GENETIC MARKERS FOR COGNITIVE HEALTH IN THE VERY OLD

by

Melissa Gonzales McNeal

A THESIS

Presented to the Department of Molecular and Medical Genetics

and the Oregon Health Sciences University

School of Medicine

in partial fulfillment of

the requirements for the degree of

Master of Science

January 2001

School of Medicine

Oregon Health Sciences University

CERTIFICATE OF APPROVAL

This is certify that the M.S. thesis of

Melissa Gonzales McNeal

has been approved

[redacted]
Professor in charge of thesis

[redacted]
Member

[redacted]
Member

[redacted]
Member

[redacted]
Member

TABLE OF CONTENTS

Title Page

Table of Contents i

Aknowledgements ii

Abstract iii

Chapter 1

Thesis Background and Rational 2

My Contributions 7

Chapter 2

Predictors of Healthy Brain Aging 9

Chapter 3

Increasing Age and the Apolipoprotein E Associated Risk of Developing Alzheimer's Disease 34

Chapter 4

Discussion 46

References 51

AKNOWLEDGEMENTS

I am grateful to all the people who made this work possible. Foremost, I would like to thank my mentor, Dr. Haydeh Payami, for her insight and friendship. I sincerely appreciate her support and guidance throughout my graduate work.

My sincere thanks to the members of my thesis advisory committee, Dr. Patty Kramer, Dr. Mike Litt, Dr. Cheryl Maslen, Dr. Haydeh Payami, and Dr. Gary Sexton for their guidance and recommendations on this project.

I thank Dr. Richard Camicioli and Dr. Jeff Kaye for their invaluable expertise and patience with my numerous questions. I also owe thanks to the ADC staff for their roles in subject recruitment, data collection, data entry, and training.

I would like to thank Sepi Zareparsi for her great friendship as well as her assistance. I also thank Lina Moses and Ranjani Ramakrishnan for the enjoyable conversations.

I owe special thanks to my family, especially my husband Joseph. They were always there for me, believing in me, encouraging me, and cheering me on. Thank you for all of your support, your sacrifices and most of all your love.

So many people have helped in so many ways; I find it impossible to list all of their contributions. To all of my friends and colleagues: thank you for your time, understanding, encouragement, inspiration and knowledge. To all of you that have answered my endless questions, offered advice, lent an ear to listen or a shoulder to cry on: thank you from the bottom of my heart.

ABSTRACT

The unremitting rise in the incidence of cognitive impairment (CI) with age, coupled with the sudden and dramatic increase in life expectancy, has created an urgent priority to address the cognitive health of the elderly. The overall objective of this thesis was to examine biological markers that modulate Alzheimer's disease (AD) onset in younger populations to determine whether the genetic risk factors continue to be important in cognitive decline in the very old. To determine if superior health at old age predicts protection against CI and AD, we prospectively studied a group of optimally healthy elderly subjects, highly selected for cognitive and physical health at age ≥85 years (oldest-old). Initially subjects represented the top 3% of the oldest-old for health. Longitudinal data was used to examine cognitive and neuropathological outcomes as well as to estimate risk. The effects of age, APOE genotype and gender were assessed. The data showed these optimally healthy elderly had lifetime risks similar to the general population, but their onset ages of cognitive decline were later. This suggests factors that delay onset of cognitive decline are key to improving cognitive health in the elderly. In this population, absence of the Apolipoprotein E (APOE) ε4 allele and male gender were associated with delayed onset of CI, while estrogen use and education had no detectable effect on cognitive outcome. To address the issue of a possible diminished association between AD and the APOE ε4 allele with increasing age, we prospectively followed a group of subjects cognitively intact at age ≥75 years. We calculated the ε4-associated risk of developing AD at subsequent time points, effectively controlling for all variables except age. Our data

confirm the notion that APOE genotype does not exert its influence with the same magnitude at very advanced ages. Continued efforts to further characterize severe dementia and to identify additional environmental and genetic risk factors in the very old are warranted.

CHAPTER 1

INTRODUCTION

THESIS BACKGROUND AND RATIONAL

By 2050 in the U.S., the number of people aged ≥65 years is expected to total 78 million and the number of people 85 years and older is expected to reach almost 18 million (Schneider 1999). With the elderly population growing at such a phenomenal rate, increasing numbers of individuals are at risk for cognitive impairment (CI). Alzheimer's disease (AD) alone, the most severe form of CI, affects up to half of people over age 85 (Evans et al. 1989). Loss of cognitive function has a devastating impact not only on the lives of patients and their families, but it also has profound implications for medical healthcare providers and future healthcare costs. In the face of the rapid increase in the proportion of very old in our society and the concomitant increase in numbers of Americans at highest risk for cognitive impairment, it is urgent that the parameters of CI be defined and ways to combat it be found.

Little is known about the pathogenesis of CI or its relationship to the aging process. The current body of knowledge has come mainly from studies on AD and related dementias. While AD has a characteristic clinical course and pathology, it is a heterogeneous condition with varied manifestations. Age at onset of AD ranges from around 30 to after 90 years. It remains unclear, however, whether CI at age ≥90 years is the same as AD at 45 years. The characteristic features of brain pathology not only differ sharply among AD patients, but brains of clinically normal individuals often show age-related changes similar to those found in the brains of AD patients. The degree of pathology, however, is usually much greater in AD. It has thus been speculated that AD

may be a form of accelerated brain aging. The uncertainty is emphasized by the fact that standard clinical criteria, set by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), only allow for an AD diagnosis up to the age of 90 years (McKhann et al. 1984). The question arises whether those who escape AD are innately immune or in preclinical stages of a process that is an inevitable consequence of aging. Two meta-analyses have shown an unremitting rise in incidence rates of dementia and AD with age, suggesting everyone is at risk if they live long enough (Gao et al. 1998; Jorm and Jolley 1998). Another study, having observed a plateau at higher ages, proposed that very-late onset AD is truly a disease (Meyer et al. 1998): one to which some individuals are vulnerable, while others are immune.

Although there are examples of early onset AD (onset <65 years), the majority of AD occurs after the age of 65 (Kokmen et al. 1996; Rocca et al. 1991). Early onset AD has a significant genetic component (St George-Hyslop 2000). Since the etiology of AD in the very old may be different, it is not known if the same genetic factors play a role. The overall objective of this thesis project was to examine biological markers that modulate AD onset in younger populations to determine whether the genetic risk factors continue to be important for CI in the very old. Identification and characterization of risk factors involved in the development of CI at advanced ages will help clarify the relationship between "normal aging", CI, dementia and AD. This has important implications for understanding the mechanisms of neurodegenerative diseases. It will also increase our

understanding of the pathogenesis of this complex disorder and may provide clues for mechanisms involved in age-related neurodegeneration.

To explore cognitive health in the very elderly, this thesis project used data from the Oregon Brain Aging Study (OBAS). OBAS is a prospective study established in 1989 with two specific aims: to define healthy brain aging, and to identify genetic and environmental factors that protect against age-related neurodegeneration. Subjects are 65 years of age or older at entry and they are extensively screened for optimal physical and cognitive health (Howieson et al. 1993; Kaye et al. 1994; Kaye et al. 1997). Once entered into the study, they are followed with frequent and detailed examinations until death, at which point most receive neuropathological examination (Green et al. 2000).

Previous reports published on OBAS show evidence that some genetic risk factors for AD onset in younger populations (i.e. family history, apolipoprotein E genotype and sex) may indeed continue to play a role in the onset and risk of CI at very advanced age (Payami et al. 1997; Payami et al. 1994a). In AD, presence of an affected first-degree relative is associated with an approximately fourfold increased risk of disease development (reviewed by Levy-Lahad et al. 1998). It is possible that positive family history is a surrogate marker for other yet to be identified genes. One study, using OBAS data, compared the occurrence of dementia in first-degree relatives of oldest-old (age ≥85 years) OBAS subjects to first-degree relatives of random controls and AD patients (Payami et al. 1994a). Risk of dementia for OBAS relatives was 1/3 the risk for control relatives and 1/11 the risk for relatives of AD patients. The results suggested that

cognitive health could aggregate in families. A second risk factor for AD is apolipoprotein E (APOE) genotype. The association between the APOE ε4 allele and increased risk of AD has been confirmed worldwide. The presence of ε4 shifts the onset of AD to a significantly earlier age and elevates the age-specific risk for developing AD (Corder et al. 1993). Another study, that used OBAS data, examined effects of family history and apolipoprotein E genotype on cognitive health in OBAS subjects cognitively healthy at age ≥75 (Payami et al. 1997). Subjects with a family history of dementia had a trend toward earlier age at onset compared to those without a family history of dementia, although the difference was not statistically significant. In addition, subjects who had the ε4 allele showed a significantly earlier age at onset and a four fold higher risk for dementia compared to subjects with no ε4 allele.

There is no question that women with AD out number men with AD (Katzman et al. 1989). Meta-analyses of incidence studies showed little difference in gender-specific dementia, but women, however, tended to have a higher incidence of AD (Gao et al. 1998; Jorm and Jolley 1998). This could be explained by the fact that men tended to have a higher incidence of vascular dementia at younger ages (Jorm and Jolley 1998). When Payami et al (Payami et al. 1994a) compared the occurrence of dementia in first-degree relatives of oldest-old OBAS subjects to first-degree relatives of random controls and AD patients, they found more women were affected with dementia than men in every group.

The first study in this thesis "Predictors of Healthy Brain Aging" was carried out to determine if superior health at old age predicts protection against CI and AD. The

subjects were initially in optimal physical and cognitive health at age ≥85 years. Longitudinal data was used to examine cognitive and neuropathological outcomes, as well as to estimate risk. The effects of age, APOE genotype, and gender on cognitive well being were assessed. This study focused primarily on the oldest-old subjects for several reasons: 1) With aging of the current baby boomers and medical advancements leading to decreased mortality, the oldest-old are the fastest growing segment of the U.S. population. 2) They have the highest risk for cognitive impairment. 3) They are the least studied. 4) This addressed the issue of ascertainment bias in OBAS where two age groups were initially recruited: young-old (65 to 84 years) and oldest-old (≥85 years). 5) They may be less genetically heterogeneous than the young-old since they are at the extreme of the onset distribution.

The second study in this thesis "Increasing Age and the Apolipoprotein E Associated Risk of Developing Alzheimer's Disease" was prompted by findings from the first study "Predictors of Healthy Brain Aging". Data from the first study of this thesis showed the effect of ε4 on risk of AD was statistically non-significant. This was in contrast to the previously published report on effects of family history and apolipoprotein E genotype in OBAS (Payami et al. 1997). Data from the published report showed that subjects with an ε4 allele had a significant four fold higher risk of developing AD than subjects with no ε4 allele, $p=0.05$. Although the association between AD and the APOE ε4 allele is confirmed, it has been suggested that the APOE ε4 effect may age dependent (Corder et al. 1994). Thus the decreased ε4 associated risk and the loss of significance

between the first study of this thesis and the previously published report could be due to aging of the cohort. The second study of this thesis, "Increasing Age and the Apolipoprotein E Associated Risk of Developing Alzheimer's Disease", was designed to explore whether the APOE ε4 effect in OBAS was age dependent. Longitudinal data on a subset of very old OBAS subjects was used to calculate the ε4 associated risk for the same individuals at subsequent time points, thereby effectively controlling for all variables except age.

My Contributions

For "Predictors of Healthy Brain Aging" I discussed the hypotheses and the study design with my advisor. I compiled clinical data from medical records, cognitive assessment scores from a large database maintained by OBAS, demographic data (e.g. sex, date of birth, ethnicity, etc.) from patient reports, and neuropathological data from autopsy reports. I decided on definitions for CI and AD, with the help of my advisor, so that the raw data could be analyzed. I reviewed autopsy reports and correlated primary and secondary neuropathological findings with clinical diagnoses. I analyzed medications in relation to health outcomes. I examined all health outcomes (e.g. hypertension, stroke, diabetes, cancer, heart disease, etc.) and determined the major risk factors competing with AD. I was directly involved in sampling the subjects and I was responsible for APOE genotyping them. I entered the compiled data and genotypes into one file for analysis. I performed all data analyses. For the first draft of the manuscript, I wrote the methods

section while my advisor wrote the rest. I edited and completed the final draft that was submitted.

For "Increasing Age and the Apolipoprotein E Associated Risk of Developing Alzheimer's Disease", I discussed the hypothesis and study design with my advisor. I did the data analyses and I wrote the manuscript.

CHAPTER 2

PREDICTORS OF HEALTHY BRAIN AGING

Melissa Gonzales McNeal, Sepideh Zareparsi, Richard Camicioli, Allison Dame, Diane Howieson, Joseph Quinn, Melvyn Ball, Jeffrey Kaye and Haydeh Payami

Submitted: *Journal of Gerontology: Biological Sciences*

ABSTRACT

To determine if superior health at old age protects against cognitive impairment (CI) and Alzheimer disease (AD), we prospectively studied 100 optimally healthy oldest-old (≥85 years) individuals. Initially, subjects represented the top 3% of the oldest-old for health. During 5.6±.3 years of follow-up, 34 subjects developed CI, 23 progressed to AD. By age 100, probability of CI and AD were .65±.09 and .49±.10. Median onset age was 97 years for CI and 100 for AD. Clearly, superior health at old age does not guarantee protection against cognitive decline. Lifetime risks were similar to the general population but onset ages were later, suggesting factors that delay onset are key to improving cognitive health in the elderly. In this population, absence of apolipoprotein E-ε4 and male gender were associated with delayed onset, while estrogen use and education had no detectable effect on cognitive outcome.

INTRODUCTION

With the rapidly growing numbers of elderly at risk for cognitive impairment (CI), greater emphasis is warranted on finding ways to improve health and quality of life for the elderly. Longevity, which science has managed to enhance considerably, is not always accompanied with health and independence. Normal cognitive function is essential for maintaining independence. Loss of cognitive function is one of the most prevalent and most feared end-of-life tragedies. The risk of CI rises sharply with increasing ages (Gao et al. 1998; Jorm and Jolley 1998). Alzheimer disease (AD) alone, one of the most severe forms of CI, affects half of all North Americans over the age of 85 (Ebly et al. 1994; Evans et al. 1989; Ritchie and Kildea 1995; Wernicke and Reischies 1994). More suffer with mild or moderate CI.

Our growing knowledge on the pathogenesis of dementia has come primarily from studies of AD and related disorders, which typically occur in the 7^{th} – 9^{th} decades of life. What appears to be AD in the 10^{th} decade, however, may not be the same disease as late-onset (60-90 years) AD. The standard clinical diagnosis of AD, by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al. 1984), excludes those whose onset is ≥90 years. Consequently, little is known about the cause of CI at higher ages, or the factors that modulate its onset and severity.

The oldest-old (age ≥85) is the fastest growing segment of the population (Schneider 1999) and has the highest risk for dementia. It is not known if dementia in the oldest-old is

a disease affecting only those who are susceptible or a condition that is an inevitable consequence of aging. Recent meta-analyses have shown that the incidence of dementia rises with age and does not level off (Gao et al. 1998; Jorm and Jolley 1998), which suggests we may all become impaired if we live long enough. In contrast, another large study concluded that only half of the elderly develop AD, while the other half is immune (Meyer et al. 1998). There were important differences in the study designs and definitions of impairment, making it difficult to draw parallel comparisons. Whether we are all at risk or a subset is innately immune, remains an issue of controversy.

Assuming that some people are "immune" to CI, we set out to identify and study individuals who seemed least likely to be at risk, hoping that they would hold the key to prevention. In 1989, we launched the Oregon Brain Aging Study (OBAS), a prospective study of the effect of aging on the central nervous system. The two primary goals were to define healthy brain aging and to identify factors associated with protection against age-related neurodegenerative disorders. To that effect, we enrolled the healthiest of the oldest individuals in the community and followed them with frequent and detailed examinations until death (Howieson et al. 1993; Kaye et al. 1994). Representing the top 1-3% of the elderly for cognitive and physical health, individuals who entered this study exemplified successful aging. We have followed them for up to 11 years (mean 5.6 years).

Although clinically and pathologically indistinguishable, it is not known if what appears to be AD in the oldest-old is the same disease as late-onset AD or a different condition. The major risk factors for late-onset AD are increasing age (Gao et al. 1998;

Jorm and Jolley 1998), allele ε4 of the apolipoprotein E (APOE) gene (Farrer et al. 1997; Strittmatter et al. 1993) and female gender (Farrer et al. 1997; Gao et al. 1998; Payami et al. 1994b). We questioned if these factors exert a similar effect on cognitive health throughout the most advanced ages. Here we report the cognitive and neuropathological outcomes, estimate risk, and assess the effects of age, APOE genotype and gender on the cognitive well being of "successful agers".

METHODS

At entry to OBAS, subjects were community dwelling, functionally independent, healthy individuals who met the strict inclusion and exclusion criteria listed in Table 1. They were cognitively healthy and free of any condition that can affect cognition (e.g., no stroke, heart disease, hypertension, cancer or diabetes).

Ascertainment

Two methods were used to identify and recruit subjects. 1) Community volunteers: The majority of subjects were recruited from the community by advertisement (print and electronic media) and direct appeals during presentations to local senior citizens groups, seeking elderly volunteers who considered themselves cognitively and physically healthy. 1207 individuals entered the initial screening process through this method. The screening process included a telephone interview followed by a review of medical records to assess inclusion and exclusion criteria. 1008 of the 1207 volunteers were excluded at the

screening stage. The remaining 199 were seen at the medical center or visited at home to obtain vital signs, perform visual and hearing screenings, and administer Mini-Mental State Examination (MMSE) (Folstein et al. 1975), Cornell Depression Scale (Alexopoulos et al. 1988), Geriatric Depression Scale (Yesavage et al. 1983), and Clinical Dementia Rating Scale (CDR) (Hughes et al. 1982). If all assessments were normal, subjects were seen at the medical center for a complete physical and neurological evaluation and laboratory studies as outlined in Table 2 (Kaye et al. 1994). Thirty-six subjects were excluded at this exam. The 163 subjects still meeting all eligibility criteria were asked to return for a neuropsychological evaluation (Howieson et al. 1993) and a magnetic resonance imaging (MRI) examination. Seven subjects were excluded based on the neuropsychological evaluation or MRI results. The 156 subjects who met all eligibility criteria were enrolled in OBAS.

2) Health Care Organizations: A database search, approved by the Institutional Review Board of Kaiser Permanante in Portland, identified 4494 health maintenance organization (HMO) members as being ≥85 years old. A computer survey of their medication records identified 3669 of the 4494 as being on exclusionary medications. Full medical record reviews for the remaining 825 members excluded an additional 698 individuals. The 127 potentially eligible individuals were invited to participate in the study. Ninety-four (74%) declined interview, 12 were found ineligible at interview, 21 underwent physical, neurological, neuropsychiatric and MRI evaluations as described above. Fourteen subjects were eligible and enrolled in OBAS. Given that 4494 oldest-old were surveyed, 127 were potentially eligible, and 74% refused participation, we estimated that the 14 who

were enrolled represented the top 1-3% of the oldest-old for health. Similar computerized surveys were performed in 1997 and 1998 at the Family Practice Clinic and the Internal Medicine Clinic of Oregon Health Sciences University (OHSU). A total of 289 individuals age ≥85 were identified. After the survey for exclusionary medications and a review of medical records, 25 subjects were potentially eligible and invited to participate in the study. Sixteen individuals (64%) did not respond or refused to participate, five were found ineligible, the remaining four underwent the full battery of examinations, met all entry criteria and were enrolled.

There was no significant difference between the community volunteers and those ascertained from health care organizations with regard to baseline MMSE, gender ratio or education level. The two groups, therefore, were pooled. One hundred seventy-four subjects have been enrolled in OBAS. For the present study, we used only those who met all entry criteria at age ≥85 years (N=100). Two were Hispanic (both female), one was Native American (female), and 97 were Caucasian (60 females, 37 males). All subjects signed informed consents approved by the OHSU Institutional Review Board. Separate consent forms were obtained for longitudinal clinical studies, genetic studies, and brain autopsy.

Genotyping

Subjects were asked to donate blood samples for extraction and storage of DNA, genetic studies, and establishment of immortalized cell lines. DNA was extracted by the

salting out method using the commercial kit PureGene. APOE genotypes were determined using a standard method (Hixson and Vernier 1990).

Follow-up Assessments

Once enrolled, every six months, subjects received a home visit for a medical history review, Older Americans Resource Scale (Fillenbaum and Smyer 1981), MMSE and CDR assessments. In addition every year, they underwent full physical, neurological, neuropsychological, and MRI examinations. The biannual assessments and annual examinations continue until death. Upon death, with prior consent, brain autopsies for neuropathological analysis were performed (Green et al. 2000). New medical conditions (e.g., stroke, heart disease, hypertension and cancer) were diagnosed either by the personal physician, which we verified by reviewing the medical records, or by us at the annual examinations.

Definitions of CI and AD

We defined CI as repeated abnormal scores on MMSE or CDR on consecutive assessments without reversion to the normal range. CI as defined here is an irreversible phenotype. All subjects entered the study cognitively intact with MMSE ≥24 and CDR=0. Subjects who maintained MMSE ≥24 and CDR=0 on all follow-up assessments up to the time of this analysis were considered cognitively intact. Subjects who scored MMSE <24 or CDR ≥0.5 on repeated assessments were categorized with CI. Subjects who received

one abnormal score (MMSE <24 or CDR ≥0.5) but recovered to the normal range on subsequent visits were classified as cognitively intact. Subjects who either had one abnormal score immediately prior to this analysis (pending follow-up) or died before their next assessment and had no autopsy were classified as indeterminate. AD was diagnosed clinically using the NINCDS-ADRDA criteria (McKhann et al. 1984), and pathologically by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (Morris et al. 1989).

Age at onset was defined as the age when the subject first scored MMSE <24 or CDR ≥0.5. Age at onset was assigned only to individuals who met the criteria for CI; that is, those who received abnormal scores on consecutive assessments and did not revert. The same definition (age at the first of consecutive abnormal scores) was used for those who progressed to AD.

Statistics

Standard statistical methods were used to calculate means ± standard error (SE). Differences in means (for age, MMSE scores, and years of education) were tested using t-tests. Age-specific cumulative incidence rates were estimated using actuarial and Kaplan Meier survival analysis. Since risk was thought to be a function of age rather than years of follow-up, age was specified for time in all survival models. For subjects who developed CI or AD, age at onset was specified as the time of event. For subjects who remained cognitively intact, current age or age at death was used. Subjects with indeterminate

cognitive status were censored at the last age when they were known to be cognitively intact. Age at onset distributions were plotted by the Kaplan Meier survival analysis method (Kaplan and Meier 1958) and were compared by log-rank statistics. Proportions (differences in allele frequencies, and gender ratios) were tested using Z statistics. Hazard ratios (HR) were calculated using the Cox proportional hazards model with 95% confidence intervals (ci) (Cox and Oakes 1984). Correlation (years of education with onset age) was tested using Pearson's correlation coefficient (r) method. SPSS software (release 10.0.5; 27Nov1999) was used for statistical analyses.

RESULTS

We screened 5990 elderly in the Portland, Oregon area and enrolled 174 subjects who met all entry criteria, including 100 oldest-old (age ≥85) and 74 young-old (65-84 years) individuals. In keeping with the focus on the oldest-old, the present study was limited to the 100 subjects who met all entry criteria at age ≥85. Table 3 lists the characteristics of these subjects.

Major Health Outcomes

At entry to study, subjects were free of cognitive impairment, stroke, heart disease, hypertension, cancer, diabetes and neurological disorders. During 5.6±.3 years of follow-up, 38% developed hypertension, 37% developed CI, 33% developed heart disease (arrhythmia, myocardial infarction), 30% developed cancer (all cancers combined), 25%

developed AD, 23% had a stroke or transient ischemic attack, 6% developed diabetes and 2% became blind. These groups are not mutually exclusive.

Cognitive Outcomes

Table 4 outlines the subjects' cognitive outcomes and their classification. At the time of this analysis, 58 of 100 subjects were classified as cognitively intact. They included 54 subjects who had normal scores (CDR=0 and MMSE ≥24) at all follow-up assessments, and four who had one or two abnormal scores but reverted and maintained normal scores at all subsequent assessments. Thirty-four of the 100 subjects were classified with CI. Eight subjects were classified as indeterminate because they had been fluctuating between the normal and the abnormal range (N=3), or had their first abnormal score at their last visit and were awaiting follow-up (N=3), or died after their first abnormal score without follow-up or autopsy (N=2).

Among the 34 subjects classified with CI, 19 had the clinical diagnosis of possible or probable AD; one was diagnosed as possible vascular dementia and one as mixed dementia. The remaining 13 were CI with questionable or incipient dementia. In addition, of the 13 subjects classified with CI, four did not meet the clinical criteria for AD but were diagnosed with definite AD at autopsy. If AD is defined as meeting clinical or neuropathological diagnosis of AD, there were 23 cases.

Neuropathological Findings

Table 5 shows the neuropathological findings with their corresponding clinical phenotypes. Twenty-seven subjects died, 20 had brain autopsy. Mean age at death was 95.6±.8 years. Neuropathological findings for 19 of the 20 subjects reported in this paper were previously published (Green et al. 2000). Here the neuropathological results are reviewed for the purposes of confirming the outcomes of the survival analyses. Of the twenty subjects with brain autopsy, only one had no AD pathology (neurofibrillary tangles and neuritic or senile plaques). She was 94.7 years old and remained cognitively intact until death. All other 19 brains had Alzheimer pathology to varying degrees. In 13 cases, the pathology was severe enough to fulfill CERAD criteria (Mirra and Heyman 1994) for a primary diagnosis of definite AD (one of the brains with AD also had a pathological diagnosis of Parkinson's disease). Two subjects had neurofibrillary tangles with no or minimal amyloid. In the other four brains, Alzheimer pathology was quantitatively insufficient to qualify for the diagnosis of pathologic AD.

Incidence of CI and AD: Age, Genotype, Gender

The cumulative probabilities of CI and AD rose with increasing ages of the subjects (Table 6). The probability that a subject would live to age 95 was .84±.05. The probability that a subject would live to age 95 cognitively intact was .59±.06. The median age at onset, estimated by the Kaplan Meier analysis, was 97.2±2.0 years for CI and 99.8±1.7 years for AD.

At baseline (Table 3), the APOE genotype and allele frequencies were similar to other published frequencies for this age group (Davignon et al. 1988a). The incident cases of CI had a higher frequency of ε4 (.13 versus .07) and a lower frequency of ε2 (.04 versus .11) than those who remained intact. When analyzed using the Cox method and taking age into account, the effect of ε4 on the risk of CI was significant (HR=2.5, p=.03). Similarly, the age at onset distribution for CI, shown by Kaplan Meier curves (Figure 1), was significantly earlier for ε4 carriers (p=.03). Cox and Kaplan Meier analyses for ε2 showed statistically insignificant trends suggesting reduced risk and delayed onset of CI in the presence of ε2. Similar trends were seen for AD (Table 7, Figure 1), although none reached statistical significance possibly due to smaller numbers of AD.

The study included 37 men and 63 women, which reflects the gender distribution after age 85 in the U.S. (U.S. Census Bureau Internet Release Date: June 15, 1999). As shown in Table 3, 41% of women developed CI as compared to 22% of men. Women had a significantly higher age-specific risk of developing CI than men (HR=3.0, p=.01, Table 7). Women also had significantly earlier age at onset than men (p=.007, see Kaplan Meier curves in Figure 1). Similar trends were seen for AD but they were not significant (Table 7, Figure 1), possibly because sample size and power were smaller for AD than CI.

We wondered if the gender difference in risk was linked to age and longer survival in women, differences in baseline MMSE scores, estrogen use in women, or higher education in men. Men and women were similar in age. At entry, the mean ages were 87.6±.7 for men and 87.4±.5 for women. Currently, men are 93.4±.7 and women are 92.9±.5 years

old. Identical proportions (76%) of men and women lived to age 90 or higher. Thirty-five percent of men and 27% of women lived to age 95 or higher. There was no evidence for increased life span in women, measured either by the Cox method (HR=1.0, p=.9) or by the Kaplan Meier and log rank statistics (p=.9). There was no significant difference in the baseline mean MMSE scores between men and women (28.0±.3 versus 27.9±.2, p=.7). Twenty-three women reported having used estrogen, 40 reported they had never used estrogen. A comparison of ever users versus never users showed no difference in the risk or the age at onset distribution for CI (p=.9) or AD (p=.9). Education level was marginally higher for men than for women (15.0±.5 versus 14.0±.3 years, p=.08) but there was no correlation between years of education and age at onset of CI (r=.07, p=.5).

DISCUSSION

We have described a population of elderly who at age 85 represented the healthiest of the oldest-old, exemplifying successful aging. They were highly selected at entry to represent those who at the time were thought to be least likely to develop CI. Yet, they proceeded to develop CI and AD at a high rate. The incidence of CI and AD was not lower in this group than others (Gao et al. 1998; Meyer et al. 1998), but the age at onset was considerably later. These data suggest that to improve cognitive health of the oldest-old, emphasis should be placed on finding factors that modify age at onset of CI.

Subjects who did not become affected were younger than those who developed CI, which begs the question: If they live long enough will they all become affected? Similarly,

subjects who had CI but did not developed AD were younger than those who progressed to AD, which raises another question: Given enough time will all the cognitively impaired elderly progress to AD? We will continue to learn more as the subjects are followed longer and reach higher ages. Seventy-three of the oldest-old are still living and being followed. Brain autopsy is critical because neuropathology may ultimately distinguish, or unify, age-related CI and AD.

A limitation of this study was sample size. Despite an extensive search since 1989, we identified only 100 individuals in the Portland Metropolitan area who were 85 or older, met the OBAS criteria for health, and were willing to participate in a life-long study that entailed biannual assessments and detailed annual examinations. Larger studies are needed to examine the present findings in more detail and to search for protective factors. Given the scarcity of optimally healthy elderly, such studies will be possible only through multi-center collaborations.

It is not known if age-related cognitive impairment leading to what appears to be AD in the oldest-old is the same as the classical late-onset AD or a different pathophysiological process. The major risk factors for late-onset AD, i.e., age, APOE genotype and gender, had a similar effect upon CI and AD in this population of oldest-old. The ε4 allele had a significant effect on risk and age onset of cognitive impairment in this group of elderly, but not on AD. The lack of a significant effect on AD may be partly due to smaller numbers of AD cases, and partly due to a weakening effect of ε4 on cognitive decline at higher ages (Corder et al. 1994). In a subset of subjects that were used in a

previous study (Payami et al. 1997), we witnessed a reduction in the effect of ε4 with longer follow-up and increasing ages. Over a 3.3 year period, the relative risk of AD as a function of ε4 dropped from 19.9 (p=.01) to 1.9 (p=.3).

Some studies suggest the ε2 allele protects against late-onset AD (Corder et al. 1994; Talbot et al. 1994; West et al. 1994), although the association of ε2 with AD is not well established (van Duijn et al. 1995). We observed a non-significant trend consistent with reduced risk and later onset in ε2-carriers. The protective effect of ε2 is difficult to assess because ε2 is the rarest of the three APOE alleles and to have sufficient power, a sample size four-times larger than the present study would be needed.

We detected a significant gender effect, which could not be explained by differences in age, survival, baseline MMSE scores or estrogen use. Men had on average one more year of education than women. It is difficult to tell if only one added year of education can result in a significant improvement in cognitive health as seen here. There was no correlation between years of education and age at onset of CI. Furthermore, the one-year difference between men and women was statistically insignificant. Thus, it is unlikely that the gender difference in cognitive outcome was solely due to education. Further studies are needed to determine if the results reflect a biological sex difference in the pathogenesis of CI, or a social or environmental gender difference that gives men an advantage. Several lines of evidence suggest sex is a factor in the pathogenesis of CI and AD. Epidemiological studies including two large meta-analyses of incidence of AD suggest that, age for age, women have a higher risk than men do (Gao et al. 1998; Jorm and Jolley

1998). Genetic studies have shown in familial late-onset AD, age at onset is earlier in women (Payami et al. 1996a). Furthermore, the APOE-associated risk for AD is sex-specific, suggesting a gene-gender interaction in the etiology of AD (Farrer et al. 1997; Payami et al. 1996b).

An alternative explanation for the gender effect seen here is related to natural selection. In mid-life, the selection pressure is higher on men than women. Men are generally more vulnerable to disease and mortality than women (Perls et al. 1993). This differential selection is reflected in the skewed sex ratio at extreme ages. Starting at approximately equal numbers at birth, by age 85 women outnumber men two to one (U.S. Census Bureau Internet Release Date: June 15, 1999). It is therefore possible that having gone through more stringent natural selection, men who reach extreme ages in optimal health have a stronger constitution overall than their age-matched female counterparts, and represent those most resistant to disease and mortality.

For research to promote cognitive health in the elderly, it is important to find the explanation to the putative male advantage. From a public health point of view, the results are alarming. Whatever the cause may be - a sex difference in disease pathogenesis, stronger selection on men, or social differences – women over the age of 85 are more likely to experience cognitive loss than men (Farrer et al. 1997; Gao et al. 1998; Henderson 1997; Jorm and Jolley 1998; Payami et al. 1996a; Payami et al. 1996b; Perls et al. 1993). These data predict a major concern for the aging population because two-thirds of all people over 85 are women.

Table 1. Subject selection criteria

Requirements For Entry	
principal language, English	has not sought evaluation for cognitive impairment
rudimentary reading and writing skills	score = 0 on Clinical Dementia Rating Scale
functionally independent	score ≥24 on Mini-Mental State Examination
gives informed consent	score ≤11 on Geriatric Depression Scale
willing and able to return for follow-up	score ≤10 on Cornell Depression Scale
willing to discuss brain autopsy	score ≤12 on Instrumental Activities of Daily Living
Major Exclusion Criteria	
MEDICAL CONDITIONS	*MAJOR SURGERIES*
diabetes mellitus	coronary bypass
hypertension (supine BP >160/95)	carotid endarterectomy
angina pectoris	*PSYCHIATRIC DISORDERS*
cardiac arrhythmia	chronic schizophrenia
myocardial infarction	major affective disorders
stroke/transient ischemic attack	phobias
chronic pulmonary disease	chronic anxiety
chronic renal disease	*VISION AND HEARING*
chronic immunosuppression	vision uncorrectable to 20/100 OU
untreated hypothyroidism	hearing loss (interferes with speech perception)
syphilis	*OTHER CONDITIONS*
vitamin deficiencies	alcohol or drug abuse
seizure disorder	significant head injury (>30 minutes unconscious)
active cancer (≤5 years no recurrence)	unexplained prolonged loss of consciousness
Parkinson's disease	use of medications impairing cognitive function

Table 2. Assessments Performed at entry and annual follow-up*

1. Personal and Family History
2. Medical History
3. Physical Exam
4. Chest x-ray†
5. Electrocardiogram†
6. Laboratory Studies†‡
7. Cortisol Saliva Test (optional)
8. Chronic Illness Rating Scale
9. Modified Ischemic Score
10. Geriatric Depression Scale
11. Cornell Depression Scale
12. Collateral Clinical Dementia Rating
13. Wechsler Memory Scale-Revised
    Visual Reproduction (I and II)

1. Wechsler Memory Scale-3
    Logical Memory (I and II)
    Letter-Number Sequencing
    Face Recognition (I and II)
    Logical Memory Recognition

1. Boston Naming Test
2. Word List Learning
3. Crossing Off Test

4. Consortium to Establish a Registry for Alzheimer's Disease Neuropsychology
    Word List Acquisition
    Word List Recall
    Word List Recognition

5. Reading Level II from the Wide Range Achievement Test-Revised†

18. Magnetic Resonance Imaging (MRI)
19. Neurological examination§
20. Modified Unified Parkinson's Disease Rating Scale
21. Oregon Gait and Balance Inventory
22. Tinetti Gait & Balance
23. Older Americans Resource Scale
    Activities of Daily Living (ADL)
    Instrumental Activities of Daily Living (IADL)
24. DSM-IIIR criteria for major affective disorder
25. Mini-Mental State Examination (MMSE)
26. Neurobehavioral Cognitive Status Examination
27. Clinical Dementia Rating (CDR)‖
28. Wechsler Adult Intelligence Scale-Revised
    Vocabulary†
    Digit Span
    Digit Symbol
    Picture Completion
    Block Design

18. Verbal Reasoning from Cognitive Competency Test
19. Temporal Orientation Test
20. Stroop Test
21. Modified Handedness Inventory†

* In addition to the annual exams listed, at six months (midway between annual assessments) follow-up interviews are conducted consisting of a review of interim medical history, ADL/IADL, MMSE and CDR.

† These assessments are performed only at entry into the study, they are not repeated during the annual follow-up exams.

‡ Laboratory studies include Chemistry Battery (serum electrolytes, glucose, blood urea nitrogen, creatinine, cholesterol, bilirubin, alkaline phosphatase, SGOT, calcium), CBC and platelet count, TSH, T_4, serum FTA, B_{12}, folate.

§ The neurological examination is a quantitated, coded examination that includes vital signs, and tests of primary sensory systems (visual acuity, audition via audioscope), motor systems (timed tests), reflexes, and balance and gait (timed tests).

‖ A collateral informant verifies the CDR.

Table 3. Characteristics of oldest-old OBAS subjects

		Cognitive Status After 5.6 Years Follow-up*		
	All	Intact	CI†	AD
Number of subjects	100	58	34	23
Women	63	33	26	17
Men	37	25	8	6
Mean entry age ± SE (years)	87.5±.4	86.9±.5	88.3±.7	88.7±.8
Mean current age ± SE (years)	93.1±.4	91.7±.5	95.3±.8	96.5±.9
Mean education ± SE (years)	14.4±.3	14.7±.4	14.2±.5	14.2±.7
Mean time followed ± SE (years)	5.6±.3	4.9±.4	7.0±.5	7.7±.5
Died during follow up (N)	27	13	12	11
Mean age at death ± SE (years)	95.6±.8	93.3±1.1	97.4±.8	97.1±.8
APOE genotype frequency				
ε2ε2	.00	.00	.00	.00
ε2ε3	.18	.22	.09	.13
ε2ε4	.00	.00	.00	.00
ε3ε3	.64	.64	.67	.70
ε3ε4	.17	.14	.21	.13
ε4ε4	.01	.00	.03	.04
APOE allele frequency ε2	.09	.11	.04	.06
ε3	.82	.82	.83	.83
ε4	.09	.07	.13	.11

* 8 subjects had indeterminate cognitive status and do not appear in any of the columns for Intact, CI or AD.

† 23 AD cases are also included among the 34 with CI.

Table 4. Clinical outcomes and classification

Clinical outcome	N	Classification
Normal at all evaluations*	54	Cognitively intact
Abnormal once or twice,		
reverted to normal on all subsequent evaluations	4	Cognitively intact
Abnormal at ≥2 consecutive evaluations, did not revert	34	CI†
Fluctuating normal/abnormal scores	3	Indeterminate
Abnormal once, awaiting follow up	3	Indeterminate
Abnormal once immediately prior to death, no autopsy	2	Indeterminate

* Normal was defined as CDR=0 and MMSE ≥24, abnormal was defined as CDR >0 or MMSE <24.

† 19 of 34 subjects with CI progressed to clinical diagnosis of AD; four did not progress to clinical AD but were pathologically proven to be AD.

Table 5. Clinical phenotypes of the autopsied cases (N=20) and their corresponding neuropathological diagnoses

Clinical Phenotype	N	Primary Neuropathologic Diagnosis
AD	7	6 AD
		1 Leukoencephalopathy
Vascular Dementia	1	Frontal Lobe Hemorrhage
CI	4	4 AD
Cognitively intact	8	3 AD
		5 "Normal"*

* Neurofibrillary tangles and neuritic plaques were present in four of the five "normal" brains but in insufficient numbers to qualify for the diagnosis of definite AD. One brain had no neurofibrillary tangles or neuritic plaques.

Table 6. Age-specific cumulative probability of survival outcomes

	Escaping Death		Escaping CI		Escaping AD	
Age	**N***	**CS ± SE†**	**N**	**CS ± SE**	**N**	**CS ± SE**
85-89	100;	.98±.02	100;	.85±.04	100;	.90±.03
90-94	76;	.84±.05	66;	.59±.06	68;	.73±.06
95-99	30;	.36±.09	17;	.35±.09	19;	.51±.10
100-104	5;	.20±.10	2;	.17±.13	3;	.30±.17
105-109	2;	.07±.08	1;	.17±.13	1;	.30±.17

* N: Number of subjects entering the interval.

† CS ± SE: Cumulative probability of survival until the end the of interval ± standard error.

Table 7. Hazard ratios for gender and APOE

	CI			AD		
	HR*	(ci)†	p	HR	(ci)	p
ε4-positive versus ε4-negative	2.5	(1.1 – 5.6)	.03	1.7	(.6 – 5.1)	ns‡
ε2-positive versus ε2-negative	.4	(.1 – 1.4)	ns	.7	(.2 – 2.5)	ns
Women versus men	3.0	(1.3 – 7.1)	.01	2.3	(.9 – 5.9)	ns

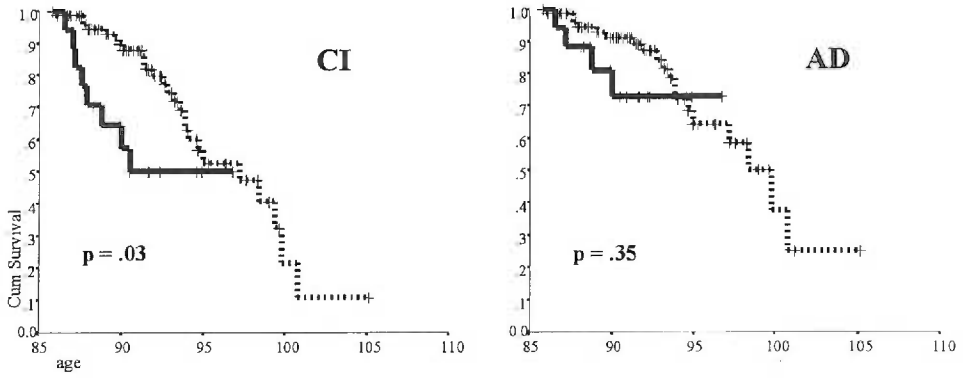
* HR: hazard ratio.

† ci: 95% confidence interval.

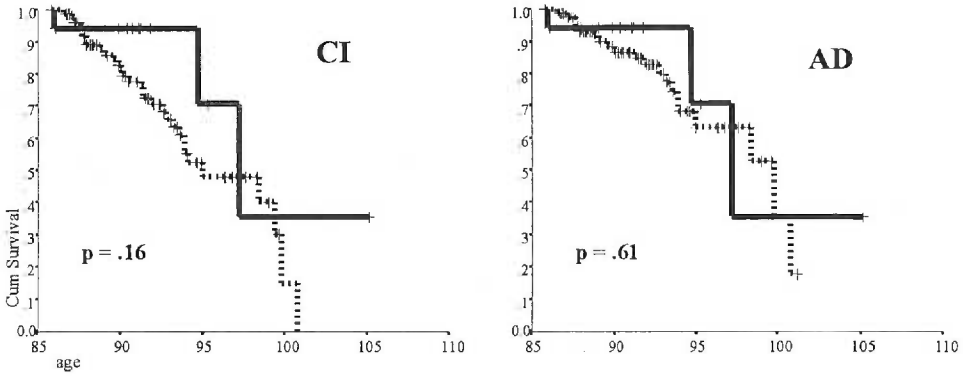
‡ ns: not significant.

Figure 1. Age at onset distributions, determined by Kaplan Meier survival analysis

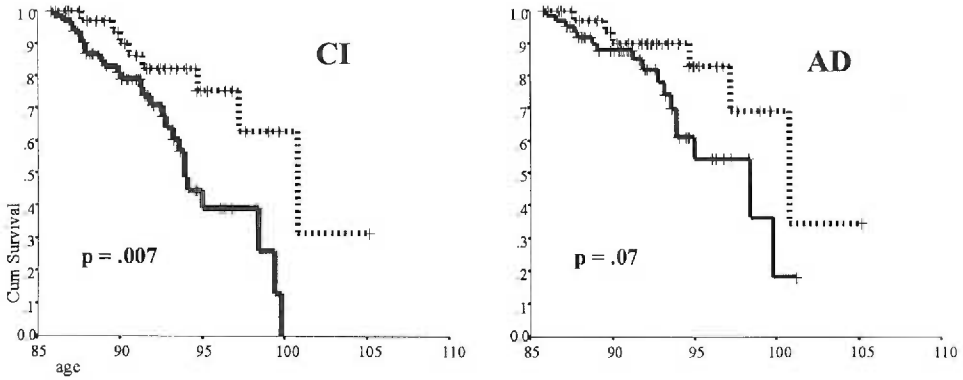
A. ε4 versus no ε4 (solid line: ε4 heterozygotes or homozygotes, dashed line: no ε4 allele)



B. ε2 versus no ε2 (solid line: ε2 heterozygotes or homozygotes, dashed line: no ε2 allele)



C. Women versus men (solid line: women, dashed line: men)



CHAPTER 3

INCREASING AGE AND THE APOLIPOPROTEIN E ASSOCIATED RISK OF DEVELOPING ALZHEIMER'S DISEASE

Melissa Gonzales McNeal, Jeffrey Kaye, Haydeh Payami

In preparation for submission to *Neuroscience Letters*

ABSTRACT

Some studies suggest the association between Alzheimer's disease (AD) and the apolipoprotein E (APOE) ε4 allele diminishes with increasing age. Investigations thus far have compared AD risk as a factor of ε4 between different populations in various age groups. The populations were not matched for all variables that influence ε4 associated risk for AD (e.g. sex, ethnicity, other causes of morbidity and mortality associated with ε4). We prospectively followed the same very old subjects and calculated their ε4 associated risk at subsequent time points, effectively controlling for all variables except age. Our data confirm the notion that APOE genotype does not exert its influence with the same magnitude at very advanced ages. Continued efforts to further characterize dementia and to identify additional risk factors in the very old are warranted.

INTRODUCTION

With the current aging of the baby boomers and medical advancements leading to decreased mortality, the elderly are the fastest growing segment of the US population. They also have the highest risk for dementia. Alzheimer's disease (AD) alone, the most common form of dementia, affects up to half of people age ≥85 (Evans et al. 1989). It, therefore, remains critical to both identify and characterize risk factors involved in AD development at very advanced ages.

Although the association between AD and the apolipoprotein E (APOE) ε4 allele is confirmed worldwide, it has been suggested that the APOE ε4 effect may be age dependent (Corder et al. 1994). Most evidence comes from case control studies based on prevalent AD cases. For example, Farrer et al performed a meta-analysis incorporating 40 AD case control studies (Farrer et al. 1997). They showed, among Caucasian subjects ascertained from clinic and autopsy based studies, increased AD risk associated with ε4 was still evident at age 90. The magnitude, however, varied such that risk increased between ages 40 and 60 but declined with age thereafter for persons with one or two ε4 alleles relative to those with ε3ε3 genotype.

Three community based prospective studies have addressed the issue of reduced association between the ε4 allele and AD with increasing age. One study followed initially non-demented 85-year-old subjects for 3 years (Skoog et al. 1998). In this group, the ε4 allele was associated with a tendency towards increased risk of AD, odds ratio (OR) 2.1, $p<.10$. While they stated this association was weaker than previously reported from

younger samples, they did not specify which younger samples or what ages. A second study used a general population of subjects initially non-demented at age ≥55 (Slooter et al. 1998). Overall, the odds of developing AD relative to ε3ε3 subjects was 1.8 (1.0-3.1) for ε3ε4 subjects and 6.2 (1.4-28.2) for ε4ε4 subjects. The OR for AD associated with ε4 heterozygosity did not reach statistical significance. When they stratified their population into three age categories of 55-75, 76-85 and >85 years, they reported the ε4 associated OR for AD was highest in the youngest age category and decreased in the two higher age categories. A third study reported the ε4 effect on risk of AD did not appear to vary with age (Evans et al. 1997). This cohort included a random sample of community residents initially free of AD at age ≥65. Overall, persons with an ε4 allele appeared at increased risk of developing AD relative to those with no ε4 allele, OR 2.27 (1.06-4.89). When the associations between AD and the ε4 allele were examined by 10-year age groups, they stated there was no consistent suggestion of any difference in effect according to age.

Although data have indicated that APOE ε4 increases risk of AD across a broad range of ages, it may be difficult to get a true picture of how the APOE ε4 effect changes with age. It is possible that the observed diminishing effect of ε4 may be confounded by other variables that were not controlled for. A sex difference in strength of the APOE ε4 association on AD has been described such that having one ε4 allele is associated with increased risk in women but not in men (Farrer et al. 1997; Payami et al. 1994b). Differences among racial groups have also been described. For example, the APOE ε4 association with AD appears weaker in some African American and Hispanic subjects

compared to Caucasian subjects (Farrer et al. 1997; Tang et al. 1996). Furthermore, a greater proportion of high-risk ε4 positive individuals may be lost to attrition thus shifting the increased risk for AD to the lower risk genotypes. Studies report the ε4 allele frequency decreases with increasing age (Davignon et al. 1988a; Kervinen et al. 1994). Increased mortality in those with this allele has been suggested (Schachter et al. 1994). The ε4 allele is also associated with other common age related disorders, such as atherosclerosis (Davignon et al. 1988b), coronary heart disease (van Bockxmeer and Mamotte 1992), and cerebrovascular disease (Pedro-Botet et al. 1992).

The study reported here followed the same subjects longitudinally and calculated their ε4 risk at different time points so they were effectively compared with themselves, thereby controlling for potential confounding variables including survival bias. Here we present data on subjects cognitively intact at age ≥75, who were prospectively followed for an average of 7 years. We assessed the ε4 associated risk on development of AD for these individuals at 5 subsequent time points during follow-up.

SUBJECTS AND METHODS

Subjects were participants in the Oregon Brain Aging Study (OBAS); a longitudinal study designed to examine effects of aging on the nervous system. Subjects were optimally healthy, cognitively intact, community volunteers age ≥65 at study entry. In addition to being functionally independent, subjects had no chronic medical conditions and were on no medications that affect cognition. Detailed exclusion and inclusion criteria

have been previously described (Kaye et al. 1994). Once enrolled, volunteers were screened biannually for both cognitive impairment, using the Mini-Mental State Exam (MMSE) (Folstein et al. 1975) and the Clinical Dementia Rating scale (CDR) (Morris 1993), and for health status. They received full physical, neurological, neuropsychological and magnetic resonance imaging examinations annually (Kaye et al. 1994). All subjects signed informed-consent forms approved by the Oregon Health Sciences University Institutional Review Board.

Not all participants entered the study during the same year since recruitment is on going. As of May 1996, 138 subjects were enrolled in OBAS. Twenty-four were excluded from this report because they were less than 75 years of age. Selecting OBAS subjects who were cognitively intact at age $\geq$75 insured that every subject who subsequently became affected represented very-late-onset AD. All 114 subjects who met this age criterion were eligible for this investigation – that is, all oldest-old subjects (age $\geq$85 at entry) and the subset of young-old subjects (age 65-84 at entry) who remained cognitively intact to age 75. Two subjects withdrew from OBAS during the study period. Their longitudinal information up to time of withdrawal is included.

Four subjects died before DNA extraction for genotyping was implemented in OBAS. Thus, APOE genotypes were determined for 110 subjects using a standard method (Hixson and Vernier 1990). Since the focus of the current report was ε4 associated risk on AD, one subject with ε2ε4 genotype was excluded from genotype-specific comparisons. This was because it is not known whether this genotype is high risk due to ε4, low risk

due to ε2, or neutral because ε4 and ε2 effects cancel out. For analyses, we grouped the 109 subjects as either ε4 positive (ε3ε4, ε4ε4) and ε4 negative (ε2ε3, ε3ε3) or ε2 positive (ε2ε3) and ε2 negative (ε3ε3, ε3ε4, ε4ε4). There were no subjects with ε2ε2 genotype.

Means are reported ± standard error (SE). Clinical AD was defined using NINCDS-ADRDA criteria, except for the upper age restriction. Hazard ratios (HR) were calculated by the Cox proportional hazards model with 95% confidence intervals (ci). Subjects given the clinical diagnosis of possible or probable AD were considered events. All other cases were considered unaffected and censored in the analyses. Since risk was thought to be a function of age rather than years of follow-up, age was specified for time in the Cox proportional hazards model. Age at onset was the age when the subject first scored <24 on MMSE or >0 on CDR. Current age or age at death was used for subjects who did not develop AD. SPSS software (release 8.0.0; 1997) was used for statistical analyses. Power calculations were performed using PS Power and Sample Size Calculations software (version 1.0.12; 1997).

RESULTS

Characteristics of the 110 subjects with APOE genotype who entered OBAS prior to May 1996 and maintained their cognitive health up to or beyond age 75 are shown in Table 1. This report includes longitudinal data collected up to September 1999. Average time followed was 6.9±.2 years. Eighteen incident cases of clinical AD occurred; five were confirmed at autopsy. To address whether the ε4 associated risk on AD was age

dependent; we calculated HR for the same 109 individuals (one ε2ε4 subject was excluded) at multiple time points during follow-up. Over a period of 3.3 years hazard ratios for ε4 allele versus no ε4 allele declined and the significance disappeared (Table 2). At the first calculation in May 1996, persons with an ε4 allele had a 19.9 fold increased risk of AD compared to subjects with no ε4 allele. At that time, their mean age was 86.1±.7 years. In September 1999, the subjects' mean age increased by 2.8 years to 88.9±.7 years and risk of developing AD for ε4 positive individuals decreased to 1.9 times that of individuals with no ε4 allele. It should be noted that the 2.8-year change in mean age is less than the follow-up period of 3.3 years because it takes into account subjects who died during follow-up.

In contrast, the protective effect of ε2 appeared to strengthen with increasing age, although it did not reach statistical significance. Power calculations revealed that given 23 subjects were ε2 positive and their median survival time free of AD was 85.6 years, the estimated power for a HR of .3 was .31. Given the same conditions that were present in this study, i.e., patient accrual time of 7.2 years with 3.3 years additional follow-up, and assuming 22% of the subjects are ε2 positive, a sample with approximately 83 ε2 positive subjects would be necessary to detect a significant HR of .3 with .80 power.

Twenty-five subjects died during follow-up. We questioned whether the declining APOE ε4 associated risk on AD was a result of differential survival. A scenario in which the unaffected ε4 positive subjects died leaving only low risk ε4 negative subjects available to develop the disease could generate the observed decline. This did not appear to be the

case since only two ε4 positive subjects died (10%), and one did so after being diagnosed with clinical AD. Conversely, 23 ε4 negative subjects died (26%).

DISCUSSION

Using prospective data, this study further validates previous reports of decreased risk associated with the ε4 allele on development of AD at very advanced age. To date, all studies have either stratified their populations into age categories and compared risk between age stratum, or compared the risk from their populations to the risk from other younger populations.

The present investigation has several advantages over most previous studies on APOE genotype and AD relative to age. First, this study examined the hypothesis of diminished risk related to the APOE locus as a function of age in such a way that all variables except for age were controlled for. By prospectively following the same subjects and comparing their risk at different time points, the individuals were essentially compared to themselves. In addition, the diagnostic work up was comprehensive and consistent for baseline and follow-up evaluations. This study had minimal loss to follow-up. Disease duration did not enter the analysis since we used age at onset of symptoms and current age, this avoided confounding by possible longer survival time. We also had neuropathological confirmation of AD on all five of the deceased patients with clinically diagnosed AD.

Seven of the subjects who were classified as clinically unaffected with AD received

a neuropathological diagnosis of AD. One subject was ε2ε3 and six were ε3ε3. It is possible that these subjects were in early stages of disease development. Four of the subjects, including the ε2ε3 subject, received abnormal cognitive scores on more than one follow-up evaluation prior to death.

Although not as universally agreed upon as the predisposing effect of ε4, there appears to be an association for the APOE ε2 allele with reduced risk and delayed onset of AD (Corder et al. 1994). In our sample, we observed a protective trend of the ε2 allele on AD development that did not reach statistical significance. The ε2 allele is the rarest of the 3 common APOE alleles, with a frequency of .08 in the general Caucasian population (Davignon et al. 1988b). To have sufficient power a sample size four times larger than the present study would be needed.

Our data suggest factors other than APOE are important determinants of risk for AD after age 75. This is reflected by the finding that 14 out of 18 individuals who developed AD lacked the ε4 allele. The fact that increased risk on development of AD associated with the APOE ε4 allele diminishes with age while the incidence of the disease appears to increase with age (Gao et al. 1998; Jorm and Jolley 1998) emphasizes the need to identify additional genetic or environmental risk factors.

Table 1. Subject characteristics

Mean age at entry ± SE (years)	81.8±.7 (range 68.9-100.5)
Women/men (N)	63/47
APOE genotype (N)	
ε2ε2	0
ε2ε3	23
ε2ε4	1
ε3ε3	66
ε3ε4	17
ε4ε4	3
APOE phenotype (N)*	
ε4 positive	20
ε4 negative	89
ε2 positive	23
ε2 negative	86

* The one ε2ε4 subject was excluded.

Table 2. ε4 and ε2 associated risk on development of AD

		ε4 positive versus ε4 negative				ε2 positive versus ε2 negative			
		affected (N)				affected (N)			
Study date	Mean Age ± SE*	ε4+/ε4-	HR	ci	p	ε2+/ε2-	HR	ci	p
May 1996	86.1±.7	3/4	19.9	2.1-193.1	.01	0/7	no events		
Oct. 1997	87.3±.7	4/8	4.5	1.3-16.0	.02	1/11	.5	.1-3.6	.46
July 1998	87.8±.7	4/9	3.6	1.0-12.2	.04	1/12	.4	.1-3.3	.42
Feb. 1999	88.5±.7	4/13	2.4	.8-7.7	.13	1/16	.3	.0-2.1	.22
Sept. 1999	88.9±.7	4/14	1.9	.6-5.7	.28	1/17	.3	.0-2.1	.22

* Mean age was calculated using current age or age at death.

CHAPTER 4

DISCUSSION

The overall objective of this thesis project was to examine biological markers that modulate Alzheimer's disease (AD) onset in younger populations to determine whether the genetic risk factors continue to be important in cognitive decline in the very old. With the growing number of elderly in our population, understanding factors that affect the onset of cognitive decline for potential use in preventing or delaying onset is extremely important. The etiology of AD may be different in the very old, and it is not known if the same genetic factors play a role.

Using a unique population of elderly, highly selected at entry to represent those thought to be least likely to develop cognitive impairment, we provided evidence that superior health at old age does not necessarily predict protection against cognitive impairment (CI) or AD. The data show these optimally healthy elderly actually have lifetime risks similar to the general population (Gao et al. 1998; Meyer et al. 1998), but their ages at onset are later. This suggests that the key to maintaining cognitive health to very advanced age lies in factors that delay the onset of impairment.

We examined the major risk factors for late-onset AD, i.e., age, Apolipoprotein E (APOE) genotype and gender, in this population of oldest-old. The ε4 allele had a significant effect on risk and age at onset of cognitive impairment, but not on AD. We reasoned the lack of a significant effect on AD could partly be due to smaller numbers of AD cases, and partly due to a weakening effect of ε4 on cognitive decline at higher ages (Corder et al. 1994). Using a subset of OBAS subjects who were initially described in a prior study (Payami et al. 1997), we witnessed a reduction in the effect of ε4 with longer follow-up and increasing ages. Over a 3.3 year period, the relative risk of AD as a function

of ε4 dropped from 19.9 (p=.01) to 1.9 (p=.3). This further validates previous reports of decreased risk associated with the e4 allele on development of AD at very advanced age (Corder et al. 1994; Farrer et al. 1997; Skoog et al. 1998; Slooter et al. 1998). This also suggests APOE is not an important determinant of risk for AD after age 75, and emphasizes the need to identify additional genetic or environmental factors.

Some studies suggest the ε2 allele protects against late-onset AD (Corder et al. 1994; Talbot et al. 1994; West et al. 1994), although the association is not universally agreed upon (van Duijn et al. 1995). We observed a non-significant trend consistent with reduced risk and later onset in ε2-carriers. The protective effect of ε2 is difficult to assess because ε2 is the rarest of the three APOE alleles and to have sufficient power, sample sizes four times larger than the ones used would be required.

In addition, we demonstrated a significant gender effect with a putative male advantage for CI. Differences in age, survival, baseline MMSE scores or estrogen use could not explain this effect. This is a major concern for the aging population since two thirds of all people over age 85 are women. It has long been recognized that there are more women with AD than men. Previous reports have shown a sex effect in familial AD (Payami et al. 1996a), and more specifically, an interaction between sex and APOE genotype (Farrer et al. 1997; Payami et al. 1994b; Payami et al. 1996b). None of these studies, however, was prospective and they could have been confounded by differential mortality rates in men and women. The OBAS data clearly demonstrate the gender effect on age at onset prospectively.

The role of gender in the development of cognitive decline warrants further research. Several possibilities could explain our results: 1) A biological sex difference in the pathogenesis of cognitive decline. The findings that gender differences in AD are genotype specific argues strongly for a pathogenic role (Farrer et al. 1997; Payami et al. 1994b; Payami et al. 1996b). This is further supported with indirect evidence. An interactive APOE and gender effect for lipid distribution has been shown (Reilly et al. 1994). The gender-specific influence of APOE polymorphism on the correlations and covariances between pairs of nine plasma lipid and apolipoprotein traits was examined. The APOE polymorphism significantly influenced 10 of 36 correlations and covariances in females and influenced only 3 of 36 in males. In addition, an interactive APOE and gender effect in response to cholinesterase therapy with tacrine has been demonstrated (Farlow et al. 1998). Farlow et al studied the effects of APOE genotype and gender on clinical response to tacrine in AD patients. The treatment effect was larger in the ε2ε3 compared with ε4 women while treatment effect size was not different between ε2ε3 and ε4 men. 2) A social or environmental gender difference that gives men an advantage. This could include education. Lower education has been associated with less favorable cognitive outcome (Jorm 1997). Although there was a one-year difference in the mean education between OBAS men and women, the lack of correlation between years of education and age at onset of CI argues against this explaining the observed gender effect. 3) A form of natural selection, created by unequal selection pressures on men and women during life. In mid-life, the selection pressure is higher on men than women. Men are generally more vulnerable to disease and mortality than women (Perls et al. 1993). This differential

selection is reflected in the skewed sex ratio at extreme ages. Starting at approximately equal numbers at birth, by age 85 women outnumber men two to one (U.S. Census Bureau Internet Release Date: June 15, 1999). It is therefore possible that having gone through more stringent natural selection, men who reach extreme ages in optimal health have a stronger constitution overall than their age-matched female counterparts, and represent those most resistant to disease and mortality.

REFERENCES

Alexopoulos, G. S., R. C. Abrams, R. C. Young, and C. A. Shomoian. 1988. Use of the Cornell Scale in non-demented patients. J Am Geriatr Soc 36 230-236.

Corder, E. H., A. M. Saunders, N. J. Risch, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, J. B. Rimmler, P. A. Locke, P. M. Conneally, K. E. Schmader, G. W. Small, A. D. Roses, J. L. Haines, and M. A. Pericak-Vance. 1994. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 7 180-184.

Corder, E. H., A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines, and M. A. Pericak-Vance. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261 921-923.

Cox, D. R., and D. Oakes. 1984. Analysis of survival data. Chapman and Hall, London.

Davignon, J., D. Bouthillier, A. C. Nestruck, and C. F. Sing. 1988a. Apolipoprotein E polymorphism and atherosclerosis: insight from a study in octogenarians. Transact Am Clin Climatol Assoc 99 100-110.

Davignon, J., R. E. Gregg, and C. F. Sing. 1988b. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8 1-21.

Ebly, E. M., I. M. Parhad, D. B. Hogan, and T. S. Fung. 1994. Prevelance and types of dementia in the very old: results from the Canadian Study of Health and Aging. Neurology 44 1593-1600.

Evans, D. A., L. A. Beckett, T. S. Field, L. Feng, M. S. Albert, D. A. Bennett, B. Tycko, and R. Mayeux. 1997. Apolipoprotein E e4 and incidence of Alzheimer disease in a community population of older persons. JAMA 277 822-824.

Evans, D. A., H. H. Funkenstein, M. S. Albert, P. A. Scherr, N. R. Cook, M. J. Chown, L. E. Hebert, C. H. Hennekens, and J. O. Taylor. 1989. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. JAMA 262 2551-2556.

Farlow, M., D. Lahiri, J. Poirier, J. Davignon, L. Schneider, and S. Hui. 1998. Treatment outcome of tacrine therapy depends on apolipoprotein genotype and gender of the subjects with Alzheimer's disease. Neurology 50 669-677.

Farrer, L. A., L. A. Cupples, J. L. Haines, B. Hyman, W. A. Kukull, R. Mayeux, R. H. Myers, M. A. Pericak-Vance, N. Risch, and C. M. van Duijn. 1997. Effects of age, sex and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. JAMA 278 1349-1356.

Fillenbaum, G. G., and M. Smyer. 1981. The development, validity and reliability of the OARS multidimensional functional assessment questionnaire. J Gerontol 36 428-434.

Folstein, M. F., S. E. Folstein, and P. R. McHugh. 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12 189-198.

Gao, S., H. C. Hendrie, K. S. Hall, and S. Hui. 1998. The relationships between age, sex, and the incidence of dementia and Alzheimer disease. A meta-analysis. Arch Gen Psychiatry 55 809-815.

Green, M. S., J. A. Kaye, and M. J. Ball. 2000. The Oregon Brain Aging Study: Neuropathology accompanying healthy aging in the oldest old. Neurology 54 105-113.

Henderson, V. 1997. Estrogen, Cognition, and a woman's risk of Alzheimer's disease. Am J Med 103 11S-18S.

Hixson, J. E., and D. T. Vernier. 1990. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res 31 545-548.

Howieson, D. B., L. A. Holm, J. A. Kaye, B. S. Oken, and J. Howieson. 1993. Neurologic function in the optimally healthy oldest old: neuropsychologic evaluation. Neurology 43 1882-1886.

Hughes, C. P., L. Berg, W. L. Danizger, L. A. Coben, and R. L. Martin. 1982. A new clinical scale for the staging of dementia. Br J Psychiatry 140 566-572.

Jorm, A. F. 1997. Alzheimer's disease: risk and protection. MJA 167 443-446.

Jorm, A. F., and D. Jolley. 1998. The incidence of dementia. A meta-analysis. Neurology 51 728-733.

Kaplan, E., and E. L. Meier. 1958. Nonparametric estimation from incomplete observations. J Am Statist Assoc 53 457-481.

Katzman, R., M. Aronson, P. Fuld, C. Kawas, T. Brown, H. Morgenstern, W. Frishman, L. Gidez, H. Eder, and W. L. Ooi. 1989. Development of dementing illnesses in an 80-year-old volunteer cohort. Ann Neurol 25 317-324.

Kaye, J. A., B. S. Oken, D. B. Howieson, J. Howieson, L. A. Holm, and K. Dennison. 1994. Neurologic evaluation of the optimally healthy oldest old. Arch Neurol 51 1205-1211.

Kaye, J. A., T. Swihart, D. Howieson, A. Dame, M. M. Moore, T. Karnos, R. Camicioli, M. Ball, B. Oken, and G. Sexton. 1997. Volume loss of the hippocampus and temporal lobe in healthy elderly destined to develop dementia. Neurology 48 1297-1304.

Kervinen, K., M. J. Savolainen, J. Salokannel, A. Hynninen, J. Heikkinen, C. Ehnholm, M. J. Koistinen, and Y. A. Kesaniemi. 1994. Apolipoprotein E and B polymorphisms -- longevity factors assessed in nonagenarians. Atherosclerosis 105 89-95.

Kokmen, E., C. M. Beard, P. C. O'Brien, and L. T. Kurland. 1996. Epidemiology of Dementia in Rochester, Minnesota. Mayo Clin Proc 71 275-282.

Levy-Lahad, E., D. Tsuang, and T. D. Bird. 1998. Recent advances in the genetics of Alzheimer's disease. J Geriatr Psychiatry Neurol 11 42-54.

McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Price, and E. M. Stadlan. 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34 939-944.

Meyer, M. R., J. T. Tschanz, M. C. Norton, K. A. Welsh-Bohmer, D. C. Steffens, B. W. Wyse, and J. C. Breitner. 1998. APOE genotype predicts when - not whether - one is predisposed to develop Alzheimer disease. Nat Genet 19 321-322.

Mirra, S., and A. Heyman. 1994. CERAD guide to the neuropathological assessment of Alzheimer's disease and other dementias. CERAD, Durham NC.

Morris, J. C. 1993. Clinical Dementia Rating: current version and scoring rules. Neurology 43 2412-2144.

Morris, J. C., A. Heyman, R. C. Mohs, and M. S. Hughes. 1989. The consortium to establish a registry for Alzheimer's disease (CERAD) Part 1. clinical and neuropsychological assessment of Alzheimer's disease. Neurology 39 1159-1165.

Payami, H., H. Grimslid, B. Oken, R. Camicioli, G. Sexton, A. Dame, D. Howieson, and J. Kaye. 1997. A prospective study of cognitive health in the elderly (Oregon Brain Aging Study): effects of family history and Apolipoprotein E genotype. Am J Hum Genet 60 948-956.

Payami, H., K. Montee, H. Grimslid, S. Shattuk, and J. Kaye. 1996a. Increased risk of familial late-onset Alzheimer disease in women. Neurology 46 126-129.

Payami, H., K. Montee, and J. Kaye. 1994a. Evidence for familial factors that protect against dementia and outweigh the effect of increasing age. Am J Hum Genet 54 650-657.

Payami, H., K. R. Montee, J. A. Kaye, T. D. Bird, C.-E. Yu, E. M. Wijsman, and G. D. Schellenberg. 1994b. Alzheimer's disease, apolipoprotein E4, and gender. JAMA 271 1316-1317.

Payami, H., S. Zareparsi, K. R. Montee, G. J. Sexton, J. A. Kaye, T. D. Bird, C.-E. Yu, E. M. Wijsman, L. L. Heston, M. Litt, and G. D. Schellenberg. 1996b. Gender difference in Apolipoprotein E-associated risk for familial Alzheimer disease: a possible clue to the higher incidence of Alzheimer disease in women. Am J Hum Genet 58 803-811.

Pedro-Botet, J., M. Senti, X. Nogues, J. Rubies-Prat, J. Roquer, L. D'Olhaberriague, and J. Olive. 1992. Lipoprotein and apolipoprotein profile in men with ischemic stroke.

Role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. Stroke 23 1556-1562.

Perls, T. T., J. N. Morris, W. L. Ooi, and L. A. Lipsitz. 1993. The relationship between age, gender and cognitive performance in the very old: the effect of selective survival. J Am Geriatr Soc 41 1193-201.

Reilly, S. L., R. E. Ferrell, and C. F. Sing. 1994. The gender-specific Apolipoprotein E genotype influence on the distribution of plasma lipids and apolipoproteins in the population of Rochester, MN. III. Correlations and covariances. Am J Hum Genet 55 1001-1018.

Ritchie, K., and D. Kildea. 1995. Is senile dementia "age-related" or "ageing-related"? - evidence from meta-analysis of dementia prevalence in the oldest old. Lancet 346 931-933.

Rocca, W. A., A. Hofman, C. Brayne, M. M. B. Breteler, M. Clarke, J. R. M. Copeland, J.-F. Dartigues, K. Engedal, O. Hagnell, T. J. Heeren, C. Jonker, J. Lindesay, A. Lobo, A. H. Mann, P. K. Mölsä, K. Morgan, D. W. O'Connor, A. da Silva Droux, R. Sulkava, D. W. K. Kay, and L. Amaducci. 1991. Frequency and distribution of Alzheimer's disease in Europe: A collaborative study of 1980-1990 prevalence findings. Ann Neurol 30 381-390.

Schachter, F., L. Faure-Delanef, F. Guenot, H. Rouger, P. Froguel, L. Lesueur-Ginot, and D. Cohen. 1994. Genetic associations with human longevity at the APOE and ACE loci. Nat Genet 6 29-32.

Schneider, E. L. 1999. Aging in the third millennium. Science 283 796-797.

Skoog, I., C. Hesse, O. Aevarsson, S. Landahl, J. Wahlstrom, P. Fredman, and K. Blennow. 1998. A population study of apoE genotype at the age of 85: relation to dementia, cerebrovascular disease, and mortality. J Neurol Neurosurg Psychiatry 64 37-43.

Slooter, A. J. C., M. Cruts, S. Kalmijn, A. Hofman, M. M. B. Breteler, C. Van Broeckhoven, and C. M. van Duijn. 1998. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: The Rotterdam Study. Arch Neurol 964-968.

St George-Hyslop, P. H. 2000. Molecular genetics of Alzheimer's disease. Biol Psychiatry 47 183-199.

Strittmatter, W. J., A. M. Saunders, D. Schmechel, M. Pericak-Vance, J. Enghild, G. S. Salvensen, and A. D. Roses. 1993. Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 90 1977-1981.

Talbot, C., C. Lendon, and N. Craddock. 1994. Protection against Alzheimer's disease with apoE e2. Lancet 343 1432-1433.

Tang, M.-X., G. Maestre, W. Tsai, X. Liu, L. Feng, W.-Y. Chung, M. Chun, P. Schofield, Y. Stern, B. Tycko, and R. Mayeux. 1996. Relative risk of Alzheimer disease and age-at-onset distributions, based on APOE genotypes among elderly African Americans, Caucasians, and Hispanics in New York City. Am J Hum Genet 58 574-584.

U.S. Census Bureau. Internet Release Date: June 15, 1999. Population Estimates for the U.S., Regions, Divisions, and States by 5-year Age Groups and Sex: Annual Time

Series Estimates, July 1, 1990 to July 1, 1998 (includes revised April 1, 1990 consus population counts) www.census.gov/population/estimates/state/st-99-08.txt.

van Bockxmeer, F. M., and C. D. S. Mamotte. 1992. Apolipoprotein ε4 homozygosity in young men with coronary heart disease. Lancet 340 879-880.

van Duijn, C., P. de Knijff, A. Wehnert, J. De Voecht, J. Bronzova, L. Havekes, A. Hofman, and C. Van Broeckhoven. 1995. The Apolipoprotein E e2 allele is associated with an increased risk of early-onset Alzheimer's disease and a reduced survival. Ann Neurol 37 605-610.

Wernicke, T. F., and F. M. Reischies. 1994. Prevalence of dementia in old age: clinical diagnoses in subjects aged 95 years and older. Neurology 44 250-253.

West, H. L., W. Rebeck, and B. T. Hyman. 1994. Frequency of Apolipoprotein E ε2 allele is diminished in sporadic Alzheimer's disease. Neuroscie Lett 175 46-48.

Yesavage, J. A., T. L. Brink, T. L. Rose, and O. Lum. 1983. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 17 37-49.