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Lack of association between BDNF rs6265 polymorphism and risk of type 2 diabetes

A protocol for meta-analysis and trial sequential analysis

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) rs6265 polymorphism has been previously suggested to be associated with the susceptibility of type 2 diabetes mellitus (T2DM), but results remained controversial. We aim to provide a more reliable conclusion about the association between BDNF rs6265 polymorphism and T2DM risk by using a meta-analysis.

Methods: Electronic databases such as Pubmed, Embase, CNKI, and Wanfang were searched for relevant articles published up to May 06, 2020. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the strength of the associations. Subgroup analysis was carried out according to source of controls and quality score of included studies. A trial sequential analysis was conducted to reduce the risk of type I error.

Results: A total of 8 case-control studies (7 conducted in China) with 1576 T2DM patients and 1866 controls were included. Overall, our results indicated no significant association between BDNF rs6265 polymorphism and T2DM risk with the random-effects model (allele model: pooled OR = 1.14, 95% CI = 0.79–1.65, homozygote model: pooled OR = 1.13, 95% CI = 0.57–2.21, heterozygote model: pooled OR = 1.07, 95% CI = 0.78–1.48, dominant model: pooled OR = 1.14, 95% CI = 0.74–1.75 and recessive model: pooled OR = 1.10, 95% CI = 0.67–1.80). Subgroup analysis by source of controls and quality score also showed no significant association between BDNF rs6265 polymorphism and T2DM risk. Trial sequential analysis results confirmed the null association and further studies were unnecessary.

Conclusion: This meta-analysis study indicated that no significant association between BDNF rs6265 polymorphism and T2DM risk.

Abbreviations: BDNF = brain-derived neurotrophic factor, CIs = confidence intervals, HWE = Hardy–Weinberg equilibrium, ORs = odds ratios, PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-analyses, RIS = required information size, T2DM = type 2 diabetes mellitus, TSA = trial sequential analysis.

Keywords: brain-derived neurotrophic factor, meta-analysis, polymorphism, type 2 diabetes mellitus

1. Introduction

Type 2 diabetes mellitus (T2DM) is an important cause of many serious life-threatening health problems, such as cancers, cardiovascular diseases, and all-cause mortality,[1] resulting in higher medical care costs and reduced quality of life.[2,3] About 1 in 11 adults have diabetes mellitus, and 90% of them have T2DM.[4] The International Diabetes Federation estimated that there were 451 million adults with diabetes worldwide in 2017, and these figures were expected to increase to 693 million by 2045.[5] The etiology of T2DM is known to be complex, and the determinants of T2DM consist of a matrix of genetic, epigenetic, lifestyle factors, polypharmacy use, cardiometabolic risk factors (such as arterial hypertension, obesity or the metabolic syndrome), oxidative stress, inflammation, etc. [4,6–8] The main drivers of the global epidemic of T2DM included the rise in obesity, a sedentary lifestyle, and energy-dense diets.[4] Genetic loci related with obesity has been identified which may associate with T2DM risk.[4]

Brain-derived neurotrophic factor (BDNF) is a protein family abundantly expressed within the brain that plays an important role in maturation, synaptic connection, neuronal repair, and plasticity in the central nervous system.[9] BDNF is highly expressed in the hypothalamus, where this neurotrophic factor has an important role in regulating metabolism of appetite.[9] It has been established that hypothalamic reduction of BDNF modulated energy homeostasis affecting food intake and promoting an anorectic signal,[10] thereby influencing energy balance and weight that increased T2DM risk. In addition, BDNF produced by monocytes was also related with inflammatory cytokines such as high-sensitivity c-reactive protein, playing a possible role in the progression of T2DM.[11]

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The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Genetic variations in BDNF have also been shown to affect the function of BDNF protein, and rs6265 polymorphism is the most extensively studied one. It is a nonsynonymous SNP resulting in a valine to methionine substitution at codon 66 in the BDNF prodomain, which is thought to interfere with intracellular trafficking and activity-dependent secretion of the BDNF protein.[2,5] To date, a number of studies had been performed to assess the association between BDNF rs6265 polymorphism and susceptibility to T2DM.[12–19] However, the results remained to be inconsistent and ambiguous. As the statistical power of an individual study may be too weak to detect association between rs6265 polymorphism and T2DM risk, a meta-analysis pooling data from all published studies may be more convincing to determine whether or not BDNF rs6265 polymorphism is a risk factor for the development of T2DM.

Hence, we aimed to conduct a meta-analysis with all available case-control studies to obtain more precise evidence for the association between BDNF rs6265 polymorphism and T2DM risk.

2. Materials and methods

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[20]

2.1. Literature search

Eligible studies investigating the association between BDNF rs6265 polymorphism and T2DM risk were searched from the public electronic databases, including PubMed, Embase, CNKI (https://www.cnki.net/), and Wanfang (http://www.wanfangdata.com.cn/index.html) with searching up to May 6, 2020, but no lower date limit was used. The following terms were utilized to identify potential related articles: (Polymorphism, Genetic[mesh] or polymorphism* or variant* or genotype*) and (BDNF or "brain derived neurotrophic factor") and (Diabetes Mellitus[MESH] or diabetes), without any restriction of publication language. Concurrently, the reference lists of included articles and reviews were manually searched to find additional relevant studies. Related articles generated by PubMed and Google scholar (https://scholar.google.com/) were also searched.

2.2. Selection criteria

After removing the duplicates by Endnote, 2 authors (XX and CD) independently reviewed titles and abstracts, as well as the full-text of the articles identified to determine their eligibility based on selection criteria. For inclusion in this meta-analysis, the eligible articles should meet the following criteria:

(1) case-control study design;
(2) evaluating the BDNF rs6265 polymorphism;
(3) the outcome was T2DM; and
(4) providing the number of individual genotypes for BDNF rs6265 polymorphism in T2DM cases and controls, respectively.

The exclusion criteria were as follows:

(1) outcomes were other types of diabetes, such as type 1 diabetes and gestational diabetes;
(2) abstract, comment, review, and editorial; and
(3) no original data of the genotype frequencies for both cases and controls.

If data were duplicated or shared in more than one article, only the most recent or complete article was included. No ethical review is needed in this study.

2.3. Data extraction and quality assessment

Data from all eligible studies were extracted by the same 2 authors independently according to the selection criteria listed above. The following data were collected from each study using a standardized form: first author's name, year of publication, country in which the study was conducted, study period, sample size, mean age, and proportion of males for cases and controls, genotyping method, genotype frequency of the BDNF rs6265 polymorphism of cases and controls, matching variables between cases and controls (if available). The reviewers resolved disagreements through discussion. For studies without enough information, corresponding authors were contacted for further information by E-mail, if possible.

The quality of each study was assessed using the Newcastle–Ottawa Scale.[21] Newcastle–Ottawa Scale has been developed for both cohort and case-control studies. For case-control studies, it contains three dimensions: selection (4 scores), comparability (2 scores), and exposure (3 scores). Accumulated score ranges from 0 to 9 points, with a score ≥ 7 indicating higher quality.

2.4. Statistical analysis

The departure of frequencies of BDNF rs6265 polymorphism from expectation under Hardy–Weinberg equilibrium (HWE) in controls was assessed by chi-square test, which can compare actual frequencies of genotype with expected value.

Odds ratios (ORs) with their 95% confidence intervals (CIs) were used to assess the strength under each genic model in overall and stratified groups to appraise the associations between the BDNF rs6265 polymorphism and T2DM risk, and Z test was used to access the significance of pooled ORs. Five models based on the allele frequency in case group and control group were used, including allele contrast model (G vs T), homozygote comparison model (G/G vs T/T), heterozygote comparison model (G/T vs T/T), dominant comparison model (G/G+G/T vs T/T), and recessive comparison model (G/G vs G/T+T/T). The subgroups were stratified by source of control and quality of the included studies.

The between-study heterogeneity was evaluated using the inconsistency index (I^2 statistic), and I^2 values of 25%, 50%, and 75% were corresponding to cut-o-points for mild, moderate, and extensive heterogeneity, respectively.[22] Due to significant between-study heterogeneity existed in this study, the random-effects model (the DerSimonian and Laird method) was chosen a priori,[23] and it was also considered as more conservative than the fixed-effects model.[24]

We also did sensitivity analyses with excluding 1 article conducted in Denmark[19] and 2 studies deviated to HWE.[14,18] Influence analysis was used to assess the stability of the results, with a single study in the meta-analysis excluding each time to reflect the influence of a single study on the summary results.[25] Furthermore, Egger regression asymmetry test and Begg funnel plot were obtained to evaluate whether there was a significant publication bias.[26]

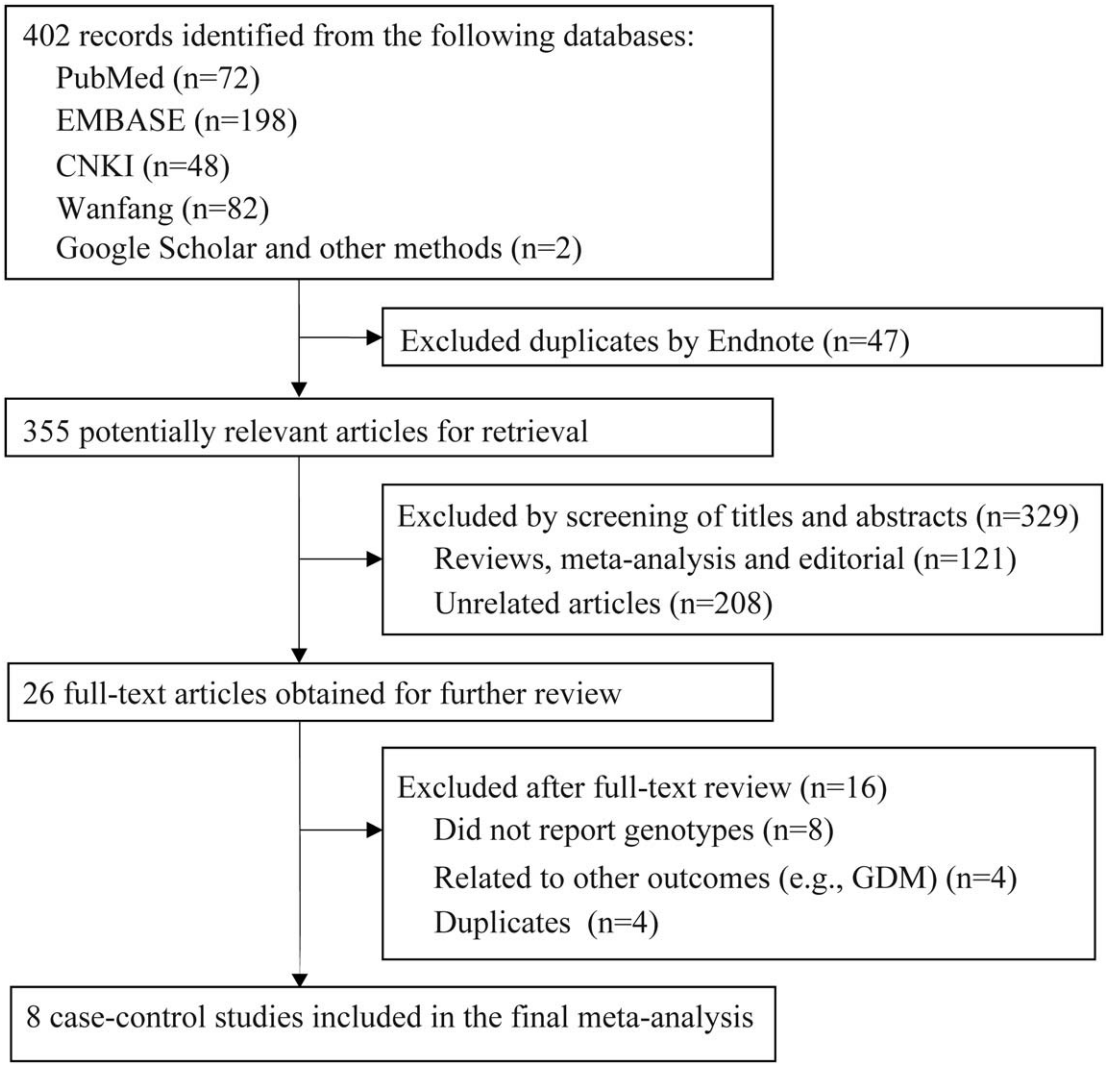

Figure 1. Flow chart of study selection.

All of the statistical analyses were performed using Stata version 12.0 (Stata Corporation, College Station, TX, USA). A 2-side P value <0.05 was considered as a statistically significant finding.

2.5. Trial sequential analysis

Cumulative meta-analyses are prone to type I and type II errors because of repeated testing of significance as trial data accumulate. Trial sequential analysis (TSA) was conducted to avoid type I error rate (α) and estimate the required sample information.[27] The required information size (RIS) was conducted by anticipating a 30% relative risk reduction for efficacy outcome, an overall 5% of a type I error, and 20% of the type II error (a statistical test power of 80%).[27] TSA was used to calculate the required number of participants (RIS), and constructed a sequential monitoring boundary to determine whether a trial could be terminated early. A cumulative Z-curve was constructed using a random-effects model, and if it crossed the trial sequential monitoring boundary suggested that the statistical evidence was conclusive. We used TSA software version 0.9 (beta) (Copenhagen Trial Unit, Copenhagen, Denmark) to conduct these analyses.

3. Results

3.1. Study selection process and characteristics

Overall, 402 articles were identified after the electronic and manual search (Fig. 1). After excluding duplicates (n=47) and unrelated articles (n=329), 26 studies were eligible for further full-text review. Among these articles, 16 articles were excluded due to:

(1) lack of information for genotype (n=8);
(2) related to other outcomes, such as GDM (n=4); and
(3) duplicate articles (n=4).

Finally, 8 articles with a total of 1576 T2DM cases and 1866 controls were included in the present meta-analysis.

Table 1 presents the main characteristics of these studies. These studies were published between 2007 and 2019. Except for 1 study conducted in Denmark,[19] all studies were

Table 1

The main features of eligible studies.

First author [ref.]	Year	Country	T2DM group			Control group			Control source	Genotyping method	Matching factors	NOS quality score
			Sample size	Age, yr	Men (%)	Sample size	Age, yr	Men (%)				
Chen Y[12]	2019	China	418	56.78±13. 8	51.9	422	56.1±11.6	55.0	Population	Fluorescence-based real-time PCR	—	6
Zhen YF[13]	2018	China	311	54.93±10.73	43.7	346	53.43±9.86	39.9	Population	PCR-RFLP	Sex, age, and education	8
Jin Y[14]	2015	China	72	60.4±4.2	32.6	208	63.5±3.2	55.8	Hospital	PCR-RFLP	—	5
Xu HY[15]	2014	China	160	55.4±3.5	50.0	80	55.1±3.3	51.2	Hospital	PCR-RFLP	—	6
Zhou JX[16]	2014	China	296	53.2±5.5	50.0	70	55.2±6.5	48.6	Hospital	PCR-RFLP	Age, gender	7
Cao Y[17]	2011	China	246	48.95±10.71	46.3	186	38.2±15.86	47.3	Population	Illumina GoldenGate	—	7
Zhou DH[18]	2010	China	144	52.54±11.03	56.9	120	51.57±9.91	59.2	Population	PCR-RFLP	—	6
Krabbe KS[19]	2007	Denmark	96	58.2±1.5	75.0	137	60.3±2.6	67.2	Population	Fluorescence-based real-time PCR	—	7

PCR = polymerase chain reaction, PCR–RFLP = PCR–restriction fragment length polymorphism.

conducted among Chinese. The sample size ranged from 233 to 840. All eligible studies had a moderate or high quality, with an overall score ranging from 5 to 8. Table 2 presents the genotype distributions for the BDNF rs6265 polymorphism of each included study. The genotype distribution of the control group was deviated with HWE in 2 studies.[14,18]

3.2. Quantitative data synthesis

Table 3 and Figure 2 show the quantitative pooled results of the meta-analysis and heterogeneity test. We did not find any statistically significant association between the BDNF rs6265 polymorphism and the risk of T2DM under all genetic models, including the allele model (OR:1.14, 95% CI: 0.79–1.65; $P=.496$), the homozygote model (OR:1.13, 95% CI: 0.57–2.21; $P=.773$), and the heterozygote model (OR:1.07, 95% CI: 0.78–1.48; $P=.677$), the dominant model (OR:1.14, 95% CI: 0.74–1.75; $P=.559$), and the recessive model (OR:1.10, 95% CI: 0.67–1.80; $P=.706$), respectively. Between-studies heterogeneity was significant under all models with I^2 ranging from 70.1% to 91.4%.

Subgroup analyses according to source of control and quality score, sensitivity analyses with excluding 1 article conducted in Denmark [19] and 2 studies deviated to HWE,[14,18] as well as influence analyses, all did not show evidence of significant findings (Table 2).

The Egger regression asymmetry test and Begg funnel plot were applied to evaluate publication bias. The funnel plot appeared symmetrical (Fig. 3), and the P values of Egger test ranged from .286 to .609. These results did not indicate a potential for publication bias.

3.3. Trial sequential analysis results

When we anticipated a 30% relative risk reduction to estimate the maximal sample size (Fig. 4), the effect-size is 5988 participants. As shown in Figure 4, the cumulative Z-curve exceeded the information size (RIS line), and the total number of cases and controls were more than the RIS. However, the cumulative Z-curve did not cross the trial sequential monitoring boundary. Therefore, our nonsignificant results were established and further relevant studies were unnecessary.

4. Discussion

This is the first meta-analysis to summarize the current evidence on the effect of BDNF rs6265 polymorphism on the development of T2DM. Our meta-analysis indicated that null associations between BDNF rs6265 polymorphism and T2DM risk under all genetic models and the TSA analysis further confirmed this finding.

The BDNF gene is located on chromosome 11, band p13.[3] It encodes BDNF protein, a neurotrophin that plays a role in the

Table 2

Brain-derived neurotropic factor rs6265 genotype distributions among T2DM cases and controls of the included studies.

First author	Year	Cases			Controls			MAF	P (HWE)*
		GG	GA	AA	GG	GA	AA		
Chen Y[12]	2019	172	213	33	176	202	44	34.4	.209
Zhen YF[13]	2018	76	165	70	88	183	75	48.1	.27
Jin Y[14]	2015	16	19	37	127	45	36	28.1	<.001
Xu HY[15]	2014	53	68	39	30	35	15	40.6	.405
Zhou JX[16]	2014	26	33	11	103	137	56	42.1	.386
Cao Y[17]	2011	106	179	73	24	98	64	60.8	.153
Zhou DH[18]	2010	22	44	25	59	82	50	47.6	.054
Krabbe KS[19]	2007	61	30	5	88	42	7	20.4	.502

HWE = Hardy-Weinberg equilibrium, MAF = minor allele frequency.

* P value of chi-square test for HWE among controls.

Table 3

Total and stratified analyses of brain-derived neurotropic factor rs6265 polymorphism and T2DM risk.

			Allelic comparison			Homozygote comparison			Heterozygote comparison			Dominant genetic model			Recessive genetic model		
Variables	N*	Cases/ Controls	OR (95%CI)	P†	I^2	OR (95%CI)	P†	I^2	OR(95%CI)	P†	I^2	OR (95%CI)	P†	I^2	OR (95%CI)	P†	I^2
Total	8	1576/1866	1.14 (0.79, 1.65)	.496	91.4	1.13 (0.57, 2.21)	.773	88.6	1.07 (0.78, 1.48)	.677	70.1	1.14 (0.74, 1.75)	.559	85.5	1.10 (0.67, 1.80)	0.706	84.4
Asian only	7	1480/1729	1.16 (0.78, 1.74)	.465	92.6	1.14 (0.55, 2.36)	.732	90.2	1.08 (0.75, 1.56)	.675	74.4	1.16 (0.71, 1.88)	.561	87.6	1.11 (0.65, 1.88)	0.704	86.7
HWE only	6	1413/1467	0.90 (0.71, 1.15)	.414	75.7	0.77 (0.46, 1.3)	.329	75.2	0.90 (0.68, 1.20)	.487	57.1	0.87 (0.63, 1.22)	.423	71.1	0.83 (0.59, 1.15)	0.259	54.5
Control source																	
Population	5	1274/1282	0.92 (0.64, 1.31)	.635	79.8	0.81 (0.38, 1.72)	.586	80.1	0.9 (0.58, 1.4)	.637	66.3	0.88 (0.53, 1.46)	.617	77.4	0.85 (0.55, 1.33)	0.480	58.5
Hospital	3	302/584	1.63 (0.78, 3.4)	.197	96.0	1.84 (0.54, 6.31)	.331	93.7	1.38 (0.84, 2.28)	.207	75.9	1.71 (0.78, 3.77)	.181	91.9	1.55 (0.57, 4.22)	0.395	92.7
Score																	
<7	4	741/901	1.01 (0.88, 1.14)	.946	0.0	0.97 (0.73, 1.29)	.824	0.0	1.06 (0.87, 1.29)	.595	0.0	1.04 (0.86, 1.25)	.712	0.0	0.96 (0.75, 1.23)	0.728	0.0
≥7	4	835/965	1.31 (0.52, 3.27)	.564	96.2	1.30 (0.26, 6.52)	.747	94.9	1.16 (0.51, 2.68)	.721	87.0	1.28 (0.43, 3.84)	.661	93.7	1.28 (0.42, 3.93)	0.665	92.8
Sensitivity analysis																	
Maximal	7	–/–	1.28 (0.90, 1.82)	.169	88.6	1.41 (0.77, 2.55)	.263	82.3	1.19 (0.94, 1.50)	.149	36.3	1.33 (0.92, 1.92)	.129	77.3	1.26 (0.77, 2.05)	0.361	79.6
Minimal	7	–/–	0.94 (0.75, 1.17)	.554	73.5	0.83 (0.52, 1.34)	.449	73.4	0.95 (0.73, 1.25)	.723	55.1	0.93 (0.68, 1.26)	.624	68.9	0.85 (0.64, 1.14)	0.290	49.6

CI = confidence interval, OR = odds ratio.

* Number of comparisons.

† *P*-value of *Z*-test for significant test.

proliferation, differentiation, and fate of neuronal cells, thus regulating plasticity and connectivity in the central nervous system.[3] BDNF has been suggested to be associated with many types of diseases such as depression and anxiety, bipolar disorder,[10] Alzheimer disease,[9] heart diseases,[4] chronic pain,[28] and T2DM.[29] BDNF rs6265 polymorphism is a common single nucleotide polymorphism in BDNF gene (c.196G>A, dbSNP: rs6265) has been identified to cause an amino-acid substitution of valine to methionine at amino-acid residue 66.[2,5] This polymorphism could alter intracellular trafficking and packaging of pro-BDNF, thus regulating secretion of the mature peptide.[2,5] The Met allele of the BDNF rs6265 polymorphism has been suggested to be associated with increased BDNF serum concentrations.[1]

Increasing evidence have been suggested the biologically plausible between BDNF protein or rs6265 polymorphism and T2DM risk. T2DM is significantly related with obesity. In the hypothalamus, this neurotrophic factor has major regulatory role in the control of appetite and metabolism, resulting in inhibition of food intake and increases energy expenditure.[9] Mice that were heterozygous for targeted disruption of BDNF was associated with a 50% reduction in BDNF expression in the hypothalamus, and consumed 47% more food than wild-type mice and are obese.[7] Chronic intracerebroventricular infusion of BDNF treatment attenuated weight gain in rats.[30] Plasma levels of BDNF decreased in humans with type 2 diabetes and obesity and plasma BDNF was inversely associated with fasting plasma glucose.[19] In addition, BDNF expression was associated with chronic inflammatory state, altered circulating inflammatory cytokines, enhanced immune system, and elevated compounds released by platelets, which was also an important process for development of T2DM.[29,31,32]

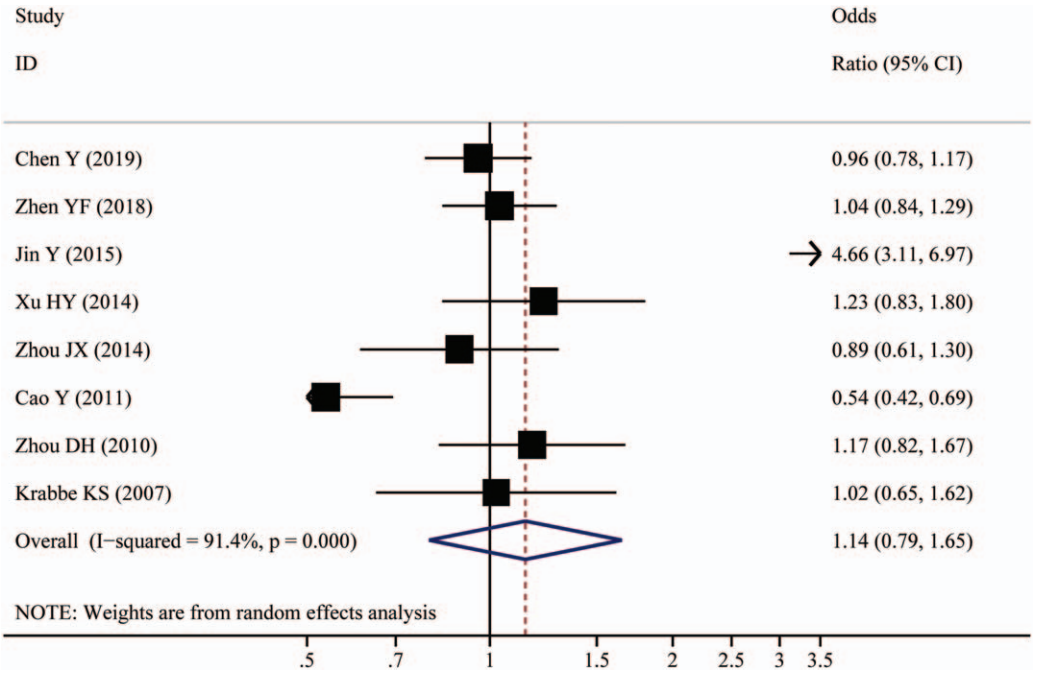

Figure 2. Forest plot of associations between BDNF rs6265 polymorphism and the risk of type 2 diabetes (allelic comparison).

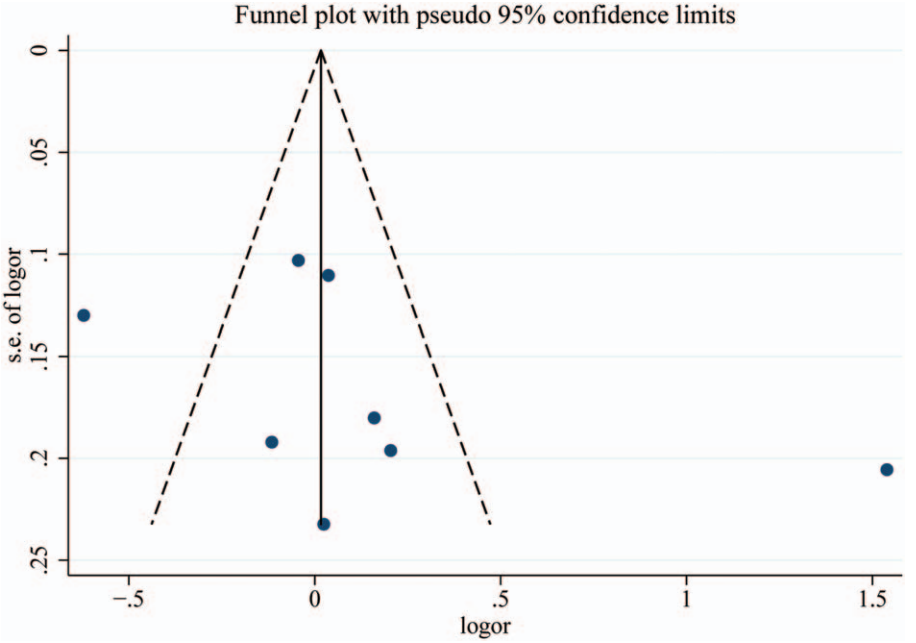

Figure 3. Funnel plot of associations between BDNF rs6265 polymorphism and the risk of type 2 diabetes (allelic comparison).

Many studies have investigated the involvement of BDNF rs6265 polymorphism in the etiology of T2DM, but with conflicting results. Meta-analysis is a powerful tool that could make the conclusion more credible, especially in analyzing conflicting associations with small sample size studies.[33] Besides, TSA was performed to effectively reduce the risk of type I error and assess whether the evidence of our results was reliable.[11] In the present meta-analysis, 8 independent case-control studies, mainly conducted in China (7 studies), with 1576 T2DM patients and 1866 controls were recruited. Our results revealed that null association was detected between BDNF rs6265 polymorphism and the risk of T2DM.

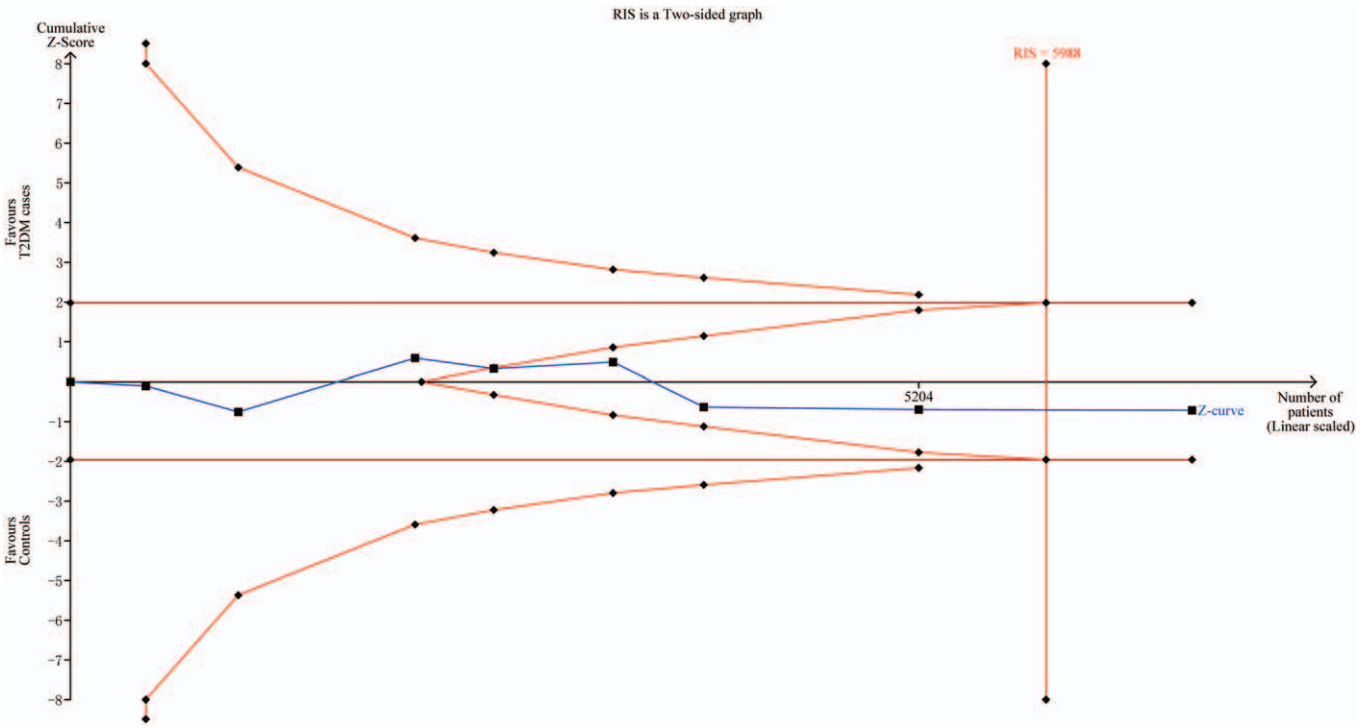

Figure 4. Trial sequential analysis of associations between BDNF rs6265 polymorphism and the risk of type 2 diabetes (allelic comparison).

Subgroup meta-analyses were performed according to different source of controls and different quality score. In the subgroup analysis by source of controls, no significant difference about such association was also observed in population-based or hospital-based controls. Besides, there were healthy population and other disease patients such as depression in the controls included.[14,15] It was likely that different individuals in the control group might have different risk of developing T2DM, thus affecting the quality of the studies. In subgroups for quality score of the included studies, there was also no association between BDNF rs6265 polymorphism and the risk of T2DM for both high- and low-quality studies. Nevertheless, we also did sensitivity analyses after excluding one study conducted in Denmark[19] and 2 studies deviated to HWE,[14,18] the null association persisted.

As we well-known, traditional meta-analysis may lead to type I and type II errors. TSA is a powerful and useful approach that could reduce the risk of type I error by estimation of RIS with an adjusted threshold for statistical significance. Just like interim analyses in a single trial, TSA decides whether additional trials were needed to evaluate for evidence.[11] If the cumulative Z-curve crosses the monitoring boundary or the RIS, it shows firm evidence for such study. Otherwise, additional studies are further needed to reach a consistent conclusion.[27] As shown in our study, the cumulative Z-curve did not cross the trial sequential monitoring boundary but reach the perpendicular line (RIS), which means that, though without significant findings, our results were robust and did not need further studies.

In this meta-analysis, we included comprehensive studies with the large sample size to estimate a null association between BDNF rs6265 polymorphism and T2DM risk, which was confirmed by TSA analysis. However, limitations included in the present should also be taken into consideration. Firstly, the prevalence of T2DM varied significantly among different ethnicities. The majority of studies included were investigated in Chinese population and only 1 study was conducted in Denmark.[19] Therefore, it was impossible for us to conduct ethnic sub-group analysis. Further studies with more data are required to investigate the association in other populations. Secondly, between-studies heterogeneity was significant under all comparisons (I^2: 70.1% ~91.4%). Due to limited number of included studies, we could only conduct subgroup analyses for source of control or quality score to explore the potential source of heterogeneity and no evidence indicated that heterogeneities could be affected by these factors. Influence analyses showed attenuated heterogeneity, but the association still persisted. Thirdly, we could not get useful data about the association between BDNF rs6265 polymorphism and the risk of T2DM in the GWAS database. However, some articles related to BDNF rs6265 polymorphism with T2DM risk were found [34–36] and no association was also observed among these studies. Fourthly, the sample size in each stratified analysis was relatively small, which might potentially limit the statistical power to explore the subgroup interaction. Finally, except for genetic factors, the development of T2DM is closely related to environment, diet, and occupational exposure, etc. Therefore, we need to control these variable factors to achieve more accurate results.

5. Conclusion

In conclusion, the results of this meta-analysis demonstrated that no evidence indicated the association between BDNF rs6265 polymorphism and T2DM risk. Our findings suggested that BDNF rs6265 polymorphism did not serve as a clinical genetic biomarker of T2DM. More importantly, further studies in various ethnic groups are needed to provide more comprehensive understanding of this association.

Author contributions

Data curation: Xian-qiong Xie.
Formal analysis: Xian-qiong Xie.
Methodology: Xian-qiong Xie.
Project administration: Dong-gui Cai.
Writing – original draft: Quan Yang.
Writing – review & editing: Quan Yang, Xian-qiong Xie, Dong-gui Cai.

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