Dysregulation of *BDNF* in prefrontal cortex of Alzheimer's disease

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Running title: *BDNF* regulation in ageing and Alzheimer's disease

Abstract

Background: Brain-derived neurotrophic factor (BDNF) is essential for neurogenesis and has been implicated in Alzheimer's disease (AD). However, few studies have investigated together the epigenetic transcriptional and translational regulation of this peptide in the brain in relation to AD.

Objective: To investigate mechanisms underlying for how *BDNF* is possibly dysregulated in the brain in relation to ageing and AD neuropathology.

Methods: Prefrontal cortex tissues were acquired from the Manchester Brain Bank (N = 67). *BDNF exon I*, and exon *IV* containing transcripts and total long 3' transcript gene expression were determined by quantitative PCR. Bisulfite pyrosequencing was used to quantify DNA methylation within promoters *I* and *IV*. Protein concentrations were quantified via an enzyme linked immunosorbent assay (ELISA). Donors were previously genotyped for the rs6265 (Val/Met) polymorphism.

Results: *BDNF exon IV* and total long 3' isoform gene expression levels negatively associated with donor's age at death (*IV*: r = -0.291, P = 0.020; *total*: r = -0.354, P = 0.004). Expression of *BDNF exon IV* containing isoform was significantly higher in Met-carriers of the rs6265 variant, compared to Val-homozygotes, when accounting for donor ages (F = 6.455, P = 0.014). *BDNF* total long 3' transcript expression was significantly lower in those with early AD neuropathology, compared to those without any neuropathology (P = 0.021). There were no associations between *BDNF* promoter I and IV methylation or protein levels with ages, rs6265 genotype or AD neuropathology status.

Conclusion: Prefrontal cortex *BDNF* gene expression is associated with ageing, rs6265 carrier status and AD neuropathology in a variant-specific manner. This dysregulation seems to be independent of DNA methylation influences at the *I* and *IV* promoters.

Key words: BDNF, DNA methylation, Alzheimer's disease, prefrontal cortex

Introduction

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that promotes neurogenesis, synaptic plasticity and long-term potentiation (LTP) in the CNS [1,2]. BDNF has been implicated in the 'age-by-disease hypothesis', in which *BDNF* expression is reduced in the ageing brain and a reduction in *BDNF* expression has been associated with multiple neurological disorders [3].

Reductions in BDNF have been widely investigated as a mediator of age-associated decline in synaptic density and cognitive function [4], with a significant association between BDNF and cognitive ageing being observed [5–7]. However, the underlying mechanisms behind age-associated BDNF declines are not completely understood.

The human BDNF gene has a complex structure involving 9 promoters and 11 exons of which only the exon IX at the 5' end contains the coding sequence [8]. The untranslated 3' exons, through alternative splicing, lead to different transcripts that still contain common coding region at the 3' end. Therefore, through the use of alternative promoters and splicing mechanisms, various different BDNF transcripts with alternative 5' untranslated regions (UTRs) can be generated that all code for the same BDNF protein. Finally, two alternative polyadenylated transcription stop sites in exon IX can lead to transcripts with either short or long 3' UTRs. A study has shown that while the short 3' UTR BDNF mRNA variant is restricted to the cell body in hippocampal neurons, the long 3' UTR mRNAs are also observed in dendrites [9]. Together, in the human brain, all exons are expressed, but to different degrees in different brain structures (for review see [10]). It is thought that these different promoters allow *BDNF* to respond to a greater variety of stimuli that further result in the generation of different transcripts that are stable in multiple intracellular environments [8].
As *BDNF* promoters mediate differential *BDNF* isoform expression in various parts of the brain, it is thought that changes to their activity could affect cellular and behavioural phenotypes [11]. Epigenetic mechanisms, predominantly promoter methylation that generally serves to silence gene expression, have been shown to regulate *BDNF* expression [12]. BDNF promoter IV is one of the most widely investigated promoters in the contest of DNA methylation changes associated with alterations in BDNF expression: For example McKinney et al. [3] found in the orbital frontal cortex that DNA methyation at promoters I, II, and IV were increased in older people and negatively correlated with BDNF expression. Keller et al. [13], for example found significant increases in DNA methylation at BDNF promoter IV in the Wernicke area from suicide subjects, when compared to controls, that correlated with lower mRNA levels for BDNF exon IV containing transcript. In the periphery, increased methylation at *BDNF* promoters I and IV have been found in blood DNA from

patients with Mild Cognitive Impairment (MCI) compared to controls and increased methylation at CpGs in promoter IV predicted conversion from MCI to AD [14]. Further studies in blood DNA have shown increased *BDNF* methylation at promoter I in AD cases compared to controls [15,16] and increased peripheral *BDNF* promoter I and IV in amnestic MCI compared to controls that further predicted the conversion from MCI to AD [14]. Interestingly, the conversion from amnestic MCI to AD depended upon an interaction of methylation with a non-synonymous single nucleotide polymorphism (SNP) in the *BDNF* gene, rs6265 [17]. However there are some conflicting reports not finding increased *BDNF* methylation in peripheral DNA in AD compared to controls [15,18]. Within the CNS, Rao and colleagues [19], studying groups of 10 AD and 10 control prefrontal cortex samples, found significant decrease in total *BDNF* mRNA in the AD brain compared to control brains together with increased promoter DNA methylation. A reduction in prefrontal cortex BDNF expression in AD has also been found in a study by Buchman et al. [19] on 535 older participants. Li et al. [29] found a reduction in temporal cortex and frontal cortex in AD, but specifically in females. They further found that rs6265 associated with transcriptional regulation only in the female brains. Garzon et al. investigating individual BDNF variants found transcript specific decreases of BDNF in AD brains. Few studies have investigated together the epigenetic, genetic, transcriptional and translational regulation of this peptide in the brain in relation to AD.

The aim of this study was to investigate mechanisms underlying the dysregulation of *BDNF* within the AD brains studying human prefrontal cortex tissue for BDNF protein levels, promoter-specific expression, promoter DNA methylation specifically at promoters for exons I and IV and the rs6265 genotype.

Methods

Study population

Fresh, frozen tissue was taken from superior frontal gyrus (Brodmann area 8). Samples were acquired from donors through the Manchester Brain Bank. Ethical approval was granted from the Manchester Brain Bank Committee. Donors were participants of a large prospective cognitive ageing cohort known as The University of Manchester Age and Cognitive Performance Research Cohort [20,21] and included all those with brain material and available neuropathological data.

Stratification into Alzheimer's disease neuropathology groups were based on the National Institute on Aging-Alzheimer's Association guidelines [22]. Briefly, the amyloid beta (Aβ) plaque score (Thal), neurofibrillary tangle stage (Braak) and neuritic plaque score (CERAD) were used to create an "ABC"

score. Four groups were determined: Not, Low, Intermediate and High AD neuropathologic change. Those with high levels of Aβ and neuritic plaques with low neurofibrillary tangle score were excluded ("ABC" score: A2-3, B0-1, C0-3), due to potential contributions by other co-morbidities.

Gene expression analysis

Brain tissue (~30 mg) was extracted for RNA using TRIsure™ (Bioline, UK), quantified using the Nanodrop 2000c (Thermo Scientific, Wilmington, USA) and qualifies using the Agilent Bioanalyser. RIN values are given in **Supplementary Table 1**. The Tetro cDNA synthesis kit (Bioline, UK) was used to reverse transcribe total RNA (2 μg), according to the manufacturer's protocol using random hexamers. Relative gene expression was analysed using qPCR with SensiFAST™ SYBR® Lo-ROX kit (Bioline), in accordance with the manufacturer's protocol using primers for *BDNF exon I* containing transcript (F: CAGCATCTGTTGGGGAGACGA; R: GCCACCTTGTCCTCGGATGT), *BDNF exon IV* containing transcript (F: TGGGAGTTTTGGGGCCGAAG; R: TGGTCATCACTCTTCTCACCTGG), *BDNF* total long 3', (F: GGACCCTTCAGAGGTGGCTC; R: GTCGGCTTGAGTGTGGTCCT), *ACTB* (F: CATCCTCACCCTGAAGTACC; R: ATAGCAACGTACATGGCTGG) and *GAPDH* (F: CCGCATCTTCTTTTGCGTCG; R: TGGAATTTGCCATGGGTGGA). qPCR was performed on a Stratagene Mx3000P qPCR system (Agilent) in duplicate. Relative gene expression, accounting for primer efficiencies and normalised to *GAPDH* and *ACTB*, were determined using the geometric averaging method described by Vandesompele and colleagues [23]. Those samples with gene expression levels were not detected (Ct ≥40) were excluded from analyses.

Genotyping

DNA samples were extracted from peripheral blood samples, as described previously [24]. Genotyping for the G196A/**Val**^{66}**Met** (rs 6265) was performed using the Kompetitive Allele Specific PCR (KASP) assay (LGC Ltd) in reaction volumes of 10ul together with 5ng of DNA that was run on a Stratagene MX3000P qPCR machine (Agilent). Fluorescence values were read by the MXPro software to enable genotype calling.

Protein quantification

Brain tissue (~100 mg) were lysed using RIPA buffer (Sigma) supplemented with 1x protease inhibitor cocktail and 0.1 M PMSF, as described previously [25]. Quantification of BDNF protein was performed using the Human/Mouse BDNF DuoSet ELISA (R&D Systems). Protein levels were normalised to total protein levels in the assay (pg/mg of total protein).

DNA methylation analysis

Genomic DNA was extracted using the Isolate II Genomic DNA kit (Bioline) and 500 ng bisulfite-converted using the EpiMark Bisulfite Conversion Kit (New England Biolabs). Primers were used to amplify regions of the BDNF promoter I (F: TGAGTGATGATTAAATGGGGATTG; R: BIO-ACTATTAACTCACATTTAAAAAACCATAAC; S: TGGGGATTGGGGGGA) and promoter IV (F: GATTTTGGTAATTCGTGTATTAGAGTGTT; R: BIO-AGATTAAATGGAGTTTTCGTTGAT; S: AATGGAGTTTTCGTTGATGGGGTGCA) using MyTaq HS mix PCR reagents (Bioline). The *BDNF* promoter I and promoter IV amplicons contained 5 and 9 CpG sites, respectively. Amplicons were processed on the Qiagen Q24 Workstation and sequenced on the Qiagen Q24 pyrosequencer. DNA methylation levels across each amplicon were averaged. See **Supplemental Figure S1** for locations of the regions analysed.

Statistical analysis

All analyses were performed using IBM SPSS Statistics (v.25). *BDNF* isoform expression, protein and DNA methylation levels were log10 transformed prior to statistical analysis. Correlations between gene expression, DNA methylation and protein levels with donor age were performed using Pearson correlation tests. Correlations between gene expression, DNA methylation and protein levels were performed using Partial correlation tests, with donor age as a covariate. Differences in gene expression, DNA methylation and protein levels between rs6265 variant groups were assessed using independent student t-tests. Further, differences between groups while controlling for donor age were assessed using a one-way ANCOVA. Differences in gene expression, DNA methylation and protein levels between AD neuropathological groups were assessed using one-way ANOVA, as well as a one-way ANCOVA to control for age. Results are presented as mean and standard deviation, unless otherwise stated. Statistical significance was accepted when $P<0.05$.

Results

Clinical and pathological characteristics of the study population can be found in Table 1.

Table 1. Clinicopathological characteristics for the donor samples.

Characteristic	Mean (SD)
Age at death (years)	87.5 (6.1)
Sex (male/female)	21/46
Post-mortem delay (hours)[a]	76.1 (43.7)
Brain weight (g)[b]	1207.4 (137.4)
BDNF rs6265 (N)	
Val/Val	39 (58%)

Val/Met	25 (37%)
Met/Met	3 (5%)
Thal score (N)	
0 (A0)	17 (25%)
1 (A1)	11 (16%)
2 (A2)	6 (9%)
3 (A2)	17 (25%)
4 (A3)	9 (13%)
5 (A3)	7 (10%)
Braak score (N)[c]	
0 (B0)	4 (6%)
I (B1)	11 (16%)
II (B1)	18 (27%)
III (B2)	13 (19%)
IV (B2)	12 (18%)
V (B3)	6 (9%)
VI (B3)	2 (3%)
CERAD score (N)	
None (C0)	18 (27%)
Sparse (C1)	19 (28%)
Moderate (C2)	18 (27%)
Frequent (C3)	12 (18%)

[a] N = 60

[b] N = 43

[c] N = 66

Relationship between *BDNF* gene expression, DNA methylation and protein levels with age

The association between *BDNF* exon I, IV and total long 3' isoform expression with the age at death of donors was investigated (Figure 1). There was a negative association between expression of exon IV containing (r = -0.291, P = 0.020; Figure 1B) and total long 3' (r = -0.354, P = 0.004; Figure 1C) *BDNF* isoforms with age, however, no relationships were evident for *BDNF* exon I containing isoform (r = -0.201, P = 0.149; Figure 1A).

There were no associations between BDNF protein levels (r = -0.143, P = 0.256) or DNA methylation levels (promoter I: r = -0.038, P = 0.761; promoter IV: r = 0.177, P = 0.156) with donor ages.

Correlations between *BDNF* gene expression, DNA methylation and protein levels

The relationships between *BDNF* gene expression, DNA methylation and protein levels can be seen in Table 2. Since donor age significantly correlated with *BDNF* gene expression, correlations were controlled for donor ages throughout.

Briefly, *BDNF exon I* isoform expression positively correlated with *BDNF exon IV* and total long 3' isoform expression. *BDNF exon IV* expression did not correlate with total long 3' variant expression. Promoter I DNA methylation negatively correlated with *BDNF exon I* isoform expression levels, however this correlation was lost (p=0.08) when account for RIN values (**Supplementary Table S2**). However, there were no associations between promoter IV methylation and *BDNF exon IV isoform* expression levels. Protein levels were not associated with either BNDF exon I, exon IV or total long 3'UTR RNA expression or DNA methylation levels.

Table 2. Partial correlation matrix, controlling for donor ages, between *BDNF* gene expression, DNA methylation and protein levels

	Gene expression			DNA methylation		
	BDNF I	*BDNF IV*	*BDNF Total*	Promoter I	Promoter IV	Protein
BDNF I						
BDNF IV	0.586***					
BDNF Total	0.327*	-0.233†				
Promoter I	-0.282*	-0.181	-0.206			
Promoter IV	-0.091	0.015	-0.007	0.383		
Protein	0.251†	0.018	0.054	0.074	0.044	

Results displayed are partial correlation coefficient values. † P < 0.10. * P < 0.05. *** P < 0.001.

Relationship between *BDNF* gene expression, DNA methylation and protein levels with rs6265 variant

The rs6265 variant was in Hardy-Weinberg equilibrium in the study population (X^2 = 0.162, P = 0.687).

To explore the differences in *BDNF* gene expression levels with the rs6265 variant, donors were stratified into Val-homozygotes and Met-allele carriers. There were no differences in *BDNF exon I* (t = -1.592, P = 0.118) and total long 3' (t = 0.122, P = 0.904) variant gene expression levels between the two groups. However, the Met-allele carriers had significantly higher *BDNF exon IV* isoform expression compared to Val-homozygotes (t = -2.640, P = 0.010) (Figure 2). This difference remained

after controlling for donor age at death (F = 6.455, P = 0.014) and age AND RIN values (F = 7.229, P = 0.009).

There were no differences in BDNF protein levels (t = 0.446, P = 0.657) or DNA methylation levels (promoter 1: -0.435, P = 0.665; promoter 4: -0.755, P = 0.453) between rs6265 variants.

Relationship between *BDNF* gene expression, DNA methylation and protein levels with Alzheimer's disease pathology

To investigate the difference in *BDNF* gene expression with AD pathology, donors were stratified based on the NIA-AA "ABC" score, which considers the amyloid plaque, neuritic plaque and neurofibrillary tangle scores. These groups were Not, Low, Intermediate and High AD neuropathological change.

Overall, there were differences in *BDNF* total long 3' isoform gene expression levels between AD pathological groups (F = 3.074, P = 0.035). Specifically, inter-group comparisons revealed a significant downregulation of BDNF total long 3', isoform expression in the Low AD group, compared to the Not AD group (P = 0.021) (Figure 3).

This difference was also apparent when accounting for donor age at death (overall comparison: F = 3.323, P = 0.026; post-hoc comparison: P = 0.021), however when including age AND RIN values, there were no differences (F = 1.909, P = 0.139).

There were no differences in *BDNF exon I* (F = 2.766, P = 0.053) or *IV* (F = 0.405, P = 0.750) isoform expression levels between the AD neuropathological groups. Further, there were no differences in protein (F = 0.953, P = 0.421) or DNA methylation (promoter 1: F = 1.019, P = 0.391; promoter 4: F = 1.009, P = 0.396) levels between groups.

Discussion

In the prefrontal cortex, *BDNF* gene expression was associated with donor age, rs6265 carrier status and early AD neuropathology in a variant-specific manner. These associations were independent of any influences of DNA methylation or protein levels. Thus, we provide further evidence to the complex mechanisms dysregulating central *BDNF* during ageing and neurodegeneration.

The majority of research investigating age associations of BDNF levels in humans has focussed on peripheral measures. Specifically, many reports suggest a gradual reduction in plasma and serum concentrations during ageing [6,26–28]. There is, however, limited knowledge of *BDNF* regulation in human brain tissue across ages. We report significant reductions of *BDNF exon IV* and total long 3', but not exon *I*, containing isoforms between the ages of 72 and 104 years old. This corroborates

findings from that of Oh and colleagues, who also reported reductions in *total* and exon *IV*-containing RNAs in the prefrontal cortex, without any differences in the exon *I*-containing transcript, between ages 16 to 96 years [7]. Because only exon IX contains the coding region, all the different exon-containing RNA transcripts will be translated to a single species of BDNF polypeptide. It is hypothesised that this sophisticated gene serves to fine-tune a dynamic transcriptional regulation in different cell types by different neuronal activities. For example, it has been shown in rodent studies that fear conditioning increased both BDNF exon I and IV containing RNA in hippocampus, but only exon IV in the CA1 region [29,30] while fear memory extinction elevated BDNF exon I and IV in prefrontal cortex [31]. Interestingly, a study on contextual fear conditioning caused a significant increase of BDNF exon I in WT hippocampus while the levels of exon IV remained unchanged [32]. This highlights that the different exons can be differently regulated. Mechanistically, within BDNF exon IV promoter three calcium responsive elements (i.e. CaRE1, 2 and 3) have been identified regulating calcium-mediated BDNF IV transcription, while in promoter I there is one CRE in promoter I that can be differently regulated by different Ca2+-stimulated protein kinases and other Ca2+-stimulated intracellular molecules [33]. The calcium hypothesis of aging [34,35], hypothesises a dysregulation of intracellular Ca2+ homeostasis is a primary factor contributing to aging-related learning and memory impairments in humans and other mammals, that may further relate to AD. Perhaps this may reflect differential regulation of BDNF transcritps. Interestingly a NF-kappaB [36] site and an E-box [37] have been identified in exon IV promoter that again allow differential regulation and may again reflect age-related changes in these regulatory factors in the brain [38](Zhang et al).

We report variant-specific associations with the rs6265 variant. Specifically, Met-carriers had an upregulation of *BDNF transcripts containing exon IV*, but not exon *I* or *total long 3'UTR* transcripts, compared to Val-homozygotes. The effect of the rs6265 polymorphism on *BDNF* gene expression is largely unknown [39]. A previous study involving over 500 prefrontal cortex donor samples revealed no differences of *BDNF* gene expression between rs6265 genotypes [40]. Despite the superior statistical power in this analysis, expression levels of different transcript variants were not reported, rather, only total expression. Given our preliminary insight suggesting the influence of the rs6265 variant may be transcript-specific in *BDNF* expression, it would be interesting to replicate our analysis in this population.

The reduction in *BDNF* expression with AD neuropathology is in agreement with other reports analysing prefrontal [40], frontal [41], parietal [42] and temporal [41] cortical tissues, as well as the hippocampus [41]. Interestingly, our results further suggest the association between expressions and neuropathology magnitude may be stage specific. Specifically, those with early AD

neuropathology having significant *BDNF* downregulation seem to be particularly affected. Increased neuroinflammation is suspected to have a major role in AD progression. The predominant hypothesis suggests levels of neuroinflammation peaks early on, possibly reflecting an initial anti-inflammatory response, followed by a second peak during conversion from MCI to AD, which may indicate a pro-inflammatory shift [43,44]. This complex relationship may be related to the microglial reaction following the deposition and propagation of amyloid and hyperphosphorylated tau pathologies [45]. Numerous studies demonstrate that neuroinflammation in turn affects the expression of BDNF within the brain; therefore, reduction of BDNF expression and function may be a key mechanism underlying the negative impact of pro-inflammatory cytokines on neuroplasticity [46].

There are a number of limitations to this study. Variations in postmortem times and RIN values (Supplementary Table 1) impacted some of the results such as BDNF promoter I methylation and exon I-containing transcript expression, that when we adjusted for, significance was lost. Also, some RNA samples were unable to clearly measured for all transcripts from the total 67 subjects (i.e. exon I, n=53; exon IV, n=64; long 3'UTR, n=66). A further confounding variable is that the prefrontal cortex samples also contain relatively heterogeneous cell populations that were not able to control for. Finally, we only investigated specific promoters and transcripts containing exon I, IV and long 3'UTR, though it would be interesting to investigate further regions of the BDNF gene and more complete coverage of all the different transcripts.

Conclusion

In conclusion, we report prefrontal cortex *BDNF* gene expression is associated with ageing, rs6265 carrier status and AD neuropathology in a variant-specific manner. This dysregulation seems to be independent of DNA methylation influences at the *I* and *IV* promoters. These results add further evidence to the complex regulation of the *BDNF* gene within the cortex.

Conflict of interest / Disclosure statement

The authors have no conflict of interests to report.

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Figure legends

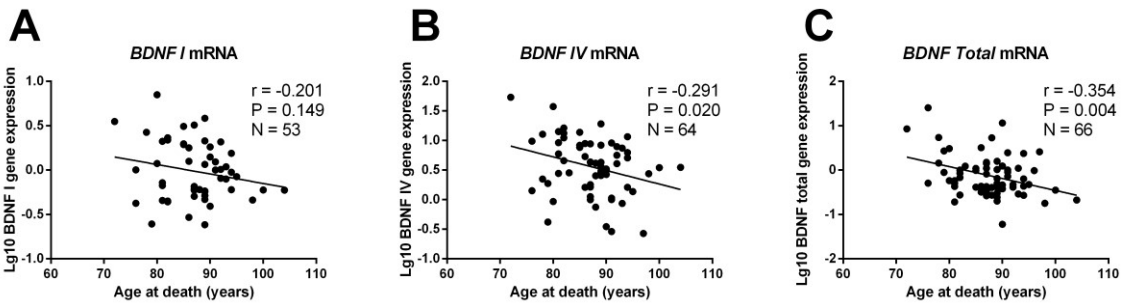


Figure 1. Associations between donor age at death with A: *BDNF I* variant, B: *BDNF IV* variant and C: *BDNF total* mRNA expression.

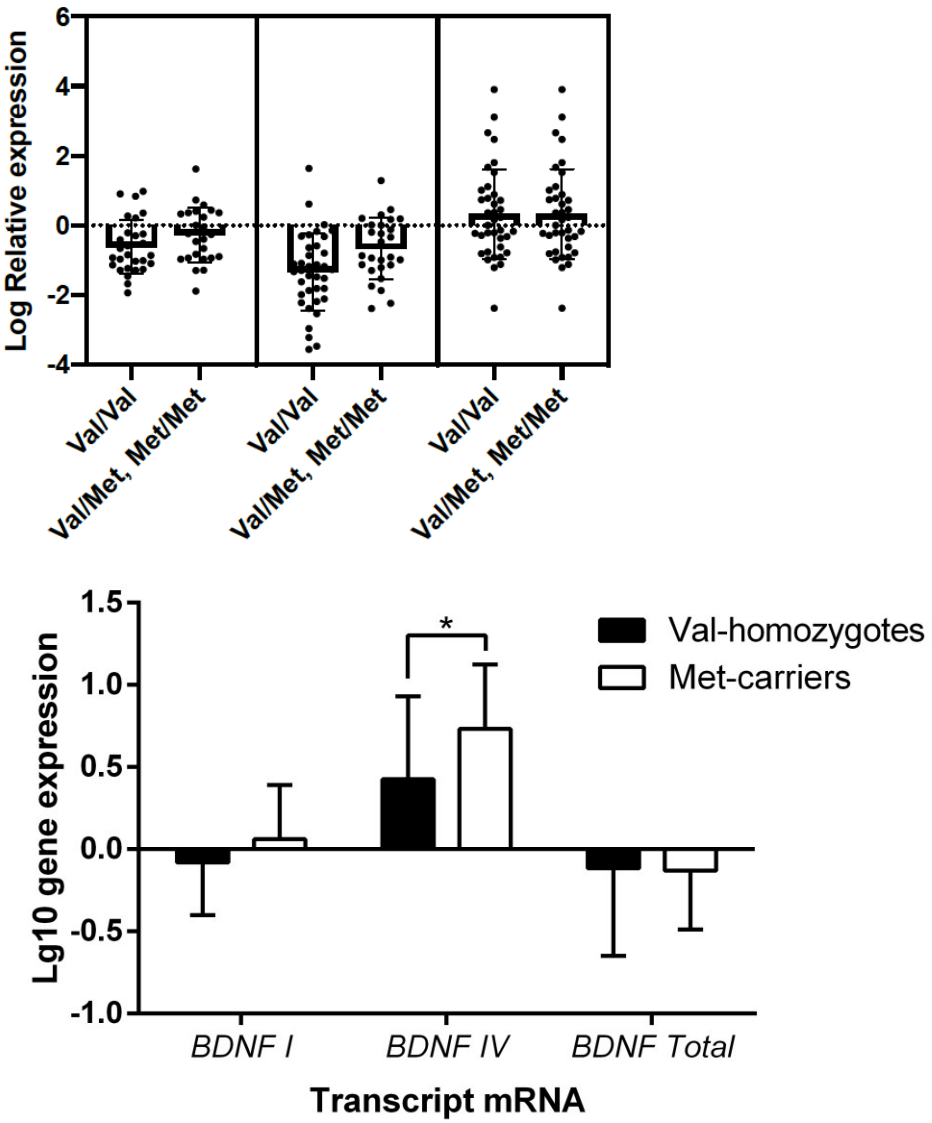


Figure 2. Difference in *BDNF* gene expression between rs6265 Val-homozygotes and Met-carriers. *BDNF I*: Val-homozygotes N = 28, Met-carriers N = 25. *BDNF IV*: Val-homozygotes N = 37, Met-carriers N = 27. *BDNF total*: Val-homozygotes N = 38, Met-carriers N = 28 * $P < 0.05$.

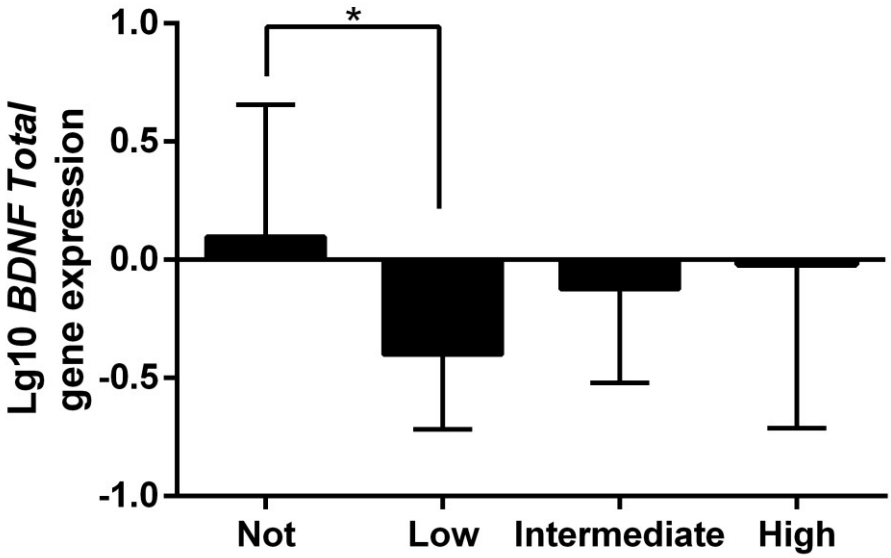
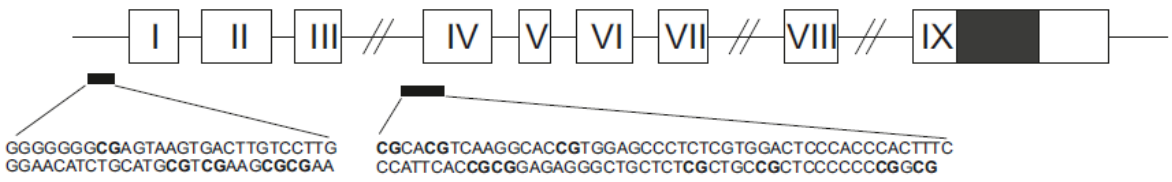


Figure 3. Difference in *BDNF total* variant gene expression between AD neuropathology groups. N = 16 (Not), 14 (Low), 26 (Intermediate), 5 (High). * P < 0.05.



Supplementary Figure 1. Schematic of the Human BDNF gene. Untranslated first exons are numbered I – VIII and the coding sequence for pro-BDNF is shaded black. Sequences of regions of promoters I and IV investigated for DNA methylation [Exon I, chr11:27,722,265-27,722,319; Exon IV, chr11:27,701,578-27,701,672 (Hg38)] are shown with CpG residues in bold.

Supplemental Table 1. Sample details.

Patient_Code	Age_at_death	Gender	Brain_Weight	PMD	Primary_Pathology_Diagnosis_Description	RIN_Value
22708	91	F	1216.00	133.0	Age changes only	3.20
11052	94	F	946.00	111.0	Age changes only	5.30
10540	98	F	1029.00	84.0	Possible AD	6.10
11383	100	F	1058.00	61.5	CAA	4.60
21337	86	F	1334.00	96.0	AD	6.10
12284	96	F	-	154.0	Moderate AD pathology	2.30
11060	83	F	1108.00	94.0	Mild AD changes in temporal lobe	2.30
11299	89	M	1070.00	36.0	Age-related tau astrogliopathy with hippocampal sclerosis and secondary TDP-43	4.90
22625	91	M	1157.00	93.0	Age changes only	4.00
10192	97	M	1252.00	120.5	Argyrophilic Grain Disease with v.mild AD-like tau	2.60
20274	94	F	1166.00	86.0	Early/Incipient AD	2.80
11618	79	F	-	116.0	Argyrophilic Grain Disease	3.40
10502	81	F	1160.00	113.5	Probable AD	5.30
10544	89	F	1450.00	144.0	CAA (?)moderate SVD	2.90
11971	81	F	1363.00	44.0	Early/incipient AD	4.50
23096	76	F	1204.00	47.0	Mild AD changes in temporal lobe	2.30
22091	82	F	1020.00	61.0	Age changes only	2.70
12762	90	F	1217.00	103.0	Age changes only	4.40
22105	89	M	-	128.0	Incipient AD	5.60
12504	90	M	-	156.0	Age changes only	#NULL!
10640	104	F	1289.00	78.0	AD	3.10
22194	88	F	1129.00	4.0	Early limbic predominant DLB	5.90
22867	93	F	1133.00	70.5	Probable AD	2.50
21297	85	M	-	187.5	Mild CAA	4.20
20088	90	F	-	41.5	Age changes only	2.30
21179	86	M	1100.00	26.0	AD	2.30
11845	89	F	-	36.0	CVD	2.30
21683	90	F	1050.00	39.0	Age changes only	6.30

22340	76	F	1359.00	129.5	Age changes only	6.50
12698	87	F	-	39.0	Age changes only	5.80
11240	89	M	-	27.0	Age changes only	3.30
11379	80	F	1000.00	81.0	Probable AD	4.60
20845	95	F	1116.00	88.0	Possible AD	3.40
22110	85	M	-	12.0	Age changes only	5.00
21493	91	M	-	-	Moderate SVD	3.30
21092	87	F	1152.00	24.0	Age changes only	2.50
11662	81	F	1250.00	-	Incipient AD	2.30
10719	72	F	1230.00	-	Incipient AD	2.60
20429	92	M	-	24.0	Moderate CVD	3.40
11802	89	F	-	134.0	Mild AD pathology	5.10
11176	82	F	1174.00	46.0	Mild DLB	4.50
11427	78	M	-	144.0	Age changes only	5.70
11550	94	M	1550.00	42.0	CAA?	6.80
20428	82	F	-	96.0	Probable AD	3.20
11341	92	M	1270.00	48.0	Early/incipient AD	2.90
22691	79	F	1290.00	-	Incipient AD	2.30
22083	87	M	-	120.0	Mild AD	2.45
20402	89	F	-	72.0	AD	4.50
20382	78	F	-	-	Probable AD	2.50
11508	94	F	1150.00	-	Age changes only	3.40
10004	86	M	-	18.0	DLB	2.70
12755	89	F	1351.00	56.0	Moderate AD pathology	2.60
22272	93	M	1348.00	39.0	Mild transitional DLB	2.30
22738	88	F	-	72.0	Mild CVD	2.70
12022	82	F	1210.00	120.0	Mild AD/PD path.	2.20
10664	90	M	1134.00	114.5	Corticobasal degeneration	4.80
12033	91	M	1520.00	43.5	Mild SVD	4.00
10772	87	F	1019.00	60.0	AD	3.10
20935	92	F	-	37.0	Age changes only	2.40
10132	81	F	1210.00	41.0	Age changes only	3.50
12221	87	M	1410.00	80.0	CVD	2.40

21664	90	F	-	6.0	Possible AD	2.40
10118	87	F	1178.00	87.0	Mild AD path. in temporal lobe	4.70
11426	88	F	-	72.0	AD	2.60
12413	80	M	1240.00	-	Incipient AD	2.20
11322	87	F	1305.00	120.0	PD	6.50
10954	85	F	-	12.0	Mild CAA	2.50

Alzheimers Disease, AD; Parkinsons Disease, PD; cerebral amyloid angiopathy, CAA; cerebrovascular disease, CVD;

Supplemental Table 2. Pearson correlations between BDNF gene expression, methylation and protein with RIN and PMD times

Correlations

		LgBDNF_Transcript1_Exp_New	LgBDNF_Transcript4_Exp_New	LgBDNF_Total_Exp_New	LgM
LgBDNF_Transcript1_Exp_New	Pearson Correlation	1	.608**	.371**	
	Sig. (2-tailed)		.000	.006	
	N	53	53	53	
LgBDNF_Transcript4_Exp_New	Pearson Correlation	.608**	1	-.106	
	Sig. (2-tailed)	.000		.406	
	N	53	64	64	
LgBDNF_Total_Exp_New	Pearson Correlation	.371**	-.106	1	
	Sig. (2-tailed)	.006	.406		
	N	53	64	66	
LgMeth_PromI_Avg	Pearson Correlation	-.268	-.162	-.179	
	Sig. (2-tailed)	.054	.205	.153	
	N	52	63	65	
LgMeth_PromIV_Avg	Pearson Correlation	-.124	-.038	-.069	
	Sig. (2-tailed)	.378	.768	.585	
	N	53	64	65	
LgBDNFprotein	Pearson Correlation	.272	.058	.101	
	Sig. (2-tailed)	.051	.652	.427	
	N	52	62	64	
RNA RIN Value	Pearson Correlation	-.058	.244	-.475**	
	Sig. (2-tailed)	.678	.052	.000	
	N	53	64	66	
Postmortum delay (hours)	Pearson Correlation	.037	-.066	-.043	
	Sig. (2-tailed)	.799	.626	.746	

N	49	57	59

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Supplemental Table 3. Partial correlation matrix, controlling for donor ages and RIN values, between *BDNF* gene expression, DNA methylation and protein levels

Correlations

Control Variables			LgBDNF_Transcript1_Exp_New	LgBDNF_ pt4_Exp
RNA RIN Value & Age at death	LgBDNF_Transcript1_Exp_New	Correlation	1.000	
		Significance (2-tailed)	.	
		df	0	
	LgBDNF_Transcript4_Exp_New	Correlation	.596	
		Significance (2-tailed)	.000	
		df	47	
	LgBDNF_Total_Exp_New	Correlation	.301	
		Significance (2-tailed)	.035	
		df	47	
	LgMeth_PromI_Avg	Correlation	-.253	
		Significance (2-tailed)	.080	
		df	47	
	LgMeth_PromIV_Avg	Correlation	-.090	
		Significance (2-tailed)	.539	
		df	47	
	LgBDNFprotein	Correlation	.266	
		Significance (2-tailed)	.065	
		df	47	