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Age-modulated association between prefrontal NAA and the *BDNF* gene

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Abstract

Brain-derived neurotrophic factor (BDNF) has been implicated in the pathophysiology of psychiatric and neurological disorders and in the mechanisms of antidepressant pharmacotherapy. Psychiatric and neurological conditions have also been associated with reduced brain levels of *N*-acetyl-aspartate (NAA), which has been used as a putative marker of neural integrity. However, few studies have explored the relationship between *BDNF* polymorphisms and NAA levels directly. Here, we present data from a single-voxel proton magnetic resonance spectroscopy study of 64 individuals and explore the relationship between *BDNF* polymorphisms and prefrontal NAA level. Our results indicate an association between a single nucleotide polymorphism (SNP) within *BDNF*, known as rs1519480, and reduced NAA level (p=0.023). NAA levels were further predicted by age and Asian ancestry. There was a significant interaction between rs1519480 and age on NAA level (p=0.031) Specifically, the effect of rs1519480 on NAA level became significant at age ≥ 34.17. NAA level decreased with advancing age for genotype TT (p=0.001) but not for genotype CT (p=0.82) or CC (p=0.34). Additional *in silico* analysis of 142 postmortem

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brain samples revealed an association between the same SNP and reduced *BDNF* mRNA expression in the prefrontal cortex. The rs1519480 SNP influences *BDNF* mRNA expression and has an impact on prefrontal NAA level over time. This genetic mechanism may contribute to interindividual variation in cognitive performance seen during normal aging, as well as contributing to the risk for developing psychiatric and neurological conditions.

Keywords

BDNF; *N*-acetyl-aspartate; proton magnetic resonance spectroscopy; SNP; genetic variation

Introduction

Brain-derived neurotrophic factor (BDNF), a widely distributed central nervous system neurotrophin, is a major neuroprotective peptide that has been implicated in the survival, differentiation, and outgrowth of neurons (Huang and Reichardt, 2001; Schinder and Poo, 2000). Various studies have demonstrated the involvement of BDNF in the pathophysiology of neurological or psychiatric disorders such as Alzheimer's disease (Durany et al., 2000), schizophrenia (Takahashi et al., 2000), and major depressive disorder (Duman et al., 1997). Preclinical studies in animal models of depression have shown that BDNF infusion produced an antidepressant-like effect and that BDNF expression increases in the hippocampus following antidepressant drug administration (Coyle and Duman, 2003; Nestler et al., 2002). Moreover, abnormally low serum levels of BDNF has been reported in major depressive disorder (Karege et al., 2002), while decreased expression of *BDNF* mRNA in the hippocampus has been observed in bipolar disorder (Thompson et al., 2011). Decreased expression of *BDNF* mRNA in the hippocampus has also been observed in Alzheimer's patients (Connor et al., 1997; Narisawa-Saito et al., 1996; Phillips et al., 1991). In addition, animal studies suggest that even normal aging is associated with decreased BDNF signaling in the brain (Mattson et al., 2004). Together, these results suggest that BDNF levels may play an important role in age-related memory deficits

Genetic variations in the *BDNF* gene have been associated with changes in brain structure, both in healthy individuals and in patients with bipolar depression and traumatic brain injury (Karege et al., 2002; Krueger et al., 2011; Liu et al., 2008). Notably, patients with these conditions also show decreased levels of *N*-acetyl-aspartate (NAA), which is a sensitive marker of neural integrity, particularly in the prefrontal cortex (PFC) (Molina et al., 2007; Olvera et al., 2007). Interestingly, proton magnetic resonance spectroscopy studies measuring hippocampal metabolites also indicate that NAA levels are diminished in Alzheimer's disease (Schuff et al., 1997) and that age-related decreases of NAA levels also occur in healthy subjects (Schuff et al., 1999).

Although serum BDNF concentration seems to be associated with cerebral NAA level (Lang et al., 2007), only few studies have explicitly investigated the relationship between NAA and BDNF (Egan et al., 2003; Gallinat et al., 2010). Egan et al. (2003) examined the effects of the BDNF val66met polymorphism on synaptic and neural activity in the hippocampal formation. Their results showed that NAA levels decreased as the number of met alleles

increased, and that heterozygous subjects (val/met) had lower NAA levels compared to homozygous val/val subjects. However, these results were limited to the analysis of the SNP rs6265 in the *BDNF* gene. To evaluate the relationship between the *BDNF* gene and NAA level, we genotyped 7 informative SNPs spanning the *BDNF* gene. We examined the effect of age on NAA level and the possible modulating effect of age on gene expression over time. We measured cerebral levels of NAA by using 3-T proton magnetic resonance spectroscopy (MRS). An additional *in silico* analysis on 142 postmortem brain samples was performed to investigate the relationship between the rs1519480 T allele and reduced *BDNF* mRNA expression in the PFC. Our results suggest that the *BDNF* SNP rs1519480 may play a key role in reducing the levels of NAA in patients with psychiatric disorders.

Methods and Materials

Participants

Participants aged between 18 and 58 years were recruited through newspaper advertisements and posters at the National Institutes of Health, Bethesda campus, according to a protocol approved by the Institutional Review Board of the National Institute of Mental Health. All participants gave informed consent. For evaluation of psychiatric disorders, all participants underwent a Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders-IV-TR (First et al., 2002) and an unstructured clinical interview with a psychiatrist. The clinical evaluation involved physical examination, electrocardiography, and laboratory tests including kidney and liver functioning, hematology profile, thyroid function tests, urinalysis, and toxicology (drug screen). Exclusion criteria were current medical or neurological disorder, pregnancy, smoking, substance abuse, and exposure to psychotropic medications within 4 weeks of scanning. All participants gave informed consent, and the study was approved by the Combined Neuroscience Institutional Review Board at the National Institute of Health (see also Hasler et al., 2007).

The selected patient group consisted of 64 individuals representing 4 diagnostic groups: healthy controls (N = 20, 5 male and 15 female patients; age [mean ± SD], 36.9 ± 13.8 years) individuals with a current episode of major depressive disorder (N = 19, 7 male and 12 female patients; mean age, 31.5 ± 9 years), individuals with major depressive disorder remission (N = 16, 4 male and 12 female patients; mean age, 40.8 ± 11.7 years), and individuals with panic disorder (N = 9, 1 male and 8 female patients; mean age, 33.8 ± 12.8 years).

We used ancestry-informative markers to estimate the degree of individual European, African, Asian, Middle East, Far East Asian and Oceanian genetic heritage. Most participants predominantly had European ancestry (N=41). Some participants predominantly had African ancestry (N=15) and few participants predominantly had ancestry from Middle East (N=3), Asia (N=3) and Far East (N=2). Predominantly means that these participants scored higher than 0.5 on the corresponding ancestry factor. Overall participants, mean ancestry score for Europe was 0.6, for Africa 0.18, for Middle East 0.09, for Asia 0.08, for Far East Asia 0.04 and for Oceania 0.01.

Magnetic Resonance Spectroscopy

We used *in vivo* proton MRS to analyze the levels of NAA in the brains of test subjects. Participants underwent 3-T whole-body magnetic resonance imaging (MRI) with a transmit–receive head coil (General Electric Medical Systems, Milwaukee, WI) that provided a homogeneous radio frequency (RF) field used to obtain spectroscopic measurements from the PFC.

Proton MRS spectra were acquired from a single voxel ($3 \times 3 \times 2$ cm^3) whose posterior edge was 1 mm anterior to the rostrum of the corpus callosum. The voxel was centered on the midline in the horizontal plane and on the bicommissural line in the sagittal plane. This voxel included portions of the perigenual anterior cingulate gyrus and the adjacent frontal polar cortex (i.e., portions of Brodmann areas 24, 32, and 10; Supplementary Figure 1).

NAA levels were measured using the unedited portion of an interleaved position resolved spectroscopy sequence-based J editing method (Hasler et al., 2005; Hasler et al., 2010b) to specifically measure γ-aminobutyric acid (GABA). The total scan time was 25.6 min and the echo time was set to 68 ms; a single scan consisted of 1024 averages at a repetition time of 1.5 s. Measured NAA levels were expressed in mmol/L (mM) and were normalized to the concentration of creatine. To reference the obtained spectra to this quantitative standard, the creatine concentration was set to 7.1 mM (the average creatine concentration in the gray and white matter of the brain). This conventional use of creatine as a concentration reference was well suited for this study because of the extremely low turnover rate of total creatine in the brain (Sanacora et al., 2003; Shen et al., 2002; Wyss and Kaddurah-Daouk, 2000).

Genotyping

Peripheral blood samples were obtained from all 64 participants, and DNA was extracted by using standard procedures. Samples were genotyped using the addiction array that was developed by Hodgkinson and colleagues to identify a panel of gene markers capable of extracting the full haplotype information of candidate genes for addiction and mood disorders (Hodgkinson et al., 2008). Our array included 9 SNPs spanning the *BDNF* gene, namely, rs1048220, rs11030121, rs12273363, rs1519480, rs6265, rs7124442, rs7934165, rs7940188, and rs908867. We excluded the SNP rs1048220 because it was a homozygous SNP. We also excluded the SNP rs908867 because sufficient data was not obtained for 57 individuals. Allele frequencies for the 7 remaining SNPs, as well as their chromosomal location, are summarized in Table 1. We observed 6 haplotypes, 5 of which accounted for the vast majority of haplotype diversity (99%) (Table 2). The linkage disequilibrium r^2 is plotted in Figure 2.

Using 186 available ancestry informative markers (AIMs), we evaluated all 64 participants for population stratification on the basis of NAA levels. Each AIM was a SNP of known allele frequency in the HapMap reference populations; a 7-factor solution was used to estimate the ancestry by using Structure version 2.0 (Falush et al., 2003), as described previously (Zhou and Wang, 2008).

In Silico Analysis of Postmortem Brain Samples

We obtained the *BDNF* genotype and mRNA expression levels of postmortem brain tissue samples from the frontal cortex of 142 subjects, as previously described (Gibbs et al., 2010). All these subjects were Caucasians from the United States and were neurologically normal at the time of death. To the best of our knowledge, subjects were medication free at time of death. The samples for this analysis were obtained from 3 tissue banks. About 31% of the subjects were women (mean age ± SD, 46.2 ± 24.1 years). Samples were collected after a mean postmortem interval of 14.3 ± 5.5 h. DNA was extracted from the cerebellum and genotyped using HumanHap550 Genotyping BeadChip (version 3; Illumina Inc., San Diego, CA). The mRNA expression levels were assayed using HumanRef-8 Expression BeadChip Kit (version 2; Illumina). The sequence of the probe was CCCTCCACCTCCTGCTCGGGGGGCTTTAATGAGACACCCACCGCTGCTGT (Probe ID 1761912).

Statistics

An additive genetic model was tested by recoding the 3 SNP genotypes as follows: 0 for the homozygous common allele, 1 for the heterozygous alleles, and 2 for the homozygous uncommon allele. Multiple linear regression analysis was used to identify significant predictors of NAA level. The factors SNP, ancestry, age, gender, and diagnosis were used as predictors.

Results

Stepwise linear regression was performed to predict NAA levels by SNPs, ancestry factors, age, gender, and diagnosis. Analysis revealed that age, rs1519480, and Asian ancestry significantly predicted NAA levels (R^2=0.31). No other factors significantly influenced NAA levels (Figure 3). The strongest predictor of measured NAA levels was age, which accounted for 16.5% of the variance (R^2=0.17, p<0.001), followed by rs1519480 (ΔR^2 = 0.08, p=0.012) and Asian ancestry (ΔR^2=0.06, p=0.028). Age was a negative predictor of NAA level (p=0.004), indicating that NAA levels tended to decrease with age. The factors rs1519480 (p=0.025) and Asian ancestry (p=0.028) were positive predictors of NAA levels (Table 3). The effect of genotype on NAA levels is shown in Figure 4.

Stepwise linear regression revealed that age, rs1519480, and Asian ancestry significantly predicted NAA levels. An analysis of covariance was calculated to show possible interactions between the predictor rs1519480 and the covariates of age and Asian ancestry. The analysis revealed that the interaction between rs1519480 and Asian ancestry was not significant (F(2, 55)=0.32, p=0.73). However, there was a significant interaction between rs1519480 and age (F(2, 55)=3.57, p<0.035).

In an attempt to further examine the influence of the interaction on NAA levels, we elucidated the specific pattern of effects of the predictor rs1519480 as a function of the moderator age using the Johnson-Neyman technique (Johnson & Fay, 1950; Johnson & Neyman, 1936; Potthoff, 1964). The Johnson-Neyman technique is an approach to identify regions of a moderator variable (age) for which the influence of the predictor (rs1519480)

on the outcome is statistically significant. We used the SPSS macro MODPROBE by Hayes and Matthes (2009) to perform this analysis. The dependent variable for this analysis was the NAA level, the focal predictor was rs1519480, and the moderator variable was age. Asian ancestry was set as an additional predictor. The overall fit of the model was highly significant (R^2=0.36, p<0.001). Including the interaction between the predictor rs1519480 and moderator age increased the value of R^2 significantly (ΔR^2=0.05, p=0.031). The moderator variable age was a negative predictor of NAA level (p=0.001), whereas rs1519480 (p=0.023) and Asian ancestry (p<0.021) were positive predictors of NAA levels (Table 4). The Johnson-Neyman method revealed −1.69 on the age scale as point of transition between the statistically non-significant and the statistically significant effect of rs1519480 on NAA levels. That is, the effect of rs1519480 reached statistical significance at age ≥ 34.17 (mean 35.86 – 1.69) but was not significant for age values < 34.17. Further analysis for the 3 genotypes revealed a significant decrease of NAA levels for TT (p=0.001) but not for CT (p=0.82) and not for CC (p=0.33) with age (Figure 5). The effects were corrected for the additional factor Asian ancestry (Table 5).

In sum, the results obtained by probing the interaction and using the Johnson-Neyman approach revealed a highly significant model to predict NAA levels. NAA levels depended on the factors age, rs1519480, and Asian ancestry. Age was negatively related to NAA level, whereas rs1519480 was positively related to NAA level. Thus, the T allele of rs1519480 was associated with a low NAA level. However, the effect of rs1519480 was moderated by the factor age: NAA level significantly decreased for genotype TT with age, but not for genotype CT or CC.

To evaluate the functional significance of rs1519480, we analyzed the genotype and mRNA levels for *BDNF* in 142 postmortem brain samples. The analysis included genome-wide SNP genotyping and transcriptome-wide mRNA expression profiling of the frontal cortex. The obtained genotype and mRNA data are publicly available (NCBI GEO Accession Number: GSE15745; dbGAP Study Accession: phs000249.v1.p1). Linear regression analysis revealed an association between the rs1519480 T allele and decreased expression of BDNF mRNA in the PFC of postmortem subjects (p=0.028, one-tailed *t*-test; Figure 6). The p value was corrected for the effects of age, gender, and site from which the tissue was sampled.

Discussion

BDNF has been implicated in the pathophysiology of mood disorders and in the mechanisms of antidepressant pharmacotherapy. These disorders have also been associated with a reduction in NAA levels in the brain (Molina et al., 2007; Olvera et al., 2010). Nevertheless, few studies have explored the relationship between *BDNF* polymorphisms and cerebral NAA levels. In this study, we report an age-dependent association between rs1519480 (a *BDNF* SNP) and NAA levels in the PFC. Moreover, *in silico* analysis of 142 postmortem brain samples revealed an association between rs1519480 and *BDNF* mRNA expression levels in the PFC, providing further evidence of a functional role for this SNP.

We demonstrated that NAA levels were significantly predicted by rs1519480, age, and Asian ancestry. Our results also indicate that the T allele of rs1519480 was associated with

low NAA levels in the PFC. Since rs1519480 is located in a highly conserved genomic region (Liu et al., 2008), Liu et al hypothesized that this SNP has a functional role. Our postmortem study revealed an association between rs1519480 and *BDNF* mRNA expression in the PFC. *BDNF* mRNA expression was lower in individuals with the T allele than in those with the C allele. The T allele was also associated with low NAA levels in the PFC. This finding provide support for the hypothesis that low levels of *BDNF* mRNA expression results in low NAA levels, although future studies would be necessary to establish a mechanistic responsible for this finding. Our results were consistent with those of a previous study (Lang et al., 2007), which reported that serum levels of BDNF were positively correlated with the NAA levels in the anterior cingulate cortex.

Our results indicate that the homozygous T allele of rs1519480 was associated with low NAA levels in the PFC and that this effect was moderated by age. Specifically, the association between rs1519480 and NAA level increased with advancing age and became significant for age values ≥ 34.17. Although age was an overall negative predictor of NAA levels, analyzing different genotypes revealed that this relationship was strongest in homozygous T individuals. Previous studies have shown an inverse correlation between NAA levels in several brain regions (particularly in the PFC) and age (Brooks et al., 2001; Gruber et al., 2008; Haga et al., 2009). NAA is a biomarker of neural function and integrity (Bjartmar et al., 2002). MRS studies on age-related changes in brain metabolites have found that NAA was significantly related to executive and cognitive functions (Charlton et al., 2007; Haga et al., 2009; Jung et al., 1999). A recent study by Rostami et al. (2011) successfully showed that the SNPs rs1519480 and rs7124442 were significantly associated with recovery of general cognitive intelligence following traumatic brain injury, indicating an important role of BDNF in neural plasticity. Taken together, these results suggest that the low expression of BDNF mRNA associated with the rs1519480 T allele could, over time, cause a reduction in NAA levels in the PFC. This change could conceivably contribute to a decrease in cognitive performance and to other age-related neuropsychological problems. Liu et al. detected a significant association between rs1519480 and the risk for developing bipolar disorder (Liu et al., 2008). Other studies using MRS have consistently shown a reduction in prefrontal NAA levels in individuals with bipolar disorder (Molina et al., 2007; Olvera et al., 2007; Winsberg et al., 2000). These findings, combined with our results, provide strong evidence that this particular SNP might play a role in the reduction of NAA levels in patients with bipolar disorders and possibly other major psychiatric conditions.

NAA may be a particularly useful marker of impaired neural integrity because reduction in NAA levels may precede more severe forms of neurotrophic changes, such as brain volume loss. Depression and bipolar disorder have been associated with volume loss of the hippocampus and other brain regions (Savitz and Drevets, 2009; Sheline et al., 2003). The neurotrophic hypothesis of mood disorders posits that decreased BDNF levels contribute to volume loss during the progression of these psychiatric conditions (Hasler, 2010). Preclinical studies have shown correlations between decreases in BDNF levels and stress-induced depressive-like behaviors, as well as enhanced expression of BDNF following antidepressant treatment (Martinowich et al., 2007). MRI morphometric measurements have shown an association between volume reduction in the anterior cingulate cortex and familial

affective disorders (Drevets et al., 1997; Hirayasu et al., 1999). This volume reduction begins early in the illness (state-independence), but appears to become more pronounced after the onset of illness, as suggested by preliminary evidence provided by studies in twins discordant for depression (Botteron et al., 1999). In clinical samples, loss in brain volume has been associated with increased risk of recurrence (Frodl et al., 2008). On the basis of these findings, it is hypothesized that genetic variation within *BDNF* is associated with reduced BDNF expression that contributes to the following: (1) the development of major psychiatric disorders because of increased stress sensitivity, and (2) increased risk of recurrence. The effects of such genetic variation may become evident only after many years or even decades.

The major limitations of this study include the relatively small sample size and the heterogeneity in the participants' psychiatric diagnoses. These factors reduced the statistical power of the analyses and may have led to type II errors, for example, the non-replication of the previously observed association between the functional *BDNF* SNP rs6265 and NAA levels (Gallinat et al., 2010). In addition, all subjects in the study sample were younger than 60 years. For this reason, we were unable to discern whether the non-existing influence of age on NAA levels in CT and CC individuals (for rs1519480) persists beyond 60 years of age. Finally, an important limitation of the mRNA measurements is the possible mismatch between mRNA and protein expression.

In summary, our results suggest that rs1519480 has an impact on the NAA level in the PFC over time due to its association with *BDNF* expression. This genetic mechanism may explain the interindividual variation observed in neuropsychological performance during normal aging, and may also contribute to the development of psychiatric conditions such as bipolar disorder and neurodegenerative neurological conditions.

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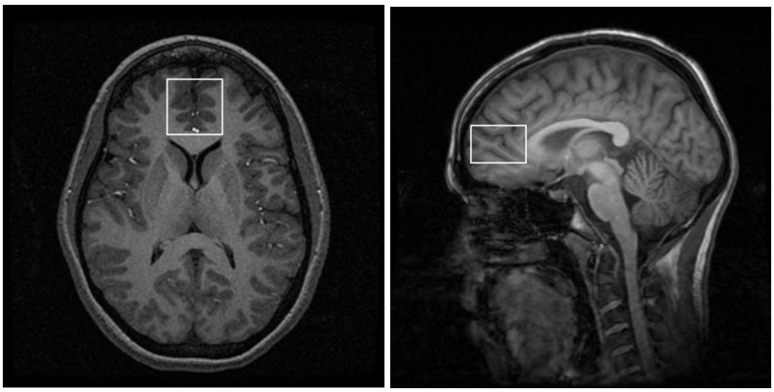

Figure 1.
Voxel placement in the ventromedial prefrontal cortex. This figure was reproduced from one of our earlier papers (Hasler et al., 2010a).

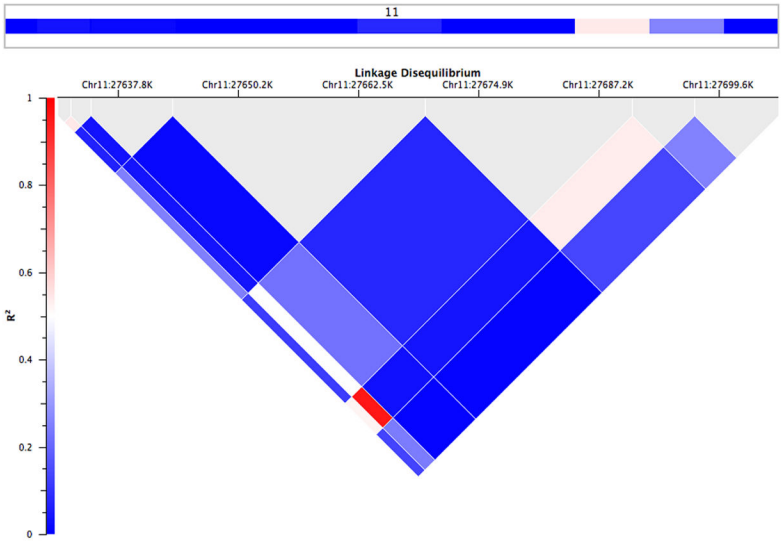

Figure 2.

Graphical overview of linkage disequilibrium r^2 among the 7 SNPs. The plot summarizes all pairs of SNPs. Strong disequilibrium is colored in red and lower levels of disequilibrium are colored in blue.

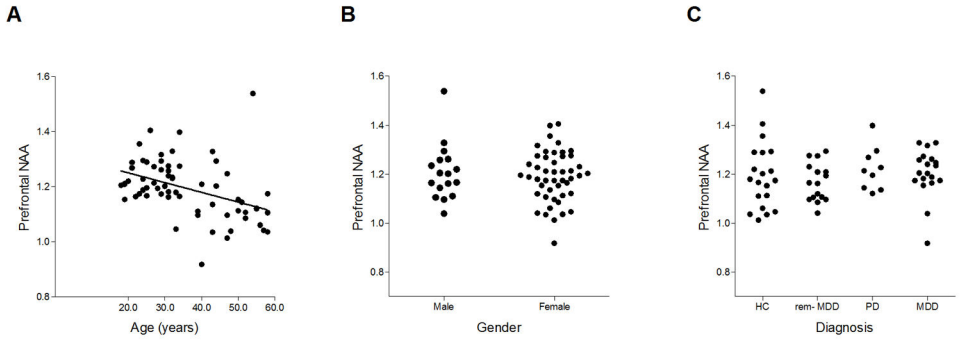

Figure 3.

(*a*) A significant effect of age on *N*-acetyl-aspartate (NAA) levels. **(*b*)** No effect of gender on the NAA levels in the prefrontal cortex. **(*c*)** No effect of psychiatric diagnosis on NAA levels in the prefrontal cortex.

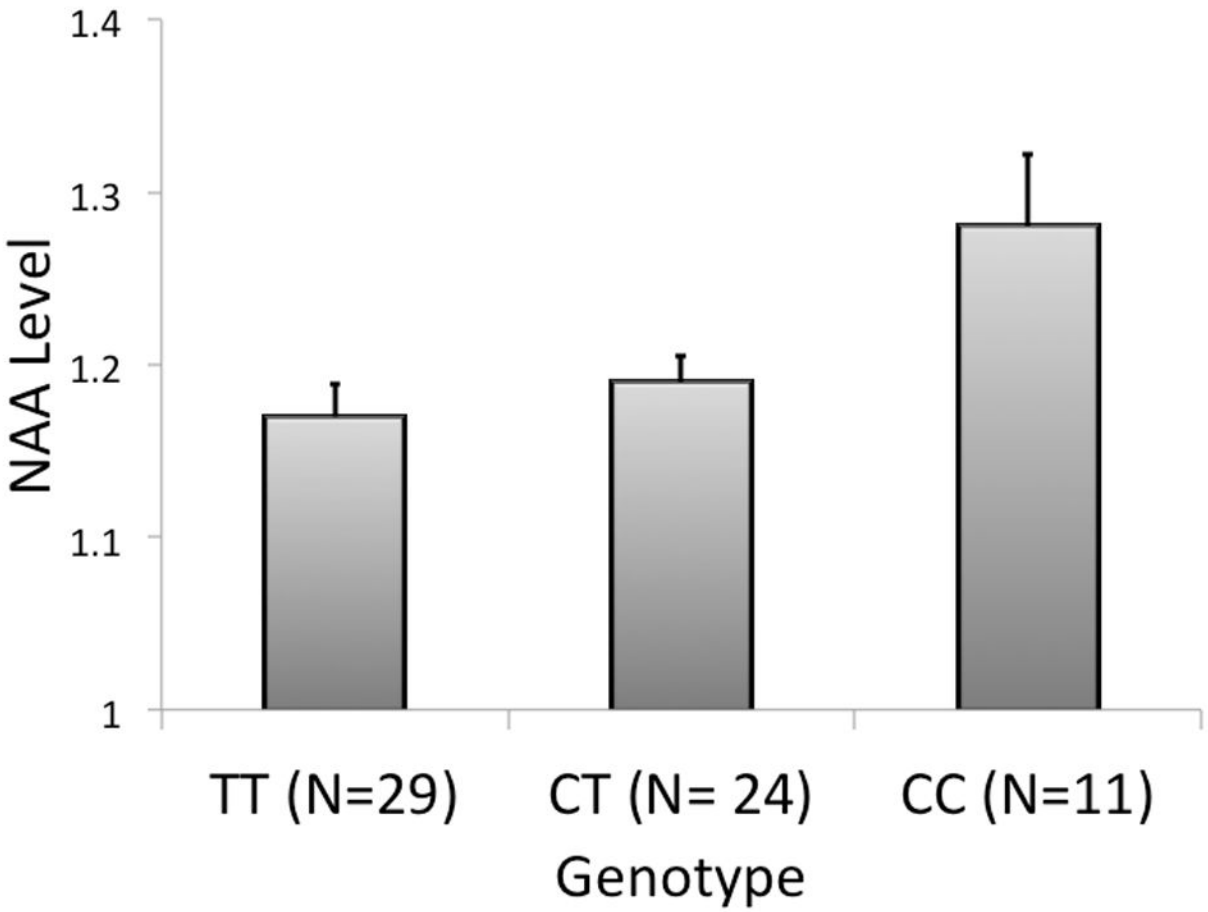

Figure 4.

Association of the single nucleotide polymorphism rs1519480 in the brain-derived neurotrophic factor (*BDNF*) with *N*-acetyl-aspartate (NAA) levels: Linear regression analysis revealed a strong positive association between the number of minor alleles and NAA levels. The effect was corrected for the covariates age and Asian ancestry.

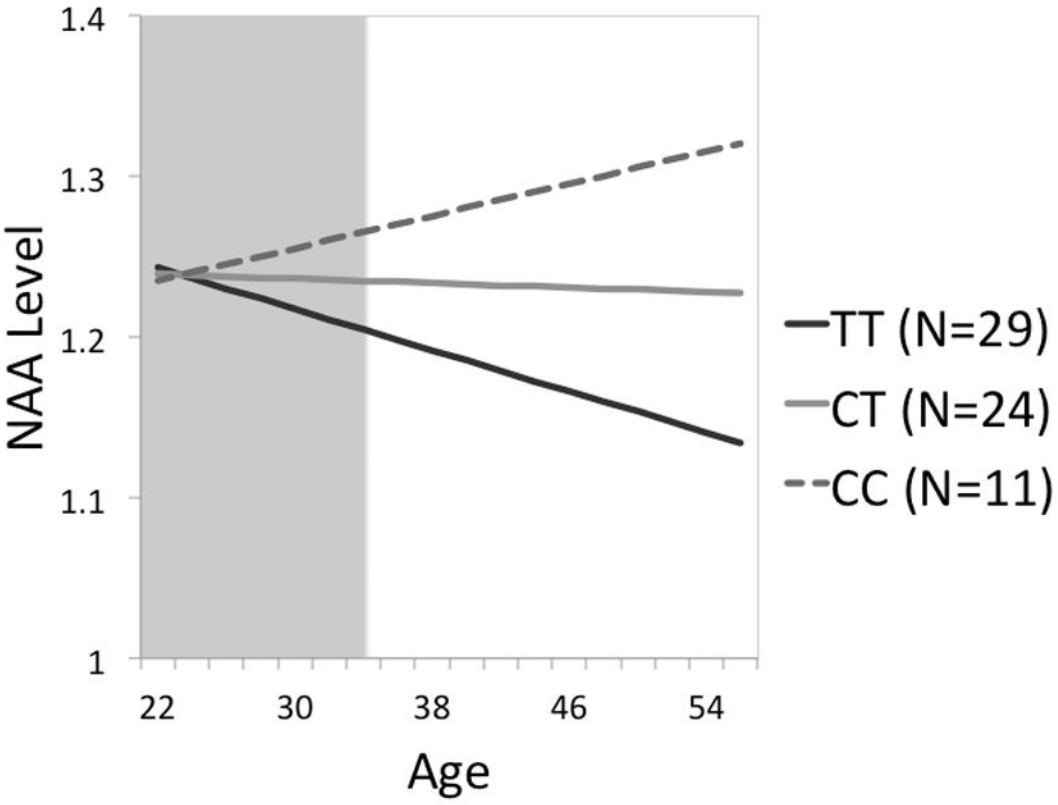

Figure 5.

The relationship between *N*-acetyl-aspartate (NAA) levels in the prefrontal cortex and age and the 3 brain-derived neurotrophic factor (*BDNF*) SNP rs1519480 genotypes. NAA levels significantly decreased for genotype TT with age, but not for genotype CT and CC. Highlighted areas indicate regions where the difference between the three genotypes reaches significance.

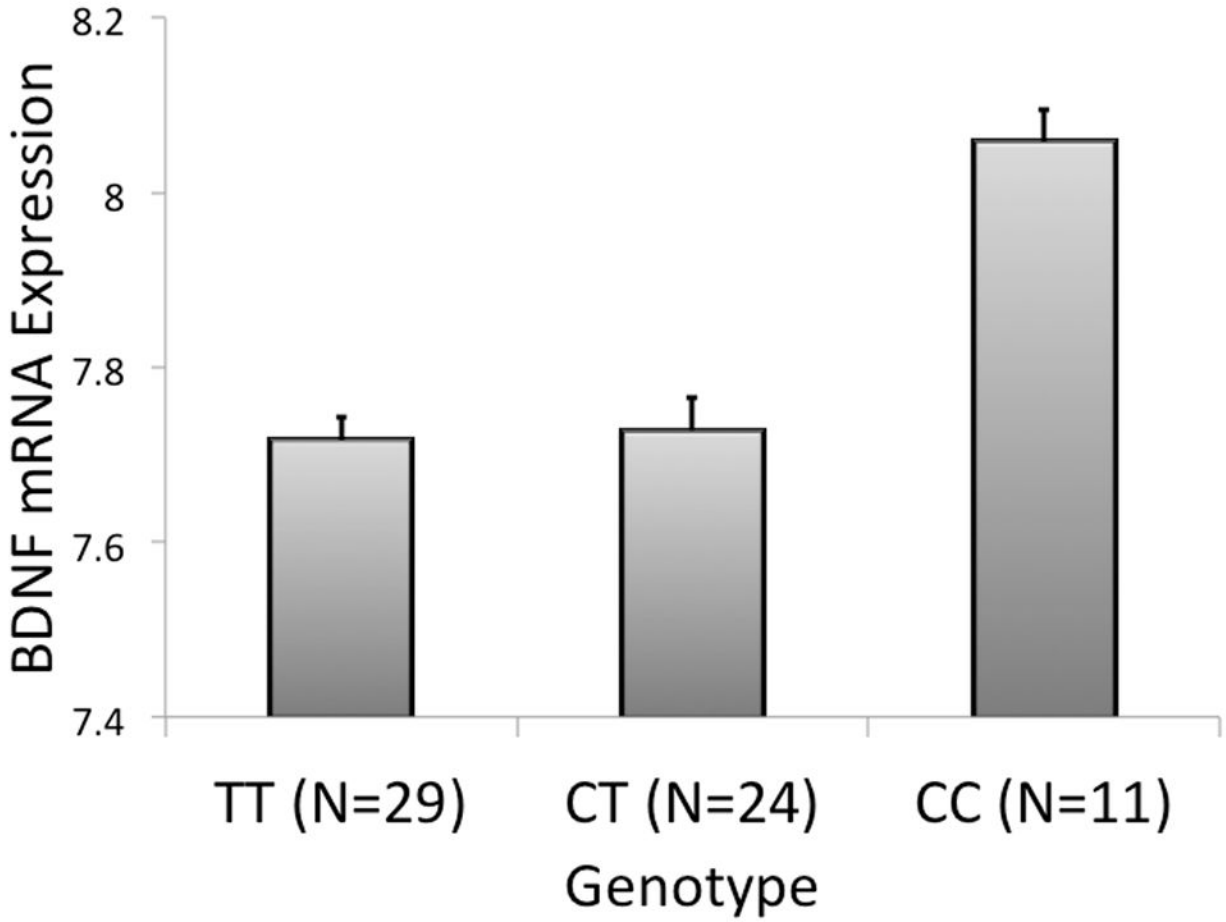

Figure 6.

Postmortem assessment of the association between the rs1519480 T allele and the expression of brain-derived neurotrophic factor (*BDNF*) mRNA in the frontal cortex tissue. Linear regression analysis revealed a decreased expression of *BDNF* mRNA with increasing numbers of rs1519480 T alleles.

Table 1

Genotype and allele frequencies in the present sample (N = 64): The SNPs are presented in the order of their chromosomal location.

SNP	Chromosome	Gene	Location	TT	CT	CC	p(T)	p(C)
rs1519480	11	BDNF	27632288	0.45	0.38	0.17	0.64	0.36
rs7124442	11	BDNF	27633617	0.58	0.38	0.05	0.77	0.23
rs6265	11	BDNF	27636492	0.02	0.19	0.80	0.11	0.89
rs7940188	11	BDNF	27650315	0.80	0.16	0.05	0.88	0.13
rs7934165	11	BDNF	27688559	0.42	0.45	0.13	0.65	0.35
rs11030121	11	BDNF	27692783	0.05	0.36	0.59	0.23	0.77
rs12273363	11	BDNF	27701435	0.86	0.14	0.00	0.93	0.07

Table 2

Haplotype sequences and their estimated probabilities. Haplotype association test revealed no effect on NAA level.

	Haplotype Frequency	Estimated probability	$p_{Bonferroni}$
1	TTCTTCT	0.52	0.28
2	CCCTCTT	0.16	0.55
3	CTCCTCT	0.13	0.11
4	TTTTCCT	0.11	1
5	CCCTCTC	0.07	1
6	TTCTCCT	0.02	1

Note: Frequency threshold was 0.01

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Table 3

Stepwise multiple linear regression to examine the predictors of NAA level. NAA level was set as a dependent variable whereas SNPs, ancestry factors, age, gender, and diagnosis were set as predictors. The factors age, rs1519480, and Asia significantly predicted NAA levels.

Model	B	SE B	t	p
1 Constant	1.32	0.04	34.48	
age	−0.004	0.001	−3.49	0.001
2 Constant	1.28	0.04	31.25	
age	−0.003	0.001	−3.13	0.003
rs1519480	0.04	0.02	2.58	0.012
3 Constant	1.26	0.04	31.46	
age	−0.003	0.001	−3.01	0.004
rs1519480	0.04	0.02	2.31	0.025
Asian ancestry	0.15	0.07	2.25	0.028

Note: $R^2 = 0.17$ for Step 1, $\Delta R^2 = 0.08$ for step 2 ($p < 0.05$) and $\Delta R^2 = 0.06$ for Step 3 ($p < 0.05$).

*$p < 0.001$

Table 4

Probing the interaction of rs1519480 and age in the linear regression model: NAA was the dependent variable, rs1519480 was a focal predictor, age was a moderator, and the factor Asian ancestry was set as an additional predictor. Linear regression analysis revealed a significant decrease of NAA levels with age and a significant increase with increasing number of T alleles in rs1519480. The factor Asian ancestry was also a positive predictor.

	B	B SE	t	p
Constant	1.19	0.01	97.82	
Asian ancestry	0.15	0.06	2.38	0.021
Age	−0.003	0.001	−3.41	0.001
rs1519480	0.04	0.02	2.34	0.023
Interact	0.003	0.001	2.22	0.031

Note: $R^2 = 0.36$, $\Delta R^2 = 0.05$ ($p < 0.05$) for the interaction.

* $p < 0.001$

Table 5

The effect of the moderator variable age on NAA level was analyzed for the 3 genotype groups. NAA level significantly decreased with age in the TT group. There was no effect for age in genotype CT or CC. The effect was corrected for the additional predictor Asia.

Genotype	B	SE B	t	p
TT	−0.003	0.001	−3.41	0.001
CT	−0.001	0.002	−0.23	0.82
CC	0.003	0.003	0.97	0.34