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Title: Anxiety symptoms in bipolar offspring with the brain-derived neurotrophic factor (BDNF) Val66Met genotype

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Keywords: Risk; Pediatric bipolar; Gene-Environment Interaction; Brain-Derived Neurotrophic Factor; Anxiety

Abstract: Several genetic and environmental factors place youth offspring of parents with bipolar disorder (BD) at high risk for developing mood and anxiety disorders. Recent studies suggest that anxiety symptoms, even at subclinical levels, have been associated with an increased risk for developing BD. The brain-derived neurotrophic factor (BDNF) gene has been implicated in the pathophysiology of both BD and anxiety disorders. We aimed to explore whether anxiety in BD offspring was associated with the BDNF Val66Met polymorphism. 64 BD offspring (mean age: 13.73 (S.D. 3.45) M=30, F=34) and 51 HC (mean age: 13.68 (S.D. 2.68) M=23, F=28) were compared on presence of the BDNF Val66Met polymorphism and on scores from the Multidimensional Anxiety Scale for Children (MASC). To assess family function, we used the Family Adaptability and Cohesion Evaluation Scales (FACES-IV). BD offspring showed higher levels of overall anxiety than did the HC group. BD offspring with the val/val genotype showed higher levels of anxiety than BD offspring with other genotypes. No significant levels of anxiety or its association with BDNF genotype were found in the HC group. BD offspring group showed significantly more family dysfunction when compared with the HC group and the family dysfunction moderated the interaction between the BDNF genotype and anxiety symptoms. This study demonstrated an association between the BDNF genotype and anxiety in BD offspring, moderated by family dysfunction. Longitudinal studies in high-risk offspring will aid in elucidating how these phenomenological, environmental, and genetic factors converge to the development of BD.

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Conflicts of interest

Dr. Chang is a consultant for GlaxoSmithKline, Bristol-Myers Squibb, Merck, and Sunovion. The other authors have nothing to disclose.

Contributors:
Drs Singh and Chang designed the study, wrote the protocol, and wrote and edited all drafts of the manuscript. Dr. Park conducted the literature search, analyses, and wrote the first draft of the manuscript. Dr. Hong contributed to the statistical analysis. Drs. Park, Chang, Hallmayer, Kim, Hong and Singh, and and Ms. Howe contributed to and approved the final manuscript.

*Cover Letter

STANFORD
UNIVERSITY

April 30, 2014

Alan Schatzberg, MD
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Dear Dr. Schatzberg,
We are submitting our manuscript, "Anxiety symptoms in bipolar offspring with the brain-derived neurotrophic factor (BDNF) Val66Met genotype," for consideration for publication in the *Journal of Psychiatric Research*. This manuscript demonstrates an association between the BDNF genotype and anxiety in youth offspring of parents with bipolar disorder, moderated by family dysfunction. We have not previously submitted this or any related manuscript for publication. None of the authors have commercial or any other financial interests in the results reported. All of the authors contributed significantly in this work so as to take public responsibility for this paper's contents. Given their expertise in the clinical at risk population or psychiatric genetics described in our study, we request the following individuals as potential reviewers for this paper:

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Thank you for your consideration. We look forward to hearing from you.

Sincerely,

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Funding for this study was provided by the NIMH (R01 MH077047 and K23 MH064460 to KDC and K23 MH085919 to MKS). The NIMH had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

Highlights

BD offspring group had higher levels of anxiety than the HC group.

BD offspring group had significantly more family dysfunction than the HC group.

BD offspring with the val/val genotype of the BDNF gene had higher anxiety than the other genotypes.

Family dysfunction moderated the association between the BDNF genotype and anxiety.

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Anxiety symptoms in bipolar offspring with the brain-derived neurotrophic factor (BDNF) Val66Met genotype

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ABSTRACT

Several genetic and environmental factors place youth offspring of parents with bipolar disorder (BD) at high risk for developing mood and anxiety disorders. Recent studies suggest that anxiety symptoms, even at subclinical levels, have been associated with an increased risk for developing BD. The brain-derived neurotrophic factor (BDNF) gene has been implicated in the pathophysiology of both BD and anxiety disorders. We aimed to explore whether anxiety in BD offspring was associated with the BDNF Val66Met polymorphism. 64 BD offspring (mean age: 13.73 (S.D. 3.45) M=30, F=34) and 51 HC (mean age: 13.68 (S.D. 2.68) M=23, F=28) were compared on presence of the BDNF Val66Met polymorphism and on scores from the Multidimensional Anxiety Scale for Children (MASC). To assess family function, we used the Family Adaptability and Cohesion Evaluation Scales (FACES-IV). BD offspring showed higher levels of overall anxiety than did the HC group. BD offspring with the val/val genotype showed higher levels of anxiety than BD offspring with other genotypes. No significant levels of anxiety or its association with BDNF genotype were found in the HC group. BD offspring group showed significantly more family dysfunction when compared with the HC group and the family dysfunction moderated the interaction between the BDNF genotype and anxiety symptoms. This study demonstrated an association between the BDNF genotype and anxiety in BD offspring, moderated by family dysfunction. Longitudinal studies in high-risk offspring will aid in elucidating how these phenomenological, environmental, and genetic factors converge to the development of BD.

Keywords: Risk; Pediatric bipolar; Gene-Environment Interaction; Brain-Derived Neurotrophic Factor; Anxiety

1. Introduction

Children of parents with bipolar disorder (BD) (BD offspring) are at higher risk for developing BD (Axelson et al., 2011; Chang et al., 2003) as well as other psychiatric disorders (Lapalme et al., 1997; Singh et al., 2007) than the general population. Anxiety disorders have attracted special attention not only because of their high prevalence in BD offspring but also because the presence of anxiety disorders may serve as a predictor for developing BD (Duffy et al., 2013; Henin et al., 2005). Even subclinical levels of anxiety can predispose individuals to developing BD, suggesting that having early anxiety symptoms may represent an endophenotype of BD (Contreras et al., 2010).

Decreased brain-derived neurotrophic factor (BDNF) levels have been implicated in both bipolar and anxiety disorders. BDNF is a member of the neurotrophin family of growth factors and is concentrated in brain regions involved in learning and memory, such as the hippocampus and amygdala (Hofer et al., 1990; Rakofsky et al., 2012). Reduced (BDNF) levels have been observed in patients with BD during manic and depressive episodes and these levels have normalized with episode recovery (Cunha et al., 2006; de Oliveira et al., 2009; Lin, 2009). Post-mortem studies have also demonstrated decreased hippocampal BDNF in patients with BD (Dunham et al., 2009; Knable et al., 2004). Similarly, reduced BDNF levels have been observed in individuals with anxiety disorders compared to those without anxiety disorders (Suliman et al., 2013). Finally, acute and chronic stress have been associated with decreased BDNF expression in the hippocampus with subsequent enhancement of anxiety-related behaviors (McEwen, 2001; Russo-Neustadt, 2003). Youth offspring of parents with BD are biologically sensitive to stress (Ostiguy et al., 2009; Ostiguy et al., 2011) and typically live in disorganized and conflictual family environments (Belardinelli et al., 2008; Chang et al., 2001).

Decreased BDNF levels in humans has also been associated with the met allele of

BDNF Val66Met polymorphism (rs6265) (Chen et al., 2006; Egan et al., 2003). However, studies on the association between the BDNF Val66Met polymorphism and BD or anxiety have been mixed, with a number of studies that have also found that the val/val allele also confers risk for anxiety or BD. This inconsistency across studies may be due to the heterogenous nature of these disorders or unidentified moderating factors between the BDNF genotype and psychiatric symptoms.

Although, anxiety may serve as a predisposing factor for BD development in BD offspring, no study has investigated the relation between anxiety and the BDNF genotype in cohorts at high-risk for BD, or the moderating effects of family dysfunction on this relation. Therefore, we aimed to compare levels of anxiety, as measured by the Multidimensional Anxiety Scale for Children (MASC) (March et al., 1997)**,** family dysfunction, measured by the Family Adaptability and Cohesion Evaluation Scales, version IV (FACES-IV) (Olson, 2011), and their associations with the BDNF genotype in BD offspring and healthy control (HC) groups. We hypothesized that (1) BD offspring would have higher levels of anxiety than the HC group; (2) levels of anxiety would interact with the BDNF Val66Met genotype in BD offspring but not in healthy controls, such that group status would moderate the relation between the BDNF Val66Met genotype and anxiety; and (3) compared to HC youth, youth offspring of parents with BD would have lower levels of family cohesion, organization, and satisfaction, and that these dysfunctional family characteristics would moderate the effect of the BDNF genotype on anxiety symptoms.

2. Materials and methods

2.1. Participants

The protocol was approved by the Stanford University Panel of Medical Research in Human Subjects. One hundred BD offspring and 60 HC were recruited from an ongoing

study of youth at risk for BD and from the community. After obtaining oral and written informed consent and assent from parents from their offspring, respectively, we conducted semi-structured interviews to assess for BD in parents and psychopathology in their offspring. Parents were diagnosed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (First et al., 1996), and were interviewed for psychiatric history of first- and second-degree relatives following the Family History-Research Diagnostic Criteria (FH-RDC) (Andreasen et al., 1977). BD offspring were assessed using the Affective Disorders Module of the Washington Schedule for Affective Disorders and Schizophrenia for School-Age Children (WASH-U-KSADS) (Geller et al., 2001). All evaluations were conducted by either a child and adolescent psychiatrist or a trained master's level research assistant with at least two years experience in psychiatric interviewing. Evaluators were aware of parental diagnosis, who were interviewed while they were euthymic at the time of their child's assessment. Inter-rater reliability was established by rating videotaped interviews, observing a trained rater, observing trained rater interviews, and performing interviews with observation by a trained rater. Diagnostic decisions were ultimately made by a board-certified child and adolescent psychiatrist (K.C. or M.S.) based on personal interview, discussion with the research assistant and written notes of parental and offspring responses to interview questions. Current and lifetime diagnoses were established according to DSM-IV criteria (American Psychiatric Association, 1994).

Youths aged 9 to 17 having at least one biological parent with BD were included in the BD offspring group. BD offspring with the following disorders or conditions were excluded: BD I or II, Tourette's disorder, substance use disorder, or a neurological condition (such as a seizure disorder). HC had no personal or parental psychiatric history and no family history of BD, and were excluded with current Young Mania Rating Scale (YMRS) (Young et al., 1978) scores greater than 8 or Childhood Depression Rating Scale (CDRS-R)

(Poznanski et al., 1985) greater than 26.

2.2. Instruments

2.2.1. Multidimensional Anxiety Scale for Children (MASC)*:* To assess anxiety symptoms, we used the MASC, a 39-item scale, distributed into four major factors: physical symptoms, social anxiety, harm avoidance, and separation anxiety. The MASC has been shown to distinguish anxiety from depression, and differentiate between various anxiety disorders (Dierker et al., 1999).

2.2.2. Family Adaptability and Cohesion Evaluation Scales, version IV (FACES-IV): To assess family function, we used the FACES-IV, a questionnaire completed by parents participating in this study. The FACES-IV includes 42 items loading on six subscales: balanced cohesion, balanced flexibility, disengaged, enmeshed, rigid, and chaotic. The family communication and family satisfaction scales are additional scales and they indicate how healthy the communication with family is and how satisfied individual's are in regard to life with their family. High family satisfaction is usually related to higher levels of balanced cohesion and flexibility and lower levels of the four unbalanced scales (disengaged, enmeshed, rigid, and chaotic).

2.3. Genotyping

DNA was obtained from peripheral venous blood (20ml). All genotyping assays were conducted within the laboratory of Dr. Joachim Hallmayer at Stanford by the same technician blinded to all clinical data. BDNF val66met genotyping was conducted as per the laboratory's standard protocol, utilizing the following primers for G196A in the BDNF gene: forward 5'-ATC CGA GGA CAA GGT GGC-3' and reverse 5'-CCT CAT GGA CAT GTT

TGC AG-3'. This generated 300 bp of polymerized chain reaction (PCR) products, subsequently digested by Pml I (New England Biolabs, Ipswich, MA) to yield either met allele (undigested, 300 bp) or val allele (digested to 180 bp þ 120 bp bands), which were visualized on 7% polyacrylamide gel using a 50 bp marker.

2.4. Statistical analysis

Since participants with the met/met were rare, the BDNF Val66Met genotype was dichotomized according to whether or not they possessed the rare met allele (Kaufman et al., 2006; Kim et al., 2007). To compare categorical variables, we conducted χ^2 tests. To compare continuous variables, we conducted independent *t* test for parametric data or Mann-Witney U test for non-parametric data. The statistical significance for all tests was set at $p < 0.05$. We corrected significance levels for multiple comparisons using a Bonferroni correction for MASC subscales(0.05／5 subscales = 0.01) and for FACES-IV subscales (0.05／8 subscales = 0.00625). We used Baron & Kenny's method to examine the moderating effect between the independent and dependent variables (Baron and Kenny, 1986).

3. Results

Among 100 BD offspring and 60 HC, 36 BD offspring and 9 HC were excluded due to incomplete MASC data, leaving 64 BD offspring (mean age: 13.73 (3.45) M=30, F=34) and 51 HC (mean age: 13.68 (2.68) M=23, F= 28) to be included for statistical analysis. Participant demographic and clinical characteristics are provided in Table 1. The BD offspring group showed significantly higher anxiety scores than the HC group in the physical subscale (p=0.001), social anxiety scale (p=0.037), and in the overall MASC score (p=0.018). Results for the physical subscale and MASC total scores remained significant after adjusting for multiple comparisons (Table 1, Fig. 1-a).

Parent reports on FACES-IV data was available for 22 BD offspring and 28 HC. There were no differences in demographic and clinical characteristics between those who provided and those who didn't provide FACES-IV data both in the BD offspring and HC groups. The BD offspring group showed significantly more family dysfunction when compared with the HC group with lower levels of cohesion ($p=0.006$), higher levels of enmeshment ($p=0.002$) and chaos ($p=0.049$), and lower levels of family satisfaction ($p=0.003$) and communication ($p=0.001$). Results for all FACES subscales remained significant after adjusting for multiple comparisons.

The val/val genotype of the BDNF Val66Met polymorphism was observed in 34 subjects (53.1 %), the val/met genotype in 27 subjects (42.2%), and the met/met genotype in 3 subjects (4.7 %) in the BD offspring group and the val/val genotype was observed in 34 subjects (66.7 %), the val/met genotype in 15 subjects (29.4%), and the met/met genotype in 2 subjects (3.9 %) in the HC group. The distributions of genotypes for the Val66Met polymorphism were consistent with expected values based on Hardy-Weinberg equilibrium in both the BD offspring ($p<0.164$) and HC groups ($p<0.997$).

When all subjects were dichotomized according to the presence or absence of the met allele (val/val vs. val/met + met/met), no significant difference was observed in the MASC scores for each BDNF genotype. There were also no significant differences in the MASC total or subscales by BDNF genotype within the HC group. However, within the BD offspring group, subjects with the val/val genotype showed significantly higher anxiety scores than subjects with the val/met or met/met genotype in the harm avoidance subscale ($p=0.005$), social anxiety subscale ($p=0.019$) and total MASC scores ($p=0.010$). Results for the harm avoidance subscale and MASC total scores remained significant after adjusting for multiple comparisons (Table 2, Fig. 1-b).

We examined the moderating effect of being BD offspring on anxiety symptoms by BDNF genotype using the Baron & Kenny method. In step 1, we conducted a regression analysis in which we analyzed the effect of the BDNF genotype and BD offspring status on the MASC total score, social anxiety subscale and harm avoidance subscale. Then, in step 2, we input the interaction variable between the BDNF genotype and BD offspring into the variables used in the step 1 analysis. The step 1 analysis revealed that the BDNF genotype did not have an effect on the harm avoidance subscale ($p>0.05$), social anxiety subscale ($p>0.05$) or total MASC scores ($p>0.05$). However, in step 2, we found a moderating effect of being BD offspring, in which BDNF genotype had an effect on the harm avoidance subscale ($B=-4.147$, $p<0.01$), social anxiety subscale ($B=-4.582$, $p<0.05$) and total MASC scores ($B=-12.500$, $p<0.05$) (Fig. 2). Thus, the BDNF genotype itself did not affect anxiety scores. However, when the BDNF genotype was combined with BD offspring status, the BDNF genotype had an effect on anxiety scores, suggesting that BD offspring status moderated the interaction between the BDNF genotype and anxiety.

We also examined the moderating effect of the FACES-IV scores on social anxiety symptoms by BDNF genotype using the Baron & Kenny method. The step 1 analysis revealed that the BDNF genotype did not have an effect on social anxiety subscale However, in step 2, we found a moderating effect of enmeshed, communication or satisfaction scores, in which BDNF genotype had an effect on social anxiety subscale ($B=2.091$, $B=1.530$ or $B=1.090$ respectively, $p<0.05$). Thus, the BDNF genotype itself did not affect social anxiety score. However, when the BDNF genotype was combined with the FACES-IV scores, the BDNF genotype had an effect on social anxiety score, suggesting that the FACES-IV scores moderated the interaction between the BDNF genotype and social anxiety.

Our main findings were not affected by the current usage of anti-anxiety or stimulant medications (Table 2). When subsequent analyses were performed excluding the subjects

who were taking anti-anxiety medication and stimulants respectively, similar results were found.

4. Discussion

In this study, we demonstrated that as hypothesized, BD offspring have higher levels of anxiety than typically developing healthy controls, and anxiety symptoms interacted with BDNF genotype in BD offspring but not in healthy controls. Specifically, BD offspring with the val/val genotype showed higher anxiety symptoms than BD offspring with other genotypes. Further, we found BD offspring group showed significantly more family dysfunction when compared with the HC group and the family dysfunction moderated the interaction between the BDNF genotype and anxiety symptoms. These findings have implications for early identification of certain genetic and environmental vulnerabilities for developing BD in youth at risk for BD.

Among the MASC subscales, the physical subscale showed the most prominent difference between the BD offspring and HC groups. The physical subscale score can be affected by elevated autonomic nervous system activity, release of cortisol and epinephrine, and amygdala excitability (McTeague and Lang, 2012). Further, the physical subscale has been shown to predict the future development of panic disorder and agoraphobia (van Gastel and Ferdinand, 2008). These findings suggest that youth offspring of BD parents may have an increased biological predisposition for anxiety disorders that manifests most prominently by physical symptoms of anxiety.

Interestingly, the harm avoidance and social anxiety subscales showed the most prominent difference between BDNF Val66Met genotype groups in the BD offspring group. The harm avoidance and social anxiety subscales have previously been related to subclinical anxiety symptoms and traits, but neither has been fully accurate in predicting a DSM-

diagnosis of an anxiety disorder (van Gastel and Ferdinand, 2008). The harm avoidance subscale has also been related to perfectionism and anxiety coping, whereas the social anxiety subscale has been related to humiliation and public performance fears (March et al., 1997). Therefore, it may be assumed that high scores on those subscales are related to subclinical range of anxiety symptoms or anxiety trait rather than an anxiety disorder.

Prior studies on the association between the BDNF genotype and anxiety have shown inconsistent results. Interestingly, most studies have not demonstrated an increased incidence of anxiety disorders by BDNF genotype, including studies of obsessive-compulsive disorder, panic disorder, generalized- anxiety disorders, or phobias (Enoch et al., 2008; Shimizu et al., 2005; Surtees et al., 2007; Wendland et al., 2007; Zhang et al., 2006). A meta-anaylsis relating anxiety to BDNF genotype in adults with anxiety or anxiety-related personality traits was also negative (Frustaci et al., 2008) An association between higher anxiety trait and the val/val genotype has been found in healthy individuals and in animals (Hünnerkopf et al., 2007; Lang et al., 2004). Others have demonstrated an association between higher anxiety trait and the met allele (Colzato et al., 2011; Jiang et al., 2005; Montag et al., 2010; Soliman et al., 2010), while yet others have shown no relation between anxiety and the BDNF genotype (Baekken et al., 2008; Tochigi et al., 2006). These inconsistencies may result from significant symptom heterogeneity among their participants and environmental factors such as family functioning.

Prior studies have also been inconsistent about the relation between BD and the Val66Met polymorphism of BDNF gene. Some family-based association studies reported significant over-transmission of the val allele in patients with BD (Geller et al., 2004; Neves-Pereira et al., 2002; Sklar et al., 2002; Strauss et al., 2005; Tang et al., 2008) whereas others have found no relation between BD and the Val66Met polymorphism of BDNF gene (Green et al., 2006; Hong et al., 2003; Kunugi et al., 2004; Nakata et al., 2003; Oswald et al., 2004;

Skibinska et al., 2004). Some studies have found that the val allele is associated with early age of onset of BD (Geller et al., 2004; Strauss et al., 2005; Tang et al., 2008), rapid cycling (Green et al., 2006; Müller et al., 2006) and suicide attempts (Vincze et al., 2008). However, other studies have linked the met allele to early age of BD onset (Skibinska et al., 2004), suicide attempts (Kim et al., 2008), and structural or functional abnormalities in areas important for the regulation of mood and anxiety, such as the hippocampus and amygdala (Bueller et al., 2006; Egan et al., 2003; Frodl et al., 2007; Hariri et al., 2003; Montag et al., 2009; Nemoto et al., 2006; Soliman et al., 2010). Thus, while BDNF genotype appears to be relevant to mood and anxiety status, the exact relationship is not clear, and it may be that environmental factors moderate the effects of BDNF genotype on behavioral outcome.

Perhaps the most significant environmental factor in BD offspring is the family environment, as families with a BD parent have been noted to have high levels of conflict and lower levels of cohesion (Barron et al., 2014; Chang et al., 2001; Romero et al., 2005). These studies found that families with a bipolar parent are chaotic, have lower family satisfaction, and lower family communication. As familial influences on defense and coping mechanisms have been identified as important mediators of psychopathology (Thienemann et al., 1998), gene by environment interaction effects may cause epigenetic modulation in BD offspring that might explain our findings in the context of exposure to family stress. For example, Gatt et al. (2009) showed a significant interaction between Val66Met polymorphism and early life stress. Animal studies have also shown that early life traumas can affect the epigenetic regulation of BDNF genes (Bazak et al., 2009; Roth et al., 2009). Such stressors have been found to cause reduced expression of BDNF, which is mediated by stress-related hormones such as catecholamines and glucocorticoids (Carbone and Handa, 2013; Duman, 2002; Hartmann et al., 2001; Rasmusson et al., 2002).

Finally, even though not many BD offspring with the val/val genotype showed clinical

levels of anxiety symptoms as defined by structured clinical interview, many of BD offspring with the val/val genotype showed subclinical anxiety symptoms. It is possible that subclinical anxiety symptoms may represent early prodromal symptoms of BD, as well as potential early forms of anxiety disorders. Future longitudinal studies would clarify the relation between these early symptoms and the development of BD.

There are some limitations in our study that are worth considering. First, this study is a cross-sectional study that will require longitudinal follow up to determine whether anxiety traits predict conversion to BD in youth at familial risk. Second, the number of subjects is relatively small for genetic analysis, and our sample contains multiple ethnic groups that could account for variability in our sample. However, our results did not change when we re-analyzed our data after excluding non-Caucasian participants.

It is important to identify comprehensive risk factors for BD development in these high-risk offspring. We found that our BD offspring group showed significantly more family dysfunction when compared with the HC group, and that the family dysfunction moderated the interaction between the BDNF genotype and anxiety symptoms. Early identification of specific environmental and genetic risk factors will enable clinicians to perform earlier interventions to prevent the onset of mood problems in high-risk youth.

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Table 1 Demographic and clinical characteristics of the participants.

		BD offspring (N=64)	Control (N=51)	p
Age		13.73 (3.45)	13.68 (2.68)	.930
Gender	male (%)	30(46.88)	23(45.10)	1.000
Race	Non-caucasian (%)	6(9.37)	20(39.22)	<.000
YMRS	mean (S.D.)	6.50(6.90)	1.10(1.34)	<.000
CDRS	mean (S.D.)	27.33(11.74)	18.85(2.24)	<.000
MASC	mean (S.D.)			
Physical		8.05 (7.01)	4.31(4.06)	.001
Harm avoidance		16.41(4.71)	15.76(4.64)	.466
Social anxiety		10.06 (6.14)	7.75(5.41)	.037
Separation anxiety		5.95(5.24)	5.51(4.35)	.628
MASC Total		40.47(17.09)	33.33(14.24)	.018

MASC, Multidimensional Anxiety Scale for Children

YMRS, Young Mania Rating Scale

CDRS, Children's Depression Rating Scale

BD, Bipolar disorder

NOS, not otherwise specified

ADHD, attention-deficit/hyperactivity disorder

ODD, oppositional defiant disorder

GAD, generalized anxiety disorder

Table 2 The demographic and clinical characteristics by BDNF genotype in bipolar offspring.

		val/val (N=34)	val/met +met/met (N=30)	p
Age		13.08 (3.34)	14.51 (3.48)	.099
Gender	Male (%)	17 (48.6)	13(44.8)	.481
Race	Non-caucasian (%)	3 (8.82)	3 (10.00)	.809
YMRS	mean (S.D.)	5.93(6.60)	7.21(7.33)	.505
CDRS	mean (S.D.)	26.40(10.28)	28.50(13.49)	.519
Mood disorder	BD.NOS (%)	9 (25.7)	2 (6.9)	.046
	MDD (%)	10(28.6)	9(31.0)	1.000
	Dysthymia (%)	1(2.9)	4(13.8)	.167
Comorbidity				
	ADHD (%)	9 (25.7)	6 (20.7)	.432
	ODD (%)	1(2.9)	3(10.3)	.321
	GAD (%)	8 (22.9)	5(17.2)	.406
	Anxiety disorder.NOS	0 (0.0)	3(10.3)	.088
	Phobia	2 (5.7)	2(6.9)	1.000
Anti-anxiety medication	Total (%)	8 (22.9)	5 (17.2)	.757
	SSRI (%)	5 (14.3)	5 (17.2)	1.000
	Antidepressant	5 (14.3)	4 (13.8)	1.000

	other than SSRI(%)			
	Anxiolytics (%)	2 (5.7)	2 (6.9)	1.000
MASC	mean (S.D.)			
Physical		8.74(6.7)	7.21(7.4)	.388
Harm avoidance		17.89(3.9)	14.62(5.0)	.005
Social anxiety		11.69(5.7)	8.10(6.2)	.019
Separation anxiety		7.09(5.6)	4.59 (4.4)	.057
MASC Total		45.40(14.7)	34.51(18.1)	.010

YMRS, Young Mania Rating Scale

CDRS, Children's Depression Rating Scale

BD, Bipolar disorder

NOS, not otherwise specified

MDD, major depressive disorder

ADHD, attention-deficit/hyperactivity disorder

ODD, oppositional defiant disorder

GAD, generalized anxiety disorder

SSRI, selective serotonin reuptake inhibitor

MASC, Multidimensional Anxiety Scale for Children

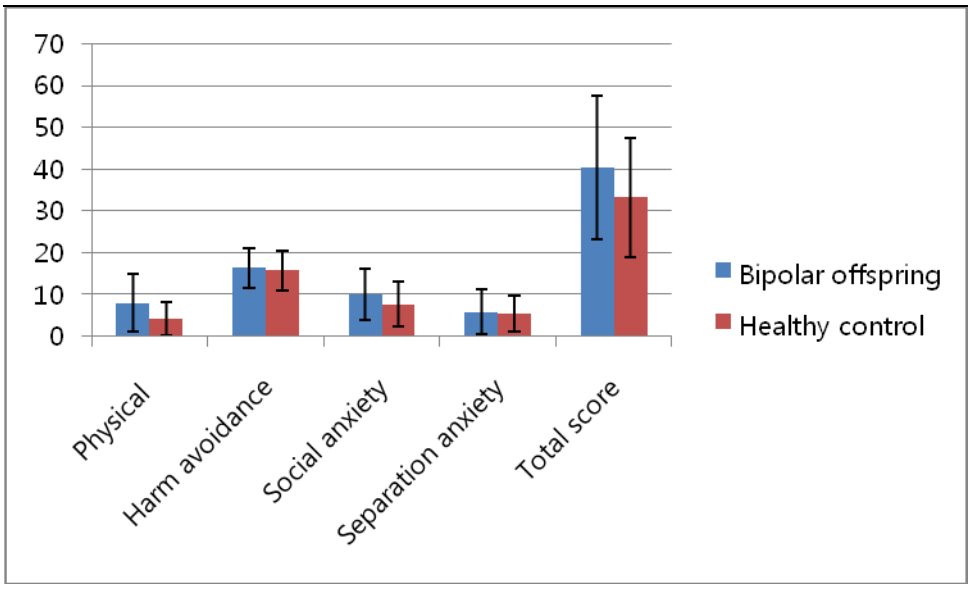
Figure Captions

Fig. 1. Anxiety symptoms differences (a) between the bipolar offspring group and healthy control group (b) by BDNF genotype in bipolar offspring.

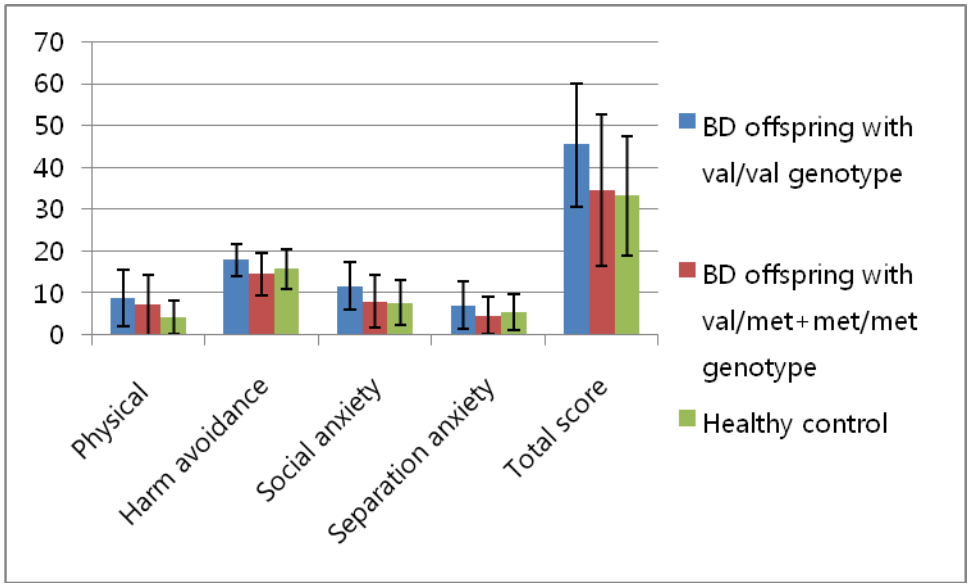
Fig. 2. The moderating effect of BD offspring status on anxiety symptoms.

Figure 1

Fig. 1. Anxiety symptoms differences (a) between the bipolar offspring group and healthy control group (b) by BDNF genotype in bipolar offspring.



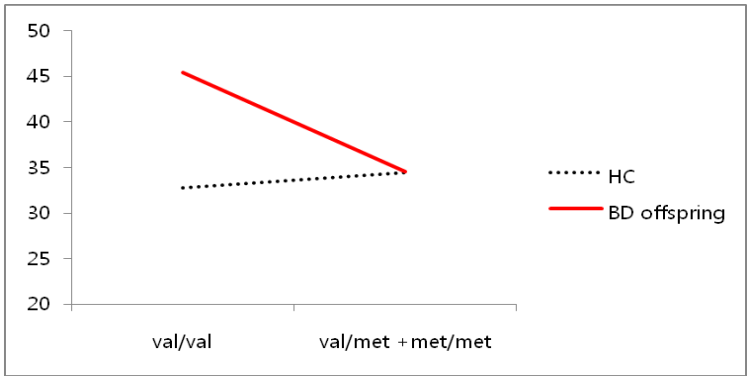
(a)



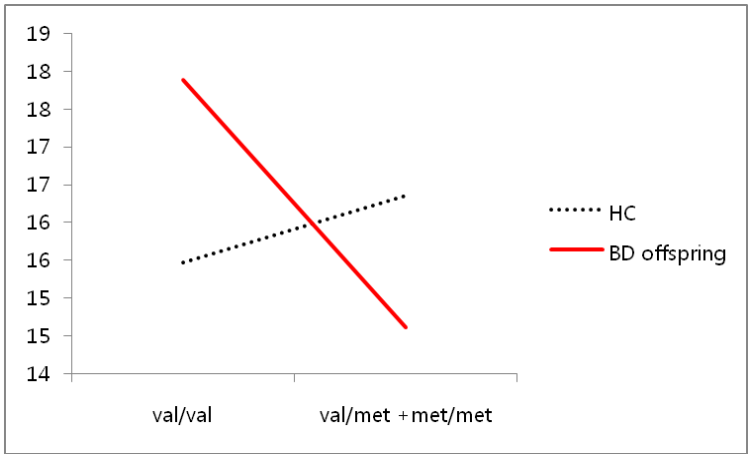
(b)

Figure 2

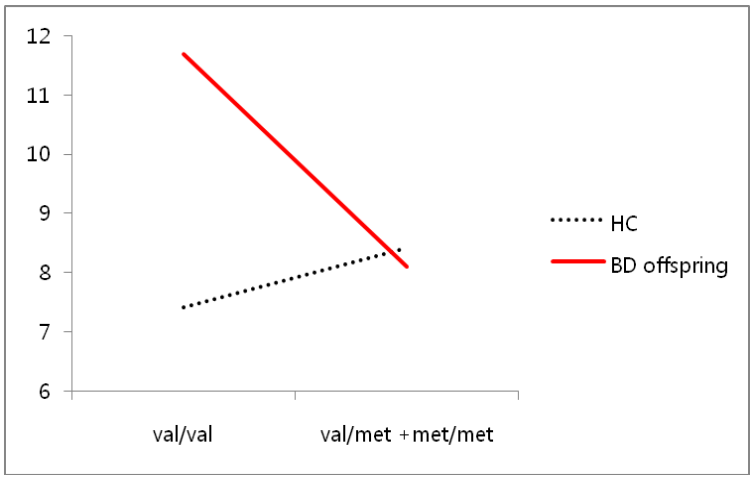
Fig. 2. The moderating effect of BD offspring status on anxiety symptoms.



(a) MASC total



(b) Harm avoidance



(c) Social anxiety