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**BDNF Variants Influence Educational Attainment  
But Not Disease Characteristics in Alzheimer's Disease**

A Thesis Submitted to the  
Yale University School of Medicine  
in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Medicine

by

Kristina Frances Victoria Zdanys

2008

## Abstract

This study aimed to determine whether brain-derived neurotrophic factor (BDNF) variants are related to premorbid educational attainment, progression of cognitive and functional decline, and associated neuropsychiatric symptoms in patients with Alzheimer's disease (AD). A sample of  $n = 341$  AD subjects was genotyped for the BDNF polymorphisms val66met, C270T, and G-712A. Subjects received tests of cognition and daily function at baseline and at multiple subsequent time points during their participation in a variety of research protocols. Cognition was measured by the Mini-Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale (ADAS-Cog). Functional performance was assessed using the Instrumental Activities of Daily Living questionnaire (IADL) as well as the Alzheimer's Disease Cooperative Study-Activities of Daily Living inventory (ADCS-ADL). Subjects were also characterized for the frequency and severity of neuropsychiatric symptoms using the Neuropsychiatric Inventory (NPI). There was a significant effect of val66met genotype on educational attainment ( $F = 7.42$ ,  $df = 2$ ,  $329$ ,  $P = .00070$ ), with met homozygotes having significantly fewer years of education than both the val/met and val/val groups. No association was observed between any BDNF polymorphism and measures of cognitive or functional decline. The C270T-T allele was associated with a higher prevalence of neuropsychiatric symptoms ( $Z = -2.11$ ,  $N = 241$ ,  $p = .035$ ) and specifically with the presence of hallucinations ( $OR = 3.25$ ,  $95\% CI = [1.22-8.62]$ ,  $p = .018$ ). In summary, the val66met polymorphism appears to be associated with lower premorbid educational attainment in AD patients. The C270T-T allele demonstrated association with total neuropsychiatric symptoms and specifically hallucinations. BDNF genotypes in this sample do not confer a more rapid rate of cognitive or functional decline.

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## **Introduction**

Neurotrophins function in neuronal development and maintenance (1, 2). Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in modulation of episodic memory that occurs in the hippocampus (3-6). It is believed that disruption of BDNF may lead to learning and memory deficits (4, 6-13). Consequently, the BDNF gene is of interest to researchers investigating neurodegenerative disease and cognitive impairment.

Decreased levels of BDNF mRNA have been observed in brains of patients with Alzheimer's disease (AD) in the parietal cortex (14) and hippocampus (15). Evidence also suggests that serum levels of BDNF reflect severity of AD, correlating with Mini-Mental State Examination (MMSE) scores (16). Measurement of serum BDNF levels in patients with vascular dementia does not demonstrate the decrease observed in AD (17). Donepezil, an acetylcholinesterase inhibitor with demonstrated cognitive and functional benefits for patients with AD, has been found to increase BDNF serum concentrations in patients to the level of healthy controls (18). The specific molecular mechanism linking acetylcholinesterase and BDNF, however, has not been elucidated. Because of the increasing evidence that BDNF may play a role in AD, polymorphisms of this gene are being studied to understand better the contribution of genetics to this complex condition.

Geneticists have recently identified several polymorphisms of BDNF: C270T, val66met (G196A), and G-712A. Previous research into the role of these polymorphisms in the pathogenesis of AD has been conflicting. The T allele of

the C270T polymorphism has been identified in several studies to be associated with AD (19-22) as well as early-onset AD in patients lacking the apolipoprotein E (ApoE)  $\epsilon$ 4 allele, an established risk factor for AD (23). However, other studies found no association (24-29).

Although the majority of studies examining val66met have found no association with AD (20, 25, 27-36), at least two studies have suggested higher frequencies of the val allele among patients with AD (19, 37). Additionally, val66met has been associated with memory performance, in which patients carrying the met allele demonstrate poorer episodic memory (38-40).

The G-712A allele was recently identified by our group (29) and was not found to have an association with AD.

Regardless of whether BDNF polymorphisms play a direct role in predisposing patients to develop AD, the role of this gene in modulating learning and memory suggests that it may influence the rate of progression and associated symptoms of this disease. The clinical presentation of AD is heterogeneous, involving not only memory deficits but also an array of neuropsychiatric comorbidities that may or may not present in a particular patient. Several investigators have suggested that distinct phenotypes may be evident in cases where AD is comorbid with psychosis (41-44) or with major depressive disorder (45-47), as compared to AD cases in which those conditions are absent.

BDNF polymorphisms have already been investigated with respect to some of these neuropsychiatric conditions in patients without AD. Extensive literature exists evaluating the role of BDNF gene variants in schizophrenia, for

which the clinical presentation is largely characterized by psychotic symptoms of hallucinations and delusions. It has been reported that the val allele is associated with schizophrenia (48), although the met/met genotype has a significantly lower age of schizophrenia onset than val/val (49). There is also evidence for preferential inheritance of the val allele among patients with psychosis and schizophrenia spectrum disorder (50). The C270T-T allele has been documented in higher frequency among patients with schizophrenia than healthy controls (51, 52). However, multiple studies suggest that BDNF polymorphisms have no association with schizophrenia (29, 53-55). Other variables have been studied in patient populations with schizophrenia to evaluate the effect of BDNF gene variants, and it has been observed that val homozygotes have higher scores of logical memory than met/val or met/met counterparts (56). Among patients with schizophrenia, those who were classified as catatonic had lower average serum BDNF than those who were paranoid or residual (57).

The association between BDNF and major depressive disorder (MDD) has also been studied. It has been reported that in a Chinese population there is an excess of the met allele of val66met among geriatric patients with depression (58). Additionally, low plasma levels of BDNF have been associated with MDD (59) and suicidal behavior in MDD (60). Concordantly, mouse models suggest that increased BDNF signaling has an antidepressant-like effect (61), and there is an increase in BDNF immunoreactivity in post-mortem tissue of patients treated with antidepressant drugs (62). A recent investigation of depression in AD found



an association between the met allele and depressive symptoms in these patients (63).

Research into the association of BDNF with other neuropsychiatric conditions has included studies of anxiety, panic disorder, affective disorders, post-traumatic stress disorder (PTSD), substance dependence, and general neurocognitive dysfunction. It has been demonstrated that val homozygotes have higher levels of anxiety than other val66met genotypes (64), and patients with panic disorder who have lower levels of serum BDNF have poorer response to cognitive behavioral therapy (65). No associations were observed between G-712A, C270T, or val66met polymorphisms and affective disorder or PTSD, although a slight association was discovered between G-712A and substance dependence (29). Additionally, among patients with systemic lupus erythematosus, the met allele of val66met was associated with better cognitive functioning in psychomotor and motor domains (66).

### **Statement of Purpose, Specific Hypothesis, and Specific Aims**

Patients with AD present with a vast array of neuropsychiatric comorbidities in addition to cognitive and functional impairment, with little evidence currently in the literature to help predict why there is such variability in these phenotypes among the AD patient population. Having observed discrepant reports in the literature regarding the role of BDNF in AD and separately in other neuropsychiatric conditions, the purpose of this investigation is to evaluate the effect of BDNF polymorphisms on cognitive, functional, and behavioral

symptoms in AD. We hypothesized that val66met met homozygotes would have an earlier age of onset and lower educational attainment than other genotypes based on the multiple studies suggesting the met allele is associated with poorer memory performance(38-40). We also hypothesized that psychotic symptoms, including hallucinations and delusions, would be more highly associated with the C270T-T allele based on studies in which this allele was more common among patients with schizophrenia (51, 52). Our study was conducted with the aim to provide insight into the specific role of BDNF in AD, and more broadly to understand the genetic basis of particular phenotypes of this disease.

## **Method**

### ***Subjects***

The study sample comprised 341 patients with probable AD (67) who enrolled in a study of the genetics of AD and were initially evaluated in the Yale Alzheimer's Disease Research Unit (ADRU). Most of these patients participated in a variety of other research protocols permitting the accumulation of longitudinal cognitive and functional data. Nineteen of these patients have subsequently died and had autopsy confirming definite AD (68). The demographics and clinical characteristics of patients are displayed in Table 1. The racial composition of the sample was: European-American ( $n=343$ ; 97.7%), African-American ( $n=6$ ; 1.7%), and Hispanic ( $n=2$ ; 0.57%).

All patients underwent a comprehensive evaluation by a research physician and ancillary staff, including cognitive assessment, medical history,

physical and neurological examinations, serum chemistries, thyroid function studies, complete blood count, B<sub>12</sub>, folate, VDRL, urinalysis, electrocardiogram, and brain MRI or CT scan. Subjects were excluded for any neurological or medical disorder (other than AD) that could produce cognitive deterioration or for significant psychiatric illness, alcohol, or substance abuse. Research protocols in which subjects participated following their initial evaluation included investigational therapeutic trials, neuroimaging studies, and neuropsychological studies. Some investigational and clinically prescribed AD treatments received by subjects—in particular acetylcholinesterase inhibitors, high-dose vitamin E ( $\geq 400$  IU daily), and psychotropic drugs—may potentially have impacted behavioral variables analyzed in this study. These treatments were assumed to be independently distributed with regard to BDNF genotype; however, this assumption was also tested statistically (see below).

Family history of AD was assessed using the Alzheimer Dementia Risk Questionnaire (69) and the Dementia Questionnaire (70) through extensive caregiver interview. Family history was considered positive if at least one first-degree relative met criteria for primary degenerative dementia. Caregiver interview was also used in conjunction with review of medical records to approximate the onset date of AD, estimated to the month of which the earliest definite symptom was noticed. All subjects (or their responsible next of kin) provided written informed consent and were studied under a protocol approved by the Yale Human Investigation Committee.

### ***Evaluation***

Subjects were evaluated using a number of cognitive, functional, and neuropsychiatric tests and rating scales at the time of initial presentation (see Tables 2 and 4). Several of these measures were repeated longitudinally, at varying frequencies, depending on the different requirements of the research protocols in which subjects subsequently participated. Many subjects enrolled in multiple studies spanning several years.

*Cognitive Performance.* The cognitive performance of subjects was measured using the Mini-Mental State Examination (MMSE) (71) and the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) (72). The MMSE evaluates cognition on a scale of 0 (maximal impairment) to 30 (no impairment) through tests of memory, orientation, and praxis. The ADAS-Cog is a neuropsychological battery that targets specific domains of cognition including memory, orientation, praxis, and language. The ADAS-Cog is much more sensitive than other testing, with a range of scores from 0 to 70 for which higher scores indicate greater cognitive impairment. This battery is frequently employed to measure efficacy of treatment in drug trials and has been shown to be a reliable predictor of cognitive decline in patients with probable AD (73, 74). Of these measures, the medical student author performed approximately 150 MMSE evaluations and 40 ADAS-Cog evaluations. The remainder was completed by ancillary staff at the ADRU.

*Functional Performance.* The functional capacity of subjects in activities of daily living was assessed through caregiver interview using both the Instrumental Activities of Daily Living (IADL) questionnaire (75) and the Alzheimer's Disease Cooperative Study-Activities of Daily Living inventory (ADCS-ADL) (76). The IADL was performed only once at baseline (and therefore included in retrospective but not prospective analyses) and evaluated everyday functioning along eight domains: using the telephone, shopping, food preparation, housekeeping, laundry, transportation, handling medications, and finances (75). A score of 1 for a given domain indicated no impairment and 0 indicated inability to complete that task. Since not all domains were valid for all subjects (e.g. men who never did laundry before AD onset), the IADL score was calculated as the sum of individual activity scores divided by the total possible valid points for that subject. The ADCS-ADL provides a comprehensive assessment of ADL performance across 28 functional domains and is scored from 0 (maximal impairment) to 78 (no impairment) (76). Use of these inventories previously was described by our group in a study of ApoE genotype and functional decline (77). Of these evaluations, approximately 35 IADLs were completed by the medical student author. The remainder of these exams was performed routinely by research staff at the ADRU.

*Neuropsychiatric symptoms.* All subjects were evaluated for behavioral and psychological symptoms using the Neuropsychiatric Inventory (NPI), a structured interview that assessed the frequency and severity of these symptoms

(78). Using scripted questions, a caregiver was asked whether the patient's behavior had changed after the onset of dementia and whether the altered behavior had been present during the month preceding the evaluation. This format therefore distinguished between psychiatric symptoms that may have been present before the onset of dementia, and those that emerged during the disease process. The NPI assessed the following behavioral domains: delusions, hallucinations, agitation, depression, anxiety, elation, apathy, disinhibition, irritability, aberrant motor behavior, sleep disturbances and eating disturbances. Specific follow-up questions were used to confirm the presence of symptoms that were reported positive. For each domain in which symptoms were confirmed, the caregiver was asked to score the frequency with which symptoms occurred as: occasionally (1), often (2), frequently (3) or very frequently (4). The caregiver was asked also to score the severity of disturbances as: mild (1), moderate (2) or marked (3). Domains absent of disturbances were scored as 0. The product of the frequency and severity score was determined for each positive item (range=1-12), and the sum of all item scores yielded the total NPI score. Possible scores ranged from 0 (no behavioral disturbances) to 144 (all behavioral disturbances maximally present). Use of the NPI was described previously by our group in a study of ApoE genotype association with psychotic symptoms (79). Of these NPI evaluations, approximately 35 were completed by the medical student author. The remainder was performed routinely by research staff at the ADRU.

Of the cognitive, functional, and neuropsychiatric measures, the MMSE was performed on the entire sample ( $N = 341$ ), whereas all other measures were

available for only certain subsets of the subject population (as detailed in Tables 2 and 4). All subject data were obtained by trained raters who were unaware of the subjects' BDNF genotypes.

### ***Determination of BDNF genotypes***

BDNF genotype determination was performed at the VA Connecticut Healthcare System, West Haven, CT, by Dr. Joel Gelernter and colleagues. As previously described by our group (29), BDNF sequence information was obtained through the public "GoldenPath" database (<http://genome.ucsc.edu>). Fifteen pairs of primers were designed to amplify part of the 5' region, the noncoding exon I, and the only coding exon V of the *BDNF* gene (29). Amplicons derived from 16-48 DNA samples of the above patients with various diagnoses and healthy controls were scanned using denaturing high performance liquid chromatography (dHPLC). A dHPLC mutation screen of the *BDNF* gene was carried out by using the "Wave" DNA Fragment Analysis System (Transgenomic, USA). Five-15 µl of the polymerase chain reaction (PCR) mixture was loaded on a "DNASep" cartridge using a linear acetonitrile gradient in a triethylamine buffer at a constant flow rate of 0.9 ml/min. The gradient was created by mixing the eluents A (0.1 M triethylamine) and B (0.1 M triethylamine and 25% Acetonitrile) at concentrations determined by the Transgenomic "Wavemaker" software. Samples that showed mobility shifts were sequenced using cycling sequencing and fluorescently labeled dideoxynucleotides on an ABI 3100 Genetic Analyzer (Applied Biosystems). A newly identified SNP, G-712A (29), and two previously

reported SNPs, C270T (21) and Val66Met (80), were genotyped in patients with AD by PCR and restriction fragment length polymorphism (PCR-RFLP) analysis. Primer pairs and PCR conditions for genotyping the three BDNF SNPs have been previously summarized (29).

### ***Statistical Analysis***

Analyses of the val66met polymorphism involved three-group comparisons (val/val, val/met, met/met). However, the C270T and G-712A polymorphisms were studied using two-group comparisons between carriers and non-carriers of the less frequent allele, given the scarcity of homozygotes for the C270T T-allele ( $n = 3$ ) and the G-712A A-allele ( $n = 2$ ). Subject characteristics (including demographics, disease characteristics, and concomitant therapies) were compared across gene dose groups using Student's *t*-test or analysis of variance (ANOVA) for continuous variables or chi-square analysis for dichotomous variables.

The effect of each BDNF polymorphism on the rate of AD progression was analyzed using both retrospective and prospective techniques similar to those employed in our previous study of ApoE  $\epsilon 4$  (77). The retrospective analyses examined cross-sectional cognitive (MMSE, ADAS-Cog) and functional (IADL, ADCS-ADL) data obtained at each subject's initial visit, while controlling for the duration of symptoms by analysis of covariance (ANCOVA). Although disease duration was the essential covariate in all retrospective analyses, age, sex, and educational attainment were also included as covariates in the ANCOVA models.



Prospective analyses of disease progression were also conducted for the MMSE, ADAS-Cog, and ADCS-ADL for all subjects who had at least two observations spanning at least six months of research participation. For these analyses, an annualized rate of change in performance on each scale was calculated by least-squares regression, using all available measurements for each subject. Rates of change were compared across BDNF genotype groups by ANCOVA, controlling for age, sex, and education.

*Behavioral Outcomes.* We derived an NPI-Psychosis score for each subject by summing the domain scores for the delusions and hallucinations sections of the full NPI. Subjects with an NPI-Psychosis score of 1 or greater were coded positively for the AD+Psychosis phenotype. These criteria reflect our conception of an AD “phenotype” as an identifiable condition in which psychotic disturbances occur with substantial frequency and/or severity. Each BDNF genotype was analyzed as a potential predictor of the AD+Psychosis phenotype using separate logistic regression models. In the AD+Psychosis model, MMSE score and ApoE  $\epsilon$ 4 genotype were included as covariates because our previous study found them significant, independent predictors of this phenotype (79).

An exploratory analysis of the association between BDNF and other behavioral disturbances in AD was conducted, using the rest of the data from the NPI. It was observed that the distribution of total NPI scores did not satisfy the assumption of normality because of a “floor effect” – many subjects scored zero. Nonparametric analyses (Mann-Whitney  $U$  or Kruskal-Wallis  $H$ ) were employed

to determine if specific genotype groups differed in total NPI score. Logistic regression analyses were performed to explore whether BDNF genotype predicted zero vs. nonzero domain scores on each item of the NPI (e.g. irritability, anxiety). The literature does not provide a clear indication of the appropriate covariates for each item of the NPI. Covariates chosen included sex, age at time of testing, MMSE score, and education level. This analysis makes no assumptions about the nature of AD phenotypes; rather, it explores whether BDNF genotype is related at all to the appearance of specific behavioral disturbances within an AD population.

## **Results**

The genotypic distribution of these three SNPs—val66met, C270T, and G-712A—was consistent with Hardy-Weinberg equilibrium expectations (data not shown).

### *Demographics*

Table 1 presents the demographic and clinical characteristics of the entire sample, as well as each genotype group. Within the AD sample, val66met genotypes were available for  $n = 332$  subjects; C270T genotypes for  $n = 332$  subjects; and G-712A genotypes for  $n = 310$  subjects. The two planned comparisons of educational attainment and age of onset among val66met groups were analyzed. There was a significant effect of val66met genotype on educational attainment ( $F = 7.42$ ;  $df = 2, 329$ ;  $P = .00070$ ) (See Figure 1). Post-hoc Tukey test revealed that the met/met homozygote group had significantly

lower education than both the val/met heterozygotes ( $P = .00062$ ) and the val/val homozygotes ( $P = .0068$ ). However, the val/met heterozygote and val/val homozygote groups did not differ from each other ( $P = .13$ ). There was no significant effect of either C270T or G-712A genotype on educational attainment. There was no significant effect of any of the three polymorphisms with regard to age of disease onset.

Few significant demographic differences were observed across genotype groups. In our sample, carriers of the A712 allele were disproportionately female ( $\chi^2 = 4.57, df = 1, P = .032$ ). Treatment history for acetylcholinesterase inhibitors, anti-psychotics, antidepressants and high-dose vitamin E was distributed independently with respect to genotype. The only exception was that our sample of met/met was significantly more likely than val/val or val/met groups to have been taking acetylcholinesterase inhibitors at the time of study assessment ( $\chi^2 = 6.21, df = 1, P = 0.045$ ). Apart from these observations, all genotype groups were well matched for demographic and disease characteristics.

#### *Retrospective Analysis of Cognitive and Functional Progression*

Table 2 presents a summary of the retrospective analyses of cognitive and functional progression in AD patients. It specifically contains the baseline cognitive and functional data according to BDNF genotypes.

#### *Val66met*

MMSE performance was analyzed in the overall sample of  $n = 332$  subjects. Gene dose groups did not differ significantly in MMSE performance ( $F = 0.65$ ;  $df = 2, 325$ ;  $P = .52$ ), controlling for disease duration, age, sex, and education.

ADAS-Cog performance was analyzed in a sub-sample of  $n = 274$  subjects. The demographic profile of this sub-sample and all others analyzed below did not differ from that of the overall sample characterized in Table 1. Gene dose groups did not differ significantly in ADAS-Cog performance ( $F = 0.75$ ;  $df = 2, 267$ ;  $P = .47$ ), controlling for disease duration, age, sex, and education.

IADL performance was analyzed in a sub-sample of  $n = 320$  subjects. Gene dose groups did not differ significantly in IADL performance ( $F = 0.01$ ;  $df = 2, 313$ ;  $P = .99$ ), controlling for disease duration, age, sex, and education.

ADCS-ADL performance was analyzed in a sub-sample of  $n = 165$  subjects. Gene dose groups did not differ significantly in ADCS-ADL performance ( $F = 0.84$ ;  $df = 2, 158$ ;  $P = .43$ ), controlling for disease duration, age, sex, and education.

### *C270T*

MMSE performance was analyzed in the overall sample of  $n = 332$  subjects. Gene dose groups did not differ significantly in MMSE performance ( $F = 0.01$ ;  $df = 1, 326$ ;  $P = .93$ ), controlling for disease duration, age, sex, and education.

ADAS-Cog performance was analyzed in a sub-sample of  $n = 275$  subjects. The demographic profile of this sub-sample and all others analyzed below did not differ from that of the overall sample characterized in Table 1. Gene dose groups did not differ significantly in ADAS-Cog performance ( $F = 0.04$ ;  $df = 1, 269$ ;  $P = .83$ ), controlling for disease duration, age, sex, and education.

IADL performance was analyzed in a sub-sample of  $n = 320$  subjects. Gene dose groups did not differ significantly in IADL performance ( $F = 0.60$ ;  $df = 1, 314$ ;  $P = .44$ ), controlling for disease duration, age, sex, and education.

ADCS-ADL performance was analyzed in a sub-sample of  $n = 165$  subjects. Gene dose groups did not differ significantly in ADCS-ADL performance ( $F = 0.40$ ;  $df = 1, 159$ ;  $P = .53$ ), controlling for disease duration, age, sex, and education.

#### *G-712A*

MMSE performance was analyzed in the overall sample of  $N = 310$  subjects. Gene dose groups did not differ significantly in MMSE performance ( $F = 0.12$ ;  $df = 1, 304$ ;  $P = .73$ ), controlling for disease duration, age, sex, and education.

ADAS-Cog performance was analyzed in a sub-sample of  $n = 261$  subjects. The demographic profile of this sub-sample and all others analyzed below did not differ from that of the overall sample characterized in Table 1. Gene dose groups did not differ significantly in ADAS-Cog performance ( $F =$

0.02;  $df = 1, 255$ ;  $P = .90$ ), controlling for disease duration, age, sex, and education.

IADL performance was analyzed in a sub-sample of  $n = 299$  subjects. Gene dose groups did not differ significantly in IADL performance ( $F = 0.78$ ;  $df = 1, 293$ ;  $P = .38$ ), controlling for disease duration, age, sex, and education.

ADCS-ADL performance was analyzed in a sub-sample of  $n = 154$  subjects. Gene dose groups did not differ significantly in ADCS-ADL performance ( $F = 1.15$ ;  $df = 1, 148$ ;  $P = .29$ ), controlling for disease duration, age, sex, and education.

#### *Prospective Analysis of Cognitive and Functional Progression*

Table 3 presents a summary of the prospective analyses of cognitive and functional progression in AD patients. It specifically contains the annualized rates of change for cognitive and functional measures according to BDNF genotypes.

#### *Val66met*

Annualized rate of change in MMSE performance was analyzed in a sub-sample of  $n = 215$  subjects. Subjects received an average of 6.7 assessments (range = 3-18) spanning 1.8 years of follow-up (range = 0.5-6.4). The rate of MMSE change did not differ significantly across gene dose groups ( $F = .00$ ;  $df = 2, 209$ ;  $P = 1.00$ ), controlling for age, sex, and education.

Annualized rate of change in ADAS-Cog performance was analyzed in a sub-sample of  $n = 179$  subjects. Subjects received an average of 9.4 assessments

(range = 3-25) spanning 1.6 years of follow-up (range = 0.5-5.8). The rate of ADAS-Cog change did not differ significantly across gene dose groups ( $F = .04$ ;  $df = 2, 173$ ;  $P = .97$ ), controlling for age, sex, and education.

Annualized rate of change in ADCS-ADL performance was analyzed in a sub-sample of  $n = 156$  subjects. Subjects received an average of 7.1 assessments (range = 3-18) spanning 1.4 years of follow-up (range = 0.5-4.9). The rate of ADCS-ADL change did not differ significantly across gene dose groups ( $F = .41$ ;  $df = 2, 150$ ;  $P = .67$ ), controlling for age, sex, and education.

#### *C270T*

Annualized rate of change in MMSE performance was analyzed in a sub-sample of  $n = 213$  subjects. Subjects received an average of 6.7 assessments (range = 3-18) spanning 1.8 years of follow-up (range = 0.5-6.4). The rate of MMSE change did not differ significantly across gene dose groups ( $F = .69$ ;  $df = 1, 208$ ;  $P = .41$ ), controlling for age, sex, and education.

Annualized rate of change in ADAS-Cog performance was analyzed in a sub-sample of  $n = 179$  subjects. Subjects received an average of 9.4 assessments (range = 3-25) spanning 1.6 years of follow-up (range = 0.5-5.8). The rate of ADAS-Cog change did not differ significantly across gene dose groups ( $F = 2.47$ ;  $df = 1, 174$ ;  $P = .12$ ), controlling for age, sex, and education.

Annualized rate of change in ADCS-ADL performance was analyzed in a sub-sample of  $n = 154$  subjects. Subjects received an average of 7.1 assessments (range = 3-18) spanning 1.4 years of follow-up (range = 0.5-4.9). The rate of

ADCS-ADL change did not differ significantly across gene dose groups ( $F = .01$ ;  $df = 1, 149$ ;  $P = .94$ ), controlling for age, sex, and education.

#### *G-712A*

Annualized rate of change in MMSE performance was analyzed in a sub-sample of  $n = 198$  subjects. Subjects received an average of 6.7 assessments (range = 3-18) spanning 1.8 years of follow-up (range = 0.5-6.4). The rate of MMSE change did not differ significantly across gene dose groups ( $F = 1.33$ ;  $df = 1, 193$ ;  $P = .25$ ), controlling for age, sex, and education.

Annualized rate of change in ADAS-Cog performance was analyzed in a sub-sample of  $n = 171$  subjects. Subjects received an average of 9.4 assessments (range = 3-25) spanning 1.6 years of follow-up (range = 0.5-5.8). The rate of ADAS-Cog change did not differ significantly across gene dose groups ( $F = 3.24$ ;  $df = 1, 166$ ;  $P = .07$ ), controlling for age, sex, and education.

Annualized rate of change in ADCS-ADL performance was analyzed in a sub-sample of  $n = 145$  subjects. Subjects received an average of 7.1 assessments (range = 3-18) spanning 1.4 years of follow-up (range = 0.5-4.9). The rate of ADCS-ADL change did not differ significantly across gene dose groups ( $F = 0.83$ ;  $df = 1, 140$ ;  $P = .40$ ), controlling for age, sex, and education.

#### *Analysis of Behavioral Symptoms*



Table 4 summarizes the behavioral outcomes measured by the NPI, including percentage of subjects with each neuropsychiatric symptom as well as odds ratios for these symptoms by genotype.

NPI data were available for  $n = 248$  subjects in our AD sample. Of these, val66met and C270T genotypes were available for  $n = 241$  subjects, and G-712A genotypes were available for  $n = 222$ . No significant differences in Total NPI score across genotype groups were found by a Kruskal-Wallis  $H$  test for val66met ( $\chi^2 = 1.61$ ,  $df = 2$ ,  $N = 241$ ,  $p = .45$ ). Carriers of the C270T-T allele had a higher mean rank for Total NPI score as revealed by Mann-Whitney  $U$  test, indicating a greater prevalence of behavioral disturbances in that group ( $Z = -2.11$ ,  $N = 241$ ,  $p = .035$ ). G-712A genotype groups did not differ in Total NPI score ( $Z = -1.66$ ,  $N = 222$ ,  $p = .098$ ).

Our selection criterion identified  $n = 86$  subjects with the Psychosis  $\geq 1$  phenotype ( $n = 164$  subjects without). This phenotype was not associated with genotype for val66met ( $OR = 2.94$ , 95% CI = [0.58, 15.04]), C270T ( $OR = 2.23$ , 95% CI = [0.88-5.63]), or G-712A ( $OR = 1.89$ , 95% CI = [0.71-4.97]).

In our exploratory logistic regression models, neither val66met nor G-712A genotype was associated with the appearance of behavioral disturbances on any NPI domain. The C270T-T allele was associated with the presence of hallucinations ( $OR = 3.25$ , 95% CI = [1.22-8.62],  $p = .018$ ) but not with disturbances in any other specific NPI domain.

## Discussion

This is the first study to evaluate the role of three BDNF polymorphisms with respect to cognitive and functional progression and behavioral symptoms in AD. Analysis of val66met found met homozygotes to have significantly lower educational attainment relative to val/met and val/val genotypes. Although retrospective and prospective analyses of cognitive and functional progression revealed no association with the val66met, C270T, or G-712A polymorphisms, analysis of behavioral symptoms demonstrated an association between the C270T-T allele and presence of hallucinations as well as increased total NPI score.

The most striking finding of this study was the highly significant effect of val66met genotype on educational attainment, as we hypothesized, demonstrating an association between met homozygosity and fewer years of education. Limited research has been conducted examining the relationship between educational attainment and BDNF genotype. Chuu et al. demonstrated within an AD population a trend towards lower educational attainment among met homozygotes (13.2 years vs. 14.0 years for val/val and 14.3 years for val/met genotypes), consistent with our finding, although this difference was not statistically significant (81). This trend was also true of a recent study by Borroni et al. in which met homozygotes had 0.99 fewer years of education than val homozygotes and 0.91 fewer years than heterozygotes (63). Taylor et al. observed no difference in education level between val homozygotes and met carriers among patients with depression and healthy controls; met homozygotes were not distinguished from

heterozygotes in this study due to small sample size (82). This is consistent with our finding inasmuch as our group demonstrated a significant difference only between met homozygotes and the other genotypes. Egan et al. demonstrated no significant effect of val66met on educational attainment among patients with schizophrenia, their siblings, or healthy controls; however, met homozygotes trended toward *more* years of education—17.7 years—when compared with val homozygotes (16.2 years) and heterozygotes (16.7 years) (38).

The discrepancies among these studies may be explained in part by the presence of AD among patients in our sample and in the Chuu et al. and Borroni et al. studies. Low level of educational attainment has been described as a risk factor for AD (83-90). It has been demonstrated that patients with higher educational attainment perform better on neuropsychological testing and are less likely to meet criteria for dementia when at the same neuropathological stage of AD as less-educated counterparts (91, 92). A systematic review of studies of “cognitive reserve” and risk of dementia found a 46% decreased risk of AD among patients with a high brain reserve (that is, those with “complex mental activity across the lifespan”) (93). Whether education provides a cognitive reserve to protect individuals from developing AD, or more highly educated people perform better on the neuropsychological testing employed to diagnose dementia, is a point of debate (90). It is possible that the effect of genotype on educational attainment observed in our study might be a consequence of the sample of AD patients, as they may have had a lower baseline cognitive reserve than a sample of non-AD counterparts.

Although the relationship of BDNF polymorphisms and educational attainment has not been studied in detail, researchers have examined the association of BDNF polymorphisms with memory performance. Several studies suggest that met carriers exhibit impaired episodic memory relative to val homozygotes (38-40). A second interpretation of our finding might be that patients who are met homozygotes (or, taking results of prior studies into consideration, met carriers, as our study may not have been sensitive enough to detect significant differences in educational attainment among heterozygotes) have impaired memory ability, consequently may have more difficulty with academic performance, and ultimately may have lower overall educational attainment. In conjunction with the cognitive reserve hypothesis, it would follow that if lower educational attainment is associated with risk of AD, one would expect met carriers to demonstrate higher rates of AD than val homozygotes. However, the vast majority of studies examining risk of AD with the val66met polymorphism demonstrate no association (20, 25-28, 30-34), or association of the val allele with AD (19, 37). Additionally, one would expect that met carriers would have a lower age of onset, as we hypothesized, because they lack the protective factor of cognitive reserve. However, our study suggests that there is no association, and this is in agreement with other studies (30, 94).

The other significant demographic findings of this study—that met homozygotes were more likely to be taking acetylcholinesterase inhibitors at the time of assessment, and that significantly more women carried the A712 allele—are more challenging to interpret. The significantly higher use of

acetylcholinesterase inhibitors may be related to the findings that the met allele predisposes to impaired episodic memory relative to other genotype groups (38-40): a drop in memory performance from a lower baseline may incite these patients to seek medication at an earlier stage than counterparts who declined from a higher baseline and therefore remain more functional. Because so little is known about the G-712A polymorphism or its effects physiologically, reasons for women to carry the A712 allele are unclear and more research should be conducted to determine whether this relationship holds in non-AD samples.

The finding that val66met, C270T, and G-712A polymorphisms are unrelated to rate of cognitive decline in AD is consistent with a previous study in which val66met was found to have no association with rate of change of MMSE score (81). While Chuu et al. determined rate of change by sequential MMSE scores, our study determined rate of change both this way and retrospectively, using approximate onset date of AD to estimate disease duration at time of testing. Cognitive decline was additionally measured by ADAS-Cog scores, following methods to evaluate rate of cognitive decline previously employed by our group (77) and others (95). To our knowledge, this is the only study to offer both retrospective and prospective analyses of cognitive decline as well as to examine ADAS-Cog scores in relation to these BDNF polymorphisms. Of note, this methodology follows our previous study of ApoE, in which no relationship was established between rate of cognitive decline and ApoE, corroborating data from several other groups (96-109), but in contrast to data from three studies suggesting  $\epsilon 4$  accelerates rate of cognitive decline (95, 110, 111). It should be

clarified that no association between ApoE and BDNF genotypes has been established (20, 21, 25, 31, 33-35, 37), although it has been suggested that the C270T-T allele is more common among individuals lacking  $\epsilon 4$  (19), and the risk of AD conferred by the C270T-T allele is stronger in patients who do not carry  $\epsilon 4$  (23).

No studies were identified in the literature examining rate of functional decline with respect to BDNF polymorphisms. Borroni et al. demonstrated no association of val66met genotype with cross-sectional IADL score for patients with AD, but this single measurement did not estimate rate of decline (63).

The association of the C270T-T allele and increased total NPI score is difficult to interpret given the variety of psychiatric domains evaluated by this scale. We hypothesized that psychotic symptoms would be more prevalent in this genotype group given previous association studies with the polymorphism and schizophrenia. However, only “hallucinations” showed a significant association with the allele; no association with “psychosis” was established when hallucinations and delusions were considered together. This was of interest to our group given our previous finding of an association between ApoE  $\epsilon 4$  and psychotic symptoms in AD, particularly from an association with delusions (79). The differences between the BDNF and ApoE studies again reinforce that these are distinct genotypes whose effects are accrued independently from one another. Several studies in the literature have examined the relationship between BDNF polymorphisms and schizophrenia. Consistent with our finding, the C270T-T allele has been documented in higher frequency among patients with

schizophrenia than healthy controls (51, 52). Our finding that hallucinations were not associated with val66met genotype is consistent with the findings of Borroni et al., who found no association of psychotic symptoms in AD patients specifically with this allele (63). Although psychotic symptoms were not associated with val66met in our study, it has been reported that the val allele is associated with schizophrenia (48). There is also evidence for preferential inheritance of the val allele among patients with psychosis and schizophrenia spectrum disorder (50). Multiple studies, however, including one from our own group, suggest that BDNF polymorphisms have no association with schizophrenia (29, 53-55). Conflicting data among these studies emphasizes the need for further examination of the role of BDNF polymorphisms among patients with psychotic symptoms, both those with AD and those with schizophrenia.

Our study did not find an association of val66met genotype with total NPI score or sub-categories of the NPI. This disputes the recent investigation by Borroni et al. that demonstrated a significant association between the met allele and increased total NPI score as well as NPI depression and apathy scores (63). One possibility for this discrepancy is that the Borroni study examined a cohort of depressed AD patients that may have contributed a disproportionately large number of patients with neuropsychiatric disturbances to the sample. Our sample included 83 patients in a sample of 241 with positive depressive symptoms on the NPI, in contrast with 93 clinically depressed patients in a sample of 264 in the Borroni study. The key difference is that “depressive symptoms” observed on the NPI do not necessarily render a diagnosis of depression, and therefore the patients

in the Borroni sample likely had higher ratings of frequency, severity, and distress than our patients. Larger numbers of more severely depressed patients may have made this genetic relationship for depression significant, and apathy is a characteristic of depression that consequently became statistically significant as well.

A notable limitation of this study is selection bias: enrolled subjects were recruited from other research protocols at an academic medical center. Many of these protocols were investigational drug trials, which may have inadvertently changed the course of AD progression for our prospective and retrospective analyses. Most of these experimental treatments were not found to impact the course of AD, however, and any small effect was likely distributed randomly with respect to genotype. Ethnically, the patients were overwhelmingly Caucasian. None of the patients were institutionalized at the time of participation. Further research should be conducted before results of this study may be elaborated to other groups. Another significant limitation is reliance upon caregiver interview, which introduces recall bias both in estimating date of disease onset for retrospective analyses and in obtaining data for the NPI (78). Nonetheless, relatives' prospective and retrospective ratings have been found to be in close agreement, particularly when the patient still resides in the community (112).

This investigation finds its place in the new era of psychiatric research, that of the genetics of mental illness. On the road to this understanding, the complexities of these conditions emerge: a particular gene may be associated with only a small part of a disease presentation. In the case of this study, the



effects of a single nucleotide substitution become apparent: the C270T-T allele contributes to hallucinations and overall neuropsychiatric symptomatology in patients with AD. A nucleotide substitution in val66met yields a new amino acid, and that met allele in turn may predict an individual's ultimate educational attainment. It is through an aggregate of these individual changes that particular phenotypes of psychiatric illnesses evolve. "Dementia" is no longer dementia alone, but dementia with psychosis, dementia with depression, dementia with agitation; "depression" becomes depression with psychotic features, depression with anxiety, depression with agitation, and so on.

The clinical applicability of genotyping patients is limited at this stage of understanding. However, once the genetic basis of psychiatric illness is better elucidated, it has the potential to revolutionize the way patients are treated. In the case of patients with the C270T-T allele who develop AD, if it is known that these patients may be predisposed to develop hallucinations, then caregivers and physicians may be more in-tune with this psychotic symptom in the patient and consequently may be able to treat the patient from an early manifestation. This is true of other neuropsychiatric comorbidities of AD that may be associated with this allele and others as well. With respect to our finding of lower educational attainment in met homozygotes, if this finding holds true universally and not just in a sample of AD patients, then theoretically if children were known to be genetically disadvantaged to achieve academically, different approaches to teaching and learning that rely less on rote memorization and more on integrating

ideas and concepts may help a young student to achieve higher levels of schooling.

That stated, the concept of genotyping remains controversial in the public eye. Many at-risk individuals already refuse available genetic tests for heritable diseases such as Huntington's disease, colon cancer, and breast cancer, stating they "don't want to know" if they are at risk. In the case of AD, neither patients with mild cognitive impairment nor healthy adults are routinely screened for well-established predisposing genetic factors such as the ApoE  $\epsilon$ 4 allele given that the genotype does not guarantee development of the disease and therefore is of limited clinical utility. Some patients worry that discovering a genotype that predisposes to illness will also impact health insurance coverage, making plans less available and more costly. However, many individuals are curious to know their own health risks, and the idea of "personalized medicine" employing techniques such as genotyping is now becoming a reality.

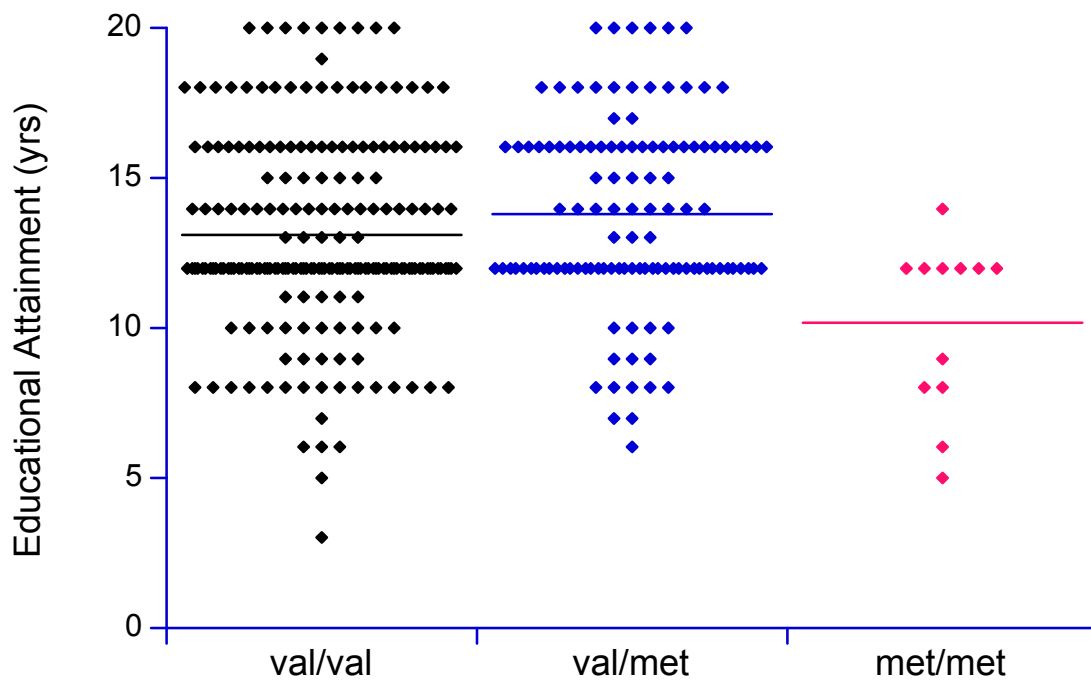
Although the findings of this study and others may not lead to immediate genotyping of patients with AD for reasons of practicality, genetic association studies are helpful in understanding the pathogenesis of the disease. As data is gathered throughout the field of genetic psychiatry, the molecular basis of psychiatric diseases is better understood. This understanding begins with the association, but from there many questions are raised: what about the protein coded by this gene is influencing the patient's symptomatology? What makes this different from the protein coded by this allele in a healthy subject? How is this different from the protein coded by a different allele in another patient with

similar symptomatology? How can we use the understanding of these proteins to develop new therapeutic strategies for our patients? Can new medications be tailored to genotype? These questions direct the next steps from this investigation: to examine BDNF genotype in non-AD samples to evaluate if met homozygotes achieve fewer years of schooling; to determine if non-AD age-matched subjects carrying the C270T-T allele are predisposed to increased neuropsychiatric disturbance; to elucidate what molecularly causes BDNF expression to decrease in AD, and whether decreased BDNF levels are a cause of the disease symptomatology or effect of some other pathogenesis; to continue to examine what other genetic factors may influence the variability of AD presentation; and to evaluate the role of BDNF as a target for AD therapeutics.

## **Conclusion**

This study demonstrates a relationship of the val66met genotype with educational attainment in which met homozygotes had significantly fewer years of education than either val/met or val/val counterparts. No association was found between cognitive and functional progression and BDNF genotype through retrospective and prospective analyses. An association between the C270T-T allele and hallucinations as well as overall NPI score was observed. This is the first study to exhibit these findings, which should be examined in a more diverse pool of subjects before generalizing the results. Additionally, more research should be conducted to understand more clearly the pathological link between genotype and cognitive, functional, and behavioral outcomes in AD.

Figure 1



Legend:

Fig. 1: Effect of val66met genotype on educational attainment. Met homozygotes exhibit significantly lower educational attainment than either val/val or val/met genotypes.

Table 1. Subject characteristics

	All Subjects	Val66Met			C270T		G-712A	
	(n=341)	Val/Val (n=203)	Val/Met (n=117)	Met/Met (n=12)	C/C (n=301)	T-carrier (n=31)	G/G (n=282)	A-carrier (n=28)
	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD	Mean SD
<b>Demographics</b>								
Age	73.8 ± 8.4	73.3 ± 8.4	74.4 ± 8.3	73.9 ± 7.4	73.6 ± 8.4	73.9 ± 8.5	73.7 ± 8.4	74.5 ± 8.1
Sex (% female)	62.8%	63.5%	58.1%	83.3%	61.1%	77.4%	59.6%	82.1% <sup>a</sup>
Education (years)	13.2 ± 3.3	13.1 ± 3.3	13.8 ± 3.2	10.2 ± 2.9 <sup>b</sup>	13.2 ± 3.4	13.1 ± 2.5	13.3 ± 3.4	12.8 ± 2.2
<b>Disease Characteristics</b>								
Onset age	69.3 ± 8.3	68.8 ± 8.3	70.0 ± 8.2	69.6 ± 8.2	69.17 ± 8.3	69.1 ± 8.2	69.3 ± 8.3	69.9 ± 8.2
Duration (years)	4.5 ± 2.0	4.5 ± 2.0	4.4 ± 2.1	4.3 ± 2.2	4.4 ± 2.0	4.8 ± 1.9	4.4 ± 2.0	4.6 ± 1.5
Family history (% positive)	48.8%	46.0%	54.7%	41.7%	49.5%	35.4%	51.2%	35.7%
ApoE e4 carrier (%)	57.4%	61.4%	52.6%	50.0%	58.3%	50.0%	57.5%	48.1%
<b>Concomitant Therapies at Baseline</b>								
Cholinesterase inhibitors (% use)	35.2%	38.4%	29.1%	58.3% <sup>c</sup>	35.5%	32.3%	33.3%	35.7%
Antipsychotics (% use)	3.5%	3.9%	3.4%	0.0%	3.3%	6.5%	2.8%	7.1%
Antidepressants (% use)	17.6%	18.2%	14.5%	41.7%	17.6%	16.1%	16.3%	21.4%
Vitamin E (≥400 IU daily) (% use)	36.1%	34.0%	40.2%	33.3%	38.2%	19.4%	37.2%	21.4%
Family history was positive if primary degenerative dementia was present in a first-degree relative.								
<sup>a</sup> A-carriers were disproportionately female ( $\chi^2=4.57$ , $P=.032$ )								
<sup>b</sup> Met/Met had significantly lower educational attainment than Val/Met ( $P=.00062$ ) and Val/Val ( $P=.0068$ ) (ANCOVA; post hoc Tukey test)								
<sup>c</sup> Met/Met was significantly more likely than Val/Val or Val/Met to be taking cholinesterase inhibitors ( $\chi^2=6.21$ , $P=0.045$ )								

**Table 2. Cognitive and functional data at baseline**

Variable	All Subjects	Val66Met			C270T		G-712A	
	(n=341) Mean SD	Val/Val (n=203) Mean SD	Val/Met (n=117) Mean SD	Met/Met (n=12) Mean SD	C/C (n=301) Mean SD	T-carrier (n=31) Mean SD	G/G (n=282) Mean SD	A-carrier (n=28) Mean SD
MMSE (n=341)	17.2±5.5	n = 203 17.3±5.4	n = 117 17.0±5.7	n = 12 17.8±5.9	n = 301 17.2±5.5	n = 31 17.2±4.7	n = 282 17.2±5.6	n = 28 17.5±4.5
ADAS-Cog (n=282)	27.1±12.0	n = 167 27.1±12.4	n = 97 27.7±11.5	n = 10 24.5±11.2	n = 252 27.2±12.1	n = 23 27.8±10.4	n = 239 27.4±12.1	n = 22 27.9±10.8
IADL (n=329)	0.65±0.19	n = 196 0.65±0.19	n = 112 0.65±0.19	n = 12 0.65±0.17	n = 289 0.65±0.19	n = 31 0.68±0.18	n = 271 0.65±0.19	n = 28 0.68±0.17
ADCS-ADL (n=170)	57.3±14.4	n = 100 57.8±13.4	n = 58 56.0±16.6	n = 7 62.0±6.7	n = 149 57.3±14.3	n = 16 57.0±12.7	n = 141 57.4±14.6	n = 13 59.5±10.7

Data displayed are baseline cognitive and functional measures adjusted for disease duration, patient age, sex, and education. MMSE = Mini-Mental State Examination; ADAS-Cog = Alzheimer's Disease Assessment Scale–Cognitive Subscale; IADL = Instrumental Ac

**Table 3. Prospective analysis of cognitive and functional data**

Variable	All Subjects	Val66Met			C270T		G-712A	
	(n=341) Mean SD	Val/Val (n=203) Mean SD	Val/Met (n=117) Mean SD	Met/Met (n=12) Mean SD	C/C (n=301) Mean SD	T-carrier (n=31) Mean SD	G/G (n=282) Mean SD	A-carrier (n=28) Mean SD
MMSE annual change	n = 220 -3.12±3.70	n = 135 -3.17±3.93	n = 73 -3.03±3.51	n = 7 -3.34±1.60	n = 197 -3.11±3.73	n = 16 -3.93±3.83	n = 184 -3.04±3.83	n = 14 -4.19±3.98
Number of observations	6.8±3.5	6.7±3.1	6.9±4.1	6.4±2.7	6.9±3.5	5.3±2.9	7.0±3.6	4.7±2.2
Duration of observation (yrs)	1.79±1.31	1.73±1.246	1.87±1.45	1.51±0.65	1.82±1.34	1.32±1.02	1.84±1.36	1.14±0.75
ADAS-Cog annual change*	n = 184 5.93±9.03	n = 107 6.13±10.34	n = 66 5.83±7.18	n = 6 5.70±3.77	n = 165 5.63±8.87	n = 14 9.53±11.18	n = 157 5.52±8.97	n = 14 9.81±11.27
Number of observations	9.4±5.1	8.9±5.0	10.1±5.3	9.7±4.4	9.3±5.0	10.4±7.2	9.5±5.0	9.2±5.8
Duration of observation (yrs)	1.63±1.22	1.51±1.23	1.78±1.24	1.61±0.63	1.63±1.22	1.55±1.27	1.66±1.25	1.36±1.07
ADCS-ADL annual change	n = 160 -10.37±11.17	n = 97 -10.10±10.84	n = 53 -10.34±11.63	n = 6 -14.12±13.03	n = 143 -10.68±11.51	n = 11 -9.55±8.49	n = 135 -10.34±11.59	n = 10 -12.41±10.02
Number of observations	7.1±3.2	6.7±2.7	7.8±4.0	6.7±2.1	7.1±3.3	5.7±1.7	7.2±3.4	5.9±1.4
Duration of observation (yrs)	1.39±1.01	1.30±0.89	1.57±1.23	1.16±0.43	1.40±1.04	0.90±0.41	1.43±1.07	0.90±0.34

Data displayed are longitudinal rates of change per year computed by linear regression.  
 \*Rates of ADAS-Cog change are positive because lower scores indicate better performance.  
 MMSE = Mini-Mental State Examination; ADAS-Cog = Alzheimer's Disease Assessment Scale–Cognitive Subscale;  
 ADCS-ADL = Alzheimer's Disease Cooperative Study–Activities of Daily Living.

**Table 4. Analysis of neuropsychiatric symptoms**

NPI Subscore	Frequency (n=248)		Val66Met (n=241)	C270T (n=241)	G712A (n=222)
	n	%	OR [95% CI] <sup>a</sup>	OR [95% CI] <sup>b</sup>	OR [95% CI] <sup>b</sup>
Delusions	76	30.6	0.64 [0.38-1.09]	1.39 [0.54-3.6]	1.44 [0.53-3.91]
Hallucinations	39	15.7	0.73 [0.38-1.40]	3.25 [1.22-8.62] <sup>c</sup>	2.76 [0.98-7.78]
Aggression	83	33.5	0.68 [0.41-1.13]	2.48 [0.97-6.33]	1.89 [0.70-5.14]
Anxiety	73	29.4	0.95 [0.58-1.55]	1.70 [0.68-4.24]	1.15 [0.41-3.22]
Depression	83	33.5	0.83 [0.51-1.33]	1.47 [0.60-3.64]	1.99 [0.78-5.10]
Elation	18	7.3	*	*	*
Apathy	95	38.3	0.82 [0.52-1.31]	1.68 [0.69-4.12]	1.55 [0.59-4.02]
Disinhibition	53	21.4	0.86 [0.49-1.50]	1.73 [0.66-4.58]	1.43 [0.47-4.33]
Irritability	80	32.3	0.94 [0.59-1.51]	1.12 [0.44-2.83]	1.41 [0.53-3.72]
Aberrant Motor Behavior	96	38.7	0.71 [0.44-1.16]	1.51 [0.61-3.78]	1.04 [0.39-2.78]
Sleep Disturbances	54	21.8	0.99 [0.57-1.70]	1.20 [0.43-3.31]	0.96 [0.30-3.11]
Eating Disturbances	50	20.2	1.42 [0.84-2.42]	0.57 [0.16-2.02]	0.48 [0.10-2.19]

<sup>a</sup>Odds Ratios for val66met are for the effect of Met-allele dose (2,1,0).

<sup>b</sup>Odds Ratios for for C270T and A712G are for carrier status of the less common allele (C270T-T, A712G-A).

<sup>c</sup>Genotype significantly increases risk for symptom, p<.05.

\*No carriers of less common allele exhibited Elation.

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