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# Decreased levels of BDNF protein in Alzheimer temporal cortex are independent of *BDNF* polymorphisms

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**Abstract**

Levels of brain-derived neurotrophic factor (BDNF) are reduced in specific brain regions in Alzheimer's disease (AD) and *BDNF* gene polymorphisms have been suggested to influence AD risk, hippocampal function, and memory. We investigated whether the polymorphisms at the *BDNF* 196 and 270 loci were associated with AD in a clinical and neuropathological cohort of 116 AD cases and 77 control subjects. To determine how BDNF protein levels relate to *BDNF* polymorphisms and AD pathology, we also measured BDNF in temporal association cortex, frontal association cortex, and cerebellum in 57 of the AD and 21 control cases. BDNF protein levels in temporal neocortex of the AD brains were reduced by 33% compared to control brains, whereas levels were unchanged in frontal and cerebellar cortex. The *BDNF* genotypes were not significantly associated with a diagnosis of AD, although the *BDNF* 270 C allele was slightly overrepresented among carriers of the *APOE*ε4 allele. Moreover, BDNF protein levels did not differ between the various *BDNF* genotypes and alleles. Neuropathologically, the loss of BDNF in AD showed a weak correlation with accumulation of neuritic amyloid plaques and loss of the neuronal/synaptic marker synaptophysin. The results suggest that the investigated *BDNF* polymorphisms are neither robust genetic risk factors nor determinants of BDNF protein levels in AD.


*Keywords:* BDNF; Alzheimer's disease; ELISA; Gene polymorphisms; Association studies; Synaptophysin

## Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is essential for neuronal survival both in the peripheral and central nervous system (CNS) (Murer et al., 2001). In CNS, BDNF is enriched in the hippocampal formation, cerebral cortex, and the amygdaloid complex (Murer et al., 2001)—regions that are widely affected by deposition of amyloid plaques and neurofibrillary tangles (NFTs) in Alzheimer's disease (AD) (Ingelsson and Hyman, 2002). In the AD brain, decreased BDNF protein levels were reported in hippocampus, entorhinal cortex, and temporal neocortex (Hock et al., 2000) while no changes were observed in areas less affected by the disease, such as the frontal, parietal, and cerebellar cortices (Hock et al., 2000; Narisawa-Saito et al., 1996). In addition, BDNF mRNA levels were found to be substantially decreased in human AD parietal cortex (Holsinger et al., 2000).

The human *BDNF* gene is located on chromosome 11 (Maisonpierre et al., 1991) and consists of 13 exons. The entire open reading frame is within the last exon and, due to alternative splicing, encodes two BDNF protein variants (GenBank accession no. AF411339). The long form of BDNF, with 247 amino acids, consists of a 5′ pro-BDNF sequence that is proteolytically cleaved to form the mature protein (Seidah et al., 1996) whereas the short BDNF form, with 153 amino acids, lacks the 5′ pro-region.

Studies of a single nucleotide polymorphism (SNP) in the 5′ pro-region of the *BDNF* gene implicated the *BDNF* 196 A

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allele to be associated with increased risk for both AD (Ventriglia et al., 2002) and bipolar disorder (Sklar et al., 2002). However, other studies failed to find an association of this *BDNF* SNP with AD (Combarros et al., 2004; Nishimura et al., 2004) or various psychiatric illnesses (Hong et al., 2003a,b; Lam et al., 2004; Tsai et al., 2003). Nevertheless, subjects possessing the *BDNF* 196 A allele had poorer episodic memory and hippocampal activation by functional MRI (relative to *BDNF* 196 GG homozygotes) (Egan et al., 2003, Hariri et al., 2003) and *BDNF* 196 GA carriers had a significantly lower mean performance IQ than subjects with the *BDNF* 196 GG genotype in a cohort of young Chinese females (Tsai et al., 2004) . Another SNP in *BDNF* exon 7 showed an association between the *BDNF* 270 T allele and AD in two different Japanese populations (Kunugi et al., 2001; Nakata et al., 2003) as well as in a German cohort (Riemenschneider et al., 2002).

It is unknown how the various polymorphic *BDNF* alleles exert their differential influence on brain function and disease processes although cell-culture studies suggest that an impaired secretion of the BDNF isoform encoded by the 196 A allele may mediate some of the observed clinical associations (Chen et al., 2004; Egan et al., 2003). Apart from detecting loss of BDNF in areas of brain affected by AD, the relation of BDNF protein to *BDNF* polymorphisms and to other biochemical and pathological changes in the AD brain has not been studied.

We investigated AD-related changes in BDNF protein levels with *BDNF* polymorphisms and AD pathology in a well-characterized pathological cohort. In particular, we hypothesized that the *BDNF* 196 A allele would be associated with decreased BDNF protein levels in affected brain regions. We also predicted that decreased levels of BDNF would be associated with a more severe disease state as indicated by clinical severity and neuropathological alterations such as synaptic loss, deposition of amyloid plaques, and formation of neurofibrillary tangles.

## Materials and methods

### *Materials*

A total of 116 AD and 77 control subjects was included for *BDNF* genotyping. Pathological specimens were investigated from 56 of the AD and 22 of the control individuals. In the AD group, the mean age was 80.4 ± 0.8 years and 32.8% were male. For control subjects, the mean age was 75.0 ± 1.0 years and 45.4% were male. All AD cases were obtained from the Alzheimer's Disease Research Center (ADRC) at Massachusetts General Hospital or from the Harvard Brain Tissue Resource Center (grant no. 1R24MH68855) and were neuropathologically diagnosed according to the NINCDS-ADRDA and Reagan criteria (Hyman and Trojanowski, 1997). Twenty-two of the control subjects were autopsied and did not demonstrate any pathology of a neurodegenerative disorder. The remaining 55 clinical control subjects were either non-affected spouses of AD patients or healthy volunteers. Of the AD brains, 106 were genotyped for apolipoprotein E (*APOE*) polymorphisms and showed an *APOEε4* allele frequency of 0.38; the corresponding frequency for the 74 genotyped control subjects was 0.10. The average PMI for the AD cases was 13.4 ± 0.8 and for controls 18.5 ± 3.2. No correlation was found between PMI and the BDNF levels.

### *Brain dissection and sample preparation*

For BDNF protein measurement, strips of gray matter from temporal, frontal, and cerebellar cortices were dissected from fresh frozen brain tissue, with care taken to avoid underlying white matter, and homogenized in the following extraction buffer: 50 mM Tris, pH 7.2, 0.1% Triton X-100, 200 mM NaCl, 2 mM EDTA, with protease inhibitors (Complete, Roche, Indianapolis, IN) and 2% protease free bovine serum albumin (BSA; Sigma, St. Louis, MO) (40 μl/mg tissue). The homogenate was centrifuged at 15,000 rpm (5 min) and the resulting supernatant was used for the BDNF ELISA.

For genetic analyses, 50–150 mg of brain tissue was dissected from each autopsied subject and the QIAamp DNA Mini Kit (QIAGEN, Los Angeles, CA) was used for DNA extraction according to the manufacturer's instructions. In brief, the tissue was homogenized, treated with Proteinase K and incubated at 56°C overnight. Next, the samples were centrifuged at 8000 rpm for absorbance through a QIAamp spin column, removed of any residual contaminants by washing in two centrifugation steps and finally eluted from the QIAamp spin column. For the clinical cases, DNA was extracted from whole blood samples, also by using the QIAamp DNA Blood Mini Kit (QIAGEN). These blood samples were subjected to the same protocol as described above, but with the incubation at 56°C for 10 min. Finally, the DNA was stored at −20°C until genotyping.

### *ELISA*

The BDNF ELISA utilized MAB848 (monoclonal mouse anti-BDNF, R&D Systems, Minneapolis, MN) as capture antibody and biotinylated BAM648 (R&D Systems) as detector antibody. Microtiter plates (96-well) (Greiner, Longwood, FL) were coated with MAB848 (75 μl/well of 0.5 μg/ml in carbonate buffer, 100 mM, pH 9.6) at 4°C overnight. The plates were washed three times with phosphate buffered saline (PBS), pH 7.0 followed by blocking with 25% BlockAce (Dai-Nippon, Osaka, Japan). The samples and BDNF standard (100 μl/well) were added to the wells containing 25 μl of the extraction buffer and incubated overnight at 4°C. Plates were washed four times with Triton wash buffer (50 mM Tris, pH 7.2, 0.1% Triton X-100, 200 mM NaCl, 2 mM EDTA) and then incubated at