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Association of Brain-Derived Neurotrophic Factor (BDNF) Gene Snps G196A and C270T with Alzheimer's Disease: A Meta-Analysis

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Abstract

Objective: To evaluate the association between diagnosis of Alzheimer's Disease (AD) and brain-derived neurotrophic factor (BDNF) gene polymorphisms 196 G/A and 270 C/T.

Methods: The authors conducted a meta-analysis along with Trial Sequential Analysis (TSA) of BDNF 196 G/A and 270 C/T polymorphisms and AD in all the available case-control studies on the topic published from 2002 to 2016.

Results: Results showed no significant association between BDNF 196 G/A and 270 C/T polymorphisms and AD in overall and ethnicity specific studies.

Conclusion: The present comprehensive meta-analysis suggested that further studies focusing on larger sample-size multi-ethnicity studies with homogeneous AD patients and well-matched controls are needed for future study to confirm the results of the study.

Keywords: BDNF; G196A; C270T; Alzheimer's disease; Polymorphism; Meta-analysis

Introduction

Alzheimer's disease (AD) is a common age-associated neurodegenerative disorder, clinically characterized by progressive memory disorder and decline in cognitive function, which typically begins with dementia [1]. The number of people with AD worldwide in 2006 was estimated at 26.6 million and is predicted to nearly quadruple by 2050 [2].

The key pathological changes associated with AD brain tissue are the accumulation of intracellular neurofibrillary tangles (NFTs) and abnormally aggregated 'reactive' proteins like β amyloid (Aβ) plaques and tau [3]. Several elements, such as senile plaques, neurofibrillary tangles (NFTs), abnormally aggregated 'reactive' proteins like β amyloid (Aβ) and tau, brain inflammation and exposure to aluminum has already shown the development of AD [4]. Brain derived neurotrophic factor (BDNF) gene is supposed to be one of the important genes, playing a significant role in AD progression [5,6]. However, as a complex disorder, the neuropathological etiology of AD mentioned above are not due to the gene itself, but are also supposed to be associated with the combined interaction between genes and environmental factors.

BDNF, a member of the neurotrophic factor family, is encoded by a gene located on chromosome 11p13 [7]. The basal physiological role of BDNF is to play an important role in the growth, development, differentiation and regeneration of various types of neurons in the central nervous system [8]. Earlier studies have already shown reduced mRNA expression of BDNF in the hippocampus of AD patients [9]. Moreover, another study has also demonstrated that BDNF and its receptor level, tyrosine receptor kinase B level, were decreased in the frontal cortex and hippocampus of AD patients [10], suggesting the possible important role of BDNF in AD pathogenesis. The possible mechanism underlying this dysregulated BDNF expression may be due to reduced transport of BDNF from the Golgi region to appropriate secretory granules in neurons, resulting in the onset of AD.

Several single nucleotide polymorphisms (SNPs) in BDNF gene, such as rs11030104, rs16917204, rs7103411, rs6265 and rs2030324 are already been reported for their association with AD. However, only rs6265 and rs2030324 have been widely studied, with no linkage disequilibrium between them. The association between AD and the G196A and C270T polymorphism in the BDNF gene are already been investigated in several individual case-control studies, producing inconsistent results, which may be the result of limited power, different ethnic backgrounds or the different processes and status in AD patients. Since, individual studies have relatively small power to confirm this association and meta-analysis can strengthen the power by combining data from different individual studies and different ethnicities also, resulting in a more comprehensive conclusion [11-13]. Moreover, we also conducted Trial Sequential Analysis (TSA) of all the published case–control studies for validating the result of meta-analysis.

Materials and Methods

Identification of eligible studies

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 guidelines for systematic review and meta-analysis and the Cochrane Collaboration definition of both terms were followed for this work [14,15]. Literature search was carried out within PubMed (Medline), EMBASE and Science Direct database up to July, 2016, using the keywords-BDNF, gene, patient, polymorphism and Alzheimer's disease. Then, potentially relevant publications and studies

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were retrieved by examining their titles and abstracts and matching the eligible criteria.

Inclusion and exclusion criteria

To facilitate the proper interpretation of results and to minimize heterogeneity, all eligible studies had to fulfill the following inclusion criteria like evaluation of BDNF gene 196 G>A and 270 C>T with AD risk; use of case control or cohort studies; recruitment of pathologically confirmed AD patients and healthy controls; and availability of genotypic frequency both in case and control. Moreover, when the case control study was included by more than one article using the same case series, then we selected the study that included the largest number of individuals.

The major reasons for exclusion of studies were overlapping data, case only studies; review articles, family-based studies and animal studies.

Data extraction and quality assessment

For each meta-analysis, the methodological quality assessment and data extraction were independently abstracted in duplicate using a standard protocol. Data accuracy was ensured using data collection form according to the inclusion and exclusion criteria listed above. In case of discrepancy on any item of the data collected from the retrieved studies, the problem was fully discussed to reach a consensus. Data extracted from each study included the name of first author, year of publication, ethnicity, number of cases and controls, types of study and genotyping methods and frequencies of the case and control.

Meta-analysis methods

The meta-analysis examined the overall association and ethnicity specific association of the A and T allele with the risk of AD relative to the G and C allele respectively, the contrast of homozygotes AA vs. GG; TT vs. CC, the contrast of heterozygotes AG vs. GG; TC vs. CC, the recessive model for the A allele: contrast AA vs. (AG+GG); TT vs. (TC+CC), and the dominant model for the A allele: contrast (AA+AG) vs. GG; (TT+TC vs. CC). All associations were indicated as odds ratios (ORs) with the corresponding 95% confidence interval (CI). A pooled OR was then estimated based on individual ORs.

Statistical Analysis

Hardy Weinberg equilibrium (HWE) was examined in the control subjects using a goodness of fit chi-square test for each study, Odds ratio (OR) with corresponding 95% confidence intervals (CI) was used to evaluate the association between the BDNF 196 G>A gene polymorphism and BDNF 270 C>T with AD risk separately. Heterogeneity was assessed by Chi-square based Q-Test [16]. If heterogeneity existed, then random effects model was used to calculate the overall pooled OR value [17]; otherwise, the fixed effect model was used [18]. Moreover, I2 statistics was used to quantify interstudy variability. It ranges between 0% and 100%, where a value of 0% indicates no observed heterogeneity, and larger values indicate an increasing degree of heterogeneity [19]. The HWE was examined in the control subjects using a goodness-of-fit chi-square test for each study. Begg's funnel plots and Egger's regression test were undertaken to evaluate the potential publication bias [20]. P-value less than 0.05 were judged significant. Publication bias was assessed by visual inspection of funnel plots in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was also assessed by the Egger's linear regression test. The significance of the intercept was determined by the t-test ($p<0.05$ was considered representative of statistically significant publication bias) [21]. All the data analysis was performed using comprehensive meta-analysis (CMA) V2 software (Biostat, USA).

Trial sequential analysis (TSA)

According to Cochrane Handbook for systematic reviews of interventions, meta-analyses and systematic reviews are considered the best available evidence if all eligible trials are included. However, the best available evidence might not always be equal to strong sufficient evidence. It is well known that meta-analysis may result in increased risk of random errors when series of sparse data are analyzed and in reduplicative significance testing when new trials are updated in cumulative meta-analysis. Therefore, keeping mind on the issues raised above, we applied the TSA to increase the robustness of current conclusions by minimizing the random errors [22-24]. The methods of using TSA were based on the 'User manual for Trial Sequential Analysis (TSA)'.

In the study, TSA was used to control the risk of random error by calculating the required information size and an adjusted threshold for statistical significance to make a robust conclusion [22,23,25]. The required information size was calculated with the assumption of a plausible relative risk of 20% with low risk bias, and the overall 5% risk for a type I error (α), 20% risk for a type II error (β) were adopted [26]. Based on required information size and risk for type I and type II errors, TSA monitoring boundaries were built. When the cumulative Z-curve crosses, the TSA monitoring boundary before the required information size is reached, a sufficient level of evidence might have been reached and further trials are not necessary. Otherwise, evidence to reach a conclusion is insufficient and further trials are necessary [27]. The software Trial Sequential Analysis Viewer (version 0.9.5.5 Beta) was used for the study and 95% CIs was adjusted for sparse data or repetitive testing, described as the TSA-adjusted 95% CIs.

Results

Eligible studies included in the meta-analysis

The literature review identified a total of 39 studies eligible for inclusion in our analysis as described in flow chart (Figure 1). Based on our preliminary search criteria, a total of 555 studies were identified in PubMed (Medline), EMBASE and Science Direct using the keywords-BDNF, gene, patient, polymorphism, Alzheimer disease and their combination. After careful review, finally, 39 potential studies were included. According to our inclusion criteria, four studies have not been included for estimating OR and 95% CI because they did not reported genotypic frequency of patients and healthy controls [28,29]. Finally, 39 eligible studies involving 9409 cases and 9522 controls were enrolled in the pooled analyses.

The populations came from 14 different countries, including Brazil, China, Colombia, Croatia, England, Finland, France, Germany, Hungary, India, Italy, Japan, Spain and USA. Detailed characteristics of all eligible studies included in meta-analysis are reported in Table 1. One overall study was conducted on BDNF 196GA polymorphism and 3 ethnicity specific studies were conducted, that includes 8 studies on American populations [30-36], 11 studies on Asian populations [37-47] and 12 studies on European populations [35,48-58]. Moreover, one overall study was also conducted on BDNF 270CT polymorphism and 3 ethnicity specific studies were also possible, that includes 5 studies on American populations [30,31,33,34,59], 7 studies on Asian populations

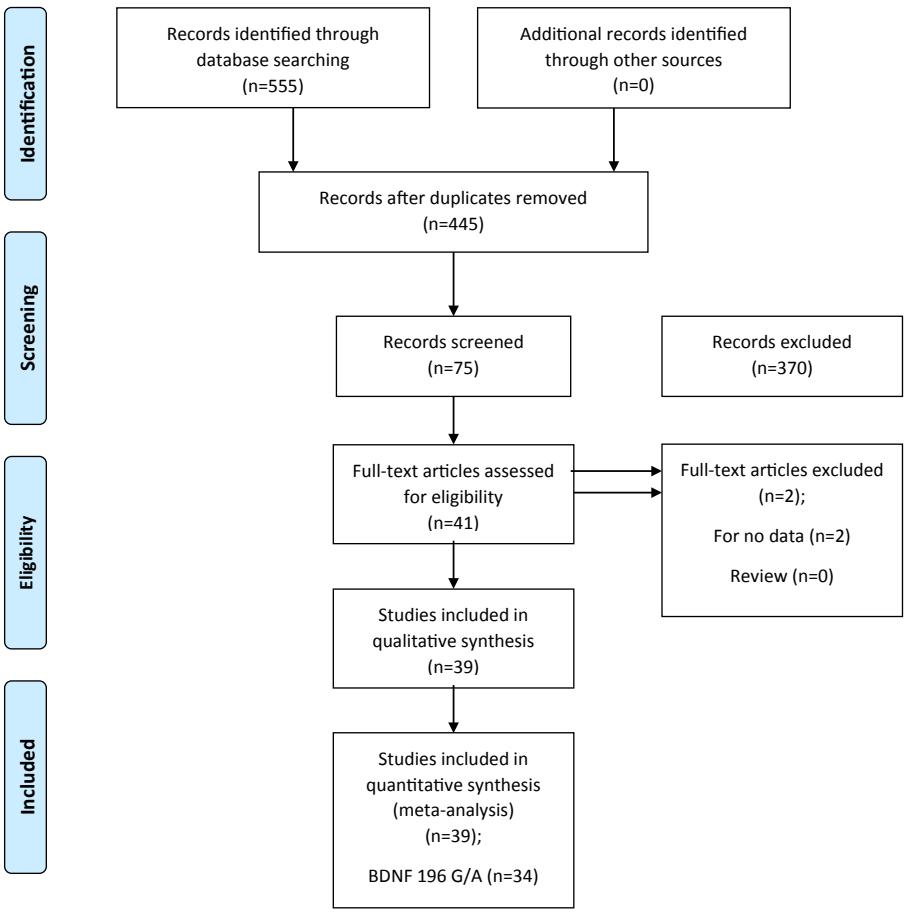

Figure 1: Study flow chart.

[37,39,41,42,44,46,60] and 7 studies on European populations [48,49,52,56,58,61,62]. Tables 2 and 3 reports genotypic distribution of G196A and C270T polymorphism of BDNF gene from each study. All studies observed HWE.

Association of BDNF SNP rs6265 polymorphisms with AD

Overall, the meta-analysis results based on different genetic models (Allelic, Homozygote, Heterozygote, Dominant and Recessive) revealed no association between BDNF 196 G/A allele in overall studies. Moreover, no association were identified between BDNF 196 G/A polymorphism and AD in ethnicity specific studies (i.e., American, Asian and European) also.

The pooled ORs of overall study analysis revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.257; OR=1.030, 95% CI=0.979 to 1.085) genetic models; homozygous (AA vs. GG: p=0.683; OR=1.039, 95% CI=0.863 to 1.251) genetic models; heterozygous (AG vs. GG: p=0.329; OR=1.052, 95% CI=0.950 to 1.165) genetic models; dominant (AA+AG vs. GG: p=0.330; OR=1.051, 95% CI=0.951 to 1.162) genetic models; and recessive (AA vs. AG+GG: p=0.847; OR=1.016, 95% CI=0.866 to 1.192) genetic models (Figure 2). All ORs were pooled through a random effect models (Table 4).

Similarly, the pooled ORs of American study analysis revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.242; OR=1.066, 95% CI=0.957 to 1.188) genetic models; homozygous (AA vs. GG: p=0.672; OR=1.280, 95% CI=0.644 to 1.328) genetic models; heterozygous (AG vs. GG: p=0.069; OR=1.127, 95% CI=0.991 to 1.282) genetic models; dominant (AA+AG vs. GG: p=0.106; OR=1.109, 95% CI=0.978 to 1.257) genetic models; and recessive (AA vs. AG+GG: p=0.493; OR=0.882, 95% CI=0.616 to 1.263) genetic models (Figure 3). All ORs were pooled through a fixed effect models (Table 5).

Additionally, the pooled ORs of Asian study analysis revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.485; OR=1.028, 95% CI=0.952 to 1.110) genetic models; homozygous (AA vs. GG: p=0.433; OR=1.104, 95% CI=0.862 to 1.413) genetic models; heterozygous (AG vs. GG: p=0.399; OR=1.055, 95% CI=0.932 to 1.193) genetic models; dominant (AA+AG vs. GG: p=0.385; OR=1.053, 95% CI=0.937 to 1.184) genetic models; and recessive (AA vs. AG+GG: p=0.777; OR=1.031, 95% CI=0.834 to 1.275) genetic models (Figure 4). All ORs were pooled through a fixed effect models except for homozygous and recessive genetic models (Table 6).

Moreover, the pooled ORs of European study analysis revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.926; OR=0.991, 95% CI=0.814 to 1.206) genetic models; homozygous (AA vs. GG: p=0.755; OR=1.070,

Authors	Country	Ethnicity	Cases (AD)	Control (HC)	Genotyping	SNP	Association
Kunugi et al. [60]	Japan	Asian	170	498	PCR	C>T	Yes
Riemenschneider et al. [62]	Germany	European	210	188	PCR	C>T	Yes
Ventriglia et al. [57]	Italy	European	130	111	PCR	G>A	Yes
Bagnoli et al. [48]	Italy	European	128 128	97 97	PCR	G>A C>T	No
Combarros et al. [51]	Spain	European	237	218	PCR	G>A	No
Nacmias et al. [54]	Italy	European	83	97	PCR-RFLP	G>A	No
Nishimura et al. [59]	Brazil	American	188	188	PCR	C>T	No
Bian et al. [38]	China	Asian	203	239	PCR	G>A	No
Bodner et al. [30]	USA	American	256 256	194 194	ABI Prism 7900HT instrument	G>A C>T	No
Desai et al. [31]	USA	American	995 719	671 523	Pyrosequencing	G>A C>T	No
Desai et al. [31]	USA	African	64 58	45 42	Pyrosequencing	G>A C>T	No
Lee et al. [34]	USA	American	95 106	70 73	PCR-RFLP	G>A C>T	No
Li et al. [35]	England	European	359	396	Allele-specific Real Time PCR	G>A	No
Li et al. [35]	USA	American	188	361	Allele-specific Real Time PCR	G>A	No
Li et al. [35]	USA	American	388	349	Allele-specific Real Time PCR	G>A	No
Matsushita et al. [41]	Japan	Asian	487 487	471 471	PCR	G>A C>T	Yes
Nishimura et al. [42]	Japan	Asian	172 172	275 275	PCR-RFLP	G>A C>T	Yes
Olin et al. [69]	USA	Mixed (Non-Hispanic American)	212	202	PCR	C>T	Yes
Vepsalainen et al. [58]	Finland	European	375 375	460 460	PCR	G>A C>T	No
Akatsu et al. [37]	Japan	Asian	95 95	108 108	PCR-RFLP	G>A C>T	No
Forero et al. [67]	Colombia	Mixed	101	168	PCR	G>A	Yes
Saarela et al. [56]	Finland	European	97 97	101 101	PCR	G>A C>T	No
Tsai et al. [46]	China	Asian	175 175	189 189	PCR	G>A C>T	Yes
Zhang et al. [68]	USA	Mixed (European-American)	295 295	250 250	PCR-RFLP	G>A C>T	No
He et al. [40]	China	Asian	513	575	Allele-specific PCR	G>A	No
Huang et al. [33]	USA	American	220 220	128 128	PCR	G>A C>T	No
Cozza et al. [52]	Italy	European	251 251	97 97	PCR	G>A C>T	No
Qian et al. [44]	China	Asian	105 105	105 105	PCR	G>A C>T	No
Yu et al. [47]	China	Asian	99	99	PCR	G>A	No
Feher et al. [53]	Hungary	European	160	164	PCR	G>A	Yes
Qi et al. [43]	China	Asian	80	86	PCR-RFLP	G>A	No
Fukumoto et al. [39]	Japan	Asian	657 657	525 525	Taqman PCR Assay	G>A C>T	Yes
Cousin et al. [61]	France	European	425	470	PCR	C>T	No
Pivac et al. [55]	Croatia	European	211	402	Taqman based Allele-specific PCR Assay	G>A	No
Borroni et al. [50]	Italy	European	234	162	PCR	G>A	Yes
Boiocchi et al. [49]	Italy	European	191 192	408 384	PCR-RFLP	G>A C>T	Yes
Sonali et al. [45]	India	Asian	57	63	PCR-RFLP	G>A	No
Vieira et al. [36]	Brazil	American	269	114	Taqman Real Time PCR Assay	G>A	No
Gomar et al. [32]	USA	American	222	175	Illumina Human610-Quad BeadChip	G>A	Yes

Table 1: Main characteristics of all studies included in meta-analysis.

First author	Cases (AD)				Controls (HC)				HWE
	Val66Met/G196A Genotype rs6265			Minor Allele Frequency (MAF)	Val66Met/G196A Genotype rs6265			Minor Allele Frequency (MAF)	
	GG	GA	AA		GG	GA	AA		P-value
Ventriglia et al. [57]	85	33	12	0.219	54	48	9	0.297	0.712
Bagnoli et al. [48]	62	60	6	0.281	55	38	4	0.237	0.414
Combarros et al. [51]	149	78	10	0.206	143	67	8	0.190	0.965
Nacmias et al. [54]	48	29	6	0.246	55	38	4	0.237	0.414
Bian et al. [38]	49	113	41	0.480	73	115	51	0.453	0.649
Bodner et al. [30]	163	85	8	0.197	126	62	6	0.190	0.623
Desai et al. [31]	662	299	34	0.184	456	197	18	0.173	0.549
Desai et al. [31]	59	5	0	0.039	42	3	0	0.033	0.817
Lee et al. [34]	45	47	3	0.278	32	30	8	0.328	0.810
Li et al. [35]	239	105	15	0.188	269	114	13	0.176	0.828
Li et al. [35]	109	73	6	0.226	235	110	16	0.196	0.497
Li et al. [35]	251	126	11	0.190	237	105	7	0.170	0.234
Matsushita et al. [41]	171	247	69	0.395	150	223	98	0.444	0.368
Nishimura et al. [42]	61	85	26	0.398	88	140	47	0.425	0.493
Vepsalainen et al. [58]	280	87	8	0.137	342	109	9	0.138	0.926
Akatsu et al. [37]	25	58	12	0.431	35	53	20	0.430	0.993
Forero et al. [67]	72	27	2	0.153	131	34	3	0.119	0.648
Saarela et al. [56]	62	32	3	0.195	81	17	3	0.113	0.095
Tsai et al. [46]	43	92	40	0.491	64	95	30	0.410	0.592
Zhang et al. [68]	178	108	9	0.213	166	74	10	0.188	0.629
He et al. [40]	155	245	113	0.459	165	285	125	0.465	0.925
Huang et al. [33]	150	66	4	0.168	98	25	5	0.136	0.050
Cozza et al. [52]	152	84	15	0.227	60	33	4	0.211	0.839
Qian et al. [44]	28	52	25	0.485	25	56	24	0.495	0.493
Yu et al. [47]	31	41	27	0.479	28	51	20	0.459	0.712
Feher et al. [53]	94	56	10	0.237	52	79	33	0.442	0.763
Qi et al. [43]	27	32	21	0.462	27	48	11	0.406	0.147
Fukumoto et al. [39]	218	319	120	0.425	197	249	79	0.387	0.982
Pivac et al. [55]	135	59	17	0.220	268	118	16	0.186	0.509
Borroni et al. [50]	128	87	19	0.267	89	63	10	0.256	0.794
Boiocchi et al. [49]	113	63	15	0.243	231	150	27	0.25	0.692
Sonali et al. [45]	3	32	22	0.666	12	23	28	0.626	0.081
Vieira et al. [36]	205	59	5	0.128	84	25	5	0.153	0.095
Gomar et al. [32]	153	69	0	0.155	120	55	0	0.157	0.013

Table 2: Genotypic distribution of BDNF gene rs6265 polymorphism included in meta-analysis.

First author	Cases (AD)				Control (HC)				HWE
	C270T Genotype rs2030324			Minor Allele Frequency (MAF)	C270T Genotype rs2030324			Minor Allele Frequency (MAF)	
	CC	CT	TT		CC	CT	TT		P-value
Kunugi et al. [60]	150	19	1	0.061	477	21	0	0.021	0.630
Riemenschneider et al. [62]	185	24	1	0.061	175	13	0	0.034	0.623
Bagnoli et al. [48]	113	14	1	0.062	83	14	0	0.072	0.443
Nishimura et al. [59]	175	13	0	0.034	170	17	1	0.050	0.429
Bodner et al. [30]	230	26	0	0.050	175	19	0	0.048	0.473
Desai et al. [31]	629	86	4	0.065	454	69	0	0.065	0.106
Desai et al. [31]	54	4	0	0.034	38	4	0	0.047	0.745
Lee et al. [34]	102	4	0	0.018	66	7	0	0.047	0.666
Matsushita et al. [41]	457	30	0	0.030	438	33	0	0.035	0.430
Nishimura et al. [42]	154	18	0	0.052	264	11	0	0.02	0.735
Olin et al. [69]	173	36	3	0.099	189	13	0	0.032	0.636
Vepsalainen et al. [58]	90	199	86	0.494	124	239	97	0.470	0.359
Akatsu et al. [37]	89	6	0	0.031	101	7	0	0.032	0.727
Saarela et al. [56]	88	9	0	0.046	81	19	1	0.103	0.922
Tsai et al. [46]	151	24	0	0.068	167	20	2	0.063	0.129
Zhang et al. [68]	271	22	2	0.044	220	30	0	0.06	0.312
Huang et al. [33]	202	16	2	0.045	113	15	0	0.058	0.481
Cozza et al. [52]	212	35	4	0.085	80	15	2	0.097	0.218
Qian et al. [44]	104	1	0	0.004	96	8	1	0.047	0.101
Fukumoto et al. [39]	611	45	1	0.035	490	34	1	0.034	0.613
Cousin et al. [61]	370	54	1	0.065	419	50	1	0.055	0.698
Boiocchi et al. [49]	55	93	44	0.471	103	192	89	0.481	0.979

Table 3: Genotypic distribution of BDNF gene rs2030324 polymorphism included in meta-analysis.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
A vs. G	0.282	-1.120 to 1.685	0.684	65.777	0.001	49.831	Random
AA vs. GG	0.035	-1.107 to 1.177	0.950	55.024	0.005	43.661	Random
AG vs. GG	0.385	-0.985 to 1.755	0.570	61.076	0.002	45.969	Random
AA+AG vs. GG	0.360	-1.052 to 1.773	0.606	64.671	0.001	48.973	Random
AA vs. AG+GG	-0.080	-1.098 to 0.936	0.872	48.938	0.021	36.655	Random

Table 4: Statistics to test publication bias and heterogeneity in meta-analysis (rs6265-Overall).

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
A vs. G	-0.983	-3.139 to 1.172	0.307	4.664	0.701	0.000	Fixed
AA vs. GG	-2.776	-5.099 to -0.452	0.027	7.660	0.264	21.671	Fixed
AG vs. GG	0.751	-1.581 to 3.084	0.460	3.218	0.617	0.000	Fixed
AA+AG vs. GG	-0.028	-2.278 to 2.220	0.975	4.784	0.729	0.000	Fixed
AA vs. AG+GG	-2.945	-5.356 to -0.533	0.025	8.386	0.211	28.451	Fixed

Table 5: Statistics to test publication bias and heterogeneity in meta-analysis (rs6265- American).

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
A vs. G	0.869	-1.485 to 3.225	0.425	15.621	0.111	35.986	Fixed
AA vs. GG	1.278	-1.164 to 3.721	0.266	19.996	0.029	49.991	Random
AG vs. GG	0.779	-1.429 to 2.988	0.445	16.666	0.082	39.996	Fixed
AA+AG vs. GG	0.989	-1.092 to 3.070	0.310	15.706	0.108	36.330	Fixed
AA vs. AG+GG	0.737	-2.142 to 3.616	0.576	20.385	0.026	50.945	Random

Table 6: Statistics to test publication bias and heterogeneity in meta-analysis (rs6265-Asian).

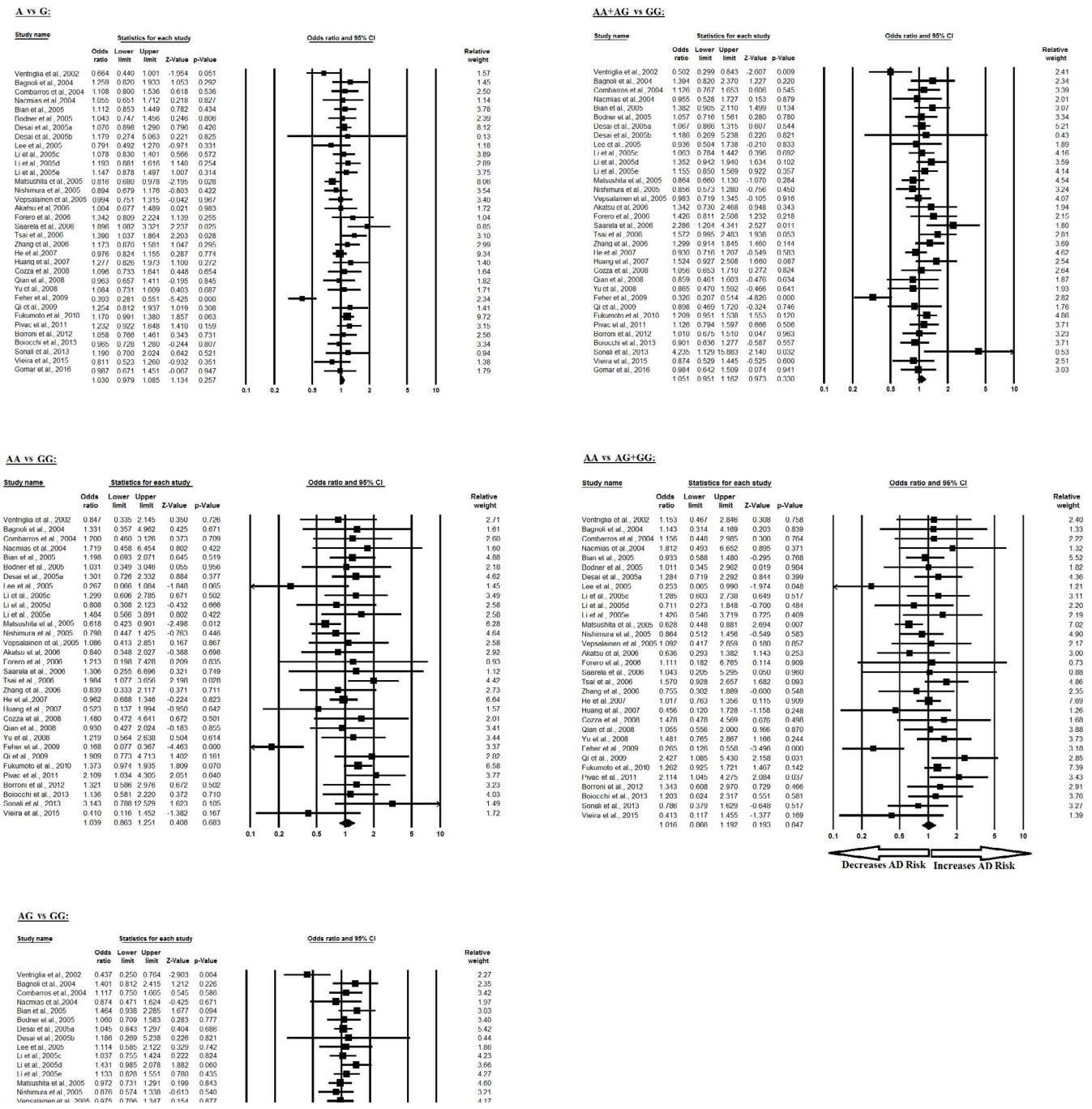

Figure 2: Forest-plot of a meta-analysis of the association between BDNF gene 196 G>A polymorphism (A vs. G; AA vs. GG; AG vs. GG; AA+AG vs. GG; AA vs. AG+GG) and overall AD risk.

95% CI=0.702 to 1.630) genetic models; heterozygous (AG vs. GG: p=0.507; OR=0.930, 95% CI=0.750 to 1.153) genetic models; dominant (AA+AG vs. GG: p=0.674; OR=0.952, 95% CI=0.758 to 1.196) genetic models; and recessive (AA vs. AG+GG: p=0.411; OR=1.113, 95% CI=0.862 to 1.438) genetic models (Figure 5). All ORs were pooled through a random effect models except for recessive genetic model (Table 7).

Association of BDNF SNP rs2030324 polymorphisms with AD

The meta-analysis results based on different genetic models revealed no association between BDNF 270 C/T allele in overall studies for all genetic models i.e. T vs. C allelic contrast, TT vs. CC homozygous genotype, TC vs. CC heterozygous genotype, dominant TT+TC vs. CC genotype and recessive TT vs. TC+CC genotype.

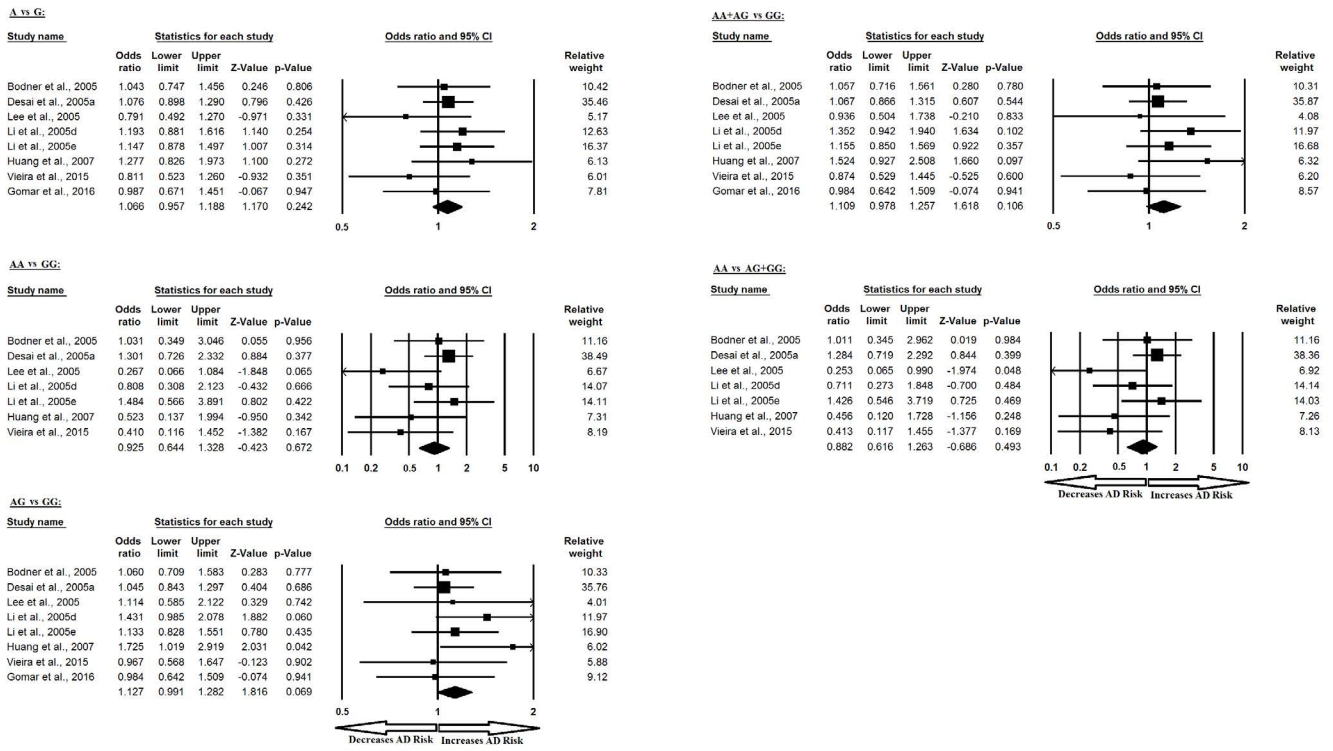

Figure 3: Forest-plot of a meta-analysis of the association between BDNF gene 196 G>A polymorphism (A vs. G; AA vs. GG; AG vs. GG; AA+AG vs. GG; AA vs. AG+GG) and American AD risk.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
A vs. G	0.935	-5.248 to 7.119	0.743	42.336	0.000	74.017	Random
AA vs. GG	0.754	-3.555 to 5.064	0.704	26.706	0.005	58.810	Random
AG vs. GG	-0.080	-5.035 to 4.873	0.971	31.298	0.001	64.854	Random
AA+AG vs. GG	-0.142	-5.701 to 5.416	0.955	38.830	0.000	71.671	Random
AA vs. AG+GG	0.517	-3.044 to 4.079	0.752	18.667	0.067	41.074	Fixed

Table 7: Statistics to test publication bias and heterogeneity in meta-analysis (rs6265-European).

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
T vs. C	-0.362	-1.780 to 1.055	0.599	52.111	0.000	59.701	Random
TT vs. CC	0.191	-0.415 to 0.797	0.509	10.175	0.809	0.000	Fixed
TC vs. CC	-0.707	-2.507 to 1.093	0.422	49.386	0.000	57.478	Random
TT+TC vs. CC	-0.723	-2.528 to 1.081	0.412	51.664	0.000	59.353	Random
TT vs. TC+CC	-0.215	-0.333 to 0.764	0.413	9.348	0.859	0.000	Fixed

Table 8: Statistics to test publication bias and heterogeneity in meta-analysis (rs2030324-Overall).

Ethnicity specific studies also not found to be associated with BDNF 270 C/T polymorphism and AD.

The pooled ORs of overall study analysis revealed that BDNF C>T gene polymorphism is not associated with AD risk in allelic (T vs. C: p=0.538; OR=1.059, 95% CI=0.881 to 1.274) genetic models; homozygous (TT vs. CC: p=0.419; OR=1.125, 95% CI=0.845 to 1.496) genetic models; heterozygous (TC vs. CC: p=0.748; OR=1.034, 95% CI=0.842 to 1.270) genetic models; dominant (TT+TC vs. CC: p=0.662; OR=1.047, 95% CI=0.852 to 1.287) genetic models; and recessive (TT vs. TC+CC: p=0.499; OR=1.088, 95% CI=0.853 to 1.387) genetic models (Figure 6). All ORs were pooled through a random effect models except for homozygous and recessive genetic models (Table 8).

Similarly, the pooled ORs of American study analysis revealed that BDNF C>T gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.358; OR=0.893, 95% CI=0.701 to 1.137) genetic models; homozygous (AA vs. GG: p=0.446; OR=1.983, 95% CI=0.340 to 11.551) genetic models; heterozygous (AG vs. GG: p=0.148; OR=0.829, 95% CI=0.643 to 1.069) genetic models; dominant (AA+AG vs. GG: p=0.226; OR=0.856, 95% CI=0.665 to 1.101) genetic models; and recessive (AA vs. AG+GG: p=0.428; OR=2.039, 95% CI=0.350 to 11.876) genetic models (Figure 7). All ORs were pooled through a fixed effect models (Table 9).

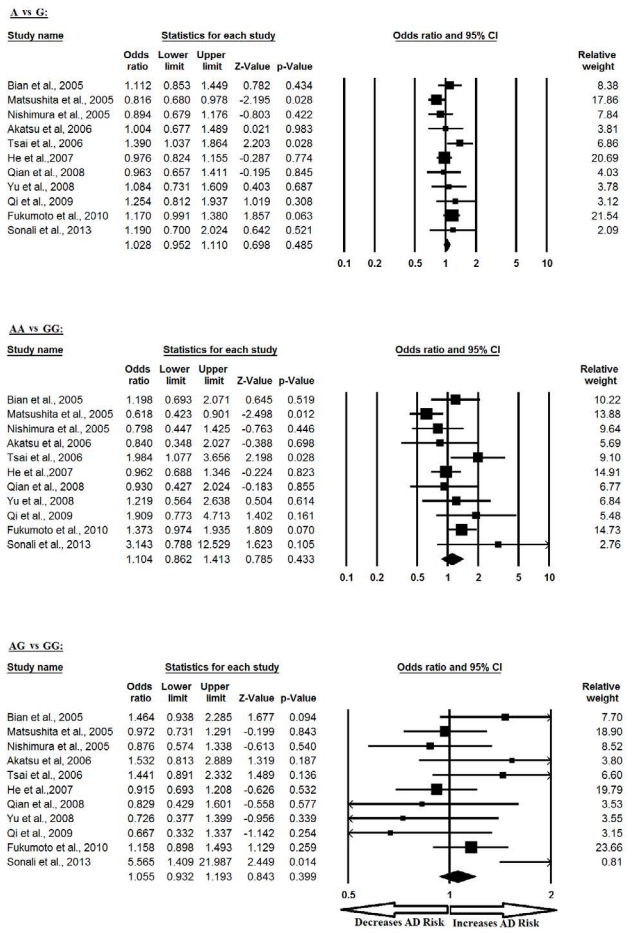

Figure 4: Forest-plot of a meta-analysis of the association between BDNF gene 196 G>A polymorphism (A vs. G; AA vs. GG; AG vs. GG; AA+AG vs. GG; AA vs. AG+GG) and Asian AD risk.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
T vs. C	-1.640	-3.489 to 0.208	0.066	3.206	0.524	0.000	Fixed
TT vs. CC	-20.859	-65.562 to 23.843	0.106	1.909	0.385	0.000	Fixed
TC vs. CC	-1.441	-3.790 to 0.906	0.145	3.135	0.535	0.000	Fixed
TT+TC vs. CC	-1.553	-3.601 to 0.494	0.094	3.083	0.544	0.000	Fixed
TT vs. TC+CC	-20.819	-68.169 to 26.529	0.112	1.905	0.386	0.000	Fixed

Table 9: Statistics to test publication bias and heterogeneity in meta-analysis (rs2030324-American).

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
T vs. C	-0.930	-6.291 to 4.429	0.673	20.906	0.002	71.300	Random
TT vs. CC	3.105	-41.558 to 47.770	0.793	3.316	0.345	9.534	Fixed
TC vs. CC	-0.780	-5.834 to 4.273	0.707	18.038	0.006	66.737	Random
TT+TC vs. CC	-0.864	-6.112 to 4.382	0.689	19.666	0.003	69.490	Random
TT vs. TC+CC	3.128	-40.505 to 46.761	0.786	3.169	0.366	5.326	Fixed

Table 10: Statistics to test publication bias and heterogeneity in meta-analysis (rs2030324-Asian).

Additionally, the pooled ORs of Asian study analysis revealed that BDNF C>T gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.382; OR=1.244, 95% CI=0.762 to 2.032) genetic models; homozygous (AA vs. GG: p=0.797; OR=0.819, 95% CI=0.179 to 3.749) genetic models; heterozygous (AG vs. GG: p=0.274; OR=1.302, 95% CI=0.812 to 2.088) genetic models; dominant (AA+AG vs. GG: p=0.327; OR=1.277, 95% CI=0.783 to 2.083) genetic models; and recessive (AA vs. AG+GG: p=0.785; OR=0.810, 95% CI=0.177 to 3.706) genetic models (Figure 8). All ORs were pooled through a random effect models except for homozygous and recessive genetic models (Table 10).

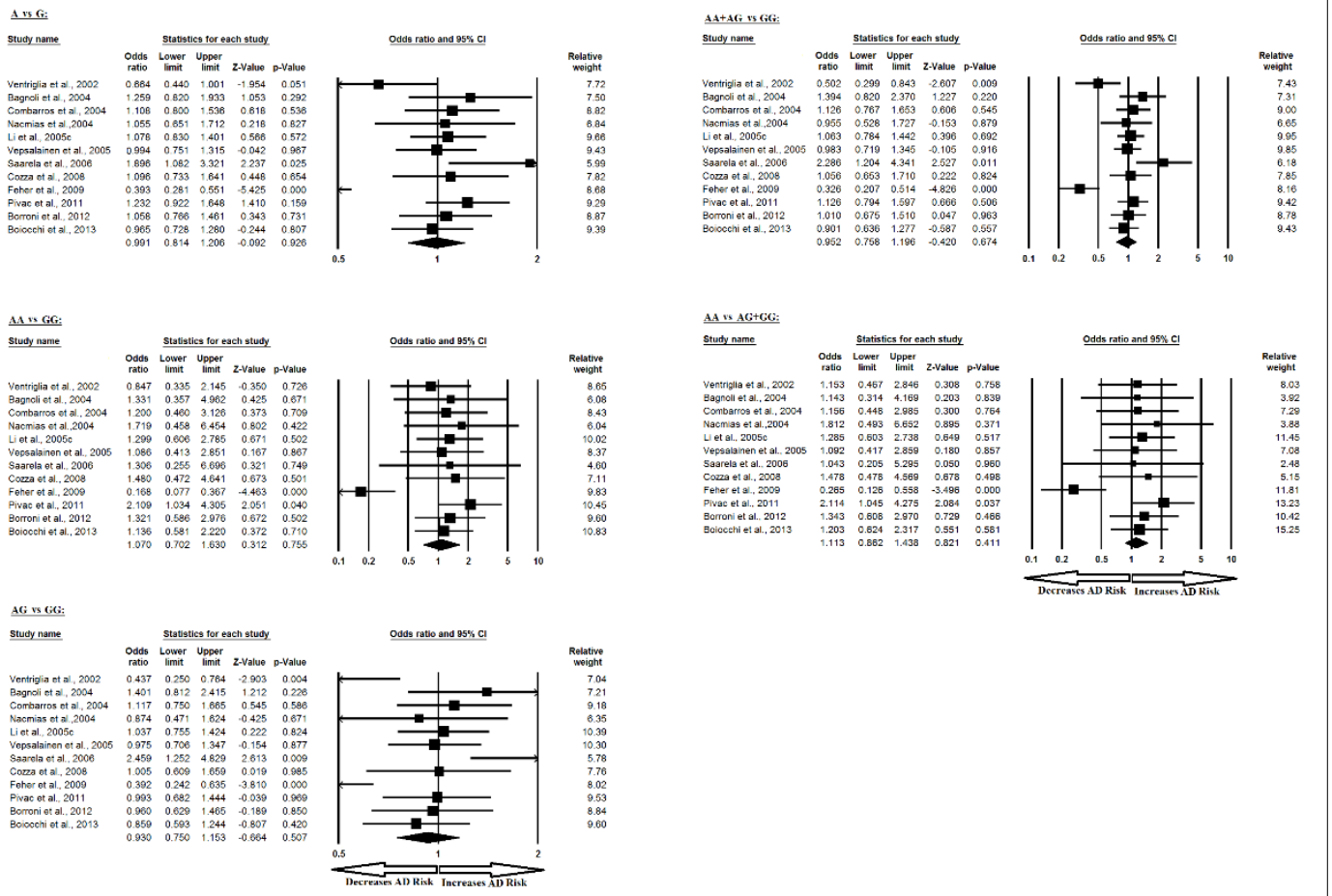

Figure 5: Forest-plot of a meta-analysis of the association between BDNF gene 196 G>A polymorphism (A vs. G; AA vs. GG; AG vs. GG; AA+AG vs. GG; AA vs. AG+GG) and European AD risk.

Comparisons	Egger's regression analysis			Heterogeneity analysis			Model used for meta-analysis
	Intercept	95% Confidence Interval	P value	Q value	$P_{heterogeneity}$	I^2 (%)	
T vs. C	-0.637	-3.265 to 1.991	0.560	9.550	0.145	37.172	Fixed
TT vs. CC	-0.057	-0.957 to 0.841	0.875	2.047	0.915	0.000	Fixed
TC vs. CC	-1.504	-4.871 to 1.862	0.302	8.455	0.207	29.038	Fixed
TT+TC vs. CC	-1.425	-4.930 to 2.078	0.343	9.412	0.152	36.249	Fixed
TT vs. TC+CC	-0.004	-0.711 to 0.702	0.988	1.363	0.968	0.000	Fixed

Table 11: Statistics to test publication bias and heterogeneity in meta-analysis (rs2030324-European).

Moreover, the pooled ORs of European study analysis revealed that BDNF C>T gene polymorphism is not associated with AD risk in allelic (A vs. G: p=0.546; OR=1.041, 95% CI=0.914 to 1.186) genetic models; homozygous (AA vs. GG: p=0.589; OR=1.086, 95% CI=0.806 to 1.462) genetic models; heterozygous (AG vs. GG: p=0.696; OR=1.038, 95% CI=0.860 to 1.253) genetic models; dominant (AA+AG vs. GG: p=0.620; OR=1.047, 95% CI=0.874 to 1.254) genetic models; and recessive (AA vs. AG+GG: p=0.656; OR=1.059, 95% CI=0.824 to 1.360) genetic models (Figure 9). All ORs were pooled through a fixed effect models (Table 11).

Evaluation of publication bias

No between study heterogeneity was found in analyses of the BDNF 196 G/A and 270 C/T polymorphisms in the overall, American, Asian or European study populations. Begg's Funnel Plot and Egger's Test were performed to evaluate the publication bias among the included studies for this meta-analysis. The shape of funnel plots did not reveal any evidence of obvious symmetry in all comparisons and the Egger's regression test was used to provide statistical evidence of funnel plot. The results of Egger's regression analysis did not show any evidence of publication bias in all genetic models except for homozygous and recessive genetic models of BDNF 196 G/A polymorphism in the American study populations (Egger's regression test p values>0.05; (Tables 4-11).

Quantitative sensitivity analysis

Sensitivity analysis was conducted to verify the robustness of our results. It is also used to ascertain whether modification of the inclusion

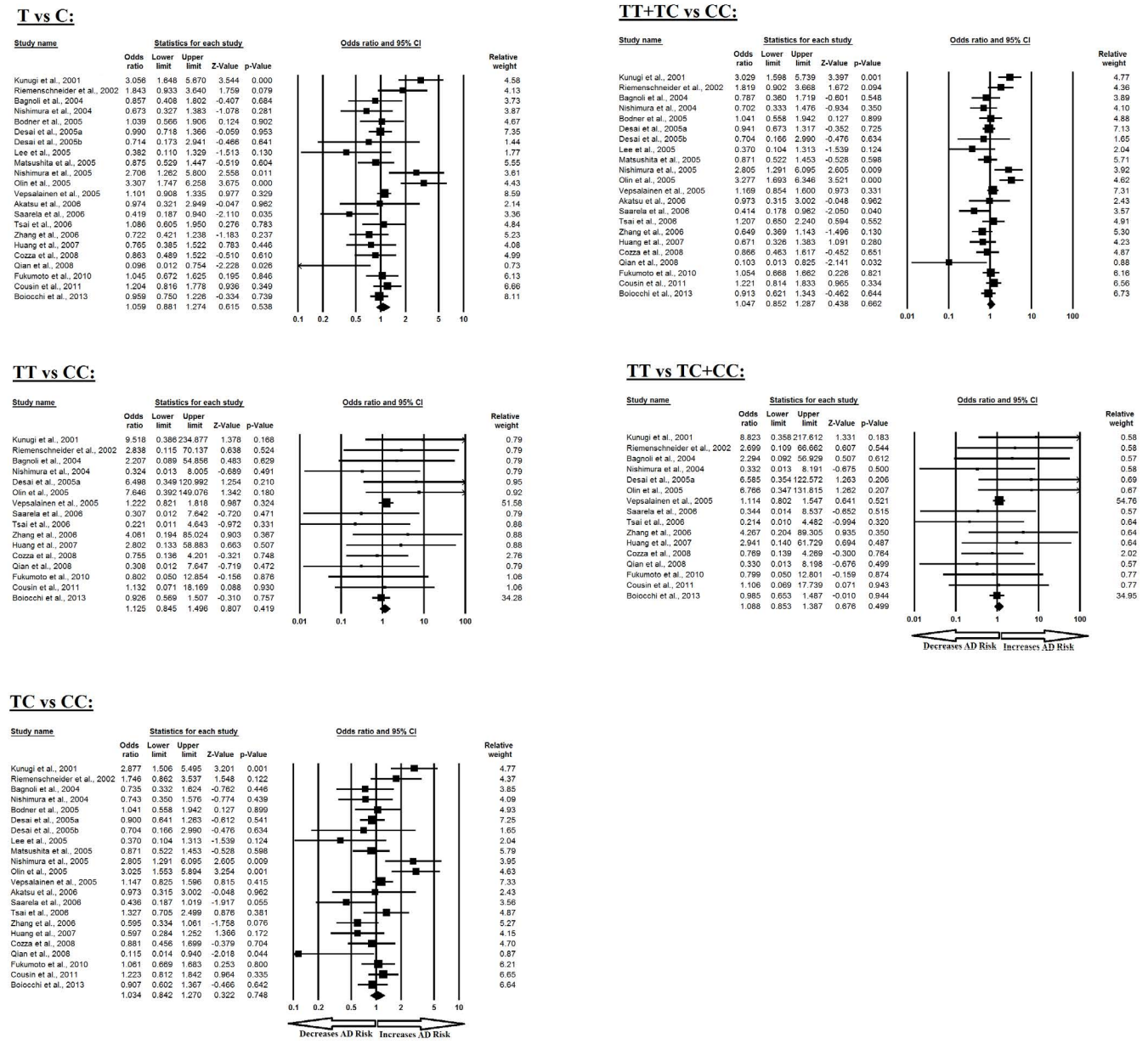

Figure 6: Forest-plot of a meta-analysis of the association between BDNF gene 270 C>T polymorphism (T vs. C; TT vs. CC; TC vs. CC; TT+TC vs. CC; TT vs. TC+CC) and overall AD risk.

criteria of the meta-analysis affected the final results. The effect of each study included in this meta-analysis assessed by sensitivity analysis of each individual study on the pooled OR by eliminating each single case-control study was done for each BDNF polymorphism [rs6265(G>A), rs2030324(C>T)] to evaluate the influence. Outcomes of sensitivity analysis revealed that no individual genetic model influenced the pooled ORs significantly in all the BDNF variants, which suggest the credibility and stability of this meta-analysis.

Trial sequential analysis (TSA)

Thirty nine trials (27329 subjects) were used to investigate the association of rs6265 and rs2030324 gene polymorphisms with AD risk. Using the data of dominant model for rs6265 (including 34 trials with 16165 subjects) as an example, the TSA was performed and found that the required information size (RIS) is 12506 subjects to demonstrate the issue. The cumulative Z-curve crosses the TSA monitoring boundary before reaching RIS, indicating that the cumulative evidence is sufficient and further trials are not necessary (Figure 10). However, the cumulative Z-curve does not crossed with TSA monitoring boundary when we performed the analysis using the data of recessive model, confirming that cumulative evidence is insufficient and further relevant trials are necessary (Figure 11).

Similarly, for rs2030324, we chose the data of five models to perform TSA. The cumulative Z-curve have not crossed with TSA monitoring

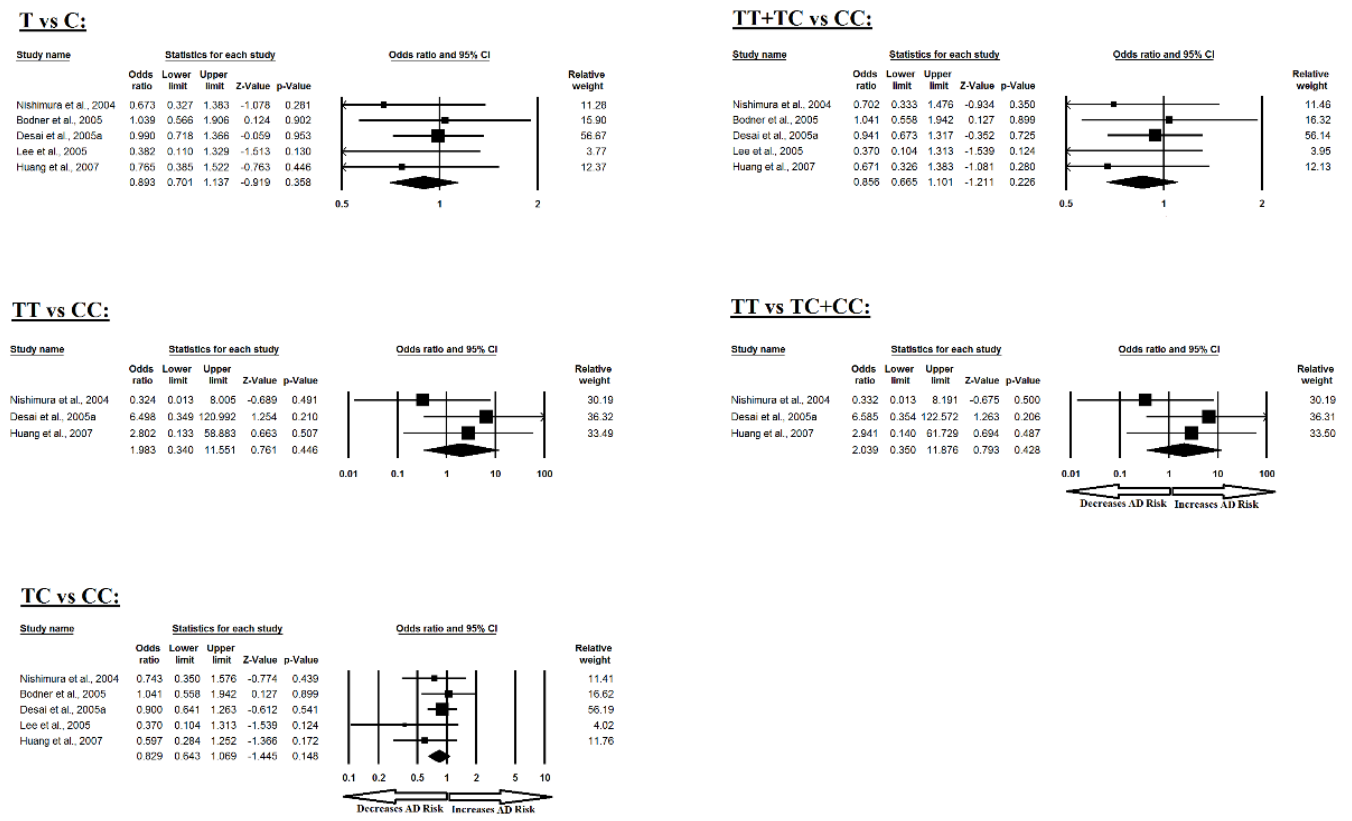

Figure 7: Forest-plot of a meta-analysis of the association between BDNF gene 270 C>T polymorphism (T vs. C; TT vs. CC; TC vs. CC; TT+TC vs. CC; TT vs. TC+CC) and American AD risk.

boundaries before the required information size is reached, indicating that cumulative evidence is insufficient and further trials are necessary (figures were not shown).

Moreover, when we performed the sub-analysis based on the ethnicity (American, Asian and European) for all models for both BDNF gene polymorphisms, the cumulative Z-curve does not crossed with TSA monitoring boundary, confirming that cumulative evidence is insufficient and further relevant trials are necessary in this regard (figures were not shown).

Discussion

In the present study, 39 studies covering 9,409 cases and 9,522 controls were carried out to investigate the association of the BDNF G196A and BDNF C270T variants with the risk of AD. 34 articles related to rs6265 and 22 articles related to rs2030324 were included in our meta-analysis. However, the results showed no significant associations between BDNF G196A and BDNF C270T with AD in overall studies along with American, Asian and European ethnic studies.

There are few evidences supporting altered mRNA and protein expression as a consequence of the gene polymorphisms 196 G/A and 270 C/T. For instance, earlier studies have shown that the BDNF Val66Met polymorphism has been shown to impair the secretion of BDNF [63], that changes brain morphology and cognitive function [64], and these polymorphisms has been shown to affect BDNF or neuronal function by reducing transport of BDNF from the Golgi region to appropriate secretory granules in neurons [65]. Moreover, the A allele of rs6265 has also found to be associated with poorer episodic memory, abnormal hippocampal activation, and lower hippocampal n-acetyl aspartate (NAA) in human subjects [66]. Individual studies have reported 10 positive results [32,39,41,42,46,49,50,53,57,67] and 22 negative results [30,31,33-38,40,45,47,48, 51,54-56,58,59,68] among the previous association studies between the BDNF G196A polymorphism and AD. In the present meta-analysis, no significant association was found between BDNF G196A and AD (P>0.05, Figures 2-5). The present meta-analysis of BDNF G196A included 34 articles. In addition, meta analyses were performed under various genetic models, including allelic, homozygous, heterozygous, dominant and recessive models. Subgroup meta-analysis by ethnicity were also conducted, although no statistically significant association results were obtained.

Similarly, literature has already reported that the BDNF C270T polymorphism is located in a non-coding region and may affect the BDNF expression in the neural soma, dendrites or axonal regions [29]. Moreover, the heterozygous carriers of the T allele of rs2030324 tend to have a greater risk of developing AD in comparison to non-carriers [49]. Individual studies have reported a total of 8 significant results [39,41,42,46,49,60,62,69] and 12 non-significant results [3 0,31,33,34,37,48,56,58,59,61,68] among the previous association studies between the BDNF C270T polymorphism and AD. In the present meta-analysis, no significant association was found between the BDNF C270T polymorphism and risk of AD (P>0.05, Figures 6-9). The present meta-analysis of BDNF G196A included 22 articles.

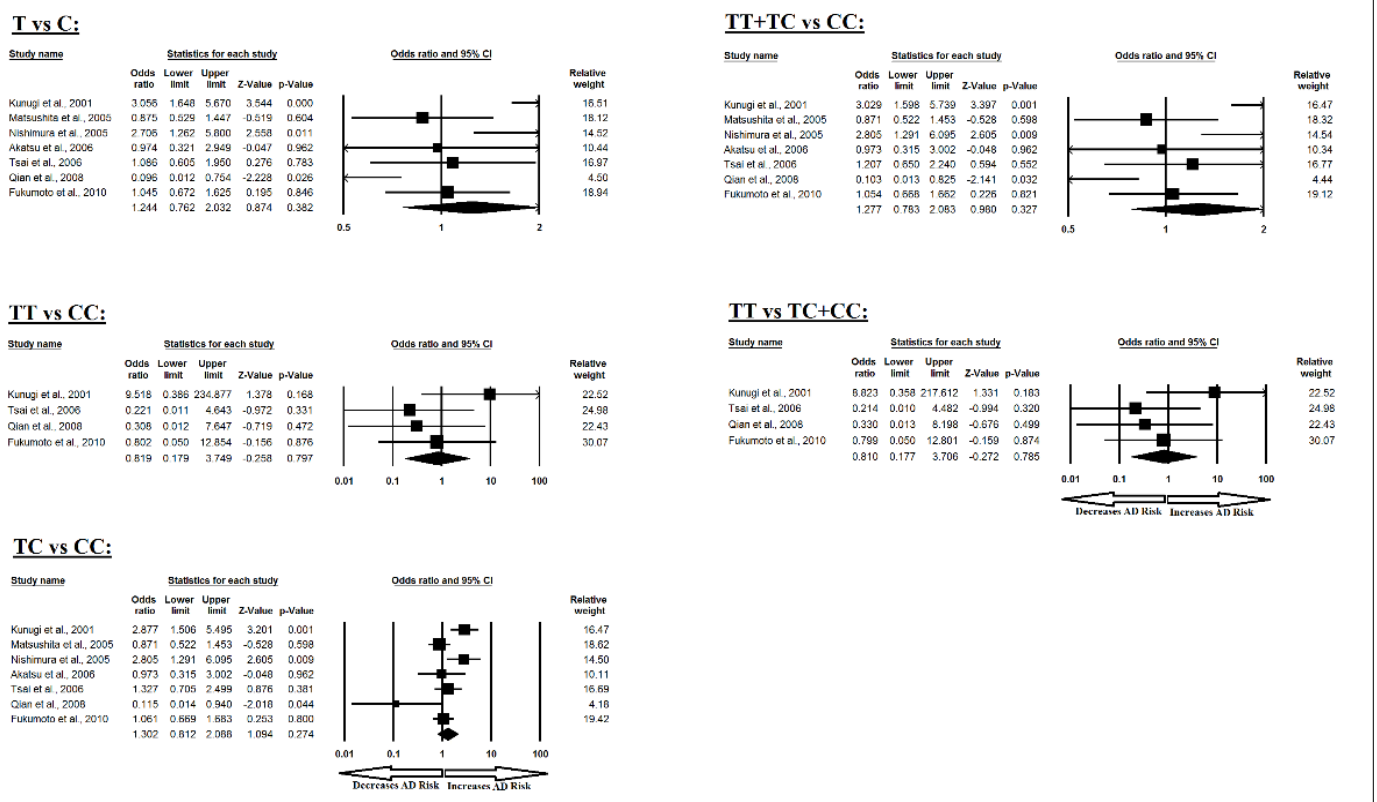

Figure 8: Forest-plot of a meta-analysis of the association between BDNF gene 270 C>T polymorphism (T vs. C; TT vs. CC; TC vs. CC; TT+TC vs. CC; TT vs. TC+CC) and Asian AD risk.

In addition, meta analyses were performed under various genetic models, including allelic, homozygous, heterozygous, dominant and recessive models. Subgroup meta-analysis by ethnicity were also conducted, although no statistically significant association results were obtained.

As we know the multifactorial nature of AD, so combined effects between gene variants and environmental factors, as well as their possible interaction, may be potential contributors to this disease. We further performed a sensitivity analysis, the results of which were consistent and strongly identified the stability of our results. Moreover, no publication bias was observed in any of the above-mentioned genetic models for both two polymorphisms except for homozygous and recessive genetic models of BDNF 196 G/A polymorphism in the American study populations. Besides, we also performed the TSA and the results of TSA showed that the conclusions in this meta-analysis are robust. All the studies included in the present meta-analysis met the HWE and were done under various genetic models with subgroup meta-analysis stratified by ethnicity. With stringent inclusion and exclusion criteria, a larger sample size and more comprehensive analysis, the present meta-analysis of BDNF G196A and C270T showed a more reliable conclusion.

Advantages

The major advantage of our meta-analysis is that the results were based on the larger number of studies, resulting into a greater chance of getting definitive conclusions. Moreover, we also performed subgroup analyses for the potential sources of heterogeneity, and sensitivity analysis for ensuring the stability of our results. However, our meta-analysis also had certain limitations. Firstly, publication bias may occur, due to the less likely published or even missed negative result studies. Secondly, limited number of ethnic studies in other populations like Africans and the most of the studies were carried out in the European, Asian and American ethnic populations. So, future studies in other ethnic populations are required in this regard. Thirdly, since AD is a complex disease, so different statuses in AD may affect the results of the study; but no detailed information of the AD diagnostic criteria was available in the previous individual studies. Therefore, there is a need for future case control studies with more comprehensive information, as different diagnostic criteria may have possible influence on the diagnosis of AD. Fourthly, there is numerous numbers of polymorphisms in BDNF and our study only focused on two polymorphisms of BDNF, which may not fully illustrate the function of this gene. Hence, studies investigating a wider range of polymorphisms are required in this regard.

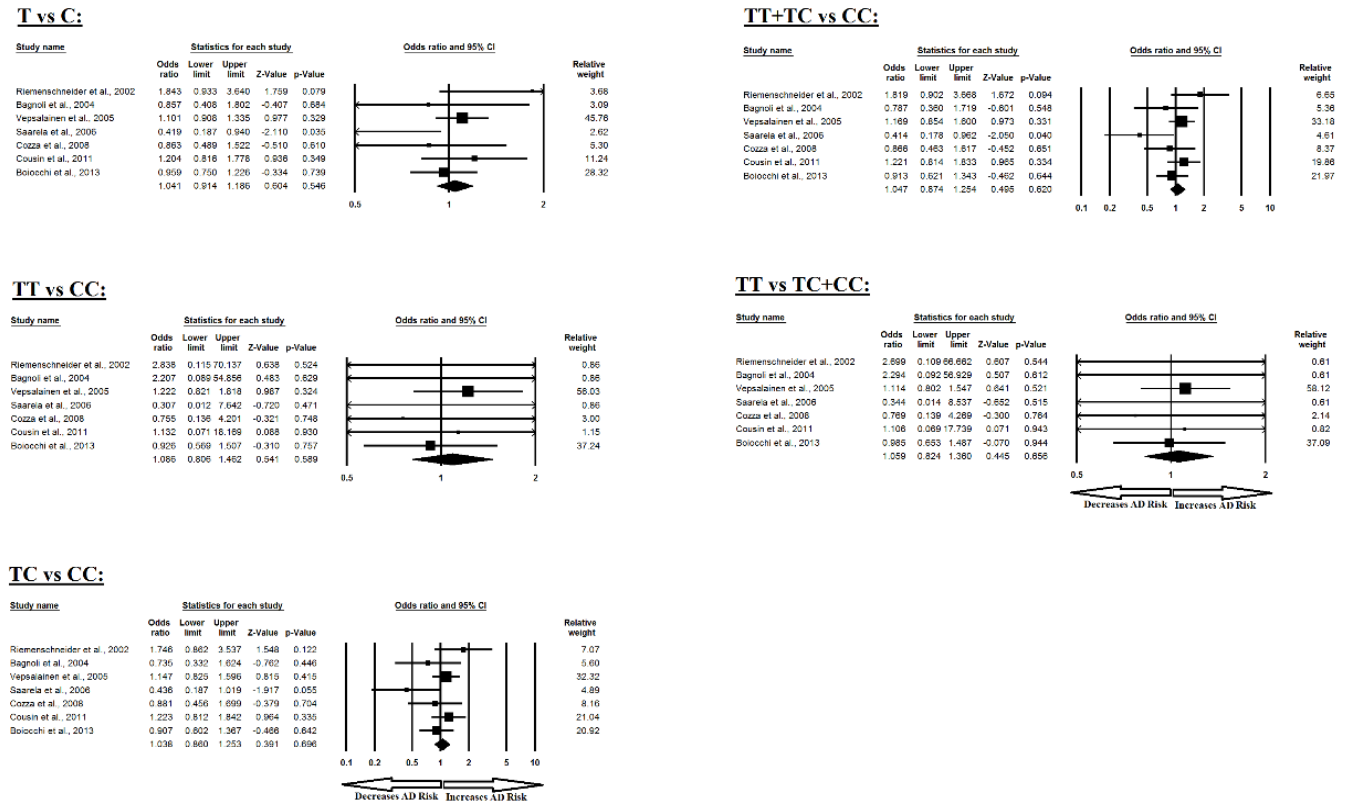

Figure 9: Forest-plot of a meta-analysis of the association between BDNF gene 270 C>T polymorphism (T vs. C; TT vs. CC; TC vs. CC; TT+TC vs. CC; TT vs. TC+CC) and European AD risk.

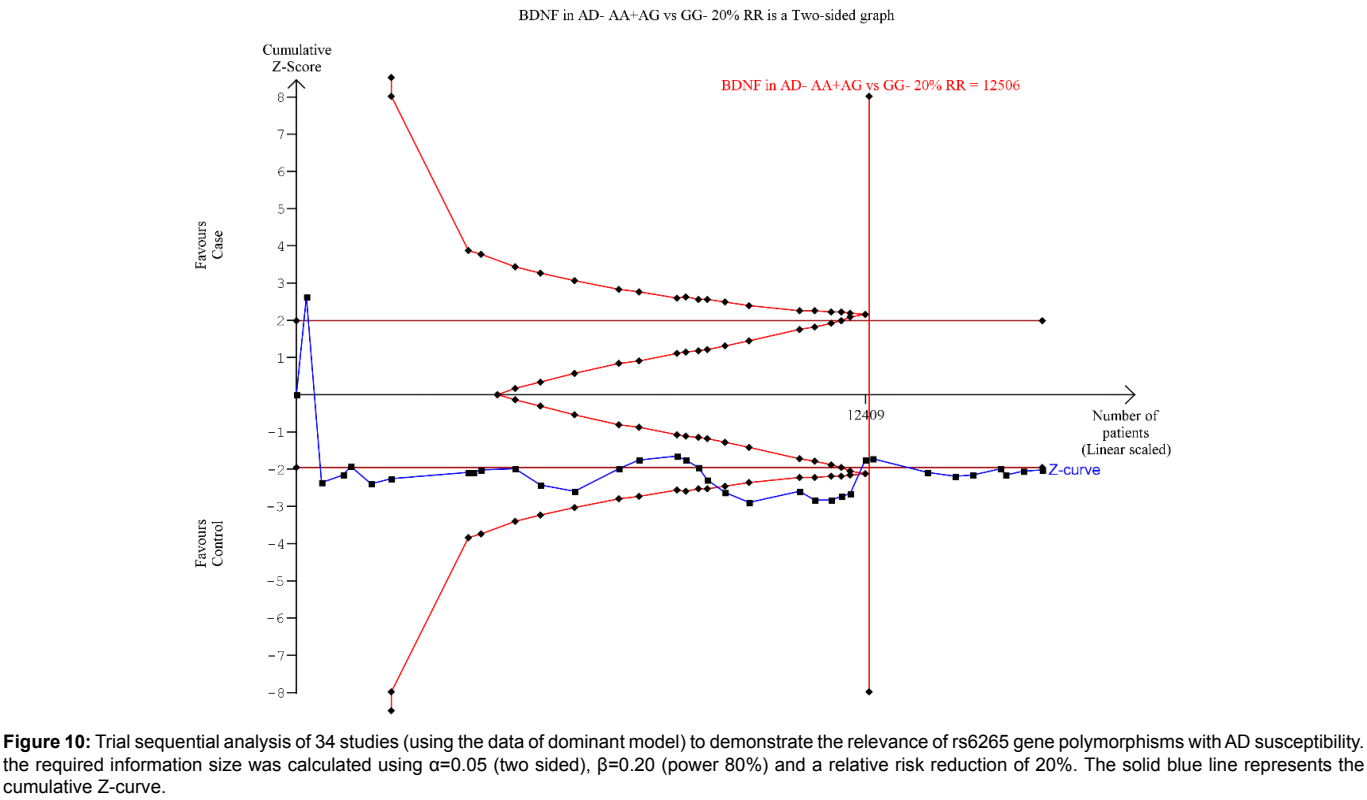

Figure 10: Trial sequential analysis of 34 studies (using the data of dominant model) to demonstrate the relevance of rs6265 gene polymorphisms with AD susceptibility. the required information size was calculated using α=0.05 (two sided), β=0.20 (power 80%) and a relative risk reduction of 20%. The solid blue line represents the cumulative Z-curve.

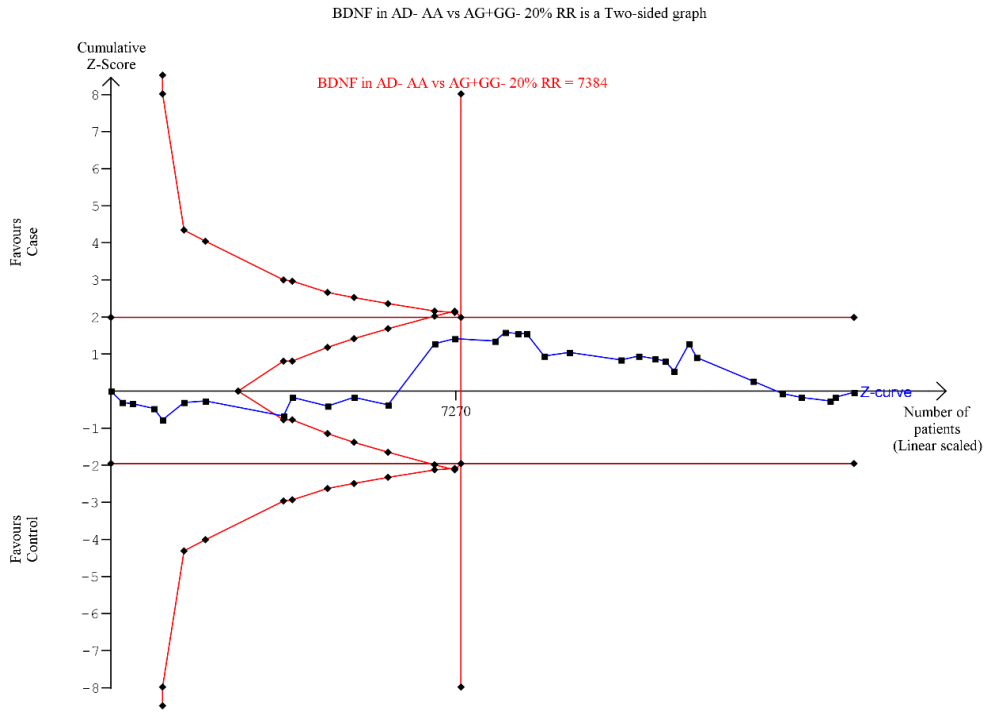

Figure 11: Trial sequential analysis of 34 studies (using the data of recessive model) to demonstrate the relevance of rs6265 gene polymorphisms with AD susceptibility. The required information size was calculated using α=0.05 (two sided), β=0.20 (power 80%) and a relative risk reduction of 20%. the solid blue line represents the cumulative Z-curve.

Conclusion

In conclusion, the present comprehensive meta-analysis revises the previous incomplete data and suggests no significant association between the BDNF G196A and BDNF C270T polymorphisms with the risk of AD under any genetic models and in any ethnic populations. Since potential biases and confounders could not be ruled out completely in this study, further studies focusing on a wider range of ethnic populations are required to confirm the results of the study.

Acknowledgement

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