BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) VAL66MET POLYMORPHISM IS NOT RISK FACTOR FOR BIPOLAR DISORDER

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Abstract: Bipolar disorder (BPD) is a psychiatric disease, characterized by the cycles of mania and depression. Several genetic studies investigated BDNF gene Val66Met polymorphism as risk factor for BPD, but results were inconclusive. Therefore, present meta-analysis was performed to re-evaluate the BDNF Val66Met polymorphism and BPD association. Four databases (Pubmed, Springer Link, Science Direct and Google Scholar) were searched for eligible studies up to March 31, 2020. Pooled odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the strength of the association. All statistical analyses were done by MetaAnalyst and Mix program. Forty studies with a total of 28,787 subjects (10,085 cases and 18,702 controls) were included in this meta-analysis. Overall, pooled analysis indicated that there was no significant association between BDNF Val66Met polymorphism and BPD risk under all five genetic models ($OR_{A vs. G}$ =0.99, 95%CI= 0.94-1.03, p=0.49; $OR_{AG vs. GG}$= 0.1.02, 95%CI= 0.95-1.07, p= 0.57; $OR_{AA vs. GG}$ = 0.98, 95%CI=0.89-1.08, p=0.75; $OR_{AA+AG vs. GG}$= 1.0, 95%CI= 0.94-1.06, p= 0.89; $OR_{AA vs. AG+GG}$= 0.96, 95%CI= 0.89-1.05, p= 0.47). Similarly, no significant association was observed in ethnicity based subgroup analysis in both Asian and Caucasian population. However, significant association was found in subtype analysis between BDNF Val66Met and BPDII ($OR_{AA+AG vs. GG}$= 1.21, 95%CI= 1.06-1.37, p= 0.003) but not with BPDI. These findings suggested that the BDNF Val66Met polymorphism confer no genetic susceptibility to BPD I but risk for BPDII.

Keywords: BDNF, Bipolar disorder, BPD, Meta-analysis, Polymorphism, Val66Met.

INTRODUCTION

Bipolar disorder (BPD) is one of the most common and severe psychiatric disorder, which is classified as a mood disorder in the Diagnostic and statistical Manual of Mental Disorders (DSM IV) (American Psychiatric Association, 2000).

World Health Organization has identified BPD as the sixth leading cause of disability (WHO, 2004). The lifetime prevalence of BPD is estimated to be between 1% and 2% (Merikangas *et al.,* 2007). The disorder is characterized by episodes of mania, with elated or irritable-angry mood and

symptoms like pressured speech, racing thoughts, grandiose ideas, increased energy, and reckless behavior, alternating with more normal periods and, in most cases, with episodes of depression. Although the etiologic mechanisms for BPD are not well known, it has been hypothesized that both environmental and genetic factors play important roles, and multiple genes contribute to risk of the disease (Goodwin, 2012).

BDNF is one of the most studied and abundant neurotrophin in central nervous system, which plays an important role in a variety of neural processes. BDNF is essential for neurogenesis, neuronal survival, and neural development pathways. In the adult, BDNF is important for synaptic plasticity, dendritic growth and long-term memory consolidation (Post, 2007). Because of the diverse functions of BDNF protein, BDNF gene is considered as a good candidate for the susceptibility to different neuropsychiatric disorders including BPD (Goldberg *et al.,* 2008).

Several polymorphisms are reported in BDNF gene, but in exon 5 G to A (G196A) transition (dbSNP: rs6265) at position 196 is most studied and clinically important. G196A polymorphism produces amino acid substitution of valine (Val) to methionine (Met) at codon 66(Val66Met) in the 5' prodomain in the BDNF protein (Cargill *et al.,* 1999). This substitution from valine (Val) to methionine (Met) at codon 66 has functional consequences (Egan *et al.,* 2003; Chen *et al.,* 2006). The Met (A) allele of the precursor peptide has been associated with impaired intracellular trafficking of pro-BDNF into dendrites and vesicles as well as a reduction inactivity-dependent secretion, the process that plays a major role in the regulation of extracellular levels of BDNF (Egan *et al.,* 2003; Chen *et al.,* 2006). The frequencies of the Val and Met alleles of BDNF Val66Met vary by ethnicity; about 80% of the European population has Val allele while only 50% of Asian has it (Pivac *et al.,* 2009). Numerous studies reported Val66Met polymorphism as a risk factor for BPD but results were inconclusive. Hence, authors performed present meta-analysis to reevaluate the association between BDNF Val66Met polymorphism and BPD risk.

MATERIALS AND METHODS
Meta-analysis was carried out according to Meta-analysis of observational studies in epidemiology (MOOSE) guidelines.

Search Strategy
A literature search was performed for available articles that were published up to March 31, 2018 from the following databases: (1) Pubmed; (2) Google Scholar; (3) Science Direct; and (4) Springer Link. The search used the following keywords: "Bipolar Disorder" or "BPD" and "brain-derived neurotrophic factor" or "BDNF" and "Val66Met". Authors also reviewed the bibliography of included articles to identify additional articles not retrieved by database search.

Inclusion and exclusion criteria
The following inclusion criteria were used: (i) studies should be original, (ii) used case control approach, and (iii) used standard diagnostic criteria for bipolar disorder patient diagnosis (Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) or the International Classification of Diseases (ICD). Studies were excluded in case of: (i) incomplete raw data /information like- number of genotype/allele, and (ii) pedigree data.

Data Extraction
Relevant information were extracted from all selected studies like- first author's name, year of publication, country name, ethnicity, sample size and number of cases and controls for BDNF Val66Met genotypes (GG, AG and AA). Mutant allele frequencies for the cases and controls were calculated from corresponding genotypes. Allele frequency was calculated by simple gene count method.

Statistical analysis
The present meta-analysis examined the overall association of Met allele with the risk of bipolar disorder. Pooled odds ratio (OR) with 95% confidence interval (CI) was used as risk association measure, which was estimated either by fixed effect (Mantel and Haenszel, 1959) or random effect (DerSimonian and Laird,1968) models depending upon heterogeneity. Heterogeneity was tested by Q-statistics and quantified by the I^2 statistic (Higgins and Thompson, 2002). If $I^2 > 50\%$ then random effect

model was used (Whitehead, 2002). Authors have also done sub-group analysis based on ethnicity and subtypes of bipolar disorder like BPDI and BPDII. In allele contrast meta-analysis, sensitivity analysis performed by exclusion of the studies in which control population was not in Hardy-Weinberg equilibrium (HWE) and studies with small sample size.

Publication bias

Publication bias was investigated by funnel plots: *viz*. funnel plot of standard error by log odds ratio and funnel plot of precision (1/standard error) by log odds ratio. Egger's regression intercept (Egger *et al.*, 1997) were adopted to assess the publication bias. All p values were two tailed with a significance level at 0.05. All statistical analyses were undertaken using program MetaAnalyst (Wallace *et al.*, 2013) and MIX version 1.7 (Bax *et al.*, 2006).

RESULTS

Characteristics of included studies

The study search workflow was shown in Figure 1. The preliminary search resulted in 187 publications from Pubmed, Springer Link, Science Direct and Google Scholar. Out of which 104 articles were excluded after title and abstract reading. Again 23 articles including review, letter to editors and comment were excluded from remaining 83 articles. Further 26 articles, which were duplicate studies, studies without genotype or allele number, studies on animal models, were excluded. Out of 34 remaining articles, 3 articles were meta-analysis, and excluded. Thus, total thirty studies that investigated the association of BDNF Val66Met polymorphism with BPD were found suitable for inclusion in the present meta-analysis (Hong *et al.*, 2003; Nakata *et al.*, 2003; Kunugi *et al.*, 2004; Oswald *et al.*, 2004; Skibinska *et al.*, 2004; Lohoff *et al.*, 2004; Nerves-Pereira *et al.*, 2005, 2011; Schumacher *et al.*, 2005; Green *et al.*, 2006; Liu *et al.*, 2007; Tramontina *et al.*, 2007; Kim *et al.*, 2008; Vincze *et al.*, 2008; Tang *et al.*, 2008; Xu *et al.*, 2009; Hosang *et al.*, 2010; Carrard *et al.*, 2011; Huang *et al.*, 2012; Pae *et al.*, 2012; Wang *et al.*, 2012; Lee *et al.*, 2012; Min *et al.*, 2012; Chang *et al.*, 2013; Lee *et al.*, 2013; Gonzalez-Castro *et al.*, 2014; Frazier *et al.*, 2014; Kenna *et al.*, 2014; Nassan *et al.*, 2015; Morales-Marín *et*

al., 2016). In nine articles, authors (Nakata *et al.*, 2003; Kunugi *et al.*, 2004; Green *et al.*, 2006; Xu *et al.*, 2009; Huang *et al.*, 2012; Wang *et al.*, 2012; Lee *et al.*, 2012; Chang *et al.*, 2013; Kenna *et al.*, 2014) investigated subtypes of bipolar disorder like BPDI and BPD II. Each case sample group (BPD I and BPD II case samples) were included as separate article so total forty article were included in the present meta-analysis.

In forty included studies, the smallest case sample size was 26 (Kenne *et al.*, 2014) and highest sample size was 864 (Green *et al.*, 2006). In included studies, total cases and controls were 10,085 and 18,702 respectively. In controls genotype percentage of GG, AG and AA were 47.73%, 40.09% and 12.18% respectively. In cases genotype percentage of GG, AG and AA were 52.63%, 36.59% and 10.23% respectively. Control population of three studies (Nerves-Pereira *et al.*, 2011; Wang *et al.*, 2012; Lee *et al.*, 2013) was not in Hardy-Weinberg equilibrium (Table 1). Case and control subjects were selected from following countries- Brazil (Tramontina *et al.*, 2007; Nerves *et al.*, 2011), Bulgaria (Oswald *et al.*, 2004), China (Hong *et al.*, 2003; Liu *et al.*, 2007; Tang *et al.*, 2008; Xu *et al.*, 2009; Wang *et al.*, 2012; Lee *et al.*, 2012; Chang *et al.*, 2013; Lee *et al.*, 2013), France (Vincze *et al.*, 2008), Germany (Schumacher *et al.*, 2005), Japan (Nakata *et al.*, 2003; Kunugi *et al.*, 2004), Korea (Kim *et al.*, 2008; Pae *et al.*, 2012; Min *et al.*, 2012), Mexico (Gonzalez-Castro *et al.*, 2014; Morales-Marín *et al.*, 2016), Scotland (Nerves-Pereira *et al.*, 2005), Taiwan (Huang *et al.*, 2012), UK (Green *et al.*, 2006; Hosang *et al.*, 2010), USA (Lohoff *et al.*, 2004; Frazier *et al.*, 2014; Kenna *et al.*, 2014; Nassan *et al.*, 2015) and Wielkopolaska (Skibinska *et al.*, 2004).
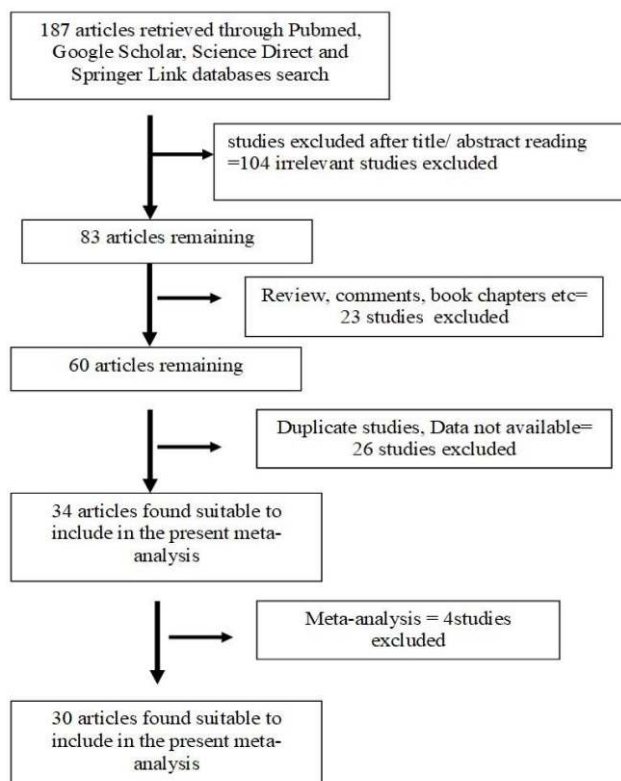
Meta-analysis

Meta-analysis with allele contrast A(Met) vs. G(Val)) showed no association with both fixed effect (OR$_{A vs.G}$ =0.99, 95%CI= 0.94-1.03, p=0.49) and random effect model (OR$_{A vs.G}$ =0.98, 95%CI= 0.93-1.03, p=0.46) (Table 1; Figure 2). Significant association was not found between BPD and mutant genotype (AA vs. GG; homozygote model) with both fixed (OR$_{AA vs. GG}$ = 0.98, 95%CI=0.89-1.08, p=0.75) and random (OR$_{AA vs. GG}$ =0.98 , 95%CI=0.87-1.10, p=0.74)

effect models (Table 2). Mutant heterozygous genotype (AG vs.GG; co-dominant model) meta-analysis did not show any significant association with BPD using fixed ($OR_{AG\ vs.\ GG}$= 0.1.02, 95%CI= 0.95-1.07, p= 0.57) and random ($OR_{AG\ vs.\ GG}$= 1.02, 95%CI= 0.93-1.13, p= 0.56) effect models (Table 2). Combined mutant genotypes (AA+AG vs. GG; dominant model) meta-analysis showed negative association with BPD using both fixed ($OR_{AA+AG\ vs.}$

$_{GG}$= 1.0, 95%CI= 0.94-1.06, p= 0.89) and random ($OR_{AA+AG\ vs.\ GG}$=1.0, 95%CI= 0.92-1.09, p= 0.86) effect models. Similarly the recessive genotypes model (AA vs. AG+GG) also did not show any association with fixed ($OR_{AA\ vs.\ AG+GG}$= 0.96, 95%CI= 0.89-1.05, p= 0.47) and random ($OR_{AA\ vs.\ AG+GG}$= 0.96, 95%CI= 0.86-1.06, p= 0.45) effect models (Table 1).

Table 1: Summary estimates for the odds ratio (OR) in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric: An overall analysis.

Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Allele Contrast (A vs. G)	0.99(0.94-1.03),0.49	0.98(0.93-1.03),0.46	0.02	34.64	0.09
Co-dominant (AG vs. GG)	1.02(0.95-1.07),0.57	1.02(0.93-1.13),0.56	<0.0001	54.68	0.49
Homozygote (AA vs. GG)	0.98(0.89-1.08),0.75	0.98(0.87-1.10),0.74	0.13	20.43	0.05
Dominant (AA+AG vs. GG)	1.00(0.94-1.06),0.89	1.00(0.92-1.09),0.86	0.0007	46.83	0.81
Recessive (AA vs. GG+AG)	0.96(0.89-1.05),47	0.96(0.86-1.06),0.45	0.09	23.92	0.02

Fig. 1: Flow diagram of study search and selection process.

Subgroup analysis

Out of 40 studies included in the present meta-analysis, 21 studies were carried out on Asian subjects, and 19 studies were carried out on Caucasian population. The subgroup analysis by ethnicity revealed that no significant association existed between BDNF Val66Met polymorphism and BPD in Asian population ($OR_{A\ vs.G}$ = 1.02, 95%CI= 096-1.07., p=0.47; co-dominant model- $OR_{AG\ vs.\ GG}$= 1.08, 95%CI= 0.99-1.18, p= 0.07; homozygote model- $OR_{AA\ vs.\ GG}$ = 1.05, 95%CI=094-1.17., p=0.34; dominant model- $OR_{AA+AG\ vs.GG}$= 1.07, 95%CI= 0.0.98-1.16, p= 0.09; recessive model-$OR_{AA\ vs.\ AG+GG}$= 1.01, 95%CI= 0.92-1.11, p= 0.79) (Table 2, Figure 3). Similarly in Caucasian subgroup analysis, heterogeneity was low and except allele contrast model, significant association was not found between C677T polymorphism and BC risk ($OR_{A\ vs.G}$ =0.93, 95%CI= 0.87-0.99, p=003.; co-dominant model- $OR_{AG\ vs.\ GG}$= 0.96, 95%CI= 0.89-1.04, p= 0.34; homozygote model- $OR_{AA\ vs.\ GG}$ = 0.80, 95%CI=0.65-0.98, p=0.03; dominant model-

$OR_{AA+AG \text{ vs. } GG}$=0.94, 95%CI= 0.87-1.02, p= 0.14;   0.65-0.95, p= 0.02) (Table 2).
recessive model-$OR_{AA \text{ vs. } AG+GG}$= 0.79 , 95%CI=

Table 2: Summary estimates for the odds ratio (OR) in various allele/genotype contrasts, the significance level (p value) of heterogeneity test (Q test), and the I^2 metric.

| Genetic Models	Fixed effect OR (95% CI), p	Random effect OR (95% CI), p	Heterogeneity p-value (Q test)	I^2 (%)	Publication Bias (p of Egger's test)
Asian studies					
Allele Contrast (A vs. G)	1.02(0.96-1.07),0.47	1.01(0.96-1.07),0.49	0.42	2.8	0.17
Co-dominant (AG vs. GG)	1.08(0.99-1.18),0.07	1.09(0.95-1.25),0.20	0.001	55.43	0.30
Homozygote (AA vs. GG)	1.05(0.94-1.17),0.34	1.05(0.94-1.17),0.34	0.56	0	0.60
Dominant (AA+AG vs. GG)	1.07(0.98-1.16),0.09	1.07(0.96-1.19),0.20	0.05	35.89	0.45
Recessive (AA vs. GG+AG)	1.01(0.92-1.11),0.79	1.01(0.91-1.11),0.77	0.35	7.62	0.13
Caucasian studies					
Allele Contrast (A vs. G)	0.93(0.87-0.99),0.03	0.91(0.82-1.01),0.08	0.009	48.77	0.18
Co-dominant (AG vs. GG)	0.96(0.89-1.04),0.34	0.95(0.83-1.08),0.48	0.004	51.91	0.92
Homozygote (AA vs. GG)	0.80(0.65-0.98),0.03	0.77(0.57-1.04),0.09	0.07	33.89	0.12
Dominant (AA+AG vs. GG)	0.94(0.87-1.02),0.14	0.93(0.82-1.05),0.26	0.004	51.96	0.63
Recessive (AA vs. GG+AG)	0.79(0.65-0.95),0.02	0.76(),0.05	0.09	31.66	0.07
BPD I studies					
Allele Contrast (A vs. G)	0.98(0.93-1.06),0.95	1.00(0.93-1.08),0.90	0.23	21.67	0.80
Co-dominant (AG vs. GG)	0.93(0.85-1.02),0.16	0.94(0.84-1.05),0.33	0.26	18.4	0.36
Homozygote (AA vs. GG)	1.06(0.91-1.23),0.43	1.06(0.91-1.23),0.43	0.77	0	0.76
Dominant (AA+AG vs. GG)	0.95(0.83-1.04),0.26	0.96(0.86-1.07),0.54	0.22	22.19	0.33
Recessive (AA vs. GG+AG)	1.08(0.0.94-1.20), 0.23	1.08(0.94-1.23),0.23	0.84	0	0.44
BPD II studies					
Allele Contrast (A vs. G)	1.10(1.01-1.13),0.04	1.08(0.93-1.14),0.04	0.29	16.58	0.85
Co-dominant (AG vs. GG)	1.31(1.06-1.60),0.03	1.3(1.1-1.7),0.02	0.14	30.23	0.52
Homozygote (AA vs. GG)	1.11(1.01.93-1.23), 0.03	1.11(0.92-1.33),0.04	0.45	0	0.93

|				
Dominant (AA+AG vs. GG)	1.21(1.06-1.37),0.003	1.21(1.01-1.46),0.03	0.07	42.67	0.27
Recessive (AA vs. GG+AG)	0.96(0.83-1.12),0.06	0.97(0.80-1.19),0.08	0.17	28.93	0.73
BPD studies					
Allele Contrast (A vs. G)	0.95(0.89-1.02),0.24	0.93(0.85-1.02),0.15	0.06	37.12	0.0008
Co-dominant (AG vs. GG)	1.02(0.93-1.12),0.63	0.98(0.86-1.13),0.87	0.03	42.87	0.22
Homozygote (AA vs. GG)	0.82(0.68-0.98),0.03	0.76(0.62-1.01)0.07	0.07	35.97	0.06
Dominant	0.99(0.90-1.08),0.88	0.95(0.84-1.08),0.48	0.03	41.18	0.08
Recessive (AA vs. GG+AG)	0.82(0.70-0.96),0.02	0.80(0.64-0.99),0.05	0.07	35.96	0.02
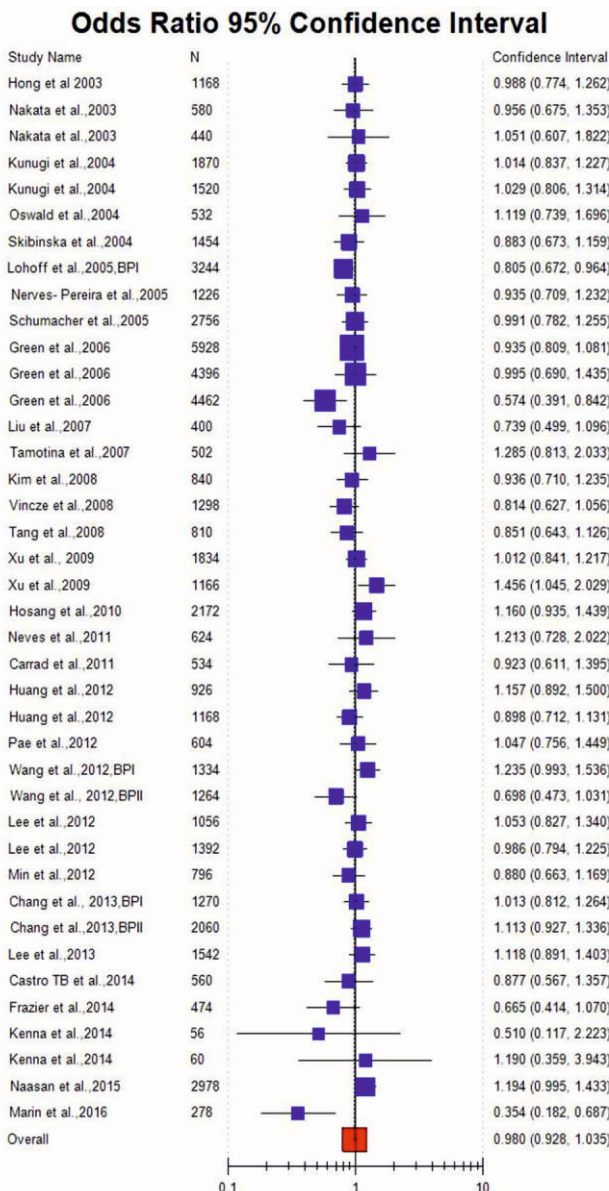
Fig. 2: Random effect Forest plot of allele contrast model (A *vs.* G) of total 40 studies of BDNF Val66Met (G196A) polymorphism.

Subgroup analysis based on subtypes of BPD like BPD I and BPDII was also done. Out of 40 studied, in 12 studies cases were of BPD I and in 10 studies, authors selected BPD II cases for their analysis. In other studies, type of bipolar disorder was not mentioned, so we grouped them in one subgroup (BPD subgroup). Meta-analysis of BPD I studies did not show any association between Val66Met and BPD I (OR$_{A vs.G}$ =0.98, 95%CI= 0.93-1.06, p=0.95; co-dominant model-OR$_{AG vs. GG}$= 0.93, 95%CI= 0.85-1.02, p= 0.16; homozygote model- OR$_{AA vs. GG}$ = 1.06, 95%CI=0.0.91, p=0.43; dominant model- OR$_{AA+AG vs. GG}$=0.95, 95%CI= 0.83-1.04, p= 0.26; recessive model-OR$_{AA vs. AG+GG}$=1.08 , 95%CI= 0.0.94-1.20, p= 0.23) (Table 2). Meta-analysis of BPD II studies showed significant association between BDNF Val66Met polymorphism and BPDII except recessive model (OR$_{A vs.G}$ = 1.10, 95%CI= 1.01, p=0.04; co-dominant model-OR$_{AG vs. GG}$= 1.31, 95%CI= 1.06-1.60, p= 0.03; homozygote model- OR$_{AA vs. GG}$ =1.11 , 95%CI= 1.01-1.23, p=0.03; dominant model-OR$_{AA+AG vs. GG}$= 1.21, 95%CI= 1.06-1.37, p= 0003; recessive model-OR$_{AA vs. AG+GG}$=0.96 , 95%CI= 0.83-1.12, p= 0.06) (Table 2;Figure 4).

Again subgroup BPD meta-analysis did not show any association between Val66Met polymorphism and BPD (OR$_{A vs.G}$ =0.95, 95%CI= 0.89-1.02, p=0.24; co-dominant model-OR$_{AG vs. GG}$= 1.02, 95%CI= 0.93-1.12, p= 0.63; homozygote model- OR$_{AA vs. GG}$ = 0.82 , 95%CI=0.68-0.98, p=0.03; dominant model-OR$_{AA+AG vs. GG}$=0.99, 95%CI= 0.90-1.08, p= 0.88;
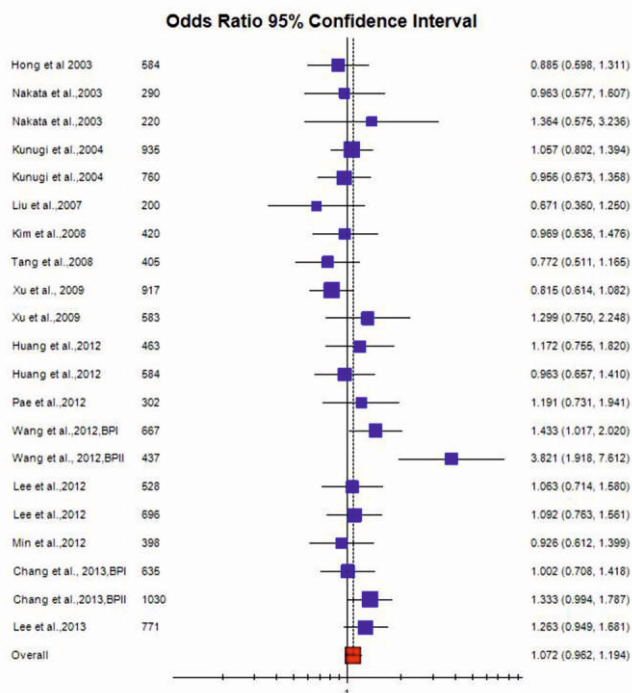
Fig. 3: Random effect Forest plot of dominant model (AA+AG *vs.* GG) of 21 studies of Asian population of BDNF Val66Met (G196A) polymorphism.
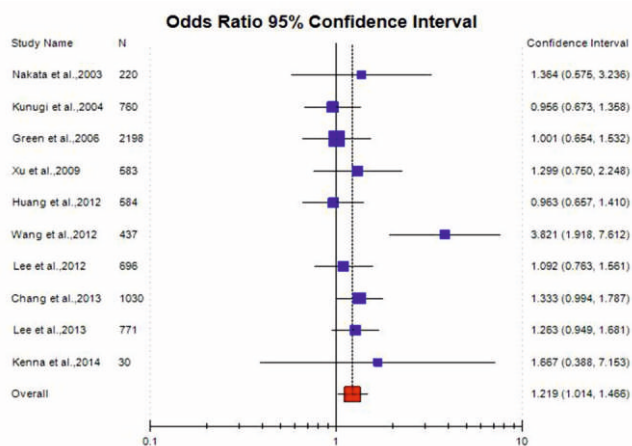

Fig. 4: Random effect Forest plot of dominant model (AA+AG *vs.* GG) of 10 studies of BPD II cases of BDNF Val66Met (G196A) polymorphism.

recessive model-$OR_{AA\ vs.\ AG+GG}$=82 , 95%CI= 0.70-0.96, p= 0.02) (Table 2).

Heterogeneity and Sensitivity analysis
Between studies heterogeneity existed, moderate heterogeneity in allele contrast ($P_{heterogeneity}$ =0.02, I^2= 34.64), high heterogeneity in co-dominant ($P_{heterogeneity}$ <0.0001, I^2= 54.68), insignificant low heterogeneity in homozygote ($P_{heterogeneity}$ =0.13, I^2= 20.43), moderate heterogeneity in dominant ($P_{heterogeneity}$ =0.0007, I^2= 46.83) and low heterogeneity in recessive ($P_{heterogeneity}$ =0.09, I^2= 23.92) comparisons (Table 1).
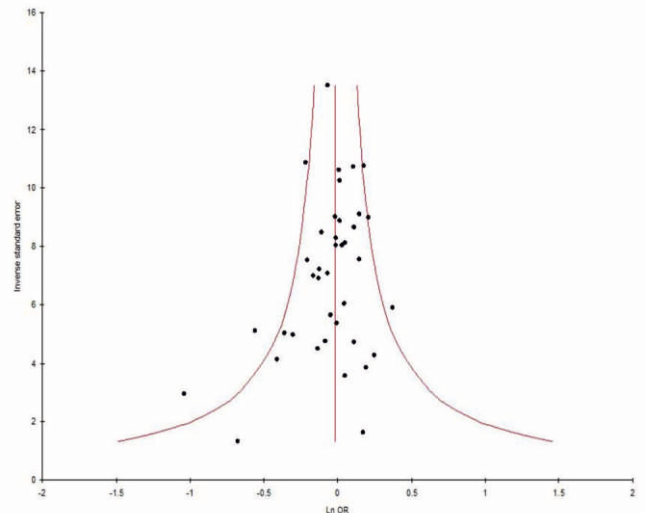
Fig. 5: Funnel plot- Precision by log odds ratio for dominant model (AA+AG *vs.* GG) of 40 studies of BDNF Val66Met (G196A) polymorphism.

Sensitivity analysis was performed by eliminating studies with small sample size (<100) and control population deviating from HWE. Control population of three studies was not in HWE (Nerves-Pereira *et al.*,2011; Wang *et al.*, 2012; Lee *et al.*, 2013) and heterogeneity was not decreased after exclusion of these studies (I^2= 34.4%; p=0.02). Sample size of six studies was less than 100 (Nakata *et al.*, 2003; Green *et al.*, 2006; Xu *et al.*, 2009; Wang *et al.*, 2012; Kenna *et al.*, 2014; Marin *et al.*, 2016) and after exclusion of these studies heterogeneity was decreased (I^2= 34.5%; p= 0.027).

Publication bias
Funnel plots and Egger's test were performed to estimate the risk of publication bias. Except recessive model, publication bias was absent (A vs. G: $P_{Egger's\ test}$= 0.09; AG vs. GG: $P_{Egger's\ test}$= 0.49; AA vs. GG: $P_{Egger's\ test}$ P= 0.05; Dominant model AA+AG vs. GG: $P_{Egger's\ test}$= 0.81; Recessive model AA vs. AG+GG: $P_{Egger's\ test}$= 0.02) (Table 1; Figure 5).

DISCUSSION
Forty case control studies with 10,085 bipolar disorder cases and 18,702 controls were included in the present meta-analysis. The results of current meta-analysis indicated that the Met (A) allele of BDNF was not associated with risk of BPD. Further, subgroup analyses based on ethnicity also did not show any association between BDNF Val66Met polymorphism and BPD in Asian as well as Caucasian populations. However, subgroup analysis based on BPD

subtypes showed significant association with BPD II (OR$_{AA+AG\ vs.\ GG}$= 1.21, 95%CI= 1.06-1.37, p= 0003) but not with BPD I. Although several evidences supported that BDNF factor is involved in BPD pathophysiology like (i) reduced BDNF levels have been observed in patients with BPD during manic and depressive episodes and these levels have normalized with episode recovery (Lin,2009), (ii) post-mortem studies have also demonstrated decreased hippocampal BDNF in patients with BPD (Dunham *et al.*, 2009), (iii) use of antidepressants and mood stabilizers, e.g. lithium and valproate, to induce expression of neurotrophins (e.g., BDNF) and synaptic changes (Duman *et al.*, 2000) and (iv) modulation of antidepressant-like affects by BDNF genotype and expression was also reported in human studies (Polyakova *et al.*, 2015). Possible explanation for the lack of association between the Val66Met (rs6265) polymorphism and BPD in present meta-analysis may be due to (i) small sample size in included genetic association studies, (ii) different clinical criteria for selecting BPD patients and (iii) BDNF factor level decreases in subtype BPD II but not in BPD I.

Meta-analysis is an acceptable powerful statistical tool which is used effectively to combine data from several similar case control studies to obtain reliable results. During past two decades, numerous meta-analysis were published which evaluated genetic polymorphism as risk factor for different diseases and disorders such as schizophrenia (Rai *et al.*, 2017), depression (Rai, 2017), alcohol dependence (Chaudhary *et al.*, 2021; Rai and Kumar, 2021), autism (Rai and Kumar, 2018), migraine (Rai and Kumar, 2021), epilepsy (Rai and Kumar, 2018), obcessive compulsive disorder (Kumer and Rai, 2020), Down syndrome (Rai *et al.*, 2017; Rai and Kumar, 2018), cleft lip and palate (Rai, 2014, 2017), osteoporosis (Yadav *et al.*, 2020), male infertility (Rai and Kumar, 2017), recurrent pregnancy loss (Rai, 2016), Uterine Leiomyoma (Kumar *et al.*, 2018), endometrial cancer (Kumar *et al.*, 2020), breast cancer (Rai, 2014; Rai *et al.*, 2017), tuberculosis (Yadav *et al.*, 2021), digestive tract cancer (Yadav *et al.*, 2018), colorectal cancer (Rai, 2015), prostate cancer (Rai and Kumar, 2020; Yadav *et al.*, 2021), ovary cancer (Rai, 2016) and MTRR A66G polymorphism distribution (Yadav *et al.*, 2021) etc.

Despite the clear strengths of present meta-analysis, including relatively large sample sizes and lack of publication bias, the interpretation of results should be done in light of few limitations like (i) crude ORs without adjustment was used as association measure, adjusted analysis could not be done due to lack of sufficient raw data about related risk factors like substance abuse, alcohol intake etc., (ii) significant heterogeneity was observed in overall meta-analysis,(iii) single gene polymorphism was considered, and (iv) gene environment interactions were not considered.

CONCLUSIONS
In present meta-analysis of all published case control studies was done to find out association between BDNF Val66Met polymorphism and bipolar disorder susceptibility. The results of present meta-analysis reported that BDNF Val66Met polymorphism is not risk factor for bipolar disorder (OR$_{A\ vs.G}$ =0.99, 95%CI= 0.94-1.03, p=0.49). According to DSM IV, bipolar disorder is a heterogeneous disease with many subtypes -BPD I, BPD II etc. and results or present study also showed that BDNF Val66Met polymorphism may be involved in the pathogenesis of BPD by influencing the susceptibility of specific subtypes such as BP II (OR=1.21, 95% CI=1.06-1.37, p=0.003). Further in subgroup analysis showed no association between Val66Met polymorphism and bipolar disorder risk either from Asian or Caucasian population. In future, studies with larger sample sizes from different ethnic population are required to reach a definitive conclusion regarding this association. Also, it is necessary to take into consideration different inheritance patterns and the interaction of the BDNF gene with the environment.

Declarations:
Ethics Approval and consent to participants: In present study, authors did not use any human sample, so there is no need of ethics approval and consent of participants. Authors declare no competing interest.

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