UNIVERSITY OF CALIFORNIA, SAN DIEGO

SAN DIEGO STATE UNIVERSITY

# APOE Genotype and Cognitive Functioning in School-Aged Children:

# A Risk Factor for Decreased Cognitive Reserve or an Example of Antagonistic Pleiotropy?

A Dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Clinical Psychology

by

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The Dissertation of Cinnamon Sue Bloss is approved, and it is acceptable in quality and form for publication on microfilm:

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2007

# EPIGRAPH

I have called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection.

Charles Darwin

How paramount the future is to the present when one is surrounded by children.

Charles Darwin

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ABSTRACT OF THE DISSERTATION

APOE Genotype and Cognitive Functioning in School-Aged Children:

A Risk Factor for Decreased Cognitive Reserve or an Example of Antagonistic Pleiotropy?

by

Cinnamon Sue Bloss

Doctor of Philosophy in Clinical Psychology

University of California, San Diego, 2007

San Diego State University, 2007

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Although the impact of the ε4 allele of the Apolipoprotein E gene (APOE-ε4) on cognitive functioning in adults has been widely studied, researchers have produced little information regarding the nature of this relationship in children. Broadly, the

current studies aimed to explore APOE-related differences in cognitive functioning in early life. Study 1 explored the relationship between APOE genotype and educational attainment in a sample of elderly adults. Results indicated that even after accounting for Alzheimer's disease (AD) diagnostic status and gender, ε4-positive individuals attained fewer years of education relative to ε2-positive individuals. Studies 2, 3, 4 and 5 examined APOE-related differences in cognitive and achievement test performances in a sample of school-aged children. Scores on group achievement tests and on a measure of visuospatial functioning were compared between the genotype groups in Study 2, and results indicated significantly worse performance among ε2-positive children relative to ε3/3 homozygotes and ε4-positive children on a test of visuospatial functioning. Notably, an increased prevalence of left-handedness was also observed among ε2-positive individuals in both Studies 1 and 2. Study 3 examined achievement test data from multiple time points, and results indicated generally stable and equivalent performances over time across APOE genotype. Study 4, which is based on findings of atypical hemispheric asymmetries in normal-functioning young adults, investigated the extent to which the ε4 allele is associated with cognitive discrepancies in school-aged children. Findings indicated that APOE genotype is associated with cognitive asymmetry in early life, but that gender is a significant moderator of this association. Finally, Study 5, a preliminary study, explored whether APOE genotype interacts with a family history of AD to further impact the development of cognitive reserve. Remarkably, results showed a synergistic effect of APOE-ε4 status and family history of AD such that children with

both risk factors showed the lowest test scores. Taken together, these studies suggest that APOE genotype is associated with early life brain and cognitive functioning. Results are discussed within the context of antagonistic pleiotropy, which suggests that APOE genotype may be associated with different effects at different developmental stages.

# Introduction

## *Overview*

There is now evidence that abnormal aging, and in particular the development of Alzheimer's disease (AD), is associated with early-life risk factors, including poor perinatal conditions, sub-optimal early-life brain development and body growth, poor early-life socioeconomic conditions, and decreased cognitive reserve, including lower educational attainment (for review see Borenstein, Copenhaver, & Mortimer, 2006). Although it has been acknowledged that these developmental risk factors may actually reflect a genetic contribution (Borenstein et al., 2006), the aging literature has placed an emphasis on understanding the extent to which these early risk factors interact with what is conceptualized as separate and *independent* genetic risk (i.e., APOE genotype) to influence the development of AD.

In contrast, there is the possibility that APOE itself is associated with the presence or absence of one or more of these early-life risk factors, an idea which has been previously proposed (Richards & Sacker, 2003). For example, instead of APOE and low educational attainment each independently contributing to the clinical expression of AD, it could be that APOE genotype independently influences educational attainment and thus itself contributes to the development of decreased cognitive reserve. In sum, the question is whether individuals at genetic risk for abnormal aging by virtue of their APOE genotype are impacted by this risk in childhood, and if so, how?

Evidence from neuropathology (Ghebremedhin, Schultz, Braak, & Braak, 1998; Morishima-Kawashima et al., 2000; Peskind et al., 2007), neuroimaging (e.g., Reiman et al., 2004), and neurocognitive (B. J. Small, Rosnick, Fratiglioni, & Backman, 2004; Teasdale, Murray, & Nicoll, 2005) studies suggests the presence of APOE-related differences at least as early as young adulthood. In addition, epidemiological studies of early-life risk factors (e.g., small head circumference, lower educational attainment) and demographic variables including gender (Corder et al., 2004; Ghebremedhin et al., 2001) have shown these variables to be differentially predictive of the development of AD depending on APOE genotype. Taken together, these studies raise the possibility that APOE genotype plays a role in brain and cognitive development starting very early in life.

Given what is already known about the role of the ε4 allele of APOE as a risk factor for the development of AD, it is tempting to assume that the effect of APOE-ε4 on early life brain and cognitive development would be negative. Indeed most, though not all (e.g., Mondadori et al., 2006), studies that have found APOE-ε4-related differences in middle-aged and young adults have interpreted these differences as "abnormalities," which is an interpretation that may very well be correct. Furthermore, one might also assume there would be a positive impact of the ε2 allele of APOE early in life, given its role as a protective factor with respect to the development of AD.

On the other hand, given the extent to which APOE-ε4 has been associated with several deleterious biological effects, from an evolutionary standpoint the

existence and persistence of this genotype among humans is a curious observation (Finch & Sapolsky, 1999). A benefit of APOE-ε4 during early life could potentially explain this observation. Antagonistic pleiotropy is a theory that posits that some individual loci or alleles have different effects on fitness at different ages (Albin, 1993). Specifically, if a locus or allele has a positive effect on fitness at a relatively early age and a deleterious effect on fitness at advanced ages, the pleiotropic allele would be favored to spread throughout the population because the early positive effect would increase total reproductive probability (Williams, 1957). Indeed, the relative frequencies of the APOE alleles suggests the possibility that natural selection may select against the ε2 allele (i.e., given its low frequency in the population) during early life in favor of the ε3 and ε4 alleles, which may confer beneficial effects during this time. Obviously this would occur at the expense of the detrimental effects of APOE-ε4 during the post-reproductive years (Becher, Keeling, McIntosh, Wyatt, & Bell, 2006; Wright et al., 2003).

In conclusion, it could very well be that APOE-ε4 is associated with negative effects in early life; however, the relative frequencies of the APOE alleles in the population raise the possibility that the opposite could also be true. Therefore, the current study sought to explore this issue by investigating the relationship between APOE genotype and cognitive test performance in school-aged children.

*APOE*

Apolipoprotein E (ApoE) is a polymorphic 299-amino acid protein, which is involved in the transport of cholesterol (Mahley, 1988). The three common ApoE

isoforms, ApoE-ε2, ApoE-ε3, and ApoE-ε4 are products of three alleles (ε2, ε3, and ε4) of the APOE gene (APOE, gene; ApoE, protein), which reside at a single locus on the long arm of chromosome 19 (Zannis, Just, & Breslow, 1981). These isoforms differ from one another at residues 112 and 158; that is, ApoE-ε3 has Cys-112 and Arg-158, while ApoE- ε4 has arginine at both positions, and ApoE-ε2 has cysteine at both positions (for review see Lahiri, Sambamurti, & Bennett, 2004). What is notable about this genetic variation is the well-known association between the ε4 allele of APOE with an increased risk of both familial and sporadic AD (Saunders et al., 1993; Strittmatter et al., 1993), a progressive and highly debilitating neurodegenerative disorder.

The three allelic variants of APOE can form six possible genotypes (i.e., APOE-ε2/2, APOE-ε2/3, APOE-ε2/4, APOE-ε3/3, APOE-ε3/4, and APOE-ε4/4). Studies have shown that in most populations the presence of at least one ε3 allele accounts for the majority of the APOE gene pool (i.e., 70 to 80 percent), and that the ε2 and ε4 alleles are observed in 5 to 10 percent and 10 to 15 percent of the population, respectively (Roses, 1996), though some studies have shown prevalence rates of ε4 as high as 26 percent (Menzel et al., 1995).

*APOE and AD*

The APOE-ε4 allele initially garnered a great deal of interest among scientists both because of the strength and reliability of its association with AD, as well as the extreme negative consequences of this disorder (Fazekas et al., 2006). Specifically, it was first demonstrated that the APOE-ε4 allele is over-represented in late-onset

familial AD patients (0.50), compared with age- and sex-matched controls (0.16) (Strittmatter et al., 1993); since then this association has been confirmed in other population-based studies. Furthermore, APOE-ε4 has been shown to have a gene-dose effect on the risk and age of onset of AD (Corder et al., 1993). Specifically, as the number of APOE-ε4 alleles increases, the inherited risk is further increased, and the mean age of onset is decreased. Notably, a number of studies have found that individuals carrying an APOE-ε2 allele are relatively protected from developing the disease (Corder et al., 1994; Graff-Radford et al., 2002; Murrell et al., 2006). However, while these studies collectively indicate that the APOE-ε4 allele is a powerful risk factor for AD, importantly, it is only a risk factor and it is neither necessary nor sufficient for the development of the disorder (Henderson et al., 1995; Hyman et al., 1996). That is, studies suggest that only about half of APOE-ε4 homozygotes develop AD by age 90 (Henderson et al., 1995), and only about 60 percent of AD patients are APOE-ε4 carriers (Mayeux et al., 1998). Overall, however, a meta-analysis of more than 15,000 cases confirmed the importance of APOE as a major susceptibility gene for AD at all ages and in all ethnic groups (Farrer et al., 1997). Furthermore, in recent years, investigations of APOE-ε4 have been expanded to include its role with respect to other neurologic disorders, including but not limited to cerebrovascular disease (Premkumar, Cohen, Hedera, Friedland, & Kalaria, 1996) and recovery from traumatic brain injury (TBI) (Han, Drake et al., 2007; Teasdale et al., 2005).

*APOE and Neurobiology*

Although the neurobiological mechanisms that underlie the association between APOE-ε4 and AD are not well understood, previous histopathological studies have reported that this allele co-localizes with neuritic plaques and neurofibrillary tangles, hallmark neuropathological lesions associated with AD (Namba, Tomonaga, Kawasaki, Otomo, & Ikeda, 1991; Tang et al., 1998; Wisniewski & Frangione, 1992). This observation suggests that the effect of the APOE-ε4 allele on AD may be mediated by an increase in the rate with which AD pathology accumulates in the brain (Ghebremedhin et al., 2001). However, other studies have suggested that APOE is involved in the normal maintenance and repair of neurons, and that APOE-ε3 is more effective than APOE-ε4 in these functions (Mahley & Huang, 1999; Weisgraber & Mahley, 1996). As such, this represents an alternative possibility with respect to the mechanism by which APOE influences the development of AD, as well as its observed association with other neurologic conditions.

*APOE in Normal-Functioning Adults*

Structural brain imaging studies have produced interesting findings with respect to brain changes in normal-functioning ε4-positive adults. An early study of a small sample of twins concordant for APOE genotype and with a mean age of 63 years found that subjects with one ε4 allele had smaller hippocampi than those without an ε4 allele (Plassman et al., 1997) despite equivalent performance on cognitive measures. Similarly, in a sample of individuals aged 50 to 62 years, Reiman and colleagues found a nonsignificant trend toward smaller hippocampal volume among ε4

homozygotes relative to individuals without a copy of the ε4 allele (Reiman et al., 1998). Another study of hippocampal and amygdalar volumes as a function of APOE genotype found that among individuals aged 60 to 90 without dementia, ε4-positive individuals had significantly smaller hippocampi and amygdalae on both the right and left side relative to ε3/3 subjects (den Heijer et al., 2002). However, it should also be noted that differences in memory performance were observed between the two groups on a word-list memory task, and that in contrast to expectations, ε2-positive individuals showed average volumes that approximated those of the ε4-positive group.

Among subjects aged 39 to 80 years, Tohgi and colleagues observed atypical decreased hippocampal asymmetry among ε4-positive individuals relative to ε4-negative individuals with comparable Mini-Mental State Examination scores (Tohgi et al., 1997). The decreased asymmetry appeared to be primarily due to reduced size of the right hippocampus among the ε4-positive subjects. These findings are similar to those of Lind et al. where among individuals aged 49 to 79 years, reduced right hippocampal volume among ε4-positive individuals was observed, and the difference was most pronounced *before* the age of 65 years (Lind, Larsson et al., 2006). However, it should be noted that ε4-positive individuals also made significantly more errors on a recognition memory task, and the number of errors was observed to correlate with right hippocampal volume. Finally, a very recent study examined gray matter density on voxel-based morphometry (VBM) in right-handed adults aged 19 to 80 (Wishart, Saykin, McAllister et al., 2006). After adjusting for age and gender, results showed that participants with one copy of the ε4 allele showed lower gray

matter density than ε3/3 participants in distributed cortical and subcortical regions, including right medial temporal, bilateral prefrontal and temporal cortex, and the cerebellum. Notably, the ε4-related differences were found to be related to individual differences in memory performance, and overall, the ε4 effect was larger in younger subjects.

Positron emission tomography (PET) studies of normal-functioning adults with and without the ε4 allele have also been conducted. In an early study that compared cognitively normal ε4 homozygotes and ε4-negative individuals aged 50 to 65 years (Reiman et al., 1996), reduced rates of glucose metabolism were observed among the ε4 homozygotes in parietal, temporal, prefrontal, and posterior cingulate regions, the same regions that have been observed to be affected in patients with probable AD. Similarly, a follow-up study of nondemented individuals aged 50 to 84 found that individuals with just one copy of the ε4 allele showed lowered inferior parietal, temporal, and posterior cingulate cortical metabolism relative to individuals without the ε4 allele (G. W. Small et al., 2000). Furthermore, this study also examined a subgroup of these individuals longitudinally and found that among the ε4-positive subjects, lower baseline metabolism predicted future cognitive decline. Remarkably, in a more recent study that included young adults aged 20 to 39 years of age (Reiman et al., 2004), one copy of the ε4 allele was associated with abnormally low rates of glucose metabolism bilaterally in the posterior cingulate, parietal, temporal, and prefrontal cortex, although the group did not differ in their neuropsychological test scores.

Functional magnetic resonance imaging studies (fMRI) have also found differences in activation in normal-functioning ε4-positive adults relative to noncarriers. Specifically, Bookheimer and colleagues were the first to demonstrate this effect (Bookheimer et al., 2000) among individuals aged 47 to 82 years of age using a verbal learning task. It was observed that both the magnitude and extent of brain activation during this task were greater among ε4-postive subjects in regions of the brain affected by AD, including the left hippocampal, parietal, and prefrontal regions. Bondi and colleagues demonstrated a similar effect among ε4-positive individuals relative to ε4-negative individuals with a mean age of 76 years using nonverbal stimuli, although the brain regions implicated were somewhat different (Bondi, Houston, Eyler, & Brown, 2005). Similarly, in a study that loaded more heavily on risk by including normal-functioning ε4-postive middle aged adults (i.e., age range 58 to 65 years) with a positive family history of AD, significantly increased activation in the left medial temporal lobe was associated with novel encoding (Fleisher et al., 2005). In a study of individuals aged 25 to 75 years of age (mean age 63 years), Wishart et al. found that one copy of the ε4 allele was associated with greater activity during a working memory task in the medial frontal and parietal regions bilaterally and in the right dorsolateral prefrontal cortex (Wishart, Saykin, Rabin et al., 2006). Finally, another study observed greater activation among ε4-positive individuals in multiple right hemisphere regions during a verbal paired-associate learning task than their demographically similar non-ε4 counterparts (Han, Houston et al., 2007).

Although a smaller number of studies have suggested that in fact the ε4 allele is associated with *decreased* activation during memory and semantic categorization tasks (Lind, Persson et al., 2006; Mondadori et al., 2006; Trivedi et al., 2006), as described above, most fMRI studies have found increased activation during task performance. This has largely been interpreted as reflective of a compensatory strategy among ε4-positive adults (i.e., these individuals require additional cognitive effort to achieve comparable performance levels on cognitive tasks) (Bondi et al., 2005; Bookheimer et al., 2000).

Findings from studies examining cognitive and neuropsychological functioning in normal-functioning ε4-positive and ε4-negative individuals have been less consistent than the neuroimaging studies reviewed above. In normal-functioning older adults, this genotype has been found to be associated with significant cognitive discrepancies in a naming versus block design test (Jacobson, Delis, Bondi, & Salmon, 2002), an auditory span versus spatial span test (Jacobson, Delis, Bondi, & Salmon, 2005), a verbal fluency versus design fluency test (Houston et al., 2005), and a global versus local processing test (Jacobson, Delis, Lansing et al., 2005). A recent study also demonstrated that normal-functioning ε4-positive elderly individuals exhibited significantly greater heterogeneity of variance on a word-list memory test than individuals without this genetic risk factor (Wetter et al., 2006).

Among middle-aged healthy adults, Flory and colleagues found that performance on learning and memory tasks was significantly reduced in ε4-positive individuals compared to non-ε4 individuals between 24 and 60 years of age (Flory,

Manuck, Ferrell, Ryan, & Muldoon, 2000). In addition, among individuals with chronic anxiety and a mean age of 50 years, decreased problem-solving ability was observed in ε4 homozygotes relative to noncarriers (Caselli, Reiman, Hentz, Osborne, & Alexander, 2004). Greenwood et al. (2000) conducted a study of the effects of APOE-ε4 genotype on performance on three experimental paradigms assessing basic attention functions and found that ε4-positive individuals showed deficits in specific components of visual attention (Greenwood, Sunderland, Friz, & Parasuraman, 2000). Specifically, results of a cued letter discrimination task and a cued visual search task demonstrated that ε4-positive individuals with a mean age of 58 years exhibited deficits in shifting spatial attention in response to invalid location cues and in adjusting the spatial scale of attention during visual search (i.e., ε4-positive individuals did not show a benefit from more precise precues).

Although the previously reviewed studies have found evidence of subtle cognitive deficits in normal-functioning ε4-positive versus ε4-negative young, middle-aged, and older adults, it should be noted that several other studies have failed to replicate these results (e.g., Plassman et al., 1997; B. J. Small, Basun, & Backman, 1998; Smith et al., 1998; Soininen & Scheltens, 1998).

*APOE in Early Life*

The few studies that have examined the effects of APOE early in life have produced somewhat mixed findings. Specifically, three previous studies of APOE-ε4 and cognitive functioning in children have produced negative findings. Turic and colleagues (2001) examined ε4 allele frequencies in two groups of children aged 6 to

15 years. This study included one group of 101 children with a mean IQ of 136 ("high *g*") and another group of 101 children with a mean IQ of 103 ("average *g*"). Results demonstrated that the frequency of the ε4 allele was not significantly different between the two groups (Turic, Fisher, Plomin, & Owen, 2001). Plomin et al. (1995) conducted a study of similar design but with fewer subjects, which examined ε4 allele frequencies of 24 children with high IQ scores (mean IQ 130) and 18 children with low IQ scores (mean IQ 82), and this study also failed to find significant differences (Plomin et al., 1995). Finally, Deary and colleagues (2002) examined the relationship between cognitive ability and APOE-ε4 genotype in individuals who were participants in the Scottish Mental Survey of 1932. Mean IQ scores at age 11 years on the Moray House Test (MHT), a test of verbal and nonverbal reasoning, were not observed to be significantly different between ε4-positive (99.4) and ε4-negative (100.8) individuals.

However, there is some support in the literature for the notion that the ε4 allele may be advantageous during early development while the ε2 allele may be detrimental. This evidence comes from a small number of developmental studies that have examined the role of APOE during the human perinatal and infancy periods of life. Specifically, one study found that the ε2 allele was over-represented in a Scottish cohort of perinatal deaths (Becher et al., 2006). In addition, another study found a decreased prevalence of the ε4 allele among spontaneous abortions (Zetterberg et al., 2002). These findings suggest, as the authors pointed out, that the presence of the ε4 allele may have a protective effect in pregnancy and that alternatively, possession of one or more ε2 alleles may be detrimental to outcome. Furthermore, study of a large

cohort of infants from an urban area found that ε4-positive babies had higher mental development index scores relative to ε4-negative babies, and that the same change in blood lead levels in these infants resulted in a significantly attenuated decrease in mental development index scores in ε4-carriers relative to non-ε4-carriers, suggesting possible protection against lead exposure associated with the ε4 allele in infants (Wright et al., 2003).

*Current Studies*

Broadly, the current studies aim to explore APOE-related differences in cognitive functioning in early life. Study 1 is a preliminary study that aims to explore the relationship between APOE genotype and educational attainment in a sample of elderly adults. Study 2 examines APOE-related differences in cognitive and achievement test performance in school-aged children. Study 3 is an investigation of whether APOE genotype influences change in achievement test performance over time among school-aged children. Study 4, a study that is based on findings of atypical hemispheric asymmetries in normal-functioning young adults, is a study of the prevalence and degree of APOE-related cognitive discrepancies in school-aged children. Finally, Study 5 is a preliminary study designed to explore whether APOE genotype interacts with family medical history, including a history of AD, to further impact the development of cognitive reserve.

## Study 1

Study 1 is a preliminary investigation of the extent to which APOE genotype is independently associated with a proxy for early cognitive development, that is, years of educational attainment, in elderly individuals. Many previous studies have found that lower educational attainment is associated with increased risk of AD (e.g., Roe, Xiong, Miller, & Morris, 2007). However, results of a recent population-based study suggest that educational attainment may also be independently associated with the presence of at least one copy of the APOE-ε4 allele, that is, even after controlling for a diagnosis of dementia (Winnock et al., 2002). In the current study, it was first hypothesized that APOE genotype would be associated with educational attainment such that ε2-positive individuals would demonstrate the highest educational attainment, followed by ε3/3 homozygotes, and with ε4-positive individuals attaining the fewest years of formal education (Hypothesis 1). Second, it was hypothesized that these differences in educational attainment would persist even after accounting for variance associated with a diagnosis of possible or probable AD (Hypothesis 2).

The second aim of Study 1 was to begin to explore the relationship between APOE genotype and handedness in elderly subjects. Specifically, previous studies have found that left-handedness is underrepresented among patients with AD compared with population norms (de Leon, la Regina, Ferris, Gentes, & Miller, 1986; Doody, Vacca, Massman, & Liao, 1999). Taken together with studies that have found the APOE-ε2 allele to also be underrepresented among patients with AD, it was hypothesized that the prevalence of left-handedness would be increased relative to

population norms among ε2-positive individuals and decreased relative to population norms among ε4-positive individuals (Hypothesis 3).

## *Method*

### *Subjects*

A total of 650 elderly subjects were initially identified from a larger pool of subjects participating in longitudinal studies of aging at the University of California, San Diego (UCSD). The reader should note that this larger pool of subjects represents a sample of convenience rather than a population-based sample. The individuals identified for participation in the current study represent a subset of subjects who, at the time of their initial visit, were at least 50 years of age, demonstrated a presentation consistent with that of either a healthy control or a diagnosis of possible or probable AD, and for whom basic demographic information was available. It was decided to exclude individuals less than 50 years of age in order to minimize the likelihood of individuals returning to school. In addition, this subset of subjects represents only English-speaking, Caucasian individuals due to well-known differential access to educational opportunities as a function of ethnic and racial background within this birth cohort.

The longitudinal studies were approved by the institutional review board at UCSD. Informed consent was obtained from the patient or his or her legal representative.

*Procedures*

*Clinical evaluation and diagnosis.* Subjects underwent extensive neurologic, medical, neuropsychiatric, and neuropsychological evaluations that have been described in detail (Galasko, Kwo-on-Yuen, Klauber, & Thal, 1990). At the initial evaluation, a final clinical diagnosis was made by two senior neurologists following review of a summary of the results from the entire evaluation (Salmon et al., 2002). The diagnosis of AD was based on National Institute of Neurological and Communication Disorders and Stroke/AD and Related Disorders Association (NINCDS/ADRDA) criteria for possible or probable AD (McKhann et al., 1984).

*DNA Samples.* DNA samples were obtained using a buccal swab technique. The samples were genotyped for APOE allele type using a polymerase chain reaction based method (Corder et al., 1993).

*Educational attainment and handedness.* Total years of educational attainment was based on participant self-report and determined according to widely accepted criteria (Heaton, Miller, Taylor, & Grant, 2004). Hand dominance for writing was based on self-report as well as on observation during the clinical evaluation. Subjects consisted of individuals who were right-hand dominant for writing, left-hand dominant for writing, or ambidextrous.

*Statistical Analyses*

All statistical analyses were conducted using SPSS statistical software. Data were screened for extreme values with respect to years of educational attainment. One-way ANOVA was used to test for mean differences in years of educational

attainment as a function of APOE genotype and as a function of AD diagnostic status. In addition, hierarchical multiple regression was employed to examine the proportion of variance in years of education attained that is accounted for by APOE genotype independent of AD diagnostic status. In addition, given statistically significant differences in age observed among the APOE genotype groups, age was included as an independent variable in all regression models. In the first model, a set of dummy-coded variables representing the 3 genotype groups (i.e., ε2-positive, ε3/3 homozygotes, and ε4-positive) were entered into the regression equation with the ε4-positive group coded as the reference group. In the second model, AD diagnostic status (i.e., healthy control or possible/probable AD) was added to the equation with healthy controls coded as the reference group. In a third model, the proportion of variance accounted for by gender was additionally examined with male participants coded as the reference group. Residuals for these data were generally normally distributed and variances were observed to be roughly equal.

In addition, chi-square tests were used to examine prevalence rates of left-hand dominance, right-hand dominance, and ambidexterity for writing as a function of APOE genotype and as a function of AD diagnostic status. Given the exploratory nature of the research questions being investigated, an alpha level of .05 was used in the interpretation of all results.

*Sample Size and Characteristics*

As previously mentioned, a total of 650 elderly subjects who met inclusion criteria were initially identified from a larger pool of subjects. Because the aim of this

study was to assess the independent effects of the APOE-ε4 allele in the absence of potential confounding effects of the APOE-ε2 allele, 15 individuals who were ε2/4 heterozygotes were excluded from further participation. Further, there were 3 individuals for whom level of educational attainment was low enough to be considered an extreme value (i.e., values fell more than three times the length of the interquartile range below the median), and thus those cases were removed from further analyses. A remaining total of 632 subjects were included in the study.

## *Results*

### *Basic Demographics*

Allele frequencies and other basic demographic data presented as a function of APOE genotype and AD diagnostic status can be found in Tables 1.1 and 1.2, respectively. The reader should note that the ε2-positive group is made up of ε2/3 heterozygotes ($n$ = 50), with the exception of 1 ε2/2 homozygote. The ε4-positive group was made up of both ε3/4 heterozygotes ($n$ = 230) and ε4/4 homozygotes ($n$ = 58). It should be noted that the allele frequencies observed in this sample of convenience are not consistent with frequencies observed in population-based studies. Specifically, the APOE-ε4 allele is overrepresented, which is not surprising given that subjects were recruited for studies of AD and other disorders of aging, and thus, this likely reflects recruitment and selection bias. With regard to the sample as a whole, there were a total of 324 men (51.2%) and 308 women (48.7%). Overall, ages ranged from 50.88 years to 101.40 years ($M$ = 74.20, $SD$ = 8.28) at the time of assessment, and the median year of birth was 1920 (range 1896 – 1947). Furthermore, given that

Table 1.1

*Subject Characteristics by Genotype*

| | ε2+ *n* = 51 | ε3/3 *n* = 293 | ε4+ *n* = 288 | *p* |
|---|---|---|---|---|
| Allele Frequency | 0.081 | 0.464 | 0.456 | n/a |
| Age at Exam in Years[a] | 76.26 (9.09) | 75.26 (8.63) | 72.75 (7.52) | <.0005[b] |
| Median Birth Year | 1920 | 1920 | 1921 | .010[b] |
| Male/Female | 33/18 | 140/153 | 151/137 | .072[c] |
| Handedness (R/L or A) | 45/6 | 279/14 | 272/16 | .017[c] |
| Education in Years[a] | 15.92 (2.75) | 14.80 (3.12) | 14.32 (2.96) | .001[b] |
| Diagnostic Status (NC/AD) | 39/12 | 147/146 | 66/222 | <.0005[c] |

[a]Data are presented as mean (standard deviation). [b]One-way ANOVA used to test group differences. [c]Chi-square used to test group differences.

Table 1.2

*Subject Characteristics by AD Diagnostic Status*

| | NC<br>*n* = 252 | AD<br>*n* = 380 | *p* |
|---|---|---|---|
| Age at Exam in Years[a] | 74.93 (9.30) | 73.71 (7.50) | .070[b] |
| Median Birth Year | 1921 | 1920 | .131[b] |
| Male/Female | 114/138 | 210/170 | .014[c] |
| Handedness (R/L or A) | 235/17 | 361/19 | .628[c] |
| Education in Years[a] | 15.36 (2.80) | 14.21 (3.11) | <.0005[b] |

[a]Data are presented as mean (standard deviation). [b]One-way ANOVA used to test group differences. [c]Chi-square used to test group differences.

statistically significant differences in age were observed between the genotype groups (see Table 1.1), age was included as an independent variable in all of the regression models.

*Educational Attainment*

One-way ANOVA revealed statistically significant differences in educational attainment between the genotype groups ($F(2, 629) = 6.625$, $p = .001$; Table 1.1) with ε4-positive individuals attaining the lowest number of years of education ($M = 14.32$, $SD = 2.96$) followed by ε3/3 homozygotes ($M = 14.80$, $SD = 3.12$) and with ε2-positive individuals attaining the highest number of years of education ($M = 15.92$, $SD = 2.75$). Furthermore, consistent with several previous reports in the literature (Katzman, 1993; Zhang et al., 1990), in the current sample of convenience, individuals with possible or probable AD attained significantly fewer years of education ($M = 14.21$, $SD = 3.11$) relative to normal controls ($M = 15.36$, $SD = 2.80$; $F(1, 630) = 22.498$, $p = <.0005$).

Although these reported mean differences in educational attainment as a function of APOE genotype and AD diagnostic status were observed, a further aim of this study was to investigate the relative proportion of variance accounted for by each of these variables. Hierarchical multiple regression was utilized for this purpose, and Table 1.3 depicts the results from all regression analyses. Model 1 ($F(3, 628) = 5.786$, $p = .001$; $R^2 = .027$) revealed that, as previously reported with ANOVA, ε4-positive individuals attained significantly fewer years of formal education relative to both ε2-positive individuals ($p <.0005$; $sr^2 = .007$) and ε3/3 homozygotes ($p = .029$; $sr^2 =$

Table 1.3

*Hierarchical Multiple Regression Models*

| Model | Model Terms | β | *p* | Adjusted R Square |
|---|---|---|---|---|
| 1 | ε3/3 versus ε4+ | .091 | .029 | |
| | ε2+ versus ε4+ | .153 | <.0005 | |
| | age | -.080 | .045 | .022 (Model 1) |
| 2 | ε3/3 versus ε4+ | .047 | .269 | |
| | ε2+ versus ε4+ | .106 | .013 | |
| | age | -.083 | .036 | |
| | AD versus NC | -.159 | <.0005 | .043 (Model 2) |
| 3 | ε3/3 versus ε4+ | .048 | .245 | |
| | ε2+ versus ε4+ | .081 | .051 | |
| | age | -.070 | .070 | |
| | AD versus NC | -.186 | <.0005 | |
| | Male versus Female | -.228 | <.0005 | .093 (Model 3) |

.021). Again, as would be expected based on findings from ANOVA, Model 2 ($F(4, 627) = 8.100$, $p < .0005$; $R^2 = .049$) revealed that individuals with possible or probable AD attained significantly fewer years of formal education relative to healthy control subjects ($p < .0005$; $sr^2 = .022$). However, in Model 2 the difference in years of education attained between ε4-positive individuals and ε2-positive individuals persisted ($p = .013$; $sr^2 = .009$), even after taking into account AD diagnostic status (Figure 1.1).

In Model 3 ($F(5, 626) = 13.890$, $p < .0005$; $R^2 = .100$) the additional proportion of variance accounted for by gender was examined, and results revealed that women in this cohort, as would be expected, attained significantly fewer years of education than men ($p < .0005$; $sr^2 = .051$). Furthermore, after taking into account gender and diagnostic category ($p < .0005$; $sr^2 = .030$), the difference in educational attainment between ε4-positive and ε2-positive individuals continued to account for a small proportion of independent variance, although strictly speaking only approached statistical significance ($p = .051$; $sr^2 = .005$).

*Handedness*

Chi-square analyses revealed significant differences in prevalence rates of hand dominance for writing as a function of APOE genotype ($\chi^2(4) = 12.033$, $p = .017$; Figure 1.2). Specifically, 6 out of 51 subjects (11.8%) were either left-hand dominant ($n = 3$) or ambidextrous ($n = 3$) in the ε2-positive group in contrast to only 14 out of 293 subjects (4.8%) in the ε3/3 homozygote group (left-hand dominant $n = 12$,

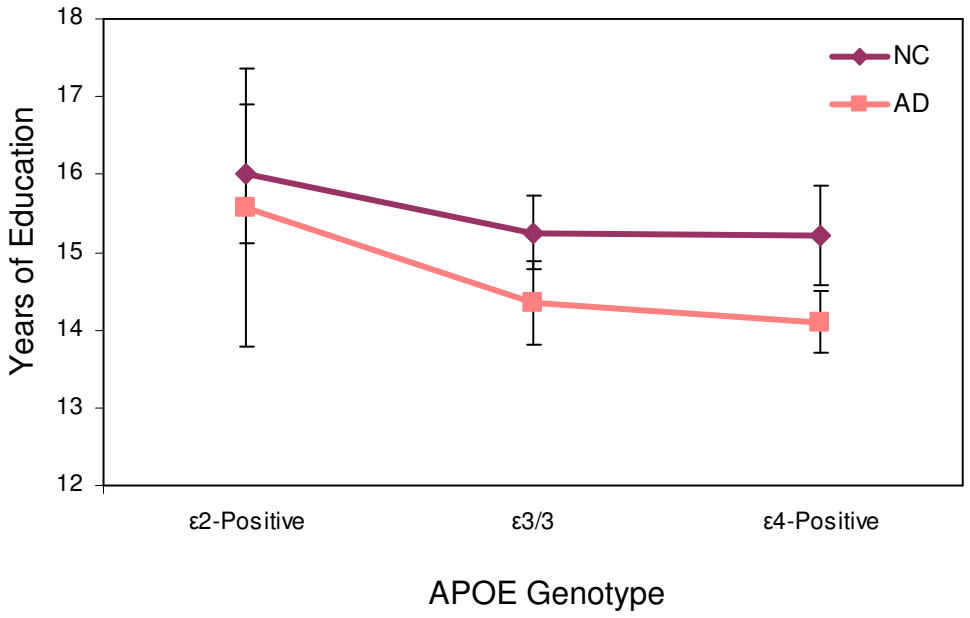


Figure 1.1. Mean years of education for normal control subjects (NC) and individuals diagnosed with possible or probable AD as a function of APOE genotype. Error bars represent 95% confidence interval.

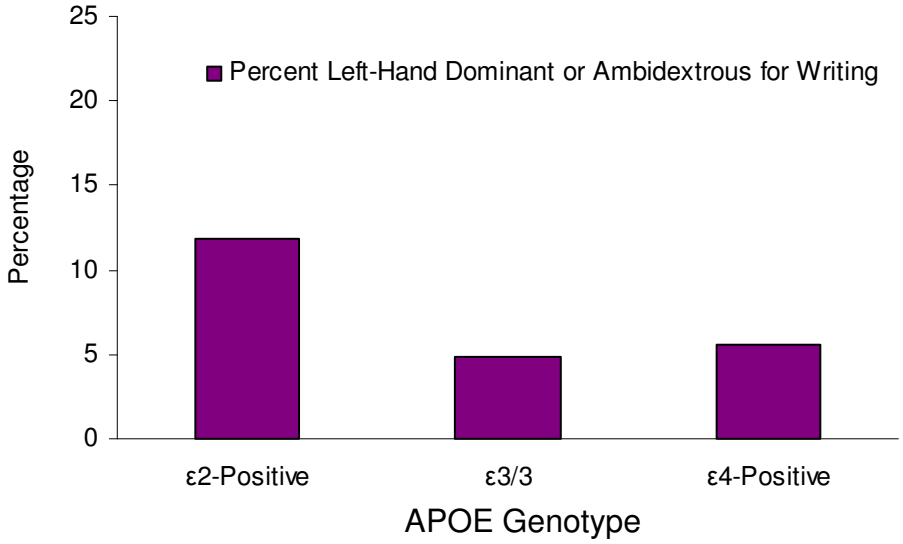


Figure 1.2. Percentage of individuals either left-hand dominant or ambidextrous for writing among a sample of elderly adults as a function of APOE genotype.

ambidextrous $n = 2$) and 16 out of 288 subjects (5.6%) in the ε4-positive group (left-hand dominant $n = 14$, ambidextrous $n = 2$). Chi-square analyses failed to find a significant difference in prevalence rates of left-hand dominance and/or ambidexterity for writing as a function of AD diagnostic status or as a function of gender.

### *Discussion*

Two primary findings emerged from Study 1. First, results suggest that there are mean differences in years of educational attainment as a function of APOE genotype in this sample of convenience such that ε4-positive individuals attained fewer years of formal education relative to ε2-positive individuals and ε3/3 homozygotes. Furthermore, regression analyses utilized to investigate the relative proportion of variance accounted for by genotype versus AD diagnostic status versus gender, raise the possibility that APOE genotype may account for a very small proportion of variance in years of educational attainment independent of AD diagnostic status and gender.

While this finding is intriguing, there are a several important caveats to be made explicit before interpreting this result. First, although ANOVA revealed significant mean differences in years of education as a function of APOE genotype (and as a function of AD diagnostic status, which is consistent with previous studies in this area), the proportion of variance accounted for by genotype independent of AD diagnostic status and gender (i.e., the results of Hierarchical Regression Model 3) was small and strictly speaking, did not reach statistical significance ($p = .051$; $sr^2 = .005$). Second, the regression model predicting educational attainment is clearly a simplified

model that did not include important covariates of educational attainment. In particular, for a cohort of this mean age, additional covariates that would likely account for variance include sociological variables (e.g., age of marriage), economic variables (e.g., economic depression, parental education/income/socioeconomic status), and historical variables (e.g., military service). Thus, although one would expect that the influence of these variables would generally be evenly distributed across the genotype groups, it is possible that after accounting for additional variables such as these, the remaining proportion of variance accounted for by APOE genotype, if any, may be trivial. Furthermore, it is also important to highlight the fact that the sample studied was a sample of convenience and included subjects who had been recruited and selected for participation in studies of aging, a process that often involved different recruitment strategies with respect to normal control subjects and individuals with AD. Specifically, there is likely a bias for subjects in this sample to be more highly educated than subjects in a population-based sample, and this bias is probably more pronounced with respect to the normal control subjects relative to the AD subjects, which is a potential confound that could have contributed to the differences in educational attainment observed. In short, before placing significant weight on this result, replication in a population-based sample, preferably taking into account additional important covariates of educational attainment as described above, is critical.

Keeping in mind these caveats, however, mean differences in educational attainment as a function of genotype were observed even after accounting for AD

diagnostic status. If it is the case that these differences represent true APOE genotype group differences, this finding raises the possibility that APOE genotype may exert a direct influence on cognitive development early in life, or it could also be the case that APOE genotype somehow influences access to educational opportunities (e.g., parental APOE genotype may influence family socioeconomic status). Alternatively, APOE may influence both factors in a synergistic fashion, which is certainly possible in light of the well-known complex interplay between the two.

The second primary finding that emerged from this study relates to handedness and suggests that prevalence rates of left-hand dominance for writing may differ as a function of APOE genotype. Specifically, a higher percentage of left-handed and ambidextrous individuals were observed among ε2-positive individuals relative to ε3/3 homozygotes and ε4-positive individuals. This finding raises the possibility that the presence of the ε2 allele may influence or be associated with factors that give rise to the development of atypical hemispheric dominance. Although quite speculative on the basis of the current study alone, it is possible that an association between atypical cerebral dominance and the ε2 allele may contribute to the mechanism by which the ε2 allele confers protection against the development of AD. That is, because cognitive abilities are more widely distributed in the brains of some left-handed persons, these individuals may be less susceptible to AD pathology or the clinical expression of AD pathology.

The finding of an increased prevalence of non-right-handedness among ε2-positive individuals may also provide clues as to the basis for the discrepant

prevalence rates of the different APOE genotypes themselves. The fact that the ε2 allele has been shown to be associated with protection against the development of AD, possibly with the absence of cognitive decline, and in the current study, with higher educational attainment, suggests that selection would favor this genetic variation. However, this selection advantage does not occur, as evidenced by the low prevalence of APOE-ε2 in the population. Some studies have found an elevated frequency of certain disorders in left-handed individuals and their families, including immune disorders, migraine, and developmental learning disorders (Geschwind & Behan, 1982). Further, another well-known theory for which there is considerable evidence is that there is an increased incidence of left-handedness (i.e., "pathological left-handedness") in children with early left hemisphere brain lesions (Satz, 1973). Given the apparent association between left-handedness and the ε2 allele, it could be that APOE-ε2 itself is associated with a high risk for disease and/or mortality, but only during infancy or early childhood. Then, if an ε2-positive child survives this period, it may be that the factors associated with survival confer later advantages such as predisposition to higher educational attainment and relative protection from the onset of pathological aging.

In addition to those caveats previously discussed, Study 1 has a number of other limitations that must be acknowledged. First, AD diagnostic status, and by extension normal control status, was not confirmed by autopsy. It is possible that a subgroup of those individuals identified as having AD may have been misdiagnosed, and of course the inclusion of both possible and probable AD patients in one group

introduces even further possibility for error in this regard. In addition, it is also possible that a subgroup of individuals initially identified as normal controls may go on to develop AD. Second, all of the participants in this study were Caucasian and English-speaking, which significantly reduces the generalizability of the findings. Furthermore, as previously stated, the fact that the sample studied was a sample of convenience and included subjects who had been recruited and selected for participation in studies of aging also decreases generalizability and introduces bias. Clearly there is a need for these findings to be replicated in population-based samples. However, it is notable that at least one other study of a community-based sample found lower educational attainment among ε4-positive individuals relative to ε4-negative individuals (Winnock et al., 2002), although other another study failed to find genotype group differences (Caselli et al., 2002), and another study found higher educational attainment among ε4-positive individuals (Hubacek et al., 2001). Finally, another significant limitation was the method used to assess handedness. Patient self-report and clinician observation of hand dominance for writing was used, but a superior study design would have involved the use of a thorough and standardized assessment of handedness (e.g., Oldfield, 1971).

Another critical consideration with respect to the findings of the current study is that the effect sizes observed are quite small, a limitation that was previously discussed with respect to educational attainment. Similarly, while differences as a function of APOE genotype in the prevalence of left-hand dominance and/or ambidexterity for writing were observed, the number and percentage of non-right-

handers among the ε2-postive group was still very low (6 out of 51 subjects or 11.8%). Therefore, while interesting in terms of understanding risk factors associated with AD, as well as factors contributing to the development of brain and cognitive reserve, the clinical significance of these findings at this point is questionable.

Overall, while this study is suggestive of a role for APOE in brain development and the development of cognitive reserve, it is clear that there are likely other factors moderating this role that were not accounted for in the present study. Furthermore, studies that examine the relationship between APOE genotype and early brain and cognitive development in children would assist in shedding additional light on these issues.

## Study 2

The first aim of Study 2 was to explore the extent to which the APOE-ε4 allele is associated with differences in cognitive and achievement test performance in school-aged children. Given that studies of APOE-ε4 and cognition in normal-functioning adults have produced mixed findings, it was first hypothesized that group differences on these measures would not emerge as a function of ε4 status (i.e., differences between ε4-positive children versus ε4-negative children) (Hypothesis 1).

However, findings from Study 1 suggested that level of educational attainment may be lower in ε4-positive adults relative to ε2-postive adults, and it has also been shown that individuals carrying an APOE-ε2 allele appear to be relatively protected from developing AD (Corder et al., 1994). Furthermore, among normal-functioning elderly, the ε2 allele has been associated with the absence of cognitive decline in some domains (Wilson, Bienias, Berry-Kravis, Evans, & Bennett, 2002). Thus, on the basis of these observations, it was further hypothesized that analysis of test performance by APOE genotype (i.e., ε2-positive children versus ε3/3 homozygotes versus ε4-postive children, rather than simply by ε4 status), would reveal small but significant differences on tests of language and reading (i.e., achievement domains that tend to be correlated with educational attainment). Specifically, it was anticipated that these differences would be characterized by better performance among ε2-positive children relative to ε4-positive children on these measures (Hypothesis 2).

The second aim of Study 2 was to explore the extent to which gender moderates the association between APOE genotype and cognitive and achievement

test performance in school-aged children. It is widely known that there are differential prevalence rates among boys and girls with respect to several neurodevelopmental disorders that impact cognition and are thought to have a genetic component (e.g., specific language impairment, attention-deficit hyperactivity disorder, autism). Further, it has been shown that the presence of a single APOE-ε4 allele appears to confer a greater risk for cognitive decline (Hyman et al., 1996) and AD (Farrer et al., 1997; Martinez et al., 1998; Payami et al., 1996) on adult women relative to men. In light of these observations, it was further hypothesized that, in general, ε4-positive girls would perform worse across measures of cognitive functioning relative to ε4-positive boys, ε4-negative girls, and ε4-negative boys (Hypothesis 3).

The third aim of Study 2 was to extend the findings of Study 1 by further examining the relationship between handedness and APOE genotype in school-aged children. As noted previously, a lower incidence of left-handedness compared to population norms has been observed among individuals with AD (de Leon et al., 1986; Doody et al., 1999). Taken together with the preliminary findings of Study 1, which showed a slightly higher percentage of individuals who were left-hand dominant or ambidextrous for writing among ε2-positive adults relative to ε3/3 homozygotes and ε4-positive adults, it is proposed that APOE genotype may also be associated with factors that give rise to handedness. Therefore it was further hypothesized that among this sample of school-aged children a higher prevalence of left-handedness would be observed among ε2-positive children relative to ε3/3-homozygoes and ε4-positive children (Hypothesis 4).

## *Method*

The study was approved by the Institutional Review Boards of the University of California, San Diego and San Diego State University. Informed consent was obtained from a parent of each participant, and informed assent was obtained from each participant.

### *Subjects*

*Recruitment.* Participants were recruited from a group of San Diego area charter middle schools and high schools. Recruitment proceeded as follows: Children at a specific school and in a specific grade were targeted for inclusion, and an email message was sent to parents of children in that grade. Concurrently, classroom presentations were made by the author to children in that grade, which typically lasted from 10 to 30 minutes and consisted of a short description of the study and a question and answer session for the students. An "informational booth" was then set up outside the school during after school hours. During this time parents and students could obtain additional information about the study and sign an informed consent agreement to participate if they chose to do so, which included a release of information providing access to the child's standardized group achievement test records.

*Screening.* Typically-developing children between the ages of 11 and 16 years were included in the study. Parents of participants were asked to complete an online demographic questionnaire pertaining to their child's developmental, medical, educational, psychiatric, and family medical history. Exclusion criteria consisted of the following: First language learned was not English, color blindness, uncorrected

visual impairment, upper extremity motor disability that may affect test performance on visual-motor tasks, genetic disorder known to affect central nervous system functioning (e.g., Fragile X), significant prenatal alcohol or drug exposure, history of head injury with loss of consciousness for greater than 10 minutes, and a diagnosed seizure disorder. The reader should note that a history of learning problems and/or attentional problems was not exclusionary. In addition, due to the fact that the children were tested in their classroom groups (see Procedures) it often occurred that a child was tested prior to their parent(s) completing the screening questionnaire. However, if it was determined after a child was tested that he or she did not meet inclusion criteria, their case was removed from further analyses. Overall, screening questionnaires were completed for approximately 80% of the sample. In the case where two or more siblings enrolled in the study, if applicable, either the male sibling and/or the sibling with achievement test data available were included.

*Procedures*

Once a number of students from a particular class had signed up to participate in the study, a lunchtime testing session was arranged with the teacher(s) of that particular class. On the specified date, children participating in the study remained in their classroom during lunch and were administered the Rey-Osterrieth Complex Figure Test (Osterrieth, 1944) Copy Condition in the group setting. Following administration of this measure, DNA samples were obtained from each child using a buccal swab technique (i.e., a mild brushing of the inside of the cheek). Then, as a group, the children received a complimentary pizza lunch for their participation (e.g.,

see Brown, Anderson, Schulte, Sintov, & Frissell, 2005). Finally, standardized group achievement test records were requested from the school for each participant. This procedure was repeated several times over the course of a five-month period during which all data were collected.

*DNA samples.* As previously stated, DNA samples were obtained using a buccal swab technique. This is a very simple, noninvasive, and widely used procedure. The samples were genotyped for APOE allele type using a polymerase chain reaction based method (Corder et al., 1993).

*Achievement tests.* Results from the California Achievement Test, Sixth Edition (CTB/McGraw-Hill, 2001) were requested from each participant's respective school. For some children the school did not have records on file, and in these cases, copies of test results were requested from parents. The California Achievement Test, Sixth Edition (CAT-6) is a standardized, multiple-choice, group achievement test that assesses basic skills in four broad domains, including Language, Reading, Spelling, and Mathematics. The Language subtest assesses skills related to vocabulary, grammar, usage, and literary analysis. The Reading subtest assesses basic reading skills, and the Spelling subtest assesses basic spelling skills. The Mathematics subtest assesses basic math skills, including computation and problem solving. The CAT-6 has been used in previous neurocognitive research; for example, it has been used to assess the effects of chronic antiepileptic drug therapy on academic achievement (Tennison et al., 1998). Standardized Normal Curve Equivalent (NCE) scores

80000provided for each subtest of the CAT-6 were used in the current study. NCE scores have a mean of 50 and a standard deviation of 21.06.

*Visuospatial test and handedness.* Standardized group achievement tests including the CAT-6, are relatively verbally-loaded tests. Therefore, in addition to examination of CAT-6 subtest scores, the Copy Condition of the Rey-Osterrieth Complex Figure Test (RCFT-CC) was administered to each child in the group setting and scored using the Taylor Scoring Criteria (Kolb & Whishaw, 1990; Waber & Holmes, 1985). A Z-score was calculated for each child, which was then transformed to a NCE score in order to facilitate direct comparisons with CAT-6 subtest scores. The RCFT-CC has been widely used to assess visuospatial perception and construction in adults and children (Fischer & Loring, 2004).

At this time, children were also asked to report their handedness for writing. Because a small number of children neglected to report their handedness, their parents were contacted via email in order to obtain this information.

*Statistical Analyses*

All statistical analyses were conducted using SPSS statistical software. RCFT-CC and CAT-6 subtest scores were found to be generally normally distributed and variances among the genotype groups were observed to be roughly equal. Data were also screened for the presence of extreme cases.

Univariate ANOVA was employed to examine differences in mean RCFT-CC and CAT-6 subtest scores as a function of APOE genotype and as a function of gender. Given that investigation of these research questions in a sample of school-

aged children can be considered exploratory, an alpha level of .05 was used in the interpretation of all results. Furthermore, significant main effects of genotype group (i.e., differences between ε2-positive children versus ε3/3 homozygotes versus ε4-postive children) were followed up by testing all pairwise comparisons utilizing Tukey's HSD procedure.

In addition, chi-square tests were used to examine prevalence rates of left- and right-hand dominance for writing as a function of APOE genotype. Differences in RCFT-CC test scores as a function of handedness were also examined, and this was done using Mann-Whitney U tests. This test is considered the nonparametric version of the t-test (Heiman, 1996). Again, an alpha level of .05 was used in the interpretation of all results.

*Sample Size and Characteristics*

A total of 196 school-aged children and adolescents were enrolled into the study. Of those, 177 participated in a lunchtime testing session, which included completion of the RCFT-CC and buccal swab testing. Genotype results were obtained for 163 of these subjects. Four female participants were excluded because each of them already had a sibling who was in the study, and 8 youth were excluded because they did not meet inclusion criteria. Also, because the aim of this study was to assess the independent effects of the APOE-ε4 allele in the absence of potential confounding effects of the APOE-ε2 allele, 4 ε2/4 heterozygotes were excluded from the analyses. In addition, there were 2 subjects for whom RCFT-CC scores were atypical and fell more than three standard deviations below the mean. These cases were considered

extreme cases and were thus removed from all subsequent analyses of RCFT-CC scores only (i.e., they were included in analyses of CAT-6 subtest scores).

Thus, the study included 145 subjects for whom basic demographic data, APOE genotype, and RCFT-CC results were obtained and included. Further, because 13 subjects who otherwise met inclusion criteria either had no group achievement test data available, or had group achievement test reports available from a test other than the CAT-6 (e.g., the Stanford Achievement Test, Ninth Edition), the study included a subset of 134 subjects on whom group achievement test data were also available.

## *Results*

### *Basic Demographics*

Allele frequencies and other basic demographic data presented as a function of genotype can be found in Table 2.1. The reader should note that the ε2-positive group consisted entirely of subjects who were ε2/3 heterozygotes, while the ε4-positive group included all ε3/4 heterozygotes with the exception of one subject who was an ε4/4 homozygote; none of the children who were genotyped were homozygous for the ε2 allele. With respect to the sample as a whole, ages ranged from 11.32 years to 16.84 years ($M$ = 13.34, $SD$ = 1.26), and there was a total of 63 boys (42.9%) and 84 girls (57.1%). The breakdown of the sample with regard to ethnicity was as follows: Asian, $n$ = 10 (6.8%); African American, $n$ = 9 (6.1%); Caucasian, $n$ = 97 (66.0%); Filipino, $n$ = 6 (4.1%); Hispanic, $n$ = 23 (15.6%); and Other, $n$ = 2 (1.4%). Parental educational attainment was assessed via parent-report, and these data were available for approximately 76.9% of the sample. Based on the information provided, years of

Table 2.1

*Basic Demographic Information by Genotype*

| | ε2+ *n* = 24 | ε3/3 *n* = 90 | ε4+ *n* = 33 | *p* |
|---|---|---|---|---|
| Allele Frequency | 0.163 | 0.612 | 0.224 | n/a |
| Age at Exam[a] | 13.54 (1.08) | 13.28 (1.30) | 13.34 (1.32) | .665[b] |
| Male/Female | 13/11 | 35/55 | 15/18 | .382[c] |
| Ethnicity | | | | .185[c] |
| Asian | 1 | 8 | 1 | |
| African American | 2 | 2 | 5 | |
| Caucasian | 17 | 60 | 20 | |
| Filipino | 2 | 4 | 0 | |
| Hispanic | 2 | 14 | 7 | |
| Other | 0 | 2 | 0 | |
| Handedness (R/L) | 17/7 | 82/8 | 31/2 | .012[c] |
| Parent Education in Years[a] | | | | |
| Mother[d] | 16.65 (1.93) | 16.28 (2.15) | 16.46 (2.17) | .769[b] |
| Father[e] | 16.75 (2.34) | 16.07 (2.44) | 16.21 (2.60) | .559[b] |

[a]Data are presented as mean (standard deviation). [b]One-way ANOVA used to test group differences. [c]Chi-square used to test group differences. [d]Based on *n* = 113. [e]Based on *n* = 112.

education were determined according to widely-accepted criteria (Heaton et al., 2004). Maternal years of education ranged from 12 to 20 ($M$ = 16.38, $SD$ = 2.10), and paternal years of education ranged from 10 to 20 ($M$ = 16.22, $SD$ = 2.45).

*Handedness*

With respect to the sample as a whole, there were a total of 17 youth (11.6%) who identified themselves as left-hand dominant for writing, which is slightly higher than overall lifetime population estimates (i.e., the prevalence of left-handedness decreases with age), but low as compared to previous estimates among children and adolescents (Annett, 2002). Notably, none of the children identified themselves as ambidextrous for writing.

Remarkably, analysis of prevalence rates of hand dominance for writing as a function of APOE genotype revealed a significantly higher prevalence rate of left-handedness among the ε2-positive children, $\chi^2(2) = 8.878$, $p = .012$ (see Table 2.1). Specifically, 7 out of 24 subjects (29.2%) were left-handed in the ε2-positive group, 8 out of 90 subjects were left-handed (8.9%) in the ε3/3 group, and 2 out of 33 subjects were left-handed (6.1%) in the ε4-positive group (Figure 2.1). Because of this observed difference in prevalence rates of hand dominance as a function of APOE genotype, separate analyses comparing left-handed and right-handed subjects with respect to observed significant genotype group differences in cognitive test performance were conducted.

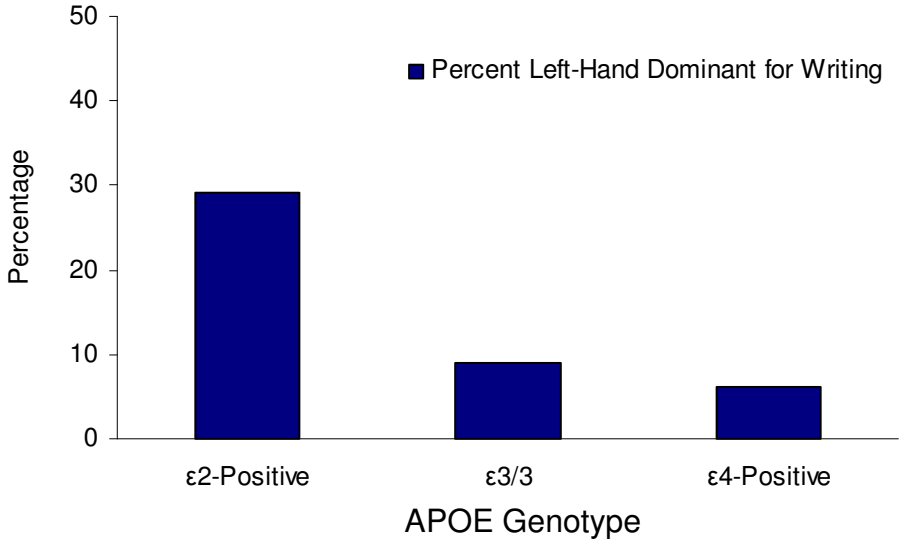


Figure 2.1. Percentage of individuals left-hand dominant for writing among a sample of school-aged children as a function of APOE genotype.

Table 2.2

*Mean Cognitive and Achievement Test Performance as a Function of APOE-ε4 Status*

| Test | ε4- | ε4+ | *p* |
|---|---|---|---|
| RCFT-CC[a] | 51.04 (13.49) | 54.56 (8.74) | .166 |
| Math[b] | 63.81 (19.72) | 61.43 (19.48) | .570 |
| Language[b] | 66.98 (20.87) | 64.79 (19.71) | .617 |
| Reading[b] | 66.60 (19.55) | 65.25 (15.06) | .734 |
| Spelling[b] | 63.85 (18.45) | 64.96 (24.95) | .793 |

[a]Based on a total of $n = 145$; data presented as mean (standard deviation). [b]Based on a total of $n = 134$; data presented as mean (standard deviation).

*Cognitive and Achievement Test Performance*

*Achievement test results.* Analysis of CAT-6 subtest scores as a function of APOE-ε4 status (i.e., ε4-positive versus ε4-negative), as predicted, failed to find a significant difference between the two groups on any of the tests (Table 2.2). Gender or the interaction between gender and ε4 status also did not significantly predict any of the CAT-6 subtest scores, although a main effect of gender on Spelling scores approached significance ($F(1, 130) = 3.698$, $p = .057$; $\eta_p^2 = .028$), with girls exhibiting a higher mean score than boys.

Follow-up analyses were performed to examine CAT-6 subtest scores as a function of APOE genotype group (i.e., ε2-positive versus ε3/3 homozygotes versus ε4-positive). Results of univariate ANOVA failed to find a significant main effect of genotype or gender, or a significant interaction between genotype and gender on any of the CAT-6 subtest scores (Table 2.3), which was contrary to predictions.

*Visuospatial test results.* Analysis of RCFT-CC scores as a function of APOE-ε4 status (i.e., ε4-positive versus ε4-negative) also failed to find a significant difference between the groups (Table 2.2). The extent to which gender or the interaction between gender and genotype predicted RCFT-CC scores was also examined, but neither effect was significant.

Again, follow-up analyses were performed to examine RCFT-CC scores among the genotype groups (i.e., ε2-positive versus ε3/3 homozygotes versus ε4-positive). Notably, results of univariate ANOVA revealed a main effect of APOE genotype ($F(2, 142) = 4.265$, $p = .016$; $\eta_p^2 = .057$) with respect to RCFT-CC scores

Table 2.3

*Mean Cognitive and Achievement Test Performance by APOE Genotype*

| Test | ε2+ | ε3/3 | ε4+ | $p$[c] |
|---|---|---|---|---|
| RCFT-CC[a] | 45.32 (16.19) | 52.58 (12.32) | 54.56 (8.74) | .016 |
| Math[b] | 67.67 (17.78) | 62.86 (20.15) | 61.43 (19.48) | .516 |
| Language[b] | 70.86 (16.46) | 66.02 (21.80) | 64.79 (19.71) | .558 |
| Reading[b] | 69.62 (18.31) | 65.86 (19.88) | 65.25 (15.06) | .673 |
| Spelling[b] | 66.52 (15.60) | 63.19 (19.11) | 64.96 (24.95) | .765 |

[a]Based on a total of $n$ = 145; data presented as mean (standard deviation). [b]Based on a total of $n$ = 134; data presented as mean (standard deviation). [c]Represents main effect of APOE genotype.

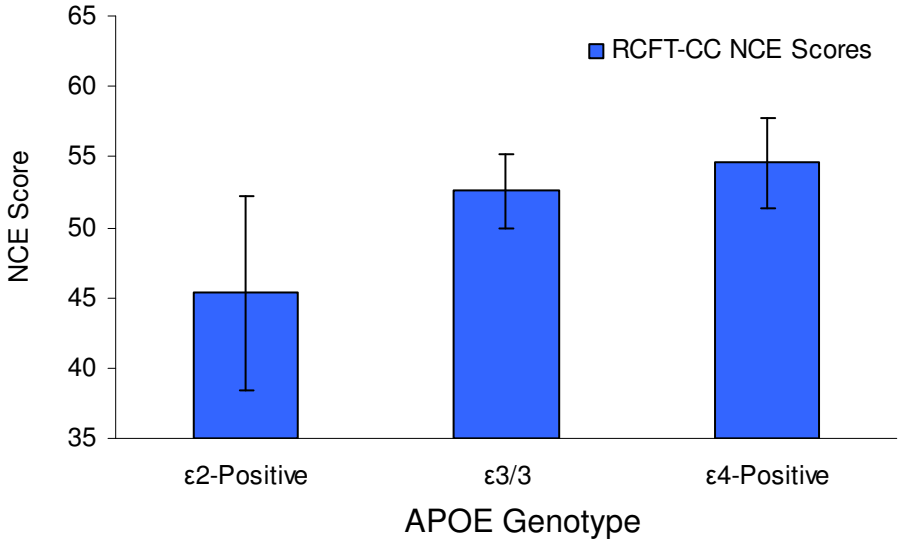


Figure 2.2. Mean RCFT-CC NCE scores for a sample of school-aged children as a function of APOE genotype. Error bars represent 95% confidence interval.

Table 2.4

*Mean Cognitive and Achievement Test Performance by APOE Genotype in Right- Versus Left-Handed Children*

| | ε2+ | | | ε3/3 | | | ε4+ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Right | Left | $p$[b] | Right | Left | $p$[b] | Right | Left | $p$[b] |
| RCFT-CC | 46.33 (16.69) | 42.87 (15.88) | .567 | 51.86 (12.49) | 59.91 (7.59) | .074 | 54.88 (8.91) | 49.80 (4.69) | .292 |
| | $n$ = 17 | $n$ = 7 | | $n$ = 81 | $n$ = 8 | | $n$ = 30 | $n$ = 2 | |
| Math[a] | 64.27 (16.73) | 76.17 (18.97) | .170 | 62.78 (19.02) | 63.71 (32.26) | .564 | 62.73 (19.40) | 44.50 (14.85) | .107 |
| | $n$ = 15 | $n$ = 6 | | $n$ = 78 | $n$ = 7 | | $n$ = 26 | $n$ = 2 | |
| Language[a] | 73.07 (16.49) | 65.33 (16.46) | .310 | 65.79 (21.64) | 68.57 (25.23) | .559 | 66.65 (18.88) | 40.50 (17.68) | .060 |
| Reading[a] | 66.33 (17.56) | 77.83 (19.07) | .242 | 65.88 (19.96) | 65.57 (20.50) | .665 | 66.54 (13.91) | 48.50 (26.16) | .264 |
| Spelling[a] | 67.40 (16.56) | 64.33 (14.02) | .724 | 63.32 (19.26) | 61.71 (18.72) | .718 | 67.73 (23.66) | 29.00 (4.24) | .032 |

Table 2.4 continued

[a]Sample sizes listed with respect to the Math subtest also apply with respect to the other achievement tests (i.e., Language, Reading, and Spelling). [b]Mann-Whitney U tests used test differences between right- and left-handed subjects within APOE genotype. Note. All data presented as mean (standard deviation).

(Table 2.3). Tukey HSD post hoc tests showed that ε2-positive subjects had significantly lower scores than both ε3/3 homozygotes ($p$ = .031) and ε4-positive subjects ($p$ = .018; see Figure 2.2). The extent to which gender or the interaction between gender and genotype predicted RCFT-CC scores was also examined, but neither effect was significant.

*Visuospatial test performance in left- versus right-handed subjects.* The potential influence of hand dominance for writing with respect to the observed genotype group differences in RCFT-CC scores was explored. Specifically, results of Mann-Whitney U tests comparing RCFT-CC scores of left- versus right-hand dominant subjects within each of the genotype groups failed to find any significant differences as a function of hand dominance (Table 2.4), although the difference in performance as a function of handedness approached significance for the ε3/3 homozygotes ($U$ = 199.500, $p$ = .074) with left-handed subjects demonstrating a higher mean score relative to right-handed subjects.

## *Discussion*

Three primary findings emerged from Study 2. First, consistent with the findings of Study 1, a higher prevalence of left-hand dominance for writing was observed among ε2-positive children (29.2%) relative to ε3/3 homozygotes (8.9%) and ε4-positive children (6.1%). Second, contrary to predictions, significant group differences as a function of APOE genotype were observed on a measure of visuospatial functioning (i.e., the RCFT-CC), with ε2-positive children performing significantly *worse* on this measure relative to both ε3/3 homozygotes and ε4-positive

children. Notably, however, a third finding is that, despite these genotype group differences with respect to handedness and performance on the RCFT-CC, the mean scores of all the genotype groups on both the RCFT-CC and each of the CAT-6 subtests were within the average range. That is, at least with respect to the limited number and types of tests used in the current study, APOE genotype is not associated with impaired test performance for any of the genotype groups in this sample of school-aged children.

The findings of an increased prevalence of left-handedness among ε2-positive children could be interpreted as providing further support for the idea that the ε2 allele may influence or be associated with factors that give rise to atypical hemispheric dominance, an idea that was initially presented in Study 1. Similarly, and as previously stated in Study 1, this finding could also provide clues as to the basis for the discrepant prevalence rates of the different APOE genotypes themselves and the fact that while the ε2 allele appears to be beneficial to survival later in life, it is paradoxically the least prevalent isoform. As previously stated, it could be that APOE-ε2 itself is associated with a higher risk for disease and/or mortality, but only during infancy or childhood. Then, if an ε2-positive child survives this period, it may be that factors associated with this early survival confer later advantages such as predisposition to higher educational attainment and relative protection from the onset of pathological aging.

The notion that APOE-ε2 may itself be a risk factor for problems in early childhood could also help explain the finding of lower performance on a measure of

visuospatial functioning in ε2-positive children relative to ε2-negative children in the current study. Although post hoc analyses suggest that the lower performance of ε2-positive children on the RCFT-CC cannot be fully accounted for by the higher prevalence of left-handedness in this group, it is possible that these phenomena have a common etiology.

Further support for the notion that the ε4 allele may be more advantageous than the ε2 allele early in life comes from a small number of developmental studies that have examined the role of APOE during the human perinatal and infancy periods of life. Specifically, one study found that the ε2 allele was over-represented in a Scottish cohort of perinatal deaths (Becher et al., 2006). In addition, another study found a decreased prevalence of the ε4 allele among spontaneous abortions (Zetterberg et al., 2002). These findings suggest, as the authors pointed out, that the presence of the ε4 allele may have a protective effect in pregnancy and that alternatively, possession of one or more ε2 alleles may be detrimental to early development. Furthermore, study of a large cohort of infants from an urban area found that ε4-positive babies had higher mental development index scores relative to ε4-negative babies at 24 months of age, and that the same change in blood lead levels in these infants resulted in a significantly attenuated decrease in mental development index scores in ε4-carriers relative to non-ε4-carriers. These findings are suggestive of possible protection against environmental toxins (i.e., lead exposure) associated with the ε4 allele in infants (Wright et al., 2003).

Notably, there have also been some studies that have suggested that the APOE-ε4 allele may have a possible advantageous effect in early adulthood (Mondadori et al., 2006) relative to the ε2 allele, which is an assertion based on the finding of disparate learning-related activation during functional neuroimaging (Mondadori et al., 2006). Furthermore, another recent study found better neuropsychological test performance following TBI among a sample of ε4-positive versus ε4-negative young adults (Han, Drake et al., 2007). However, there have been other studies of individuals in this age range (Reiman et al., 2004; Scarmeas et al., 2005) that have concluded that the ε4 allele is associated with abnormalities in young adulthood.

This study has a number of limitations. First, although this was not a significantly underpowered study, statistical power was not optimal for detecting the small effect sizes observed; for example, the main effect of genotype group on RCFT-CC scores was small ($\eta_p^2 = .057$, $d = .24$, observed power = .737). In addition, some characteristics of the sampling method (e.g., the majority of parents who were contacted regarding the study elected not to participate, coupled with the fact that the nonresponding group was not characterized) suggest that the sample could be biased in some way. Furthermore, characteristics of the sample itself (e.g., inclusion of a small number of subjects for whom complete demographic information was not available) suggest the possibility that other, unaccounted for variables could have contributed to the pattern of results. In addition, the method used to assess handedness (i.e., child- or parent-report) was not ideal, and hand dominance was only assessed for writing. A superior study design would have involved the use of a thorough and

standardized assessment of handedness (e.g., Oldfield, 1971). Finally, the test measures used in this study largely represent tests of convenience (e.g., existing group achievement test data). In light of the fact that significant genotype group differences emerged, individual administration of a comprehensive test battery that assessed multiple cognitive domains may have provided more sensitive measures to test the hypothesis that the APOE-ε2 allele may be detrimental with respect to cognitive functioning in childhood.

In conclusion, results of the current study failed to find lower mean test performance among ε4-positive children relative to ε2-positive children, as predicted. In fact, findings were opposite the prediction, and suggest that the ε2-allele may be a risk factor for lower test performance in childhood. This is further supported by the higher prevalence of left-hand dominance for writing observed among ε2-positive children. These findings are generally consistent with a small number of developmental studies that suggest that the presence of the ε4 allele may have a protective effect in pregnancy and early development, and that alternatively possession of one or more ε2 alleles may be detrimental to early development.

The current study adds to emerging findings in the literature of antagonistic pleiotropy with respect to APOE genotype. Antagonistic pleiotropy refers to selection of a gene that confers an advantage at one age and a disadvantage at another depending on how it affects total reproductive probability (Williams, 1957). It may be that natural selection selects against the ε2 allele during the perinatal and infancy periods in favor of the ε4 allele, which may confer beneficial effects during this time.

This early advantage occurs at the expense of detrimental effects of APOE-ε4 during the post-reproductive years (Becher et al., 2006; Wright et al., 2003). Further study is needed to first replicate and then determine the nature and extent to which the ε2 allele may be associated with poorer cognitive functioning in childhood. For example, it could be that only certain cognitive domains are affected. In addition, longitudinal data are needed to determine when the switch may occur and the protective effects of APOE-ε2 coupled with the deleterious effects of APOE-ε4 begin to "kick in."

## Study 3

The first aim of Study 3 was to explore cognitive and achievement test performance over time as a function of APOE genotype in school-aged children. Specifically, it is notable that in some studies, significant longitudinal changes in cognitive performance have been observed among ε4-positive relative to ε4-negative middle-aged and elderly adults (Blair et al., 2005; Bretsky, Guralnik, Launer, Albert, & Seeman, 2003; Caselli, Reiman, Osborne et al., 2004; Dik et al., 2001; Swan, Lessov-Schlaggar, Carmelli, Schellenberg, & La Rue, 2005). Furthermore, among normal-functioning elderly, the ε2 allele has been associated with the absence of decline in some domains (Wilson et al., 2002), which is consistent with the observation that it confers some degree of protection from the development of AD. Overall, these changes among ε4-positive adults have largely been interpreted as changes associated with either normal age-related decline or preclinical AD.

While it is possible that APOE genotype could be associated with different patterns of achievement test performance over time among school-aged children (e.g., significant variability in scores from year to year among ε4-positive children, which could contribute to the development of lower cognitive reserve), there is as yet no evidence for this notion. Thus, with respect to the current study it was hypothesized that test performance would be observed to be generally stable over a 1- to 3-year period among school-aged children irrespective of APOE genotype (Hypothesis 1).

## *Method*

The study was approved by the Institutional Review Boards of the University of California, San Diego and San Diego State University. Informed consent was obtained from a parent of each participant, and informed assent was obtained from each participant.

### *Subjects*

Recruitment and screening procedures were previously described within the context of Study 2.

### *Procedures*

Cognitive testing and DNA collection and analysis procedures were also previously described within the context of Study 2. With respect to the current study, only children who had CAT-6 testing reports available from at least two time points were included. Furthermore, RCFT-CC scores were obtained at only a single time point and thus these data were not included in Study 3. Again, standardized NCE scores provided for each subtest of the CAT-6 (i.e., Language, Reading, Spelling, and Math) were used in all data analyses. NCE scores have a mean of 50 and a standard deviation of 21.06.

### *Statistical Analyses*

All statistical analyses were conducted using SPSS statistical software. Scores on each of the CAT-6 subtests were found to be generally normally distributed with roughly equal variances among each of the APOE genotype groups. No extreme cases were observed. Initially, one-way ANOVA was used to examine mean CAT-6 subtest performance as a function of APOE genotype separately at Time 1 and Time 2.

As the purpose of this study was to examine the test-retest stability of CAT-6 subtest scores as a function of APOE genotype, both the association between and the absolute agreement of scores at Time 1 and Time 2 were examined within APOE genotype group using Pearson (*r*) and Intraclass correlational (ICC) analyses, respectively. Pearson's *r* was used as a simple measure of association and the ICC was used as a measure of true agreement (Wilk et al., 2002).

Because differentially significant correlations were observed among the APOE genotype groups with respect to correlations between Time 1 and Time 2 Language and Reading test scores, respectively, two sets of follow-up analyses were conducted. First, Fisher Z-score transformations were used to determine whether or not the observed differences between correlations (i.e., among the genotype groups) in these domains (i.e., Language and Reading) represented statistically significant differences. Second, repeated measures ANOVA was employed to further explore possible differences in Language and Reading test scores between Time 1 and Time 2, as well as the effect of gender as a potential moderating variable. In addition, preliminary analyses investigating differences in test performance in these domains across three time points were also conducted using repeated measures ANOVA with Greenhouse-Geisser correction.

Given that investigation of these research questions in a sample of school-aged children can be considered exploratory, an alpha level of .05 was used in the interpretation of all results.

*Sample Size and Characteristics*

As described previously, after exclusions, there was a subgroup of 134 children for whom basic demographic data, genotype, and CAT-6 data at a single time point were available. A subset of 104 children had CAT-6 data available at a minimum of two time points, and a subset of 50 children had CAT-6 data available at a minimum of three time points. Therefore, longitudinal analysis of data at two time points is based on a total of 104 children, and longitudinal analysis of data at three time points is based on a total of 50 children.

## *Results*

*Basic Demographics*

Allele frequencies and other basic demographic data presented as a function of genotype can be found in Table 3.1. The reader should note that the ε2-positive group consisted entirely of subjects who were ε2/3 heterozygotes, while the ε4-positive group included all ε3/4 heterozygotes with the exception of one subject who was an ε4/4 homozygote; none of the children who were genotyped were homozygous for the ε2 allele. With respect to the sample as a whole, at Time 1 ages ranged from 8.57 years to 13.12 years ($M$ = 10.74, $SD$ = 1.30), and at Time 2 ages ranged from 9.57 to 14.11 ($M$ = 12.13, $SD$ = 1.46). There was a total of 47 boys (45.2%) and 57 girls (54.8%). The breakdown of the sample with regard to ethnicity was as follows: Asian, $n$ = 9 (8.7%); African American, $n$ = 7 (6.7%); Caucasian, $n$ = 63 (60.6%); Filipino, $n$ = 5 (4.8%); Hispanic, $n$ = 19 (18.3%); and Other, $n$ = 1 (1.0%). Parental educational attainment was assessed via parent-report, and these data were available

Table 3.1

*Basic Demographic Information by APOE Genotype*

| | ε2+ | ε3/3 | ε4+ | |
|---|---|---|---|---|
| | *n* = 18 | *n* = 67 | *n* = 19 | *p* |
| Allele Frequency | 0.173 | 0.644 | 0.183 | n/a |
| Age at Time 1[a] | 11.00 (1.06) | 10.56 (1.34) | 11.14 (1.28) | .143[b] |
| Age at Time 2[a] | 12.50 (1.21) | 11.94 (1.54) | 12.46 (1.32) | .196[b] |
| Male/Female | 9/9 | 27/40 | 11/8 | .358[c] |
| Ethnicity | | | | .190[c] |
| Asian | 1 | 8 | 0 | |
| African American | 1 | 2 | 4 | |
| Caucasian | 12 | 40 | 11 | |
| Filipino | 2 | 3 | 0 | |
| Hispanic | 2 | 13 | 4 | |
| Other | 0 | 1 | 0 | |
| Handedness (R/L) | 12/6 | 61/6 | 18/1 | .012[c] |
| Parent Education in Years[a] | | | | |
| Mother[d] | 16.47 (2.00) | 16.04 (2.34) | 16.53 (2.17) | .656[b] |
| Father[e] | 16.47 (2.30) | 16.08 (2.40) | 16.60 (2.44) | .706[b] |

Table 3.1 continued

[a]Data are presented as mean (standard deviation). [b]One-way ANOVA used to test group differences. [c]Chi-square used to test group differences. [d]Based on $n = 81$. [e]Based on $n = 80$.

for approximately 77.9% of the sample. Based on the information provided, years of education were determined according to widely-accepted criteria (Heaton et al., 2004). Maternal years of education ranged from 12 to 20 ($M$ = 16.21, $SD$ = 2.17), and paternal years of education ranged from 12 to 20 ($M$ = 16.25, $SD$ = 2.37). With respect to the sample as a whole, there were a total of 13 youth (12.5%) who identified themselves as left-hand dominant for writing. None of the children identified themselves as ambidextrous for writing.

*Longitudinal Achievement Test Performance Across Two Time Points*

*Mean test performance by APOE genotype at time 1 and time 2.* One-way ANOVA was used to examine mean CAT-6 subtest performance as a function of APOE genotype separately at Time 1 and Time 2. These analyses failed to find a significant main effect of APOE genotype at Time 1 or Time 2 with respect to any of the CAT-6 subtests (Table 3.2).

*Stability of test performance over time.* Both the association between and the absolute agreement of CAT-6 scores at Time 1 and Time 2 were examined within APOE genotype using Pearson ($r$) and Intraclass correlational (ICC) analyses, respectively (Table 3.3). Results showed significant Pearson and ICC correlations between Time 1 and Time 2 Math scores, as well as Time 1 and Time 2 Spelling scores, among all of the genotype groups. In addition, significant Pearson and ICC correlations between Time 1 and Time 2 Language scores were observed among ε3/3 homozygotes and ε4-positive children, but not ε2-positive children. In contrast, significant Pearson and ICC correlations between Time 1 and Time 2 Reading scores

Table 3.2

*Mean Achievement Test Performance by APOE Genotype*

| | ε2+ *n* = 18 | ε3/3 *n* = 67 | ε4+ *n* = 19 | *p*[b] |
|---|---|---|---|---|
| Math[a] | | | | |
| Time 1 | 76.33 (16.90) | 68.75 (18.91) | 69.37 (15.32) | .281 |
| Time 2 | 68.94 (17.93) | 62.30 (20.00) | 63.16 (16.26) | .422 |
| Language[a] | | | | |
| Time 1 | 71.06 (14.13) | 68.64 (17.36) | 70.47 (18.09) | .827 |
| Time 2 | 73.00 (15.88) | 67.70 (19.77) | 67.53 (17.27) | .548 |
| Reading[a] | | | | |
| Time 1 | 72.61 (18.90) | 66.62 (17.43) | 64.74 (16.81) | .345 |
| Time 2 | 69.89 (18.13) | 67.18 (18.34) | 66.37 (12.76) | .803 |
| Spelling[a] | | | | |
| Time 1 | 68.72 (16.82) | 66.15 (19.51) | 63.58 (23.62) | .735 |
| Time 2 | 67.78 (15.88) | 65.85 (17.65) | 65.32 (22.11) | .905 |

[a]Data are presented as mean (standard deviation). [b]Represents main effect of APOE genotype.

Table 3.3

*Association Between and Reliability of Time 1 and Time 2 Achievement Test Scores*

| | ε2+ | | ε3/3 | | ε4+ | |
|---|---|---|---|---|---|---|
| | *n* = 18 | | *n* = 67 | | *n* = 19 | |
| Test | *r* | ICC | *r* | ICC | *r* | ICC |
| Math | .762** | .761** | .744** | .743** | .526* | .525** |
| Language | .298 | .296 | .496** | .492** | .623** | .622** |
| Reading | .599** | .599** | .599** | .599** | .375 | .362 |
| Spelling | .776** | .775** | .602** | .599** | .745** | .743** |

*Correlation is significant at the .05 level (2-tailed). **Correlation is significant at the .01 level (2-tailed).

were observed among ε3/3 homozygotes and ε2-positive children, but not ε4-positive children.

*Post-hoc analysis of differences in longitudinal achievement performance as a function of APOE genotype.* The finding of differentially significant correlations among the APOE genotype groups with respect to Time 1 and Time 2 Language and Reading test scores was further explored using Fisher Z-score transformations and significance testing. Specifically, the observed differences in correlations of Time 1 and Time 2 Language test scores between ε2-positive versus ε3/3 homozygotes, as well as between ε2-positive versus ε4-positive children were tested, but neither observed difference was statistically significant (in both cases $p > .05$). In addition, the observed differences in correlations of Time 1 and Time 2 Reading test scores between ε4-positive versus ε3/3 homozygotes, as well as between ε4-positive versus ε2-positive children were tested, but again, neither observed difference was statistically significant (in both cases $p > .05$).

The finding of differentially significant correlations was also additionally explored using repeated measures ANOVA. However, results failed to find a significant within subjects main effect of time, a between subjects main effect of APOE genotype, or a significant interaction between time and APOE genotype with respect to either Language or Reading test scores.

Given that APOE genotype has been found to be associated with different cognitive profiles in normal-functioning adult men and women (Swan et al., 2005), the role of gender as a potential moderating variable was also examined with respect to

the observed genotype group differences in Language and Reading scores. However, again, repeated measures ANOVA failed to find a significant gender effect with respect to either Language or Reading test scores, although with respect to Reading scores the three-way interaction between time, APOE genotype, and gender approached significance ($F(2, 98) = 2.546$, $p = .084$; $\eta_p^2 = .049$). Although this finding did not reach statistical significance, the data are depicted in Figure 3.1.

*Preliminary Analysis of Differences in Longitudinal Achievement Test Performance Across Three Time Points*

Given the finding of differentially significant correlations as a function of APOE genotype between Time 1 and Time 2 CAT-6 test scores (i.e., albeit only with respect to Language and Reading scores), preliminary analyses were conducted to begin to examine the relationship between APOE genotype and patterns of achievement test performance over three time points. These analyses are considered preliminary given the relatively small sample sizes studied (i.e., $n = 50$). Furthermore, because sample sizes were small, the effect of gender was not examined.

Although not of primary interest in the current study, repeated measures ANOVA revealed a significant within subjects main effect of time with respect to both Language ($F(2, 88) = 3.845$, $p = .027$; $\eta_p^2 = .076$) and Math ($F(2, 88) = 4.699$, $p = .013$; $\eta_p^2 = .091$) scores. Specifically, Language scores tended to increase then decrease over time across APOE genotype, while Math scores tended to consistently decrease over time across genotype. In addition, a within subjects main effect of time approached significance with respect to Spelling scores ($F(2, 84) = 3.019$, $p = .060$;

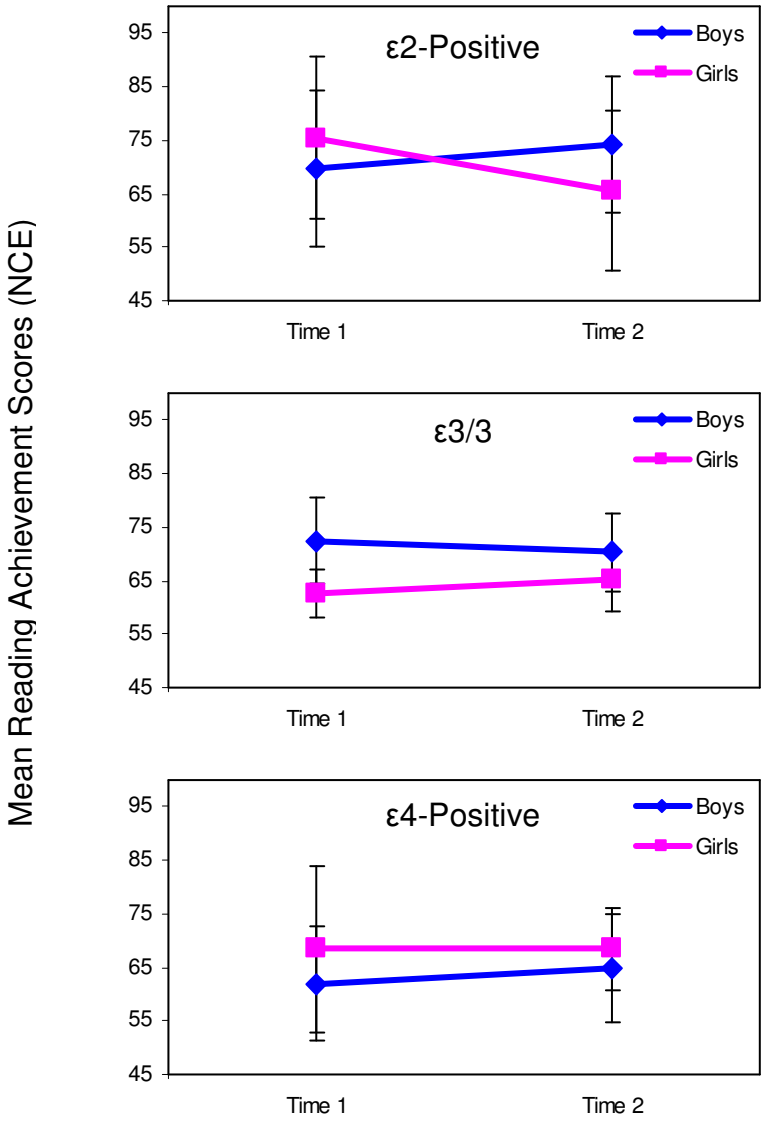


Figure 3.1. Depiction of the nonsignificant three-way interaction between time, APOE genotype, and gender with respect to mean CAT-6 Reading scores. Error bars represent 95% confidence interval.

$\eta_p^2$ = .060) with scores consistently decreasing over time across genotype. There were no significant effects observed with respect to Reading scores.

*Discussion*

The results of Study 3 suggest that, overall, among school-aged children achievement test scores are relatively stable over a 1- to 3-year period regardless of APOE genotype. This is consistent with what would be expected given that APOE-related changes in cognitive status over time that have been reported in adults are generally interpreted as being associated with either age-related decline or preclinical AD. Hence, based on the existing literature, APOE genotype would not necessarily be expected to be associated with differential change in achievement test performance in children, and overall, the results of the current study provide no strong evidence to suggest that this is the case. Consistent with results from Study 2, another finding is that the mean achievement subtest scores of all the genotype groups at each time point studied were within the average range, suggesting that APOE genotype is not associated with impaired achievement test performance for any of the genotype groups in this sample of school-aged children.

However, results did reveal some differentially significant correlations as a function of APOE genotype in Time 1 and Time 2 Language and Reading test scores that should be addressed. Specifically, while significant correlations were observed with respect to Time 1 and Time 2 Language scores among ε4-postive children, these correlations were not significant among ε2-positive children; in contrast, while significant correlations were observed with respect to Time 1 and Time 2 Reading

scores among ε2-positive children, these correlations were not significant among ε4-positive children. Although these results were observed despite the nearly equal sample sizes of the 2 groups, the nonsignificant correlations in each case (Table 3.3) still represent relatively strong positive associations (i.e., for Language scores among ε2-positive children $r = .298$ and for Reading scores among ε4-positive children $r = .375$). With a larger sample size, these correlations would likely be significant, which suggests that the differential significance likely does not represent true genotype group differences in these domains. Additional evidence for this is that post hoc testing using Fisher Z-score transformations failed to find that the correlations themselves were significantly different between the genotype groups.

Furthermore, although follow-up analyses examining the possible moderating role of gender with respect to this finding showed differences in Reading change scores between boys and girls and between the genotype groups (i.e., the three-way interaction approached significance; see Figure 3.1), the effect size was small ($\eta^2 = .049$, $d = .23$, observed power = .498). In addition, preliminary analyses of differences in achievement test performance across three time points failed to find any significant effects of APOE genotype, or even any effects that approached significance. Taken together, these findings suggest a minimal effect of APOE genotype on achievement test performance over time, although further exploration of the role of gender with respect to change over time, particularly among ε2-positive children would be warranted. Taken together with the findings of Study 2, it could be that significant variability in scores from year to year is actually characteristic of ε2-

positive children and that this variability is moderated by gender. This could be consistent with the theory that the ε2 allele is associated with detrimental effects early in life and that the ε4 allele may be beneficial during this time (i.e., the theory of antagonistic pleiotropy).

This study, however, has a number of limitations that must be acknowledged. First, the study is primarily based on data from two time points, and the developmental window between Time 1 and Time 2 was relatively small (i.e., 1 to 3 years). Specifically, examination of achievement test performance over a wider interval, and of course with data from more than two or three time points, would better enable any true "developmental" effects of genotype and gender, as well as the potential interaction between the two, to be assessed. Another possible weakness relates to the possibility that while APOE may indeed be associated with cognitive functioning in childhood, the expression of the phenotype may depend on other developmental changes and events (e.g., puberty) (Schork, Greenwood, & Braff, 2007). Thus, a better study design would have examined data that were representative of as many key developmental stages as possible (e.g., well before puberty and well after puberty). This is especially true given that it is already well-established that APOE-ε4 is associated with an "age-specified" phenotype in adulthood (Schork et al., 2007), that is, the development of AD. Accordingly, similar age-specified phenotypes associated with APOE could be present early in life.

Other limitations of the current study are that, as illustrated above, statistical power was not optimal for detecting the observed effect sizes. In addition, some

characteristics of the sampling method, as previously mentioned with respect to Study 2 (e.g., the majority of parents who were contacted regarding the study elected not to participate, coupled with the fact that the non-responding group was not characterized), suggest that the sample could be uniformly biased in some way. Furthermore, characteristics of the sample itself (e.g., inclusion of a small number of subjects for whom complete demographic information was not available, significant group differences in the prevalence of left-hand dominance for writing as a function of APOE genotype) suggest the possibility that other unaccounted for variables could have contributed to the pattern of results. Finally, only achievement functioning was assessed in the current study. It is quite possible that cognitive functioning in other domains (e.g., episodic memory, visuoperception and construction) (B. J. Small et al., 2004) is moderated by APOE genotype in children.

In conclusion, results of the current study failed to find evidence that APOE genotype influences changes in achievement test performance over a 1- to 3-year period among school-aged children in a statistically or clinically significant manner; rather, test performance appears to be relatively stable across achievement domains regardless of genotype. Specifically, it is possible that with larger sample sizes, assessment at additional time points, assessment at time points representative of "key" developmental stages, and more comprehensive assessment of multiple cognitive domains, true APOE-related differences in cognitive development may emerge. In sum, this possibility cannot be ruled out on the basis of this study.

## Study 4

The first aim of Study 4 was to examine the relationship between APOE genotype and cognitive asymmetries or discrepancies in children. Neuroimaging studies have produced findings suggesting that APOE-ε4-positive individuals as young as 20 years of age show evidence of atypical hemispheric asymmetry (e.g., Reiman et al., 2004). Further, neuropsychological studies of normal-functioning elderly have found a higher incidence of discrepant scores on pairs of lateralized cognitive tasks in which one task is more likely to be affected than the other in an early dementia (Houston et al., 2005; Jacobson et al., 2002; Jacobson, Delis, Bondi et al., 2005; Jacobson, Delis, Lansing et al., 2005). Based on evidence of an association between APOE-ε4 and hemispheric asymmetry in young, middle-aged, and elderly adults, it is predicted that ε4-positive children will also demonstrate cognitive asymmetry. Specifically, it was hypothesized that ε4-positive children will show higher mean discrepancy scores on pairs of lateralized cognitive tests (Hypothesis 1) and a higher incidence of asymmetric cognitive profiles (Hypothesis 2), relative to ε3/3-positive children and ε2-positive children.

The second aim of Study 3 was to explore the extent to which gender may moderate an association between APOE-ε4 and cognitive discrepancies in children. As previously stated, there are differential prevalence rates among boys and girls with respect to several neurodevelopmental disorders that are thought to have a genetic component and are often characterized by discrepant cognitive profiles (e.g., specific language impairment, attention-deficit hyperactivity disorder, autism). In addition, it

has been shown that the presence of a single APOE-ε4 allele appears to confer a greater risk for cognitive decline (Hyman et al., 1996) and AD (Farrer et al., 1997; Martinez et al., 1998; Payami et al., 1996) on adult women relative to men. Thus, with respect to the current study it was further hypothesized that ε4-postive girls would exhibit higher mean discrepancy scores and a higher incidence of asymmetric cognitive profiles relative to ε4-postive boys, ε3/3 homozygotes, and ε2-positive children (Hypothesis 3).

## *Method*

The study was approved by the Institutional Review Boards of the University of California, San Diego and San Diego State University. Informed consent was obtained from a parent of each participant, and informed assent was obtained from each participant.

### *Subjects*

Recruitment and screening procedures were previously described within the context of Study 2.

### *Procedures*

Cognitive testing and DNA collection and analysis procedures were also previously described within the context of Study 2. With respect to the current study, only right-handed children who had both RCFT-CC and CAT-6 data from at least a single time point were included. Notably, because some individuals who are left-handed have been shown to have more widely distributed, mixed, or atypical hemispheric dominance for language, cognitive tasks that are normally representative

of right (e.g., visuospatial tasks) or left (e.g., language tests) hemispheric functioning in right-handed individuals with typical brain organization, may not be similarly representative in left-handed individuals (Howieson, Loring, & Hannay, 2004). Thus, given that the current study is an examination of lateralized cognitive discrepancies, inclusion of left-handed subjects introduces a potential confounding variable. Therefore, consistent with previous studies that have found evidence of hemispheric asymmetries associated with APOE genotype (Jacobson, Delis, Bondi et al., 2005; Scarmeas et al., 2005; Wishart, Saykin, McAllister et al., 2006), left-handed subjects were excluded from the current study.

*Statistical Analyses*

*Discrepancy scores.* RCFT-CC and CAT-6 NCE scores were analyzed both individually and in terms of a discrepancy score that represented the absolute value of the difference in NCE scores between selected pairs of tests (see Table 4.1 for a list of the discrepancies that were examined). The reader should note that these pairs of tests were selected a priori. The absolute value was used because the magnitude of the difference score was of primary interest in these analyses irrespective of its direction, which is consistent with previous work in this area (Houston et al., 2005; Jacobson et al., 2002; Jacobson, Delis, Bondi et al., 2005; Jacobson, Delis, Lansing et al., 2005). Univariate ANOVA was employed to examine differences in mean scores on individual tests and mean discrepancy scores, both as a function of APOE genotype and as a function of gender.

Table 4.1

*Cognitive Discrepancy/Asymmetry Scores Examined*

| Tests Used in Discrepancy Comparison | Abbreviation |
|---|---|
| Rey Complex Figure Test – Copy Condition Discrepancies | |
| RCFT-CC – CAT-6 Math | RCFT – MT |
| RCFT-CC – CAT-6 Language | RCFT – LA |
| RCFT-CC – CAT-6 Reading | RCFT – RD |
| RCFT-CC – CAT-6 Spelling | RCFT – SP |
| CAT-6 Math Subtest Discrepancies | |
| CAT-6 Math – CAT-6 Language | MT – LA |
| CAT-6 Math – CAT-6 Reading | MT – RD |
| CAT-6 Math – CAT-6 Spelling | MT – SP |
| CAT-6 Verbal Subtests Discrepancies | |
| CAT-6 Language – CAT-6 Reading | LA – RD |
| CAT-6 Language – CAT-6 Spelling | LA – SP |
| CAT-6 Reading – CAT-6 Spelling | RD – SP |

*Asymmetric profiles.* In addition to mean discrepancy scores, the relative frequency of an asymmetric profile was also examined. Also in accordance with past studies (Demadura, Delis, Jacobson, & Salmon, 2001; Finton et al., 2003; Jacobson et al., 2002; Jacobson, Delis, Bondi et al., 2005), an asymmetric profile was operationally defined as a Z-score difference greater than 1 standard deviation (i.e., NCE score difference greater than 21.06). The relative frequency of this profile as a function of APOE genotype and as a function of gender was examined using chi-square tests.

All statistical analyses were conducted using SPSS statistical software. Data were also screened for extreme cases. Mild positive skewness was observed with respect to all of the discrepancy scores. However, given that ANOVA is generally robust to violations of the normality assumption (Maxwell & Delaney, 2000) and the degree of skewness was relatively minimal, it was decided that scores would not be transformed in favor of maximizing ease of interpretation. However, as a safeguard, all analyses with discrepancy scores were additionally performed using Mann-Whitney U tests (results not shown), which is considered the nonparametric version of the t-test (Heiman, 1996). Notably, no differences in statistical significance were observed between the two methods.

Given that investigation of these research questions in a sample of school-aged children can be considered exploratory, an alpha level of .05 was used in the interpretation of all results. Furthermore, significant main effects of genotype group (i.e., mean differences between ε2-postive children versus ε3/3 homozygotes versus

ε4-positive children) were followed up by testing all pairwise comparisons utilizing Tukey's HSD procedure.

*Sample Size and Characteristics*

As described previously, after exclusions, there was a subgroup of 134 children for whom basic demographic data, APOE genotype, RCFT-CC data, and CAT-6 test data were available. Of these 134 youth, a total of 15 were left-hand dominant for writing and were thus excluded from the study as described above. Furthermore, there were 2 subjects for whom RCFT-CC scores were atypical and fell more than three standard deviations below the mean. These were considered extreme cases and were thus removed from all subsequent analyses that involved RCFT-CC scores (although these cases were included in analyses that involved only CAT-6 subtest scores). Thus, analyses that involved RCFT-CC scores included a total of 117 subjects, and analyses that involved only CAT-6 subtest scores included a total of 119 subjects.

## *Results*

*Basic Demographics*

Allele frequencies and other basic demographic data presented as a function of genotype can be found in Table 4.2. The reader should note that the ε2-positive group consisted entirely of subjects who were ε2/3 heterozygotes, while the ε4-positive group included all ε3/4 heterozygotes with the exception of one subject who was an ε4/4 homozygote; none of the children who were genotyped were homozygous for the ε2 allele. With respect to the sample as a whole, ages ranged from 11.38 years to 16.84 years ($M$ = 13.31, $SD$ = 1.28) and there was a total of 51 boys (42.9%) and 68

Table 4.2

*Basic Demographic Information by APOE Genotype*

| | ε2+ | ε3/3 | ε4+ | |
|---|---|---|---|---|
| | *n* = 15 | *n* = 78 | *n* = 26 | *p* |
| Allele Frequency | 0.126 | 0.655 | 0.218 | n/a |
| Age at Exam[a] | 13.46 (0.96) | 13.23 (1.32) | 13.46 (1.33) | .652[b] |
| Male/Female | 7/8 | 30/48 | 14/12 | .370[c] |
| Ethnicity | | | | .042[c] |
| Asian | 1 | 8 | 0 | |
| African American | 0 | 2 | 5 | |
| Caucasian | 10 | 50 | 15 | |
| Filipino | 2 | 3 | 0 | |
| Hispanic | 2 | 13 | 6 | |
| Other | 0 | 2 | 0 | |
| Parent Education in Years[a] | | | | |
| Mother[d] | 16.54 (1.98) | 16.32 (2.23) | 16.40 (2.06) | .946[b] |
| Father[e] | 16.77 (2.28) | 16.00 (2.44) | 16.55 (2.68) | .487[b] |

[a]Data are presented as mean (standard deviation). [b]One-way ANOVA used to test group differences. [c]Chi-square used to test group differences. [d]Based on *n* = 92. [e]Based on *n* = 91. *Note.* All subjects are right-hand dominant for writing.

girls (57.1%). The breakdown of the sample with regard to ethnicity was as follows: Asian, $n$ = 9 (7.6%); African American, $n$ = 7 (5.9%); Caucasian, $n$ = 75 (63.0%); Filipino, $n$ = 5 (4.2%); Hispanic, $n$ = 21 (17.6%); and Other, $n$ = 2 (1.7%). Parental educational attainment was assessed via parent-report, and these data were available for approximately 77.3% of the sample. Based on the information provided, years of education were determined according to widely-accepted criteria (Heaton et al., 2004). Maternal years of education ranged from 12 to 20 ($M$ = 16.37, $SD$ = 2.14), and paternal years of education ranged from 10 to 20 ($M$ = 16.23, $SD$ = 2.47). As previously stated, only participants who identified themselves as right-hand dominant for writing were included in the study.

*Performance on Individual Cognitive and Achievement Tests*

*Achievement test results.* Univariate ANOVA was used to test for differences in CAT-6 subtest scores as a function of APOE genotype and as a function of gender. Results failed to find a significant main effect of genotype or gender, or a significant interaction between genotype and gender with respect to any of the CAT-6 subtest scores (Table 4.3).

*Visuospatial test results.* Similarly, univariate ANOVA was used to test for differences in RCFT-CC scores as a function of APOE genotype and as a function of gender. Notably, analyses failed to find a significant main effect of APOE genotype with respect to RCFT-CC scores ($F(2, 111) = 0.651$, $p = .524$; $\eta_p^2 = .012$; Table 4.3) among this smaller and more homogenous sample of right-handed children (i.e., relative to the larger, mixed-handedness sample included in Study 2), although the

Table 4.3

*Mean Cognitive and Achievement Test Performance by APOE Genotype*

| Test | ε2+ | ε3/3 | ε4+ | $p^c$ |
|---|---|---|---|---|
| RCFT-CC[a] | 48.94 (14.14) | 51.55 (12.65) | 53.40 (8.85) | .524 |
| Math[b] | 64.27 (16.73) | 62.78 (19.02) | 62.73 (19.40) | .953 |
| Language[b] | 73.07 (16.49) | 65.79 (21.64) | 66.65 (18.88) | .500 |
| Reading[b] | 66.33 (17.56) | 65.88 (19.96) | 66.54 (13.91) | .995 |
| Spelling[b] | 67.40 (16.56) | 63.32 (19.26) | 67.73 (23.66) | .419 |

[a]Based on a total of $n = 117$; data presented as mean (standard deviation). [b]Based on a total of $n = 119$; data presented as mean (standard deviation). [c]Represents main effect of APOE genotype.

same pattern of results was observed (i.e., ε2-positive children obtained a lower mean RCFT-CC score relative to both ε3/3 homozygotes and ε4-positive children), which suggests decreased statistical power in the current study relative to Study 2. Furthermore, analyses failed to find a significant main effect of gender, or a significant interaction between gender and genotype with respect to RCFT-CC scores.

*Discrepancy Score Analysis*

While somewhat inconsistent with the results of Study 2, the reader should note that the failure to find a significant main effect of genotype with respect to individual RCFT-CC scores among this sample of right-handed children provides an important rationale for examination of discrepancy scores and the relative frequency of asymmetric profiles among these children. Specifically, as stated previously, it has been proposed that examination of mean differences on individual cognitive tests as a function of APOE genotype will mask subtle asymmetry characteristically present in ε4-positive individuals (e.g., Jacobson, Delis, Bondi et al., 2005). Thus, with respect to the current study, if results had revealed significant mean differences on any of the individual tests, discrepancy/asymmetric profile analyses would be inappropriate, as these analyses would produce results driven by the initial observed differences on the individual tests.

*RCFT – Achievement discrepancy scores.* Univariate ANOVA was used to test for differences in RCFT – CAT-6 subtest discrepancy scores as a function of APOE genotype and as a function of gender. Results failed to find a significant main effect of genotype or gender, or an interaction between genotype and gender on any of

the RCFT – CAT-6 subtest discrepancy scores (Table 4.4). However, the main effect of genotype ($F(2, 111) = 2.364$, $p = .099$; $\eta_p^2 = .041$) and the interaction between genotype and gender ($F(2, 111) = 2.774$, $p = .067$; $\eta_p^2 = .048$) with respect to RCFT-CC – Reading discrepancy scores approached significance with both ε2-positive and ε3/3 homozygote boys exhibiting higher discrepancy scores than ε2-positive and ε3/3 homozygote girls, and ε4-positive girls exhibiting higher discrepancy scores than ε4-positive boys.

*Achievement test discrepancy scores.* Univariate ANOVA was also used to test for differences in CAT-6 subtest discrepancy scores as a function of APOE genotype and as a function of gender. Although results failed to find a significant main effect of genotype or gender with respect to any of the CAT-6 subtest discrepancy scores (Table 4.4), the main effect of genotype with respect to Language – Math discrepancy scores approached significance ($F(2, 113) = 2.408$, $p = .095$; $\eta_p^2 = .041$) with ε4-positive children exhibiting a higher mean discrepancy score relative to both ε2-positive children and ε3/3 homozygotes. In addition, a significant interaction was observed between genotype and gender with respect to Reading – Math discrepancy scores ($F(2, 113) = 4.978$, $p = .008$; $\eta_p^2 = .081$) with ε4-positive girls exhibiting the highest mean discrepancy scores relative to children in all of the other groups (Figure 4.1). Similarly, a significant interaction was also observed between genotype and gender with respect to Spelling – Math discrepancy scores ($F(2, 113) = 4.672$, $p = .011$; $\eta_p^2 = .076$) with the same pattern of performance observed among girls and boys as with Reading – Math discrepancy scores (Figure 4.2).

Table 4.4

*Mean Discrepancy Scores by APOE Genotype*

| | ε2+ | ε3/3 | ε4+ | *p*[c] |
|---|---|---|---|---|
| Rey Complex Figure Test – Copy Condition Discrepancies[a] | | | | |
| RCFT-CC – MT | 21.26 (11.77) | 16.50 (12.81) | 14.77 (13.62) | .246 |
| RCFT-CC – LA | 24.84 (12.64) | 20.46 (15.11) | 17.13 (12.60) | .272 |
| RCFT-CC – RD | 20.42 (17.10) | 20.23 (13.28) | 13.92 (11.07) | .099 |
| RCFT-CC – SP | 20.17 (14.67) | 19.13 (12.86) | 22.95 (16.00) | .506 |
| CAT-6 Math Subtest Discrepancies[b] | | | | |
| MT – LA | 12.27 (8.49) | 12.37 (11.03) | 17.08 (10.38) | .095 |
| MT – RD | 11.13 (8.06) | 11.95 (8.08) | 11.04 (9.62) | .913 |
| MT – SP | 17.53 (11.22) | 16.38 (10.67) | 19.92 (15.02) | .358 |
| CAT-6 Verbal Subtests Discrepancies[b] | | | | |
| LA – RD | 12.07 (6.83) | 12.27 (8.54) | 9.58 (7.14) | .211 |
| LA – SP | 13.40 (11.84) | 15.24 (10.23) | 14.31 (11.79) | .805 |
| RD – SP | 15.33 (9.31) | 13.72 (11.00) | 16.27 (10.39) | .542 |

[a]Based on a total of $n = 117$; data presented as mean (standard deviation). [b]Based on a total of $n = 119$; data presented as mean (standard deviation). [c]Represents main effect of APOE genotype.

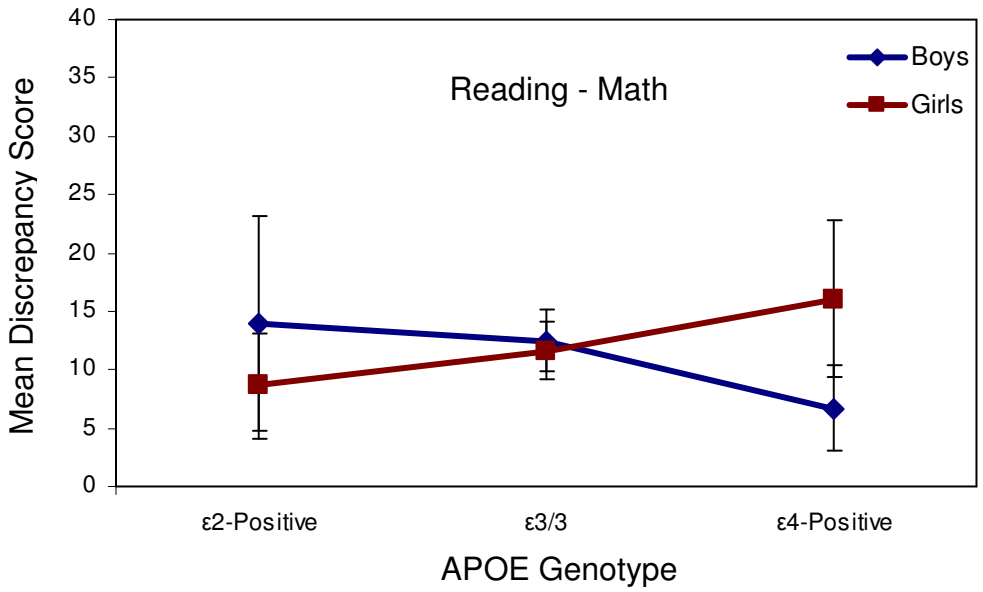


Figure 4.1. Mean CAT-6 Reading – Math discrepancy scores in school-aged boys and girls as a function of APOE genotype. Error bars represent 95% confidence interval.

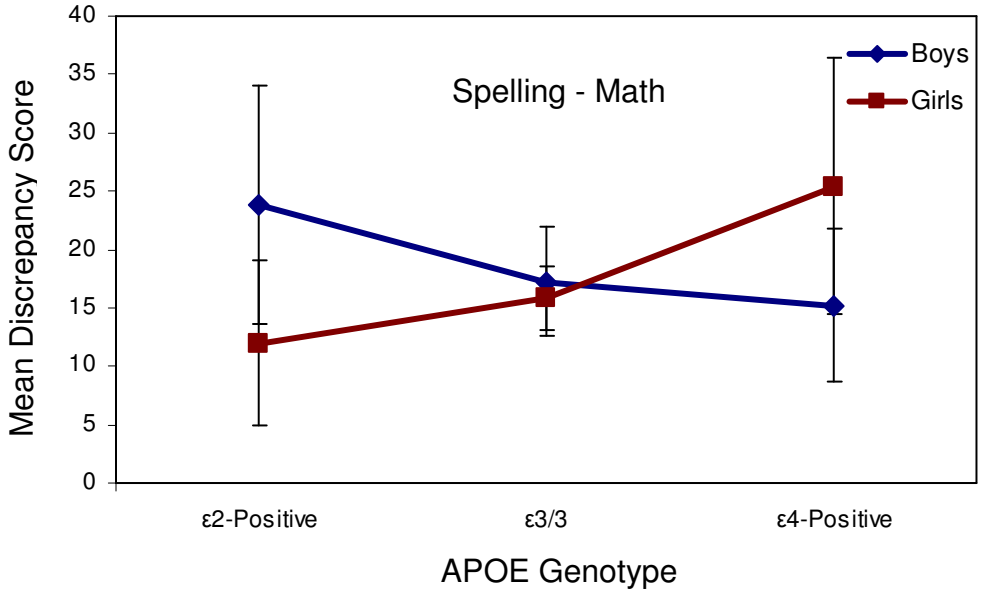


Figure 4.2. Mean CAT-6 Spelling – Math discrepancy scores in school-aged boys and girls as a function of APOE genotype. Error bars represent 95% confidence interval.

*Frequency of Asymmetric Profiles*

*RCFT – Achievement asymmetry.* Chi-square analyses were used to examine differences in the frequency of an asymmetric profile as a function of APOE genotype and as a function of gender. Although not in the predicted direction, analysis by genotype revealed a significant difference in the frequency of RCFT – Reading asymmetry ($\chi^2(2) = 7.252$, p = .027) with 46.7% ($n = 7$) and 45.5% ($n = 35$) of ε2-positive and ε3/3 homozygote children, respectively, exhibiting an asymmetric profile versus only 16.0% ($n = 4$) of ε4-positive children. Similarly, a difference in RCFT – Math asymmetry approached significance ($\chi^2(2) = 4.714$, $p = .095$) with 60.0% ($n = 9$) of ε2-positive children exhibiting an asymmetric profile versus only 31.2% ($n = 24$) and 32.0% ($n = 8$) of ε3/3 homozygote and ε4-positive children, respectively, exhibiting an asymmetric profile.

Analysis within gender revealed that among girls, there were no significant differences in the frequency of RCFT – CAT-6 asymmetry scores as a function of genotype. However, among the boys, there was a significant difference in the frequency of RCFT – Reading asymmetry ($\chi^2$ (2) = 7.115, $p = .029$) with 71.4% ($n = 5$) and 51.7% ($n = 15$) of ε2-positive and ε3/3 homozygote boys, respectively, exhibiting an asymmetric profile versus only 15.4% ($n = 2$) of ε4-positive boys. Similarly, the frequency of RCFT – Math asymmetry was significant among boys ($\chi^2$ (2) = 6.866, $p = .032$) with 85.7% ($n = 6$) of ε2-positive boys exhibiting asymmetry versus only 34.5% ($n = 10$) and 30.8% ($n = 4$) of ε3/3 homozygotes and ε4-positive boys, respectively, exhibiting an asymmetric profile.

*Achievement test asymmetry.* With respect to the sample as a whole, there were no significant differences in the frequency of CAT-6 subtest asymmetry scores as a function of APOE genotype. However, analyses within gender revealed a significant difference in the frequency of Spelling – Math asymmetry ($\chi^2$ (2) = 8.506, $p$ = .014) among girls, with only 12.5% ($n$ = 1) and 27.1% ($n$ = 13) of ε2-positive and ε3/3 homozygote girls exhibiting asymmetry versus 66.7% ($n$ = 8) of ε4-positive girls. Among the boys, there were no significant differences in the frequency of CAT-6 subtest asymmetry scores as a function of genotype.

## *Discussion*

Three tentative conclusions can be drawn from the results of the current study, which is an examination of cognitive asymmetry as a function of APOE genotype among school-aged children. What is perhaps most notable with respect to the findings of Study 4 is the observation that APOE genotype does appear to be associated with significant asymmetry early in life. However, findings also suggest that gender moderates the association between measures of asymmetry and APOE genotype such that while the APOE-ε4 allele may be associated with significant asymmetry in school-aged girls, the APOE-ε2 allele may be associated with significant asymmetry in school-aged boys. Notably, these results were observed after exclusion of children who were identified as left-hand dominant for writing.

The gender difference in asymmetry is interesting in light of three other lines of research. The first line of research supports the notion that the ε4 allele may be advantageous early in life while the ε2 allele may be detrimental. This idea comes

from a small number of developmental studies that have examined the role of APOE during the human pre-, perinatal, and infancy periods of life. Specifically, one study found that the ε2 allele was over-represented in a Scottish cohort of perinatal deaths (Becher et al., 2006). Another study found a decreased prevalence rate of the ε4 allele among spontaneous abortions (Zetterberg et al., 2002). These findings suggest that the presence of the ε4 allele may have a protective effect in pregnancy and that, alternatively, the possession of one or more ε2 alleles may be detrimental to early development.

Second, differential prevalence rates and susceptibility have been observed among boys and girls with respect to several neurodevelopmental disorders that are thought to have a genetic component and are often characterized by discrepant cognitive profiles (e.g., specific language impairment, attention-deficit hyperactivity disorder, autism). Specifically, prevalence rates are generally much higher in boys. The third notable line of research is that studies of aging have shown that the presence of a single APOE-ε4 allele appears to confer a greater risk for cognitive decline (Hyman et al., 1996) and AD (Farrer et al., 1997; Martinez et al., 1998; Payami et al., 1996) on adult women relative to men. These lines of research suggest gender-specific effects with respect to neurodevelopment (i.e., with boys being more susceptible to atypical or delayed cognitive development), as well as gender-specific effects of APOE with respect to neurodegeneration and the onset of AD (i.e., with women being more susceptible to APOE-ε4-related cognitive decline).

Taken together, these three lines of research raise the possibility that boys may be more susceptible than girls to the "neurodevelopmental" risk associated with the ε2 allele, given their apparent heightened susceptibility to neurodevelopmental disorders in general. This could be why the ε2-positive boys are showing increased cognitive asymmetry during childhood relative to ε2-positive girls. Likewise, it may be that girls are more susceptible than boys to the "neurodegenerative" risk associated with the ε4 allele, given reports of greater susceptibility of women to ε4-related cognitive decline. This could be why the ε4-positive girls are showing increased cognitive asymmetry during childhood relative to ε4-positive boys. These differences could be the result of differential gene-gene or gene-environment interactions with gender.

In interpreting these findings, it is also important to acknowledge a relatively large body of research that has accumulated with adults at genetic risk for AD by virtue of their APOE-ε4 status. Specifically, a number of neuroimaging studies (e.g., Reiman et al., 1996) have found evidence of atypical hemispheric asymmetries in brain metabolism in healthy ε4-positive adults. Furthermore, another group of studies has shown an association between the APOE-ε4 allele and lateralized cognitive discrepancies in normal-functioning elderly individuals (Houston et al., 2005; Jacobson et al., 2002; Jacobson, Delis, Bondi et al., 2005; Jacobson, Delis, Lansing et al., 2005). These findings, taken together with results from the current study, suggest the possibility that hemispheric and/or degree of cognitive asymmetry is in fact at least partially moderated by APOE genotype throughout the lifespan. However, in children this relationship may be significantly influenced by gender, and in early life the

APOE-ε2 allele may also put individuals at risk for discrepant cognitive profiles. Notably, the above-referenced studies of cognitive discrepancies in adults did not examine the effects of the ε2 allele separate from the ε3 allele, and thus it is difficult to determine whether asymmetries may be associated with APOE-ε2 in adults.

This study has a number of limitations. First, as with Studies 2 and 3, the test measures employed largely represent tests of convenience (i.e., existing group achievement test data) and a measure of visuospatial perception and construction that could be administered in a group setting. This limitation manifests in two significant ways with respect to the current study. Most notably, this represents a significant weakness in that the RCFT-CC and CAT-6 subtests, although used together to construct a single discrepancy score, were administered to children at different time points (i.e., on average they were administered 1 year apart). This was unforeseen at the time of data collection. Furthermore, NCE scores from these two measures were calculated based on different normative samples, making comparisons less precise. Finally, it should be acknowledged that, overall, lateralized cognitive discrepancies would also have been better assessed using an individually-administered comprehensive test battery with multiple measures of putative right- and left-hemisphere functioning.

In addition, although the present studies had adequate power, a larger sample may have been more sensitive for detecting the small effect sizes observed. For example, the interaction effect of genotype and gender on Reading – Math discrepancy scores (i.e., the largest effect observed) was relatively small ($\eta_p^2 = .081$, $d = .28$,

observed power = .803). Further, limitations associated with the sampling method and characteristics of the sample itself that were discussed with respect to Studies 2 and 3, also apply in the current study.

However, these limitations notwithstanding, results of the current study, taken together with existing research in this area, suggest that APOE genotype may be associated with significant cognitive asymmetry across the lifespan. Contrary to what has been observed in adults, it appears that in childhood, both the APOE-ε4 *and* APOE-ε2 alleles may be associated with cognitive asymmetries and that this association may depend on gender.

## Study 5

Study 5 is a preliminary study, the aim of which is to explore the relationship between the APOE-ε4 allele, a family history of AD, and cognitive and achievement test performance in school-aged children. While the influence of APOE genotype with respect to brain structure and function has been well-studied, the influence of family history of AD has not (Fratiglioni, Ahlbom, Viitanen, & Winblad, 1993). This is notable given that the presence of a first-degree relative with AD has been found to double the lifetime risk of developing the disease, and the presence of an additional affected family member may increase the risk by as much as 3-fold (Devi et al., 2000; Fratiglioni, 1993; van Duijn et al., 1991). Finally, what is perhaps even more remarkable is that existing data suggest *additive* effects of family history and APOE-ε4 (Cupples et al., 2004; Payami et al., 1997) with respect to risk.

Elucidation of the relative influence of family history of AD and APOE genotype on AD risk, as well as on brain structure and function across the lifespan in normal-functioning individuals is important given that these risk factors co-occur in individuals quite often (Sager, Hermann, & La Rue, 2005). To date, neuroimaging (Bassett et al., 2006) and neuropsychological (Hom, Turner, Risser, Bonte, & Tintner, 1994; Rice, Abraham, Rudrasingham, Owen, & Williams, 2003) studies of individuals at genetic risk for AD by virtue of their family history status have typically either not accounted for APOE genotype or have examined family history and APOE genotype together, defining the presence of both as high risk and the absence of both as low risk (e.g., Fleisher et al., 2005). However, even though few studies have evaluated family

history as an explicit risk factor separate from APOE genotype (Johnson et al., 2006), findings with respect to this risk factor in and of itself have been suggestive of the presence of differences in brain activation patterns and subtle differences in cognitive test performance (Bondi et al., 1994; Rice et al., 2003) associated with a positive family history of AD. Therefore, it remains an empirical question as to whether or not there is an additive influence of these risk factors with respect to cognition in normal-functioning individuals, as well as when, during the lifespan, this influence may emerge (e.g., in childhood).

Thus, with respect to the current study and based on the above-described prior findings, it is hypothesized that children with a positive family history of AD will exhibit lower performance on cognitive and achievement tests relative to children without a family history of AD (Hypothesis 1). Furthermore, given data suggesting additive risk of a positive family history and APOE-ε4 on the lifetime risk of AD, it is also proposed that each of these factors independently contributes to diminutions in cognitive reserve across the lifespan (Richards et al., 2003). Therefore, it is further hypothesized that ε4-positive children with a positive family history of AD will show the lowest performance on cognitive and achievement tests relative to ε4-positive children without a positive family history, as well as ε4-negative children with and without a positive family history (Hypothesis 2).

## *Method*

The study was approved by the Institutional Review Boards of the University of California, San Diego and San Diego State University. Informed consent was

obtained from a parent of each participant, and informed assent was obtained from each participant.

*Subjects*

Recruitment and screening procedures were previously described within the context of Study 2.

*Procedures*

Cognitive testing and DNA collection and analysis procedures were also previously described within the context of Study 2. With respect to the current study, only children for whom a completed online demographic questionnaire was available (i.e., a questionnaire pertaining to the child's developmental, medical, educational, psychiatric, and family medical history) were included. Again, standardized NCE scores for the RCFT-CC and each subtest of the CAT-6 (i.e., Language, Reading, Spelling, and Math) were used in all data analyses. NCE scores have a mean of 50 and a standard deviation of 21.06.

*Family history data.* In addition, data from the family history section of the online demographic questionnaire described in Study 2 was utilized in the current study. Specifically, in the "Family History" section the parent was asked to indicate whether or not the child's biological family history was significant for any of the following: (a) Parkinson's disease, (b) AD, (c) significant memory problems, (d) strokes or Transient Ischemic Attacks/"mini-strokes," (e) heart attacks, (f) hypertension, (g) learning or reading difficulty, (h) grade retention, and (i) attention problems. In addition, for each instance in which the parent endorsed a positive

family history, he or she was asked to indicate how the affected individual(s) is related to the child. With respect to the family history variables of interest in the current study (i.e., a history of AD and/or a history of memory problems), children were grouped based on the above-described parent-report data, which indicated the presence or absence of a history of AD and the presence or absence of a history of significant memory problems (MP).

*Statistical Analyses*

All statistical analyses were conducted using SPSS statistical software. Scores on the RCFT-CC and on each of the CAT-6 subtests were found to be generally normally distributed with roughly equal variances among the genotype and family history groups. No extreme cases were observed.

Initially one-way ANOVA was used to examine mean RCFT-CC and CAT-6 subtest scores separately as a function of APOE-ε4 status (i.e., ε4-positive or ε4-negative). In addition, mean scores as a function of family history (FH) status (i.e., FH-positive or FH-negative) were also examined using one-way ANOVA. Univariate ANOVA was then employed to examine the interaction between APOE-ε4 and FH status. Given that investigation of these research questions among a sample of school-aged children can be considered exploratory, as well as to allow for maximal statistical power in this preliminary study, an alpha level of .05 was used in the interpretation of all statistical tests.

*Sample Size and Characteristics*

As described in Study 2, after exclusions, there was a subgroup of 147 children for whom basic demographic data, genotype, and RCFT-CC data were available. Furthermore, 110 of these children had family history data available, and a subset of 102 of these children had group achievement test data available. In addition, there was 1 subject who had an RCFT-CC score that fell more than 3 standard deviations below the mean and was considered an extreme case and thus removed from subsequent analyses of RCFT-CC scores only. Therefore, analyses of RCFT-CC data within the context of Study 4 will be based on a total of 109 subjects, and analyses of Achievement test data within the context of Study 4 will be based on 102 subjects.

## *Results*

*Basic Demographics*

Allele frequencies and other basic demographic data presented as a function of APOE-ε4 status can be found in Table 5.1. The reader should note that the ε4-negative group consisted entirely of subjects who were ε2/3 heterozygotes and ε3/3 homozygotes, while the ε4-positive group included all ε3/4 heterozygotes with the exception of one subject who was an ε4/4 homozygote; none of the children who were genotyped were homozygous for the ε2 allele. With respect to the sample as a whole, age at the time of study participation ranged from 11.38 to 16.70 years ($M$ = 13.42, $SD$ = 1.25). There was a total of 50 boys (45.5%) and 60 girls (54.5%). The breakdown of the sample with regard to ethnicity was as follows: Asian, $n$ = 5 (4.5%); African American, $n$ = 7 (6.4%); Caucasian $n$ = 80 (72.7%); Filipino, $n$ = 4 (3.6%); Hispanic

Table 5.1

*Basic Demographic Information by APOE-ε4 Status*

| | ε4- | ε4+ | |
|---|---|---|---|
| | *n* = 86 | *n* = 24 | *p* |
| Allele Frequency | 0.782 | 0.218 | n/a |
| Age at Exam[a] | 13.42 (1.22) | 13.42 (1.40) | .985[b] |
| Male/Female | 38/48 | 12/12 | .613[c] |
| Ethnicity | | | .123[c] |
| Asian | 5 | 0 | |
| African American | 3 | 4 | |
| Caucasian | 64 | 16 | |
| Filipino | 4 | 0 | |
| Hispanic | 9 | 4 | |
| Other | 1 | 0 | |
| Handedness (R/L) | 74/12 | 22/2 | .465[c] |
| Parent Education in Years[a] | | | |
| Mother | 16.35 (2.08) | 16.46 (2.17) | .822[b] |
| Father[d] | 16.24 (2.41) | 16.21 (2.60) | .962[b] |
| AD Family History (Yes/No) | 23/63 | 9/15 | .305[c] |

[a]Data are presented as mean (standard deviation). [b]One-way ANOVA used to test group differences. [c]Chi-square used to test group differences. [d]Based on *n* = 109.

*n* = 13 (11.8%); and Other *n* = 1 (0.9%). Parental educational attainment was assessed via parent-report. Based on the information provided, years of education were determined according to widely-accepted criteria (Heaton et al., 2004). Maternal years of education ranged from 12 to 20 (*M* = 16.37, *SD* = 2.10), and paternal years of education ranged from 10 to 20 (*M* = 16.23, *SD* = 2.44). With respect to the sample as a whole, there was a total of 14 youth (12.7%) who identified themselves as left-hand dominant for writing. None of the children identified themselves as ambidextrous for writing.

*Family history status.* With respect to the sample as a whole, there was a total of 32 children (29.1%) for whom a family history of AD was endorsed. The breakdown with regard to the relation of the reportedly affected relative(s) was as follows: grandparent, *n* = 18; great-grandparent, *n* = 10; great-aunt, *n* = 1; uncle, *n* = 1; and two or more relatives, *n* = 2. In addition, there was a total of 22 children (20.0%) for whom a family history of memory problems was endorsed. The breakdown with regard to the relation of the reportedly affected relative(s) was as follows: grandparent, *n* = 15; great-grandparent, *n* = 4; and parent, *n* = 3. Furthermore, considering cases of overlap, there was a total of 43 children (39.1%) for whom a family history of AD and/or memory problems was endorsed.

When examining the combined effect of genotype and family history status (i.e., testing the interaction), two sets of analyses were completed. First, analyses were run with a positive family history defined as the presence of a relative with AD.

Second, analyses were rerun with a positive family history defined as the presence of a relative with AD, significant memory problems, or both.

*Cognitive and Achievement Test Performance*

*Influence of AD family history status.* One-way ANOVA was used to examine the extent to which a positive family history of AD (FH-positive) was associated with differences in RCFT-CC and CAT-6 subtest performance. Contrary to expectations, results failed to find any significant differences in test performance between FH-positive and FH-negative children on any of the measures examined.

*Influence of APOE-ε4 status.* One-way ANOVA was also used to examine the extent to which the presence of the APOE-ε4 allele was associated with differences in cognitive and achievement test performance in this sample of children. However, results again failed to find any significant differences in test performance between ε4-positive and ε4-negative children.

*Combined influence of AD family history and APOE-ε4 status.* Univariate ANOVA was used to examine the extent to which the combined effects of a positive family history of AD and the presence of the APOE-ε4 allele are associated with decreased cognitive and achievement test performance in school-aged children (i.e., a test of the interaction). Consistent with results presented above, analyses failed to find a significant main effect of AD family history status or APOE-ε4 status with respect to RCFT-CC or any of the Achievement test scores (Table 5.2). However, remarkably, a significant interaction between AD family history status and APOE-ε4 status was observed with respect to RCFT-CC scores ($F(1, 105) = 4.292$, $p = .041$; $\eta_p^2 = .039$)

Table 5.2

*Mean Cognitive and Achievement Test Performance by APOE-ε4 Status and Family History of AD*

| | ε4- | | ε4+ | | APOE | AD | Interaction |
|---|---|---|---|---|---|---|---|
| | FH+ | FH- | FH+ | FH- | $p^b$ | $p^c$ | $p^d$ |
| RCFT-CC | 56.01 (10.67) | 50.67 (14.03) | 48.74 (9.14) | 56.03 (8.63) | .755 | .748 | .041 |
| | $n = 22$ | $n = 63$ | $n = 9$ | $n = 15$ | | | |
| Math[a] | 68.90 (19.79) | 63.35 (18.80) | 54.25 (19.93) | 64.79 (13.74) | .167 | .600 | .093 |
| | $n = 20$ | $n = 60$ | $n = 8$ | $n = 14$ | | | |
| Language[a] | 71.40 (15.94) | 69.92 (19.80) | 54.88 (29.74) | 72.57 (12.64) | .163 | .103 | .055 |
| Reading[a] | 71.00 (17.00) | 68.42 (18.54) | 57.00 (18.64) | 71.00 (11.54) | .206 | .206 | .067 |
| Spelling[a] | 66.45 (17.92) | 64.82 (17.49) | 63.25 (35.47) | 67.79 (22.05) | .982 | .778 | .549 |

[a]Sample sizes listed with respect to the Math subtest also apply with respect to the other achievement tests (i.e., Language, Reading, and Spelling). [b]Represents main effect of APOE-ε4 status in univariate ANOVA. [c]Represents main effect of AD

Table 5.2 continued

family history status in univariate ANOVA. [d]Represents interaction between APOE-ε4 status and AD family history status in univariate ANOVA. Note. All data presented as mean (standard deviation).

such that FH-positive children possessing a copy of the APOE-ε4 allele obtained the lowest scores on the RCFT-CC. Furthermore, interactions between AD family history status and APOE-ε4 status approached significance for Math ($F(1, 98) = 2.875$, $p = .093$; $\eta_p^2 = .028$), Language ($F(1, 98) = 3.783$, $p = .055$; $\eta_p^2 = .037$), and Reading ($F(1, 98) = 3.423$, $p = .067$; $\eta_p^2 = .034$) scores, again, with FH-positive children who also possess a copy of the APOE-ε4 allele obtaining the lowest scores with respect to each measure.

*Additional influence of a family history of memory problems (MP).* In an effort to both further explore the influence of a family history of memory problems on cognitive test performance in children, as well as increase statistical power in the current preliminary study, analyses were rerun such that the FH-positive group now included children whose parents endorsed either a positive family history of AD, a positive family history of memory problems (MP), or both (Table 5.3). Univariate ANOVA again failed to find a significant main effect of AD/MP family history status or APOE-ε4 status with respect to RCFT-CC or any of the Achievement test scores, although a main effect of AD/MP family history status approached significance with respect to Language ($F(1, 98) = 3.004$, $p = .086$; $\eta_p^2 = .030$) scores (i.e., FH-positive children exhibited lower scores than FH-negative children, as would be predicted). However, consistent with findings presented above, significant interactions between AD/MP family history status and APOE-ε4 status were observed with respect to RCFT-CC ($F(1, 104) = 6.181$, $p = .015$; $\eta_p^2 = .056$; Figure 5.1), Language ($F(1, 98) =$

Table 5.3

*Mean Cognitive and Achievement Test Performance by APOE-ε4 Status and Family History of AD and/or Memory Problems*

| | ε4- | | ε4+ | | APOE | AD/MP | Interaction |
|---|---|---|---|---|---|---|---|
| | FH+ | FH- | FH+ | FH- | $p$[b] | $p$[c] | $p$[d] |
| RCFT-CC | 55.48 (11.40) | 50.67 (13.61) | 48.32 (9.19) | 57.51 (7.42) | .956 | .439 | .015 |
| | $n$ = 31 | $n$ = 53 | $n$ = 11 | $n$ = 13 | | | |
| Math[a] | 68.39 (18.51) | 62.77 (19.26) | 56.30 (19.34) | 64.83 (13.60) | .270 | .748 | .120 |
| | $n$ = 28 | $n$ = 52 | $n$ = 10 | $n$ = 12 | | | |
| Language[a] | 71.21 (16.29) | 69.79 (20.19) | 56.50 (26.47) | 74.17 (12.98) | .273 | .086 | .044 |
| Reading[a] | 71.14 (15.69) | 67.94 (19.32) | 57.60 (16.91) | 72.83 (10.95) | .310 | .159 | .032 |
| Spelling[a] | 66.21 (16.30) | 64.69 (18.25) | 62.00 (31.98) | 69.58 (22.78) | .945 | .535 | .352 |

Table 5.3 continued

[a]Sample sizes listed with respect to the Math subtest also apply with respect to the other achievement tests (i.e., Language, Reading, and Spelling). [b]Represents main effect of APOE-ε4 status in univariate ANOVA. [c]Represents main effect of AD/MP family history status in univariate ANOVA. [d]Represents interaction between APOE-ε4 status and AD/MP family history status in univariate ANOVA. Note. All data presented as mean (standard deviation).

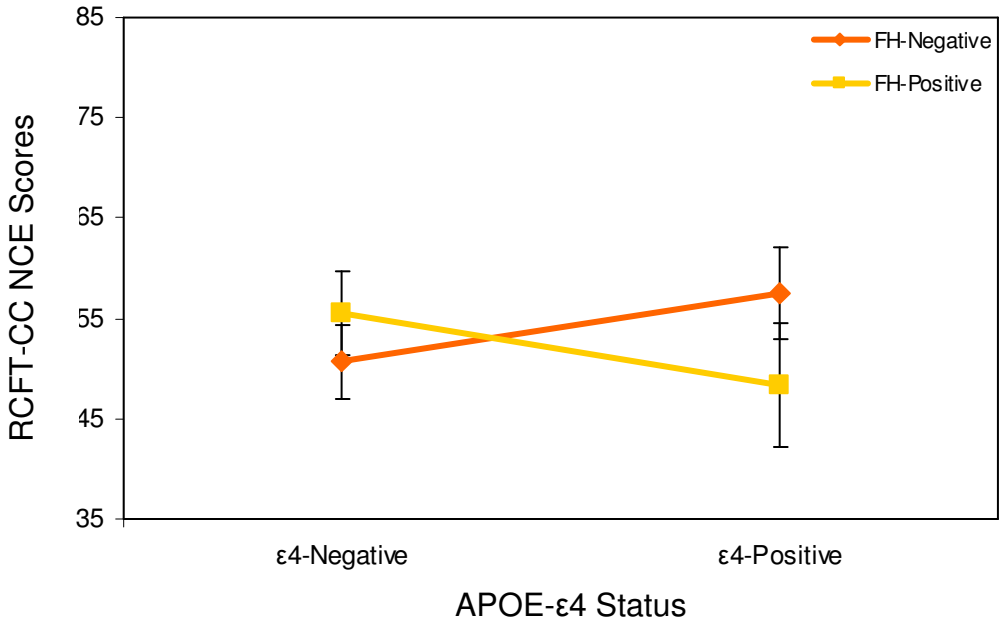


Figure 5.1. Mean RCFT-CC NCE scores for children with and without a family history of AD and/or memory problems as a function of APOE-ε4 status. Error bars represent 95% confidence interval.

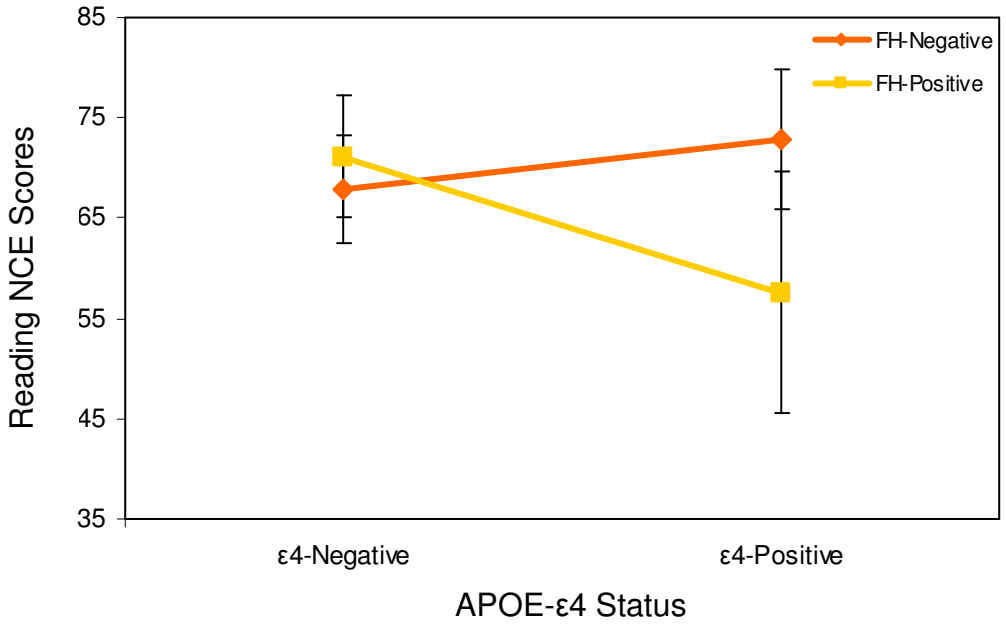


Figure 5.2. Mean CAT-6 Reading NCE scores for children with and without a family history of AD and/or memory problems as a function of APOE-ε4 status. Error bars represent 95% confidence interval.

4.151, $p = .044$; $\eta_p^2 = .041$), and Reading ($F(1, 98) = 4.726$, $p = .032$; $\eta_p^2 = .046$; Figure 5.2) scores such that FH-positive children who possessed a copy of the APOE-ε4 allele obtained the lowest scores on each of these measures.

*Discussion*

The results of Study 5 suggest that the combined effects of a positive family history of AD and/or memory problems, coupled with the presence of an APOE-ε4 allele, predict lower cognitive test performance in school-aged children. These findings suggest that together, these risk factors may contribute to early-life diminutions in cognitive reserve. However, notably, children with both risk factors still obtained scores within the average range on each of the measures examined. With respect to cognitive functioning in childhood, these findings suggest a synergistic effect of these risk factors, rather than an additive effect given that no significant main effects with respect to FH status or APOE-ε4 status were observed.

From these data it appears that as yet unknown factor(s) embodied in a family history of AD may influence the impact of the ε4 allele on cognitive functioning as early as childhood. Specifically, these factors could consist of other genetic factors (Borroni et al., 2004) and/or environmental factors (i.e., gene-gene and/or gene-environment interactions). Notably, research has suggested that several early-life risk factors, seemingly environmental in nature but that could also reflect a genetic contribution, have been found to be associated with the development of AD. These include but are not limited to poor perinatal conditions (i.e., birth weight and prenatal nutrition, intrauterine environment, and sibship size and birth order), sub-optimal

early-life brain development (i.e., head growth, nutrition), poor early-life socioeconomic conditions (i.e., low parental SES, birth order, sibship size, household size), decreased environmental enrichment, and history of traumatic brain injury (for review see Borenstein et al., 2006). It is possible that while some proportion of the variance with respect to these factors may be shared with APOE-ε4 status, there may be a significant proportion that is independent of APOE-ε4 and accounted for by FH. These "environmental" factors may then interact with APOE-ε4 to produce lower cognitive test performance in children with both risk factors.

There are noteworthy limitations to the current study. First, assessment of family history status with respect to both AD and memory problems consisted only of parent-report data. A more thorough and reliable method would have been to obtain family medical records to confirm clinical diagnosis of AD, or even more conservatively, include only those children with a family history of autopsy-confirmed AD. Second, this study did not distinguish between children with second- or third-degree relatives with AD or memory problems. Rather, inclusion of a child in the FH-positive group was based only on parent-report of the presence or absence of any relative with a history of AD or memory problems. Furthermore, "memory problems" was not operationally defined. In addition, like the previous studies reported, although this was not a significantly underpowered study, statistical power was not optimal for detecting the small effect sizes observed.

For example, the interaction effect of FH and APOE-ε4 status on RCFT-CC scores (i.e., the largest effect observed) was relatively small ($\eta^2 = .056$, $d = .24$,

observed power = .693). In addition, sample sizes were not large enough to examine the influence of APOE genotype (i.e., compare performance among ε2-positive versus ε3/3 heterozygotes versus ε4-positive children) or gender, and the previous studies reported here have found these to be important explanatory variables. Another limitation is the potential error introduced by performing multiple comparisons, and limitations associated with the sampling method and characteristics of the sample itself that were previously discussed with respect to Studies 2, 3, and 4 also apply in the current study.

As this study is a preliminary study, findings suggest a number of areas for further study. First, more thorough and reliable assessment of family history status is critical before any notable weight can be placed on these results. Second, because the effect sizes observed appear to be relatively small, studies with larger sample sizes are necessary. In addition, larger sample sizes would allow for analysis of other explanatory variables, including APOE genotype (i.e., ε2-positive versus ε3/3 heterozygotes versus ε4-positive children) and gender. Finally, examination of multiple cognitive domains, in particular memory, is critical given that most studies that have found differences between individuals with and without a family history of AD have primarily observed these differences with respect to learning and memory (Bondi et al., 1994; La Rue, O'Hara, Matsuyama, & Jarvik, 1995). Use of a comprehensive individually-administered battery would achieve this goal. Finally, assessment of additional early-life variables (e.g., head circumference, sibship size) and genetic factors (e.g., catechol-O-methyltransferase gene) that have been

implicated in AD pathogenesis may shed additional light on the complex interplay between environmental and genetic risk factors for AD and their impact on cognitive functioning in early life.

## Conclusion

The studies presented here have aimed to explore the relationship between APOE genotype and cognitive functioning in early life. Study 1 was a study of the relationship between APOE genotype, AD diagnostic status, educational attainment, and handedness in a sample of older adults. Results of this study suggest that there may be an association between educational attainment and APOE genotype such that ε4-positive individuals attain fewer years of formal education relative to ε2-positive individuals even after accounting for variance associated with AD diagnostic status. Specifically, initial hypotheses with respect to this relationship were supported.

Study 2 examined cognitive test performance and handedness as a function of APOE genotype in a sample of school-aged children. Results of this study found that, contrary to predictions, the ε2 allele was associated with decreased performance on a measure of visuospatial perception and construction. Furthermore, consistent with predictions, these studies together suggest a higher prevalence rate of left-handedness among ε2-positive individuals relative to ε3/3 homozygotes and ε4-positive individuals (i.e., in both adults and children). Finally, although it was hypothesized that gender would be a significant moderator of test performance in children, this prediction was not supported.

Results of these initial studies were surprising in that they found some evidence that could be consistent with the possibility that the APOE-ε2 allele, contrary to expectations, may be a risk factor for problems during infancy or early childhood. Specifically, the finding of an increased prevalence of left-handedness among ε2-

positive individuals could reflect an association with factors that give rise to atypical hemispheric dominance. This finding could provide clues as to the basis for the discrepant prevalence rates of the different APOE genotypes themselves (i.e., the fact that while the ε2 allele appears to be quite beneficial to survival later in life, it is paradoxically the least prevalent isoform). Taken together with a small number of developmental studies that have found evidence for an advantageous effect of the ε4 allele during the human pre-, perinatal, and infancy periods of life, and possibly a detrimental effect of the ε2 allele, these findings add to evidence beginning to accumulate in the literature of antagonistic pleiotropy with respect to APOE genotype.

Antagonistic pleiotropy refers to selection of a gene that confers an advantage at one age and a disadvantage at another depending on how it affects total reproductive probability (Albin, 1993; Williams, 1957). It may be that natural selection selects against the ε2 allele during the perinatal and infancy periods in favor of the ε4 allele, which may confer beneficial effects during this time. This then occurs at the expense of detrimental effects of APOE-ε4 during the post-reproductive years. Additional evidence that could be consistent with this notion comes from studies that have suggested that the APOE-ε4 allele may have a possible advantageous effect in early adulthood (e.g., Mondadori et al., 2006) relative to the ε2 allele, as well as the finding of better neuropsychological test performance following TBI among a sample of ε4-positive versus ε4-negative young adults (Han, Drake et al., 2007).

Although the finding of higher educational attainment among ε2-positive individuals relative to ε4-positive individuals seemingly contradicts this theory, there

are a number possible explanations for this inconsistency. First, the elderly ε2-positive individuals included in the sample of Study 1 represent a subgroup of individuals who have survived to old age. Thus, it follows that those ε2-positive individuals most adversely affected during early development may not be represented in the study; thus, findings may reflect this bias. Second, given that this is a sample of individuals recruited for studies of AD and abnormal aging, it could also be the case that a significant proportion of the ε4-postive individuals who were initially diagnosed as normal control, may actually go on to develop AD. Thus, despite attempting to partial out variance associated with APOE genotype and variance associated with AD diagnostic status, findings, to some extent, may still reflect differences in educational attainment that are primarily a function of AD diagnostic status rather than APOE genotype.

In addition, results from Study 5 suggest a third possible explanation for the finding of higher educational attainment among ε2-positive individuals relative to ε4-positive individuals, a finding which seemingly contradicts the theory of antagonistic pleiotropy with respect to APOE genotype. Specifically, as previously mentioned, this sample of elderly individuals was recruited for studies of AD and abnormal aging, and as such, it is possible that many subjects may have a positive family history of AD. Unfortunately, this variable was not accounted for in Study 1. However, if it is the case that a disproportionately high number of the ε4-positive elderly subjects have positive family history of AD, taken together with findings from Study 5, it would follow that educational attainment may in fact be lower in this group of subjects. That

is, the theory of antagonistic pleiotropy holds, except when APOE genotype interacts with factors associated with a positive family history of AD.

Study 3 was an examination of longitudinal test performance in school-aged children as a function of APOE genotype. While as predicted, findings generally showed stable test performance over time across APOE genotype, follow-up analyses showed differences that approached significance in Reading subtest change scores between boys and girls as a function of genotype. Although minimal importance can be placed on this finding, it is somewhat notable in light of the fact that it was the ε2-positive boys and girls who exhibited the largest differences between performance at Time 1 and Time 2. Specifically, this could be consistent with the idea that the ε2 allele is associated with detrimental effects early in life, and the overall theory of antagonistic pleiotropy with respect to APOE genotype.

Study 4 aimed to examine the relationship between APOE genotype and cognitive asymmetry in children. Somewhat contrary to predictions, results suggested that APOE genotype is in fact associated with significant cognitive asymmetry (i.e., mean discrepancy scores and incidence of asymmetric cognitive profiles) in children, but that gender fully moderates this association. Specifically, while the APOE-ε4 allele was found to be associated with significant asymmetry in school-aged girls, the APOE-ε2 allele was found to be associated with significant asymmetry in school-aged boys. Taken together, these findings, in conjunction with multiple other lines of research, raise the possibility that boys may be more susceptible than girls to the "neurodevelopmental" risk that may be associated with the ε2 allele (i.e., given their

heightened susceptibility to neurodevelopmental disorders in general), and that girls may be more susceptible than boys to the "neurodegenerative" risk associated with the ε4 allele (i.e., given reports of greater susceptibility of women to ε4-related cognitive decline). However, this explanation is speculative and not entirely consistent with the notion of antagonistic pleiotropy with respect to APOE genotype. Further study that takes into account gender-related developmental variables (e.g., pubertal stage) may help clarify this relationship, especially given that some studies have shown that hormone levels (e.g., estrogen) can influence the development and clinical expression of AD.

Finally, Study 5, a preliminary study, was an effort to begin to explore the relationship between APOE-ε4 status and a family history of AD. While there are several limitations to this study, the finding of a synergistic effect between family history of AD and APOE-ε4 status (i.e., children with both risk factors exhibited the lowest performances on cognitive and achievement tests), as predicted, raises the possibility that as yet unknown factor(s) embodied in a family history of AD may influence the impact of the ε4 allele on cognitive functioning as early as childhood. Furthermore, these factors could consist of other genetic factors and/or environmental factors (i.e., gene-gene and/or gene-environment interactions). Specifically, results suggest that the theory of antagonistic pleiotropy holds, except when APOE genotype interacts with factors associated with a positive family history of AD. The hypothesis that a positive family history of AD in and of itself would be associated with

significantly lower test performance in this sample of school-aged children was not supported.

In conclusion, while interesting findings have emerged from this work, overall, the present studies have created more questions than answers. Reasons for this stem, in large part, from the limitations associated with these studies, which were outlined within the context of each report. However, most notably, future studies in this area should employ larger samples that will increase statistical power to detect relatively small effects (i.e., effect sizes observed were generally between .20 and .25). Furthermore, since it appears that the ε2 allele itself may be associated with a unique neurocognitive phenotype, future studies should continue to examine the effect of each APOE genotype rather than APOE-ε4 status alone (i.e., the presence or absence of the ε4 allele). In addition, assessment of multiple cognitive domains will be critical in order to fully understand the nature of the relationship between APOE genotype and cognitive functioning in children, as well as the clinical significance of this relationship, if any. Finally, better assessment of family history variables, as well as assessment of additional early life variables and genetic factors that have been implicated in AD pathogenesis, may shed additional light on the complex interplay between genetic and environmental risk factors for AD and their impact on cognitive functioning in early life.

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