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ZNF804A Gene Variants Have a Cross-diagnostic Influence on Psychosis and Treatment Improvement in Mood Disorders

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Objective: Genetic variations in the gene encoding zinc finger protein 804A gene (*ZNF804A*) have been associated with major depression and bipolar disorder. In this work we focused on the potential influence of *ZNF804A* variations on the risk of developing specific sub-phenotypes as well as the individual response to available treatments.
Methods: We used two samples of different ethnic origin: a Korean sample, composed by 242 patients diagnosed with major depression and 132 patients diagnosed with bipolar disorder and 326 healthy controls; an Italian sample composed 151 major depression subjects, 189 bipolar disorder subjects and 38 outpatients diagnosed for a primary anxiety disorder.
Results: Our analyses reported an association of rs1344706 with psychotic phenotype in the cross-diagnostic pooled sample (geno $p = 4.15 \times 10^{-4}$, allelic $p = 1.06 \times 10^{-4}$). In the cross-diagnosis Italian sample but not in the Korean one, rs7597593 was involved with depressive symptoms improvement after treatment (geno $p = 0.025$, allelic $p = 0.007$).
Conclusion: The present study evidenced the role of *ZNF804A* alterations in symptoms improvement after treatment. Both manic and depressive symptoms seem to be modulated by *ZNF804A*, though the latter was observed in the bipolar pooled sample only. The role of this factor is likely related to synaptic development and maintenance; however, further analyses will be needed to better understand the molecular mechanics involved with *ZNF804A*.

KEY WORDS: *ZNF804A*; Bipolar disorder; Major depressive disorder; Symptoms improvement; Psychotic disorders.

INTRODUCTION

Several studies evidenced that the genetic background can significantly contribute to the risk of mood disorders [1-5] as well as the individual response to available treatments [6,7]. The specific biological aspects involved in complex disorders are however difficult to identify since every genetic variation seems to give a small contribution to the pathologic phenotype [8]. However a growing body of evidence is suggesting that the genetic variants associated with psychiatric disorders may have a trans-diagnostic effect on specific sub-phenotypes rather than the general disease risk [9].

In this study, we analyzed the potential influence of a gene previously associated with unipolar (major depressive disorder, MDD) or bipolar disorder (BPD) on discrete phenotypic effects of mood disorders [10-12]. The gene we considered is zinc finger protein 804A (*ZNF804A*, OMIM: 612282), located on chromosome 2q32.1. *ZNF804A* encodes for a protein containing a C2H2-type

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zinc finger domain supposed to act as a transcription factor [13,14]. The exact functions of this protein remain unknown so far [15], but some studies suggested that a variant in *ZNF804A* (rs1344706) can influence brain structure and activity [16-19]. ZNF804A protein is commonly detected through the human brain, especially in the medial temporal lobe and cortex areas [13,15,20]. *ZNF804A* was associated with the risk for BPD and psychosis [10,11], MDD [12], schizophrenia [21-24], as well as efficacy of antidepressant [25] and antipsychotic drugs [26,27]. These findings were replicated [22,28,29] and confirmed in meta-analyses [24,30] suggesting *ZNF804A* as a cross-diagnosis risk gene.

In this study, we investigated 7 single nucleotide polymorphisms (SNPs) within *ZNF804A* in two independent samples of Asian (Korean) and Caucasian (Italian) ancestry. We focused especially on rs1344706, an intronic SNPs solidly associated with psychiatric disorders [31-36], but we also evaluated other SNPs to increase the coverage of the gene.

METHODS

Subjects

The Korean sample was composed by 242 patients diagnosed with MDD and 132 patients diagnosed with BPD according to the Diagnostic and Statistical Manual for Mental Disorders, 4th edition (DSM-IV) criteria [37] and 326 healthy controls. Recruitment details and exclusion criteria have been previously reported [38,39]. All the patients were recruited at the Department of Psychiatry of Seoul St. Mary's Hospital for treatment. Controls were collected among hospital staff and patients; controls did not have to meet criteria for any current or past psychiatric condition. Subjects with severe or unstable medical and/or neurological conditions, in treatment with a long-acting antipsychotic, with current or recent (past six months) comorbidity with alcohol and substance abuse disorders were excluded from the study. All individuals were Koreans of Korean ancestry.

The Italian sample included 151 MDD, 189 BPD, and 38 outpatients diagnosed for a primary anxiety disorder (AD) (including DSM-IV panic disorder, generalized anxiety and social phobia), but presenting with a comorbid clinical depressive episode. Sixty-five healthy controls were also recruited among volunteers and clinical staff. The patients were recruited among subjects admitted to two university tertiary psychiatric care centers in Italy; the psychiatric department of the local health unit in Bologna (University of Bologna) and the psychiatric department of the "A. Gemelli" General Hospital in Rome (Catholic University of Rome). Inclusion criteria for the patients were as follows; age 18 to 75 years, presence of a DSM-IV diagnosis of MDD, BPD or primary AD with comorbid depression, eligibility for a pharmacological treatment with antidepressants. Exclusion criteria were represented by presence of severe or unstable medical conditions, neurological disorders and/or cognitive impairment. All individuals were Italian of Italian ancestry. A subsample of Italian MDD patients (n = 88) was already analyzed with regard to antidepressant response [40]. The ethical committee of The Catholic University of Korea, Bucheon St. Mary's Hospital approved the study procedures (No. HC10TISI0031); all the subjects were included after they had signed an informed consent.

Evaluations

Based on an agreement prior to the study, rather homogeneous assessment methods were employed in the different centers. Patients and controls were evaluated for psychiatric disorders by the Mini-International Neuropsychiatric Interview (MINI) [37]. Demographic and clinical variables, including age at first illness episode (onset), family history for psychiatric disease, history of suicide attempt and comorbidity with alcohol/substance dependence, were collected by clinical interview and review of clinical charts. Alcohol/substance disorder was evaluated on Italian patients only since alcohol/substance use represented exclusion criteria in the Korean center. Patients with MDD (both Italians and Koreans), those with BPD in current depressive episode (Italians only) and AD patients with comorbid depression (Italians only) were evaluated for symptoms severity at baseline and after 6−8 weeks of treatment by the Hamilton Rating Scale for Depression (HDRS) [41]. In Koreans only, total baseline and endpoint HDRS scores were available for this analysis, while on Italian samples we also had single HDRS items scores. For these subsamples, antidepressant response, remission to treatments was assessed according to Schosser *et al.* [42] Response to treatment was defined as a 50% improvement of HDRS scores from baseline to endpoint. Remission was defined as a HDRS score of ≤ 7 at the endpoint; re-

sistance as non-response to at least two adequate consecutive antidepressant trials (including the present) [42]. A total of 32 AD, 121 MDD, and 172 BPD patients (all Italian) were also evaluated at baseline for anxiety symptoms by the Hamilton Anxiety Rating Scale [43]. Korean BPD patients, all in a manic episode, were evaluated by the Young Mania Rating Scale (YMRS) over a period of 3 to 6 weeks of treatment [44]. Response was defined as a $\geq$ 50% reduction from baseline in YMRS score. Remission defined as YMRS total score $\leq$ 12 [44].

Genetic Analysis

The choice of the SNPs was performed through a literature check of the data available on *ZNF804A* and its correlation with psychiatric disorders [12,25,35,45-47]. The list of SNPs was further enriched to guarantee its maximum possible coverage of the gene, according to the resources available. Out of 10 SNPs, only 2 SNPs were analyzed in both samples (rs1344706 and rs7597593). Five were genotyped in Koreans only (rs359895, rs1021043, rs17508706, rs1987025, and rs7588907), and 3 on Italians only (rs7605689, rs39317905, and rs7603001) due to ethnic specificity.

For the Korean sample, high-throughput genotyping using a pyrosequencer (Biotage AB, Uppsala, Sweden) was employed to genotype the genomic DNA from subjects blood. Polymerase chain reaction (PCR) primers (Bioneer, Daejeon, Korea) and sequencing primers (Bioneer) used for the pyrosequencing assay were designed through Pyrosequencing Assay Design software ver. 1 (Biotage), 1 primer of each primer set was biotinylated. Genomic DNA of Italian samples was extracted from blood thorough an automated magnetic-beads based nucleic acids extractor (Maxwell; Promega, Madison, WI, USA). Presence of the investigated SNPs within each sample was checked by a multiplex Sequenom MassArray platform (Sequenom Inc., San Diego, CA, USA). Sequenom's MassARRAY Designer software was used to designs PCR and extension primers (sequences available on request) for each investigated SNP. All analyses on the chosen SNPs were performed by two independent investigators blind to clinical information of the subjects. Samples showing ambiguous alleles were discarded if they showed the same features on repeated genotyping.

Statistical Analysis

Main statistical analyses were performed using the IBM SPSS package for Windows ver. 20.0 (http://www.ibm.com/analytics/us/en/technology/spss/; IBM Co., Armonk, NY, USA). Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested by Haploview 3.2 software for Windows (Broad Institute of MIT and Harvard, Cambridge, MA, USA) [48]. Haplotypes' analysis was performed in "R" environment (http://cran.r-project.org/), using the statistics package "haplo.score". To control for multiple testing we used Bonferroni correction to evaluate the significance of our findings. For the correction we took in consideration the number of variables tested for significance. Significance was considered for $p < 0.05/23 = 0.0022$.

RESULTS

Socio-demographic tables summarizing the characteristics of the samples under investigation are reported below (Tables 1 and 2). All the SNPs were in HWE (data not shown). Frequencies of alleles for rs7597593 did not vary significantly between the two samples ($\chi^2 = 0.009$, $p = 0.925$). A significant difference was detected for rs1344706 ($\chi^2 = 82.641$, $p < 0.001$), whose allelic distribution is widely known to be different between European and Asian populations (see Hap-Map frequencies on the site of National Center for Biotechnology Information; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1310647/).

Psychiatric Diagnosis

ZNF804A SNPs were not associated with psychiatric risk in the cross-diagnosis samples. However, some significant associations were evidenced for rs1344706 with BPD risk in the BPD merged sample (against controls, geno $p = 5.77 \times 10^{-6}$ and allelic $p = 2.78 \times 10^{-3}$). Interestingly, the same SNP, rs1344706, was also correlated as a trend with MDD risk (against controls, geno $p = 0.007$ and allelic $p = 0.014$) in the MDD merged sample (pooling the data from Korean and Italian). Table 3 summarizes our findings. No correlations between *ZNF804A* polymorphisms and AD with comorbid depression were evidenced by single SNP analyses.

ZNF804A polymorphisms were also tested to evidence possible differences between the two main mood dis-

Table 1. Sociodemographic data of the Korean sample

	MDD subsample (n = 242)	BPD subsample (n = 132)	Controls (n = 326)
Age (yr)	43.57 ± 14.81	36.36 ± 11.61	45.36 ± 13.06
Age at onset (yr)	39.75 ± 13.76	26.58 ± 10.20	–
Baseline HDRS score	22.75 ± 7.30	7.70 ± 4.03	–
Baseline YMRS score	–	33.27 ± 9.09	–
Sex (%)			
Female	62.0	34.1	54.9
Male	38	65.9	45.1
ND	0	0	0
Family history (%)			
Yes	19.4	31.8	–
No	80.2	29.6	–
ND	0.4	38.6	–
Suicide history (%)			
Yes	22.3	16.7	–
No	77.3	67.4	–
ND	0.4	15.9	–
Psychosis			
Yes	–	55.3	–
No	–	43.2	–
ND	–	1.5	–
Remission, HDRS (%)		–	
Yes	45.9	–	–
No	54.1	–	–
ND	0	–	–
Response, HDRS (%)			
Yes	37.6	–	–
No	62.4	–	–
ND	0	–	–
Remission, YMRS (%)			
Yes	–	90.2	–
No	–	9.9	–
ND	–	0	–
Response, YMRS (%)			
Yes	–	95.5	–
No	–	4.6	–
ND	–	0	–

Values are presented as mean ± standard deviation or percent only. MDD, major depressive disorder; BPD, bipolar disorder; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale; ND, not detected.

orders (MDD + depressed AD vs. BPD). However, none of the analyzed samples and subsamples evidenced any significant data. Finally, haplotype analysis did not reveal any significant association with diagnoses.

Clinical Features

Several variables were tested for association with *ZNF804A* SNPs, including age of onset, family history for psychiatric disease, suicide attempt, comorbidity with alcohol/substance disorder (Italian sample only), psychotic phenotype, depressive, manic and anxiety severity at baseline.

In the cross-diagnosis sample (BPD + MDD + AD), pooling Italian and Korean samples, we observed an association between rs1344706 and the psychotic phenotype (geno $p = 4.15 \times 10^{-4}$, allelic $p = 1.06 \times 10^{-4}$). Haplotype analysis did not reveal any significant association between *ZNF804A* SNPs and clinical features. Table 3 summarizes our findings.

Antidepressant Treatment Efficacy

ZNF804A SNPs were not associated with antidepressant treatment outcome, either in the subsamples of Italian depressed BPD and Italian AD with comorbid depression, or in pooled samples.

However, trends of association were observed between rs7597593 and remission to treatment (allelic $p = 0.025$), rs7597593 and response to treatment (geno $p = 0.025$, allelic $p = 0.007$), and rs7605689 (geno $p = 0.012$, allelic $p = 0.003$) in the cross-diagnostic Italian sample. Table 3 summarizes our findings.

Antimanic Treatment Efficacy

The efficacy of antimanic treatment was evaluated in the BPD subsamples of Korean and Italian groups. The pooled data from the two groups was also tested for association.

In the merged sample, our findings evidenced no significant correlations with remission. However, we observed some associations with response. In particular, rs1344706 (geno $p = 1.22 \times 10^{-3}$, allelic $p = 7.38 \times 10^{-4}$) was correlated with this phenotype in the merged sample. Further, the same SNP was also significantly associated with manic symptoms at baseline (geno $p = 2.16 \times 10^{-7}$, allelic $p = 1.10 \times 10^{-6}$) and their improvement (geno $p = 3.15 \times 10^{-7}$, allelic $p = 2.54 \times 10^{-6}$) in the pooled BPD sample. Table 3 summarizes our findings.

DISCUSSION

The main aim of this work was to investigate the possible role of *ZNF804A* variants on the modulation specific sub-phenotypes within psychiatric subjects in a cross-diagnostic setting. While the biological role of *ZNF804A* is still not entirely known, alterations within this gene ap-

Table 2. Sociodemographic data of the Italian sample

	MDD subsample (n = 151)	BPD subsample (n = 189)	AD subsample (n = 38)	Controls (n = 65)
Age (yr)	54.30 ± 15.11	47.27 ± 12.38	41.89 ± 14.56	42.08 ± 12.66
Age at onset (yr)	38.58 ± 18.68	27.05 ± 11.08	31.91 ± 14.66	–
Baseline HDRS score	19.08 ± 7.91	15.67 ± 6.64	13.33 ± 5.98	–
Baseline YMRS score	–	3.97 ± 5.71	–	–
Baseline HARS score	17.90 ± 7.79	16.38 ± 6.86	18.97 ± 6.15	–
Sex (%)				
Female	65.6	50.3	63.2	53.9
Male	34.4	49.7	36.8	44.6
ND	0	0	0	1.5
Family history (%)				
Yes	57.6	14.3	47.4	–
No	29.1	11.1	31.6	–
ND	13.3	74.6	21.1	–
Suicide history (%)				
Yes	24.5	9.0	0	–
No	58.9	15.9	68.4	–
ND	16.6	75.1	31.6	–
Psychosis				
Yes	3.3	0.5	0	–
No	96.7	30.2	100	–
ND	0	69.3	0	–
Remission, HDRS (%)				
Yes	27.2	54.0	60.5	–
No	31.8	21.2	18.4	–
ND	41.1	24.9	21.1	–
Response, HDRS (%)				
Yes	15.2	14.3	26.3	–
No	43.1	60.9	47.4	–
ND	41.7	24.9	26.3	–
Remission, YMRS (%)				
Yes	–	65.1	–	–
No	–	1.6	–	–
ND	–	33.3	–	–
Response, YMRS (%)				
Yes	–	14.8	–	–
No	–	18.0	–	–
ND	–	67.2	–	–
Remission, HARS (%)				
Yes	–	37.6	–	–
No	–	29.1	–	–
ND	–	33.3	–	–
Response, HARS (%)				
Yes	–	5.29	–	–
No	–	61.4	–	–
ND	–	33.3	–	–

Values are presented as mean ± standard deviation or percent only.
MDD, major depressive disorder; BPD, bipolar disorder; AD, primary anxiety disorder; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale; HARS, Hamilton Anxiety Rating Scale; ND, not detected.

pear to have a role in neurodevelopment: data available in literature links alleles of *ZNF804A* with alterations in neural activity and connectivity, cognitive effects, and neuroanatomical changes [18].

According to our data, the psychotic phenotype seems to be influenced by *ZNF804A* rs1344706 genotype in the pooled sample, regardless of the disorder. Analyses on the BPD pooled subsample confirmed the association of this

Table 3. Summary of the significant results from analyses

SNP	Variable	Type of analysis	Raw *p* value	Analysis details		
				Mean	Statistic value	95% CI
Cross-diagnosis merged sample (MDD + AD + BPD - Kor + Ita)						
rs1344706	Psychosis	Genotypic	4.154E-04	GG vs. TT	B = 1.398, SE = 0.409, *p* = 0.001, OR = 4.045	1.814−9.020
				TG vs. TT	B = 1.165, SE = 0.366, *p* = 0.001, OR = 3.207	1.566−6.570
		Allelic	1.063E-04	not G vs. G	B = −1.239, SE = 0.351, *p* = 0.000, OR = 0.290	0.145−0.577
Cross-diagnosis Italian sample (MDD + AD + BPD - Ita)						
rs7597593	Symptom improvement (HDRS)	Allelic	5.299E-04	C: μ = 13.364	Err. = 0.484	12.406−14.322
				not C: μ = 13.463	Err. = 0.927	11.629−15.297
Bipolar merged sample (BPD - Kor + Ita)						
rs1344706	Disease	Genotypic	5.769E-06	GG vs. TT	B = −1.054, SE = 0.219, *p* = 0.000, OR = 0.349	0.227−0.535
				TG vs. TT	B = −0.564, SE = 0.186, *p* = 0.002, OR = 0.569	0.396−0.819
		Allelic	2.783E-05	not G vs. G	B = 0.727, SE = 0.175, *p* = 0.000, OR = 2.069	1.469−2.913
	Psychosis	Allelic	2.009E-03	not G vs. G	B = −1.197, SE = 0.413, *p* = 0.004, OR = 0.302	0.134−0.679
	Antimanic response	Genotypic	1.215E-03	GG vs. TT	B = 1.862, SE = 0.599, *p* = 0.002, OR = 6.435	1.990−20.815
				TG vs. TT	B = 1.072, SE = 0.395, *p* = 0.007, OR = 2.920	1.346−6.334
		Allelic	7.377E-04	not G vs. G	B = −1.278, SE = 0.375, *p* = 0.001, OR = 0.279	0.134−0.581
rs1344706	HDRS score at baseline	Genotypic	7.872E-04	GG: μ = 9.750	Err. = 0.897	7.985−11.515
				TG: μ = 11.836	Err. = 0.567	10.719−12.952
				TT: μ = 13.931	Err. = 0.665	12.623−15.240
		Allelic	1.210E-03	G: μ = 11.240	Err. = 0.482	10.292−12.188
				not G: μ = 13.931	Err. = 0.668	12.617−15.246
	YMRS score at baseline	Genotypic	2.159E-07	GG: μ = 26.811	Err. = 2.148	22.582−31.041
				TG: μ = 19.992	Err. = 1.393	17.249−22.735
				TT: μ = 11.571	Err. = 1.706	8.212−14.931
		Allelic	1.102E-06	G: μ = 22.011	Err. = 1.182	19.683−24.339
				not G: μ = 11.571	Err. = 1.726	8.173−14.970
	Symptom improvement (YMRS)	Genotypic	3.153E-07	GG: μ = 17.363	Err. = 1.290	14.822−19.904
				TG: μ = 12.738	Err. = 0.821	11.122−14.355
				TT: μ = 8.259	Err. = 1.024	6.243−10.275
		Allelic	2.537E-06	G: μ = 14.071	Err. = 0.703	12.685−15.456
				not G: μ = 8.259	Err. = 1.040	6.211−10.307
Depressed bipolar Italian sample (BPD.D - Ita)						
rs7597593	Symptom improvement (HDRS)	Allelic	1.636E-03	C: μ = 14.799	Err. = 0.437	13.931−15.666
				not C: μ = 14.674	Err. = 0.826	13.036−16.312

SNP, single nucleotide polymorphism; 95% CI, 95% confidential interval; MDD, major depressive disorder; AD, depressed subsample of anxiety disorder; BPD, bipolar disorder; BPD.D, depressed subsample of BPD; Kor, Korean; Ita, Italian; SE, standard error; OR, odds ratio; HDRS, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale.

SNP with psychosis; moreover, in this subsample rs1344706 was also associated with BPD risk; further, it may play a role in manic symptoms severity (YMRS) and response to antimanic treatment. This SNP also showed an association with depressive symptoms improvement (HDRS), but only in the pooled bipolar sample.

Literature data report several evidences supporting the role of rs1344706 in psychiatric disorders [12,31-36,46,49], and with antidepressant response [25,40]. Regarding its association with psychosis, there were already some evidences indicating a possible correlation between this SNP and the psychotic phenotype [31], that indicated how rs1344706 may be able to mediate one of *ZNF804A* mRNA isoform, *ZNF804AE3E4*, during fetal life [31]. This mediation is the mechanic most likely involved in psychosis development. An *in-silico* analysis on the effect of this mutation on Human Splicing Finder (HSF) [50] (http://www.umd.be/HSF3) revealed that the change T > G may indeed create an enhancer consensus sequence and at the same time the loss of a potential branch point which may affect splicing mechanics. This alteration may be correlated to an early modification of neural nets during fetal neurodevelopment.

Another interesting result we obtained is the rs7597593 association with depressive symptoms improvement (HDRS) in the cross-diagnostic Italian sub-group. This SNP was associated with MDD in literature [12]; however, to our knowledge, it was the first time it was associated with depressive symptoms improvement. Despite the lack of data of this specific SNP, alterations within *ZNF804A* were already associated with antidepressant response to treatment, even by our group in another sample (only partially overlapping with this one) [25]. Of particular interest, is that our association this time was extended to include a BPD sample (in a cross-diagnostic setting). Also, this association was evidenced even in a BPD subsample (BPD depressed Italian subgroup). Overall, this data imply how *ZNF804A* action may have an effect on depressive symptoms improvement during treatment, irrespectively of the psychiatric disorder. Indeed, *ZNF804A* seems able to influence emotion perception mechanics [51], linking its variants with mood alterations.

This SNP is an intron variant, as such it does not directly cause any amminoacidic changes on ZNF804A protein. Also, it does not seem to have any relevant impact on splicing, at least according to HSF [50] (http://www.umd.be/HSF3). However, the same software, indicated that the change T > C cause the loss of a silencer consensus sequence, which may somewhat alter the transcriptional regulation of this gene, thus relevantly impacting with its function. It has to be noted that the significant data obtained, derived from our Italian sample. In the Korean sample rs7597593 did not show any relevant association or trend. Ethnic differences may play a role in this discrepancy; however, further analyses should be done on different sample in order to confirm this possibility.

Both the significant SNPs we found seem to exert their influence through a modification of ZNF804A isoforms concentrations rather than an actual modification of the protein. In particular, from *in-silico* data, these SNPs likely alter mRNA maturation mechanics. This data may hint to the importance of regulating ZNF804A isoforms concentration in neurodevelopment and synaptic maintenance. However, the precise molecular mechanics need further data to be correctly identified.

The main limit of our study is the choice of SNPs between the two laboratories, since only two of them overlapped between Korean and Italian samples. This selection was due to ethnic and logistic issues and somewhat limited our analyses, nevertheless the data obtained may be helpful in the analysis of *ZNF804A* role in psychiatric disorders and their treatment. Although we have tested two independent samples, the sample size is still relatively small compared to the large scale genetic studies. Secondly, our analyses tested some common SNPs within *ZNF804A*, thus SNPs other than the ones investigated, rare missense mutations and CNVs were not investigated. As such, we cannot guarantee a complete coverage of this Gene. Further, the trends underlined in this work have to be considered as exploratory and more analyses in larger sample would be needed to further evaluate our data. Also, ethnic differences should be taken in consideration while evaluating these results. Many results observed in the pooled samples were not observed in the ethnic homogenous ones, but trends emerged, probably for the reduction in sample size. It has to be noted, though, that rs1344706 distribution between the two samples did vary significantly. This variation should be taken in consideration when interpreting the results. Finally, the presence of false positives should always be taken in consideration even when statistical correction is applied.

The present study evidenced the role of *ZNF804A* alterations in symptoms improvement after treatment. Both manic and depressive symptoms seem to be modulated by *ZNF804A*, though the latter was observed in the bipolar pooled sample only. Its role on the psychotic phenotype was also observed, irrespectively of ethnic origin and psychiatric disorder. Unfortunately, the lack of a biological understanding of *ZNF804A* function, limit our capacity of correctly evaluate our data in the greater picture that is the neural nets (and the brain in general) physiology. Further efforts should be done to understand the biological functions of *ZNF804A*.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Conception and design of the study: Chi-Un Pae and Alessandro Serretti. Acquisition and analysis of data: Chi-Un Pae, Marco Calabrò, Laura Mandelli, Concetta Cristafulli, Soo-Jung Lee and Tae-Youn Jun. Original draft: Marco Calabrò. Final editing of manuscript and intellectual comments: Marco Di Nicola, Roberto Colombo, Luigi Janiri, Sheng-Min Wang, Prakash S. Masand, Ashwin A. Patkar, Changsu Han, Chi-Un Pae and Alessandro Serretti.

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