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ORIGINAL RESEARCH ARTICLE

Brain-derived neurotrophic factor val66met polymorphism and volume of the hippocampal formation

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Magnetic resonance (MR) imaging studies have identified hippocampal structural alterations in the pathogenesis of schizophrenia. Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins that is widely expressed in the hippocampal formation and has been implicated in the neurobiology of schizophrenia. Polymorphisms in the BDNF gene may therefore confer risk for schizophrenia through hippocampal pathogenesis and/or making the hippocampus more susceptible to environmental insults. In this study, we investigated whether val66met, a functional and abundant missense polymorphism in the coding region of the BDNF gene, was associated with the volume of the hippocampal formation in 19 patients with first-episode schizophrenia and 25 healthy volunteers. A total of 124 contiguous T1-weighted coronal MR images (slice thickness = 1.5 mm) were acquired through the whole head using a 3D Fast SPGR IR Prep sequence on a 1.5 T GE imaging system. Volumes of the right and left hippocampal formation were measured manually by an operator blind to group status and genotype. All participants were genotyped for the BDNF val66met locus. Mixed model analyses revealed a main effect of BDNF val66met genotype such that in the combined sample of patients and healthy volunteers, val/val homozygotes ($N = 27$) had larger volumes of the hippocampal formation compared to val/met heterozygotes ($N = 17$). In separate analyses by group, however, val66met genotype accounted for a greater proportion of the variance in the volume of the hippocampal formation in patients compared to healthy volunteers. These findings implicate genetic involvement of BDNF in variation of human hippocampal volume and suggest that this effect may be greater among patients compared to healthy volunteers.
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Temporolimbic abnormalities have been hypothesized to play an important role in the pathophysiology of schizophrenia. Post-mortem studies have reported pyramidal cell disarray,[1] reduced pyramidal cell density[2] and number,[3] smaller neurons,[4] as well as reduced volume[5] among patients with schizophrenia. In addition, *in vivo* magnetic resonance (MR) imaging studies have provided further evidence that patients with schizophrenia have temporolimbic abnormalities.[6,7] Moreover, functional MR imaging studies have yielded further evidence for hippocampal dysfunction in schizophrenia.[8]

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Brain-derived neurotrophic factor (BDNF) plays an important role in the development and maintenance of adult neurons and are important regulators of synaptic plasticity within the brain.[9,10] In particular, BDNF is widely expressed in the hippocampus[11] and plays an important role in the long-term potentiation of hippocampal neurons.[12] Animal studies suggest that BDNF within the hippocampus may be affected by variations in maternal care,[13] environmental enrichment,[14] exercise,[15] stress[16] and diet.[17] Other studies have identified a role for BDNF in memory processes that have been linked to the hippocampus. For example, rapid and selective induction of BDNF expression has been observed during hippocampus-dependent contextual learning.[18] Moreover, Lee *et al*[19] recently reported a role for BDNF in hippocampal memory consolidation through infusion of antisense oligodeoxynucleotides into the rat hippocampal formation.

Several studies have reported that polymorphisms in the BDNF gene may contribute to the pathogenesis of schizophrenia.[20–22] In addition, polymorphisms in this gene have relevance for several aspects of the phenomenology of schizophrenia including negative symptoms[23] and treatment responsiveness.[24,25] Moreover, post-mortem studies indicated that BDNF levels are abnormal in patients with schizophrenia[26–28] and several studies reported an association between neonatal rat hippocampal lesions and BDNF mRNA.[29,30] It is plausible that polymorphisms in the BDNF gene could play a role in the neurobiology of schizophrenia through hippocampal pathogenesis and/or making the hippocampus more susceptible to environmental insults.[31]

The *BDNF* gene has been mapped to chromosome 11p13 and contains an evolutionarily recent missense mutation at position 66 resulting in a valine to methionine substitution. Prior work suggests that the BDNF val66met polymorphism may be associated with impaired cognitive functioning and, in particular, worse memory functioning in humans. Specifically, healthy humans homozygous for the *val* allele exhibit better memory functioning[32] greater mean performance IQ,[33] higher hippocampal *N*-acetylaspartate (NAA)[32] and increased functional activity within the hippocampus during a memory task[34] compared to *met* carriers. Although hippocampal volume is significantly heritable,[35,36] the relationship between polymorphisms in the BDNF gene and hippocampal structure has not been well-studied. In this study, we investigated the relationship between the val66met polymorphism and volume of the hippocampal formation in patients experiencing a first-episode of schizophrenia and healthy volunteers. Based on previously published studies,[32,34] we hypothesized that (1) individuals who are val/met heterozygotes would have smaller volumes of the hippocampal formation compared to val/val homozygotes and (2) that these effects would be more pronounced among patients compared to healthy volunteers given previous evidence of both hippocampal structural pathology and BDNF involvement in schizophrenia.

Material and methods

Subjects

The 19 (14 males, five females; mean age = 26.2 years, SD = 5.8) patients with schizophrenia included in this study were recruited from The Zucker Hillside Hospital in Glen Oaks, NY. All patients were in their first-episode of illness and had little prior exposure to antipsychotic medications overall (median = 0 weeks; range = 0–23 weeks). In all, 11 patients were antipsychotic drug-naïve at the time of the scan. Mean age at onset for patients was 22.7 (SD = 5.1) years. Diagnoses were based on the Structured Clinical Interview for Axis I DSM-IV Disorders[37] and included schizophrenia ($N = 17$), schizoaffective ($N = 1$) or schizophreniform ($N = 1$) disorders. Additional comorbid major diagnoses included OCD ($N = 4$) and substance abuse and/or dependence ($N = 7$). A total of 25 (10 males, 15 females; mean age = 27.1 years, SD = 6.7) healthy volunteers were recruited from local newspaper advertisements and through word of mouth in the community and had no history of psychiatric illness as determined from the SCID-NP.[38] Exclusion criteria for all individuals included serious neurological or endocrine disorder, any medical condition or treatment known to affect the brain, or meeting DSM-IV criteria for mental retardation. We restricted subject enrollment to Caucasians to control for potential population effects. All procedures were approved by the North Shore-Long Island Jewish Health System IRB and written informed consent was obtained from all participants.

MR imaging procedures

MR imaging exams were conducted at Long Island Jewish Medical Center and acquired in the coronal plane using a 3D Fast SPGR with IR Prep (TR = 12.7. or 14.7, TE = 4.5 or 5.5 ms, matrix = 256 × 256, FOV = 22 cm) on a 1.5 T whole body superconducting imaging system (General Electric, Milwaukee, WI, USA) producing 124 contiguous images (slice thickness = 1.5 mm) through the whole head. All scans were reviewed by a neuroradiologist and a member of the research team. Any scan with significant artifacts was repeated. Patients typically received 1–2 mg of lorazepam orally prior to the scan.

Measurement procedures

All measurements were completed in MEDx[39] by an operator (CM) blind to group membership, hemisphere and genotype following alignment of the images along the anterior and posterior commissures for standardization across subjects. Scans of patients and healthy volunteers were mixed together randomly and no identifying information was available to the operator from the scan.

Total intracranial contents

Total intracranial contents included the cerebrum, cerebrospinal fluid, cerebellum and brainstem. Interrater reliability between two raters as assessed by intraclass correlations in nine cases was 0.99.

Hippocampal formation

Two contiguous portions of the hippocampal formation (posterior and anterior) were measured in each hemisphere by a single operator (CM) as described previously.[7] The posterior boundary of the hippocampus was the slice where an ovoid mass of gray matter appeared inferiomedially to the trigone of the lateral ventricle. The Fasciola cinerea, Gyrus Fasciolaris, isthmus and crus of the fornix were excluded from measurement. Following the interruption of the pulvinar by the crus of the fornix, all CA-segments (CA1, CA2, CA3, CA4), dentate gyrus, alveus, parasubiculum, presubiculum, subiculum proper and prosubiculum were included in the measurements.

The anterior boundary was the coronal slice posterior to the one where the cisterna pontis became clearly visible. The posterior boundary of the anterior hippocampal formation was the slice where the cisterna pontis became clearly visible and included all the segments that were measured for the posterior hippocampus as well as the uncus and intralimbic gyrus (including the dentate gyrus and Ammon's horn). Intraclass correlations between three operators for nine cases were: (right, left): posterior hippocampus (0.87, 0.88) and anterior hippocampus (0.94, 0.87). An illustration of the delineation criteria are provided elsewhere.[7]

DNA procedures

Blood samples were collected from all individuals and DNA subsequently extracted. *BDNF* val66met genotypes were determined using a 5′-exonuclease allelic discrimination (Taqman) assay using Reference SNP ID: rs6265 (ABI Assay on Demand C_11592758_10, Applied Biosystems, Foster City, CA, USA), on an ABI7900 instrument. Genotyping error rate for this assay was determined by replicate genotyping of samples, and was <0.005.

Handedness

Handedness was determined using a modified 20-item Edinburgh Inventory.[40] The total number of right- and left-hand items was scored, and the laterality quotient was computed as [(total right−total total left)/(total right + total left)]100. Subjects with a laterality quotient greater than 0.70 were classified as dextral; the rest were classified as nondextral.[41]

Statistical analyses

Statistical testing was conducted using mixed models (SAS; v8.02).[42] Group (patient *vs* healthy volunteer), and val66met genotype (*met/val vs val/val*) were between subjects factors. We used sex as a statistical covariate given the imbalance in sex distribution between the groups. Intracranial contents was also used as a statistical covariate to control for non-specific differences in hippocampal size among individuals. The genotype-by-hemisphere-by-region interaction was not statistically significant for either patients or healthy volunteers and we thus pooled right and left posterior and anterior hippocampal segments for analysis. We used either independent groups *t*-tests or χ^2 analyses to examine group differences in sample characteristics. Genotype frequencies were investigated with Fisher's exact test to test for Hardy–Weinberg equilibrium.

Results

Patients and healthy volunteers did not differ significantly in genotype, age or handedness (P's >0.05), but did differ in sex distribution ($\chi^2=4.94$, df $=1$, $P=0.03$). Genotype distributions were as follows (patient, healthy volunteer): *val/met* (7,10) and *val/val* (12,15); these distributions did not differ significantly from each other or from Hardy–Weinberg equilibrium (P's >0.05). There were no *met/met* homozygotes in this sample.

Using total intracranial contents and sex as covariates there was a significant main effect of genotype (F $=8.72$, df $=39$, $P=0.005$) such that *val/val* homozygotes had significantly larger volumes of the hippocampal formation compared to *val/met* heterozygotes (see Table 1). Individual hippocampal volumes are illustrated by genotype in Figure 1. Exclusion of the *val/val* homozygote with the largest hippocampal volume from analysis did not alter the finding of a significant main effect of genotype. The group-by-genotype interaction was not statistically significant ($P>0.05$).

Given the main effect of BDNF val66met genotype, we examined possible differences in sample characteristics between *val/val* homozygotes and *val/met* heterozygotes (see Table 1). There were no significant differences between the two groups in distributions of sex, handedness, clinical diagnosis of schizophrenia, comorbid diagnoses or total intracranial volume. *Val/val* homozygotes were significantly older compared to *val/met* heterozygotes, however. Thus, given the age differences between the genotype groups and that the groups were not matched exactly for diagnosis, we repeated the analysis using age and diagnosis as statistical covariates (along with intracranial contents). The main effect of genotype remained statistically significant for total hippocampal volume (F $=9.40$, df $=39$, $P=0.004$) with significant effects evident in both the right (F $=13.44$, df $=39$,

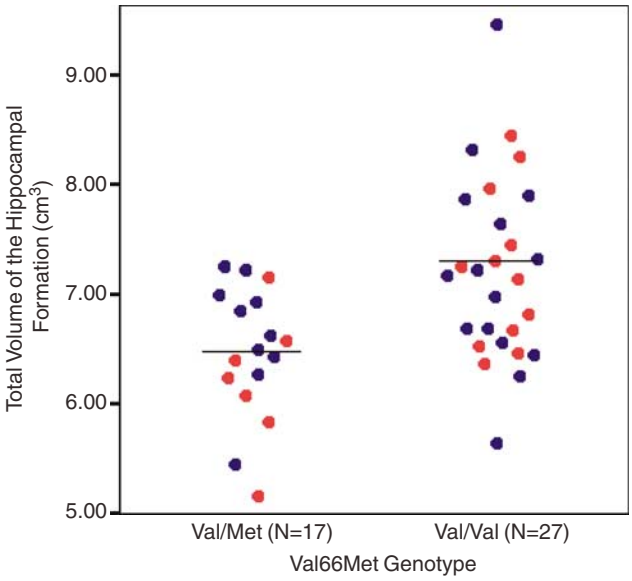

Figure 1 Total volume of the hippocampal formation by genotype. Red-colored circles denote patients and blue-colored circles denote healthy volunteers. Horizontal lines represent the mean volume.

Table 1 Sample characteristics and brain volume data by gentotype

	Val/Val (N = 27)	*Val/Met (N = 17)*	*Statistic*	*df*	*P*
Sample characteristics					
Group (patients, healthy volunteers)	12/15	7/10	$\chi^2 = 0.21$	1	0.65
Sex (male, female)	14/13	10/7	$\chi^2 = 0.05$	1	0.83
Handedness (right, left)	23/4	15/2	$\chi^2 = 0.08$	1	0.77
Age (years)	28.5 (6.8)	23.9 (4.3)	$t = -2.45$	42	0.02
Education (years)	14.7 (2.2)	14.4 (1.7)	$t = -0.38$	42	0.71
Comorbid diagnoses among patients					
Obsessive-compulsive disorder	3	1	$\chi^2 = 0.35$	1	0.56
Substance abuse/dependence	4	3	$\chi^2 = 0.06$	1	0.80
Brain volume data[a]					
Total hippocampus volume	7.22 (0.83)	6.47 (0.60)	$t = -3.22$	42	0.002
Right hippocampus	3.73 (0.48)	3.26 (0.24)	$t = -3.74$	42	0.001
Left hippocampus	3.49 (0.39)	3.21 (0.39)	$t = -2.30$	42	0.027
Total intracranial volume	1379 (160)	1323 (119)	$t = -1.24$	42	0.221

Data are presented as mean ± SD in parentheses, unless otherwise indicated.
[a]Brain volume data presented as cm^3.

$P = 0.0007$) and left hippocampal ($F = 4.58$, $df = 39$, $P = 0.039$) formation. Excluding patients with a diagnosis of schizophreniform ($n = 1$) or schizoaffective ($n = 1$) disorder or patients treated with antipsychotic medications ($n = 8$) did not alter these findings substantively. Lastly, we examined whether the relationship between genotype and hippocampal volume was comparable across patients and healthy volunteers in a *post hoc* analysis. These analyses revealed that genotype accounted for a larger percentage of the variance in hippocampal volume among patients ($F = 11.72$, $df = 15$, $P = 0.004$; partial eta squared = 0.44) compared to healthy volunteers ($F = 2.30$, $df = 21$, $P = 0.14$; partial eta-squared = 0.10).

Discussion

These findings provide evidence for an association between a functional *BDNF* polymorphism, val66met, and volume of the hippocampal formation in humans. Specifically, in a combined sample of patients with schizophrenia and healthy volunteers, met carriers had smaller hippocampus volumes compared to *val/val* homozygotes. This effect was, however, more pronounced among patients compared to healthy volunteers. It is noteworthy that *val*/*val* homozygotes did not differ significantly from *val/met* heterozygotes in total intracranial volume suggesting that the observed relationship did not effect global brain development.

Despite its role in neuronal development and maintenance, few studies have investigated the relationship between BDNF genetic variation and brain structure and function in humans. In a structural MR imaging study, Wassink *et al*[43] reported an association between a microsatellite marker located 1 kb upstream from the *BDNF* transcription initiation site and parietal lobe volume in schizophrenia. This study used a nonfunctional marker, however, and did not examine the val66met polymorphism, for which met66 represents a loss-of-function allele.[32] Other studies specifically investigating the BDNF val66met polymorphism reported an association between this polymorphism and hippocampal structural and functional integrity. Egan *et al*[32] identified decreased NAA, a putative measure of neuronal integrity, in the hippocampus and abnormal hippocampal activation as assessed via functional magnetic resonance imaging (fMRI) in association with the met allele. Similarly, in an fMRI study that assessed the relationship between BDNF val66met genotype and hippocampal activity during episodic memory processing, Hariri *et al*[34] reported that met carriers exhibited decreased hippocampal engagement compared to *val/val* homozygotes during both encoding and retrieval of information. The results of our study thus extend the findings of Egan *et al*[32] and Hariri *et al*[34] by implicating a role for genetic variation in the val66met polymorphism in association with hippocampal volume.

There may be several mechanisms through which BDNF could be associated with volumetric alterations of the hippocampal formation. Egan *et al*[32] reported that cultured hippocampal neurons transfected with the *met* allele exhibited lower depolarization-induced secretion compared to those transfected with the *val* allele suggesting that this gene may impact intracellular trafficking, which could be associated with changes in synaptic plasticity and morphology.[44] Other studies have provided evidence that exogenous administration of BDNF is linked with changes in hippocampal morphology. Shetty and Turner[45] reported that BDNF supports survival of hippocampal stem cell-derived neurons, which may induce cell differentiation into pyramidal-like neurons. Murphy *et al*[46] reported that estradiol downregulates BDNF

in cultured hippocampal neurons, which in turn, decreases inhibition and increases excitatory tone in pyramidal neurons, leading to a two-fold increase in dendritic spine density. Additionally, growth properties of cultured neonatal dentate granule cells are influenced by exogenously applied BDNF.[47] Lastly, Suzuki *et al*[48] have demonstrated that injection of kainate into the hippocampus is associated with a long-lasting global trophic response of granule cells involving somatic and dendritic growth, which is correlated with a specific overexpression of BDNF.

A potential limitation of this study is that case–control association studies can be influenced by undetected population stratification.[49] We restricted the study group to Caucasians, however, and focused on a polymorphism with demonstrated functional effects. Larger studies adequately powered for 'genomic control' analysis or family-based designs would be useful to confirm these findings. Other possible study limitations include the small sample size and heterogeneity of subjects. Moreover, we were unable to investigate potential epistatic differences between patients and healthy volunteers as well as the possible role of gene–environment interactions on the observed findings.

In summary, our data suggest that variation in the size of the hippocampal formation may be partially explained by the val66met polymorphism in the *BDNF* gene. Future work could address the potential relationship between structural and functional modalities among genotypes, including the effects of the BDNF met66/met66 genotype, which was not observed in any of the individuals in this study, but expected in 3–4% of the population.

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