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# Genetic Variation in the Brain Derived Neurotrophic Factor Gene in Alzheimer's Disease

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**Genes known to contribute to the genetic predisposition to Alzheimer's disease (AD) are active in pathways of neurodegeneration but explain only a minority of the genetic contribution to AD. A protein of importance in cerebral neurodegeneration is the brain-derived neurotrophic factor (BDNF). Variations in two single-nucleotide polymorphisms (SNPs) within the *BDNF* gene have previously been associated with AD, and one of these SNPs has also been associated with memory loss and affective disorders. We performed a case control study of three BDNF SNPs in 250 neuropathologically confirmed cases of AD and 194 unrelated controls. We did not find a significant association between the three BDNF SNPs studied and AD when evaluated individually or with haplotype analysis. Nor did BDNF genotype appear to affect the APOE ε4 association with AD. The three SNPs studied were closely linked ($D' = 0.99$ across the region). We discuss possible reasons for our failure to confirm the previously reported associations.** © 2005 Wiley-Liss, Inc.

**KEY WORDS: Alzheimer's disease; BDNF; dementia; association study; APOE ε4**

## INTRODUCTION

Although in some cases Alzheimer's disease (AD) can be attributed to rare mutations in specific genes, the majority of the genetic risk for AD is not yet characterized [Finckh, 2003]. Many genes associated with AD, including *APOE ε4*, the presenilins 1 and 2, and *APP* appear to participate in a pathway of increased accumulation of beta amyloid with attendant neurotoxicity and neurodegeneration [Hardy, 2003]. The brain-derived neurotrophic factor (BDNF) protein promotes survival of neurons vulnerable to neurodegeneration in AD [Whitehouse et al., 1982] and BDNF levels are altered in AD [Holsinger et al., 2000]. Three studies have been published associating BDNF single-nucleotide polymorphisms (SNPs) with AD, [Kunugi et al., 2001; Riemenschneider et al., 2002; Ventriglia et al., 2002], however, two studies were unable to confirm this association [Bagnoli et al., 2004; Tsai et al., 2004]. None of these studies evaluated autopsy proven cases of AD, or performed haplotype analysis.

Three SNPs in the *BDNF* gene chosen for investigation in this study are approximately equidistant along the length of the gene (Fig. 1). The most terminal polymorphism studied (rs6265 dbSNP NCBI) is a G to A change resulting in a valine to methionine substitution at codon 66 (Val66Met) in the terminal exon of the gene and has functional consequences for protein secretion [Egan et al., 2003]. The Val66Met polymorphism has been associated with AD [Ventriglia et al., 2002], bipolar disorder [Neves-Pereira et al., 2002; Sklar et al., 2002], OCD [Hall et al., 2003], altered episodic memory [Egan et al., 2003], and personality traits [Sen et al., 2003].

The "C270T" polymorphism is an untranslated polymorphism in a 5′exon that contributes to BDNF mRNA transcript 4 (NM_001709 NCBI) and is located more than 40 kb upstream from Val66Met (Fig. 1). The "C270T" polymorphism has been associated with AD in both Japanese and European populations [Kunugi et al., 2001; Riemenschneider et al., 2002]. The final SNP evaluated in this study (rs7103411 dbSNP NCBI) is a frequent intronic polymorphism (minor allele frequency in Caucasians of approximately 31%, http://www.appliedbiosystems.com) located approximately halfway between "C270T" and Val66Met.

Late onset Alzheimer's disease (LOAD), or AD with onset after about 63–65 years of age or older, is a multigenic disorder [Tanzi and Bertram, 2001] and combinations of genes may increase vulnerability to LOAD [Pastor et al., 2003]. The major genetic risk factor identified in LOAD is APOE ε4 which has also been reportedly associated with the "C270T" genotype in AD [Riemenschneider et al., 2002]. Therefore, we also evaluate BDNF genotypes for an interaction with the APOE ε4 allele in this study.

## MATERIALS AND METHODS

### Study Design

Association studies utilizing candidate genes and evaluating linkage disequilibrium may be more effective tools to evaluate complex multilocus disorders as these methods have greater statistical power, compared to linkage to detect genes of small effect [Risch, 2000]. The diagnosis of AD may be difficult due to similarities with other dementias or psychiatric disorders, thus in this study only autopsy proven cases of AD were

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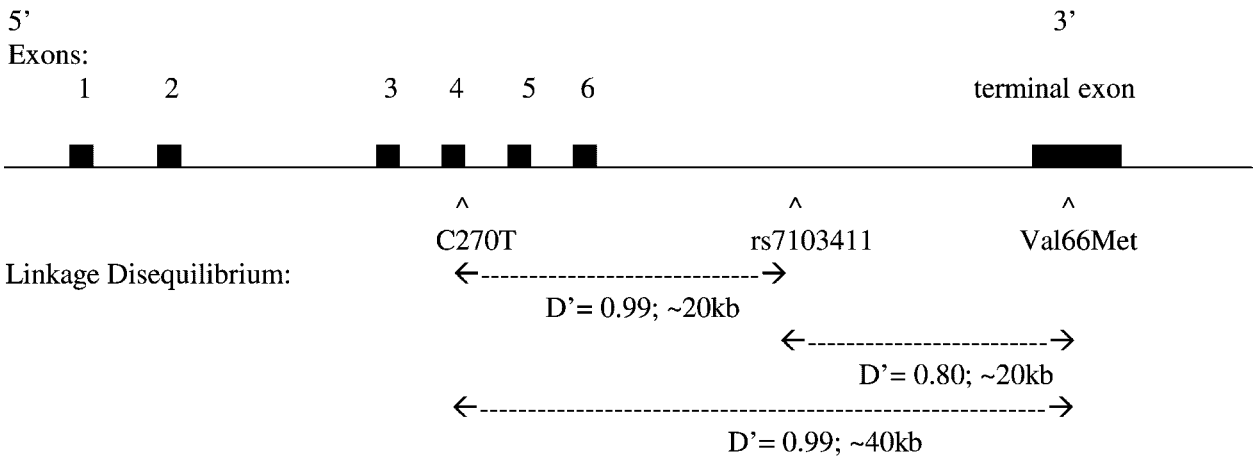


Fig. 1. Diagram of the brain-derived neurotrophic factor (*BDNF*) gene which is located along approximately 65 kb of the minus strand of chromosome 11p13. Exons are represented by solid rectangles. The terminal exon contains the coding region including the prodomain. The locations of the three single-nucleotide polymorphisms (SNPs) genotyped in this study are shown as C270T, rs7103411, and Val66Met. Linkage disequilibrium is diagrammed beneath with double-headed arrows indicating appropriate regions on the diagram.

included. Restricting our study population to Caucasians of European extraction guided the choice of informative markers. The three polymorphisms chosen for study were located at approximately 20 kb intervals within the gene (Fig. 1).

### Study Samples

All cases of AD were Caucasians clinically diagnosed with AD by NINDS criteria and with neuropathological confirmation of the diagnosis at autopsy. The AD cases and controls in this study were obtained from three different sources: (1) the Rush Religious Orders Study (ROS), a longitudinal clinical-pathologic study of aging and AD among men and women Catholic clergy [Bennett et al., 2002], (2) The National Alzheimer's Disease Tissue Repository at Indiana University, a national tissue bank derived from families with multiple members with AD and spousal controls (http://ncrad.iu.edu), and (3) the Brain Bank of the Center for Neurodegenerative Diseases (CNDR), a tissue bank derived from autopsy subjects followed at the University of Pennsylvania prior to death [Baba et al., 1998].

The mean age of onset of AD was in the seventies or above for all three cohorts while earliest age of onset was 63 years old. In this study, we define LOAD as AD with onset at 63 or older. Control cases were free of dementia and were roughly matched for age with a mean age at death for all controls of 81 years while the mean age at death in AD subjects was 83 years. AD is more common in females therefore the sex ratio of our affected population was skewed toward females (Table I).

### Power Analysis

Power calculations employed Quanto Beta Version 0.5.1 [Gauderman et al., 2001]. An example of these calculations are available (website).

### DNA Extraction and Genotyping

DNA was extracted from brain tissue frozen at autopsy (ROS and UPenn cohorts) using Trizol (Invitrogen, Carlsbad, CA) or was obtained directly from the National Repository (Indiana). SNP analysis was performed on an ABI Prism 7900HT instrument using "Assays-On-Demand" (Val66Met

TABLE I. Cohort Characteristics

| Cohort | Number of individuals | Mean age at death (SD) | Sex (%male) | Mean age of onset (range) [SD] |
|---|---|---|---|---|
| All subjects | 450 | 83 (7.4) | 42 | |
| AD subjects—all | 256 | 83 (7.1) | 38 | 77 (63–99) [8.2] |
| Control subjects—all[a] | 194 | 81 (7.7) | 46 | |
| Religious Orders Study (ROS) | 188 | 86 (6.7) | 44 | |
| ROS AD | 72 | | 38 | 85 (68–99) [6.4] |
| ROS controls | 116 | | 47 | |
| Indiana repository | 156 | 83 (6.2) | 38 | |
| Indiana AD | 99 | | 27 | 72 (63–92) [5.9] |
| Indiana controls | 57 | | 40 | |
| UPENN—CNDR | 106 | 79 (8.5) | 50 | |
| UPenn AD | 85 | | 48 | 73 (63–89) [5.9] |
| UPenn controls | 21 | | 57 | |

[a]Controls were evaluated for dementia on average within 7 months of death (ROS cases), within an average of 7 months of yearly informant report (spousal controls) or prior to death (UPenn cases). When information on the cause of death was available (e.g., spousal controls might still be alive) the most common causes of death listed at Indiana were cancer, renal failure and stroke, at UPenn were renal failure, cancer cardiovascular disease, and sepsis and cause of death was not available in the ROS cohort. Sex differences overall and within individual groups are non-significant at $P = 0.05$ by Chi-square analysis.

and rs7103411) or "Assays By Design"("C270T") reagents from Applied Biosystems, Inc. (Foster City, CA) resulting in more than 99% reproducibility in our samples. The APOE ε4 genotype was determined by restriction fragment length polymorphism analysis [Addya et al., 1997].

### Data Analysis

Allele frequencies, genotype frequencies, and Hardy–Weinberg equilibrium were calculated from the primary data and Hardy–Weinberg equilibrium was verified with Chi-square analysis. Association was evaluated by Chi-square analysis and verified with odds ratio (OR) calculations using the cocaphase program http://linkage.rockefeller.edu/ott [Dudbridge, 2003]. Haplotype analysis was performed using the Estimate Haplotype (EH) program http://linkage.rockefeller.edu/ott/eh.htm [Zhao et al., 2000]. Linkage disequilibrium was evaluated using pairwise comparisons with the 2 Locus Linkage Disequilibrium calculator (2LD) program http://linkage.rockefeller.edu/ott and verified manually [Lewontin, 1964; Zhao et al., 2000]. Interactions between individual BDNF SNPs and APOE ε4 as independent factors affecting AD were evaluated with bivariate regression analysis (SPSS, Chicago, IL) [Sinquefield, 1976].

## RESULTS

Allele frequencies, genotype frequencies, and haplotype frequencies are given in Table II. The three SNPs are in Hardy–Weinberg equilibrium. Allele frequencies did not differ significantly between patients and controls or between cohorts for any of the three SNPs. Haplotype frequencies calculated by the EH program indicate a significant degree of linkage disequilibrium.

Linkage disequilibrium calculated between "C270T" and rs7103411 is $D' = 0.99$ ($P < 0.001$); while between rs7103411 and Val66Met is $D' = 0.80$ ($P < 0.0001$). Across the region the linkage disequilibrium between "C270T" and Val66Met is $D' = 0.99$ ($P < 0.001$) (Fig. 1).

Chi-square analysis and cocaphase analysis did not reveal a significant association between genotype and AD for any of the SNPs when analyzed independently or when analyzed together using haplotype analysis. Nor was any association or strong tendency toward association noted in any of the three cohorts when they were evaluated separately. Analysis for APOE ε4 revealed an association AD with an OR of 5, however, no interaction of APOE ε4 genotype and BDNF genotype was identified.

TABLE II. Frequency of Alleles, Genotypes, and Haplotypes at Three Brain-Derived Neurotrophic Factor (BDNF) Single-Nucleotide Polymorphisms (SNPs) Studied

| | AD (%), (n = 256) | Controls (%), (n = 194) | All (%), (n = 450) |
|---|---|---|---|
| Alleles | | | |
| C270T | | | |
| Minor allele-T | 5.08 | 4.82 | 5 |
| rs7103411 | | | |
| Minor allele-G | 21.5 | 20.9 | 21.3 |
| Val66Met | | | |
| Minor allele-A | 19.6 | 19.2 | 19.4 |
| Genotypes | | | |
| C270T | | | |
| CC | 89.83 | 90.35 | 90.0 |
| CT | 10.17 | 9.65 | 10.0 |
| rs7103411 | | | |
| AA | 61.25 | 62.8 | 61.8 |
| AG | 34.6 | 32.6 | 33.9 |
| GG | 4.17 | 4.65 | 4.34 |
| Val66Met | | | |
| GG | 63.8 | 64.7 | 64.1 |
| GA | 33.2 | 32.2 | 32.9 |
| AA | 3.02 | 3.00 | 3.01 |
| Haplotypes | | | |
| C270T-C | | | |
| rs7103411-A | | | |
| Val66Met-G | 72.7 | 70.8 | 71.9 |
| Val66Met-A | 1.96 | 2.36 | 2.81 |
| rs7103411-G | | | |
| Val66Met-G | 3.08 | 4.41 | 4.35 |
| Val66Met-A | 17.7 | 17.9 | 16.2 |
| C270T-T | | | |
| rs7103411-A | | | |
| Val66Met-G | 4.20 | 0.60 | 4.51 |
| Val66Met-A | 0 | 4.30 | 0.098 |
| rs7103411-G | | | |
| Val66Met-G | 0.30 | 0 | 0.133 |
| Val66Met-A | 0 | 0.009 | 0.003 |

Frequencies are given in percentages (%). Minor allele frequencies are given at the three SNP sites and genotype frequencies are given for all genotypes encountered. Haplotype frequencies were determined with the Estimate Haplotype (EH) Program assuming the presence of an association between SNPs.

## DISCUSSION

This study differs from previous work as it examines three SNPs within the *BDNF* gene, includes haplotype analysis, evaluates only autopsy proven cases of AD and is larger than previous studies. Our findings on SNP allele frequencies and linkage disequilibrium within the *BDNF* gene are similar to previous findings [Sklar et al., 2002; Hall et al., 2003] but our results do not support previously noted associations between BDNF polymorphisms and AD [Kunugi et al., 2001; Riemenschneider et al., 2002; Ventriglia et al., 2002]. Previously noted associations have not been uniformly confirmed [Bagnoli et al., 2004; Tsai et al., 2004].

The minor allele frequency of the BDNF Val66Met polymorphism was 19% in our study and was similar to previous findings in European populations (Table III), although the minor allele frequency may vary in different populations such as the Chinese (Table III). In our study, the frequency of the BDNF "C270T" SNP polymorphism was low as in previously reported control populations, however, in two of three studies the frequency of the polymorphism was significantly increased in AD patients (Table III).

In our study, linkage disequilibrium calculated between "C270T" and rs7103411 is $D' = 0.99$, between rs7103411 and Val66Met $D' = 0.80$ and between "C270T" and Val66Met $D' = 0.99$ (Fig. 1). These values indicate extensive linkage disequilibrium within the *BDNF* gene as found in previous studies of bipolar disorder [Sklar et al., 2002] and obsessive compulsive disorder [Hall et al., 2003].

Thus, although our findings are generally consistent with previous findings of allelic frequencies and linkage disequilibrium we were not able to confirm previous associations of BDNF polymorphisms and LOAD. One possible explanation of the discrepancies between studies may be inadequately matched control populations. Our control populations were mainly derived from a large well characterized community based population (ROS) or from spousal controls (Indiana Repository), designed to minimize population differences. A second reason for the discrepancy in results could be phenotypic heterogeneity, however, our affected subjects were well characterized as they were both clinically diagnosed and neuropathologically confirmed to have AD. A third reason for

TABLE III. Association Studies of BDNF in AD

| Study author, population, number of patients | Val66Met | | | "C270T" | | | Odds ratio (OR) (95% CI) | Interaction with ApoE |
|---|---|---|---|---|---|---|---|---|
| | Minor allele, frequency% | Genotype, frequency% | | Minor allele, frequency% | Genotype, frequency% | | | |
| Bodner, American Caucasian, AD (256), controls (194) | AD, 19.6%; controls, 19.2% | AD | Controls | AD, 5.08%; controls, 4.82% | AD | Controls | | (–) |
| | | GG-63.3 | GG- | | CC-89.8 | CC-90.3 | | |
| | | GA-33.2 | GA- | | CT-10.2 | CT-9.6 | | |
| | | AA-3.02 | AA- | | TT-0 | TT-0 | | |
| Kunugi, Japanese, AD (270), EOAD (51), LOAD (119), controls (498) | ND | ND | ND | EOAD, T-3%; LOAD, T-7.6%; controls, T-2.5% | AD | EOAD | LOAD | Controls | 3.2 (1.3–7.7) | (–) |
| | | | | | CC-88 | CC-94 | CC-86 | CC-96 | | |
| | | | | | CT-11 | CT-5.9 | CT-13 | CT-4.2 | | |
| | | | | | TT-0.6 | TT-0 | TT-0.8 | TT-0 | | |
| Riemenschneider, German, AD (210), EOAD (98), LOAD (109), controls (188) | ND | ND | ND | AD, T-12%; controls, T-7% | AD | EOAD | LOAD | Controls | 2.16 (1.04–4.48) | (+) |
| | | | | | CC-88 | CC-85 | CC-91 | CC-93 | | |
| | | | | | CT-12 | CT-14 | CT-9 | CT-6.9 | | |
| | | | | | TT-1 | TT-1 | TT-0 | TT-0 | | |
| Ventriglia, Italian, AD (130), controls (111) | AD, A-22%; controls, A-30% | AD | Controls | ND | ND | ND | | 1.99 (1.15–3.46) | (–) |
| | | GG-65 | GG-49 | | | | | | |
| | | GA-25 | GA-43 | | | | | | |
| | | AA-9 | AA-8 | | | | | | |
| Tsai, Chinese, AD (163), controls (89) | AD, A-49%; controls, A-49% | AD | Controls | ND | ND | ND | | | |
| | | GG-25 | GG-25 | | | | | | |
| | | GA-52 | GA-52 | | | | | | |
| | | AA-24 | AA-24 | | | | | | |
| Bagnoli, Italian, AD, controls (97) | AD, 28%; controls, 24% | AD | Controls | AD, 6%; controls, 7% | AD | Controls | | | |
| | | GG-48 | GG-57 | | CC-88 | CC-86 | | | |
| | | GA-47 | GA-39 | | CT-14 | CT-14 | | | |
| | | AA-5 | AA-4 | | TT-1 | TT-0 | | | |

the discrepancy could be population stratification, however, two previous studies performed in Italian populations yielded opposite results (Table III).

Three previous studies demonstrated an association of BDNF SNPs with AD (Table III). In one report the V66M allele was associated with AD at an OR of 1.99 in an Italian population. Our study is expected to have greater than 95% power to detect this strength of association if inheritance is dominant or log additive but less detection power occurs in the case of a recessive inheritance pattern. (Power calculations with Quanto Beta Version 0.5.1, assuming case control design, disease frequency of 15%, and a two-sided type one error rate of 0.05.) Thus, lack of replication may have been due to a recessive mode of inheritance. The two other studies finding an association investigated the "C270T" SNP. In a Japanese study (Table III) an OR of 3.2 was obtained, and we would expect to detect this association at a power of greater than 99% in our study if the inheritance were dominant or log additive (assumptions for power calculations as above), thus lack of replication may be due to different populations or recessive inheritance. The final study, performed in a German population (Table III) detected an association of "C270T" at an OR of 2.16. Our study has greater than 80% power to detect this association if inheritance is dominant or log additive (assumptions for power calculations as above), thus in this case our inability to replicate may have been due to recessive inheritance or inadequate power.

The clinical ascertainment of AD can be problematic due to confusion with depression, other forms of dementia and other medical illness. Depression can be indistinguishable from dementia clinically and cognitive impairment may respond to treatment with antidepressants [Alexopoulos et al., 1993]. Depression is common in AD, occurring at a rate of 30–50% in some studies [Olin et al., 2002] thus risk factors associated with depression could make a significant impact in studies of AD. Furthermore affective disorders have a genetic component [Johansson et al., 2001] and have been associated with the BDNF Val66Met polymorphism [Sklar et al., 2002]. Neither previous studies nor this study have considered depression in their study populations. In the absence of this information it is possible that accurate matching of control groups and comparison across different studies has been confounded, at least in part, by variability in the amount of depression, leading to difficulties in replication or spurious associations.

In summary, we did not find an association between the *BDNF* gene and LOAD in a large autopsy based case control association study limited to individuals of European descent. This may not be surprising since the major peaks on chromosome 11 associated with AD are in the 11p15 or 11q23 regions, not the 11p13 region where BDNF is located (www.alzgene.org). Nor could we identify an interaction between the BDNF SNPs evaluated and the APOE ε4 allele effect in LOAD. We were able to generate allele and genotype frequencies on three SNPs within the *BDNF* gene and demonstrate extensive linkage disequilibrium in the 65 kb *BDNF* gene region. Our study was sufficiently large to have a good power to detect a dominant or log additive effect; however, lack of replication may be due to a lesser power to detect association with a recessive inheritance pattern and the relatively infrequent "C270T" polymorphism. In addition, the discrepancy between studies may have been due to population stratification or ascertainment issues such as an inability to reliably distinguish AD and affective disorders, inability to identify comparable populations equally weighted for depression in different studies or an inability to obtain control populations appropriately matched for depressive disease. Additional studies clarifying the role of depression in AD as well as other clinical factors and risk factors may be indicated.

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