Meta-analytic method reveal a significant association of the BDNF Val66Met variant with smoking persistence based on a large samples

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Abstract
Although numerous genetic studies have reported the link between Val66Met in BDNF gene with smoking, the findings remain controversial, mainly due to small-to-moderate sample sizes. The main aim of current investigation is to explore whether the variant of Val66Met has any genetic functions in the progress of smoking persistence. The Val-based dominant genetic model considering Val/* (namely, Val/Val + Val/Met) and Met/Met as two genotypes with comparison of the frequency of each genotype in current smokers and never smokers. There were seven genetic association articles including eight independent datasets with 10,160 participants were chosen in current meta-analytic investigation. In light of the potent effects of ethnicity on homogeneity across studies, we carried out separated meta-analyses according to the ancestry origin by using the wide-used tool of Comprehensive Meta-analysis software (V 2.0). Our meta-analyses results indicated that the Val66Met polymorphism was significantly linked with smoking persistence based on either all the chosen samples (N = 10,160; Random and fixed models: pooled OR = 1.23; 95% CI = 1.03–1.46; P value = 0.012) or Asian samples (N = 2,095; Fixed model: pooled OR = 1.25; 95% CI = 1.01–1.54; P value = 0.044; Random model: pooled OR = 1.25; 95% CI = 1.001–1.56; P value = 0.049). No significant clue of bias in publications or heterogeneity across studies was detected. Thus, we conclude that the Val66Met (rs6265) variant conveys genetic susceptibility to maintaining smoking, and smokers who carry Val/* genotypes have a higher possibility of maintaining smoking than those having Met/Met genotype.

Introduction
Cigarette smoking, a chronic and complex brain-related disorder, has been documented to lead to many diseases, including various cancers [1–3]. Notably, smoking conveys highly susceptibility to lung cancer, ranging from fivefold to tenfold. However, the smoking prevalence is still high or increasing in some Asian countries. Recently, an authoritative report from World Health Organization [4] demonstrated that there were about six million deaths worldwide each year resulting from smoking. In China, the largest tobacco-consuming country in the world, suffering a huge health hazard threatening from cigarette smoking, which leads to ~1.4 million people to die in the year of 2010 [5–7]. Badly, the number of smoking-related deaths is estimated to reach to two million in the year of 2030 and about three million in the year of 2050 if the patterns of smoking in China are unchanged [6–8]. Thus, it is necessary to develop powerful and effective approaches for promotion of smoking cessation and prevention of smoking initiation.

Twin- and family-based genetic studies [9–13] have documented various behaviors relevant to smoking, including the initiation of smoking, persistence in smoking, dependence of nicotine, attempt to stop smoking, and cessation, are affected by both environmental factors and genetic components. In total, the average heritability of dependence of nicotine is 0.56 in both male and female
have demonstrated that variants in BDNF conferring susceptibility to nicotine dependence [30]. The chromosome 11p13 region is likely to harbor genes relevant to the etiology or treatment of several conditions and stress reduce its secretion [28]. These previously reported findings showed that BDNF might regulate the responsiveness of dopamine in such a vital way that may be modulated nicotine on enhancing cognitive functions, which potentially modulate dopamine reward [22, 23].

BDNF, encoded by BDNF gene in the chromosome 11p13–15, shows highly expression in the mammalian brain, and involves in regulation of multiple biological functions of neurons, such as neuron development, regeneration, survival, and maintenance [24]. BDNF is a required element for modulating the neuron development and molecular regulation of dopaminergic reward pathways [25–27]. Animal and human studies have reported that cognitive stimulation, antidepressant treatment, and physical activity improve BDNF secretion, whereas mood disorders and stress reduce its secretion [28]. These previously reported suggestions that BDNF might regulate the responsiveness of dopamine in such a vital way that may be relevant to the etiology or treatment of several conditions implicating dopamine. Earlier studies [29] have documented that chronic nicotine could increase BDNF expression in the rat hippocampus, whereas acute nicotine prominently decreases the gene expression of BDNF. Genome-wide linkage investigations have suggested that the chromosome 11p13 region is likely to harbor genes conferring susceptibility to nicotine dependence [30]. Recent genetics-based association studies [19, 23, 31, 32] have demonstrated that variants in BDNF implicated in vulnerability to smoking behaviors.

In particular, the genetic variant of Val66Met (i.e., rs6265) has been investigated extensively to influence the BDNF system. The functional polymorphism of Val66Met was observed to change BDNF intracellular packaging and trafficking, which affects the activity-dependent secretion of BDNF protein [33]. Since Beutten et al. first [34] found a significant link between genetic variant in BDNF and dependence of nicotine in male smokers based on European-American origin, a growing number of genetics-based association researches [23, 35–42] have been performed to reveal the genetic correlation of rs6265 with cigarette smoking-related traits. Nevertheless, these results are still equivocal. Furthermore, more relevant studies are essential for better determining the functional role of rs6265 in smoking behaviors and whether harboring the variant of rs6265 may have clinical therapeutic implications. To our best knowledge, no meta-analysis has performed a combined analysis of the effect of rs6265 in BDNF on smoking persistence. Thereby, we conducted a meta-analysis based on a large-scale sample sizes of existing studies for the association between rs6265 in BDNF and smoking persistence.

Materials and methods
Effective search strategy and stringent inclusion criteria
We interrogated the public database of NCBI PubMed (https://www.ncbi.nlm.nih.gov/pubmed) for studies with regard to the association of variants in BDNF with smoking behaviors prior to June 25, 2018. The searching keywords used were “susceptibility”, “polymorphism”, “genetics”, “variant”, “BDNF”, “brain-derived neurotrophic factor”, “smoking”, “nicotine”, “cigarette”, and “tobacco”. Abstracts were tested for possible related papers in consistent with the normal criteria for inclusion and exclusion proposed by Moher et al. [43]. The references cited in these full text papers were hand-checked for underlying other-related publications missed by the original search. Duplication studies were dumped and articles reported in previous data were removed.

Data extraction
According to the “Quality of Reporting of meta-analysis and PRISMA guidelines” [43], nine studies [23, 35–42] were read carefully for potent eligible dataset according to a strictly systematic and comprehensive review (see Supplementary Fig. S1 for details). Only studies or datasets meeting the five strict criteria as following were chosen: (1) a case-control-based studies (namely, family-based studies
were abandoned); (2) a peer-reviewed publication; (3) data were independent from other previous studies; (4) raw genotype or allele frequency could be accessed for use; and (5) sufficient data could be employed to reckon an odds ratio (OR) and 95% confidence interval (CI). For each chosen study, two authors (Wu Xubiao and Hailong Zhao) used a standardized forms to extract the following data: author names and publication years, paper based on English or based on other kinds of languages, used samples’ ancestry, types of the chosen studies, ethnicity, the number of participants in each chosen study, gender ratio, P values of Hardy–Weinberg equilibrium (HWE), criteria of diagnosis for defining smoking phenotype, and the count of subjects with distinct smoking status stratified by the genotypes of rs6265.

### Classification of phenotypes and genotypes

As documented in previous studies [15, 18, 44], we defined the elements of smoking behaviors as comparing the status of smoking: (1) use ever smoking versus (vs.) never smoking to assess initiation of smoking, (2) use current smoking vs. never smoking to assess maintaining smoking (i.e., smoking persistence), and (3) use current smoking vs. ex-smoking to assess smoking cessation. In the accepted studies, the data for smoking was mainly concentrated on smoking persistence. Thus, we carried out our meta-analysis for the association of Val66Met genotypes in BDNF under a dominant model (Val/Val and Val/Met genotypes vs. Met/Met genotypes) with smoking persistence.

### Statistical analysis

Firstly, we combined all datasets from the included articles to conduct an overall meta-analysis. Further, we performed separated meta-analyses through taking into account the underlying influence of different race: Asian ancestry and Caucasian ancestry. Since there is only one study for African ancestry, no stratified meta-analysis for this race. All meta-analyses were conducted by employing the wide-used tool of Comprehensive Meta-analysis software package (V. 2.0; Biostat Inc., Englewood, NJ). The significance of a combined OR is calculated by a Z-score examination, and P value < 0.05 is thought to be significant.

We used both random and fixed models for meta-analyses. With respect the random model based on DerSimonian and Laird methods [45], which assumes that the heterogeneity across distinct chosen studies is attributed to variation within- and between-study, the effect size of each study was computed to create a combined OR and 95% CI. The effect size of each single study using the fixed model was combined with the use of Mantel–Haenszel methods [45], which assumes that genotype effect is constant across

### Table 1 Characteristics of each study chosen in current meta-analysis (N = 10,160 participants)

| Study name | Publication year | Sample Size | % Caucasian | % male | Current smoking | Never smoking | Val/* genotype frequency (%) |
|------------|------------------|-------------|-------------|--------|-----------------|---------------|-----------------------------|
|            |                  |             |             |        | Val/Val         | Val/Met       | Met/Met                     |
| Lang et al. | 2007             | 253         | 100         | 48.4   | 105             | 30            | 4                           |
| Wang et al. | 2007             | 149         | 100         | 51     | 12              | 20            | 16                          |
| Montag et al. | 2008           | 554         | 100         | 47     | 264             | 86            | 136                         |
| Landi et al. | 2009             | 238         | 100         | 53     | 48              | 136           | 277                         |
| Zhang et al. | 2012             | 628         | 100         | 51     | 85              | 8            | 4                           |
| Zhang et al. | 2015             | 1318        | 100         | 76     | 114             | 73            | 175                         |
| Jiang et al. (EA) | 2017          | 1616        | 100         | 51     | 215             | 235           | 308                         |
| Jiang et al. (AA) | 2017          | 3404        | 100         | 51     | 1643            | 518           | 1543                        |

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different researches and the observed variation is attributed to chance of random. Compared with the fixed model, the random model that generating a wider CI is more conservative. Thus, the fixed model appears to be more encouraged when no heterogeneity across studies exists; otherwise, the random model should be acceptable.

We used both Cochran’s $Q$ and $I^2$ test [46] to evaluate the potential heterogeneity between different chosen studies. The across-all-studies heterogeneity is of significance when $P_Q < 0.05$, and further it can be assessed by the value of $I^2$ ($I^2 = Q - df/Q$), which is employed for determining the percentage of evaluated variation across different chosen studies resulting from heterogeneity instead of that by chance of random. For a typical example, when $I^2$ value is equal to 50%, the outcomes from meta-analyses can inflate evaluated variation percentage not explained by genotype to 50% [15, 18]. Further, we also utilized two kinds of approaches of funnel-shaped distribution and Egger-regression test to assess publication bias. Funnel plot uses a method of linear regression to visualize the funnel-shaped distribution asymmetry based on the natural logarithm of the OR. When several chosen studies were out of the funnel distribution, the plot tends to be asymmetrical, indicating there exists a possibility of bias in publication.

Results

Basic characteristics on the chosen studies

In current study, we searched ten studies reported between 2007 and 2017 (Supplementary Table S1). All these chosen studies contain a total of 11,348 subjects, ranging from 149 to 3404 samples in each study. There are two main populations based on Asian ancestry and Caucasian ancestry. Among these studies, different sex ratios were detected (Table 1). Except for the study of Zhang et al. [37], the genotypes of all studies were HWE (Supplementary Table S1). Since other three typical genetic-based models, i.e., allelic, additive, and recessive genetic model, showed no evidence of a significant association between Val66Met and smoking persistence (data not included), we thus adopted a genetic model of Val/* genotype dominant for all the subsequent analyses. There are seven eligible studies with a total of 10,160 samples for meta-analysis (Table 1). Genotype distributions of Val/* (i.e., Val/Val and Val/Met genotypes) of these eight studies are shown in Table 1.

Meta-analysis based on all included studies

We first carried out an overall meta-analysis to reveal whether the link of Val66Met genotypes in BDNF with maintaining smoking by incorporating all the chosen datasets. The dataset from the chosen studies reported by Jiang et al. [35] was stratified into two datasets based on Asian and Caucasian ancestry (Table 1). The pooled OR was 1.23 ($P$ value = 0.021) with the 95% CI from 1.03–1.46 in both the fixed and random models (Fig. 1 and Table 2), suggesting that the Val/* genotypes convey a higher susceptibility to smoking persistence. The Cochran’s $Q$ and $I^2$ test demonstrated no evidence of significant heterogeneity across all the chosen studies existed ($I^2 = 0.0; P_Q = 0.49$).

Meta-analysis based on different ethnicities

By considering the Val/* genotypes frequency differences between different populations, we conducted two stratified meta-analyses, incorporating the datasets depended on Asian population and Caucasian population. With respect to the Asian ancestry, the combined OR was 1.25 (95% CI = 1.01–1.54; $P = 0.044$) in the fixed model (Fig. 2). Similar with the outcomes from overall meta-analysis, no evidence of significant across-study heterogeneity was observed in Asian population ($I^2 = 5.6; P_Q = 0.35$; see Table 2). When the meta-analysis for Asian population conducted in the random model, the association is still significant with the pooled OR of 1.25 (95% CI = 1.01–1.56; $P = 0.049$). Although there was no evidence of significant heterogeneity between studies of Caucasian population ($I^2 = 18.9; P_Q = 0.296$), no significant association was detected (Table 2 and Supplementary Fig. S2).

Sensitivity-based and accumulative-based analysis for reliability of current meta-analysis

A sensitivity-based analysis was carried out for datasets from all the chosen articles in the fixed model to test whether the detected effect of Val66Met variant on smoking
persistence was significantly affected by kicking out one independent article each time. As presented in Fig. 3, the combined ORs fluctuated faintly ranging from 1.196 to 1.303, suggesting our current findings from meta-analytic approach were not prominently impacted by any single dataset from chosen study. All calculated P values for sensitivity-based test were detected to be statistically significant (Fig. 3a and Supplementary Table S2). To determine whether Val66Met polymorphism showing significant association with smoking persistence alters with the increase of years of publication, we also conducted an accumulative-based analysis for all the datasets from our current chosen studies in the fixed model. The combined ORs reduced from 1.422 in 2007 to 1.207 in 2009, and went to relatively stable after the year of 2009 to now (Fig. 3b). Please refers to Supplemental Table S3 for the specific P values and other detailed relevant information.

Assessment of possible bias in publication

For all these performed meta-analyses in the current investigation, we employed the approaches of Egger-regression analysis and funnel-shaped distribution to evaluate the existing bias in publication. As shown in Table 2, all the calculated P values from the Egger-regression analysis were >0.05, suggesting that there existed no significant evidence of publication bias in all three meta-analyses performed in the present investigation. Consistently, the funnel-shaped plots for meta-analyses based on all chosen studies, Asian population, and Caucasian population tend to be of symmetry with no study deviated out of the funnel-shaped plot (Fig. 4, Supplementary Figs. S3 and S4), which provides further supportive evidence of no publication bias for all these conducted meta-analyses.

**Table 2** Results from the meta-analysis for smoking persistence stratified by different datasets

| Dataset of meta-analysis | Cohorts (n) | Sample size (N) | Estimate of Heterogeneity | Fixed-effects model | Random-effects model |
|--------------------------|------------|-----------------|---------------------------|---------------------|---------------------|
|                          |            |                 | F (%)  P (Q)              | Pooled OR 95% CI    | P (Z) Pooled OR 95% CI |
| All samplesa             | n = 8      | N = 10,490      | 0.0  0.49                | 1.23  1.03–1.46 0.021 | 1.23  1.03–1.46 0.021 |
| Asian samplesb           | n = 3      | N = 2,095       | 5.6  0.35                | 1.25  1.01–1.54 0.044 | 1.25  1.00–1.56 0.049 |
| Caucasian samplesc       | n = 4      | N = 4,991       | 18.9 0.296               | 1.18  0.86–1.61 0.31  | 1.14  0.78–1.66 0.51 |

Current results were based on Val/* dominant model

aMeta-analysis of all samples from all chosen studies

bMeta-analysis of all Asian samples from chosen studies

cMeta-analysis of all Caucasian samples from chosen studies

dlEgger’s regression test for publication bias

**Fig. 2** Forest plot shows the results from current meta-analysis for the association of the polymorphism of Val66Met in *BDNF* with smoking persistence based on Asian population-based studies. The specific odds ratio, lower limit, upper limit, Z-score, and P value of each individual study are shown by rows. As presented in Fig. 3, the combined ORs fluctuated faintly ranging from 1.196 to 1.303, suggesting our current findings from meta-analytic approach were not prominently impacted by any single dataset from chosen study. All calculated P values for sensitivity-based test were detected to be statistically significant (Fig. 3a and Supplementary Table S2). To determine whether Val66Met polymorphism showing significant association with smoking persistence alters with the increase of years of publication, we also conducted an accumulative-based analysis for all the datasets from our current chosen studies in the fixed model. The combined ORs reduced from 1.422 in 2007 to 1.207 in 2009, and went to relatively stable after the year of 2009 to now (Fig. 3b). Please refers to Supplemental Table S3 for the specific P values and other detailed relevant information.

Discussion

In current investigation, we first conducted a meta-analysis to identify the association between Val66Met (rs6265) in *BDNF* and smoking persistence, and detected a prominent
effect of Val66Met polymorphism on smoking persistence based on a large-scale samples. Since there exist no significant evidence of heterogeneity across all chosen studies or publication bias identified, we thus concluded that the Val allele play a crucial role in the pathology of the persistence of smoking, suggesting that carriers of Val/Val and Val/Met genotypes have a higher likelihood of maintaining smoking than individuals carrying the homozygous genotype of Met/Met.

The dopaminergic reward system functionally response to the stimulation of nicotine is regulated by BDNF protein, which could regulate dopamine D3 receptor (DRD3) expression through a regulation system of stimulating the function of the DRD1 [47]. The encoding BDNF gene, located on chromosome 11, has been well-reported to be correlated with substance abuses and psychiatric diseases, such as nicotine dependence [19, 31], alcohol dependence [31, 48], schizophrenia [49], personality [28], and attention deficit hyperactivity disorder [50]. The well-studied polymorphism of Val66Met, located in the region of exon 9 that codes for the pro-BDNF, was observed to change BDNF intracellular packaging and trafficking, which affects the activity-dependent secretion of BDNF protein [33]. The rs6265 variant has been demonstrated to be functionally linked with the impaired function of memory and hippocampal [51] and increased anxiety-related behaviors [52]. Consistently, multiple lines of evidence have documented that the genetic variant of rs6265 is significantly associated with various psychiatric diseases, including schizophrenia, bipolar, and eating disorders [53, 54]. Furthermore, a number of genetic researches have reported a potential association between the common
Val66Met variant and susceptibility to smoking-related phenotypes [23, 35–42]. For example, a large-scale GWAS [19] identified the polymorphism of rs6265 was significantly associated with smoking initiation (OR = 1.06, 95% CI 1.08–1.18, \( P = 3.6 \times 10^{-8} \)). However, the pattern of the results for smoking persistence is inconsistent, which primarily because of the small sample size or sampling bias in each investigation. It remains an open question whether the genetic variant of rs6265 has an effect on smoking persistence. The meta-analytic method is extensively thought to be an effective tool for improving the power of our statistical analysis through combining various studies on the same topic.

In view of recessive, additive, and allelic genetic models have no significant results, the Val-dominant model was mainly applied in current meta-analytic investigation. The rs6265 polymorphism was detected to be significantly correlated with maintaining smoking based on all chosen samples and Asian ancestry-based samples. As for Caucasian population, we did not detect any evidence for the link. The allele frequencies of Val allele of rs6265 variant show highly differences across different population, with Val allele estimated to be ~78.4% in Caucasian population but only about 51.5% among Asian populations, which is in consistent with the findings reported by previous articles [28, 55]. As such, the different frequencies of rs6265 allele

![Fig. 5 The BDNF gene expression in different 53 human tissues and rs6265-eQTL analysis.](image-url)
may contribute to the nonsignificant association of smoking persistence among Caucasian population. However, we observed on significant clue of bias in publication or between-study heterogeneity in all the present meta-analyses. Further, no study fell noticeably out of the funnel-shaped distribution. After applying of the method of Duval and Tweedie trim and fill by adding missing negative studies, our meta-analyses-based results remain to be significant. In addition, from sensitivity-based and accumulative-based analysis, the findings from meta-analysis based on all the chosen studies showed that the combined OR was not substantially vulnerable to any single study and gradually tended to steady as the years of relevant publications increased. Our interesting results offer strong evidence that the effect of rs6265 polymorphism contribute susceptibility to maintaining smoking.

Although a significant association between rs6265 and smoking persistence was observed, the findings of current meta-analytic investigation should be cautiously explained in light of underlying flaws as listed in the following. First, smoking is a common brain disorder with a complex etiology influenced by multiple factors and commonly show a comorbidity with other neuropsychiatric disorders or drug addictions, which appears to have a highly likelihood to share common genetic susceptibility components in the dopamine-related reward circuit. Further, the vast majority of the chosen studies did not offer relevant data that allows us to include or exclude subjects with comorbidity diseases in the selected samples. Thus, these comorbidity diseases in included smokers or never smokers might confuse the results of current meta-analysis. Second, there existed distinct gender ratios of subjects among these chosen articles, which might contribute to the existing limitations, as indicated by many previous reported studies [15, 18, 56, 57]. Third, in the present study, we did not compute the inter-rater-reliability for choosing published studies, which might cause some selecting biases. Nevertheless, if such bias existed, it would be minimized by using two authors independently reviewed all these published studies. Finally, due to there exist a deficient number of published articles on other variants, we could not test the biological interactions of gene by gene, which confer risk to the etiology of addictions or addictive behaviors, including smoking persistence. For example, Terracciano et al. [28] have documented that *BDNF* Val66Met show genetic interaction with 5-HTTLPR variant in the serotonin transporter gene. They demonstrated that individuals with BDNF Met variant and 5-HTTLPR LL have a higher scores of neuroticism, but with BDNF Val variant and 5-HTTLPR LL have a lower scores of neuroticism.

In sum, our current meta-analyses show the Val66Met variant has a moderate effect on smoking persistence in a large-scale samples. Compared with individuals with Met/Met genotype, individuals with Val/* genotypes have a 23% greater possibility of maintaining smoking, which suggests that the dopamine-relevant functions of Val66Met variant is implicated in mediating the biological process of smoking persistence. More related molecular experiments are needed to explore the biological function and regulation mechanism of the rs6265 variant on smoking persistence and reveal the genetic interactions with other genes. Current investigation advances our understanding of genetic components underlying smoking persistence, and would contribute to the development of effective strategies for smoking cessation.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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