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Gender Specific Effect of Brain-Derived Neurotrophic Factor (BDNF) Gene SNP G196A on Susceptibility to Alzheimer's Disease: A Meta-Analysis

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Abstract:
Alzheimer's Disease (AD) is one of the most common age-associated neurodegenerative disorders worldwide. The pathogenesis of AD has been found to be associated to genetic polymorphisms in several case-control and cohort studies for the BDNF -196G>A (rs6265) gene polymorphism but have yielded inconsistent results. In this study, PubMed, EMBASE, and Science Direct web-databases were searched for relevant reports, showing association of BDNF -196G>A gene with AD risk. A total of 19 reports involving 5238 AD cases and 5492 controls were included. Significant associations in five genetic models, i.e. allelic (A vs G: p = 0.001; OR = 1.139, 95% CI = 1.051 to 1.233); homozygous (AA vs GG: p = 0.016; OR = 1.264, 95% CI = 1.044 to 1.530); heterozygous (AG vs GG: p = 0.015; OR = 1.146, 95% CI = 1.027 to 1.279) ; and dominant (AA+AG vs GG: p = 0.003; OR = 1.175, 95% CI = 1.058 to 1.305) were found in overall analysis in case of female subjects but not in male subjects. The results suggest that BDNF -196G>A gene polymorphism significantly contributes to AD susceptibility in females. Trial Sequential Analysis (TSA) was also conducted and results showed that the conclusions in this meta-analysis are robust.

Keywords: BDNF, Alzheimer's disease, polymorphism, meta-analysis, sex difference.

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 (Mucke, 2009). Atrophied basal forebrain cholinergic neurons and the limbic structures are the main neuropathological characters of AD patients (Mattson, 2004). The number of people with AD worldwide in 2006 was estimated at 26.6 million, and is predicted to nearly quadruple by 2050(Brookmeyer, Johnson, Ziegler-Graham, and Arrighi, 2007).

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 (Reitz, Brayne, and Mayeux, 2011). 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(Armstrong, 2013). Mutations in several genes, i.e. gene encoding Amyloid Precursor Protein (APP) (Goate et al., 1991), presenilin-2 (Levy-Lahad et al., 1995), Epsilon 4 allele of the Apolipoprotein E (APOE) (Saunders et al., 1993) are known to increase susceptibility to familial AD or sporadic AD or both. However, as AD is a genetically 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.

As we know that neurotrophins like Nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF) and Neurotrophin-3 (NT-3) are responsible for growth, development, differentiation and regeneration of various types of neurons in the central nervous system. So, polymorphism in these genes may increase the susceptibility to AD (Reichardt, 2006). Several findings have suggested that Brain-derived neurotrophic factor (*BDNF*) gene is believed to be one of the important candidate genes for AD risk (Borroni, 2010 and Serretti, 2007). Studies have already shown reduced mRNA expression of BDNF in the hippocampus and temporal cortices of AD patients (Connor et al., 1997). Moreover, another study has also revealed decrease in BDNF/Tyrosine receptor kinase B (TrKB) neurotrophic signaling pathway in the frontal cortex and hippocampus of AD patients (Ferrer et al., 1999), suggesting the possible important role of BDNF in AD pathogenesis.

The association between AD and the G196A polymorphism in the BDNF gene has already been investigated in several individual case-control studies, producing inconsistent results but very few studies are there for gender specific effect of this polymorphism in BDNF. Since, there are many evidences in support of gender specific effects of BDNF in different disorders like major depressive disorders (Verhagen et al., 2008), PD (Foltynie, 2005), etc. So, we have tried to perform the comprehensive meta-analysis for the association between G196A polymorphism and AD in both sexes separately by combining data from different individual studies and different ethnicities also. Moreover, we also conducted Trial Sequential Analysis (TSA) of all the published case–control studies in the hope of validating the results 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 (Moher et al., 2009 and Green and McDonald, 2007). Literature search was carried out within PubMed (Medline), EMBASE and Science Direct database up to December, 2019, using the keywords- bdnf, patient, bdnf level, bdnf gene, polymorphism and Alzheimer's disease. Then, potentially relevant publications and studies 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 in both genders 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 would be 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 allele with the risk of AD relative to the G allele, the contrast of homozygotes AA vs GG, the contrast of heterozygotes AG vs GG, the recessive model for the A allele: contrast AA vs (AG+GG), and the dominant model for the A allele: contrast (AA+AG) vs GG in both genders separately. 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 in both genders with AD risk separately. Heterogeneity was assessed by Chi-square based Q-Test (Wu, R. and B. Li, 1999). If heterogeneity existed, then random effects model was used to calculate the overall pooled OR value (DerSimonian, R. and N. Laird, 1986); otherwise, the fixed effect model was used (Mantel, N. and W. Haenszel, 1959). Moreover, I^2 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 (Higgins et al., 2003). 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 (Harbord et al., 2006). p value less than 0.05 was 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) (Eggeret al., 1997). 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 (Wetterslev et al., 2008; Brok et al., 2009 and Xie et al. 2014). 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 (Wetterslev et al., 2008; Brok et al., 2009 and Turner et al. 2013). 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 (Wetterslev et al., 2009). 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 (Holst et al., 2015). 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 19 studies eligible for inclusion in our analysis as described in Flow Chart (Figure 1). Based on our preliminary search criteria, a total of 234 studies were identified in PubMed (Medline), EMBASE and Science Direct using the keywords- bdnf, patient, bdnf level, bdnf gene, polymorphism, Alzheimer disease and their combination. After careful review, finally, 19 potential studies were included. According to our inclusion criteria, 13 studies have not been included for estimating OR and 95% CI because they didn't report genotypic frequency of patients and healthy controls of male and female separately (Ventriglia et al., 2002; Bagnoli et al., 2004; Bodner et al., 2005; Nishimura et al., 2005; Vepsalainen et al., 2005; Zhang et al., 2006; Huang et al., 2007; Cozza et al., 2008; Feher et al., 2009; Borroni et al., 2012;Sonali et al., 2013; Vieira et al., 2015; and Gomar et al., 2016). Finally, 19 eligible studies involving 5238 cases and 5492 controls were enrolled in the pooled analyses.

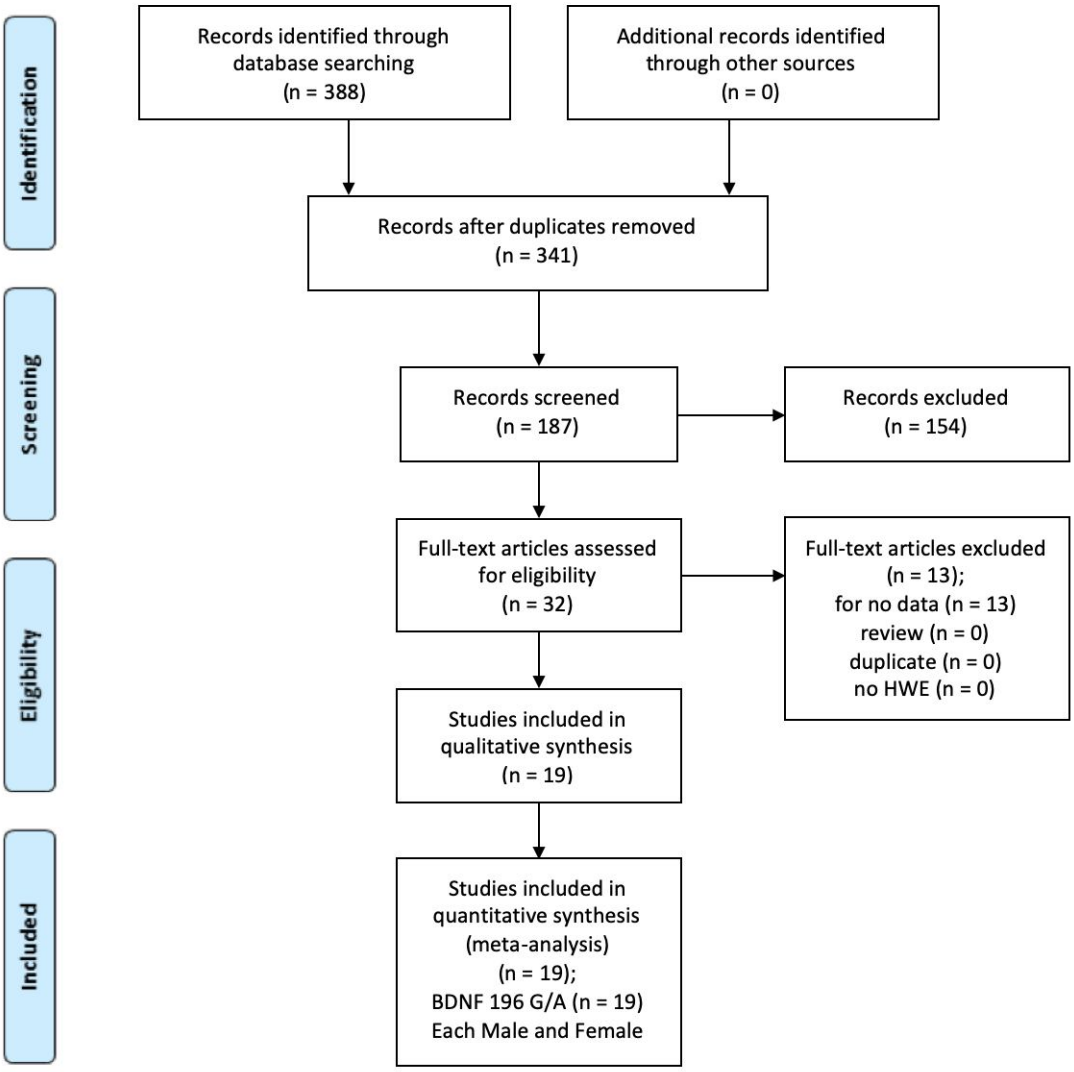

Figure 1: PRISMA flow chart showing the selection process (inclusion/exclusion) of the relevant studies of BDNF-196 G > A (rs6265) polymorphism and AD risk.

Table 1: Summary of main Characteristics of all the studies included in the present meta-analysis

First Author & Year	Country	Ethnicity	Cases	Control	Genotyping Technique Used	SNP	Association Yes/No
Combarros et al., 2004	Spain	European	237	218	PCR	G >A	No
Nacmias et al., 2004	Italy	European	83	97	PCR-RFLP	G >A	No
Bian et al., 2005	China	Asian	203	239	PCR	G >A	No
Desai et al., 2005[a]	USA	American	995	671	Pyrosequencing	G >A	No
Desai et al., 2005[b]	USA	African	64	45	Pyrosequencing	G >A	No
Lee et al., 2005	USA	American	95	70	PCR-RFLP	G >A	No
Li et al., 2005[c]	England	European	359	396	Allele-specific Real Time PCR	G >A	No
Li et al., 2005[d]	USA	American	188	361	Allele-specific Real Time PCR	G >A	No
Li et al., 2005[e]	USA	American	388	349	Allele-specific Real Time PCR	G >A	No
Matsushita et al., 2005	Japan	Asian	487	471	PCR	G >A	Yes
Akatsu et al, 2006	Japan	Asian	95	108	PCR-RFLP	G >A	No
Forero et al., 2006	Colombia	Mixed	101	168	PCR	G >A	Yes
Saarela et al., 2006	Finland	European	97	101	PCR	G >A	No
Tsai et al., 2006	China	Asian	175	189	PCR	G >A	Yes
He et al.,2007	China	Asian	513	575	Allele-specific PCR	G >A	No
Yu et al., 2008	China	Asian	99	99	PCR	G >A	No
Fukumoto et al., 2010	Japan	Asian	657	525	Taqman PCR Assay	G >A	Yes
Pivac et al., 2011	Croatia	European	211	402	Taqman based Allele-specific PCR Assay	G >A	No
Boiocchi et al., 2013	Italy	European	191	408	PCR-RFLP	G >A	Yes

[a]American Whites sample from University of Pittsburgh, Alzheimer's Disease Research Center (ADRC).
[b]American Blacks sample from University of Pittsburgh, Alzheimer's Disease Research Center (ADRC).
[c]UK sample from Cardiff University, Wales College of Medicine and King's College London.
[d]UCSD sample from the University of California, San Diego.
[e]WashU sample from the Washington University.

The populations came from 9 different countries, including China, Colombia, Croatia, England, Finland, Italy, Japan, Spain and USA. Detailed characteristics of all eligible studies included in meta-analysis are reported in Table 1. In both genders i.e. male and female separately, one overall study was conducted on BDNF 196GA polymorphism and 3 ethnicity specific studies were conducted, that includes 4 studies on American populations (Desai et al., 2005; Lee et al., 2005; and Li et al., 2005), 7 studies on Asian populations (Bian et al., 2005; Matsushita et al., 2005; Akatsu et al., 2006; Tsai et al., 2006; He et al., 2007; Yu et al., 2008; and Fukumoto et al., 2010), and 6 studies on European populations (Li et al., 2005; Combarros et al., 2004; Nacmias et al., 2004;Saarela et al., 2006; Pivac et al., 2011; and Boiocchi et al., 2013). Table 2 and Table 3 reports genotypic distribution of G196A polymorphism of BDNF gene in both male and female subjects separately from each study. All studies observed HWE.

Table 2: Genotypic distribution of BDNF -196 G>A gene polymorphism in male subjects included in the present meta-analysis.

First Author & Year	Cases				Controls				HWE
	Genotype			Minor Allele	Genotype			Minor Allele	
	GG	GA	AA	MAF	GG	GA	AA	MAF	p-value
Combarros et al., 2004	42	31	3	0.243	38	23	2	0.214	0.504
Nacmias et al.,2004	12	10	3	0.32	16	16	4	0.333	1.000
Bian et al., 2005	29	46	20	0.452	37	68	29	0.470	0.829
Desai et al., 2005[a]	216	98	15	0.194	169	82	9	0.192	0.805
Desai et al., 2005[b]	17	1	0	0.027	11	1	0	0.041	0.880
Lee et al., 2005	14	19	1	0.308	12	16	4	0.375	0.706
Li et al., 2005[c]	46	26	0	0.180	56	28	5	0.213	0.551
Li et al., 2005[d]	54	38	2	0.223	81	39	6	0.202	0.643
Li et al., 2005[e]	88	45	7	0.210	87	45	2	0.182	0.151
Matsushita et al., 2005	54	77	16	0.370	46	69	35	0.463	0.358
Akatsu et al, 2006	9	22	6	0.459	5	11	6	0.522	0.992
Forero et al., 2006	21	7	0	0.125	41	11	1	0.122	0.795
Saarela et al., 2006	16	3	0	0.078	35	7	3	0.144	0.012
Tsai et al., 2006	24	42	25	0.505	31	45	12	0.392	0.495
He et al.,2007	63	93	39	0.438	68	115	60	0.483	0.413
Yu et al., 2008	18	30	14	0.467	17	24	9	0.42	0.916
Fukumoto et al., 2010	76	114	40	0.421	75	106	39	0.418	0.883
Pivac et al., 2011	32	20	2	0.222	122	59	6	0.189	0.725
Boiocchi et al., 2013	44	21	6	0.232	101	69	13	0.259	0.796

MAF: Minor Allele Frequency; HWE: Hardy Weinberg Equilibrium.

Table 3: Genotypic distribution of BDNF -196 G>A gene polymorphism in female subjects included in the present meta-analysis.

First Author & Year	Cases				Controls				HWE
	Genotype			Minor Allele	Genotype			Minor Allele	
	GG	GA	AA	MAF	GG	GA	AA	MAF	p-value
Combarros et al., 2004	107	47	7	0.189	105	44	6	0.180	0.609
Nacmias et al.,2004	36	19	3	0.215	39	22	0	0.180	0.085
Bian et al., 2005	20	67	21	0.504	36	47	22	0.433	0.364
Desai et al., 2005[a]	449	201	19	0.178	287	115	9	0.161	0.522
Desai et al., 2005[b]	42	4	0	0.043	31	2	0	0.030	0.857
Lee et al., 2005	31	28	2	0.262	20	14	4	0.289	0.519
Li et al., 2005[c]	178	73	14	0.190	192	73	5	0.153	0.518
Li et al., 2005[d]	51	32	4	0.229	150	67	9	0.188	0.660
Li et al., 2005[e]	163	81	4	0.179	150	60	5	0.162	0.727
Matsushita et al., 2005	117	170	53	0.405	104	154	63	0.436	0.659
Akatsu et al, 2006	16	36	6	0.413	30	42	14	0.406	0.913
Forero et al., 2006	51	20	2	0.164	90	23	2	0.117	0.708
Saarela et al., 2006	45	21	2	0.183	46	10	0	0.089	0.463
Tsai et al., 2006	19	50	15	0.476	33	50	18	0.425	0.900
He et al.,2007	92	152	74	0.471	97	170	65	0.451	0.539
Yu et al., 2008	13	11	13	0.5	11	27	11	0.5	0.475
Fukumoto et al., 2010	142	205	80	0.427	122	143	40	0.365	0.850
Pivac et al., 2011	103	39	15	0.219	146	59	10	0.183	0.212
Boiocchi et al., 2013	69	42	9	0.25	130	81	14	0.242	0.771

MAF: Minor Allele Frequency; HWE: Hardy Weinberg Equilibrium.

Association of BDNF SNP rs6265 polymorphism in male subjects 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 in male subjects. Moreover, no associations were identified between *BDNF* 196 G/A polymorphism and AD in ethnicity specific studies (i.e. American, Asian and European) in male subjects also.

The pooled ORs of overall study analysis in male subjects revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs G: $p = 0.551$; OR = 0.970, 95% CI = 0.877 to 1.073) genetic models; homozygous (AA vs GG: $p = 0.403$; OR = 0.904, 95% CI = 0.714 to 1.145) genetic models; heterozygous (AG vs GG: $p = 0.853$; OR = 1.014, 95% CI = 0.879 to 1.169) genetic models; dominant (AA+AG vs GG: $p = 0.911$; OR = 0.992, 95% CI = 0.866 to 1.137) genetic models; and recessive (AA vs AG+GG: $p = 0.382$; OR = 0.910, 95% CI = 0.735 to 1.125) genetic models. All ORs were pooled through a fixed effect model.

Similarly, the pooled ORs of American study analysis in male subjects revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs G: $p = 0.636$; OR = 1.050, 95% CI = 0.857 to 1.288) genetic models; homozygous (AA vs GG: $p = 0.692$; OR = 1.141, 95% CI = 0.593 to 2.197) genetic models; heterozygous (AG vs GG: $p = 0.755$; OR = 1.041, 95% CI = 0.810 to 1.338) genetic models; dominant (AA+AG vs GG: $p = 0.661$; OR = 1.056, 95% CI = 0.829 to 1.345) genetic models; and recessive (AA vs AG+GG: $p = 0.728$; OR = 1.122, 95% CI = 0.587 to 2.145) genetic models. All ORs were pooled through a fixed effect model.

Additionally, the pooled ORs of Asian study analysis in male subjects revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs G: $p = 0.353$; OR = 0.939, 95% CI = 0.821 to 1.073) genetic models; homozygous (AA vs GG: $p = 0.648$; OR = 0.903, 95% CI = 0.582 to 1.401) genetic models; heterozygous (AG vs GG: $p = 0.916$; OR = 0.988, 95% CI = 0.797 to 1.225) genetic models; dominant (AA+AG vs GG: $p = 0.639$; OR = 0.953, 95% CI = 0.778 to 1.167) genetic models; and recessive (AA vs AG+GG: $p = 0.607$; OR = 0.901, 95% CI = 0.607 to 1.339) genetic models. All ORs were pooled through a fixed effect model except for homozygous and recessive genetic model.

Moreover, the pooled ORs of European study analysis in male subjects revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs G: $p = 0.765$; OR = 0.964, 95% CI = 0.757 to 1.227) genetic models; homozygous (AA vs GG: $p = 0.838$; OR = 0.932, 95% CI = 0.474 to 1.831) genetic models; heterozygous (AG vs GG: $p = 0.929$; OR = 1.014, 95% CI = 0.748 to 1.373) genetic models; dominant (AA+AG vs GG: $p = 0.899$; OR = 0.981, 95% CI = 0.734 to 1.313) genetic models; and recessive (AA vs AG+GG: $p = 0.948$; OR = 0.978, 95% CI = 0.505 to 1.896) genetic models. All ORs were pooled through a fixed effect model.

Association of BDNF SNP rs6265 polymorphism in female subjects with AD

The meta-analysis results based on different genetic models revealed association between BDNF 196 G/A allele in overall studies in female subjects for A vs G allelic contrast, AA vs GG homozygous genotype, AG vs GG heterozygous genotype and dominant AA+AG vs GG genotype. Ethnicity specific studies also showed association with *BDNF* 196 G/A polymorphism and AD in European female subjects for A vs G allelic contrast, AA vs GG homozygous genotype and recessive AA vs AG+GG genotype. However, no associations were identified for American and Asian female subjects.

The pooled ORs of overall study analysis in female subjects revealed that BDNF G>A gene polymorphism is associated with AD risk in allelic (A vs G: $p = 0.001$; OR = 1.139, 95% CI = 1.051 to 1.233) genetic models; homozygous (AA vs GG: $p = 0.016$; OR = 1.264, 95% CI = 1.044 to 1.530) genetic models; heterozygous (AG vs GG: $p = 0.015$; OR = 1.146, 95% CI = 1.027 to 1.279) genetic models; and dominant (AA+AG vs GG: $p = 0.003$; OR = 1.175, 95% CI = 1.058 to 1.305) genetic models; but not associated with recessive (AA vs AG+GG: $p = 0.077$; OR = 1.169, 95% CI = 0.983 to 1.390) genetic models. All ORs were pooled through a fixed effect model.

Similarly, the pooled ORs of American study analysis in female subjects revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs G: $p = 0.157$; OR = 1.130, 95% CI = 0.954 to 1.338) genetic models; homozygous (AA vs GG: $p = 0.899$; OR = 1.038, 95% CI = 0.587 to 1.834) genetic models; heterozygous (AG vs GG: $p = 0.081$; OR = 1.196, 95% CI = 0.978 to 1.463) genetic models; dominant (AA+AG vs GG: $p = 0.092$; OR = 1.183, 95% CI = 0.973 to 1.439) genetic models; and recessive (AA vs AG+GG: $p = 0.911$; OR = 0.968, 95% CI = 0.550 to 1.703) genetic models. All ORs were pooled through a fixed effect model.

Moreover, the pooled ORs of Asian study analysis in female subjects revealed that BDNF G>A gene polymorphism is not associated with AD risk in allelic (A vs G: $p = 0.084$; OR = 1.101, 95% CI = 0.987 to 1.228) genetic models; homozygous (AA vs GG: $p = 0.140$; OR = 1.186, 95% CI = 0.945 to 1.489) genetic models; heterozygous (AG vs GG: $p = 0.237$; OR = 1.202, 95% CI = 0.886 to 1.630) genetic models; dominant (AA+AG vs GG: $p = 0.071$; OR = 1.164, 95% CI = 0.987 to 1.373) genetic models; and recessive (AA vs AG+GG: $p = 0.367$; OR = 1.096, 95% CI = 0.898 to 1.338) genetic models. All ORs were pooled through a fixed effect model except for heterozygous genetic model.

However, the pooled ORs of European study analysis in female subjects revealed that BDNF G>A gene polymorphism is associated with AD risk in allelic (A vs G: $p = 0.024$; OR = 1.214, 95% CI = 1.026 to 1.436) genetic models; homozygous (AA vs GG: $p = 0.010$; OR = 1.848, 95% CI = 1.161 to 2.943) genetic models and recessive (AA vs AG+GG: $p = 0.009$; OR = 1.838, 95% CI = 1.161 to 2.908) genetic models but not associated with heterozygous (AG vs GG: $p = 0.610$; OR = 1.056, 95% CI = 0.857 to 1.301) genetic models and dominant (AA+AG vs GG: $p = 0.165$; OR = 1.151, 95% CI = 0.944 to 1.404) genetic models. All ORs were pooled through a fixed effect model.

Evaluation of publication bias

No between-study heterogeneity was found in analyses of the *BDNF* 196 G/A polymorphism in both male and female subjects 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.

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 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)] in both male and female subjects separately 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)

Nineteen trials (10730 subjects) were used to investigate the association of rs6265 gene polymorphism with AD risk in both male and female separately. Using the data of dominant model for rs6265 in female (including 19 trials with 10730 subjects) as an example, the TSA was performed and found that the required information size (RIS) is 3611 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 (Figure2). 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 (Figure3).

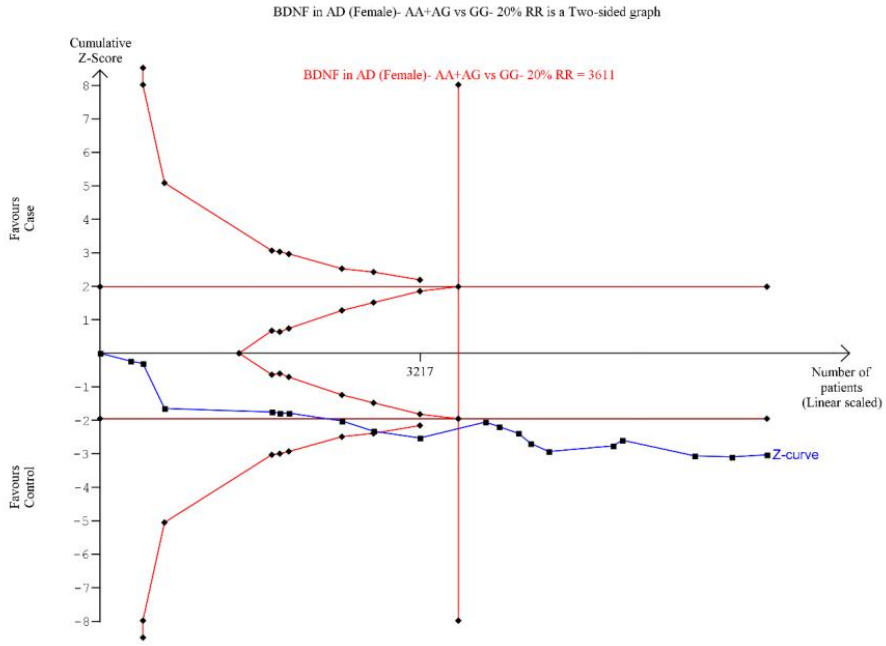

Figure 2: Trial sequential analysis of 19 studies (using the data of dominant model) to demonstrate the relevance of rs6265 gene polymorphisms with AD susceptibility in female subjects

Note: The required information size was calculated using $\alpha = 0.05$ (two sided), $\beta = 0.20$ (power 80%) and a relative risk reduction of 20%. The solid blue line represents the cumulative Z-curve.

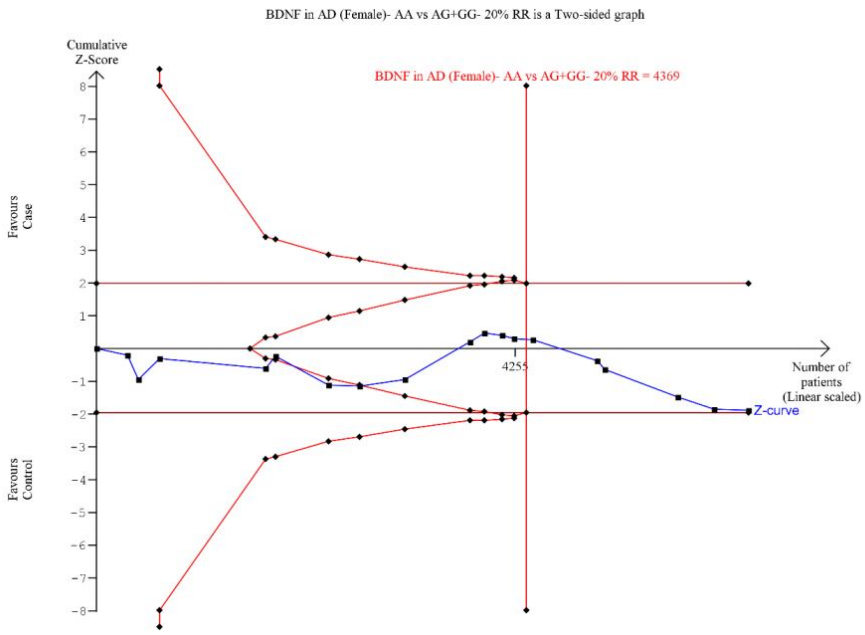

Figure 3: Trial sequential analysis of 19 studies (using the data of recessive model) to demonstrate the relevance of rs6265 gene polymorphisms with AD susceptibility in female subjects

Similarly, for male subjects, we chose the data of all models to perform TSA. The cumulative Z-curve have not crossed with TSA monitoring boundaries before the required information size is reached, indicating that cumulative evidence is insufficient and further trials are necessary.

Moreover, when we performed the sub-analysis based on the ethnicity (American, Asian and European) for all models for both male and female subjects, the cumulative Z-curve crossed with TSA monitoring boundary for allelic model of European female only, confirming that cumulative evidence is sufficient and further relevant trials are not necessary.

DISCUSSION

In the present study, 19 studies covering 5,238 cases and 5,492 controls have been carried out to investigate the association of the *BDNF* G196A variant with the risk of AD in both male and females separately. 16 articles related to rs6265 were included in our meta-analysis. However, the results showed significant associations between *BDNF* G196A with AD in overall studies along with European ethnic studies also in females. In contrast, no such association was observed in case of males. These results led to evidence that this G196A or Val66Met allelic polymorphism has increasing AD risk in females, but not in males.

Earlier, studies have shown that the *BDNF* Val66Met polymorphism has been shown to be associated with reduced transport of BDNF from the Golgi region to appropriate secretory granules in neurons (Egan et al., 2003; and del Toro et al., 2006). This might be associated with the impaired or decreased BDNF secretion (McHughen et al., 2010), resulting in the change of brain morphology, decreased brain structures (e.g., hippocampus) and cognitive function (Pezawas et al., 2004). Also, it has been reported that this polymorphism had shown more widespread age associated volume reduction in the dorsolateral prefrontal cortices (Nemoto et al., 2006). 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 (Egan et al., 2003).

There are many evidences in support of the gender specific effects of BDNF like sexually dimorphic effects in depression and anxiety related behavior in BDNF knockout mice (Monteggia et al., 2007); more important role of Val66 Met polymorphism in the development of major depressive disorder in men than in women (Verhagen et al., 2008); and gender specific effect of BBNF in Parkinson's disease (Foltynie et al., 2005). Lot of earlier studies have already reported higher occurrence of AD in women than in men (Fratiglioni et al., 1997). These findings support with our observations of the gender specific effect of BDNF on AD. It might be probable as estrogen plays an important role in the BDNF expression like in a study, it has been found that estrogen receptors co-localize with BDNF synthesizing neurons in the forebrain (Miranda et al., 1993) and estrogen induces BDNF expression through the estrogen response element (Sohrabji et al., 1995).

Individual studies have reported 5 positive results (Matsushita et al., 2005; Tsai et al., 2006; Fukumoto et al., 2010; Boiocchi et al., 2013; and Forero et al., 2006)and 14 negative results (Desai et al., 2005; Lee et al., 2005; Li et al., 2005; Bian et al., 2005; Akatsu et al., 2006; He et al., 2007; Yu et al., 2008; Combarroset al., 2004;Nacmias et al., 2004; Saarela et al., 2006; and Pivac et al., 2011) among the previous association studies between the *BDNF* G196A polymorphism and AD. In the present meta-analysis, significant association was found between *BDNF* G196A and AD (P>0.05 in females. The present meta-analysis of *BDNF* G196A included 19 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, and European females were found to be significantly associated.

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 in both genders showed a more reliable conclusion.

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. So, 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 are numerous numbers of polymorphism in *BDNF* and our study only focused on single polymorphism of *BDNF*, which may not fully illustrate the function of this gene. So, studies investigating a wider range of polymorphisms are required in this regard.

In conclusion, the present comprehensive meta-analysis revises the previous incomplete data and suggests the meta-analytic evidence that *BDNF* G196A polymorphism has gender specific effect on AD susceptibility and is found to be significantly associated with the risk of AD in females and in European female populations also. 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. Further studies explaining molecular mechanisms for this association are also required.

Conflict of Interest

The author declares no conflict of interest.

REFERENCES

1. Mucke, L., (2009). Neuroscience: Alzheimer's disease. Nature, 461(7266), 895-897.
2. Mattson, M. P., (2004). Pathways towards and away from Alzheimer's disease. Nature, 430(7000), 631-639.
3. Brookmeyer, R., E. Johnson, K. Ziegler-Graham and H. M. Arrighi, (2007). Forecasting the global burden of Alzheimer's disease. Alzheimers Dement, 3(3), 186-191.
4. Reitz, C., C. Brayne and R. Mayeux, (2011). Epidemiology of Alzheimer disease. Nat Rev Neurol, 7(3), 137-152.
5. Armstrong, R. A., (2013). What causes alzheimer's disease? Folia Neuropathol, 51(3), 169-188.
6. Goate, A., M. C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James and et al., (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature, 349(6311), 704-706.
7. Levy-Lahad, E., W. Wasco, P. Poorkaj, D. M. Romano, J. Oshima, W. H. Pettingell, C. E. Yu, P. D. Jondro, S. D. Schmidt, K. Wang and et al., (1995). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science, 269(5226), 973-977.
8. Saunders, A. M., W. J. Strittmatter, D. Schmechel, P. H. George-Hyslop, M. A. Pericak-Vance, S. H. Joo, B. L. Rosi, J. F. Gusella, D. R. Crapper-MacLachlan, M. J. Alberts and et al., (1993). Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology, 43(8), 1467-1472.
9. Reichardt, L. F., 2006. Neurotrophin-regulated signalling pathways. Philos Trans R SocLond B Biol Sci, 361(1473), 1545-1564.
10. Borroni, B., C. Costanzi and A. Padovani, (2010). Genetic susceptibility to behavioural and psychological symptoms in Alzheimer disease. Curr Alzheimer Res, 7(2), 158-164.
11. Serretti, A., P. Olgiati and D. De Ronchi, (2007). Genetics of Alzheimer's disease. A rapidly evolving field. J Alzheimers Dis, 12(1), 73-92.
12. Connor, B., D. Young, Q. Yan, R. L. Faull, B. Synek and M. Dragunow, (1997). Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Brain Res Mol Brain Res, 49(1-2), 71-81.
13. Ferrer, I., C. Marin, M. J. Rey, T. Ribalta, E. Goutan, R. Blanco, E. Tolosa and E. Marti, (1999). BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. J NeuropatholExpNeurol, 58(7), 729-739.
14. Verhagen, M., A. van der Meij, P. A. van Deurzen, J. G. Janzing, A. Arias-Vasquez, J. K. Buitelaar and B. Franke, (2008). Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. Mol Psychiatry, 15(3), 260-271.
15. Foltynie, T., S. G. Lewis, T. E. Goldberg, A. D. Blackwell, B. S. Kolachana, D. R. Weinberger, T. W. Robbins and R. A. Barker, (2005). The BDNF Val66Met polymorphism has a gender specific influence on planning ability in Parkinson's disease. J Neurol, 252(7), 833-838.
16. Moher, D., A. Liberati, J. Tetzlaff and D. G. Altman, (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ, 339: b2535.
17. Green, S. and S. McDonald, (2005). Cochrane Collaboration: more than systematic reviews? Intern Med J, 35(1), 3-4.
18. Wu, R. and B. Li, (1999). A multiplicative-epistatic model for analyzing interspecific differences in outcrossing species. Biometrics, 55(2), 355-365.
19. DerSimonian, R. and N. Laird, (1986). Meta-analysis in clinical trials. Control Clin Trials, 7(3), 177-188.
20. Mantel, N. and W. Haenszel, (1959). Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst, 22(4), 719-748.
21. Higgins, J. P., S. G. Thompson, J. J. Deeks and D. G. Altman, (2003). Measuring inconsistency in meta-analyses. BMJ, 327(7414), 557-560.
22. Harbord, R. M., M. Egger and J. A. Sterne, (2006). A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. Stat Med, 25(20), 3443-3457.

23. Egger, M., G. Davey Smith, M. Schneider and C. Minder, (1997). Bias in meta-analysis detected by a simple, graphical test. BMJ, 315(7109), 629-634.
24. Wetterslev, J., K. Thorlund, J. Brok and C. Gluud, (2008). Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. Journal of clinical epidemiology, 61(1), 64-75.
25. Brok, J., K. Thorlund, J. r. Wetterslev and C. Gluud, (2009). Apparently conclusive meta-analyses may be inconclusive-trial sequential analysis adjustment of random error risk due to repetitive testing of accumulating data in apparently conclusive neonatal meta-analyses. International journal of epidemiology, 38(1), 287-298.
26. Xie, S., X.-F. Shan, K. Shang, H. Xu, J. He and Z.-G. Cai, (2014). Relevance of LIG4 gene polymorphisms with cancer susceptibility: evidence from a meta-analysis. Scientific reports, 4: 6630.
27. Turner, R. M., S. M. Bird and J. P. T. Higgins, (2013). The impact of study size on meta-analyses: examination of underpowered studies in Cochrane reviews. PLoS One, 8(3), e59202.
28. Wetterslev, J., K. Thorlund, J. Brok and C. Gluud, (2009). Estimating required information size by quantifying diversity in random-effects model meta-analyses. BMC medical research methodology, 9(1), 86.
29. Holst, L. B., M. W. Petersen, N. Haase, A. Perner and J. r. Wetterslev, (2015). Restrictive versus liberal transfusion strategy for red blood cell transfusion: systematic review of randomised trials with meta-analysis and trial sequential analysis. Bmj, 350: h1354.
30. Ventriglia, M., L. BocchioChiavetto, L. Benussi, G. Binetti, O. Zanetti, M. A. Riva and M. Gennarelli, (2002). Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. Mol Psychiatry, 7(2), 136-137.
31. Bagnoli, S., B. Nacmias, A. Tedde, B. M. Guarnieri, E. Cellini, C. Petruzzi, A. Bartoli, L. Ortenzi and S. Sorbi, (2004). Brain-derived neurotrophic factor genetic variants are not susceptibility factors to Alzheimer's disease in Italy. Ann Neurol, 55(3), 447-448.
32. Bodner, S. M., W. Berrettini, V. van Deerlin, D. A. Bennett, R. S. Wilson, J. Q. Trojanowski and S. E. Arnold, (2005). Genetic variation in the brain derived neurotrophic factor gene in Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet, 134B(1), 1-5.
33. Nishimura, M., S. Kuno, R. Kaji and H. Kawakami, (2005). Brain-derived neurotrophic factor gene polymorphisms in Japanese patients with sporadic Alzheimer's disease, Parkinson's disease, and multiple system atrophy. MovDisord, 20(8), 1031-1033.
34. Vepsalainen, S., E. Castren, S. Helisalmi, S. Iivonen, A. Mannermaa, M. Lehtovirta, T. Hanninen, H. Soininen and M. Hiltunen, (2005). Genetic analysis of BDNF and TrkB gene polymorphisms in Alzheimer's disease. J Neurol, 252(4), 423-428.
35. Zhang, H., F. Ozbay, J. Lappalainen, H. R. Kranzler, C. H. van Dyck, D. S. Charney, L. H. Price, S. Southwick, B. Z. Yang, A. Rasmussen and J. Gelernter, (2006). Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. Am J Med Genet B Neuropsychiatr Genet, 141B (4), 387-393.
36. Huang, R., J. Huang, H. Cathcart, S. Smith and S. E. Poduslo, (2007. Genetic variants in brain-derived neurotrophic factor associated with Alzheimer's disease. J Med Genet, 44(2), e66.
37. Cozza, A., E. Melissari, P. Iacopetti, V. Mariotti, A. Tedde, B. Nacmias, A. Conte, S. Sorbi and S. Pellegrini, (2008). SNPs in neurotrophin system genes and Alzheimer's disease in an Italian population. J Alzheimers Dis, 15(1), 61-70.
38. Feher, A., A. Juhasz, A. Rimanoczy, J. Kalman and Z. Janka, (2009). Association between BDNF Val66Met polymorphism and Alzheimer disease, dementia with Lewy bodies, and Pick disease. Alzheimer Dis AssocDisord, 23(3), 224-228.
39. Borroni, B., M. Bianchi, E. Premi, A. Alberici, S. Archetti, B. Paghera, C. Cerini, A. Papetti and A. Padovani, (2012). The brain-derived neurotrophic factor Val66Met polymorphism is associated with reduced hippocampus perfusion in frontotemporal lobar degeneration. J Alzheimers Dis, 31(2), 243-251.
40. Sonali, N., M. Tripathi, R. Sagar and S. Vivekanandhan, (2013). Val66Met polymorphism and BDNF levels in Alzheimer's disease patients in North Indian population. Int J Neurosci, 123(6), 409-416.

41. Vieira, R. N., J. D. Magalhaes, J. Sant'Anna, M. M. Moriguti, J. J. de Paula, M. T. Cintra, D. M. de Miranda, L. De Marco, E. N. de Moraes, M. A. Romano-Silva and M. A. Bicalho, (2015). The GAB2 and BDNF polymorphisms and the risk for late-onset Alzheimer's disease in an elderly Brazilian sample. IntPsychogeriatr, 27(10), 1687-1692.
42. Gomar, J. J., C. Conejero-Goldberg, E. D. Huey, P. Davies and T. E. Goldberg, (2016). Lack of neural compensatory mechanisms of BDNF val66met met carriers and APOE E4 carriers in healthy aging, mild cognitive impairment, and Alzheimer's disease. Neurobiol Aging, 39: 165-173.
43. Desai, P., R. Nebes, S. T. DeKosky and M. I. Kamboh, (2005). Investigation of the effect of brain-derived neurotrophic factor (BDNF) polymorphisms on the risk of late-onset Alzheimer's disease (AD) and quantitative measures of AD progression. Neurosci Lett, 379(3), 229-234.
44. Lee, J., H. Fukumoto, J. Orne, J. Klucken, S. Raju, C. R. Vanderburg, M. C. Irizarry, B. T. Hyman and M. Ingelsson, (2005). Decreased levels of BDNF protein in Alzheimer temporal cortex are independent of BDNF polymorphisms. ExpNeurol, 194(1), 91-96.
45. Li, Y., C. Rowland, K. Tacey, J. Catanese, J. Sninsky, J. Hardy, J. Powell, S. Lovestone, J. C. Morris, L. Thal, A. Goate, M. Owen, J. Williams and A. Grupe, (2005). The BDNF Val66Met polymorphism is not associated with late onset Alzheimer's disease in three case-control samples. Mol Psychiatry, 10(9), 809-810.
46. Bian, J. T., J. W. Zhang, Z. X. Zhang and H. L. Zhao, (2005). Association analysis of brain-derived neurotrophic factor (BDNF) gene 196 A/G polymorphism with Alzheimer's disease (AD) in mainland Chinese. Neurosci Lett, 387(1), 11-16.
47. Matsushita, S., H. Arai, T. Matsui, T. Yuzuriha, K. Urakami, T. Masaki and S. Higuchi, (2005). Brain-derived neurotrophic factor gene polymorphisms and Alzheimer's disease. J Neural Transm (Vienna), 112(5), 703-711.
48. Akatsu, H., H. D. Yamagata, J. Kawamata, K. Kamino, M. Takeda, T. Yamamoto, T. Miki, I. Tooyama, S. Shimohama and K. Kosaka, (2006. Variations in the BDNF gene in autopsy-confirmed Alzheimer's disease and dementia with Lewy bodies in Japan. Dement Geriatr Cogn Disord, 22(3), 216-222.
49. Tsai, S. J., C. J. Hong, H. C. Liu, T. Y. Liu and Y. J. Liou, (2006). The brain-derived neurotrophic factor gene as a possible susceptibility candidate for Alzheimer's disease in a chinese population. Dement Geriatr Cogn Disord, 21(3), 139-143.
50. He, X. M., Z. X. Zhang, J. W. Zhang, Y. T. Zhou, M. N. Tang, C. B. Wu and Z. Hong, (2007). Lack of association between the BDNF gene Val66Met polymorphism and Alzheimer disease in a Chinese Han population. Neuropsychobiology, 55(3-4), 151-155.
51. Yu, H., Z. Zhang, Y. Shi, F. Bai, C. Xie, Y. Qian, Y. Yuan and L. Deng, (2008). Association study of the decreased serum BDNF concentrations in amnestic mild cognitive impairment and the Val66Met polymorphism in Chinese Han. J Clin Psychiatry, 69(7), 1104-1111.
52. Fukumoto, N., T. Fujii, O. Combarros, M. I. Kamboh, S. J. Tsai, S. Matsushita, B. Nacmias, D. E. Comings, H. Arboleda, M. Ingelsson, B. T. Hyman, H. Akatsu, A. Grupe, A. L. Nishimura, M. Zatz, K. M. Mattila, J. Rinne, Y. Goto, T. Asada, S. Nakamura and H. Kunugi, (2010). Sexually dimorphic effect of the Val66Met polymorphism of BDNF on susceptibility to Alzheimer's disease: New data and meta-analysis. Am J Med Genet B Neuropsychiatr Genet, 153B(1), 235-242.
53. Combarros, O., J. Infante, J. Llorca and J. Berciano, (2004). Polymorphism at codon 66 of the brain-derived neurotrophic factor gene is not associated with sporadic Alzheimer's disease. Dement GeriatrCognDisord, 18(1), 55-58.
54. Nacmias, B., C. Piccini, S. Bagnoli, A. Tedde, E. Cellini, L. Bracco and S. Sorbi, (2004). Brain-derived neurotrophic factor, apolipoprotein E genetic variants and cognitive performance in Alzheimer's disease. Neurosci Lett, 367(3), 379-383.
55. Saarela, M. S., T. Lehtimaki, J. O. Rinne, H. Huhtala, R. Rontu, A. Hervonen, M. Roytta, J. P. Ahonen and K. M. Mattila, (2006). No association between the brain-derived neurotrophic factor 196 G>A or 270 C>T polymorphisms and Alzheimer's or Parkinson's disease. Folia Neuropathol, 44(1), 12-16.

56. Pivac, N., M. Nikolac, G. Nedic, M. Mustapic, F. Borovecki, S. Hajnsek, P. Presecki, M. Pavlovic, N. Mimica and D. Muck Seler, (2011). Brain derived neurotrophic factor Val66Met polymorphism and psychotic symptoms in Alzheimer's disease. Prog NeuropsychopharmacolBiol Psychiatry, 35(2), 356-362.
57. Boiocchi, C., E. Maggioli, M. Zorzetto, E. Sinforiani, C. Cereda, G. Ricevuti and M. Cuccia, (2013). Brain-derived neurotrophic factor gene variants and Alzheimer disease: an association study in an Alzheimer disease Italian population. Rejuvenation Res, 16(1), 57-66.
58. Egan, M. F., M. Kojima, J. H. Callicott, T. E. Goldberg, B. S. Kolachana, A. Bertolino, E. Zaitsev, B. Gold, D. Goldman, M. Dean, B. Lu and D. R. Weinberger, (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell, 112(2), 257-269.
59. del Toro, D., J. M. Canals, S. Gines, M. Kojima, G. Egea and J. Alberch, (2006). Mutant huntingtin impairs the post-Golgi trafficking of brain-derived neurotrophic factor but not its Val66Met polymorphism. J Neurosci, 26(49), 12748-12757.
60. McHughen, S. A., P. F. Rodriguez, J. A. Kleim, E. D. Kleim, L. Marchal Crespo, V. Procaccio and S. C. Cramer, (2010). BDNF val66met polymorphism influences motor system function in the human brain. Cereb Cortex, 20(5), 1254-1262.
61. Pezawas, L., B. A. Verchinski, V. S. Mattay, J. H. Callicott, B. S. Kolachana, R. E. Straub, M. F. Egan, A. Meyer-Lindenberg and D. R. Weinberger, (2004). The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. J Neurosci, 24(45), 10099-10102.
62. Nemoto, K., T. Ohnishi, T. Mori, Y. Moriguchi, R. Hashimoto, T. Asada and H. Kunugi, (2006). The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. Neurosci Lett, 397(1-2), 25-29.
63. Monteggia, L. M., B. Luikart, M. Barrot, D. Theobold, I. Malkovska, S. Nef, L. F. Parada and E. J. Nestler, (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biol Psychiatry, 61(2), 187-197.
64. Fratiglioni, L., M. Viitanen, E. von Strauss, V. Tontodonati, A. Herlitz and B. Winblad, (1997). Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen Project, Stockholm. Neurology, 48(1), 132-138.
65. Miranda, R. C., F. Sohrabji and C. D. Toran-Allerand, (1993). Neuronal colocalization of mRNAs for neurotrophins and their receptors in the developing central nervous system suggests a potential for autocrine interactions. Proc Natl Acad Sci U S A, 90(14), 6439-6443.
66. Sohrabji, F., R. C. Miranda and C. D. Toran-Allerand, (1995). Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. Proc Natl Acad Sci U S A, 92(24), 11110-11114.
67. Forero, D. A., B. Benitez, G. Arboleda, J. J. Yunis, R. Pardo and H. Arboleda, (2006). Analysis of functional polymorphisms in three synaptic plasticity-related genes (BDNF, COMT AND UCHL1) in Alzheimer's disease in Colombia. Neurosci Res, 55(3), 334-341.