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Val66Met Polymorphism in BDNF Has No Sexual and *APOE* ε4 Status-Based Dimorphic Effects on Susceptibility to Alzheimer's Disease: Evidence From an Updated Meta-Analysis of Case–Control Studies and High-Throughput Genotyping Cohorts

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Abstract

Some studies showed that Val66Met polymorphism of brain-derived neurotrophic factor (BDNF) conveys susceptibility to Alzheimer's disease (AD) in females only. However, the confounding effects of some risk factors for AD were omitted in these studies. The aim of this meta-analysis comprising 19 604 patients with AD and 26 333 controls was to reexamine the association between Val66Met and AD by conditioning the effects of age, sex, and/or apolipoprotein E (*APOE*) ε4 status. In agreement with the previous meta-analysis, Val66Met was associated with AD in females without confounding adjustment (odds ratio [OR], 1.08; 95% confidence interval [CI], 1.03-1.14; $P = .003$). Nevertheless, after adjusting for age and *APOE* ε4 status, Val66Met was not associated with AD in females (OR, 1.02; 95% CI, 0.94-1.11; $P = .57$). This comprehensive meta-analysis with the largest sample size demonstrated no association could be observed between Val66Met and AD in general or by dividing samples based on sex or *APOE* ε4.

Keywords

Alzheimer's disease, brain-derived neurotrophic factor, Val66Met polymorphism, meta-analysis, high-throughput genotyping

Introduction

Alzheimer's disease (AD) is the most common form of dementia and pathologically characterized by senile plaques, comprising amyloid β-peptide (Aβ), and neurofibrillary tangles, which in turn is consisted of hyperphosphorylated tau. These pathological changes are accompanied by deficits in axonal transport and neuronal loss.

Neurotrophins, such as brain-derived neurotrophic factor (BDNF), can promote the development, regeneration, survival, and functioning of neurons.[1] Reduced BDNF messenger RNA (mRNA) level and BDNF protein level were observed in cerebral cortices of patients with AD.[2,3] Moreover, BDNF/Tropomyosin receptor kinase B (TrkB) neurotrophic signaling pathway is selectively decreased in frontal cortex and hippocampus of patients with AD.[4,5] Arancibia et al showed potential protective effect of BDNF against Aβ-induced neurotoxicity in vitro and in vivo.[6] Furthermore, social interaction can rescue memory deficit in an AD mouse model by increasing mRNA and protein levels of BDNF in the hippocampus.[7] Recently, BDNF gene therapy was shown to prevent neuronal degeneration and to stimulate neuronal function in

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patients with AD.[8] The body of evidence demonstrates dysfunction of BDNF is critical in the development of AD and suggests polymorphisms of BDNF may confer risk of AD.

Val66Met is a functional single-nucleotide polymorphism (SNP) of *BDNF*. G>A substitution at nucleotide 196 of *BDNF* results in the Val66-to-Met amino acid change in the human BDNF protein.[9] Since a decade ago, many studies have been performed to evaluate the association between Val66Met and AD. Except a few case–control studies that showed either Val or Met allele of the SNP was associated with AD,[10-12] most studies reported no association.[13-15] One recent review argued that population stratification and uncontrolled gene–gene or gene–environment interactions were likely to account for the inconsistency.[16] Therefore, these conflicting results may be ascribed to the omitted confounding factors, such as age, sex, and apolipoprotein E (*APOE*) ε4, the strongest genetic risk factor for AD. Furthermore, some studies examined interactive effects of either sex or *APOE* ε4 with Val66Met,[17-20] but the findings are still mostly negative and conflicting. One probable explanation is that the results were underpowered and biased by limited sample sizes.

Fukumoto et al conducted a sex-based meta-analysis on the association between Val66Met and AD with a large sample size (4711 cases and 4537 controls).[21] They revealed sexually dimorphic effect of Val66Met in AD—Met allele confers susceptibility to AD in females but not in males. Recently, 1 study also reported female-specific effect of Val66Met on susceptibility to AD in 1 of their 2 independent Chinese Han cohorts.[20] However, these studies neglected to adjust for other confounding factors for AD, such as age and *APOE* ε4.

In recent years, a few genes were reported to interact with *APOE* ε4 on AD risk. Jun et al revealed *PICALM*, 1 of genome-wide association study (GWAS) identified genes, confers risk predominantly in *APOE* ε4 noncarriers.[22] Reiman et al reported *GAB2* modifies late onset AD risk in *APOE* ε4 carriers only.[23] In addition, some other genes have interactive effects with *APOE* ε4.[24] Therefore, it is necessary to evaluate whether Val66Met can confer AD risk by interacting with *APOE* ε4.

In this study, we incorporated Val66Met data from high-throughput genotyping data, which is different from ordinary meta-analysis on polymorphism. This is the first meta-analysis with the largest sample size to date to comprehensively examine the association between Val66Met and AD by introducing age, sex and *APOE* ε4 status as the confounding factors.

Methods

Search Strategy

PubMed and EmBase databases were searched for all published case–control association studies of the Val66Met polymorphism with AD. Various combination of these search terms were used: "BDNF," "brain-derived neurotrophic factor," "polymorphism," "Val66Met," "rs6265" and "Alzheimer." Furthermore, reference list of Val66Met from the AlzGene database (www.alzgene.org) were referred to.[25]

Inclusion Criteria

The titles and abstracts of all articles identified by the search strategy were retrieved for further review. Inclusion criteria for all potentially relevant articles were (1) diagnosis of AD according to the *Diagnostic and Statistical Manual of Mental Disorders* and the National Institute of Neurological Disorders and Stroke–Alzheimer Diseases and Related Disorders working group criteria,[26] (2) case–control studies reporting genotype or allele frequencies of the *BDNF* Val66Met polymorphism in patients with AD and healthy controls, and (3) genotype frequencies in Hardy-Weinberg equilibrium (HWE) for the healthy controls ($P > .05$).

Val66Met Data From High-Throughput Genotyping Data

In this study, we used Val66Met data extracted from case–control high-throughput genotyping data for AD. Twelve unrelated high-throughput genotyping cohorts were collected from NIA Genetics of Alzheimer's Disease Data Storage Site (NIAGADS). For detailed information on the 12 cohorts from NIAGADS, please refer to Supplementary Table. We also collected genotyping data from Alzheimer's Disease Neuroimaging Initiative (ADNI; the ADNI database http://www.loni.ucla.edu/ADNI/).[27] The ADNI genotyping data were generated as previously described We did not include high-throughput genotyping cohorts of which Val66Met data were imputed.

Data Extraction

Two investigators (Q.Z. and Y.S.) performed the literature search and reviewed all the results independently. Full articles were examined for further assessment if the information in the title or abstract suggested the study is possibly eligible. Data from each study were extracted independently by 2 investigators (Y.Z. and L.S.), using a standardized protocol. In case of disagreement of study inclusion, a third investigator (S.J.) was involved. The following information were extracted: first author name, year of publication, ethnicity, sex, presence or absence of *APOE* ε4, and full genotyping data of Val66Met of the studied patients. Both S.J. and Y.Q. extracted the Val66Met data from the high-throughput genotyping cohorts.

Statistical Analysis

We used R package *meta* (version 4.8-4) to perform the meta-analysis.[28] The odds ratio (OR) and 95% confidence interval (CI) of AD for the Met allele compared with the Val allele were assessed in each study using logistic regression. The study-specific ORs were then pooled with adjustment for study. Between-study heterogeneity was examined using the Cochran's Q-test by calculating the I^2 statistics. A fixed-effects model using the Mantel-Haenszel method was applied when no statistically significant heterogeneity was detected. Otherwise, a random effects DerSimonian and Laird model was applied. For ORs after adjusting for covariates, the inverse variance weighting is used for pooling.

To explore the possible sex-specific or *APOE* ε4 status-specific effect of Val66Met polymorphism on AD, 4 subgroups (female,

Table 1. Characteristics of Included Published Studies.

First Author, Year[reference number]	Ethnicity	Cases/Controls	Female (%)	Presence of *APOE* ε4 (%)
Bagnoli et al, 2004[29]	Caucasian	128/97	NA	NA
Bodner et al, 2005[30]	Caucasian	256/195	NA	NA
Combarros et al, 2004[31]	Caucasian	237/218	69.50	37.10
Cozza et al, 2008[32]	Caucasian	251/97	NA	NA
Desai et al, 2005[33]	African American	64/45	72.50	NA
Desai et al, 2005[33]	Caucasian	995/671	64.80	NA
Fehér et al, 2009[34]	Caucasian	160/164	NA	NA
Fukumoto et al, 2010[21]	Asian	657/525	61.90	NA
Giedraitis et al, 2009[35]	Caucasian	84/385	NA	NA
Huang et al, 2007[36]	Caucasian	220/128	NA	52.30
Li et al, 2005[17] (UCSD)[a]	Caucasian	188/361	57	36.40
Li et al, 2005[17] (WashU)[b]	Caucasian	388/349	62.80	41.50
Li et al, 2005[17] (UK)[c]	Caucasian	359/396	70.90	41.60
Li et al, 2008[37]	Caucasian	692/682	NA	NA
Li et al, 2017[20]	Asian	715/760	58.23	47.48
Nacmias et al, 2004[38]	Caucasian	83/97	66.10	NA
Reiman et al, 2007[23]	Caucasian	859/551	NA	NA
Saarela et al, 2006[11]	Caucasian	97/101	62.60	NA
Ventriglia et al, 2002[9]	Caucasian	130/111	NA	NA
Vepsäläinen et al, 2005[13]	Caucasian	372/464	NA	NA
Zhang et al, 2006[39]	Caucasian	295/250	NA	NA
Akatsu et al et al, 2006[18]	Asian	95/108	70.90	NA
Bian et al, 2005[40]	Asian	203/239	48.20	29.40
He et al, 2007[14]	Asian	513/575	59.70	NA
Matsushita et al, 2005[10]	Asian	487/471	69	NA
Nishimura et al, 2005[41]	Asian	172/275	NA	NA
Tsai et al, 2006[12]	Asian	175/189	50.80	NA
Forero et al, 2006[42]	Mixed	101/168	69.90	NA
Lee et al, 2005[43]	Unknown	95/70	60	NA
Pivac et al, 2011[15]	Caucasian	211/402	60.70	NA
Boiocchi et al, 2013[19]	Caucasian	191/408	57.60	NA

Abbreviations: NA, not available; *APOE*, apolipoprotein E.
[a]UCSD samples from the University of California, San Diego.
[b]WashU samples from the Washington University.
[c]UK samples from Cardiff University, Wales College of Medicine and King's College London.

male, *APOE* ε4 carrier, and *APOE* ε4 noncarrier) were created. Pooled OR and 95% CI with or without adjusting for covariates were calculated for each subgroup after excluding studies of genotype frequencies that are not in HWE in healthy controls.

Sensitivity analysis was performed by sequential exclusion of individual studies (leave-one-out analysis) for meta-analysis of total samples and subgroup meta-analyses. Publication bias was evaluated graphically using funnel plot. Begg's rank correlation test was conducted to evaluate the publication bias quantitatively.

Results

Characteristics of Published Studies Included and High-Throughput Genotyping Cohorts

After literature search and review, 31 published association studies of AD with Val66Met were included. Characteristics of the 31 studies are shown in Table 1. Characteristics of the 13 unrelated high-throughput genotyping cohorts genotyped Val66Met are shown in Table 2. Finally, 19 604 cases and 26 333 controls were included.

Val66Met Polymorphism and AD Risk

For the overall association between Val66Met and AD, phenomenal heterogeneity ($I^2 = 43\%$) was observed across studies (Supplementary Figure S1). Therefore, random effects meta-analysis was used. Val66Met was not associated with AD before (OR = 1.02, 95% CI = 0.97-1.07; $P = .40$; Supplementary Figure S1) and after adjusting for age, sex, and *APOE* ε4 status (OR, 1.00; 95% CI, 0.94-1.06; $P = .97$; Figure 1). Sensitivity analysis showed OR and P value were not statistically altered after each leave-one-out analysis. Funnel plot and Begg's test showed no evidence of publication bias (Supplementary Figure S2).

Interaction Between Val66Met Polymorphism and Sex on AD Risk

We divided samples into male and female subgroups and assessed the associations separately between Val66Met and AD in the 2 subgroups. In agreement with previous meta-analysis findings,[21] Met allele is significantly associated with

Table 2. Characteristics of Included High-Throughput Genotyping Cohorts.

Abbreviated Cohort Name[a]	Ethnicity	Cases/Controls	Female, %	Presence of *APOE* ε4, %
NIA-LOAD	Mixed	993/884	62.30	52
ADC1	Caucasian	1574/527	55.30	57.90
ADC2	Caucasian	745/165	54.20	55.10
ADC3	Caucasian	862/618	54.20	40.70
UPITT	Mixed	1424/996	63.80	42.20
TGEN II	Caucasian	1013/585	54.20	48.50
ROSMAP	Caucasian	368/1326	69.10	23.30
WashU1	Caucasian	403/225	58.10	43.60
MIRAGE	Caucasian	603/885	59.70	38.90
ACT	Unknown	567/1701	57.70	26.10
UMVUMSSM	Unknown	1240/1230	62.50	37.90
MAYO	Caucasian	841/1253	53.40	42.70
ADNI	Mixed	213/347	47	41.60

Abbreviation: *APOE*, apolipoprotein E.
[a]Cohort full names: NIA-LOAD, National Institute on Aging Genetics Initiative for Late-Onset Alzheimer's Disease; ADC1, Alzheimer's Disease Center Dataset 1; ADC2, Alzheimer's Disease Center Dataset 2; ADC3, Alzheimer's Disease Center Dataset 3; UPITT, University of Pittsburgh; TGEN II, Translational Genomics Research Institute II; ROSMAP, Religious Orders Study and Memory and Aging Project; WashU1, Washington University Dataset 1; MIRAGE, Multi Institutional Research on Alzheimer Genetics Epidemiology; ACT, Adult Changes in Thought; UMVUMSSM, University of Miami (UM), Vanderbilt University (VU) and Mount Sinai School of Medicine (MSSM); MAYO, Mayonnaise; ADNI, Alzheimer's Disease Neuroimaging Initiative.

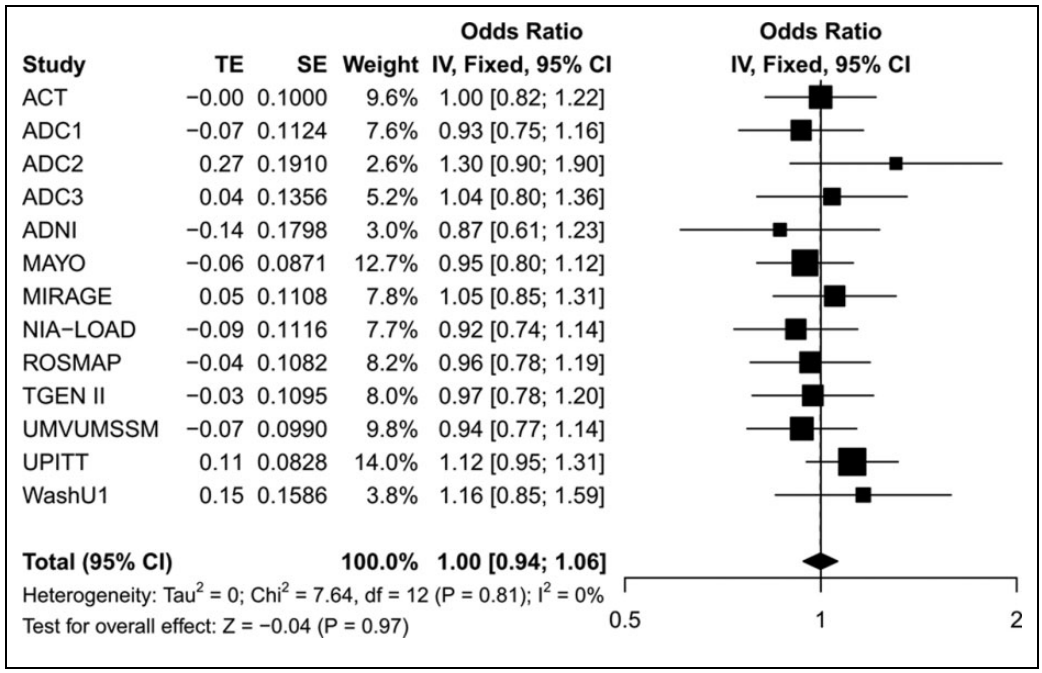

Figure 1. Forest plot of meta-analysis on the association between Val66Met polymorphism and AD after adjusting for age, sex, and *APOE* ε4 status. AD indicates Alzheimer's disease; *APOE*, apolipoprotein E.

AD in females (OR = 1.08, 95% CI = 1.03-1.14; $P = .003$; Supplementary Figure S3) without confounding adjustment. However, after adjusting for age and *APOE* ε4 status, Met allele is not associated with AD in females (OR, 1.02; 95% CI, 0.94-1.11; $P = .57$; Figure 2A). Met allele is not associated with AD in males before (OR, 0.96; 95% CI, 0.90-1.02; $P = .17$; Supplementary Figure S4) and after adjusting for age and *APOE* ε4 status (OR, 0.94; 95% CI, 0.86-1.04; $P = .22$; Figure 2B). Sensitivity analysis showed OR and P value were not statistically altered after each leave-one-out meta-analysis for each subgroup. Funnel plots and Begg's tests showed no evidence of publication bias in either female (Supplementary Figure S5) or male (Supplementary Figure S6) subgroup.

Interaction Between Val66Met Polymorphism and APOE ε4 Status on AD Risk

Likewise, we divided samples into *APOE* ε4 carrier and *APOE* ε4 noncarrier subgroups and assessed the associations between Val66Met and AD in the 2 subgroups separately. Val66Met is

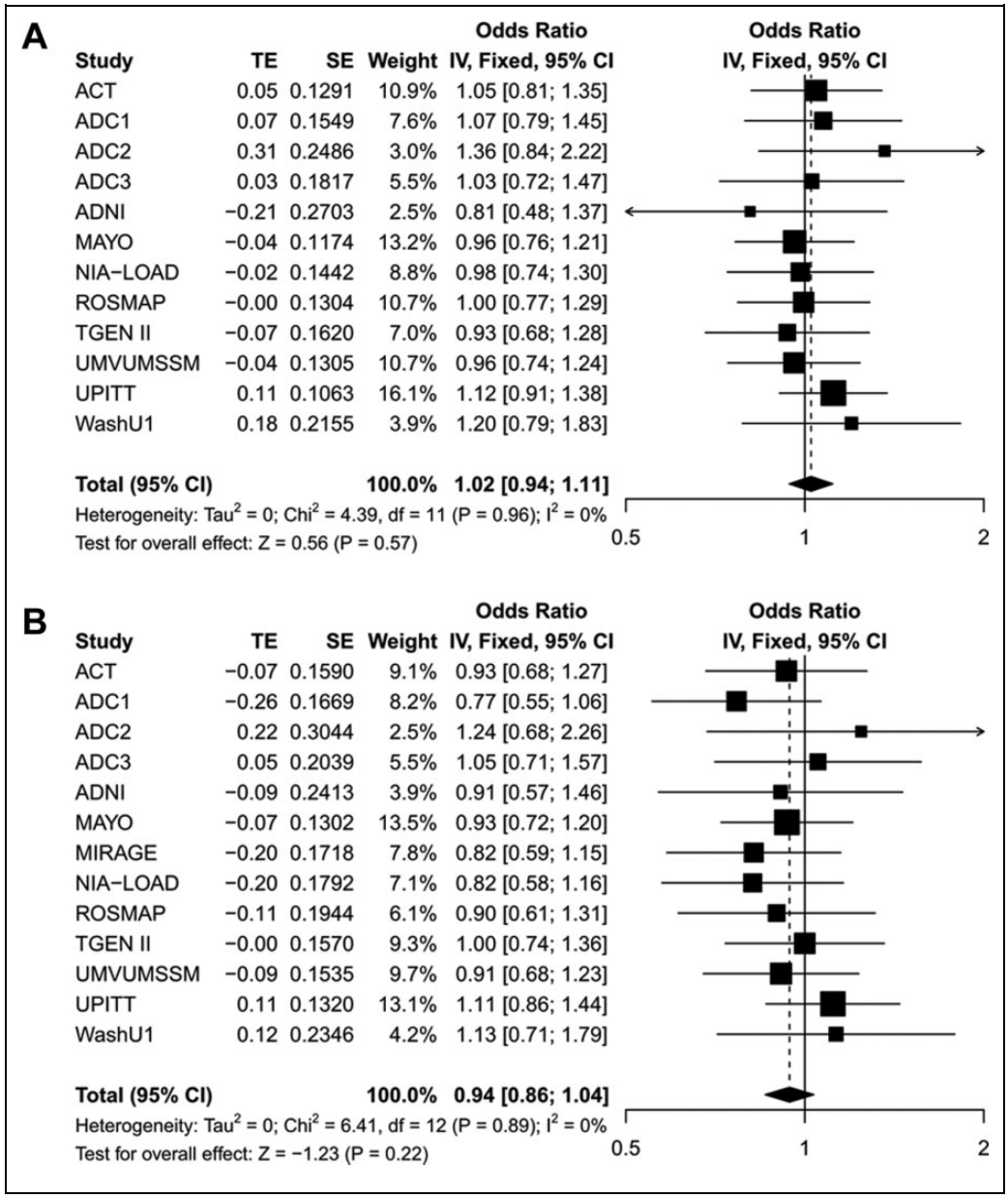

Figure 2. Forest plots of subgroup meta-analyses on the associations between Val66Met polymorphism and AD in females (A) and males (B) after adjusting for age and *APOE* ε4 status. AD indicates Alzheimer's disease; *APOE*, apolipoprotein E.

not associated with AD in *APOE* ε4 carriers before (OR = 1.02, 95% CI = 0.93-1.10; $P = .72$; Supplementary Figure S7) and after adjusting for age and sex (OR = 0.97, 95% CI = 0.88-1.07; $P = .56$; Figure 3A). Similarly, Val66Met is not associated with AD in *APOE* ε4 noncarriers before (OR = 1.05, 95% CI = 0.98-1.12; $P = .18$; Supplementary Figure S8) and after adjusting for age and sex (OR = 1.02, 95% CI = 0.94-1.11; $P = .64$; Figure 3B). Sensitivity analysis showed OR and P value were not statistically altered after each leave-one-out meta-analysis for each subgroup. Funnel plots and Begg's tests showed a slight publication bias in *APOE* ε4 noncarrier subgroup meta-analysis without adjusting for age and sex (Supplementary Figure S9A) but not in *APOE* ε4 noncarrier subgroup meta-analysis after adjusting for age and sex (Supplementary Figure S9B). No evidence of publication bias was observed in *APOE* ε4 carrier subgroup (Supplementary Figure S10).

Discussion

We conducted, for the first time, a comprehensive meta-analysis to assess the association between the Val66Met of *BDNF* and AD by introducing age, sex, and *APOE* ε4 as confounding factors. We included published studies and high-throughput genotyping cohorts with Val66Met data in this meta-analysis.

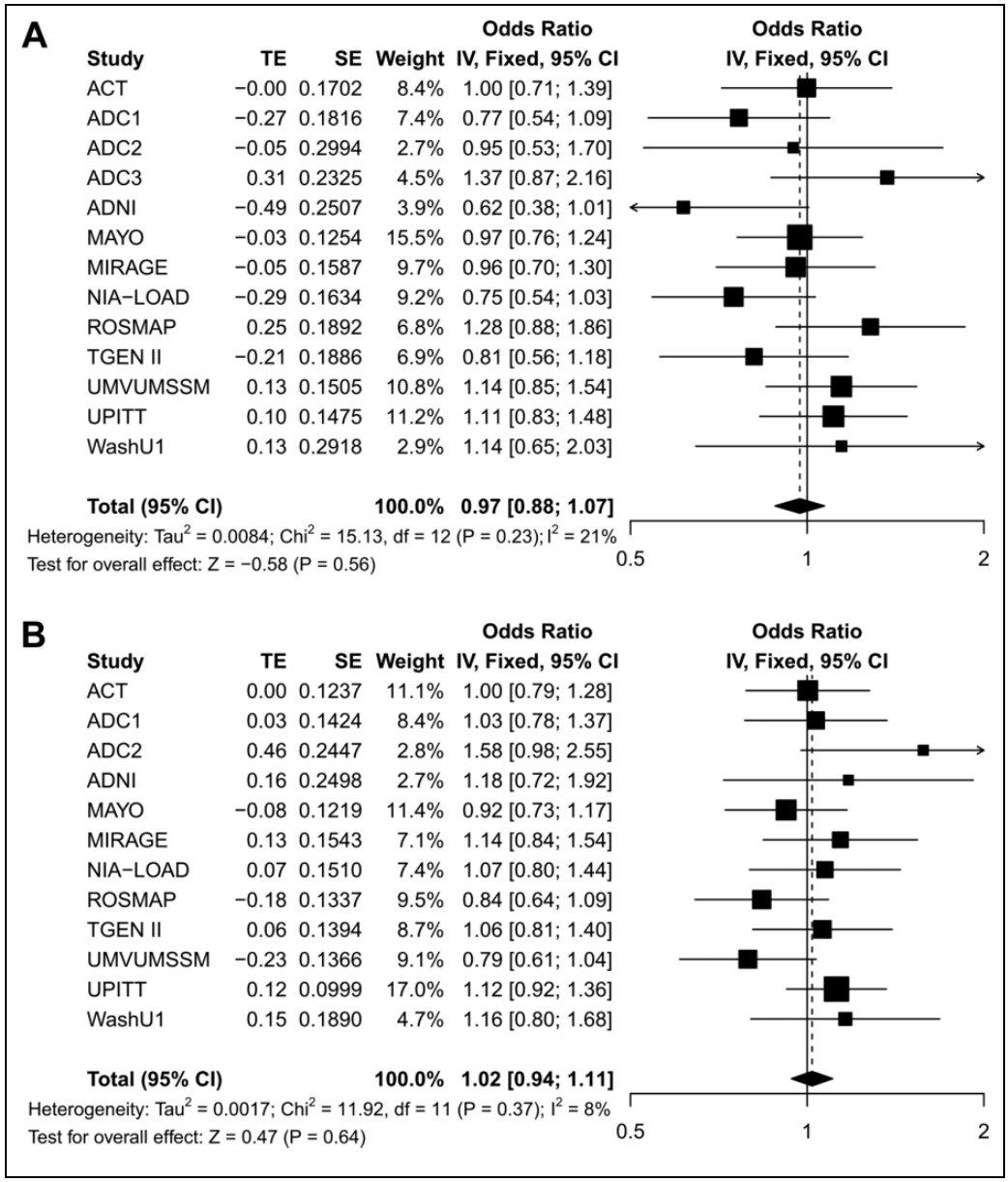

Figure 3. Forest plots of subgroup meta-analyses on the associations between Val66Met polymorphism and AD in *APOE* ε4 carriers (A) and *APOE* ε4 noncarriers (B) after adjusting for age and sex. AD indicates Alzheimer's disease; *APOE*, apolipoprotein E.

Neurotrophins, of which BDNF is a member,[44] are evolutionarily young and do not exist in invertebrate species.[45] The late evolutionary appearance of neurotrophins suggests that these molecules are necessary for both the development and functioning of a more complex nervous system.[46,47] Transgenic mice deficient in either neurotrophins or neurotrophic receptors can result in neonatal death.[45,48]

Moreover, BDNF has also emerged as an important regulator of synaptogenesis and synaptic plasticity underlying learning and memory in adult central nervous system.[49] These evidences demonstrate BDNF is critical in the development and functioning of nervous system neonatally and in adults. However, no GWAS signal can be identified in region encompassing *BDNF* for neurological or psychiatric diseases, suggesting variants in region encompassing *BDNF* could not disturb its function significantly or their consequences can be compensated. We hypothesize that fatal variants in region encompassing *BDNF* were discriminated against by natural selection because of its indispensable role in nervous development.

Biased samplings involving confounding factors may explain the heterogeneous results in previous association studies. In the present study, overall meta-analysis assessing association between Val66Met and AD showed an extraordinary heterogeneity across studies. However, heterogeneity was profoundly reduced after adjusting for age, sex, and *APOE* ε4

status. After subdividing samples based on sex or *APOE* ε4 status, no cross-study heterogeneity was observed even in confounding effect-unadjusted subgroup meta-analysis. For female subgroup meta-analysis, in agreement with the previous meta-analysis and the recent study on Chinese Han population that omitted confounding adjustment, [20,21], Met allele was associated with AD in females without adjusting for covariates. Nevertheless, after adjusting for age and *APOE* ε4 status, Met allele was not associated with AD in females. It suggests that the female-specific association between Val66Met and AD identified in the previous meta-analysis may be ascribed to the effects of age and *APOE* ε4 status. These facts underlie the necessity of confounding adjustment for research on Val66Met and even other polymorphisms in AD.

In conclusion, we showed Val66Met polymorphism was not associated with and had no sexual or *APOE* ε4 status-based dimorphic effect on susceptibility to AD. Our study demonstrates that confounding adjustment is necessary for research of Val66Met and even other polymorphisms on AD or AD-related trait.

Authors' Note

The authors Qingnan Zhao and Yaqi Shen contributed equally and share the first authorship. Data for this study were prepared, archived, and distributed by the National Institute on Aging Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania (U24-AG041689), funded by the National Institute on Aging.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplementary material for this article is available online.

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