BDNF Val66Met polymorphism modulatory effects on prefrontal regions in major depressive disorder

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Abstract

Background: Brain derived neurotrophic factor (BDNF) Val66Met polymorphism contributes to development of depression (MDD), but it is unclear whether neural effects observed in healthy individuals are sustained in MDD

Aims: To investigate BDNF Val66Met effects on key regions in MDD neurocircuitry: amygdala, anterior cingulate, middle frontal and orbitofrontal regions.

Method: MRI scans were acquired in 79 MDD (mean age 49 years) and 74 healthy volunteers (HV) (mean 50 years). Effects on surface area and cortical thickness were examined with multiple comparison correction.

Results: Met allele carriers showed reduced caudal middle frontal thickness in both MDD and HV. Significant interaction effects were found in the anterior cingulate and rostral middle frontal regions in which Met MDD carriers showed the greatest reduction in surface area.

Conclusions: Modulatory effects of the BDNF Val66Met polymorphism on distinct subregions in the prefrontal cortex in MDD support the neurotrophin model of depression.

Declaration of Interest: None.

Introduction

Heritability estimates in major depressive disorder (MDD) are in the order of 48-75% (1). A potential candidate gene is the brain derived neurotrophic factor (BDNF) polymorphism which has been linked with an increased incidence of MDD (2). BDNF is the most common of the neurotrophins and has an important role in synaptic plasticity, neurogenesis, neural growth and differentiation (3-4). A common single nucleotide polymorphism (SNP) at codon 66 of the BDNF gene results in a valine to methionine (Val66Met) substitution which has a functional impact on cellular packaging, transportation, and secretion, and the neurotrophic model proposes that decreased BDNF expression contributes to the development of depression (5). BDNF is widely distributed within key regions in the neural circuitry of affective processing and in major depressive disorder, including in the anterior cingulate, prefrontal regions, hippocampus, and amygdala (6). Effects of the BDNF Val66Met polymorphism in healthy subjects though have been variable. In subcortical limbic regions, reduced volumes of the hippocampus (7-8) and amygdala (8) as well as no significant differences (9-10) have been reported in Met carriers relative to Val homozygotes. In prefrontal regions, Met carriers have shown reduced middle (MFC) (7,11) and inferior frontal (7) volumes, though no significant differences in orbitofrontal (12) volumes.

Few studies have examined the effect of the BDNF polymorphism in depression, and the main region of interest to date has been the hippocampus which has shown mixed findings with reduced volume in Met carriers (10,13), as well as reduced (14) and increased (15) volume in Val homozygotes, while previously we found no differences between genotypes (16). However, studies in depression have been limited in their sample characteristics: absence of a healthy control group (13); specified regions of interest: namely, hippocampus (10,14-16) and amygdala (10); and measures of white matter tracts only (17-18). Moreover, the majority of studies have measured regional

gray matter, including gray matter density. The determinants of the gray matter volume of a given cortical region are the surface area and cortical thickness, which have distinct developmental and genetic origins (19). Volumetric alterations are thus a product of independent or concurrent disparities in the two constituents. In healthy subjects, Yang et al. (20) found a broad distribution of reduced cortical thickness in Met homozygotes compared to Val homozygotes in Chinese adults. To our knowledge, BDNF Val66Met modulation of cortical thickness and surface area has not been investigated in MDD.

We sought to examine the effects of the BDNF Val66Met polymorphism on the neurocircuitry of depression, in particular the amygdala and the prefrontal regions of the anterior cingulate (ACC), middle frontal and orbitofrontal cortices. We investigated the modulatory effects on regional brain volume and its components of surface area and cortical thickness. We expected to observe an effect of Met carrier status in reduced cortical volume in the middle frontal cortices (7,11) and perhaps in the corresponding cortical thickness (20) in healthy subjects, while it was uncertain whether the impact of the polymorphism would be sustained in MDD.

Method

The study was approved by the Ethics Research Committee, Institute of Psychiatry, King's College London, UK, and all participants provided written informed consent. All participants had previously participated in genetic association studies (21) and were of white European ancestry. A total of 153 subjects were included: 79 patients with a diagnosis of recurrent major depressive disorder (MDD) and 74 healthy controls (HC) matched by age, sex, handedness and IQ (Table 1). All MDD patients met criteria for recurrent MDD as characterised by the DSM-IV-TR using the Schedules for Clinical Assessment in Neuropsychiatry interview (22), and healthy controls were screened to ensure they had never experienced a depressive episode. All participants were

screened for contraindications to MRI, as well as any indication of neurological disorder such as head injury leading to loss of consciousness or conditions known to effect brain structure, such as alcohol or drug abuse. Subjects were excluded if they or a first degree relative had ever experienced an episode of mania, hypomania, schizophrenia or mood incongruent psychosis. IQ was measured using the Wechsler Abbreviated Scale of Intelligence (23) and depressive symptoms with the Beck Depression Inventory (24).

Most patients were taking at least one antidepressant medication (n=58), and some were not taking any medications at the time of the MRI scan (n=21) (Table 2). The antidepressant medications encompassed selective serotonin reuptake inhibitors, serotonin-noradrenergic reuptake inhibitors, tricyclic antidepressants, and other antidepressant classes. In addition, 7 patients were taking additional medication for augmentation of the antidepressant medication: mood stabilisers, benzodiazepine, antipsychotic medication, and thyroxine.

Genotyping

Val66Met BDNF genotyping was performed using Taqman 5' exonuclease assay (16). BDNF genotypes were divided into three groups: Val/Val, Val/Met and Met/Met. For the purpose of the present analysis, the groups were combined into Val homozygotes (Val/Val) and Met carrier (Met-allele) groups due to the small number of Met homozygotes subjects.

MRI data acquisition

Magnetisation-Prepared Rapid Gradient Echo (MP-RAGE) T1-weighted scans were acquired at 1.5T (General Electric, WI, USA) with the parameters: TE = 3.8 ms, TR = 8.59 ms, flip angle = 8◦, field of view = 24 × 24 cm, slice thickness = 1.2 mm, number of

slices = 180 and image matrix = 256 × 256. The MPRAGE volume was acquired using the ADNI custom pulse sequence, with full brain and skull coverage (25).

Data analysis

Gray matter volumes, surface area and average cortical thickness measurements were measured using the Freesurfer pipeline version 5.1.0 (http:// surfer.nmr.mgh.harvard. edu/). The analysis involved removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures, intensity normalization, tessellation of the grey matter-white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. Once the cortical models are complete, registration to a spherical atlas takes place which utilizes individual cortical folding patterns to match cortical geometry across subjects. This is followed by parcellation of the cerebral cortex into units based on gyral and sulcal structures. The pipeline generated 68 cortical thickness, cortical volume, surface area, mean curvature, gaussian curvature, folding index and curvature index measures (34 from each hemisphere) and 46 regional subcortical volumes. All volumetric measures from each subject were normalized by the subject's intracranial volume, while cortical thickness measures were not normalized.

As *a priori* hypotheses, only the following regions were examined: amygdala, anterior cingulate (rostral and caudal ACC), middle frontal cortex (rostral and caudal), and orbitofrontal cortex (medial and lateral) bilaterally. A multivariate ANOVA (SPSS version 21) was used to assess differences between both MDD patients and healthy controls and between Val homozygotes and Met carriers (Val/Met and Met/Met genotypes). False

discovery rate (FDR) was used to adjust for multiple comparisons resulting from the ANOVA models with alpha = 0.05 (26).

Results

A significant effect of genotype which survived FDR correction for multiple comparisons was found in the left caudal middle frontal cortex (Brodmann's area (BA) 6) in cortical thickness in which Met carriers showed the greatest reduction in cortical thickness as compared to Val homozygotes in both the MDD and healthy control groups (Val/Val MDD: 2.474 (0.138) mm; Met-allele MDD: 2.434 (0.177) mm; Val/Val HC: 2.491 (0.158) mm; Met-allele HC: 2.361 (0.141) mm ($F (1, 149) = 11.029$, $p = 0.001$)) (Figure 1).

Significant interaction effects which survived multiple comparison correction were found in the surface area in 3 regions: right caudal anterior cingulate (Val/Val MDD: 782.48 (140.172) mm^2; Met-allele MDD: 711.00 (150.610) mm^2; Val/Val HC: 764.02 (153.134) mm^2; Met-allele HC: 869.26 (186.699) mm^2, ($F (1, 149) = 11.729$, $p = 0.001$)); right rostral middle frontal cortex ((Val/Val MDD: 5975.30 (667.014) mm^2; Met-allele MDD: 5592.39 (611.784) mm^2; Val/Val HC: 5673.02 (754.373) mm^2; Met-allele HC: 6125.70 (671.124) mm^2 ,($F (1, 149) = 13.489$, $p = 0.0003$)); and left rostral middle frontal cortex ((Val/Val MDD: 5723.28 (633.790) mm^2; Met-allele MDD: 5383.06 (473.553) mm^2; Val/Val HC: 5477.19 (677.134) mm^2; Met-allele HC: 5890.04 (711.311) mm^2, ($F (1, 149) = 12.869$, $p = 0.0005$)). In all of these regions, reduced surface area was associated with Met carrier status relative to Val homozygotes in MDD, while the inverse was observed in healthy subjects.

No other main or interaction effects remained significant following multiple comparison correction, including in the amygdala (MDD left amygdala: 1385.24 (219.404) mm^3, MDD right: 1482.92 (231.585) mm^3; HC left: 1433.81 (257.164) mm^3, HC right: 1552.50

(271.312) mm^3; Val/Val left: 1432.30 (249.516) mm^3, Val/Val right: 1537.49 (250.534) mm^3, Met-allele left: 1372.20 (218.359) mm^3, Met-allele right: 1484.20 (255.904) (all $p > 0.3$).

Discussion

Modulatory effects of the BDNF Val66Met polymorphism as well as genotype by MDD interactions were revealed in key nodes in the neurocircuitry of MDD. Distinct effects were observed in the anterior cingulate and subregions of the middle frontal cortices implicating converging yet separate influences of the disease process, genetic modulation, and their interaction.

Pezawas et al. (7) provided the first report of an effect of the BDNF Val66Met polymorphism in the caudal middle frontal cortex, revealing reduced gray matter volume in healthy Met carriers compared to Val homozygotes. The potential contribution of cortical thinning to the volumetric reductions has recently been added (20), and reduced middle frontal activity has also been observed (9) in healthy Met carriers. In the present study, we found a main effect of Met carrier status on cortical thinning in the caudal middle frontal cortex in both healthy subjects and in patients with depression. Our study extends both the observation by Yang et al. (20) in healthy Chinese adults and the original finding (7) by localising the contribution of cortical thinning to the reductions in caudal middle frontal volume.

Moreover, this is the first report of the extent that Met carrier status leads to middle frontal cortical thinning as the effect of the Met allele appears to supersede the disease process effects of MDD on cortical thickness. Gray matter reductions in the caudal middle frontal region have been frequently observed in MDD, which show further reductions in recurrent MDD (27). Our findings indicate that the impact of the BDNF

Val66Met polymorphism on cortical thinning in the caudal middle frontal region is even greater than that of recurrent MDD. It is possible that the influence of the Met allele in this region may not necessarily be causal in itself for MDD, but may be a predisposing, contributory factor in association with other neurogenic effects. Stress is linked an increase in cortisol, which in turn causes a reduction of BDNF (28). Met carriers are less able to compensate to this BDNF reduction due to the deficient transport of the BDNF preprotein which causes clumping around the nucleus, perhaps leading to neuronal atrophy in response the reduced BDNF levels (4-5).

We also observed a significant interaction effect in the surface area in the caudal anterior cingulate and rostral middle frontal cortices. The anterior cingulate and middle frontal cortices are key regions in the neurocircuitry of mood disorders, and the anterior cingulate is a well replicated predictive marker of clinical response in MDD (29) which is evident at the individual level (30). Surface area and cortical thickness have independent genetic and developmental origins (19). The radial unit hypothesis proposes that cortical thickness is determined by the number of cells within a neuronal column and cortical surface area is comprised of the number of neuronal columns (31). There is support for a general regional expansion of surface area from childhood into adolescence, particularly in boys (32), followed by subsequent decreases in adulthood with increasing age (33), while cortical thinning is a pronounced feature of adolescence which continues into adulthood (32). There is though some notable variability in the regional changes (32-33), for example the surface area of the anterior cingulate cortex may show relatively fewer changes in adulthood (32). Modulation of grey matter density by the BDNF Val66Met polymorphism in both the anterior cingulate and middle frontal cortices has been found in bipolar disorder (34), in the anterior cingulate in healthy subjects with a history of childhood abuse (12), while no prefrontal regional effects have been reported in schizophrenia (35). The present study localises the genetic influence to cortical surface area in its contribution to grey matter volume, which had not been

examined in previous studies in patient populations (34-35). It is possible that the surface area reductions observed in the present study is an endophenotype for MDD, or more generally for mood disorders including bipolar disorder, or it may also be a feature in schizophrenia which has not been well captured by the morphometric studies to date. The lack of an association of the BDNF gene with schizophrenia (36) though suggests that the effect may be more strongly expressed in mood disorders.

Contrary to our hypothesis, no significant interaction effects were found in the grey matter volume of the amygdala. Our observations are consistent with findings in healthy BDNF Met carriers with a history of childhood adversity (12) and in depression (10), but there have also been reports of reduced amygdala volume in healthy subjects with (37) and without (8) a history of stressful events. Similarly, studies of amygdala responsivity have been mixed with reports of significant (38-39) but more frequently of no impact (9,40-41) of the BDNF polymorphism. The literature on amygdala volume in depression though is inconsistent with some suggestion that amygdala volume is reduced in more chronic forms of depression (42). In the present study, patients had a history of recurrent depression characterised by discrete acute depressive episodes with periods of euthymia rather than a more chronic treatment resistant type of depression. Our findings are most comparable with Frodl et al. (10) who similarly did not observe any effects of diagnosis or BDNF genotype on amygdala volume.

We had limited our analysis to *a priori* defined regions in the prefrontal cortex and the amygdala within the neurocircuitry of depression. However, the sample size in the present study is relatively modest and replication in an independent sample is required. Cortical thinning in the medial orbitofrontal region has been reported in first episode depression (46) and in a younger cohort of MDD patients (mean age 34 years) than in the present study (47). Although there was evidence of left medial orbitofrontal cortical thinning in MDD patients relative to healthy controls in the current sample, this difference

did not survive correction for multiple comparisons. Furthermore, BDNF Val66Met effects have also been observed in the temporal and parietal cortices in healthy Chinese adults (20). There may also be a modulatory effect of early life stress as healthy Met carriers with a history of greater stressful events have shown reduced grey matter volume in the amygdala (37), hippocampus (37) and anterior cingulate cortex (12) as compared to Val homozygotes. Another limitation of the present study is the absence of data on the history of possible childhood trauma in our subjects, though it is unclear whether these effects would persist alongside the pathophysiological effects of the illness. As well, most of the patients were taking antidepressant medication, which may have an effect on BDNF levels (5) and in turn potentially on neural volumes (43). The anterior cingulate region has been consistently identified as a predictive marker of clinical response (29), and the BDNF polymorphism has shown an association with treatment response (44). Furthermore, an interaction effect of BDNF and its high-affinity receptor, neurotrophic tyrosine kinase receptor 2 (NTRK2), gene polymorphisms has been associated with the development of treatment-resistant depression (45). How these potentially complementary markers may interact at a neural level in predicting clinical response requires further investigation. Neural correlates of BDNF associations with clinical response in a patient sample which is medication free and perhaps in their first episode of depression would elucidate the effects of medication, recurrent episodes of depression, and prediction of clinical response.

In summary, the present study demonstrated sustained effects of the BDNF Val66Met polymorphism on distinct subregions in the prefrontal cortex in depression. The effects in the caudal middle frontal regions exceeded those of the illness as Met carrier status was associated with greater cortical thinning in both MDD and healthy subjects. Effects in the anterior cingulate and rostral middle frontal regions revealed an interaction with BDNF Val66Met genotype, in which MDD Met carriers showed the greatest reduction in

surface area. Our findings specify the anterior cingulate and middle frontal regions as key regions within the neurotrophin hypothesis of depression.

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Figure Legends

Figure 1.

A significant main effect of BDNF Val66Met polymorphism which survived correction for multiple comparisons was found in the left caudal middle frontal region (Brodmann's area 6). Met allele carriers showed the greatest reduction in cortical thickness in both the MDD and healthy subject groups. Units are mm. Boxplots indicate interquartile range, median, and range. For Met carriers, the boxes are colored in green with diagonal lines and Val homozygotes are colored in blue with horizontal lines.

Figure 2.

The significant interaction effect in the right caudal anterior cingulate is presented. Met carriers with depression showed the greatest reduction in surface area as compared to MDD and healthy Val homozygotes as well as healthy Met carriers. Units are mm^2. Boxplots indicate interquartile range, median, and range. For Met carriers, the boxes are colored in green with diagonal lines and Val homozygotes are colored in blue with horizontal lines.

Table 1. Demographic Features

	MDD **(n = 79)**	**Healthy Controls** **(n = 74)**	**Test statistic**
Age (years)	49.09 (8.96)	50.92 (7.82)	F=1.803, p=0.181
Gender	27 M: 52 F	34 M: 40 F	χ^2= 2.207, p=0.137
Verbal IQ	117.44 (11.59)	119.04 (8.74)	F=0.917, p=0.340
Handedness	69 R: 8 L	63 R: 10 L	χ^2= 0.390, p=0.823
BDNF Met carriers Val/Val	 33 46	 27 47	χ^2= 0.448, p=0.503

Mean values are presented with standard deviation in parenthesis. Abbreviations: MDD, Major Depressive Disorder; M, male; F, female; R, right handed; L, left handed. One subject in each group was ambidextrous, and data were missing from one subject in the MDD group.

Table 2. Clinical Features of MDD Participants

	MDD	
	Met-carrier (n = 33)	**Val/Val (n = 46)**
BDI	15.64 (11.58)	15.61 (11.10)
STAI	39.2 (11.07)	38.58 (10.15)
Age of onset (years)	20.28 (9.54)	20.30 (9.67)
Number of previous episodes	4.41 (3.16)	4.18 (3.24)
History of suicide attempts	14 (42.4%)	19 (41.3%)
History of ECT	2 (6.1%)	4 (8.7%)
History of hospital admissions	9 (27.3%)	13 (28.3%)
Current antidepressant medication	26 (78.8%)	32 (69.6%)

Mean values and parentheses present the standard deviation or percentages as indicated. Mean number of MDD subjects are presented for number of previous episode, clinical history, and current medications. Abbreviations: MDD, Major Depressive Disorder; BDI, Beck Depression Inventory; STAI, State Trait Anxiety Inventory. There were no significant differences between the MDD Met-carriers and Val/Val homozygotes in any of the measures.

Figure 1.

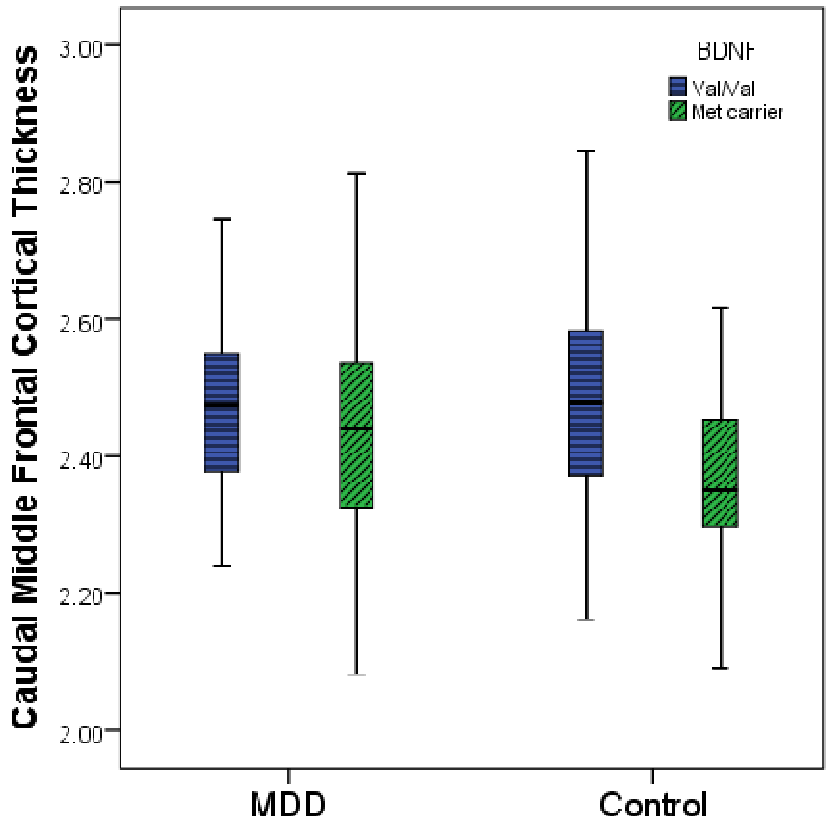
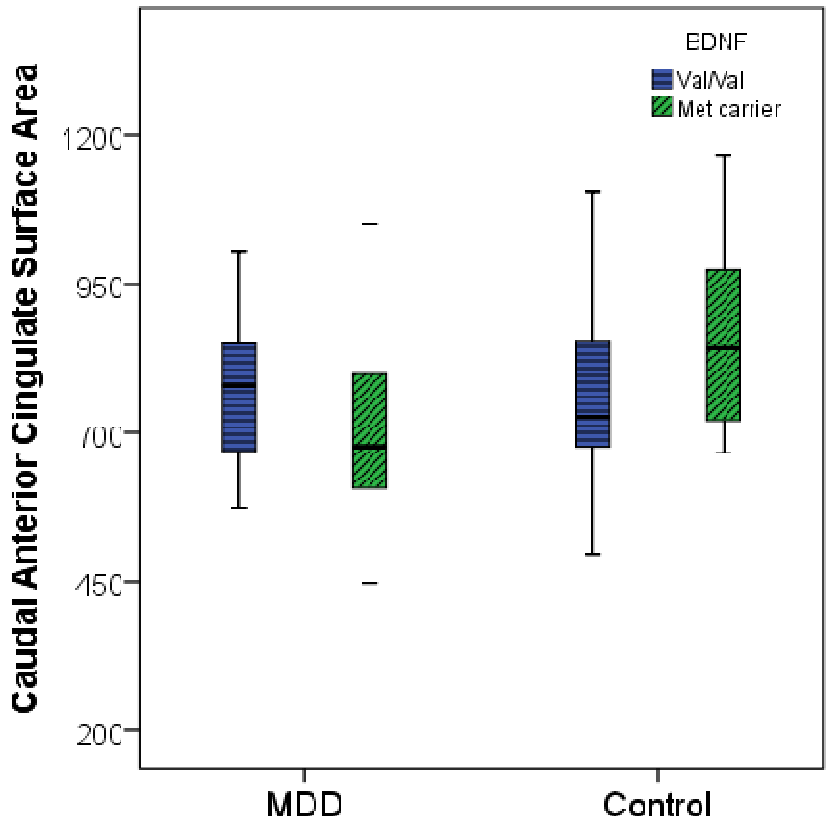
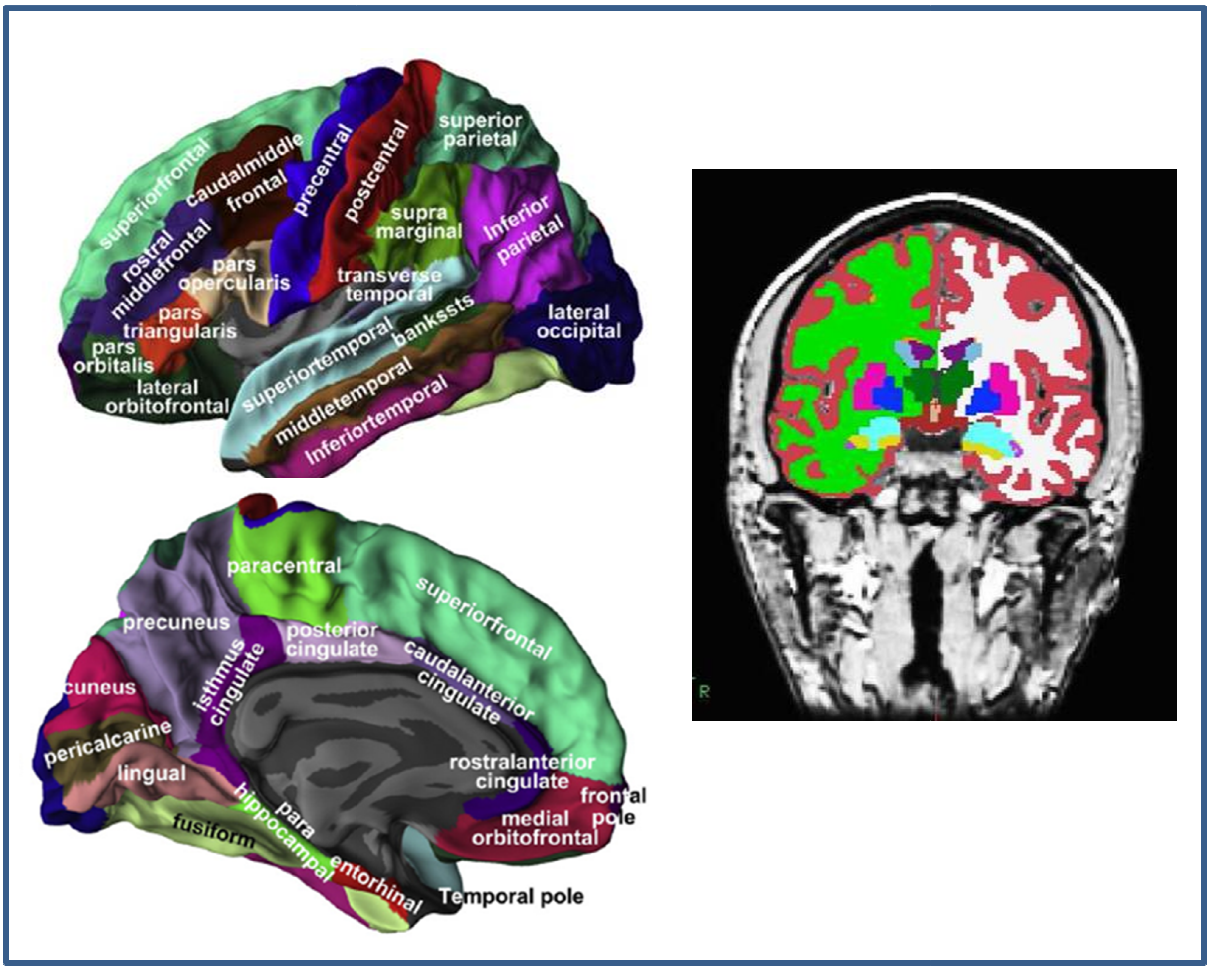

Caudal Middle Frontal Cortical Thickness
3.00
2.80
2.60
2.40
2.20
2.00
BDNF
Val/Val
Met carrier
MDD
Control

Figure 2.


EDNF
Val/Val
Met carrier
Caudal Anterior Cingulate Surface Area
1200
950
700
450
200
MDD
Control

Supplementary Figure DS1.

Depiction of the parcellation of cerebral regions onto lateral and medial hemispheres and coronal cross section indicating location of subcortical regions and the grey matter-white matter boundary using Freesurfer version 5.1.0 (http://surfer.nmr.mgh.harvard.edu/) (Supplementary references 1-3).



Supplementary References:

1. Fischl B, Sereno MI, Tootell RB, Dale AM. High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum Brain Mapp* 1999; **8**:272–284.

2. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002; **33**:341–355

3. Desikan RS, Se´gonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 2006; **31**:968–980