Evidence for an Association between Brain-Derived Neurotrophic Factor Val66Met Gene Polymorphism and General Intellectual Ability in Early-Onset Schizophrenia

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ABSTRACT

Background: Brain-derived neurotrophic factor (BDNF) plays a crucial role in the survival, development and maintenance of neuronal systems, and the Val66Met polymorphism has been implicated in memory functions.

Method: We examined the association of BDNF with general intellectual ability in 161 individuals including 53 early-onset patients with schizophrenia (EOS), 91 healthy biological relatives, and 17 relatives with major depressive disorder (MDD), using the Wechsler Intelligence Scales (WISC).

Results: Regardless of diagnosis, individuals with the Met66 allele had a significantly higher performance score than those homozygous for Val66 on vocabulary, block design and object assembly subtests of the WISC. EOS probands showed poor performance on all IQ subtests compared with relatives Val/Val genotype.

Limitations: Relatively smaller sample size of individual genotypes.

Conclusions: BDNF genotype may play a role in specific cognitive functions and dimensions of intelligence. The Met allele appears to be associated with superior performance in IQ compared with relatives Val/Val genotype.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a glutamate neurotrophic factor, is a member of the neurotrophin family that plays a crucial role in the survival and differentiation of particular neuronal systems, and has a strong involvement in promoting brain growth and development (1-3). BDNF also appears to mediate activity-dependent synaptic plasticity and neuronal development (4-7), and is more widely distributed and expressed in the central nervous system (CNS) and has survival-promoting functions on various CNS neurons including human hippocampus and cerebral cortex (8).

Association studies have implicated BDNF as a strong candidate gene in bipolar disorder (9-11), schizophrenia (12, 13), Alzheimer’s disease (14, 15), eating disorders (16), neuroticism (17), and obsessive-compulsive disorder (18). BDNF has been implicated in schizophrenia based on its effects on neurotransmitter systems that are dysregulated during the illness and its involvement in the mechanism of action of antipsychotic drugs (19, 20). BDNF is involved in the development and survival of dopaminergic and serotonergic neurons (21). An increase in BDNF mRNA levels has been reported in the hippocampus in schizophrenia (22). High concentrations of BDNF in cortical areas and reductions of neurotrophins in the hippocampus have been reported in patients with schizophrenic psychosis in comparison with normal...
controls (23). In contrast, two further studies showed reduced BDNF levels in schizophrenia (24, 25). However, Shimizu et al. (26) showed no significant differences in serum BDNF levels between antipsychotic-naïve and medicated patients with schizophrenia and healthy individuals. In addition, there was no correlation between BDNF levels and duration of illness, age of onset, clinical symptoms.

The BDNF gene, located on 11p13, has a non-conservative exonic single nucleotide polymorphism (SNP) at nucleotide 196 (dbSNP number rs6265, G/A), which results in a valine (Val) to methionine (Met) substitution within the 5’ proBDNF protein at codon 66 (Val66Met). This SNP may be related to hippocampus-mediated memory performance in humans. Several studies have reported that BDNF Met66 carriers with a diagnosis of schizophrenia may be at a substantially greater risk of hippocampal dysfunction (27, 28). Pezawas et al. (29) reported 12-15% volume reductions in the hippocampus in Met66 carriers relative to Val66 homozygotes; significant reductions were observed in the dorsolateral prefrontal cortex. The association of BDNF and hippocampal function raised concerns about whether the Val66Met polymorphism influenced intelligence scores in schizophrenia.

The Val allele of the BDNF Val66Met polymorphism is expressed more frequently in patients with psychosis (13), schizophrenia (30), and schizoaffective disorder or other affective disorders (31), linking genetic variation of the BDNF polymorphism with symptoms of psychosis. In a Chinese cohort study, Tsai and colleagues (32) examined the association of BDNF gene polymorphism with general intellectual ability in a healthy female population (aged 18-21 years, n=114) using the Wechsler Adult Intelligence Scale - Revised version (WAIS-R). Individuals with the Val/Val genotype showed modest increases in performance intelligence quotient (IQ) scores in comparison with individuals with the Met/Met genotype and the heterozygous group, suggesting a relationship between BDNF and dimensions of general intellectual ability. Egan et al. (27) reported that the Met allele of the functional polymorphism was associated with poor performance on human episodic memory and abnormality in hippocampal function in patients with schizophrenia (n=106), their unaffected siblings (n=138) and healthy controls (n=59). However, the authors reported no association of BDNF Val66Met polymorphism on IQ. Whalley et al. (33) reported no difference in a sentence completion task between Val/Met and Met carriers in young subjects at high risk of developing schizophrenia, although the former group showed relatively increased activation of the anterior cingulate cortex. Rosa et al. (13) showed a preferential transmission of the Val allele from heterozygous parents to offspring affected with psychosis, suggesting a possible role of this gene in the vulnerability to schizophrenia spectrum disorder.

Individuals with early-onset and adult-onset schizophrenia show intellectual deficits across the lifespan (34-38). Early onset schizophrenia (EOS; onset before age 18 years) is a rare, chronic, and relatively severe variant of the adult-onset counterpart of the disorder (39, 40). Intelligence scores in patients with EOS often range between 80 and 90 (approximately 1-1.5 standard deviations below the normative mean), which is significantly lower than adult-onset cases (41-44). Individuals with a genetic predisposition to schizophrenia show a significant difference in IQ scores in comparison to normal controls (45-51), including premorbid periods (50). One study showed an association of BDNF Val55Met polymorphism with general intellectual ability in healthy individuals (32), but no studies to date have investigated the association of this polymorphism with intelligence in schizophrenia. The lower than average IQ scores of EOS patients informed our choice of examining the association of the BDNF Val66Met polymorphism and general intellectual ability in EOS probands and their first-degree relatives.

**METHODS**

**PARTICIPANTS**

Patients were identified by clinicians’ referrals from secondary care services within the South London and Maudsley NHS Trust and were included if they: (a) were aged between 13-18 years; (b) fulfilled Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (52) criteria for schizophrenia; and (c) had at least one first-degree relative unaffected by schizophrenia spectrum disorders. Eligible first-degree relatives were invited to participate, with the patients’ consent, if aged 13-65 years and without a personal history of schizophrenia spectrum disorders.

Exclusion criteria for the entire sample (patients and healthy relatives) included: (a) head injury leading to a loss of consciousness for > 1 hour; (b) a per-
sonal history of neurological or medical disorders; (c) a family history of hereditary neurological disorders; and (d) fulfilling DSM-IV criteria for lifetime drug or alcohol dependence and drug or alcohol abuse in the preceding six months.

All participants were recruited as part of the Vulnerability Indicators in Psychosis Study (VIPS) from January 2003 to January 2007. This study examines clinical, cognitive, and genetic liability and disease expressivity in schizophrenia. The study procedures were in accordance with the Joint South London and Maudsley and the Institute of Psychiatry NHS Research Ethics Committee. Written informed consent or assent was obtained from all participants.

**CLINICAL EVALUATION AND NEUROPSYCHOLOGICAL ASSESSMENT**

All participants underwent an interview by a trained child and adolescent psychiatrist who was initially blind to diagnosis but not family status, using the Structured Clinical Interview for DSM-IV (SCID) for Axis I disorders (patient and non-patient version) (53, 54).

General intellectual ability was measured using age-appropriate Wechsler Intelligence Scales. The Wechsler Adult Intelligence Scale–Revised edition (WAIS-R) (55) was used for individuals aged 16 years and above and the Wechsler Intelligence Scale for Children–Third edition (WISC-III) (56) was used for younger subjects. Five healthy relatives and eight EOS probands were assessed using the WISC-III, and the WAIS-R was performed on 86 healthy relatives, 45 EOS probands and 17 relatives with major depressive disorder (MDD). All participants were assessed on the following IQ subtests of the Wechsler Intelligence Scales (WISC-III or WAIS-R): vocabulary, comprehension, similarities, object assembly, and block design. The age-appropriate scaled scores for each subtest were inputted for statistical analysis.

**GENOTYPING PROCEDURES**

Buccal swab DNA was obtained from 161 participants. The G→A SNP encoding the amino acid substitution Val66Met (dbSNP rs6265) was genotyped. Genotype for rs6265 was determined by a TaqMan SNP genotyping assay (assay ID: C_11592758_10) (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, U.S.A.). 10 ng DNA was used in a 10-μl reaction, according to the manufacturers’ instructions. Endpoint analysis was performed on an AB Prism 7900HT Sequence Detection System (SDS) and a probability > 95% was attained for the SDS package. Genotype frequencies for rs6265 were 0.22, 0.76, and 0.02 for Val66Met, Val66Val, and Met66Met, respectively. The genotype frequencies were comparable with Hap-Map population genome build dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=6265). The genotype distribution of the rs6265 SNP in BDNF was in Hardy-Weinberg equilibrium. There were 21 undetermined BDNF genotypes in the full sample, owing to amplification failure. One hundred and seven subjects had the Val/Val genotype, 33 the Val/Met genotype, and three the Met/Met genotype. Owing to the low frequency of the Met66 homozygotes (~2%), the

**Table 1. Group comparisons of IQ subtest scores**

<table>
<thead>
<tr>
<th>Variable</th>
<th>EOS Patients</th>
<th>Relatives with MDD</th>
<th>Healthy Relatives</th>
<th>Test Statistic</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 53)</td>
<td>(n = 19)</td>
<td>(n = 91)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>17.2(1.31)</td>
<td>43.27(8.16)</td>
<td>34.87(14.67)</td>
<td>Wald $\chi^2=610.36$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Years of Education</td>
<td>10.47(1.04)</td>
<td>12/0(2.59)</td>
<td>12.02(2.41)</td>
<td>Wald $\chi^2=40.32$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>29/24</td>
<td>4/13</td>
<td>42/29</td>
<td>$\chi^2=7.73$</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>GAF score</td>
<td>56.2±1.99</td>
<td>65.9±1.77</td>
<td>81.6±0.81</td>
<td>Wald $\chi^2=89.34$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IQ subtests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vocabulary</td>
<td>7.2±3.07</td>
<td>9.6±3.51</td>
<td>8.6±3.57</td>
<td>Wald $\chi^2=10.02$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Comprehension</td>
<td>7.1±3.49</td>
<td>10±3.21</td>
<td>10±2.72</td>
<td>Wald $\chi^2=18.38$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Similarities</td>
<td>8.1±2.59</td>
<td>10±2.92</td>
<td>9.4±3.05</td>
<td>Wald $\chi^2=7.14$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Block Design</td>
<td>8.6±3.39</td>
<td>9.2±3.03</td>
<td>10.1±3.61</td>
<td>Wald $\chi^2=3.31$</td>
<td>0.19</td>
</tr>
<tr>
<td>Object Assembly</td>
<td>8.0±2.75</td>
<td>7.8±2.64</td>
<td>8.2±3.02</td>
<td>Wald $\chi^2=5.42$</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Uncorrected P values.
genotypes Val/Met and Met/Met were combined for the analysis (n = 36), and known as “Met carriers.”

**Statistical Analysis**

Statistical analyses were carried out using the Statistical Package for the Social Science (SPSS) version 18. To study the effect of genotype and diagnosis on IQ we used generalized estimating equation (GEE) with an exchangeable within-subject working correlation structure and robust standard errors covariates (57, 58) approach to account for familial inter-correlation between the EOS probands and their first-degree relatives. The Hubert White sandwich estimator was used which provides standard errors that are robust to possible misspecification of the correlation matrix. Unlike repeated measurement ANOVA a GEE allows for several observations per case (i.e., observations of two parents) and uses a full case analysis in the presence of missing data. We used a factorial design to study genotype (Val/Val vs. Met carriers) × diagnosis (EOS vs. healthy relatives vs. MDD) interaction on IQ subtests. Following a significant main effect of genotype or genotype × diagnosis interaction, Bonferroni correction (p-value multiplied by the number of pairwise comparisons) was performed. We calculated the effect sizes using Cohen’s d for measures that survived Bonferroni correction.

**Results**

**Sample Characteristics**

One hundred and one families of schizophrenia patients were assessed. The age entry criterion was between 13 and 65 years for all participants. Fourteen families did not meet the inclusion criteria for the study, and 23 families refused to participate. In total, 64 patients with schizophrenia and 179 relatives were enrolled into the study. Of these, 32 relatives were excluded for not meeting the inclusion criteria (substance abuse), 39 relatives withdrew or were unavailable to participate in the study, and 11 patients with schizophrenia did not meet the criteria for remission. The total sample used in the analysis constituted 161 individuals: 53 patients with schizophrenia and 108 of their first-degree relatives. Seventeen relatives were diagnosed with MDD (two relatives had bipolar disorder), and 91 relatives had no psychiatric disorder.

The majority of schizophrenia patients were prescribed antipsychotics, most commonly atypical agents (n=46); one was taking typical medication and six were unmedicated at the time of assessment. The mean time since onset of positive symptoms, as documented in the medical notes, to study entry was 1.77 years (SD 0.93).

**Group Comparisons**

Group differences across IQ subtests are presented in Table 1. As there was a significant difference between groups for age and years of education, these variables were inputted as covariates. As shown in Table 1, there was a significant main effect of diagnosis prior to Bonferroni correction (uncorrected p-values presented) on all IQ subtests, except the block design subtest. Specifically, there was a main effect of diagnosis on vocabulary, where EOS probands performed less well than healthy relatives (P=0.07, Cohen’s d=0.40) and MDD relatives (P=0.01, Cohen’s d=0.77). Similarly, EOS probands performed significantly less well on the comprehension subtest than healthy relatives (P<0.001, Cohen’s d=0.82) and MDD relatives (P<0.05, Cohen’s d=0.03). A trend was observed for a diagnosis effect on similarities in which EOS probands showed below average performance than healthy relatives (P=0.095, Cohen’s d=0.44) and MDD relatives (P=0.07, Cohen’s d=0.39). There was no difference in performance IQ across groups.

**Effect of Genotype**

Table 2 shows the means and standard deviations of IQ subtests in relation to BDNF genotype. There was a significant main effect of genotype on vocabulary (Wald $\chi^2=5.66$, df=1, P=0.017), with Met carriers outperforming Val/Val genotype (Cohen’s d=0.39). With regard to performance IQ measures, there was a significant main

<table>
<thead>
<tr>
<th>IQ subtest</th>
<th>BDNF (Val66Met)</th>
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<tbody>
<tr>
<td></td>
<td>Val/Val (n = 109)</td>
</tr>
<tr>
<td>Vocabulary</td>
<td>7.8±0.3*</td>
</tr>
<tr>
<td>Comprehension</td>
<td>8.8±0.3</td>
</tr>
<tr>
<td>Similarities</td>
<td>8.9±0.2</td>
</tr>
<tr>
<td>Block Design</td>
<td>9.3±0.3*</td>
</tr>
<tr>
<td>Object Assembly</td>
<td>7.8±0.2*</td>
</tr>
</tbody>
</table>

*p < 0.05
effect of genotype on block design (Wald $\chi^2=4.48$, df=1, $P=0.034$, Cohen's $d=0.40$) and object assembly scaled score (Wald $\chi^2=5.42$, df=1, $P=0.02$, Cohen's $d=0.39$), revealing significantly higher scores for Met carriers compared with the Val/Val genotype. Figure 1 depicts the association of BDNF on these IQ subtests. As shown in Table 2, there was no evidence for any association between the BDNF Val66Met polymorphism and comprehension (Wald $\chi^2=2.03$, df=1, $P=0.15$) or similarities (Wald $\chi^2=2.53$, df=1, $P=0.11$).

**Genotype by Diagnosis Interaction**

We found no significant genotype × diagnosis interactions for vocabulary (Wald $\chi^2=3.86$, df=2, $P=0.145$), comprehension (Wald $\chi^2=2.19$, df=2, $P=0.33$), similarities (Wald $\chi^2=2.47$, df=2, $P=0.29$), block design (Wald $\chi^2=0.93$, df=2, $P=0.62$) or object assembly (Wald $\chi^2=0.36$, df=2, $P=0.83$).

**Discussion**

The BDNF Val66Met gene polymorphism has been found to be associated with performance on specific IQ subtests in EOS probands and their first-degree relatives. Regardless of diagnosis, individuals with the Met/Met or Val/Met genotype demonstrated enhanced performance on the vocabulary, block design, and object assembly subtests of the Wechsler Intelligence scale. Our findings support previous studies showing an association of the BDNF polymorphism with intelligence (32) and generalized cognitive functioning (27, 59).

Rosa and colleagues (13) recently showed that individuals with the Met/Met genotype performed better on prefrontal-cortical related tasks (as measured using the Wisconsin card sorting task and the Trail Making Test) in comparison with the Val/Met and Val/Val genotypes. With respect to intelligence scores, our results follow a similar trend in which individuals with the Met allele are associated with better performance on specific IQ measures compared with the Val/Val genotype. Our findings harmonize with those of Harris et al. (60) who observed an association of the BDNF Met66 allele with enhanced verbal reasoning ability, which correlated with IQ, in community-dwelling elderly volunteers. In contrast, Tsai et al. (32) reported that Val66 homozygotes had significantly higher performance IQ scores than heterozygotes; a parsimonious explanation of these findings may reflect the cohort studied: the cohort involved only females, they were all ethnically Han Chinese, and they were all young healthy nursing students who were not predisposed to a lower IQ. Previous studies have shown that the Met allele of the Val66Met polymorphism is associated with poor IQ scores (27, 61, 62). Our findings are supported by previous studies that demonstrate an association of the Met allele with better performance on general intellectual ability and other cognitive functions (13, 33, 62). However, owing to the small sample size and the family-based design of this investigation, further studies with larger sample sizes are needed to confirm these associations.

Previous studies showed an association between BDNF and hippocampal function, which led to investigations into the influence of BDNF on intelligence in schizophrenia (27, 32). Semantic memory has been shown to be associated with verbal IQ in clinical and research settings (63), although some reports consider semantic memory to be hippocampally-independent (64, 65). Our findings showed that, compared with the Val/Val genotype, Met66 carriers achieved the highest score in the vocabulary subtest, which is a measure of an individual's general mental ability articulately to describe the meaning of words. Egan and colleagues (27) reported an association of the BDNF genotype on episodic memory in patients with schizophrenia, their siblings, and normal controls. Although the hippocampal system is considered to be a common pathway between semantic memory and episodic memory (66), the expressivity of BDNF and the accumulation
of different effects on cognitive functions in different psychotic disorders points to a general effect of the polymorphism on cognitive abilities, and more specifically to memory performance and IQ. However, further replication is warranted to confirm this result.

The lack of a genotype × diagnosis interaction may at least partly result from a lack of diagnostic specificity of BDNF genotype in relation to general intellectual ability. Indeed, it has been argued that the nosological classification of DSM-IV-TR (67) may be somewhat arbitrary (68). As noted by Owen and colleagues (68), there is an absence of a distinctive “zone of rarity” between schizophrenia and mood disorders, which may explain our findings. In addition, the additive actions of several susceptibility genes, which could either be multiple risk genes or protective gene variants, may be associated with the differential expression of general intelligence.

There are some limitations in this study. In view of the critical role of activity-dependent secretion of BDNF in hippocampus-based synaptic plasticity and learning and memory (27), antipsychotic medication could have contributed to impairments in general intellectual ability in schizophrenia. Our EOS patients and their first-degree relatives were clearly related and the comparison sample was relatively small for a genetic association study. Age and years of education were included as covariates in the analysis, although we cannot exclude potential stratification entirely. Therefore, our findings should be taken with caution, and considered as a preliminary investigation, which may be useful for future studies incorporating larger sample sizes. We dealt with the issue of inter-correlation by using the GEE method and a within-subjects design. Indeed, our EOS sample was small however this reflects the rare and severe form of the illness in comparison with adult-onset schizophrenia (69, 70). Similar to previous studies (13, 32, 59), we lacked a control group, however our aim was to study the influence of BDNF on intelligence in EOS and investigate the possibility of genetic differentiation of the BDNF gene polymorphism in families of schizophrenia patients. Future larger studies should elucidate the critical role of BDNF on these specific cognitive measures in schizophrenia and healthy non-relatives, and fully evaluate the validity of these conclusions.

In conclusion, the BDNF Val66Met polymorphism appears to be associated with general intellectual ability. The IQ subtests used in our study suggests an association of the BDNF polymorphism with vocabulary, block design, and object assembly, which provides support to the hypothesis that BDNF may play a critical role in the neurodevelopment of brain circuitry involved in general cognitive abilities, and more specifically to memory and dimensions of IQ. Further investigation is warranted to provide a more validated conclusion.

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Contributions
NSV was involved in the design, analysis and interpretation of data, and wrote the first draft of the manuscript. BKP was involved in the interpretation of the data and critically revised the manuscript. Both authors approved the final version.

Conflicts of Interest
None.

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