

Family-Based Association Study Between Brain-Derived Neurotrophic Factor Gene Polymorphisms and Attention Deficit Hyperactivity Disorder in UK and Taiwanese Samples

Xiaohui Xu,^{1*} Jonathan Mill,¹ Kaixin Zhou,¹ Keeley Brookes,¹ Chih-Ken Chen,² and Philip Asherson¹

¹MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, United Kingdom

²Department of Psychiatry, Chang Gung Memorial Hospital, Taiwan

Brain-derived neurotrophic factor (BDNF) plays an important role in normal neuronal development. Several lines of evidence implicate the involvement of BDNF in attention-deficit hyperactivity disorder (ADHD). This study investigated the role of two common BDNF variants (Val66Met, C270T) in two samples of ADHD probands from the United Kingdom (n = 180) and Taiwan (n = 212). We found evidence of increased transmission of the C allele of the C270T in Taiwanese samples (TDT: $\chi^2 = 6.78$, $P = 0.009$) and the two samples pooled together (TDT: $\chi^2 = 7.24$, $P = 0.007$). No association was found between the Val66Met polymorphism and ADHD in either of the two populations. Analysis of haplotypes demonstrated a significant decreased transmission of haplotypes containing the Val66 allele and the 270T allele in the Taiwanese samples (TDT: $\chi^2 = 4.57$, $P = 0.032$) and the pooled sample set (TDT: $\chi^2 = 5.82$, $P = 0.016$). This study provides evidence for the possible involvement of BDNF in susceptibility to ADHD. © 2006 Wiley-Liss, Inc.

KEY WORDS: brain-derived neurotrophic factor (BDNF); attention-deficit hyperactivity disorder (ADHD); association studies; single nucleotide polymorphism (SNP)

Please cite this article as follows: Xu X, Mill J, Zhou K, Brookes K, Chen C-K, Asherson P. 2007. Family-Based Association Study Between Brain-Derived Neurotrophic Factor Gene Polymorphisms and Attention Deficit Hyperactivity Disorder in UK and Taiwanese Samples. *Am J Med Genet Part B* 144B:83–86.

Attention-deficit hyperactivity disorder (ADHD) is a common and heritable childhood behavioral disorder characterized by developmentally inappropriate levels of hyperactivity,

impulsivity, and inattentiveness. Polymorphic variants in several genes involved in regulation of dopamine and related neurotransmitter pathways are reported to be associated with ADHD [Asherson, 2004]. Here we investigate a possible role for brain-derived neurotrophic factor (BDNF), a protein that is widely expressed in the adult mammalian brain and plays a critical role in the development, survival, and functional maintenance of neurons [Lewin and Barde, 1996].

The human BDNF gene is located on the short arm of chromosome 11 at the boundary of 11p13 and 11p14 [Hanson et al., 1992]. Several lines of evidence suggest that BDNF could play a role in the etiology of ADHD [Tsai, 2003]. Stimulant drugs, which are widely used in the treatment of ADHD, have been shown to elevate BDNF expression in the brain [Meredith et al., 2002]. Furthermore, BDNF knockout mice have been shown to be hyperactive [Rios et al., 2001] and have brain size deficits similar to those seen in children with ADHD [Jones et al., 1994]. Finally, variants in the BDNF gene have been widely linked to a number of other psychiatric phenotypes that may share genetic risk factors with ADHD [Neves-Pereira et al., 2002, 2005; Hong et al., 2003; Nanko et al., 2003; Szekeres et al., 2003; Craddock et al., 2005].

Several polymorphisms of BDNF gene have been reported and studied in psychiatric disorders. The most frequent are the Val66Met (rs6265) polymorphism [Cargill et al., 1999] and the C270T SNP [Kunugi et al., 2001]. The Val66Met SNP has been identified at nucleotide 196 within the 5' precursor peptide (proBDNF) sequence that causes an amino acid substitution of valine to methionine at codon 66. Chen et al. [2004] reported that the Val66Met polymorphism alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. The C270T polymorphism is in the 5'-noncoding region of intron 1 (GenBank accession #X60202). The functional significance of this SNP or other variants tagged by C270T has not yet been investigated.

The Met (A) allele of Val66Met is reported to be associated with abnormal hippocampal activation and impaired episodic memory in humans [Egan et al., 2003; Hariri et al., 2003]. The Val (G) allele has been reported to be associated with bipolar disorder in several family-based association studies [Neves-Pereira et al., 2002; Sklar et al., 2002; Geller et al., 2004]. In addition, the Val allele showed significant association with schizophrenia in a Scottish population [Neves-Pereira et al., 2005]. The Met allele has been reported to be associated with the restrictive type of anorexia nervosa [Ribases et al., 2003] and was found to have a protective effect in depression [Sen et al., 2003].

The T allele of C270T was associated with schizophrenia [Szekeres et al., 2003; Nanko et al., 2003] and late-onset Alzheimer's disease [Kunugi et al., 2001; Olin et al., 2005],

Grant sponsor: The Wellcome Trust; Grant sponsor: UK Medical Research Council (MRC).

*Correspondence to: Dr. Xiaohui Xu, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, SE5 8AF, UK.
E-mail: x.xu@iop.kcl.ac.uk

Received 19 December 2005; Accepted 29 June 2006

DOI 10.1002/ajmg.b.30406

while the C allele was associated with bulimia nervosa and older age of onset of weight loss, with the 270C-Met66 haplotype found to be more frequent in anorexia nervosa and bulimia nervosa compared to controls ($P \leq 0.02$ and $P \leq 0.002$, respectively) [Ribases et al., 2004].

To our knowledge, two out of three studies have reported a positive association between the Val66Met in BDNF and ADHD. The first study reported association of the Val allele using both a within family test of association and case control analysis of adults with ADHD [Lanktree et al., 2004]. The second study also found the Val allele was associated with ADHD in a family based-sample of 341 ADHD probands and their parents ($P \leq 0.02$). In addition, they found a strong preference for paternal transmission of the putative risk allele ($P \leq 0.0005$) [Kent et al., 2005]. The third study investigated both the Val66Met and C270T polymorphisms in patients with obesity, eating disorder, and ADHD, but found no association with either marker [Friedel et al., 2005]. In this study, we examine the role of the two BDNF polymorphisms (Val66Met and C270T) in two clinical ADHD samples from the UK and Taiwan.

For the UK sample, DNA was collected from 180 DSM-IV ADHD combined subtype probands, from both parents for 116 of the ADHD probands and from the mother alone for 64 of the probands. Cases were recruited from child behavior clinics in South-East England and referred for assessment if they were thought by experienced clinicians to have a diagnosis of the combined subtype of ADHD under DSM-IV criteria, with no significant Axis I co-morbidity apart from oppositional defiant disorder (ODD) and conduct disorder (CD) and IQ greater than 70. Only those individuals fulfilling the recruitment criteria after completion of research assessments were included in the study. Ninety-six percent of the sample was male. The age range was 5–15 years at the time of assessment (mean 10.41, SD 2.34). Parents were interviewed with a modified version of the Child and Adolescent Psychiatric Assessment (CAPA) [Angold et al., 1995]. Information on ADHD symptoms at school was obtained using the long form of the Conner's questionnaire [Conners, 1995]. HYPESHEME data sheets were completed using these data plus review of case notes. HYPESHEME is a computerized DSM-IV and ICD-10 operational criteria checklist and diagnostic algorithm [Curran et al., 2000]. HYPESHEME diagnoses were checked against researcher applied DSM-IV criteria and discrepancies reviewed by two researchers. Where consensus could not be reached, cases were brought to case conference and final consensus agreement made with a senior clinical researcher. All probands were of European-Caucasian origin.

For the Taiwanese sample, DNA sample was collected from 212 DSM-IV ADHD probands, from both parents for 135 of the ADHD probands and from the mother alone for 35 of the probands. ADHD cases were ascertained from child psychiatric clinics in the Chang Gung Memorial Hospital in Taipei area, Taiwan. Following completion of a standard maternal interview, the Kiddie-SADS [Kaufman et al., 1997] and completion of parent and teacher Conner's revised rating scales [Conners, 1995] diagnosis was applied according to DSM-IV criteria. Seventy-eight percent had the combined type and 22% the inattentive subtype of ADHD with no co-morbid disorders apart from oppositional defiant disorder; conduct disorder, and possible Tourettes syndrome in four cases. Eighty-nine percent of the sample was male, 13% had IQ between 50 and 69 (87% >69), and the age range was 5–15 years (mean 8.96, SD 2.60).

Two SNPs in the BDNF gene were genotyped using enzyme restriction as previously described. The Val66Met SNP changes a *PmlI* restriction site [Matsushita et al., 2005] and the C270T SNP changes a *HinI* restriction site [Kunugi et al., 2001]. Genotype data were analyzed using the transmission

TABLE I. Parental Allele Frequency for BDNF Polymorphisms

	Val66Met		C270T	
	Allele	Allele frequency	Allele	Allele frequency
UK	G	0.78	C	0.94
Samples	A	0.22	T	0.06
Taiwanese	G	0.47	C	0.96
Samples	A	0.53	T	0.04

disequilibrium test (TDT) [Spielman et al., 1993], the haplotype-based haplotype relative risk test (HHRR) [Terwilliger and Ott, 1992], and TRANSMIT for the analysis of haplotypes [Clayton, 1999].

Population frequencies for the two markers were estimated from parental genotypes and can be seen in Table I. Evidence for linkage disequilibrium (LD) between the two markers was examined by calculating D' and r^2 statistics. Significant LD was found between the two markers in both UK and Taiwanese samples ($D' = 1$, $r^2 = 0.3$ and $D' = 1$, $r^2 = 0.2$, respectively). Allele frequency differences between the two markers in both populations account for relatively low r^2 values despite the very high levels of LD measured by the D' statistic. For C270T polymorphism due to the similarity of allele frequency and LD relationship in the two distinct populations, we have included analysis of the combined dataset in addition to each sample independently.

For SNP Val66Met, no significant preferential transmission of any allele was found in either UK samples or Taiwanese samples (Table II). Furthermore, there was no evidence of preferential parental transmission in the two populations (paternal transmission in UK and Taiwanese samples: $\chi^2 = 0.57$, $P = 0.45$; $\chi^2 = 0.02$, $P = 0.89$, respectively; maternal transmissions in UK and Taiwanese samples: $\chi^2 = 1.79$, $P = 0.18$; $\chi^2 = 0.09$, $P = 0.77$, respectively).

For SNP C270T, there was evidence of increased transmission of the C allele in the Taiwanese sample (HHRR: OR = 3.9, 95% CI = 1.28–11.90, $P = 0.008$; TDT: $P = 0.009$; TRANSMIT: $P = 0.008$). There was also a weak non-significant trend for association of the same allele in the UK sample, while combining the two datasets together also gave significant evidence for association (HHRR: OR = 2.3, 95% CI = 1.22–4.23, $P = 0.007$; TDT: $P = 0.007$; TRANSMIT: $P = 0.037$) (Table III).

TABLE II. TDT, HHRR, and TRANSMIT Analysis of Val66Met

	UK samples		Taiwanese samples	
	Allele		Allele	
	G	A	G	A
TDT				
Transmitted	45	41	63	60
Non-transmitted	41	45	60	63
χ^2 (P -value)	0.186 (0.666)		0.073 (0.788)	
HHRR				
Transmitted	210	53	121	132
Non-transmitted	206	57	118	135
χ^2 (P -value)	0.184 (0.668)		0.071 (0.789)	
TRANSMIT				
Transmitted	280	72	196	216
Expected	273	79	195	217
χ^2 (P -value)	1.673 (0.196)		0.044 (0.835)	

TABLE III. TDT, HHRR, and TRANSMIT Analysis of C270T

	UK samples		Taiwanese samples		Combined samples	
	Allele		Allele		Allele	
	C	T	C	T	C	T
TDT						
Transmitted	17	10	15	4	32	14
Non-transmitted	10	17	4	15	14	32
χ^2 (<i>P</i> -value)	1.836 (0.176)		6.783 (0.009)		7.235 (0.007)	
HHRR						
Transmitted	271	11	287	4	558	15
Non-transmitted	264	18	276	15	540	33
χ^2 (<i>P</i> -value)	0.798 (0.180)		6.998 (0.008)		7.213 (0.007)	
TRANSMIT						
Transmitted	333	21	405	9	738	30
Expected	332	22	398	16	730	38
χ^2 (<i>P</i> -value)	0.163 (0.686)		7.067 (0.008)		4.329 (0.037)	

Given the strong LD relationships between the two markers, we performed haplotype analyses and found evidence for decreased transmission of the Val66/270T haplotype in the Taiwanese sample and the pooled Taiwan/UK sample (TDT: $P = 0.032$; HHRR: $P = 0.012$; TRANSMIT: $P = 0.014$ for Taiwanese samples and TDT: $P = 0.016$; HHRR: $P = 0.013$, TRANSMIT: $P = 0.07$ for pooled samples).

An alternative haplotype analysis program, WHAP (<http://statgen.iop.kcl.ac.uk>) was used to test the contribution of the individual SNPs to the haplotype association. WHAP allows the user to drop one or more markers to test whether they contribute significantly to the haplotype association. The results of this analysis in the Taiwanese and combined datasets showed that the Val66Met could be dropped and was not making a significant contribution to the haplotype association. In contrast, the Taiwanese data suggests that the C270T marker makes a significant contribution to the haplotype association ($P = 0.003$); a finding that was significant at a trend level in the combined dataset ($P = 0.07$). These data suggest that the haplotype association does not better explain the association with C270T.

In summary, polymorphisms in BDNF have been implicated in a number of psychiatric phenotypes including ADHD. Several lines of evidence suggest that BDNF could play a role in the etiology of ADHD [Tsai, 2003] and two previous studies found association with the Val allele of Val66Met [Lanktree et al., 2004; Kent et al., 2005]. In our study, no association was found between Val66Met and ADHD in either of the two populations. Furthermore, we did not find evidence for preferential paternal transmission suggested in the earlier study of Kent and colleagues. We did however find evidence for increased transmission of the C allele of C270T in Taiwanese samples ($P = 0.009$) and the Taiwanese and UK samples pooled together ($P = 0.007$) with an average odds ratio of 2.29.

We therefore failed to support the previous association reported between Val66Met in BDNF and ADHD. Although we do find evidence for association with the C allele of C270T we must treat this finding with caution for several reasons. First, this is the first study to report this association and replication of association findings is a key requirement. Second, the significance levels we report have not been adjusted for genetic variation spanning the entire BDNF locus (gene-wide significance) or the entire genome (genome-wide significance) and many nominal significance values at the level we observed will exist by chance alone throughout the genome. Third, a functional effect of C270T or other variants tagged by C270T

has not been investigated. Further investigations with this gene in ADHD need to be performed before any firm conclusions can be made.

ACKNOWLEDGMENTS

This research was funded by The Wellcome Trust and UK Medical Research Council (MRC). The study was also supported by the Department of Psychiatric of Chang Gung Memorial Hospital and the Taipei City Psychiatric Centre in Taiwan. We thank the families who took part in this research.

REFERENCES

- Angold A, Prendergast M, Cox A, Harrington R, Simonoff E, Rutter M. 1995. The child and adolescent psychiatric assessment (CAPA). *Psychol Med* 25(4):739–753.
- Asherson P, the IMAGE Consortium. 2004. Attention-deficit hyperactivity disorder in the post-genomic era. *Eur Child Adolesc Psychiatry* 13:50–70.
- Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, daley GQ, Lander ES. 1999. Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nature Genetics* 22:231–238.
- Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS. 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci* 24(18):4401–4411.
- Clayton D. 1999. A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. *Am J Hum Genet* 65:1170–1177.
- Conners CK. 1995. The Conners rating scales: Instruments for assessments of childhood psychopathology. Duke University: Durham, USA.
- Craddock N, O'donovan MC, Owen MJ. 2005. The genetics of schizophrenia and bipolar disorder: Dissecting psychosis. *J Med Genet* 42(3):193–204.
- Curran S, Newman S, Taylor E, Asherson P. 2000. Hypescheme: An operational criteria checklist and minimum data set for molecular genetic studies of attention deficit and hyperactivity disorders. *Am J Med Genet* 96(3):244–250.
- Egan MF, Kojimam M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B. 2003. The BDNF Val/Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112:257–269.
- Friedel S, Fontenla Horro F, Wernter AK, Geller F, Dempfle A, Reichwald K, Smidt J, Brönnner G, Konrad K, Herpertz-Dahlmann B, Warnke A, Hemminger U, Linder M, Kiefl H, Goldscmidt HP, Siegfried W, Remschmidt H, Hinney A, Hebebrand J. 2005. Mutation screen of the

- brain derived neurotrophic factor gene (BDNF): Identification of several genetic variants and association studies in patients with obesity, eating disorders and attention-deficit/hyperactivity disorder. *Am J Med Genet Part B* 132B:96–99.
- Geller B, Badner JA, Tillman R, Christian SL, Bolhofner K, Cook EH Jr. 2004. Linkage disequilibrium of the brain-derived neurotrophic factor Val66-Met polymorphism in children with a prepubertal and early adolescent bipolar disorder phenotype. *Am J Psychiatry* 161(9):1698–1700.
- Hanson IM, Seawright A, van Heyningen V. 1992. The human BDNF gene maps between FSHB and HVBS1 at the bound-ary of 11p13-p14. *Genomics* 13:1331–1333.
- Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. 2003. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci* 23:6690–6694.
- Hong CJ, Yu YW, Lin CH, Tsai SJ. 2003. An association study of a brain-derived neurotrophic factor Val66Met polymorphism and clozapine response of schizophrenic patients. *Neurosci Lett* 349:206–208.
- Jones KR, Farinas I, Backus C, Reichardt LF. 1994. Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell* 76(6):989–999.
- Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moneci P, Williamson D, Ryan N. 1997. Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): Initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* 36:980–988.
- Kent L, Green E, Hawi Z, Kirley A, Dudbridge F, Lowe N, Raybould R, Langley K, Bray N, Fitzgerald M, Owen MJ, O'donovan MC, Gill M, Thapar A, Craddock N. 2005. Association of the paternally transmitted copy of common Valine allele of the Val66Met polymorphism of the brain-derived neurotrophic factor (BDNF) gene with susceptibility to ADHD. *Mol Psychiatry* 10:939–943.
- Kunugi H, Ueki A, Otsuka M, Isse K, Hirasawa H, Kato N, Nabika T, Kobayashi S. 2001. A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. *Mol Psychiatry* 6:83–86.
- Lanktree M, Muglia P, Squassina A, Krinsky M, Jain U, Macciadi F, Kennedy JL. 2004. A potential role for brain derived neurotrophic factor (BDNF) in adult ADHD. Abstract in *Am J Med Genet Part B* 130B:96–97.
- Lewin GR, Barde YA. 1996. Physiology of the neurotrophins. *Annu Rev Neurosci* 19:289–317.
- Matsushita S, Arai H, Matsui T, Yuzuriha T, Urakami K, Masaki T, Higuchi S. 2005. Brain-derived neurotrophic factor gene polymorphisms and Alzheimer's disease. *J Neural Transm* 112:703–711.
- Meredith GE, Callen S, Scheuer DA. 2002. Brain-derived neurotrophic factor expression is increased in the rat amygdala, piriform cortex and hypothalamus following repeated amphetamine administration. *Brain Res* 949(1–2):218–227.
- Nanko S, Kunugi H, Hirasawa H, Kato N, Nabika T, Kobayashi S. 2003. Brain-derived neurotrophic factor gene and schizophrenia: Polymorphism screening and association analysis. *Schizophr Res* 62:281–283.
- Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F, Kennedy JL. 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: Evidence from a family-based association study. *Am J Hum Genet* 71:651–655.
- Neves-Pereira M, Cheung JK, Pasdar A, Zhang F, Breen G, Yates P, Sinclair M, Cromie C, Walker N, St Clair DM. 2005. BDNF gene is a risk factor for schizophrenia in a Scottish population. *Mol Psychiatry* 10:208–212.
- Olin D, MacMurray J, Comings DE. 2005. Risk of late-onset Alzheimer's disease associated with BDNF C270T polymorphism. *Neurosci Lett* 381:275–278.
- Ribases M, Gratacos M, Armengol L, de Cid R, Badia A, Jimenez L, Solano R, Vallejo J, Fernandez F, Estivill X. 2003. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol Psychiatry* 8:745–751.
- Ribases M, Gratacos M, Fernandez-Aranda F, Bellodi L, Boni C, Anderluh M, Cavallini MC, Cellini E, Di Bella D, Erzegovesi S, Foulon C, Gabrovsek M, Gorwood P, Hebebrand J, Hinney A, Holliday J, Hu X, Karwautz A, Kipman A, Komel R, Nacmias B, Remschmidt H, Ricca V, Sorbi S, Wagner G, Treasure J, Collier DA, Estivill X. 2004. Association of BDNF with anorexia, bulimia and age of onset of weight loss in six European populations. *Hum Mol Genet* 13:1205–1212.
- Rios M, Fan G, Fekete C, Kelly J, Bates B, Kuehn R, Lechan RM, Jaenisch R. 2001. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol* 15:1748–1757.
- Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burmeister M. 2003. A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology* 28:397–401.
- Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, Schaffner S, Kirov G, Jones I, Owen M, Craddock N, DePaulo JR, Lander ES. 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neurotrophic factor. *Mol Psychiatry* 7:579–593.
- Spielman RS, McGinnis RE, Ewens WJ. 1993. Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 52:506–516.
- Szekeres G, Juhasz A, Rimanoczy A, Keri S, Janka Z. 2003. The C270T polymorphism of the brain-derived neurotrophic factor gene is associated with schizophrenia. *Schizophr Res* 65:15–18.
- Terwilliger JD, Ott J. 1992. A haplotype-based 'haplotype relative risk' approach to detecting allele association. *Hum Hered* 42:337–346.
- Tsai SJ. 2003. Attention-deficit hyperactivity disorder and brain-derived neurotrophic factor; a speculative hypothesis. *Med Hypotheses* 60:849–851.