Val/Val genotype of brain-derived neurotrophic factor (BDNF) Val\textsuperscript{66}Met polymorphism is associated with a better response to OROS-MPH in Korean ADHD children

Bung-Nyun Kim\textsuperscript{1}, Tarrant D. R. Cummins\textsuperscript{2}, Jae-Won Kim\textsuperscript{1}, Mark A. Bellgrove\textsuperscript{2}, Soon-Beom Hong\textsuperscript{3}, Sook-Hyung Song\textsuperscript{1}, Min-Sup Shin\textsuperscript{1}, Soo-Churl Cho\textsuperscript{1}, Ji-Hoon Kim\textsuperscript{3}, Jung-Woo Son\textsuperscript{4}, Yun-Mi Shin\textsuperscript{5}, Un-Sun Chung\textsuperscript{6} and Doug-Hyun Han\textsuperscript{7}

\textsuperscript{1}Division of Child and Adolescent Psychiatry, Department of Psychiatry, College of Medicine, Seoul National University, Seoul, Korea
\textsuperscript{2}Queensland Brain Institute and School of Psychology, The University of Queensland, Brisbane, Australia
\textsuperscript{3}Department of Psychiatry, Pusan National University Hospital, Pusan, Korea
\textsuperscript{4}Department of Psychiatry, Chungbuk National University Hospital, Cheongju, Korea
\textsuperscript{5}Department of Psychiatry, Ajou University Hospital, Suwon, Korea
\textsuperscript{6}Department of Psychiatry, Kyungpook National University Hospital, Daegu, Korea

Abstract

Research on psychostimulants, analysis of animal models and genetic association studies all suggest that the brain-derived neurotrophic factor gene (BDNF) may be a good candidate for pharmacogenetic studies of attention deficit hyperactivity disorder (ADHD). Yet to date there have been no pharmacogenetic studies of BDNF in ADHD. A total of 102 drug-naive ADHD children (8.7±2.1 yr) were treated with osmotic release oral system-methylphenidate (OROS-MPH) for 12 wk, and four kinds of response criteria were applied, based first, on a combined threshold of the ADHD Rating Scale – IV (ARS) and the Clinical Global Impression – Improvement scale (CGI-I); second, on scores of 1 or 2 vs. 3–7 on the CGI – Severity scale; third, on a >50% reduction in ARS scores; and fourth, on satisfaction of all of the aforementioned criteria. The Val\textsuperscript{66}Met polymorphism of BDNF and six single nucleotide polymorphisms from the SLC6A2, ADRA2A and NTF-3 genes were tested for association with each criterion. Relative to other genotypes, homozygosity for the Val allele of the BDNF Val\textsuperscript{66}Met polymorphism was associated with a greater relative frequency of good response under all four response criteria (after controlling for baseline ARS score, age, gender, final dose (mg/kg) of OROS-MPH at 12 wk, and level of academic functioning). This association was significant at the uncorrected level for the first and third response criteria (\(p = 0.013\) and \(p = 0.018\), respectively) and significant at a Bonferroni-corrected level for the second and fourth response criteria (\(p = 0.0002\), \(p = 0.003\), respectively). Our findings support an association between homozygosity for the Val allele of BDNF and better response to OROS-MPH in Korean ADHD children as assessed by four different response criteria.

Received 4 January 2011; Reviewed 11 April 2011; Revised 10 May 2011; Accepted 23 May 2011; First published online 28 June 2011

Key words: ADHD, BDNF, methylphenidate, pharmacogenetics, Val\textsuperscript{66}Met polymorphism.

Introduction

Attention deficit hyperactivity disorder (ADHD) is a multifactorial neurodevelopmental disorder that is characterized by inattention, hyperactivity and impulsiveness (Faraone & Biederman, 1998). Many association and linkage studies have shown that genetic factors are important in the pathogenesis of ADHD (Banaschewski et al. 2010; Faraone & Mick, 2010) yet the precise genetic causes are still largely unknown. Genetic research has focused mainly on candidate genes of the dopaminergic, noradrenergic and serotonergic systems (Gizer et al. 2009). However because ADHD is a neurodevelopmental disorder, it is...
reasonable to expect that neurotrophic factors, which participate in the development, survival and functional maintenance of neurons, may also contribute to a genetic predisposition. Children with ADHD show delayed maturation of the cerebral cortex, particularly in the prefrontal regions, which are important for the control of attention and motor planning (Rapoport & Gogtay, 2008). Further, different trajectories of cortical thickness development are reported to be associated with clinical outcomes and treatment response (Shaw et al. 2006, 2009). As modification of gene expression appears to be required for brain development, a child’s neurodevelopment may be specifically affected by a variety of neurotrophic genetic polymorphisms (Rapoport & Gogtay, 2008). In line with this proposal, it has been reported that genetic polymorphisms which affect neuroplasticity and/or neural development occur in neurotrophic factor genes such as BDNF and are related to the pathophysiology of ADHD (Kent et al. 2005).

Pharmacological data, analysis of animal models and association studies also suggest that the BDNF gene is a plausible candidate gene for ADHD with several lines of evidence implicating this gene in its pathogenesis. Heterozygous BDNF knockout mice show learning deficiencies, aggressiveness, anxiety, and hyperactive locomotor behaviour compared to wild-type littermates (Chourbaji et al. 2004; Rios et al. 2001). In addition, animal studies indicate that BDNF is critical for working memory (Mizuno et al. 2000) and that central BDNF mRNA levels (and its specific receptor; NTRK2) are modulated by psychostimulant and antidepressant drugs used for ADHD treatment (Meredith et al. 2002; Nibuya et al. 1995).

In humans the BDNF gene, located at chromosome 11p14.1 (Hanson et al. 1992), codes for a precursor peptide (proBDNF), which is proteolytically cleaved to form the mature protein (Mowla et al. 2001). BDNF G196A Val66Met (dbSNP number rs6265) is a single nucleotide polymorphism (SNP) in the gene where adenine and guanine alleles vary, resulting in a variation between valine and methionine at codon 66 (Bath & Lee, 2006; Egan et al. 2003). BDNF G196A Val66Met is probably the most investigated SNP of the BDNF gene. Allelic variants at this locus have been shown to be associated with mood disorders, obsessive-compulsive disorder, and many other neurodevelopmental and neurodegenerative disorders (Arancio & Chao, 2007; Brunoni et al. 2008; Mossner et al. 2005; Zeev et al. 2009).

Several polymorphisms in BDNF have been studied in ADHD; however, there has been inconsistency in the results thus far. Kent et al. (2005) reported that the common valine allele of the BDNF Val66Met polymorphism was preferentially transmitted by fathers to ADHD probands. Taiwanese researchers showed preferential transmission of the C allele of the C270T polymorphism in an ADHD group (Xu et al. 2007) and recently we found a positive association between a BDNF polymorphism and girls with ADHD in a Korean sample (Cho et al. 2010). Yet, one German study and two meta-analyses using diverse populations with ADHD failed to replicate preferential transmission of specific BDNF alleles (Forero et al. 2009; Sanchez-Mora et al. 2010; Schimmelmann et al. 2007). Further, several other association studies have tested the possible involvement of other BDNF polymorphisms in ADHD with conflicting results (Conner et al. 2008; Kim et al. 2007; Lanktree et al. 2008; Lasky-Su et al. 2007; Lee et al. 2007; Ribases et al. 2008).

Nevertheless, a recent association study between BDNF Val66Met genotype and psychostimulant response supports a role for BDNF as a candidate gene in methylphenidate (MPH) pharmacogenetics in ADHD (Flanagin et al. 2006). This research indicates that BDNF potentiates neurotransmitters that are strongly linked to psychostimulant actions. Specifically, Flanagin et al. (2006) found that the genotype at the BDNF Val66Met polymorphism is associated with inter-individual variations in the response to psychostimulants in healthy adults; Val/Val homozygotes showed increased activity-dependent secretion of BDNF by neurons and increased intracellular trafficking of synaptic vesicles in comparison with carriers of the Val/Met or Met/Met genotypes. Hence they argued that inter-individual variation originates from changes in dopamine potentiation in the synapse according to BDNF Val66Met genotype.

However, there have been no pharmacogenetic studies that relate DNA variation in BDNF to MPH-induced symptom changes in children with ADHD. Osmotic release oral system-methylphenidate (OROS-MPH) has been reported to alleviate ADHD symptoms in approximately 70% of children with ADHD (Biederman et al. 2007; Wolraich et al. 2001). Among responding subjects, the proportion of full remission of symptoms is reported to be about 70–80% (Steele et al. 2006a, b). Clinical trial data shows that while 30% of ADHD children receive very little benefit from MPH, about 50% of children show symptom attenuation during the MPH treatment period. Pharmacogenetic approaches may play an important role in differentiating good from poor responders to MPH and may allow for individualized therapeutic approaches that maximize clinical benefit in ADHD.
As MPH potentiates dopamine availability in the synapses, many pharmacogenetic studies have focused on dopamine-related genes (Cheon et al. 2007; Franke et al. 2010; McGough et al. 2009). However, for the reasons outlined above we believe that a more diverse range of genes should be examined, including neurotrophic factor genes such as the brain-derived neurotrophic factor (BDNF) and neurotrophic factor (NTF-3) genes. Along with these, the current study also examines polymorphisms in the norepinephrine transporter (SLC6A2) and α2A-adrenergic receptor genes (ADRA2A).

Thus here we investigate the hypothesis that MPH response is associated with BDNF G196A Val*Met genotype. Specifically, we hypothesize that ADHD children with the Val/Val genotype will show a higher remission rate to treatment with OROS-MPH than ADHD children with the Met/Met or Val/Met genotypes. We also test whether MPH response is related with genotype at NTF-3, SLC6A2 and ADRA2A polymorphisms. The objective of the present study is to test these hypotheses with different response criteria, thereby providing converging sources of evidence and maximizing the validity of the results.

Materials and methods

Subjects

The present sample comprised 102 ADHD children (8.7 ± 2.1 yr, 82 boys) recruited from child psychiatric clinics at six university hospitals in South Korea. Inclusion criteria were (1) diagnosis of ADHD according to DSM-IV criteria, (2) age 6–12 yr, (3) severity of symptom score of ≥ 4 (moderate degree) on the Clinical Global Impression – Severity scale (CGI-S) and severe enough to receive medication treatment, and (4) the absence of any history of exposure to psychostimulants such as MPH. Exclusion criteria were (1) any other mental disorders except for transient tic disorder, oppositional defiant disorder, mild anxiety disorder and enuresis, (2) a past or present history of brain damage or convulsive disorder, (3) mental retardation, autism, language difficulties, or developmental problems including learning disability.

The study was approved by the institutional review board for human subjects at the Seoul National University Hospital and other hospitals. Parents/guardians provided written informed consent, and the children or adolescents provided verbal assent regarding participation in this study.

Diagnostic tools

Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-SADS-PL; Kaufman et al. 1997). The Korean version of K-SADS-PL was translated and its validity and reliability for ADHD, tic disorder, and oppositional defiant disorder were established before use (Kim et al. 2004).

Clinical Global Impression (CGI) scale (National Institute of Mental Health, 1970). This scale was standardized in Korean and its validity and reliability were established for ADHD (Cheon et al. 2009). Furthermore, we established strong inter-rater reliability before the start of medication (kappa = 0.89).

ADHD Rating Scale – IV (ARS). Composed of a total of 18 items, ARS is the ADHD symptom severity scale designed by DuPaul (1991) according to DSM-IV criteria. Each item has a 4-point scale from 0 to 3. The 18 items are composed of nine items reflecting the symptoms related to inattention and nine items reflecting the symptoms related to hyperactivity and impulsivity. The Korean version of ARS (K-ARS) was standardized by So et al. (2002). The K-ARS has good reliability and validity among Korean children and a high inter-rater reliability was established before the current study commenced (kappa = 0.92).

OROS-MPH administration and treatment response

Participants took part in a prospective 12-wk, open-label study to achieve symptomatic remission by OROS-MPH. All of the ADHD subjects were administered OROS-MPH for 12 wk. The dosages were increased for 9 wk until doses were reached that were sufficient to achieve therapeutic effect, as determined by the investigators’ assessment of symptoms and side-effects, and then these doses were maintained until week 12. Clinical assessment was conducted by certified child and adolescent psychiatrists who had completed the inter-rater reliability establishment courses at baseline prior to medication. Four methods of assessing response to OROS-MPH were applied.

Response criterion 1. The strict symptomatic remission criteria of Steele et al. (2006a, b) was applied: (1) scores ≤ 18 on the investigator-rated ARS; (2) a score of 0 or 1 on each item of the ARS; (3) a score of ‘very much/much improvement’ on the CGI – Improvement (CGI-I) after 12 wk of treatment. If the subject satisfied all the above criteria, they were categorized
as a member of the remission group, otherwise they were categorized as a member of the non-remission group.

Response criterion 2. Global severity of each patient was assessed on the basis of investigator-rated CGI-S. Subjects with scores of 1 (not ill) or 2 (very mild) on the CGI-S after treatment were regarded as ‘good responders’, whereas subjects with scores of 3–7 were regarded as ‘poor responders’. All response assessment was performed by investigators blind to the results of the genotyping.

Response criterion 3. Stimulant response was assessed on the basis of the reduction in ARS achieved after 12 wk of treatment. Following Cheon et al. (2007) an improvement of >50% in the ARS scores after 12 wk of treatment compared to baseline ARS scores, was considered a ‘good response’, whereas an improvement of <50% was considered a ‘poor response’.

Unified criterion. Under the strict unified criterion, a child was regarded as a responder if he/she satisfied all the above three response criteria.

Preparation of genomic DNA

Genomic DNA was extracted from blood (stored frozen) using the G-DEXTM II Genomic DNA Extraction kit (Korea) according to the manufacturer’s protocol. A 20 ml (2 × 10 ml) EDTA anticoagulated venous blood sample was collected from each patient in the Clinical Research Institute at the Seoul National University Hospital. Samples were coded for blinding and confidentiality. DNA was then extracted in the core laboratory in Lab Genomics* using a standard DNA isolation kit. Concentrations were determined using the PicoGreen* dsDNA Quantification kit (Molecular Probes, USA).

Genotyping

BDNF genotyping was based upon analysis of primer extension products generated from previously amplified genomic DNA using a chip-based MALDI-TOF mass spectrometry platform (Sequenom Inc., USA). The general procedures were performed according to the manufacturer’s standard protocol.

The PCR reaction was performed in a volume of 5 μl containing 1 × PCR buffer (Solgent, Korea), 2.5 mM MgCl₂, 0.2 mM each of dNTP and 0.1 U hot-start Taq polymerase (Solgent), 200 nM of each primer (F: 5'-ACG TTG GAT GCA TCA TTG GCT GAC ACT TTC; R: 5'-ACG TTG GAT GCT TCA TTG GGC CGA ACT TTC) and 4.0 ng genomic DNA. The reaction consisted of denaturation at 95 °C for 15 min, followed by 45 cycles at 95 °C for 20 s, 56 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 3 min.

Following PCR, unincorporated dNTPs were removed by adding 0.3 U of Shrimp alkaline phosphatase (Sequenom Inc.) and incubating for 40 min at 37 °C, followed by 5 min at 85 °C for enzyme inactivation.

In the homogenous MassEXTEND (hME) reaction, the total volume of each reaction was 9 μl, which included Thermosequenase (Sequenom Inc.), ACG termination mix, and 5 μM extension primer (5’-GGC TGC CAC TTT CGA ACA C). The primer extension protocol was started at 94 °C for 2 min and followed by 55 cycles of 94 °C for 5 s, 52 °C for 5 s, and 72 °C for 5 s. After desalting of the reaction product with SpectroCLEAN (Sequenom Inc.), samples were dispensed on 384-well SpectroCHIPs (Sequenom Inc.), using SpectroJET (Sequenom Inc.). The SpectroCHIPs were analysed in the fully automated mode with the MALDI-TOF MassARRAY system (Sequenom Inc.). After automatic overall measurement, assays which had bad peaks were checked again manually. All primers in the PCR and hME reactions were designed using Assay Designer 3.1 (Sequenom Inc.).

The SNPs from the SLC6A2 (rs28386840, rs5569), α₂-adrenergic receptor (ADRA2A: rs1800544 and rs553668) and neurotrophin-3 (NTF-3: rs6332, rs6489630) genes were genotyped as described previously (Cho et al. 2008a, b, 2010).

Statistical analysis

Allele frequencies were determined and Hardy–Weinberg equilibrium was calculated for all markers, using goodness-of-fit χ² tests. The distribution of the genotypes for each polymorphism was in agreement with the expected values of the Hardy–Weinberg equilibrium (p > 0.05).

Group differences in the clinical variables involving continuous data were computed using independent t tests or one-way analyses of variance (ANOVAs). Between-group comparisons involving categorical data were assessed using the χ² test or the likelihood ratio test.

We used additive, dominant and recessive model regressions to assess the association between genotype at each SNP and remission group (criterion 1) after 12 wk of treatment with OROS-MPH (controlling for age, gender, baseline ARS score, daily dose of MPH and academic function scale score in SNPstats; Sole et al. 2006). The same regression models were used to assess associations between genotype and CGI-S
between genotype and the unified response criterion. SPSS software (SPSS Inc., USA) was used to perform a likelihood ratio test to assess association between genotype at each SNP and the change in the ARS score (criterion 3) after OROS-MPH treatment. The Bonferroni-corrected significance level was set at $p = 0.05/7$ (SNPs) $\times 4$ (number of response criteria) = 0.0017.

Results

**Demographic, clinical and genetic characteristics**

The average score of overall ADHD symptoms according to investigator-measured ARS decreased from $33.5\pm 9.6$ at baseline to $12.5\pm 5.2$ after 12 wk OROS-MPH treatment. Of the DSM-IV subtypes of ADHD, the combined subtype was the most common in our subjects (67.6%), followed by the inattentive (26.4%) and hyperactive-impulsive (5.8%) subtypes. With regard to comorbidity, anxiety disorder (8.8%) was the most common, followed by oppositional defiant disorder (6.8%), transient tic disorder (6.8%) and enuresis (5.9%) (Table 1). Among the 102 ADHD children, the A (Met) allele of BDNF Val$^66$Met polymorphism was identified in 107/204 chromosomes (52%) and the G (Val) allele was identified in 97 (48%). The Val/Val genotype was observed in 21 ADHD children (21%), the Val/Met genotype in 55 (54%) and the Met/Met genotype in 26 (25%). The genotypic distribution for the BDNF Val$^66$Met polymorphism is very similar to those of other studies in Korea (Choi et al. 2006; Kang et al. 2010). There were no significant differences between subjects with the Val/Val genotype and those with other genotypes with regard to age, sex, IQ, ADHD subtype, comorbid conditions and dose of MPH. As no significant associations were found between the response criteria and the NTF-3, SLC6A2 and ADRA2A polymorphisms they will not be discussed further.

**Association between the Val/Val genotype at the BDNF Val$^66$Met polymorphism and remission after OROS-MPH treatment (response criterion 1)**

Following the first remission criteria, there was a significant association at an uncorrected level between the BDNF genotype and the relative frequency of remission post-treatment after controlling for baseline ARS score, age, gender, final dose (mg/kg) of OROS-MPH at 12 wk and level of academic functioning ($p = 0.013$) (Table 2). Among 21 ADHD children with the Val/Val genotype, 95.2% showed symptom remission and 4.8% showed non-remission. Whereas among 81 children with the Val/Met or Met/Met genotypes, 74.1% showed remission and 25.9% showed non-remission.

**Association between the Val/Val genotype at the BDNF Val$^66$Met polymorphism and response to OROS-MPH treatment as assessed by CGI-S (response criterion 2)**

A significant association at the Bonferroni-corrected level was found between the relative frequency of CGI-S 1 or 2 status post-treatment and homozygosity of the Val allele of the BDNF Val$^66$Met polymorphism ($p = 0.0002$) (Table 2). Among 21 children with the Val/Val genotype 81.0% were assessed as ‘not ill (CGI 1)’ or ‘very mild (CGI 2)’, whereas among 81 ADHD children with the Val/Met or Met/Met genotype, only 37.0% were assessed as ‘not ill’ or ‘very mild’ (Fig. 1).

**Association between the Val/Val genotype at the BDNF Val$^66$Met polymorphism and reduction in ARS after OROS-MPH treatment (response criterion 3)**

Based on the response criterion of a 50% reduction in investigator-rated ARS, we found that while 95.2% of the subjects with homozygosity for the
Val allele showed $\geq 50\%$ improvement with OROS-MPH treatment, only 74.1% of the subjects with Val/Met and Met/Met genotypes showed $\geq 50\%$ improvement. This difference in the relative occurrence of a good response was significant at an uncorrected level, $p = 0.018$ (Table 2).
Association between the Val/Val genotype at the BDNF Val<sup>66</sup>Met polymorphism and the response to OROS-MPH treatment under the unified response criterion

Based on the unified response criterion, we found a significant association at a Bonferroni-corrected level between drug response and homozygosity of the Val allele of the BDNF Val<sup>66</sup>Met polymorphism ($p=0.0003$). Of children with the Val/Val genotype 76.2% were assessed as responders, while only 38.2% of children with the Val/Met or Met/Met genotypes were assessed as responders.

Discussion

To our knowledge, this is the first pharmacogenetic study to use and find a role for the BDNF Val<sup>66</sup>Met polymorphism in stimulant response in ADHD. Assessment of response is one of the most important components for valid and reliable pharmacogenetic studies. In previous studies, the assessment of response was generally performed by parent and teacher ratings which can be subjective and unreliable. Experienced and well-trained child and adolescent psychiatrists who have spent adequate time with both the children and their mothers can assess symptoms more reliably and can gather comprehensive information from parents for the best assessment of response. In the current study, investigators with high inter-rater reliability status, who were blind to the genotypes, rated the response to OROS-MPH treatment. In addition, we applied four different kinds of threshold to strictly assess the response and maximize the validity of our results. Another strength of our study was the use of OROS-MPH rather than an MPH-immediate release form; OROS-MPH may be a better option in the current context because it allows for a full range of dose conditions and better drug adherence for eliciting pharmacogenetic effects than shorter-acting formulae of MPH (Froehlich et al. 2010).

Previous association studies have reported a possible role for the BDNF gene in ADHD. Kent et al. (2005) demonstrated a preferential paternal transmission of the Val allele in 341 ADHD trios and Xu et al. (2007) found evidence for a decreased transmission of the −270 T-Val<sup>66</sup> haplotype in two independent samples of ADHD probands. In addition, two studies considered the BDNF gene in adulthood ADHD. While Lanktree et al. (2008) found evidence for a contribution of the Val allele, Conner et al. (2008) showed no association between this polymorphism and adult ADHD scores. Similarly no association between the Val<sup>66</sup>Met SNP and ADHD was detected by Schimmelmann et al. (2007) who studied 468 children from 294 families (comprising one or more affected siblings) or by Lee et al. (2007) who examined 315 ADHD children from 266 nuclear families. Three genome-wide association studies (GWAS) have been reported for ADHD so far, one used a DNA pooling approach with 343 adult ADHD patients and 304 controls (Lesch et al. 2008), another used 958 affected family trios with child ADHD siblings (Neale et al. 2008) and the other, 896 cases with DSM-IV ADHD and 2455 controls (Neale et al. 2010a). In each case, the dependent variable was ADHD as a categorical trait or a related quantitative measure such as ADHD symptom level, age of onset of ADHD symptoms or conduct problems (Anney et al. 2008; Lasky-Su et al. 2008; Neale et al. 2008). These studies included the Val<sup>66</sup>Met variation (rs6265) and other polymorphic sites within the BDNF gene, but failed to identify association at a genome-wide significance level. In addition a recent meta-analysis study of GWAS also failed to find a significant genome-wide association (Neale et al. 2010b).

In spite of conflicting results from genetic association studies of ADHD, the results of the present study provide support for the hypothesis that genetic variations in BDNF can influence behavioural responses to MPH in ADHD children. With respect to the role of BDNF in dopaminergic systems, recent studies show that BDNF facilitates maturation of mesenchymal stem cell-derived dopamine progenitors to functional neurons (Trzaska et al. 2009) and modulates cell death in the substantia nigra (Oo et al. 2009). BDNF therefore seems to play a key role in the development of dopamine system in the brain. Further, some animal studies have suggested that dopamine-enhancing drugs like MPH induce marked changes of BDNF mRNA in hippocampal and frontal regions of juvenile rodents (Banerjee et al. 2009). Psychostimulant administration to animals affects BDNF levels in the brain which in turn induces behavioural changes. A single intraperitoneal injection of amphetamine in a mouse increases striatal BDNF by approximately 5-fold for up to 12 h (Thomas et al. 2004). Exogenous infusions of BDNF enhance the behavioural effects of psychostimulants on experimental animals (Horger et al. 1999; Meredith et al. 2002). BDNF changes in the brain could be induced by diverse psychostimulants, antidepressants, mood stabilizers and atypical antipsychotics (Jornada et al. 2010; Kang et al. 2010; Meredith et al. 2004). BDNF may therefore play a key role in the psychostimulant and psychotropic related plastic changes in the brain.
Recent animal studies also indicate that BDNF could be important for working-memory and long-term memory. Li et al. (2010a) reported that impaired spatial working memory in DAT1 knockout mice may be related to decreased frontal cortical BDNF in mice and documented apparent roles for BDNF in a short-term memory process. Using an ADHD animal model (6-OHDA-induced lesion mouse model), Sadan et al. (2009) reported that stem cells producing BDNF could regenerate the dopaminergic neurons in the striatum. Furthermore, BDNF also appears to play a role in potentiating dopamine action in these areas, an effect which might be increased in response to psychostimulants (amphetamine) via increased mobilization and/or docking of synaptic vesicles to presynaptic active zones (Narita et al. 2003). The Val/Val genotype has been shown to significantly increase activity-dependent secretion of BDNF by neurons in comparison with the Met/Met genotype (Flanagin et al. 2006). In human studies using cognitive tests, the BDNF polymorphism affected backward recall in healthy adults or older adults with Met allele carriers recalling fewer items than Val homozygotes as a result of reduced BDNF signalling (Li et al. 2010b). Such studies provide evidence of solid links between BDNF, dopamine systems in the brain and the cognitive-behavioural effects of psychostimulants. This clear link between BDNF and psychomotor stimulants raises the possibility that genetic variations in this protein may contribute to individual differences in response to psychomotor stimulants in children with ADHD.

Additionally, genetic imaging studies indicate that Met allele carriers of the BDNF gene show decreased volume in the dorsolateral prefrontal cortex, an area associated with planning and higher-order cognitive functioning in ADHD, as well as in subcortical regions, such as the caudate nucleus, which are related to behavioural inhibition (Pezawas et al. 2004). Recently it was also found that Met allele carriers have smaller temporal and occipital lobar grey-matter volumes (Ho et al. 2006). These genetic imaging studies indicate that better response to MPH in ADHD individuals with Val/Val homozygosity may be related to a lower degree of brain anatomical deficit and functional impairment in these children.

The results of the current study indicate that the BDNF gene is a probable candidate for explaining variations in response to MPH in ADHD children. However, the present results must be interpreted cautiously, as differences in MPH response in Korean ADHD children may be explained by genetic variations in the many pharmacokinetic and dynamic processes that mediate the effects of MPH. The mechanism of MPH action is a multifactorial phenotype, the expression of which may be affected by several different neurotransmitter systems involving various signal transduction elements (Froehlich et al. 2010). Although the BDNF gene may affect response to MPH, the influence of, and interactions between, many target genes should be considered particularly those genes that are related to neural and synaptic plasticity such as SNAP-25, NTF-3, ciliary neurotrophic factors (CNTF) and G-protein-coupled receptor kinase-interacting protein-1 (GIT-1).

We recently found both a significant association with a GIT-1 gene polymorphism in ADHD children and, ADHD-like behaviour and MPH response in a GIT-1-deficient animal model (Won et al. 2011). Other candidate genes involved in neural plasticity like SNAP-25 and CNTF have also been reported to increase the risk of ADHD (Forero et al. 2009; Ribases et al. 2008).

It is important to note the limitations of this study. The total number of subjects studied (N = 102) was relatively small for a genotypic analysis and as serum levels of MPH were not determined we cannot be entirely sure that there were no differences among the serum concentrations of MPH between the genotype groups. Another limitation is that we evaluated only a single SNP on the BDNF gene. However as the BDNF gene is mapped to a highly conserved region of the genome, the marker analysed in the current study gave reasonable coverage of the entire gene with linkage disequilibrium relations (D’) with all other SNPs in the gene ranging between 88% and 100% (International HapMap Consortium, 2003). Finally we cannot exclude the presence of a population stratification bias; however, as the Korean population is characterized by a relatively high genetic homogeneity (Kim, 2003), a false-positive due to the presence of ethnic subgroups is unlikely in our sample. Moreover, in this study a diverse range of response criteria were tested and consistent positive results were demonstrated, such consistency in results across different assessments supports the reliability of our results.

In sum, this study provides evidence that genetic variation in the BDNF gene is related to remission induced by chronic oral administration of OROS-MPH in Korean ADHD children. Finding the genetic basis for variation in treatment response to commonly used therapeutic drugs like MPH may help researchers and clinicians to apply more individualized treatments to ADHD children.
Acknowledgements

This work (B. N. Kim) was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (RIF-2011-003-E00026) and by the Korean Janssen Pharmaceutical Company. Mark A. Bellgrove is supported by a National Health, Medical Research Council (NHMRC, Australia) Career Development Australia (NHMRC Project 569636, 569533, 569532).

Statement of Interest

None.

References


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 Research* 949, 218–227.


