Association study of candidate variants from brain-derived neurotrophic factor and dystrobrevin-binding protein 1 with neuroticism, anxiety, and depression

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Objectives **Association of the valine/methionine variant at codon 66 (Val66Met) of brain derived neurotrophic factor (BDNF) has been reported inconsistently across a spectrum of psychiatric disorders. Haplotypes of six tagging single nucleotide polymorphisms (SNPs) of a 37-kb region of dystrobrevin-binding protein 1 (DTNBP1) were found to be associated with schizophrenia. These haplotypes have not been studied extensively for other psychiatric disorders but are plausible candidates for anxiety and depression disorders. Here, association between variants of BDNF and DTNBP1, and multiple anxiety and depression phenotypes is explored.**

Methods **Study participants were selected as sibling pairs that were either concordant or discordant for extreme neuroticism scores from a total sample of 18 742 Australian twin individuals and their siblings. All participants completed detailed Composite International Diagnostic Interview from which diagnoses of *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-IV depression and anxiety disorders were determined. Six hundred and seventy-four participants had a diagnosis of anxiety and/or depression from 492 families. The BDNF Val66Met and six DTNBP1 (rs3213207, rs1011313, rs2619528, rs760761, rs1018381, rs2619538) SNPs were genotyped on samples from study participants ($n=2045$ from 987 families) and, where possible, their parents ($n=787$). Family-based association tests were conducted between the individual SNPs and the DTNBP1 six SNP haplotypes and anxiety, depression, and neuroticism.**

Results **We found no convincing evidence for association between any of the variants studied and anxiety, depression, or neuroticism.**

Conclusion **This study sample is relatively large but our results do not support an association between BDNF Val66Met and anxiety, depression, or neuroticisim. DTNBP1 haplotypes, which have been found to be risk factors for schizophrenia, are unlikely to be risk factors for anxiety and depression. *Psychiatr Genet* 18:219–225 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Psychiatric Genetics 2008, **18**:219–225

Keywords: anxiety, association, brain-derived neurotrophic factor, dystrobrevin binding protein 1, major depression, neuroticism

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Received 19 November 2007 **Accepted** 6 February 2008

Introduction

Variants in the genes encoding brain-derived neurotrophic factor (BDNF) and dystrobrevin binding protein 1 (DTNBP1) have been studied extensively for association with schizophrenia. Replication of reported associations has been inconsistent, but meta-analyses (Mutsuddi *et al.*, 2006; Gratacos *et al.*, 2007) favor the existence of associations, albeit of low increased risk, and likely multiple causal variants. Associations with other psychiatric disorders and personality traits have been studied but to a lesser extent. Depression and anxiety are considered to have a common genetic basis (Levinson, 2006) and are often comorbid: a prospective longitudinal cohort study found that 72% of lifetime generalized anxiety disorder cases had a history of depression and 48% of lifetime depression cases had anxiety (Moffitt, 2007). The personality trait of neuroticism is defined as a tendency to experience psychological distress and individuals with high neuroticism scores are characterized by emotional instability, low self-esteem, anxiety, and depression (Eaves *et al.*, 1989). Neuroticism scores are found to be high in those suffering from psychiatric disorders such as major depression and anxiety disorders (Hirschfeld and Klerman, 1979) and this association seems to be reciprocal and at least partly of genetic origin with the genetic correlations between neuroticism and depression or anxiety ranging from 0.5 to 0.8 (Jardine *et al.*, 1984; Fanous *et al.*, 2002; Kendler *et al.*, 2006). Less recognized is the comorbidity between anxiety disorders and schizophrenia, but 62% of patients receiving treatment for schizophrenia (Huppert and Smith, 2005) and 25% of patients being discharged after treatment for schizophrenia (Seedat *et al.*, 2007) were found to qualify for diagnoses of anxiety disorders, although the extent to

0955-8829 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/YPG.0b013e3283050aee

which this is a genetic relationship, is unclear. In this study, we investigate variants in BDNF and DTNBP1, which have been found to be associated with schizophrenia and we test for association in a large study sample measured for anxiety, depression, and neuroticism. First, we review the association studies and meta-analyses conducted for BDNF and DTNBP1 with these traits.

Brain-derived neurotrophic factor

BDNF plays an important role in neuronal differentiation in the development as well as in synaptic plasticity, and neuronal survival in the adult brain (Thoenen, 1995; Angelucci *et al.*, 2005) making variants of BDNF plausible causal candidates for association with a spectrum of psychiatric disorders. A nonsynonymous single nucleotide polymorphism (SNP), G→A, at codon 66 (Val66Met) within the *BDNF* gene results in an amino acid substitution of valine (Val)→methionine (Met). Allele frequencies differ between populations; pooling control populations from several studies, the frequency of the Val variant was estimated to be 0.81 in Caucasian and 0.56 in Asian populations (Gratacos *et al.*, 2007). Association studies of the Val66Met polymorphism have been conducted for a wide range of psychiatric-related disorders but with mixed and often conflicting results. Even meta-analyses conducted on largely the same primary resources have led to contradictory conclusions (Gratacos *et al.*, 2007; Kanazawa *et al.*, 2007). In the most comprehensive meta-analysis conducted to date (Gratacos *et al.*, 2007), 39 case–control studies of five psychiatric phenotypes were examined, comprising 15 mood disorder studies (including eight studies of bipolar and six studies of major depression), four eating disorder studies, 14 schizophrenia and other psychotic disorder studies, and six substance abuse studies. The meta-analysis showed no evidence for association with mood disorders, but the authors note that family-based studies not included in their analysis (Neves-Pereira *et al.*, 2002; Sklar *et al.*, 2002; Geller *et al.*, 2004) and which control for population stratification have shown consistent evidence for preferential transmission of the Val variant. The meta-analysis did find association with the Val66Met variant and the other three phenotypes, but with association to different genotypes: Risk increased for schizophrenia with the Met/Met genotype, for eating disorders with Val/Met and Met/Met genotypes, and for substance abuse with the Val/Val genotypes. Association studies of working memory tests have been reviewed (Hansell *et al.*, 2007) and show consistent evidence that the presence of Met is associated with poorer scores. Only a handful of studies have been conducted on anxiety-related disorders: in a study of 164 triads with obsessive compulsive disorder (OCD) probands, significant overtransmission was found for the Val variant ($P = 0.0005$) (Hall *et al.*, 2003). However, three other studies of OCD (Mossner *et al.*, 2005; Zai *et al.*, 2005; Wendland *et al.*, 2008) and two studies of panic disorder (Lam *et al.*, 2004; Shimizu *et al.*, 2005) found no evidence of association. In addition, no evidence of association was found with the Val66Met variant in a large-scale study of neuroticism (Willis-Owen *et al.*, 2005).

Dystrobrevin-binding protein 1

DTNBP1 is a 140-kb gene encoding dystrobrevin-binding protein 1 or dysbindin and is considered to be one of the best candidate genes for risk of schizophrenia (Straub *et al.*, 2002; Williams *et al.*, 2005). Reduced protein (Talbot *et al.*, 2004) and gene (Weickert *et al.*, 2004) expression of DTNBP1 has been observed in schizophrenia cases compared with controls and a functional role of DTNBP1 may be through binding with snapin at synaptic sites (Talbot *et al.*, 2006). Comparison of studies has been hampered by the genotyping of different variants in different studies. By using HapMap CEU trio genotype data, Mutsuddi *et al.* (2006) identified a set of six tagging SNPs that define the associated region, but show that there is inconsistency between studies as all major haplotypes from the 37-kb region showing association with schizophrenia in different studies, perhaps implying multiple underlying causal variants. In the single study conducted so far for major depressive disorder (MDD), no evidence for association with DTNBP1 variants was found (Zill *et al.*, 2004), but an association has been reported between DTNBP1 variants and response to antidepressant treatment in a small sample of patients with MDD (Pae *et al.*, 2007). DTNBP1 variants have also been found to be associated with general cognitive ability (Burdick *et al.*, 2006) and prefrontal brain function (Fallgatter *et al.*, 2006) in healthy individuals.

In this study, we investigate association between variants of *BDNF* and *DTNBP1* and neuroticism, major depression and anxiety disorders and seek to replicate associations with specific alleles and/or haplotypes reported in other studies.

Materials and methods

Ascertainment

All participants were adult twins and their families recruited through the Australian Twin Registry and were predominantly of North European ancestry. All provided written informed consent under the study protocols approved by the Queensland Institute of Medical Research Human Research Ethics Committee. Over the period 1980–1995, the participants completed self-report questionnaires, which included either the full 90-item Eysenck Personality Questionnaire revised (EPQ-R) (Eysenck *et al.*, 1985) with a 23-item neuroticism scale or a shortened questionnaire (EPQ-R-S) with a 12-item neuroticism scale. EPQ-R or EPQ-R-S neuroticism scores were available for 18 742 Australian twin individuals and their siblings. Sibling pairs that were either concordant or discordant for extreme EPQ scores (one sibling in the top

or bottom decile, the other sibling in the top or bottom quintile and excluding monozygotic twin pairs) were recruited to complete a more detailed personality questionnaire. Using these criteria, multiple siblings were selected from some families. This extreme discordant and concordant (EDAC) (Risch and Zhang, 1995) design is a cost-efficient strategy for obtaining an informative data set for genetic studies (Purcell *et al.*, 2001). Blood (or buccal) samples were obtained where possible from the selected siblings and their parents. Full details of the recruitment procedure for study, including response rates and incidence of *Diagnostic and Statistical Manual of Mental Disorder*s (DSM)-IV diagnoses for anxiety and depression-related disorders are given elsewhere (Kirk *et al.*, 2000).

Phenotypes

EPQ-R neuroticism scores ($n = 1968$) were analyzed as sex-standardized residuals of the averaged angular transformation (Freeman and Tukey, 1950) after regression of the transformed neuroticism scores on age, sex, age $\times$ sex, age^2, and age$^2 \times$ sex calculated using the population sample from which the EDAC sample was selected (Wray *et al.*, 2007). EDAC study participants completed a questionnaire that included a shortened Composite International Diagnostic Interview (CIDI, 1997), which provided DSM-IV (DSM-IV, 1994) life-time diagnoses of depression (296.2: MDD, single episode, 296.3: MDD, recurrent episode or 300.4: dysthymic disorder) and anxiety (300.23: social phobia, 300.02: generalized anxiety disorder, 300.01: panic with agoraphobia, 300.21: panic without agoraphobia, 300.22: agoraphobia without panic, and 300.03: OCD). Standard clinical significance exclusion criteria (Andrews *et al.*, 2001) were applied, which helps to ensure accurate prevalence rates of DSM-IV diagnoses (Kirk *et al.*, 2000; Andrews *et al.*, 2001). Diagnoses were coded as: 2 = affected, 1 = unaffected for all DSM-IV diagnoses, 0 = not scored or affected for a different DSM-IV diagnosis. The DSM-IV phenotypes used for association analysis were DEP (any depression diagnosis, $n = 518$, $n_{\text{families}} = 399$), ANX (any anxiety diagnosis, $n = 382$, $n_{\text{families}} = 310$) and DEP or ANX (any depression or anxiety diagnosis, that is, all cases in ANX and/or DEP, $n = 674$, $n_{\text{families}} = 492$) and two specific disorders, anxiety OCD ($n = 114$, $n_{\text{families}} = 105$), and PDAG (panic disorder and/or agoraphobia, $n = 105$, $n_{\text{families}} = 101$). This study sample comprises 2045 study participants and a total of 2832 genotyped individuals from 987 families. A description of the structure of the data with respect to family size and number of DEP or ANX-affected individuals is listed in Table 1. Although not selected as part of the EDAC design, 68 MZ twin pairs were included in the study when an additional sibling had been selected. Only one individual from each of these MZ twin pairs were used in the analysis, preferentially selecting the one with any DSM-IV diagnosis of anxiety or depression if they were discordant for affected status.

Single nucleotide polymorphism genotyping

The Val66Met (rs6265) from BDNF and six SNPs from DTNBP1 (rs3213207, rs1011313, rs2619528, rs760761, rs1018381, rs2619538) were genotyped by primer extension reaction and MALDI-TOF mass spectrometry (MassARRAY, Sequenom Inc., San Diego, California, USA). Five of the DTNBP1 SNPs were chosen from the six SNPs identified by Mutsuddi *et al.* (2006) as tagging all the reported associations with schizophrenia. We replaced their SNP 5 (rs2005976) with their SNP 4 (rs2619528) because these SNPs are in high linkage disequilibrium (LD), $r^2 = 0.88$ (Van den Oord *et al.*, 2003) and because the latter (but not the former) is included in the HapMap build 35 (HapMap, 2003). Genotypes from an additional 81 polymorphic markers were used to verify the pedigree relationships between study participants. Genotyping success rates were more than 99% for all SNPs after exclusion of any Mendelian errors.

Statistical power

This study is designed to be a replication study and therefore we could declare significance at the type I error rate to be 0.05. Even within this replication study we,

Table 1 Description of the full data set of 986 families with 2042 siblings with both genotypes and phenotypes; genotypes were available on an additional 788 parents

			% Families							% Families		
			Number of affected							Number of parents genotyped		
		Number of families	0	1	2	3	4	5		0	1	2
Number measured per family	1	221	67	33						63	19	18
	2	548	48	36	16					52	23	25
	3	159	40	29	24	8				38	26	35
	4	44	37	31	22	11	0			33	32	36
	5	13	39	15	23	15	8	0		23	39	39
	6	1	0	0	0	0	0	100		0	0	100
Total	Number	987[a]	495	334	137	19	1	1	Overall %	51	23	26

[a]908 families were simple nuclear families, for the remaining 79 families the study participants were the children of a twin pair.
Affecteds are those with any diagnosis of anxiety or depression.

however, are conducting multiple tests. At the other extreme our study sample has been, and will be, used for other association studies and so genome-wide type I error rate of 5×10^{-8} would be most conservative. Using these two extremes we consider the power of a family-based study with 492 families assuming a transmission disequilibrium test design of a single affected offspring per family. Such a study has more than 80% power to detect a causal variant with heterozygous genotype relative risk of 1.5 (or 2.3) under a genetic model of multiplicative allelic action of frequency 0.1 using a genotyped marker of frequency 0.1 which is in complete LD with the causal variant assuming a type I error rate of 0.05 (or 5×10^{-8}). These calculations provide a baseline indication of power as our study design includes many families with multiple affected sibs and/or unaffected sibs plus an additional 495 families contributing unaffected/control individuals; both these factors should result in increased power (Martin *et al.*, 2000). Associated DTNBP1 haplotype was estimated to have a frequency of 0.06, relative risk of 3.11 (heterozygous or homozygous) (Van den Oord *et al.*, 2003); we have 84% to detect a variant with an effect size of this magnitude at the most conservative type I error rate of 5×10^{-8}.

Statistical analysis

Departures from the Hardy–Weinberg equilibrium for each SNP using unrelated individuals ($n = 637$) from families with no affected offspring were tested using the program *PedStats* (Wigginton and Abecasis, 2005). Association analysis was undertaken using logistic regression as implemented in UNPHASED v3.10 (Dudbridge, 2003). UNPHASED optimally combines all the information available (which differs between families, for example, sibship size, number of parents genotyped, number of affected sibs per family including none) generating frequencies for 'case' and 'control' haplotypes from all parental chromosomes. The default settings of UNPHASED allow estimation of uncertain haplotype frequencies by the expectation-maximization algorithm and assume that transmissions are not independent in families with multiple siblings. Options used were 'missing', to allow imputation of missing parental genotypes, – 'rare' 0.01 to require haplotypes to have frequency greater than 1% in either cases or controls; – 'window' to specify the number of SNPs used in haplotype analyses; – 'individual' to generate P values for each individual haplotype in addition to a global P value, which tests if the distribution of haplotype frequencies differs between 'cases' and 'controls'. The option – 'permutation' 10 000 was used to generate empirical P values from 10 000 simulation permutations in situations where the association was less than 0.05 as a check that, given our family structure or, in the case of rare variants, that the test statistic follows asymptotic distribution. For analysis of individual SNPs the option – 'genotype' was also used to allow association tests of individual single SNP genotypes, in which 'control' genotypes are derived from the pair of nontransmitted alleles. Pairwise $|D'|$ and r^2 measures of LD among variants within genes were estimated from 'control' chromosomes using the – 'window 2-LD' option.

On the basis of associations published for BDNF Val66Met, in this study we conducted an association analysis on ANX or DEP, DEP and ANX, PDAG, and OCD. For the DTNBP1 variants association has only been reported for schizophrenia and our hypothesis was that risk variants for schizophrenia may be common to anxiety and depression disorders. Therefore, for DTNBP1 we investigate the association with the diagnostic groups ANX or DEP, DEP, and ANX.

Results

Brain-derived neurotrophic factor

Allele and genotype frequencies for the Val66Met BDNF were in good agreement with other studies of Caucasian populations (Gratacos *et al.*, 2007) and the genotype frequencies were in the Hardy–Weinberg equilibrium (Table 2). Association analysis revealed evidence for rejecting the null hypotheses of no association only for OCD. The frequency of the Met allele was higher for 'cases' than 'controls' (frequency 0.24 vs. 0.19, $P = 0.05$). The genotype test was also significant (global $P = 0.022$), where the frequency of transmission of the Met/Met genotype to OCD cases was higher (frequency 0.09 in 'case' vs. 0.04 in 'control' genotypes, $P = 0.029$). No significant overtransmission of the Met/Met genotype was found for the anxiety disorder sample, which included the OCD cases, together with PDAG and generalized anxiety disorder. No association was found with neuroticism (results not presented).

Dystrobrevin-binding protein 1

Allele frequencies for all the DTNBP1 SNPs agreed well with those reported from the HapMap CEU trios and genotype frequencies did not deviate from the Hardy–Weinberg equilibrium for any of the SNPs. Measures of LD between the SNPs (Table 3) also agreed well with those reportedly calculated from HapMap CEU genotypes; the high r^2 between SNPs r2619528 and rs760761 (0.97 in our data, 0.94 in HapMap samples) means that these SNPs provide virtually the same information for association. Therefore, association results for rs760761 are excluded from Table 4, but the SNP is included in the six-SNP haplotype association reported in Table 5 to allow unambiguous comparison with the haplotypes reported in Mutsuddi *et al.* (2006). As expected from the high LD between SNPs rs2005976 and rs2619528, the frequencies of the six-SNP haplotypes agreed well with those of Mutsuddi *et al.* (2006) despite our substitution of rs2005976 with rs2619528. Association analysis showed a difference in allele frequencies of

Table 2 Allele and genotype frequencies for the Val66Met BDNF SNP and association analysis *P* values

Both sexes	Frequencies				*P* values			
rs6265	A Met	A/A	A/G	G/G	Allele	A/A	G/G	Genotype
Control[a]	0.19	0.04	0.31	0.65	0.37[a]			
ANXorDEP	0.20	0.05	0.29	0.66	0.60	0.07	0.75	0.18
DEP	0.20	0.05	0.28	0.67	0.93	0.06	0.27	0.09
ANX	0.21	0.06	0.31	0.64	0.10	0.13	0.30	0.16
PDAG	0.19	0.05	0.28	0.68	0.24	0.84	0.18	0.43
OCD	0.23	0.09	0.28	0.63	**0.05**	**0.022**	0.47	**0.029**

P values are asymptotic except when the asymptotic *P* value <0.05 that are in bold typeface and are replaced by empirical *P* values based on 10 000 simulation permutations.
[a]'Control' frequencies reported are from the DEP or ANX analysis and may differ by ± 0.01 for the other analyses.
BDNF, brain-derived neurotrophic factor; SNP, single nucleotide polymorphism.

Table 3 Measures of linkage disequilibrium between the genotyped DTNBP1 SNPs

			Distance (kb)	LD: $\|D'\|$ and r^2					
dbSNP ID	Alternative name	Alleles	from rs3213207	rs3213207	rs1011313	rs2619528	rs760761	rs1018381	rs2619538
rs3213207	P1635	A/G	0		**0.00**	**0.43**	**0.48**	**0.01**	**0.06**
rs1011313	P1325	A/G	5.3	0.60		**0.03**	**0.02**	**0.01**	**0.11**
rs2619528	P1765	A/G	21.7	0.95	1.00		**0.97**	**0.43**	**0.13**
rs760761	P1320	C/T	23.0	1.00	0.79	1.00		**0.41**	**0.13**
rs1018381	P1578	C/T	29.0	1.00	1.00	1.00	1.00		**0.06**
rs2619538	SNP A	A/T	37.1	0.86	0.86	0.86	0.86	0.86	

DTNBP1, dystrobrevin binding protein 1; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

Table 4 Allele frequencies, genotype frequencies from the case–control sample and association analysis *P* values for five genotyped SNPs from DTNBP1

	Frequencies				*P* values			
rs3213207	G	G/G	A/G	A/A	Allele	G/G	A/A	Genotype
Control	0.12	0.01	0.21	0.78				
ANX or DEP	0.12	0.01	0.21	0.78	0.13	0.58	0.17	0.32
DEP	0.12	0.01	0.21	0.77	0.07	0.37	0.12	0.17
ANX	0.12	0.01	0.22	0.76	0.11	0.63	0.08	0.20
rs1011313	A	A/A	A/G	G/G	Allele	A/A	G/G	Genotype
Control	0.09	0.01	0.16	0.83				
ANX or DEP	0.09	0.01	0.17	0.82	0.86	0.91	0.88	0.88
DEP	0.09	0.01	0.17	0.83	0.54	0.67	0.46	0.46
ANX	0.10	0.01	0.18	0.81	0.62	0.46	0.50	0.51
rs2619528	A	A/A	A/G	G/G	Allele	A/A	G/G	Genotype
Control	0.20	0.04	0.32	0.64				
ANX or DEP	0.20	0.04	0.32	0.64	0.15	0.44	0.19	0.32
DEP	0.21	0.04	0.33	0.64	0.08	0.35	0.11	0.19
ANX	0.21	0.05	0.31	0.64	0.16	0.14	0.36	0.21
rs1018381	T	T/T	T/C	C/C	Allele	T/T	C/C	Genotype
Control	0.09	0.01	0.16	0.83				
ANX or DEP	0.09	0.01	0.16	0.83	0.59	0.73	0.52	0.51
DEP	0.09	0.01	0.17	0.83	0.47	0.93	0.42	0.42
ANX	0.08	0.01	0.15	0.84	0.90	0.23	0.90	0.93
rs2619538	T	T/T	T/A	A/A	Allele	T/T	A/A	Genotype
Control	0.43	0.19	0.47	0.34				
ANX or DEP	0.41	0.16	0.50	0.34	0.12	0.06	0.58	0.17
DEP	0.41	0.16	0.51	0.33	**0.036**	**0.013**	0.49	**0.036**
ANX	0.40	0.15	0.50	0.35	0.31	0.11	1.00	0.28

SNP rs760761 is not listed because the results are similar to those of rs2619528 because of the high r^2 between these SNPs.
ANX, any anxiety diagnosis; DEP, any depression diagnosis; DTNBP1, dystrobrevin binding protein 1; SNP, single nucleotide polymorphism.

rs2619538 (Table 4) and DEP 'cases' and DEP 'controls' (0.41 vs. 0.43, empirical $P = 0.036$) and also in genotype frequencies (empirical $P = 0.031$) resulting from the undertransmission of A/A (frequency 0.16 in DEP 'cases', 0.20 in DEP 'controls', $P = 0.013$). Association tests for the 6 SNP haplotypes could only be conducted without the – 'missing' option (unknown parental haplotypes have not been imputed so the sample size is reduced) and showed no evidence for the association in global tests (Table 5). Haplotype frequencies derived from control

Table 5 DTNBP1 six-SNP (rs3213207, rs1011313, rs2619528, rs760761, rs1018381, rs2619538) haplotype frequencies and association analysis *P* values

	Frequency in Mutsuddi *et al.*[a]	Control	Frequency			*P* value		
			ANX or DEP	DEP	ANX	ANX or DEP	DEP	ANX
G-G-A-T-C-T	–	0.01	0.01	0.02	0.01	0.21	0.21	0.10
G-G-A-T-C-A	0.11	0.10	0.08	0.07	0.08	0.42	0.64	0.58
G-A-G-T-C-A	–	0.00	0.02	0.03	0.02	0.07	**0.060**	0.09
A-G-A-T-T-T	–	0.01	0.01	0.01	0.00	0.96	0.52	0.10
A-G-A-T-T-A	0.08	0.08	0.08	0.07	0.09	0.99	0.88	0.61
A-G-G-C-C-T	0.33	0.32	0.31	0.32	0.30	0.39	0.21	0.33
A-G-G-C-C-A	0.42	0.39	0.40	0.40	0.40	0.84	0.84	0.65
A-A-G-C-C-T	0.06	0.09	0.09	0.08	0.10	0.68	0.39	0.59
A-A-G-C-C-A	–	0.00	0.00	0.00	0.00	0.91	0.90	0.22

[a]We replaced SNP rs2005976 by SNP rs2619528 [these SNPs are in high LD (van den Oord *et al.*, 2003), $r^2 = 0.88$].
ANX, any anxiety diagnosis; DEP, any depression diagnosis; DTNBP1, dystrobrevin binding protein 1; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

chromosomes agree well with those derived from HapMap CEU genotypes and reported in figure 1 of Mutsuddi *et al.* (2006) (with the qualifications discussed in the Methods section), but there was no evidence of association. No association was found with neuroticism for any analyses (results not presented).

Discussion

We did not find any evidence of association between the BDNF Val66Met alleles and depression. For depression our results agree with the meta-analysis of six case–control studies of major depression (Gratacos *et al.*, 2007). Overtransmission of the Val variant has been reported in other family-based studies of depression (Neves-Pereira *et al.*, 2002; Sklar *et al.*, 2002; Geller *et al.*, 2004), but was not found here. As in other studies (Lam *et al.*, 2004; Shimizu *et al.*, 2005) of PD we found no evidence of association, but the number of cases in this study, as in others, is not high. For OCD we found overtransmission of the Met/Met genotype ($P = 0.029$, global genotype $P = 0.022$). It would be prudent to treat the significance of this association with caution given the number of tests conducted, even though this is a replication study. Indeed, the direction of the overtransmission does not agree with the report of the only study of OCD to report an association with Val66Met, which found overtransmission of the Val variant (Hall *et al.*, 2003).

Analysis of DTNBP1 tag SNPs and haplotypes found no conclusive evidence of association with any anxiety or depression disorders. The A/A genotype was found to be transmitted less frequently to depression cases than controls (frequency 0.16 vs. 0.19) but the weak association ($P = 0.013$) will not withstand any correction for multiple testing and the direction of the association does not support results reported for association with schizophrenia (Williams *et al.*, 2004).

In conclusion, we have conducted an association study using a relatively large study sample with a study design that allowed us to probe associations with anxiety and depression and neuroticism. Our results do not support a hypothesis that variants of BDNF and DTNBP1 are common causal variants for a spectrum of psychiatric disorders. Our results have been presented so that future meta-analyses can use information generated in this study. Family-based studies are often excluded from meta-analyses; our results could be included in case–control meta-analyses by conservatively using the number of case and control families, together with the reported allele, genotype and haplotype frequencies and *P* values.

Acknowledgements

This study was approved by the QIMR Human Research Ethics Committee. The authors thank Lorna Peters for her role in preparing the CIDI computer-driven telephone interview and the scoring algorithm; our interviewers and clerical and administrative support staff supervised by Dixie Statham; Scott Gordon and David Smyth for computer support; and our laboratory staff, especially Megan Campbell, Anjali Henders and Leanne McNeil. Phenotype collection was funded by grants from the Australian National Health and Medical Research Council (971232, 339450). Most of all the authors thank the twins and their relatives for their willing participation in the study.

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