose tolerance test was no less than 11.1 mmol/L. Furthermore, no genetic relationship existed between the subjects in the individual studies. Only the data drawn from officially published manuscripts with case-control or cohort studies could be adopted. The HWE should be followed by genotype member of the control group in the individual studies.

Data extraction. The informed consent was obtained from all subjects. The studies data was extracted in the light of a standard protocol. Three researchers carried out the meta-analysis; two of whom searched out the studies duplicately, and the third researcher acted as the arbitrator to resolve the conflict between the two researchers and come to an agreement. The current meta-analysis rejected the studies that did not follow the selection criteria, that were repeatedly published, or that provided insufficient data. If similar data was rooted in different manuscripts by the same authorship, the data was only once adopted. The following items including the first author's name, publication year, region, continents, number of genotypes, matching criteria and total number of cases and controls should be shown in the extracted data.

Statistical analyses. Five genetic models as the allelic (G allele distribution frequency), recessive (GG vs. AA + AG), dominant (GG + AG vs. AA), homozygous (GG vs. AA), and heterozygous (AG vs. AA) genetic models were used in the present meta-analysis. The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to compare the association of *CDKAL1* gene rs7756992 A/G polymorphism and T2DM. The heterogeneity among the studies was calculated by Chi-square-based Q-tests with significance set at $P < 0.05$ level[27]. If heterogeneity existed, the random-effect model (DerSimonian and Laird method) would be used[28]. Or else, the fixed-effect model was adopted (the Mantel–Haenszel method)[29]. Z test was used to estimate the pooled OR with significance set at $P < 0.05$ level.

The HWE was assessed by using Fisher's exact test with significance set at $P < 0.05$ level. The potential publication bias was estimated by adopting the funnel plot. The funnel plot symmetry was evaluated by using Egger's linear regression test on the natural logarithm scale of the OR and significance was set at $P < 0.05$ level[30]. The statistical analyses were performed by Stata 12.0 software (StataCorp, College Station, TX, USA).

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Author contributions

Conceived and designed the experiments: Y.L. Performed the experiments: Y.L., L.W. Analyzed the data: Y.L., C.Z., Z.Y. Contributed reagents/material/analysis tools: Y.L., J.X. Wrote the manuscript: Y.L., Y.Q. Reference collection and data management: Y.L., X.W. Statistical analyses and paper writing: Y.L., X.L. Study design: Y.L., A.C.

Additional information

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