The Brain-Derived Neurotrophic Factor Val66Met Polymorphism and Rate of Decline in Alzheimer’s disease

Jenny Y-J Chuu, Joy L. Taylor, Jared Tinklenberg, Art Noda, Jerome Yesavage and Greer M. Murphy, Jr.*

Abstract. It is largely unknown why some patients with Alzheimer’s disease (AD) decline cognitively more rapidly than others. Genetic differences among patients could influence rate of decline. Brain-derived neurotrophic factor (BDNF) is a neurotrophin important in the survival neurons and in memory function. BDNF levels are reduced in the brain in AD. The Val66Met polymorphism in the BDNF gene modifies neuronal BDNF secretion, and affects hippocampal function and memory performance. We tested the hypothesis that the BDNF Val66Met polymorphism influences rate of cognitive decline in AD. In a sample of 149 AD patients followed for an average of 3.9 years, we found no effect of BDNF Val66Met genotype on rate of change in the Mini Mental State Examination. Results were similar when we excluded patients taking an acetylcholinesterase inhibitor, those placed in a nursing home during the study, or those with a neuropathological diagnosis that included AD plus an entity other than AD. We also found no evidence that the effects of the BDNF Val66Met genotype depend on APOE genotype, which itself had no effect on rate of cognitive change. These findings suggest that the functional BDNF Val66Met variant is not a major determinant of rate of cognitive decline in AD.

Keywords: Alzheimer’s disease, brain-derived neurotrophic factor, Mini Mental State Examination, cognitive decline, Apolipoprotein E, single nucleotide polymorphism, genotype

1. Introduction

Some patients with Alzheimer’s disease (AD) experience rapid cognitive decline, whereas others decline much more slowly [19,38]. The biological basis, if any, for these differences is not understood. The most prevalent genetic risk factor for Alzheimer’s disease, the apolipoprotein E ε4 allele [9], has been tested as a predictor of rate of decline in AD, with generally negative results [10,16,28]. A biological marker for rapid cognitive decline would be valuable because at-risk patients could be targeted for pharmacologic, medical, and psychosocial interventions designed to slow deterioration.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that supports the survival and differentiation of neurons [18]. BDNF is a small dimeric protein that is abundantly and widely expressed in the adult mammalian brain. BDNF has survival promoting actions on a variety of central nervous system neurons including those in the hippocampus and cortex [5]. BDNF is also important for learning and memory, an effect that may be mediated through enhancement of hippocampal synaptic plasticity [2,27]. Decreased BDNF mRNA and protein levels have been reported in AD brain [33,35]. These findings suggest that BDNF could be important in neuronal survival, hippocampal function, and cognitive decline in AD.
There is a single nucleotide polymorphism (SNP) at nucleotide 196 (rs6265; G to A), which produces a valine (Val) to methionine (Met) change at codon 66 of the human BDNF propeptide. Neurons transfected to express the Met variant show decreased BDNF secretion when depolarized in comparison with cells harboring the Val variant [13,17]. Subjects homozygous for the Met variant show decreased performance on tests of memory function [11,13], and impaired hippocampal activity and decreased hippocampal volumes as demonstrated by brain imaging in comparison with carriers of the Val form of BDNF [13,34]. Recently, associations have been reported between the BDNF Val66Met polymorphism and age at onset in Huntington’s disease [1], as well as cognitive function in patients with Parkinson’s disease [15]. The Val66Met variant has also been associated with neuropsychiatric conditions such as bipolar disease and other mood disorders [24,30,37], and with cognitive function in these patients [36]. Some studies have reported positive associations between risk for AD and the BDNF Val66Met polymorphism [25,40], but others have not [3,4,6,8,12,23,31,39].

Because BDNF expression levels are decreased in AD, because the BDNF Val66Met variant has been associated with risk for AD, and because this variant has been shown to predict BDNF secretion, hippocampal and memory function, and the phenotypic features of certain neurodegenerative disorders, we hypothesized that this variant might affect the rate at which cognitive decline occurs in patients with AD. Specifically, carriers of the Met/Met genotype with decreased BDNF secretion might be expected to decline more rapidly.

2. Materials and methods

2.1. Subjects

Our cohort consisted of 149 patients who were part of a longitudinal study of probable AD conducted at the Stanford NIA Alzheimer’s Disease Core Center and the Stanford Alzheimer’s Research Center of California. Patients were diagnosed as having probable AD using NINCDS-ADRDA criteria [26], and reassessed longitudinally until they were too impaired or too ill for further cognitive testing. Of the 149 patients, 51 had complete postmortem neuropathologic evaluations with a diagnosis of definite AD (n = 39) or definite AD plus another diagnosis (n = 12). The cases with additional neuropathologic diagnoses included AD with Parkinson’s disease, AD with Lewy bodies (Lewy body variant of AD) and mixed dementia (AD with vascular dementia). The accuracy of the clinical diagnosis of probable AD is 89% at our institution, based on a series of 120 autopsy cases of probable AD.

All patients had contributed a blood or buccal mucosa sample suitable for DNA extraction and genotyping, and all had at least two Mini-Mental State Examination (MMSE) [14] scores between 23 and 0 taken at least 11 months apart that could be used to estimate cognitive decline. Both clinical and DNA protocols had been approved by the Stanford University Administrative Panel on Human Subjects.

The sample consisted of 70 men and 79 women; 135 were Caucasians; the others were ethnic minorities. Subjects had a mean age at symptom onset of 68.9 years (SD = 9.1) and had a mean symptom duration of 4.5 years (SD = 2.8) between onset and first cognitive evaluation in our clinic. Clinical data on 86 subjects were previously reported in a study of APOE ε4 and cognitive decline [28].

2.2. Clinical data

The Mini-Mental State Examination (MMSE) was used to assess cognitive function approximately every 6 months. Subjects were followed for an average of 3.9 years (SD = 2.0). To estimate rate of cognitive decline, we determined slopes by using a linear regression of MMSE scores on age for each individual subject as described previously [28]. Slopes were expressed as change in MMSE points per year. To avoid MMSE ceiling and floor effects, we included only data in the window of follow-up in which scores declined from 23 to the first instance of a zero score. The mean number of MMSE scores used to calculate the slopes was 6.5 (SD = 2.8), and all subjects had at least two MMSE scores that could be used in this calculation.

2.3. Genotyping

Genomic DNA was extracted from frozen ethylenediaminetetraacetic acid-containing whole blood or buccal mucosa samples. BDNF G196A genotypes for SNP rs6265, corresponding to Val66Met, were obtained using Taqman allelic discrimination (Assay on Demand ID C\textsubscript{1} 1592758\_J0, Applied Biosystems, Foster City, CA) following the manufacturer’s instructions. APOE genotyping was performed as previously described [28]. APOE genotypes were obtained on 146 patients. BDNF and APOE genotype assays and calls were performed with researchers blinded as to clinical data.
years of education, first MMSE score included in the slope calculation, number of MMSE examinations, or number of years followed (Table 2). There was a trend towards a shorter time between disease onset and first cognitive testing in the clinic among Met/Met carriers ($F = 2.8$, $df = 2$, 146, $p < 0.07$). The mean for the Met/Met group was 2.1 years, whereas the mean for the Val/Met group was 5.0 years, and that for the Val/Val group was 4.4 years.

Because rate of decline in AD can be affected by initiation or discontinuation of treatment with acetylcholinesterase (AChE) inhibitors, we performed analyses that excluded these subjects. There were 93 patients who did not take an AChE inhibitor in the interval during which MMSE data were collected. In the subsample of untreated patients, there was a trend towards a difference among the genotype groups in MMSE slope, with the 4 Met/Met subjects in this subgroup showing a faster rate of decline ($F = 2.9$, $df = 2$, 90, $p = 0.06$).

The mean change in MMSE slope per year was $-5.1$ for the Met/Met group, whereas for the Val/Met group the mean was $-2.9$ and for the Val/Val group the mean slope was $-3.9$. Among subjects not taking an AChE inhibitor, there were no significant differences among the genotype groups in education, age, number of years included in the slope calculations, number of MMSE scores, or time from onset to first cognitive testing.

Rate of decline in AD may also be affected by nursing home placement. We examined the effects of the BDNF Val66Met polymorphism on change in MMSE score in those subjects who had not been treated with an AChE inhibitor, and who had not been placed in a nursing home during the study period. Among the 50 untreated non-institutionalized patients, there were no significant differences among the BDNF Val66Met genotypes ($F = 2.0$, $df = 2$, 47, NS). There were only two cases with the Met/Met genotype among the patients who had neither been placed in a nursing home nor had received an AChE inhibitor. For these two cases, the mean MMSE slope per year was $-5.67$, versus $-3.86$ for the Val/Val group and $-2.47$ for the Val/Met group. However, cases with a rapid rate of decline were not confined to the Met/Met group. There were cases in the Val/Val and the Val/Met groups with rates of decline greater than the mean $-5.67$ MMSE units per year observed in the Met/Met group.

The presence of other neurodegenerative processes such as Lewy body disease can affect rate of cognitive decline in AD [20,32]. Hence, we performed an analysis in which the 12 patients with a neuropathologic diagnosis of AD plus another entity were excluded. In

### Table 2

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N (%)</th>
<th>MMSE slope per year Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val/Val</td>
<td>96 (64.4)</td>
<td>$-3.7$ (2.5)</td>
</tr>
<tr>
<td>Val/Met</td>
<td>48 (32.2)</td>
<td>$-3.1$ (2.0)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>5 (3.4)</td>
<td>$-4.2$ (2.7)</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td></td>
</tr>
</tbody>
</table>

#### 2.4. Statistical analysis

One-way analyses of variance (ANOVA) and independent sample $t$ tests were performed to test the effects of BDNF genotype on rate of decline. To test for an interaction between BDNF genotype and APOE genotype, a two-way ANOVA was performed using a general linear model approach.
this subgroup of 137 patients, all 39 patients with neuropathologic examinations had diagnoses of definite AD only. ANOVA showed there were no significant differences among the BDNF genotype groups in rate of cognitive decline ($F = 1.2, df = 2, 134, NS$).

Finally, we tested whether the effects of the BDNF Val66Met genotype might depend on APOE ε4 allele carrier status. ANOVA showed no significant difference among the BDNF genotype groups in either the APOE ε4 carrier ($F = 1.6, df = 2, 97, NS$) or noncarrier groups ($F = 0.21, df = 2, 43, NS$; see Table 3).

4. Discussion

Our results show that the BDNF Val66Met polymorphism has no significant effect on rate of MMSE decline in AD. These findings suggest that although the Val66Met variant has functional effects on BDNF secretion in the brain [7], and may also influence hippocampal function and memory performance [11], these effects do not influence how rapidly global cognitive function deteriorates in patients with AD. Results were the same regardless of whether subjects with neuropathologic diagnoses of AD plus another diagnostic entity were included or excluded. We did detect a trend toward a faster rate of decline in subjects with the Met/Met genotype among those who did not take an AChE inhibitor during the study, although there were only 4 Met/Met cases in this subgroup.

This study is the first to directly test the effects of the Val66Met variant on rate of cognitive change in AD using clinical data collected longitudinally for up to 12.8 years (mean 3.9 years). One study reported no effect of the Val66Met variant on age of onset in AD [39]. Another study examined the effect of the BDNF Val/Met variant on memory function in AD using a cross sectional design, but found no effect [29].

In a cohort of late onset AD cases, Desai et al. [12] found no differences among BDNF Val66Met genotype groups in age at onset, disease duration, first MMSE score, and last MMSE score. However, they did not test the effects of the Val66Met variant on rate of change in MMSE. Rates of change calculated using multiple data points more accurately reflect disease progression than do cross sectional comparisons of scores for the different genotype groups at study entry and termination. A number of studies have examined the BDNF Val66Met variant as a risk factor for AD. Some have reported a positive association [25,40]. However, Li et al. [23] recently studied three case-control samples with a total of 935 AD patients and 1106 controls, and found no association of the Val66Met variant with risk for AD. Similarly, other recent studies found no association between the Val66Met polymorphism and risk for AD among Chinese, Spanish, Italian, US and Japanese samples [3,4,6,8,25,39]. BDNF levels are decreased in AD brain, but it was shown that the Val66Met polymorphism does not affect BDNF concentrations in AD brain (although the sample included only three Met/Met AD subjects) [22]. This finding may explain the lack of correlation between BDNF Val66Met genotype and rate of cognitive decline.

We previously found no effect of the APOE ε4 allele, an established risk factor for AD, on rate of cognitive decline in a sample of 86 patients [28]. The present study included these 86 original cases, plus another 60 subsequently evaluated subjects genotyped for APOE. Even with this larger sample, we detected no difference between ε4 carriers and noncarriers on rate of decline in AD. Further, we found no difference in the effects of the BDNF Val66Met variant between ε4 carriers and noncarriers, suggesting that these two functional polymorphisms do not interact to affect rate of cognitive decline in AD.

There was a trend for carriers of the Met/Met genotype to have a shorter duration between age at onset and first evaluation in the clinic. This could reflect a more rapid initial early decline, or other factors, such as behavioral disturbance that could bring the patient...
Table 3
Val66Met BDNF genotype and rate of MMSE change stratified by ApoE ε4 carrier status

<table>
<thead>
<tr>
<th>APOE ε4 non-carriers</th>
<th>N (%)</th>
<th>MMSE slope points per year Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF Val66Met genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>26 (56.5)</td>
<td>−3.5 (2.5)</td>
</tr>
<tr>
<td>Val/Met</td>
<td>18 (39.1)</td>
<td>−3.4 (1.6)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>2 (4.4)</td>
<td>−4.5 (1.7)</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>APOE ε4 carriers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF Val66Met genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>68 (68)</td>
<td>−3.8 (2.6)</td>
</tr>
<tr>
<td>Val/Met</td>
<td>29 (29)</td>
<td>−2.8 (2.2)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>3 (3)</td>
<td>−4.1 (3.6)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

to the clinic earlier. As additional Met/Met subjects are evaluated for rate of decline, it will be interesting to see if this trend is validated. Moreover, it is yet to be seen in subjects with mild cognitive impairment if the BDNF Val66Met variant affects rate of cognitive change or rate of conversion to a diagnosis of AD.

There are limitations to our study. First, there were only five carriers of the Met/Met genotype. This problem arises in almost all studies of this polymorphism due to the infrequency of the Met allele in populations of largely European origin. Although mean rate of decline for those carrying the Met/Met genotype was greater than that for other genotype groups in our sample, we may have lacked sufficient statistical power to detect a significant difference. Further, standard deviations were large with considerable variation within genotype groups. It is possible that some of the patients in our cohort without neuropathologic diagnoses did not have AD, which might increase variability and decrease power to detect a specific effect of the BDNF Val66Met variant on AD progression. Finally, the MMSE may lack sensitivity to subtle changes in memory function. Yet, the MMSE is a good indicator of overall cognitive performance in the range between 23 and 0 that we evaluated for genetic effects.

There are various statistical approaches to modeling rate of decline in AD. We used a linear model, but cognitive decline in AD can be nonlinear. To take this into account, we eliminated the initial upper flat portion of the decline function, and also the flat end-stage by including only those MMSE scores between 23 (a widely used criterion score for the onset of dementia) and zero as previously described [28]. This increases the fit of the remaining data by eliminating highly variable ceiling and floor effects. Other statistical methods such as random effects regression [21] may be more powerful than our approach in modeling decline in AD under certain circumstances. However, these methods impose strong requirements on the population that may produce false positive and false negative results. Our approach is more conservative and does not involve strong assumptions about the underlying population structure.

In conclusion, although BDNF expression levels are reduced in AD brain, and the BDNF Val66Met variant affects neuronal BDNF secretion, this marker does not predict rate of decline in AD, nor does it interact with the APOE ε4 allele to affect cognitive change. Although the APOE ε4 allele is a documented risk factor for AD, we found no evidence that ε4 carriers have a different rate of decline than do ε4 non-carriers. Thus, neither of these variants is likely to become a clinically useful DNA marker for identifying those AD patients likely to experience rapid cognitive deterioration. As more patients take medications designed to slow decline in AD, it will be difficult to identify sizeable study cohorts in which medication history does not confound rate of decline measures. On the other hand, cohorts consisting entirely of subjects treated with anti-Alzheimer’s medications may be suitable for pharmacogenetic studies of rate of cognitive decline.

Acknowledgments

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