ASSOCIATION STUDIES OF 22 CANDIDATE SNPS WITH LATE-ONSET ALZHEIMER'S DISEASE

by

Jessica Figgins

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This thesis was presented

by

Jessica Figgins

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and approved by

Thesis Advisor:
M. Ilyas Kamboh, Ph.D.
Professor and Acting Chair
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh

Committee Member:
F. Yesim Demirci, M.D.
Research Assistant Professor
Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh

Committee Member:
Steven T. DeKosky, M.D.
Professor and Chair, Department of Neurology
Chief, Division of Cognitive and Behavioral Neurology
Director, Alzheimer's Disease Research Center
University of Pittsburgh

M. Ilyas Kamboh, Ph.D.

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Jessica Figgins, M.S.

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Alzheimer's disease (AD) is a complex and multifactorial disease with the possible involvement of several genes. Complex diseases such as AD have a large affect on the public health. It was estimated in 2007 that over 5 million Americans had AD, and more than $91 billion dollars was spent by medicare on AD and other dementias. Genetics plays a significant role in the etiology of the disease, therefore, it is of public health importance that the genetics of AD be investigated. With the exception of the *APOE* gene as a susceptibility marker no other genes have been identified for late-onset AD (LOAD). A recent genome wide association study of 17,343 gene-based putative functional single nucleotide polymorphisms (SNPs) found 19 significant variants, including 3 linked to *APOE*, showing association with LOAD in several population samples. We have set out to replicate the 16 new significant associations in a large case-control cohort of American Whites. Additionally we examined six variants present in positional and/or biological candidate genes for AD. We genotyped the 22 SNPs in up to 1,009 Caucasian Americans with LOAD and up to 1,010 age matched older healthy Caucasian Americans. All variants were genotyped using 5' nuclease assays. We did not observe a statistically significant association between the SNPs with the risk of AD, either individually or stratified by *APOE*. Our data suggest that the association of the studied variants with LOAD, if it exists, is not statistically significant in our population study.

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PREFACE

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1.0 BACKGROUND

1.1 INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder most commonly found in individuals over the age of 65. AD can be classified by the age at onset (AAO), and family history. Familial AD is classified by a significant family history of AD, and sporadic AD is classified by a lack of significant family history. These categories can be further delineated by early-onset (<60 years of age) and late-onset (≥60 years of age). Sporadic AD is the most common form, making up approximately 90% of cases, familial AD makes up the remaining ~10%. Three genes have been implicated in the etiology of early onset AD with an autosomal dominant mode of inheritance: *amyloid precursor protein (APP), presenilin 1 (PSEN1)* and *presenilin 2 (PSEN2)*. These genes have been found to make up approximately 50% of early onset AD, and they explain <1% of all AD cases (Kamboh, 2004). Even with the investigation of these three genes the exact etiology of early onset AD is unknown. Late onset Alzheimer's disease (LOAD) has been particularly difficult to research due to its complexity, it is thought to be a multifactorial condition in which genes and environment play a role. The only known genetic risk factor for LOAD is apolipoprotein E (*APOE)* (Kamboh, 2004).

1.2 EPIDEMIOLOGY OF ALZHEIMER'S DISEASE

In 2007 it was estimated that 5.1 million Americans had AD, 4.9 million of these individuals are age 65 or older (Alzheimer's association, 2007). Advances in medicine have lead to more individuals living into their 80s and 90s. The risk of AD increases with age, therefore the number of persons with the disease is expected to grow as individuals continue to live longer. In 2005, Medicare spent $91 billion on AD and other dementias, projected to increase to $160 and $189 billion in 2010 and 2015 (Alzheimer's Association, 2007). State and federally funded Medicaid spent $21 billion in 2005 on AD and other dementias. This is projected to increase to $24 and $27 billion in 2010 and 2015 respectively (Alzheimer's Association, 2007). One study looked at the cost of institutional care in AD. The median nursing home length of stay was 2.75 years (mean 2.95, 95% CI = 2.5, 3.4), over 10 times the national median length of stay for all diagnoses (Welch et al. 1992). Nursing home charges for the cohort were estimated to be $35,000-$52,000 per patient. A prolonged period of institutionalization combined with the high expense of nursing home care makes the costs of AD extensive.

1.3 DIAGNOSIS AND PATHOGENESIS

In order to study AD it is vital to determine the AAO. It is difficult to determine the exact age of diagnosis. Less severe symptoms may be present for a considerable amount of time before a diagnosis is made. Diagnosis can be classified as possible or probable with a clinical diagnosis. Probable AD is when the individual has no other illness that may contribute to the symptoms. Possible AD is when the individual meets the criteria for another illness that may contribute to

his or her cognitive decline. Clinical diagnosis is made by assessing clinical characteristics such as; progressive decline in memory and intellectual ability, declining language and speech skills and loss of orientation. Most but not all diagnoses can be made clinically, a definitive diagnosis can only be made after death by a brain biopsy. There are two pathological features of AD; extra-cellular senile plaques and the presence of intracellular neurofibrillary tangles.

Extra-cellular senile plaques mainly consist of a 42 amino-acid amyloid β peptide (Aβ) derived from amyloid precursor protein (APP). APP is a transmembrane protein that can be cleaved by β or α and γ secretase. If APP is cleaved by α and γ secretase a harmless soluble protein is produced. If APP is cleaved by β and γ secretase a peptide of 39-43 amino acids is produced. The Aβ peptide-42 which is approximately 10% of the total product is considered harmful, and may lead to the generation of damaging plaques (Maccioni et al., 2001). These plaques can occur outside and between neurons, affecting brain function or in the cerebral blood vessels, causing vascular damage such as senile cerebral amyloid angiopathy. Cerebral amyloid angiopathy is a type of disease of the blood vessels, in which Aβ peptide-42 deposits in the walls of the blood vessels of the brain. This build up makes these blood vessels more likely to fail, which increases the risk of stroke. The deposition of Aβ peptide also interferes with calcium regulation and neurotransmission, generates free radicals which cause mitochondrial damage and causes inflammation, all of which lead to cell death. (Chen et al., 2007).

Intracellular neurofibrillary tangles occur within neurons and involve the protein, Tau. The normal function of Tau is to associate with microtubules, which function to support the cell and transport nutrients through the cytoplasm. Microtubules are made of the protein tubulin, Tau associates with tubulin and provides stability to microtubules in the cytoplasm. Tau's common

state is hypophosphorylation, however in AD Tau is hyperphosphorylated. This hyperphosphorylation causes self aggregation of Tau, and inhibits Tau's ability to associate with tubulin and microtubules. This leads to the death of neurons due to lack of cellular stability and inability to transport nutrients. (Maccioni et al., 2001).

1.4 ENVIRONMENTAL RISK FACTORS FOR LOAD

LOAD is considered a multifactorial condition in which interaction of genes and environmental factors are involved in the etiology of the condition. While investigating genetic factors in a multifactorial condition it is important to take into account environmental factors that may be confounding results. Some possible environmental risk factors for LOAD include education, tobacco usage, alcoholism, head trauma, cerebrovascular disease, higher cholesterol level, etc. Gender also seems to affect the risk of AD with the disease being more common in females than males. Although there is a multitude of possible risk factors, most of the data is questionable. The only proven risk factor for AD is age.

1.4.1 Education:

It has been proposed that a low level of education is a risk factor for sporadic LOAD. In contrast a higher level of education may be associated with later onset of the disease. One study indicates that up to a 4-5 year delay in the onset of AD is possible with a higher level of education (Katzman et al., 1993). One theory is that those who are more educated are better equipped to compensate for neurological damage. Therefore, they can sustain damage for longer without

showing symptoms of the disease. There are confounding factors that make it difficult to specifically look at education and onset of AD. Those who are less educated may have jobs that involve manual labor, these types of occupations may make them more likely to be exposed to toxic substances, and head trauma (Letenneur et al., 1999). However, other studies have refuted this hypothesis that lower education level makes one more susceptible to Alzheimer's disease. Moritz and Petitti (1993) reported results in which those with a lower education level had a later onset of disease. However, once the clinical symptoms manifested they were severe. This data may have been confounded by the fact that those with lower education were diagnosed at a later time due to failure to detect symptoms. A recent study contradicts the hypothesis that level of education is positively correlated with an earlier onset of disease. Mejia et al. (2003) reported that 8% of individuals examined with high level education (7-14 years) were found to have had an early onset of their disease. However, other factors that influence education level may be confounding the data. Studies may classify AAO by the first appearance of symptoms reported by the patient's family. A lower or higher education level may confound the time at which symptoms are detected. For example, it may be more difficult to detect cognitive delay in patients that have less education. Symptoms may not be noticed as soon in those with lower education and less cognitive and occupational demands. Family members of those with a higher education level may notice neurologic deficits sooner, due to the fact that they may have greater occupational and non-occupational demands (Mejia et al., 2003). Studies have noted that the years of education may not be affecting the onset of AD. Rather, when an individual passes a threshold of education their risk may decrease (Letenneur et al., 1999). Any study looking at education and its risk on AD is confounded by participant rate according to education.

1.4.2 Gender:

One study found a higher incidence of AD in women than men after 80, however the incidence was higher in men before the age of 80 (Letenneur et al., 1999). This study looked at other dementias and did not see this same trend. Incidence of these conditions decreased in both sexes after the age of 85. When the subjects were corrected for educational level the difference between the onset and sex remained. Another study reported a higher incidence of dementia or AD in women, however, the population only consisted of subjects over 85 and 75 years of age (Gussekloo et al., 1995). Other studies have found no association in the incidence of AD and the sex of the individual (Bachman et al., 1993). Some possible explanations as to why women seem to be at a higher risk for AD are biological such as: genetics and hormonal differences.

1.4.3 Tobacco and Alcohol Usage:

Epidemiological studies have found conflicting tobacco affects on the risk of AD. There are many confounding factors that come into play when studying AD and tobacco use. Many studies that found smoking to have a protective effect on AD, did not control for sex, occupational categories and educational level (Letenneur et al., 2004). When these confounding factors are taken into account tobacco use has been found to be a slight risk factor or have no affect on the

risk of AD. A study conducted on 3,770 subjects looked at current smokers, past smokers and never smokers. A small association was seen, the percentage of subjects with probable or possible AD was almost the same in current and past smokers with never smokers have he lowest incidence of AD. However, when confounding factors were taken into account such as education level and occupation the association disappeared (Letenneur et al., 2004). Alcohol consumption in relation to AD has been examined in several studies. A prospective study in Sweeden on 402 subjects found a reduction of risk when individuals were light to moderate drinkers compared to non drinkers (alcohol included beer, wine and hard alcohol)(Letenneur et al., 2004). When *APOE* genotyping was taken into account the reduction was more evident in those that carry the *APOE*4* risk allele. Compared to non-drinkers the risk of developing AD was decreased 1.8-fold for light drinkers and by 4-fold for mild drinkers (Letenneur et al., 2004). Observation does not prove that alcohol is a causal link to AD. Selection bias may skew the results of the studies. It is possible that only the healthier drinkers were captured by the study. This confounding factor could explain the slight protective association observed. Other factors such as lifestyle and diet were also not controlled for.

1.4.4 Head Trauma:

A study done in 2000 by Guo et al. (2000) examined head injury and its affect on individual's risk for AD. Their results indicate that severity of head injury is related to the magnitude of AD risk. Head injury with loss of consciousness was seen to have a greater effect than head injury without loss of consciousness. Contrary to other studies the results from Guo et al. (2000) indicate that head injury had a greater affect on risk among those without the *APOE*4* allele

compared with those having one or two *APOE*4* alleles. This study also looked at the affect of head trauma and sex. They did not find a significantly higher association of head trauma and risk of AD between women and men. Although this study found an increased risk for AD, with severe head trauma, they did not observe a difference in the AAO between patients with and without head injury. There is a possibility that these results may be the confounded by recall bias. Another study examined association between recurrent concussions and AD in professional football players (Guskiewicz et al., 2005). This study indicated an association between AAO and head injury, but did not find an association between previous concussions and lifetime onset of AD. This study found that concussions sustained by players may promote an earlier expression of AD, however, the factor of age eventually overwhelms this factor and prevents it from becoming an independent predictor of lifetime onset of AD (Guskiewicz et al., 2005). This study also stated that cognitive status of the athletes with repeated trauma is influenced by age, *APOE* status, and cumulative exposure to head trauma. Another study looked at chronic traumatic brain injury in boxing and risk of AD. This study found that boxers with low exposure to brain injury had low brain injury scores irrespective of *APOE*. However, in the group with high exposure to brain injury, those with *APOE**4 showed a higher amount of brain injury than those without *APOE**4. The *APOE**4 allele was associated with an increased severity of neurological deficits in the high exposure boxers in this study (Jordan et al., 1997).

1.4.5 Vascular Disease

Studies have suggested an association between vascular factors pre-disposing to cerebrovascular disease and AD. Cerebrovascular disease is any disease in which the arteries in the brain or

arteries connected to the brain are blocked or defective. Stroke and other events that lead to a restriction of blood supply have been seen to increase an individual's risk of AD by up to three times (Kalaria et al., 2003). This is most likely due to the increased stress on the brain caused by these events. Some predisposing factors to cerebrovascular disease include: hypertension, atrial fibrillation, carotid thickening, aortic sclerosis and diabetes.

1.4.6 Cholesterol Levels

Lipids may play an important role in APP processing which leads to production of the 42 amino-acid Amyloid β peptide (Aβ). The action of γ secretase takes place in the lipid membrane. Therefore, the lipid environment of the membrane may play a role in enzyme activity and the development of Aβ and AD (Hartmann et al., 2007). The brain is the most cholesterol rich organ in the body, synthesis, removal, storage or transport within the brain is strictly regulated (Hartmann et al., 2007). Hypercholesterolemia was found to be an early risk factor for the development of Aβ in a longitudinal, population-based study (Kivipelto et al., 2001). Although the exact role of cholesterol in AD pathogenesis is unknown, there is evidence that there is a dose dependent relationship between the level of cholesterol and the average onset of AD. A study in which cholesterol synthesis was inhibited in animals or extracted, the amount of Aβ was seen to decrease in cerebrospinal fluid and the brain (Fassbender et al., 2001). An increase in cholesterol levels is a risk factor to cerebrovascular disease which is also a possible risk factor for the onset of AD.

1.5 GENETIC SUSCEPTIBILITY

1.5.1 Genetics of Early Onset Alzheimer's Disease

Alzheimer's disease is most commonly seen in individuals age 65 or older. However, AD still accounts for 30% of all nontraumatic dementia in individuals 30-64 years of age (Filley et al., 2007). In early onset AD patients an autosomal dominant inheritance has been observed with mutations in *PSEN1*, *PSEN2* or *APP*. *PSEN1* mutations are the most commonly observed, and make up approximately 50% of familial early onset AD cases. However, they only account for about 1% of all AD cases (Filley et al., 2007). *APP* mutations have been observed in approximately 5-7% of familial early onset AD, and *PSEN2* accounts for less than 1% (Filley et al., 2007).

PSEN1 is located at chromosome 14q24.3, *PSEN2* at 1q41, and *APP* at 21q21.2. There have been a number of mutations reported in these genes; over 164 in *PSEN1*, ~28 in *APP* and ~10 in *PSEN2,* as of October, 2007 (Alzheimer Disease & Frontotemporal Dementia Mutation Database).

PSEN1 and *PSEN2* are both located in the tissues of the brain. They are comparable in size and are both located in similar intracellular compartments such as the endoplasmic reticulum and cytoplasm. Different studies have investigated the function of these proteins. *PSEN1* has been hypothesized to play a roll in cell adhesion, and *PSEN1* and *PSEN2* have been visualized in association with interphase kinetochores and centromeres, which may suggest that the proteins play a role in chromosome organization and segregation (Filey et al., 2007). It has been suggested that mutations in *PSEN1* and *PSEN2* may interfere with normal *APP* processing and lead to the AD. *PSEN 1* has been reported to be necessary for the activation of γ secretase. It

remains to be determined whether PSEN1 is a transmembrane aspartyl protease, γ secretase co-factor, or assists in the colocalization of γ secretase and APP. It has been hypothesized that gain of function mutations in *PSEN1* lead to an increased γ secretase activity, and therefore a higher rate of formation of senile plaques (online mendelian inheritance of man).

1.5.2 Genetics of Late Onset Alzheimer's Disease

Late onset AD does not show a clear inheritence pattern, and therefore indicates multifactorial inheritance. Most cases of LOAD are sporadic. However, first degree relatives of an individual with AD are at a higher risk than the general population. Survey and data from Cupples et al. (2004) found that substantial portions of relatives of those with AD, are interested in more fully understanding their risk. Liddell et al. (2001) reported a predicted risk of developing AD in the first degree relatives of probands with AD of 15-19% compared with 5% in the general population, or a 3 to 4 fold increased risk.

The only susceptibility maker found to be associated with LOAD has been apolipoprotein E (*APOE*), located on chromosome 19q13. *APOE* consists of three common alleles: *APOE*2*, *APOE*3* and *APOE*4*, with variation at codon 112 and 158. The *APOE*3* allele is the most common in the general population. The *APOE*4* allele has been found to significantly increase one's risk to AD, while the *APOE*2* allele seems to be protective. *APOE*4* is common in the U.S. population with a frequency of about 14% (Cupples et al., 2004). The *APOE*4* allele has a dose related relationship with LOAD, two copies lead to an earlier age of onset (mean age before 70), and one copy leads to a latter age of onset (means age after 70) (Nussbaum et al., 2004). The odds of AD are increased 3 times among those that carry

one copy of the *APOE*4* allele, and 15 times among those that carry two copies of the *APOE*4* allele (Merikangas et al., 2003).

APOE plays a vital role in the transport of cholesterol and other lipids through the body, and has been linked to the central nervous system. It is hypothesized to be involved with the mobilization and redistribution of cholesterol, and in the repair, growth and maintenance of myelin and neuronal membranes during development and injury (Panza, 2004). Studies have found that in AD patients, *APOE*4* tends to co-localize with Aβ plaques in the brain. In vitro studies have also found *APOE*4* to have a stimulatory role in the formation of Aβ plaques. Although the exact pathology of the relationship between *APOE*4* and LOAD is unknown, it has been suggested that *APOE*4* may bind with greater affinity to Aβ plaques and serve as a chaperone in their formation (Panza, 2004). *APOE*3* and *APOE*2* may bind with less affinity, this could explain why these individuals do not show in increase in disease. The degree to which *APOE* status affects one's risk is also dependent on age. The older an individual is the less the affect of *APOE*4* on AD, with little to no affect by the age of 90 (Kamboh, 2004).

1.5.3 Genome Wide Association Studies

Since sequencing of the human genome has been completed, SNP-based genome wide association studies have become possible. These studies examine the whole genome for SNP association to specific diseases, instead of examining SNPs that are biological and/or positional candidates. Alleles in genes across the whole genome can now be studied for significant association to multifactorial conditions (Coon et al., 2007).

In a genome wide association study examining 17,343 putative functional SNPs located in 11221 unique genes, 19 SNPs were found to have a statistically significant association with AD (Grupe, et al., 2007). Of these 3 SNPs were present in the *APOE* ancestry genes and thus reflect the known association of *APOE* with AD. Our goal was to replicate the other new 16 associations in a large case-control sample of white Americans. Additionally we examined six positional and/or biological candidates as risk factors for AD (SNP 17-22). Five of these SNPs were identified in a paper investigating association with risk of coronary artery disease (Samani, et al., 2007). It is possible that these SNPs are also biological candidates for risk of AD. This is exemplified by *APOE*, which is a known risk factor for both coronary artery disease and AD.

SNP17 is located between 10q11.1 and 10q11.2, in close proximity to *choline acyltransferase* (CHAT) a biological candidate gene that has been implicated in association with AD, due to its role in cholinergic neurotransmission (Kamboh, 2004). Cholinergic neurotransmission is part of the nervous system that releases or is activated by acetylcholine or a related compound. One of the earliest pathologic events in AD is thought to be the degeneration of cholinergic neurons of the basal forebrain (Auld et al., 2002). Research showing a decrease in choline acetyltransferase (ChAT) activity (30-90%), ChAT mRNA (~50%) in the temporal lobe and frontal and parietal corticies of the AD brain, has further implicated CHAT in AD risk (Auld et al., 2002). This gene has been previously investigated for a possible association with LOAD. Harold et al. (2003) indicated that polymorphisms in CHAT were probably not the primary cause of LOAD. The authors of this study also commented that their results do not rule out CHAT as a possible risk factor for AD, but further investigation is needed. SNP18 is located between 2q36 and 2q37, with the closest gene located at 2q36.3. SNP19 is located in the SMAD family member 3 gene (*SMAD3*), at 15q22.33. Scott et al. (2003) found a linkage peak for AD on

chromosome 15q22 with a peak LOD score of 2.8 in 38 families with minimum age at onset >79 years, and a peak LOD score of 3.1 in 43 families with mean age at onset >80 years. SMAD3 is known to be associated with transforming growth factor beta 1 (TGF-β1). TGF-β1 is known to be associated with factors that may affect phosphorylation of tau (Ueberham et al., 2006). Ueberham et al. (2006) suggests that SMAD proteins may play a critical role in the pathogenesis of AD. SNP20 is located between 9p21 and 9p22, with the closest gene located at 9p21.3. Several studies have identified linkage peaks for AD at 9p22 (Kehoe et al., 1999; Meyers et al., 2004; Pericak-Vance et al., 2000; Haines et al., 2001), and at 9p21 (Blacker et al., 2003). SNP21 is located in the methylenetetrahydrofolate dehydrogenase gene (MTHFD1L) at 6q25.1. A genome wide scan for AD in 466 families, Pericak-Vance et al. (2000) reported a linkage peak at the telomeric end of chromosome 6 spanning the region of 6q25.1. MTHFD1L is involved in the enzymatic reaction transforming homocysteine to methyionine, and when not functioning correctly has been linked as a possible risk factor of AD. Homocysteine is thought to exhibit pro-oxidative activity, and oxidative stress is one factor implicated as a risk for AD (Dorszewska et al., 2007). Studies have also shown that homocysteine can pass the blood/brain barrier to have a larger affect on the CNS (Agnati et al., 2005). Ho et al. (2002) hypothesized that elevated homocysteine levels may cause vascular damage, deteriorate function of the blood/brain barrier and cause abnormal nitrogen oxide production, all of which may increase the risk of AD. SNP 22 is located in the *transcription factor7-like 2 gene* (*TCF7L2*) at 10q25.3. Several studies have found linkage peaks for AD spanning the 10q25.3 region (Kehoe et al., 1999; Li et al., 2002; Blacker et al., 2003). *TCF7L2* has been studied as a possible biological risk factor of Diabetes (Grant et al., 2006). Research has also been done in order to determine if there is a link between diabetes and AD. Although an exact link has not been identified, the possible link of *TCF7L2*

with diabetes, and the possible link of diabetes and AD makes *TCF7L2* a possible biologic candidate for AD risk (Sima et al., 2006).

2.0 PATIENTS AND METHODS

2.1 SUBJECTS

2.1.1 Sample Population

Subjects were 2,029 Caucasian American individuals. The cases included 1,009 Caucasian Americans diagnosed with LOAD. In this group 67.69% were female and 7.8% were autopsy confirmed. The mean AAO was 72.85 ± (SD) 6.24 years. The controls included 1,010 age-matched healthy Caucasian Americans. In this group 59.80% were female and 1,29% were autopsy confirmed. The mean age at baseline was 74.07 ± 6.20 years. The subjects were recruited with informed consent and the study was approved by the University of Pittsburgh Institute Review Board.

DNA was isolated from blood using the QIAamp blood DNA Maxi kit protocol (Qiagen, Valencia CA), and from brain tissue using the QIAamp DNA Mini kit protocol. A small number of samples with a low amount of DNA were amplified using the GenomiPhi kit (GE Healthcare).

2.2 GENOTYPE DETERMINATION

2.2.1 TaqMan Assay

The genotypes were determined using TaqMan SNP Genotyping Assays (Applied Bio Sysytems, Foster City, CA). The assay identification numbers for each SNP are listed in Table 2.1.

Table 2.1 TaqMan SNP genotyping assays

SNP	Gene	location	allele	reference number	TaqMan Genotyping Assay
1	*GALP*	19q13.42	C / G	rs3745833	C___1936853_1_
2	*PCK1*	20q13.31	A / G	rs8192708	C___2508722_10
3	*TNK1*	17p13.1	A / T	rs1554948	C___8717889_1_
4	*SERPINA13*	14q31-14q32	A / T	rs11622883	C___2188996_10
5	*PGBD1*	6p22.1	A / G	rs3800324	C__25942411_20
6	*LMNA*	1q21.2-1q21.3	A / G	rs505058	C____6303747_10
7	*UBD*	6p21.3	C / G	rs444013	C____588691_20
8	UBA52P1 Closest gene 7p15.2	7p15	C / T	rs1859849	C__11827509_10
9	*BCR*	22q11.2-22q12	C / T	rs9608099	C___2447571_1_
10	*MYH13*	17p13	C / T	rs2074877	C___2179030_1_
11	*EBF3*	10q26.3	C / T	rs11016976	C__10057645_10
12	*AGC1*	15q26.1	G / T	rs2882676	C___1834284_10
13	*CTSS*	1q21	A / G		C__15746640_10
14	*FAM63A*	1q21.2	A / T		C__22274641_10
15	*TRAK2*	2q33	C / T	rs13022344	C___1226613_10
16	LOC442265 Closest gene 6q24.1	6q24	A / G	rs6907175	
17	near *CHAT*	10q11.1-10q11.2	A / G	rs501120	C___1033658_10
18	LOC646736 Closest gene 2q36.3	2q36-2q37	A / C	rs2943634	C__15949769_10
19	*SMAD3*	15q22.33	C / T	rs17228212	C__33991364_10
20	LOC729983 Closest gene 9p21.3	9p22-9p21	C / G	rs1333049	C__1754666_10
21	*MTHRD1L*	6q25.1	A / G	rs6922269	C__29894051_10
22	*TCF7L2*	10q25.3	C / T	rs7901695	C____384583_10

The TaqMan analysis was performed after all samples were placed on 384 well plates. Every plate had a mixture of cases and controls, and ten percent of the samples were repeated in order to assess error rate. Formula and cycling conditions were followed according to Applied BioSystems TaqMan protocol.

The biochemical principle of TaqMan is outlined briefly as follows. TaqMan involves first the amplification of the DNA followed by reading using the7900HT Fast Real-Time PCR system machine (Applied Biosystems). The samples are placed on a 384 well plate. A mixture is then added to the samples which contains master mix, genotyping assay and water. The genotyping assay consists of sequence specific forward and reverse primers, one probe labeled at the 5' end with VIC dye and one probe labeled at the 5' end with FAM dye. A nonfluorescent quencher is attached to the 3' end of each of these probes. The probes lie between the two primers and overlay the SNP of interest.

AmpliTaq Gold® Polymerase with 5' exo-nuclease activity is used in the PCR reaction. During the annealing process the polymerase binds to the primers and begins elongation of the new DNA strand. The polymerase removes DNA downstream impeding its ability to synthesize a new strand. Therefore, when the polymerase reaches the attached probe during elongation the 5' exo-nuclease activity cleaves the probe. This is only true for probes that bind with the target sequence. Those probes that do not bind with the target sequence remain in tact in the solution, these probes are not able to fluoresce due to the fact that the VIC/FAM dye remains close to the quencher. The probes that are cleaved, allow for the VIC/FAM dye to separate from the quencher. This allows for the dye to fluoresce. This reaction is illustrated in Figure 1.

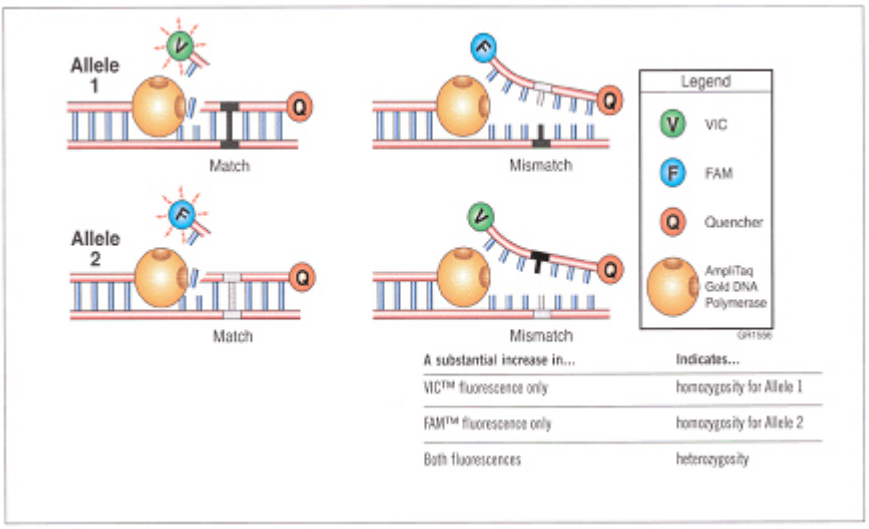

Figure 1TaqMan Chemical Reaction

The product is analyzed using the 7900HT Fast Real-Time PCR system. The fluorescence of each product is measured, and placed on a graph accordingly. The output allows us to distinguish between the different clusters of fluorescence intensity that are seen for each genotype. An example of the results from a TaqMan analysis can be viewed in Figure 2.

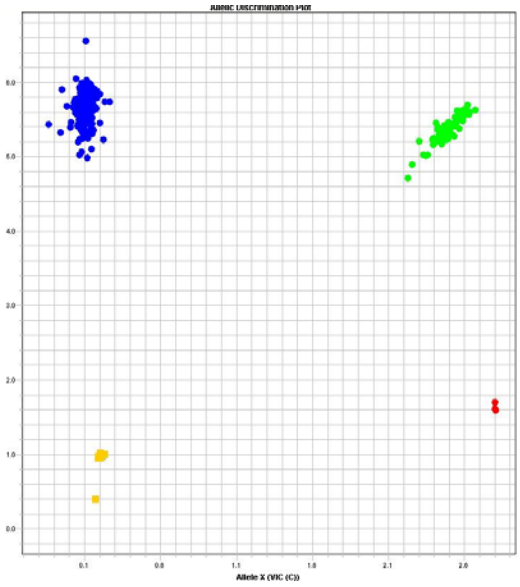

Figure 2 Results From TaqMan Analysis

2.3 STATISTICAL METHODS

Allele and genotype frequencies were calculated by the direct allele-counting method. Goodness of fit to Hardy-Weinberg equilibrium was tested using the X^2 test. Differences between genotype and allele frequencies in case and controls were tested with the X^2 or Fisher's exact tests as appropriate. These statistics were calculated using R 2.2.0 with the genetics package attached (R Development Core Team, 2005).

Genotype and allele frequencies for cases and controls were stratified for: *APOE* status, age at onset (AAO), diagnosis method (autopsy vs clinical), and classification of probable vs possible AD. Analysis of variance for AAO, disease duration (age at death – age at diagnosis), and Mini-Mental state examination (MMSE) at baseline and change in score was calculated.

We tested the hypothesis of Grupe et al. (2007) that the 16 identified SNPs were risk loci for AD. We used the risk alleles identified in the paper and coded our population in order to determine how many risk alleles they carried total (assuming a dosage model) and how many loci contained a risk allele (assuming a dominance model). We carried out a linear regression for each model adjusting for age, sex and *APOE* status. These calculations, as well as linear regression for odds ratios, were done using R 2.2.0 with the genetics package attached. Power estimation was made by calculating for a population size of 1,000 cases and 1,000 controls.

In our analysis we chose not to examine interactions between the SNPs. This was due to the fact that no specific association was seen with any of the SNPs individually. Since we were examining so many SNPs a large amount of tests would have to be run. It is quite possible that interactions may be found by chance due the number of SNPs. Using the Bonferroni correction every time we do another test the p value gets smaller and smaller, making it more difficult to find a significant association.

3.0 RESULTS

3.1 *APOE* POLYMORPHISM IN AD CASES AND CONTROLS

APOE genotype frequencies are presented in Table 3.1. In cases the 3/4 genotype was the most frequent making up 46% of the total case genotypes. The 3/3 genotype was the second most frequent making up 38% of the total case genotypes. The rest of the genotypes in the cases were below 10%. In controls the 3/3 genotype was the most frequent making up 65% of the total control genotypes. The rest of the genotypes in the controls were below 20%. While the frequency of the *APOE*4* allele was significantly higher in cases than controls ($p<0.001$) the frequency of the *APOE*2* allele was lower in cases than controls ($p<0.001$).

Table 3.1. APOE polymorphism AD cases and controls

APOE				
	AD Cases		Controls	
	n	%	n	%
3/3	386	0.38	656	0.65
3/4	467	0.46	168	0.17
2/3	39	0.04	143	0.14
2/4	27	0.03	23	0.02
4/4	87	0.09	14	0.01
2/2	2	0.002	5	0.005
Total (n)	1,008		1,009	
APOE*3	(1278) 0.63		(1623) 0.80	p< 0.001
APOE*4	(668) 0.33		(219) 0.11	p<0.001
APOE*2	(70) 0.004		(176) 0.009	p<0.001

3.2 GENOTYPE RATE

The genotyping error rate for all the SNPs but SNP 18 was estimated to be <1%, the estimated error rate for SNP 18 was 2.8%. The genotyping failure rate for all SNPs besides SNP16 was <1.14%, the failure rate for SNP16 was 4%.

3.3 HARDY WEINBERG EQUILIBRIUM FOR 22 SNPS

P-values based on Hardy Weinberg (HWE) equilibrium analysis are listed in Table 3.2. Twenty-one SNPs were in HWE, however, SNP2 *PCK1* was found to be out of HWE with a p value of 2.569 E-36 for cases and 1.401 E-29 for controls. A search through NCBI found three groups reporting genotype and allele frequencies for this SNP. These groups include HapMap (ss44233834), Perlegen (ss24451444) and University of Washington headed by Debra Nickerson (ss66859094) (dbSNP). The allele frequencies with these studies, as well as Grupe et al. (2007) were similar to ours, but the genotype frequencies were not. The data from NCBI and the Grupe et al. (2007) were in HWE. When we examined the plots read from TaqMan, the clusters were distinct, and the calls were conservative. Each plate individually was also not within HWE, the magnitudes of the *p* values were similar for each of the plates. The cases and controls showed the same effect with similar *p* values. The pattern in all of the plates in cases and controls, was an excess of both homozygotes and a shortage of heterozygotes in our data.

Table 3.2. P-values based on HWE for cases and controls

	p value cases	p value controls
SNP1 *GALP* rs3745833	0.501	0.645
SNP2 *PCK1* rs8192708	2.569E-36	1.401E-29
SNP3 *TNK1* rs1554948	0.570	0.131
SNP4 *SERPINA13* rs11622883	0.871	0.713
SNP5 *PGBD1* rs3800324	0.168	0.308
SNP6 *LAMIN AC* rs505058	0.054	0.790
SNP7 *UBD* rs444013	0.189	0.117
SNP8 rs1859849	0.433	0.200
SNP9 *BCR* rs9608099	0.175	0.911
SNP10 *MYH13* rs2074877	0.429	0.132
SNP11 *EBF3* rs11016976	0.672	0.187
SNP12 *AGC1* rs2882676	0.330	0.842
SNP13 *CTSS*	0.429	0.063
SNP14 *FAM63A*	0.325	0.057
SNP15 *TRAK2* rs13022344	0.550	0.910
SNP16 rs6907175	0.002	0.914
SNP17 rs501120	0.449	0.660
SNP18 rs2943634	0.173	0.253
SNP19 *SMAD3* rs17228212	0.259	0.108
SNP20 rs1333049	0.435	0.090
SNP21 *MTHRD1L* rs6922269	0.831	0.957
SNP22 *TCF7L2* rs7901695	0.778	0.327

3.4 DISTRIBUTION OF 22 SNPS IN CASES AND CONTROLS

Allele and genotype frequencies for 22 SNPs for cases and controls are given in Table 3.3. No significant difference was found except for SNP 2 *PCK1* rs3745833 which showed a marginal difference between genotype frequencies (p=0.057) and allele frequencies (p=0.015).

Table 3.3. Distribution of 22 SNPs in cases and controls

			Genotype n(%)				Allele		
		n	CC	CG	GG	*p*	C	G	*p*
SNP1 *GALP* rs3745833	AD	1008	426 (0.423)	466 (0.462)	116 (0.115)	0.424	0.654	0.346	0.198
	C	1005	401 (0.399)	473 (0.471)	131 (0.130)		0.634	0.366	
		n	AA	AG	GG	*p*	A	G	*p*
SNP2 *PCK1* rs8192708	AD	1009	807 (0.800)	139 (0.138)	63 (0.115)	0.057	0.869	0.131	0.015
	C	1008	762 (0.756)	173 (0.172)	73 (0.130)		0.842	0.158	
		n	AA	AT	TT	*p*	A	T	*p*
SNP3 *TNK1* rs1554948	AD	1009	187 (0.185)	505 (0.500)	317 (0.314)	0.101	0.436	0.564	0.115
	C	1006	225 (0.224)	476 (0.473)	305 (0.303)		0.460	0.540	
		n	AA	AT	TT	*p*	A	T	*p*
SNP4 *SERPINA13* rs11622883	AD	1007	216 (0.214)	498 (0.495)	293 (0.291)	0.931	0.462	0.538	0.955
	C	1004	212 (0.211)	505 (0.503)	287 (0.286)		0.463	0.537	
		n	AA	AG	GG	*p*	A	G	*p*
SNP5 *PGBD1* rs3800324	AD	1009	0 (0.000)	84 (0.083)	925 (0.917)	0.411	0.042	0.958	0.974
	C	1002	3 (0.003)	77 (0.077)	922 (0.920)		0.041	0.959	
		n	CC	CT	TT	*p*	A	T	*p*
SNP6 *LAMIN AC* rs505058	AD	1009	16 (0.185)	173 (0.500)	820 (0.314)	0.101	0.436	0.564	0.115
	C	1006	10 (0.224)	173 (0.473)	822 (0.303)		0.460	0.540	
		n	CC	CG	GG	*p*	C	G	*p*
SNP7 *UBD* rs444013	AD	1009	255 (0.253)	525 (0.520)	229 (0.227)	0.049	0.513	0.487	0.168
	C	996	297 (0.298)	471 (0.473)	228 (0.229)		0.535	0.465	
		n	CC	CG	GG	*p*	C	G	*p*
SNP 8 rs1859849	AD	1009	59 (0.058)	389 (0.386)	561 (0.556)	0.276	0.251	0.749	0.491
	C	1003	76 (0.076)	371 (0.370)	556 (0.554)		0.261	0.739	
		n	TT	TC	CC	*p*	T	C	*p*
SNP9 *BCR* rs9608099	AD	1005	96 (0.096)	459 (0.457)	450 (0.448)	0.354	0.324	0.676	0.319
	C	996	96 (0.096)	424 (0.426)	476 (0.478)		0.309	0.691	
		n	CC	CT	TT	*p*	C	T	*p*
SNP10 *MYH13* rs2074877	AD	1009	408 (0.214)	458 (0.495)	143 (0.291)	0.931	0.462	0.538	0.955
	C	1004	381 (0.211)	493 (0.503)	130 (0.286)		0.463	0.537	
		n	CC	CT	TT	*p*	C	T	*p*
SNP11 *EBF3* rs11016976	AD	1009	24 (0.024)	252 (0.250)	733 (0.726)	0.354	0.149	0.851	0.192
	C	1005	23 (0.023)	224 (0.223)	758 (0.754)		0.134	0.866	
		n	AA	CA	CC	*p*	A	C	*p*
SNP12 *AGC1* rs2882676	AD	107	145 (0.144)	455 (0.452)	407 (0.404)	0.674	0.370	0.630	0.476
	C	997	130 (0.130)	456 (0.457)	411 (0.412)		0.359	0.641	

Table 3.3 continued:

		n	AA	AG	GG	*p*	A	G	*p*
SNP13 *CTSS*	AD	1009	859 (0.851)	142 (0.141)	8 (0.008)	0.121	0.922	0.078	0.924
	C	1005	848 (0.844)	155 (0.154)	2 (0.002)		0.921	0.079	
		n	AA	AT	TT	*p*	A	T	*p*
SNP14 *FAM63A*	AD	1008	6 (0.006)	117 (0.116)	885 (0.878)	0.059	0.064	0.936	0.924
	C	1007	1 (0.001)	139 (0.138)	867 (0.861)		0.070	0.930	
		n	CC	CT	TT	*p*	C	T	*p*
SNP15 *TRAK2* rs13022344	AD	1009	108 (0.107)	457 (0.453)	444 (0.440)	0.912	0.333	0.667	0.797
	C	1001	108 (0.108)	444 (0.444)	449 (0.449)		0.330	0.670	
		n	AA	AG	GG	*p*	A	G	*p*
SNP16 rs6907175	AD	967	266 (0.275)	435 (0.450)	266 (0.275)	0.061	0.500	0.500	0.542
	C	969	251 (0.259)	486 (0.502)	232 (0.239)		0.510	0.490	
		n	CC	CT	TT	*p*	C	T	*p*
SNP17 rs501120	AD	1008	19 (0.019)	260 (0.258)	729 (0.723)	0.899	0.148	0.852	0.688
	C	1008	19 (0.019)	251 (0.249)	738 (0.732)		0.143	0.857	
		n	AA	AC	CC	*p*	A	C	*p*
SNP18 rs2943634	AD	1000	127 (0.127)	431 (0.431)	442 (0.442)	0.793	0.343	0.658	0.497
	C	996	118 (0.118)	426 (0.428)	452 (0.454)		0.332	0.668	
		n	CC	CT	TT	*p*	C	T	*p*
SNP19 *SMAD3* rs17228212	AD	1009	76 (0.075)	376 (0.373)	557 (0.552)	0.945	0.262	0.738	0.954
	C	1004	79 (0.079)	369 (0.368)	556 (0.554)		0.262	0.738	
		n	CC	CG	GG	*p*	C	G	*p*
SNP20 rs1333049	AD	1009	251 (0.249)	492 (0.488)	266 (0.264)	0.201	0.493	0.507	0.704
	C	1007	225 (0.223)	530 (0.526)	252 (0.250)		0.487	0.513	
		n	AA	AG	GG	*p*	A	G	*p*
SNP21 *MTHRD1L* rs6922269	AD	1008	75 (0.074)	395 (0.392)	538 (0.534)	0.704	0.270	0.729	0.414
	C	1004	67 (0.067)	386 (0.384)	551 (0.549)		0.259	0.742	
		n	CC	CT	TT	*p*	C	T	*p*
SNP22 *TCF7L2* rs7901695	AD	1009	95 (0.094)	423 (0.419)	491 (0.487)	0.282	0.304	0.696	0.125
	C	1007	114 (0.113)	429 (0.426)	464 (0.461)		0.326	0.674	

3.5 STRATIFICATION BY *APOE*

APOE stratified data for 22 SNPs are presented in Table 3.4. Among non-*APOE*4* carriers, 3 significant association were observed. SNP3 *TNK1*/rs1554948 showed a significant difference in genotypes (p=0.029) and allele frequencies (p=0.017) between cases and controls. SNP7 *UBD*/rs444013 (p=0.044) and SNP18 rs2943634 (p=0.039) showed a significant difference in allele frequencies. SNP16 rs6907175 showed a significant difference in genotype frequencies (p=0.032). Among *APOE*4* carriers, SNP2 *PCK1*/rs8192708 showed a significant difference in genotype frequencies (p=0.018).

Table 3.4. Distribution of 22 SNPs, based on *APOE*4* carriers and non *APOE*4* carriers

				Genotype n (%)				Allele		
			n	CC	CG	GG	*p*	C	G	*p*
SNP1 *GALP* rs3745833	No E4	AD	427	169 (0.396)	198 (0.464)	60 (0.141)	0.948	0.628	0.372	0.782
		C	799	320 (0.401)	372 (0.466)	107 (0.134)		0.633	0.367	
	E4	AD	580	257 (0.443)	267 (0.460)	56 (0.097)	0.535	0.673	0.327	0.276
		C	202	81 (0.401)	98 (0.485)	23 (0.114)		0.644	0.356	
			n	AA	AG	GG	*p*	A	G	*p*
SNP2 *PCK1* rs8192708	No E4	AD	427	332 (0.778)	68 (0.159)	27 (0.063)	0.629	0.857	0.143	0.285
		C	801	608 (0.759)	131 (0.164)	62 (0.077)		0.841	0.159	
	E4	AD	581	475 (0.818)	70 (0.120)	36 (0.062)	0.018	0.878	0.122	0.097
		C	204	152 (0.745)	41 (0.201)	11 (0.054)		0.846	0.154	
			n	AA	AT	TT	*p*	A	T	*p*
SNP3 *TNK1* rs1554948	No E4	AD	427	67 (0.157)	212 (0.496)	148 (0.347)	0.029	0.405	0.595	0.017
		C	798	175 (0.219)	377 (0.472)	246 (0.308)		0.456	0.544	
	E4	AD	581	119 (0.205)	293 (0.504)	169 (0.291)	0.568	0.457	0.543	0.515
		C	205	49 (0.239)	97 (0.473)	59 (0.288)		0.476	0.524	
			n	AA	AT	TT	*p*	A	T	*p*
SNP4 *SERPINA13* rs11622883	No E4	AD	426	88 (0.207)	230 (0.540)	108 (0.254)	0.270	0.477	0.523	0.485
		C	797	171 (0.215)	394 (0.494)	232 (0.291)		0.462	0.538	
	E4	AD	580	128 (0.221)	268 (0.462)	184(0.317)	0.203	0.452	0.548	0.688
		C	204	40 (0.196)	109 (0.534)	55 (0.270)		0.463	0.537	
			n	AA	GA	GG	*p*	A	G	*p*
SNP5 *PGBD1*	No E4	AD	427	0 (0.000)	44 (0.103)	383 (0.897)	0.132	0.052	0.948	0.282
		C	797	3 (0.004)	61 (0.077)	733 (0.920)		0.042	0.958	

Table 3.4 continued:

rs3800324	E4	AD	581	0 (0.000)	40 (0.069)	541 (0.931)		0.034	0.966	0.629
		C	202	0 (0.000)	16 (0.079)	186 (0.921)		0.040	0.960	
			n	CC	CT	TT	*p*	C	T	*p*
SNP6	No	AD	427	7 (0.016)	71 (0.166)	349 (0.817)	0.416	0.100	0.900	0.974
LAMIN AC	E4	C	797	7 (0.009)	144 (0.181)	646 (0.811)		0.099	0.901	
rs505058	E4	AD	581	9 (0.015)	102 (0.176)	470 (0.809)	0.441	0.103	0.897	0.190
		C	204	2 (0.010)	29 (0.142)	173 (0.848)		0.081	0.919	
			n	CC	CG	GG	*p*	C	G	*p*
SNP7	No	AD	427	104 (0.244)	215 (0.504)	108 (0.253)	0.108	0.495	0.505	0.044
UBD	E4	C	790	236 (0.299)	378 (0.478)	176 (0.223)		0.538	0.462	
rs444013	E4	AD	581	150 (0.258)	310 (0.534)	121 (0.208)	0.132	0.525	0.475	0.789
		C	203	59 (0.291)	92 (0.453)	52 (0.256)		0.517	0.483	
			n	CC	CG	GG	*p*	C	G	*p*
SNP8	No	AD	427	24 (0.056)	167 (0.391)	236 (0.553)	0.372	0.252	0.748	0.596
rs1859849	E4	C	797	61 (0.077)	295 (0.370)	441 (0.553)		0.262	0.738	
	E4	AD	581	35 (0.060)	222 (0.382)	324 (0.558)	0.718	0.251	0.749	0.923
		C	203	15 (0.074)	73 (0.360)	115 (0.567)		0.254	0.746	
			n	CC	TC	TT	*p*	C	T	*p*
SNP9	No	AD	426	43 (0.101)	193 (0.453)	190 (0.446)	0.420	0.327	0.673	0.244
BCR	E4	C	793	75 (0.095)	333 (0.420)	385 (0.485)		0.305	0.695	
rs9608099	E4	AD	578	53 (0.092)	266 (0.460)	259 (0.448)	0.855	0.322	0.678	0.763
		C	2000	21 (0.105)	90 (0.450)	89 (0.445)		0.330	0.670	
			n	CC	CT	TT	*p*	C	T	*p*
SNP10	No	AD	427	172 (0.403)	199 (0.466)	56 (0.131)	0.810	0.636	0.364	0.550
MYH13	E4	C	797	306 (0.384)	382 (0.479)	109 (0.137)		0.624	0.376	
rs2074877	E4	AD	581	236 (0.406)	258 (0.444)	87 (0.150)	0.034	0.628	0.372	0.934
		C	203	73 (0.360)	110 (0.542)	20 (0.099)		0.631	0.369	
			n	CC	TC	TT	*p*	C	T	*p*
SNP11	No	AD	427	10 (0.023)	113 (0.265)	304 (0.712)	0.387	0.156	0.844	0.247
EBF3	E4	C	798	19 (0.024)	183 (0.229)	596 (0.747)		0.138	0.862	
rs11016976	E4	AD	581	14 (0.024)	139 (0.239)	428 (0.737)	0.481	0.144	0.856	0.233
		C	204	4 (0.020)	41 (0.201)	159 (0.779)		0.120	0.880	
			n	AA	CA	CC	*p*	A	C	*p*
SNP12	No	AD	427	64 (0.150)	199 (0.466)	164 (0.384)	0.326	0.383	0.617	0.136
AGC1	E4	C	793	104 (0.131)	351 (0.443)	338 (0.426)		0.352	0.648	
rs2882676	E4	AD	579	81 (0.140)	256 (0.442)	242 (0.418)	0.218	0.361	0.639	0.378
		C	201	26 (0.129)	103 (0.512)	72 (0.358)		0.386	0.614	
			n	AA	AG	GG	*p*	C	G	*p*
SNP13	No	AD	427	365 (0.855)	57 (0.133)	5 (0.012)	0.091	0.922	0.078	0.998
CTSS	E4	C	797	674 (0.846)	121 (0.152)	2 (0.003)		0.922	0.078	
	E4	AD	581	493 (0.849)	85 (0.146)	3 (0.005)	0.471	0.922	0.078	0.747
		C	204	170 (0.833)	34 (0.167)	0 (0.000)		0.917	0.083	
			n	AA	TA	TT	*p*	A	T	*p*
SNP14	No	AD	426	3 (0.007)	50 (0.117)	373 (0.876)	0.158	0.066	0.934	0.727
FAM63A	E4	C	799	1 (0.001)	109 (0.136)	689 (0.862)		0.069	0.931	
	E4	AD	581	3 (0.005)	67 (0.115)	511 (0.880)	0.300	0.063	0.937	0.452
		C	204	0(0.000)	30 (0.147)	174 (0.853)		0.074	0.926	
			n	CC	TC	TT	*p*	C	T	*p*

Table 3.4 continued:

SNP15 *TRAK2* rs13022344	No E4	AD	427	48 (0.112)	199 (0.466)	180 (0.422)	0.678	0.345	0.655	0.492
		C	796	88 (0.111)	352 (0.442)	356 (0.447)		0.332	0.668	
	E4	AD	581	60 (0.103)	258 (0.444)	263 (0.453)	0.957	0.325	0.675	0.958
		C	202	20 (0.099)	92 (0.455)	90 (0.446)		0.327	0.673	
			n	AA	AG	GG	*p*	A	G	*p*
SNP16 rs6907175	No E4	AD	412	106 (0.257)	181 (0.439)	125 (0.303)	0.032	0.477	0.523	0.084
		C	776	205 (0.264)	388 (0.500)	183 (0.236)		0.514	0.486	
	E4	AD	554	160 (0.289)	253 (0.457)	141 (0.255)	0.299	0.517	0.483	0.352
		C	192	45 (0.234)	98 (0.510)	49 (0.255)		0.490	0.510	
			n	CC	CT	TT	*p*	C	T	*p*
SNP17 rs501120	No E4	AD	427	6 (0.014)	108 (0.253)	313 (0.733)	0.742	0.141	0.859	0.587
		C	804	16 (0.020)	207 (0.257)	581 (0.723)		0.149	0.851	
	E4	AD	580	13 (0.022)	152 (0.262)	415 (0.716)	0.310	0.153	0.847	0.128
		C	204	3 (0.015)	44 (0.216)	157 (0.770)		0.123	0.877	
			n	AA	CA	CC	*p*	A	C	*p*
SNP18 rs2943634	No E4	AD	425	56 (0.132)	179 (0.421)	190 (0.447)	0.701	0.342	0.658	0.967
		C	794	96 (0.121)	353 (0.445)	345 (0.435)		0.343	0.657	
	E4	AD	574	71 (0.124)	251 (0.437)	252 (0.439)	0.074	0.342	0.658	0.039
		C	201	21 (0.104)	73 (0.363)	107 (0.532)		0.286	0.714	
			n	CC	TC	TT	*p*	C	T	*p*
SNP19 *SMAD3* rs17228212	No E4	AD	427	26 (0.061)	153 (0.358)	248 (0.581)	0.434	0.240	0.760	0.218
		C	801	63 (0.079)	295 (0.368)	443 (0.553)		0.263	0.737	
	E4	AD	581	50 (0.086)	223 (0.384)	308 (0.530)	0.803	0.278	0.722	0.511
		C	203	16 (0.079)	74 (0.365)	113 (0.557)		0.261	0.739	
			n	CC	GC	GG	*p*	C	G	*p*
SNP20 rs1333049	No E4	AD	427	106 (0.248)	215 (0.504)	106 (0.248)	0.633	0.500	0.500	0.480
		C	803	180 (0.224)	419 (0522)	204 (0.254)		0.485	0.515	
	E4	AD	581	144 (0.248)	277 (0.477)	160 (0.275)	0.251	0.486	0.514	0.823
		C	204	45 (0.221)	111 (0.544)	48 (0.235)		0.493	0.507	
			n	AA	AG	GG	*p*	A	G	*p*
SNP21 *MTHRD1L* rs6922269	No E4	AD	427	30 (0.070)	171 (0.400)	226 (0.529)	0.688	0.270	0.730	0.444
		C	800	54 (0.068)	302 (0.378)	444 (0.555)		0.256	0.744	
	E4	AD	580	44 (0.076)	224 (0.386)	312 (0.538)	0.723	0.269	0.731	0.939
		C	203	13 (0.064)	84 (0.414)	106 (0.522)		0.271	0.729	
			n	CC	CT	TT	*p*	C	T	*p*
SNP22 *TCF7L2* rs7901695	No E4	AD	427	46 (0.108)	173 (0.405)	208 (0.487)	0.641	0.310	0.690	0.351
		C	803	94 (0.117)	340 (0.423)	369 (0.460)		0.329	0.671	
	E4	AD	581	49 (0.084)	250 (0.430)	282 (0.485)	0.796	0.299	0.701	0.528
		C	204	20 (0.098)	89 (0.436)	95 (0.466)		0.316	0.684	

3.6 STRATIFICATION BY AUTOPSY VS CLINICAL

Autopsy confirmed cases vs clinically diagnosed cases were stratified for 22 SNPs, the data is presented in Table 3.5. The analysis did not reveal any SNPs with statistically significant difference. The Alzheimer's Disease Research Center has found a high correlation (>95%) between clinically diagnosed AD cases and those confirmed at autopsy. This 5% uncertainty may explain the inability of our study to detect a significant difference in cases diagnosed by autopsy vs clinically.

Table 3.5. Distribution of 22 SNPs, based on autopsy vs clinical diagnosis in cases

			Genotype n (%)				Allele		
		n	CC	CG	GG	*p*	C	G	*p*
SNP1	AUT	79	40(0.506)	29(0.367)	10(0.127)	0.202	0.69	0.31	0.32
GALP	CLIN	929	386(0.416)	437(0.470)	106(0.114)		0.651	0.349	
SNP2		*n*	AA	AG	GG	*p*	A	G	*p*
PCK1	AUT	79	64(0.810)	14(0.177)	1(0.013)	0.112	0.899	0.101	0.244
rs8192708	CLIN	930	743(0.799)	125(0.134)	62(0.067)		0.866	0.134	
SNP3		*n*	AA	AT	TT	*p*	A	T	*p*
TNK1	AUT	79	14(0.177)	44(0.577)	21(0.266)	0.545	0.456	0.544	0.595
rs1554948	CLIN	930	173(0.186)	461(0.496)	296(0.318)		0.434	0.566	
SNP4		*n*	AA	AT	TT	*p*	A	T	*p*
SERPINA13	AUT	79	21(0.266)	29(0.367)	29(0.367)	0.062	0.449	0.551	0.745
rs11622883	CLIN	928	195(0.210)	469(0.505)	264(0.284)		0.463	0.537	
SNP5		*n*	AA	GA	GG	*p*	A	G	*p*
PGBD1	AUT	79	0(0.000)	7(0.089)	72(0.911)	N/A	0.044	0.956	0.861
rs3800324	CLIN	930	0(0.000)	77(0.083)	853(0.917)		0.041	0.959	
SNP6		*n*	CC	CT	TT	*p*	C	T	*p*
LAMIN AC	AUT	79	0(0.000)	11(0.139)	68(0.861)	0.346	0.07	0.93	0.168
rs505058	CLIN	930	16(0.017)	162(0.74)	752(0.809)		0.104	0.896	
SNP7		*n*	CC	CG	GG	*p*	C	G	*p*
UBD	AUT	79	20(0.253)	43(0.544)	16(0.203)	0.852	0.525	0.475	0.745
rs444013	CLIN	930	235(0.253)	482(0.518)	213(0.229)		0.512	0.488	
SNP8		*n*	CC	CG	GG	*p*	C	G	*p*
rs1859849	AUT	79	6(0.076)	27(0.342)	46(0.582)	0.613	0.247	0.753	0.894
	CLIN	930	53(0.057)	362(0.389)	515(0.554)		0.252	0.748	
SNP9		*n*	CC	TC	TT	*p*	C	T	*p*

Table 3.5 continued:

BCR	AUT	78	6(0.077)	34(0.436)	38(0.487)	0.712	0.295	0.705	0.42
rs9608099	CLIN	927	90(0.097)	425(0.458)	412(0.444)		0.326	0.674	
SNP10		*n*	CC	CT	TT	*p*	C	T	*p*
MYH13	AUT	79	25(0.316)	42(0.532)	12(0.152)	0.242	0.582	0.418	0.183
rs2074877	CLIN	930	383(0.412)	416(0.447)	131(0.141)		0.635	0.365	
SNP11		*n*	CC	TC	TT	*p*	C	T	*p*
EBF3	AUT	79	3(0.038)	21(0.266)	55(0.696)	0.631	0.171	0.829	0.413
rs11016976	CLIN	930	21(0.023)	231(0.248)	678(0.729)		0.147	0.853	
SNP12		*n*	AA	CA	CC	*p*	A	C	*p*
AGC1	AUT	79	8(0.101)	41(0.519)	30(0.380)	0.355	0.361	0.639	0.804
rs2882676	CLIN	928	137(0.148)	414(0.446)	377(0.406)		0.371	0.629	
SNP13		*n*	AA	AG	GG	*p*	A	G	*p*
CTSS	AUT	79	68(0.861)	11(0.139)	0(0.000)	0.708	0.93	0.07	0.672
	CLIN	930	791(0.851)	131(0.141)	8(0.009)		0.921	0.079	
SNP14		*n*	AA	TA	TT	*p*	A	T	*p*
FAM63A	AUT	79	0(0.000)	8(0.101)	71(0.899)	0.7	0.051	0.949	0.475
	CLIN	929	6(0.006)	109(0.117)	814(0.876)		0.065	0.935	
SNP15		*n*	CC	TC	TT	*p*	C	T	*p*
TRAK2	AUT	79	6(0.076)	39(0.494)	34(0.430)	0.575	0.323	0.677	0.766
	CLIN	930	102(0.110)	418(0.449)	410(0.441)		0.334	0.666	
SNP16		*n*	AA	AG	GG	*p*	A	G	*p*
rs6907175	AUT	75	21(0.280)	32(0.427)	22(0.293)	0.904	0.493	0.507	0.865
	CLIN	892	245(0.275)	403(0.452)	244(0.274)		0.501	0.499	
SNP17		*n*	CC	CT	TT	*p*	C	T	*p*
rs501120	AUT	79	0(0.000)	27(0.342)	52(0.658)	0.108	0.171	0.829	0.395
	CLIN	929	19(0.020)	233(0.251)	677(0.729)		0.146	0.854	
SNP18		*n*	AA	CA	CC	*p*	A	C	*p*
rs2943634	AUT	78	14(0.179)	27(0.346)	37(0.474)	0.178	0.353	0.647	0.783
	CLIN	922	113(0.123)	404(0.438)	405(0.439)		0.342	0.658	
SNP19		*n*	CC	TC	TT	*p*	C	T	*p*
SMAD3	AUT	79	1(0.013)	30(0.380)	48(0.608)	0.084	0.203	0.797	0.078
rs17228212	CLIN	930	75(0.081)	346(0.372)	509(0.547)		0.267	0.733	
SNP20		*n*	CC	GC	GG	*p*	C	G	*p*
rs1333049	AUT	79	22(0.278)	31(0.392)	26(0.329)	0.193	0.475	0.525	0.64
	CLIN	930	229(0.246)	461(0.496)	240(0.258)		0.494	0.506	
SNP21		*n*	AA	AG	GG	*p*	A	G	*p*
MTHRD1L	AUT	79	5(0.063)	27(0.342)	47(0.595)	0.524	0.234	0.766	0.286
rs6922269	CLIN	929	70(0.075)	368(0.396)	491(0.529)		0.273	0.727	
SNP22		*n*	CC	CT	TT	*p*	C	T	*p*
TCF7L2 rs7901695	AUT	79	6(0.076)	30(0.380)	43(0.544)	0.547	0.266	0.734	0.28
	CLIN	930	89(0.096)	393(0.423)	448(0.482)		0.307	0.693	

3.7 AGE STRATIFIED ANALYSIS

In order to examine a possible affect of age we stratified the data in to three age groups: 60-69, 70-79 and >80 years of age (Table 3.6). The cases were categorized by AAO, and the controls were categorized by the age at which they were brought into the study. SNP2 *PCK1/* rs8192708 showed a statistically significant difference in allele frequency between cases and controls in the >80 age group (p=0.012). SNP3 *TNK1*/rs1554948 showed a statistically significant difference in genotype frequencies between cases and controls in the 70-79 age group (p=0.024). SNP10 *MYH13*/rs2074877 showed a statistically significant difference in genotype frequencies between cases and controls in the >80 age group (p=0.008). SNP12 *AGC1*/rs2882676 showed a statistically significant difference in allele frequencies between cases and controls in the >80 age group (p=0.036). SNP21 MTHRD1L/rs6922269 showed a statistically significant difference in allele frequencies between cases and controls in the 60-69 age group (p=0.023).

Table 3.6. 22 SNPs stratified by three age groups

				Genotype n (%)				Allele		
			n	CC	CG	GG	*p*	C	G	*p*
SNP1 *GALP* rs3745833	60-69	AD	301	128(0.425)	141(0.468)	32(0.106)	0.508	0.659	0.341	0.260
		C	230	87(0.378)	114(0.496)	29(0.126)		0.626	0.374	
	70-79	AD	526	218(0.414)	243(0.462)	65(0.124)	0.989	0.645	0.355	0.908
		C	601	248(0.413)	277(0.461)	76(0.126)		0.643	0.357	
	>80	AD	143	67(0.469)	60(0.420)	16(0.112)	0.225	0.678	0.322	0.102
		C	169	63(0.373)	82(0.485)	24(0.142)		0.615	0.385	
			n	AA	AG	GG	*p*	A	G	*p*
SNP2 *PCK1* rs8192708	60-69	AD	302	245(0.811)	40(0.132)	17(0.056)	0.291	0.877	0.123	0.134
		C	230	174(0.757)	41(0.178)	15(0.065)		0.846	0.154	
	70-79	AD	526	414(0.787)	72(0.137)	40(0.076)	0.422	0.856	0.144	0.617
		C	602	461(0.766)	99(0.164)	42(0.070)		0.848	0.152	
	>80	AD	143	116(0.811)	22(0.154)	5(0.035)	0.084	0.888	0.112	0.012
		C	171	123(0.719)	33(0.193)	15(0.088)		0.816	0.184	
			n	AA	AT	TT	*p*	A	T	*p*

Table 3.6 continued:

SNP3 *TNK1* rs1554948	60-69	AD	302	65(0.215)	143(0.474)	94(0.311)	0.485	0.452	0.548	0.828
		C	230	45(0.196)	121(0.526)	64(0.278)		0.459	0.541	
	70-79	AD	526	91(0.173)	280(0.532)	155(0.295)	0.024	0.439	0.561	0.204
		C	601	140(0.233)	280(0.466)	181(0.301)		0.466	0.534	
	>80	AD	413	22(0.154)	68(0.476)	53(0.371)	0.191	0.392	0.608	0.185
		C	170	40(0.235)	71(0.418)	59(0.347)		0.444	0.556	
			n	AA	AT	TT	*p*	A	T	*p*
SNP4 *SERPINA13* rs11622883	60-69	AD	302	61(0.202)	143(0.474)	98(0.325)	0.652	0.439	0.561	0.517
		C	230	54(0.235)	103(0.448)	73(0.317)		0.459	0.541	
	70-79	AD	524	117(0.223)	262(0.500)	145(0.277)	0.622	0.473	0.527	0.529
		C	600	120(0.200)	312(0.520)	168(0.280)		0.460	0.540	
	>80	AD	143	28(0.196)	78(0.545)	38(0.259)	0.770	0.469	0.531	0.789
		C	169	38(0.225)	86(0.509)	45(0.266)		0.479	0.521	
			n	AA	AG	GG	*p*	A	G	*p*
SNP5 *PGBD1* rs3800324	60-69	AD	302	0(0.000)	18(0.060)	284(0.940)	0.137	0.030	0.970	0.310
		C	230	3(0.013)	13(0.057)	214(0.930)		0.041	0.959	
	70-79	AD	526	0(0.000)	52(0.099)	474(0.901)	0.469	0.049	0.951	0.437
		C	599	0(0.000)	51(0.085)	548(0.915)		0.043	0.957	
	>80	AD	143	0(0.000)	11(0.077)	132(0.923)	1.000	0.038	0.962	0.856
		C.	168	0(0.000)	12(0.071)	156(0.929)		0.036	0.964	
			n	AA	AG	GG	*p*	A	G	*p*
SNP6 *LAMIN AC* rs505058	60-69	AD	302	6(0.020)	55(0.182)	241(0.798)	0.214	0.111	0.889	0.074
		C	230	3(0.013)	30(0.130)	197(0.857)		0.078	0.922	
	70-79	AD	526	8(0.015)	90(0.171)	428(0.814)	0.480	0.101	0.899	0.647
		C	600	6(0.010)	116(0.193)	478(0.797)		0.107	0.893	
	>80	AD	143	2(0.014)	23(0.161)	118(0.825)	0.746	0.094	0.906	0.596
		C	170	1(0.006)	26(0.153)	143(0.841)		0.082	0.918	
			n	CC	CG	GG	*p*	C	G	*p*
SNP7 *UBD* rs444013	60-69	AD	302	77(0.255)	161(0.533)	64(0.212)	0.213	0.522	0.478	0.152
		C	228	74(0.325)	110(0.482)	44(0.193)		0.566	0.434	
	70-79	AD	526	134(0.255)	272(0.517)	120(0.228)	0.278	0.513	0.487	0.572
		C	594	172(0.290)	280(0.471)	142(0.239)		0.525	0.475	
	>80	AD	143	34(0.238)	72(0.503)	37(0.259)	0.647	0.490	0.510	0.482
		C	169	48(0.284)	79(0.467)	42(0.249)		0.518	0.482	
			n	CC	CG	GG	*p*	C	G	*p*
SNP8 rs1859849	60-69	AD	302	14(0.046)	111(0.368)	177(0.586)	0.905	0.230	0.770	0.943
		C	230	9(0.039)	87(0.378)	134(0.583)		0.228	0.772	
	70-79	AD	526	35(0.067)	207(0.394)	284(0.540)	0.600	0.263	0.737	0.896
		C	598	46(0.077)	220(0.368)	332(0.555)		0.261	0.739	
	>80	AD	143	7(0.049)	60(0.420)	76(0.531)	0.061	0.259	0.741	0.221
		C	170	21(0.124)	61(0.359)	88(0.518)		0.303	0.697	
			n	CC	TC	TT	*p*	C	T	*p*
SNP9 *BCR* rs9608099	60-69	AD	300	25(0.083)	140(0.467)	135(0.450)	0.851	0.317	0.683	0.882
		C	229	22(0.096)	103(0.450)	104(0.454)		0.321	0.679	
	70-79	AD	525	52(0.099)	232(0.442)	241(0.459)	0.906	0.320	0.680	0.715
		C	593	58(0.098)	255(0.430)	280(0.472)		0.313	0.687	
	>80	AD	143	15(0.159)	71(0.497)	57(0.399)	0.091	0.353	0.647	0.077
		C	169	16(0.095)	65(0.385)	88(0.521)		0.287	0.713	

Table 3.6 continued:

			n	CC	CT	TT	*p*	C	T	*p*
SNP10 *MYH13* rs2074877	60-69	AD	302	126(0.417)	145(0.480)	31(0.103)	0.235	0.657	0.343	0.106
		C	229	83(0.362)	113(0.490)	33(0.144)		0.609	0.391	
	70-79	AD	526	206(0.392)	247(0.471)	73(0.139)	0.908	0.626	0.374	0.696
		C	599	239(0.399)	282(0.471)	78(0.130)		0.634	0.366	
	>80	AD	143	57(0.399)	56(0.392)	30(0.210)	0.008	0.594	0.406	0.616
		C	171	58(0.339)	94(0.550)	19(0.111)		0.614	0.386	
			n	CC	CT	TT	*p*	C	T	*p*
SNP11 *EBF3* rs11016976	60-69	AD	302	8(0.026)	79(0.262)	215(0.712)	0.442	0.157	0.843	0.260
		C	230	3(0.013)	55(0.239)	172(0.748)		0.133	0.867	
	70-79	AD	526	10(0.019)	130(0.240)	386(0.734)	0.142	0.143	0.857	0.498
		C	599	18(0.030)	123(0.205)	458(0.765)		0.133	0.867	
	>80	AD	143	4(0.028)	40(0.280)	99(0.692)	0.551	0.168	0.832	0.457
		C	171	2(0.012)	46(0.269)	123(0.719)		0.146	0.854	
			n	AA	AC	CC	*p*	A	C	*p*
SNP12 *AGC1* rs2882676	60-69	AD	301	41(0.136)	141(0.468)	119(0.395)	0.912	0.370	0.630	0.846
		C	229	32(0.140)	103(0.450)	94(0.410)		0.365	0.635	
	70-79	AD	525	73(0.139)	234(0.446)	218(0.415)	0.889	0.362	0.638	0.756
		C	592	82(0.139)	272(0.459)	238(0.402)		0.368	0.632	
	>80	AD	143	26(0.182)	63(0.441)	54(0.378)	0.062	0.402	0.598	0.036
		C	171	16(0.094)	78(0.456)	77(0.450)		0.322	0.678	
			n	AA	AG	GG	*p*	A	G	*p*
SNP13 *CTSS*	60-69	AD	302	255(0.844)	46(0.152)	1(0.003)	0.903	0.921	0.079	0.661
		C	230	191(0.830)	38(0.165)	1(0.004)		0.913	0.087	
	70-79	AD	526	446(0.848)	74(0.141)	6(0.011)	0.082	0.918	0.082	0.994
		C	600	503(0.838)	96(0.160)	1(0.002)		0.918	0.082	
	>80	AD	143	125(0.874)	17(0.119)	1(0.007)	0.550	0.934	0.066	0.695
		C	170	150(0.882)	20(0.118)	0(0.000)		0.941	0.059	
			n	AA	AT	TT	*p*	A	T	*p*
SNP14 *FAM63A*	60-69	AD	302	0(0.000)	37(0.123)	265(0.877)	0.470	0.061	0.939	0.495
		C	230	1(0.040)	31(0.135)	198(0.861)		0.072	0.928	
	70-79	AD	525	5(0.010)	62(0.118)	458(0.872)	0.037	0.069	0.931	0.973
		C	602	0(0.000)	83(0.138)	519(0.862)		0.069	0.931	
	>80	AD	143	1(0.007)	14(0.098)	128(0.895)	0.286	0.056	0.944	0.456
		C	170	0(0.000)	24(0.141)	146(0.859)		0.071	0.929	
			n	CC	TC	CC	*p*	C	T	*p*
SNP15 *TRAK2* rs13022344	60-69	AD	302	30(0.099)	143(0.474)	129(0.427)	0.437	0.336	0.664	0.211
		C	230	18(0.078)	102(0.443)	110(0.478)		0.300	0.700	
	70-79	AD	526	59(0.112)	236(0.449)	231(0.439)	0.536	0.337	0.668	0.671
		C	599	72(0.120)	249(0.416)	278(0.464)		0.328	0.672	
	>80	AD	143	16(0.112)	60(0.420)	67(0.469)	0.091	0.322	0.678	0.149
		C	167	18(0.108)	90(0.539)	59(0.353)		0.377	0.623	
			n	AA	AG	GG	*p*	A	G	*p*
SNP16 rs6907175	60-69	AD	115	37(0.322)	50(0.435)	28(0.243)	0.453	0.539	0.461	0.674
		C	226	61(0.270)	114(0.504)	51(0.226)		0.522	0.478	
	70-79	AD	512	145(0.283)	227(0.443)	140(0.273)	0.302	0.505	0.495	0.981
		C	571	148(0.259)	280(0.490)	143(0.250)		0.504	0.496	
	>80	AD	340	84(0.247)	158(0.465)	98(0.288)	0.155	0.479	0.521	0.330

Table 3.6 continued:

		C	167	40(0.240)	91(0.545)	36(0.216)		0.512	0.488	
			n	CC	CT	TT	*p*	C	T	*p*
SNP17 rs501120	60-69	AD	121	4(0.033)	32(0.264)	85(0.702)	0.248	0.165	0.835	0.348
		C	227	2(0.009)	59(0.260)	166(0.731)		0.139	0.861	
	70-79	AD	530	8(0.015)	128(0.242)	394(0.743)	0.732	0.136	0.864	0.965
		C	588	12(0.020)	135(0.230)	441(0.750)		0135	0.865	
	>80	AD	343	7(0.020)	94(0.274)	242(0.706)	0.771	0.157	0.843	0.537
		C	171	5(0.029)	49(0.287)	117(0.684)		0.173	0.827	
			n	AA	AC	CC	*p*	A	C	*p*
SNP18 rs2943634	60-69	AD	115	9(0.078)	52(0.4520	54(0.470)	0.323	0.304	0.696	-.929
		C	221	26(0.118)	84(0.380)	111(0.502)		0.308	0.692	
	70-79	AD	507	74(0.146)	209(0.412)	224(0.442)	0.301	0.352	0.648	0.264
		C	577	66(0.114)	248(0.430)	263(0.456)		0.329	0.671	
	>80	AD	330	40(0.121)	144(0.430)	146(0.442)	0.742	0.339	0.661	0.508
		C	165	21(0.127)	77(0.467)	67(0.406)		0.361	0.639	
			n	CC	CT	TT	*p*	C	T	*p*
SNP19 *SMAD3* rs17228212	60-69	AD	121	12(0.099)	46(0.380)	63(0.521)	0.982	0.289	0.711	0.947
		C	228	22(0.096)	89(0.390)	117(0.513)		0.292	0.708	
	70-79	AD	526	42(0.080)	196(0.373)	288(0.548)	0.656	0.266	0.734	0.348
		C	583	41(0.070)	208(0.357)	334(0.573)		0.249	0.751	
	>80	AD	344	21(0.061)	128(0.372)	195(0.567)	0.962	0.247	0.753	0.957
		C	169	11(0.065)	61(0.361)	97(0.574)		0.246	0.754	
			n	AA	AG	GG	*p*	A	G	*p*
SNP20 rs1333049	60-69	AD	120	23(0.192)	60(0.500)	37(0.308)	0.712	0.442	0.558	0.419
		C	229	50(0.218)	117(0.511)	62(0.271)		0.474	0.526	
	70-79	AD	532	133(0.250)	259(0.487)	140(0.263)	0.215	0.493	0.507	0.518
		C	593	126(0.212)	317(0.535)	150(0.253)		0.480	0.520	
	>80	AD	338	91(0.269)	161(0.476)	86(0.254)	0.545	0.507	0.493	0.404
		C	171	48(0.281)	87(0.509)	36(0.211)		0.535	0.465	
			n	AA	AG	GG	*p*	A	G	*p*
SNP21 *MTHRD1L* rs6922269	60-69	AD	118	11(0.093)	52(0.441)	55(0.466)	0.063	0.314	0.686	0.023
		C	229	15(0.066)	77(0.336)	137(0.598)		0.234	0.766	
	70-79	AD	528	43(0.081)	211(0.4000	274(0.519)	0.795	0.281	0.719	0.505
		C	590	43(0.073)	231(0.392)	316(0.536)		0.269	0.731	
	>80	AD	340	18(0.053)	127(0.374)	195(0.574)	0.573	0.240	0.760	0.681
		C	167	7(0.042)	70(0.419)	90(0.539)		0.251	0.749	
			n	AA	AG	GG	*p*	A	G	*p*
SNP22 *TCF7L2* rs7901695	60-69	AD	122	10(0.0820	52(0.426)	60(0.492)	0.751	0.295	0.705	0.869
		C	230	22(0.096)	89(0.387)	119(0.517)		0.289	0.711	
	70-79	AD	528	53(0.100)	224(0.424)	251(0.475)	0.562	0.313	0.688	0.280
		C	593	68(0.115)	260(0.438)	265(0.447)		0.334	0.666	
	>80	AD	341	32(0.094)	136(0.399)	173(0.507)	0.290	0.293	0.707	0.112
		C	168	22(0.131)	71(0.423)	75(0.446)		0.342	0.658	

3.8 ANALYSIS OF VARIANCE OF 22 SNPS FOR AGE-AT-ONSET (AAO)

APOE-adjusted mean value of AAO among AD cases for each SNP are given in Table 3.7. Analysis of variance (ANOVA) was used in order to determine if the mean of a variable was significantly different between genotypes. The ANOVA calculation for AAO showed a statistically significant difference between genotypes for SNP10 *MYH13*/rs2074877 (p=0.00196) and SNP20 rs1333049 (p=0.0417). The genotype TT for SNP10 *MYH13*/rs2074877, and CC for SNP 20 rs1333049 had the latest AAO. The heterozygotes (CT and GC) did not seem to have similar ages at onset, suggesting a possible recessive pattern.

Table 3.7. Analysis of variance of 22 SNPs for AAO, taking into account *APOE* status and gender

	Mean age of onset according to Genotypes			*p-value*
SNP1 *GALP*	CC(413)	CG (444)	GG(113)	*p*
rs3745833	73.13 ± 6.34	72.62 ± 5.89	72.79 ± 5.69	0.480
SNP2 *PCK1*	AA(775)	AG(134)	GG(62)	*p*
rs8192708	72.83 ± 6.13	72.98 ± 6.18	72.64 ± 5.01	0.961
SNP3 *TNK1*	AA(178)	AT(491)	TT(302)	*p*
rs1554948	72.34 ± 6.00	73.00 ± 6.16	72.91 ± 5.99	0.459
SNP4 *SERPINA13*	AA(206)	AT(483)	TT(280)	*p*
rs11622883	72.84 ± 6.28	72.90 ± 5.90	72.75 ± 6.24	0.948
SNP5 *PGBD1*	AA(0)	AG(81)	GG(890)	*p*
rs3800324	NA	73.43 ± 5.88	72.80 ± 6.09	0.366
SNP6 *LAMIN AC*	CC(16)	CT(68)	TT(787)	*p*
rs505058	70.45 ± 6.83	72.74 ± 6.27	72.92 ± 6.01	0.265
SNP7 *UBD*	CC(245)	CG(505)	GG(221)	*p*
rs444013	72.57 ± 5.95	72.75 ± 6.03	73.37 ± 6.29	0.313
SNP8	CC(56)	CG(378)	GG(537)	*p*
rs1859849	73.93 ± 5.92	73.11 ± 6.19	72.54 ± 6.00	0.147
SNP9 *BCR*	CC(92)	CT(443)	TT(433)	*p*
rs9608099	73.68 ± 5.80	72.76 ± 6.11	72.79 ± 6.09	0.399
SNP10 *MYH13*	CC(389)	CT(448)	TT(134)	
rs2074877	72.65 ± 6.14	72.50 ± 5.88	74.55 ± 6.27	0.00196
SNP11 *EBF3*	CC(22)	CT(249)	TT(700)	*p*
rs11016976	74.19 ± 7.16	73.02 ± 5.98	72.74 ± 6.07	0.4703
SNP12 *AGC1*	AA(140)	AC(438)	CC(391)	*p*
rs2882676	73.28 ± 5.89	72.61 ± 6.18	72.95 ± 6.03	0.4806

Table 3.7 continued:

SNP13 *CTSS*	AA(826)	AG(137)	GG(8)	*p*
	72.94 ± 6.09	72.25 ± 5.96	72.99 ± 7.01	0.468
SNP14 *FAM63A*	AA(6)	AT(113)	TT(851)	*P*
	75.32 ± 4.49	72.14 ± 5.79	72.92 ± 6.12	0.265
SNP15 *TRAK2* rs13022344	CC(105)	CT(439)	TT(427)	*p*
	73.13 ± 6.61	72.58 ± 6.02	73.05 ± 6.00	0.469
SNP 16 rs6907175	AA(255)	AG(421)	GG(256)	*p*
	72.42 ± 5.81	73.19 ± 6.24	73.05 ± 6.16	0.272
SNP17 rs501120	CC(18)	CT(249)	TT(703)	*p*
	72.66 ±. 6.85	73.26 ± 6.28	72.71 ± 5.98	0.466
SNP18 rs2943634	AA(124)	CA(410)	CC(428)	*p*
	73.33 ± 5.98	72.66 ± 6.06	72.90 ± 6.11	0.547
SNP19 *SMAD3* rs17228212	CC(73)	TC(363)	TT(535)	*p*
	72.54 ± 5.91	72.94 ± 5.99	72.82 ± 6.16	0.869
SNP20 rs1333049	CC(241)	GC(471)	GG(259)	*p*
	73.64 ± 6.45	72.43 ± 5.85	72.87 ± 6.05	0.0417
SNP21 *MTHRD1L* rs6922269	AA(69)	AG(381)	GG(520)	*p*
	71.74 ± 5.31	72.75 ± 6.08	73.07 ± 6.16	0.216
SNP22 *TCF7L2* rs7901695	CC(92)	CT(409)	TT(470)	*p*
	72.64 ± 6.08	72.72 ± 6.04	73.00 ± 6.11	0.751

3.9 ANALYSIS OF VARIANCE OF 22 SNPS FOR BASELINE MINI MENTAL STATE EXAM (MMSE) SCORE

APOE-adjusted mean value of baseline MMSE score among AD cases and controls for each SNP are given in Table 3.8. Analysis of variance (ANOVA) was used in order to determine if the mean of a variable was significantly different between genotypes. The ANOVA calculation for baseline MMSE score showed a statistically significant difference between genotypes for SNP20 rs133049 (p=0.025). The genotype GG had the lowest baseline MMSE score, the data does not seem to support a dosage effect model.

Table 3.8. Analysis of variance of 22 SNPs for baseline MMSE score, taking into account disease and *APOE* status, gender and AAO

	MMSE according to Genotypes			*p-value*
SNP1 *GALP*	CC(787)	CG(878)	GG(224)	*p*
rs3745833	23.10 ± 6.02	23.13 ± 6.16	23.60 ± 5.88	0.243
SNP2 *PCK1*	AA(1468)	AG(293)	GG(133)	*p*
rs8192708	23.04 ± 6.12	23.57 ± 6.11	23.91 ± 5.42	0.711
SNP3 *TNK1*	AA(387)	AT(913)	TT(592)	*p*
rs1554948	23.77 ± 6.13	22.93 ± 6.08	23.18 ± 5.99	0.552
SNP4 *SERPINA13*	AA(405)	AT(939)	TT(544)	*p*
rs11622883	22.89 ± 6.27	23.19 ± 6.09	23.36 ± 5.92	0.409
SNP5 *PGBD1*	AA(3)	AG(152)	GG(1734)	*p*
rs3800324	25.30 ± 1.59	22.94 ± 6.32	23.19 ± 6.06	0.999
SNP6 *LAMIN AC*	CC(23)	CT(323)	TT(1545)	*p*
rs505058	21.44 ± 6.24	23.44 ± 5.84	23.15 ± 6.12	0.306
SNP7 *UBD*	CC(504)	CG(954)	GG(434)	*p*
rs444013	23.36 ± 6.05	23.04 ± 6.04	23.19 ± 6.21	0.857
SNP8	CC(131)	CG(711)	GG(1048)	*p*
rs1859849	24.65 ± 5.28	22.94 ± 6.21	23.14 ± 6.05	0.233
SNP9 *BCR*	CC(181)	CT(837)	TT(861)	*p*
rs9608099	23.11 ± 6.02	23.02 ± 6.13	23.32 ± 6.05	0.817
SNP10 *MYH13*	CC(743)	CT(890)	TT(257)	*P*
rs2074877	22.81 ± 6.30	23.50 ± 5.91	23.09 ± 5.94	0.159
SNP11 *EBF3*	CC(45)	CT(449)	TT(1398)	*p*
rs11016976	23.35 ± 6.87	23.10 ± 5.80	23.20 ± 6.14	0.323
SNP12 *AGC1*	AA(263)	AC(855)	CC(764)	*p*
rs2882676	23.05 ± 5.72	23.22 ± 6.20	23.15 ± 6.06	0.853
SNP13 *CTSS*	AA(1602)	AG(279)	GG(10)	*p*
	23.16 ± 6.04	23.50 ± 603	17.24 ± 10.02	0.086
SNP14 *FAM63A*	AA(7)	AT(239)	TT(1646)	*P*
	18.27 ± 8.12	23.79 ± 5.87	23.12 ± 6.08	0.228
SNP15 *TRAK2*	CC(208)	CT(844)	TT(836)	*p*
rs13022344	23.58 ± 5.78	23.09 ± 6.25	23.15 ± 5.97	0.421
SNP 16	AA(206)	AG(333)	GG(200)	*p*
rs6907175	20.93 ± 5.36	21.41 ± 5.44	21.14 ± 5.94	0.845
SNP17	CC(18)	CT(187)	TT(565)	*p*
rs501120	21.46 ± 4.79	21.02 ± 5.35	21.13 ± 5.73	0.828
SNP18	AA(93)	CA(314)	CC(358)	*p*
rs2943634	21.16 ± 5.17	20.97 ± 5.39	21.20 ± 5.91	0.796
SNP19 *SMAD3*	CC(59)	TC(298)	TT(414)	*p*
rs17228212	21.83 ± 4.98	21.10 ± 5.44	21.02 ± 5.81	0.807
SNP20	CC(193)	GC(386)	GG(192)	*p*
rs1333049	22.02 ± 5.39	20.92 ± 5.64	20.58 ± 5.69	0.025
SNP21 *MTHRD1L*	AA(70)	AG(303)	GG(397)	*p*
rs6922269	20.59 ± 5.94	21.36 ± 5.62	21.01 ± 5.55	0.135

Table 3.8 continued:

SNP22 *TCF7L2* rs7901695	CC(72)	CT(307)	TT(392)	*p*
	21.33 ± 4.63	21.19 ± 5.76	21.01 ± 5.66	0.772

3.10 ANALYSIS OF VARIANCE OF 22 SNPS FOR CHANGE IN MINI MENTAL STATE EXAM (MMSE) SCORE

APOE-adjusted mean value of change in MMSE score among AD cases and controls for each SNP are given in Table 3.9. Change in MMSE score was calculated as the final – initial MMSE score. The length of time between the final and initial assessment varied between individuals, this was adjusted for in the model. Analysis of variance (ANOVA) was used in order to determine if the mean of a variable was significantly different between genotypes. The ANOVA calculation for change in MMSE score did not reveal any SNPs with statistically significant difference between genotypes.

Table 3.9. Analysis of variance of 22 SNPs for MMSE score change, taking into account disease and *APOE* status, gender and AAO

	MMMSE change according to Genotypes			*p-value*
SNP1 *GALP* rs3745833	CC(313)	CG(338)	GG(92)	*p*
	-4.19 ± 5.17	-4.68 ± 5.29	-4.64 ± 4.78	0.267
SNP2 *PCK1* rs8192708	AA(584)	AG(110)	GG(49)	*p*
	-4.45 ± 5.15	-3.99 ± 4.94	-5.76 ± 5.89	0.122
SNP3 *TNK1* rs1554948	AA(150)	AT(372)	TT(222)	*p*
	-4.63 ± 5.53	-4.26 ± 5.08	-4.70 ± 5.11	0.548
SNP4 *SERPINA13* rs11622883	AA(158)	AT(367)	TT(218)	*p*
	-4.56 ± 4.66	-4.63 ± 5.47	-4.12 ± 5.04	0.788
SNP5 *PGBD1* rs3800324	AA(1)	AG(59)	GG(684)	*p*
	-0.652 ± NA	-4.21 ± 5.17	-4.49 ± 5.18	0.687
SNP6 *LAMIN AC* rs505058	CC(7)	CT(142)	TT(595)	*p*
	-4.01 ± 3.33	-4.48 ± 4.96	-4.47 ± 5.25	0.800
SNP7 *UBD*	CC(183)	CG(389)	GG(169)	*p*

Table 3.9 continued:

rs444013	-4.43 ± 4.75	-4.45 ± 5.21	-4.62 ± 5.59	0.782
SNP8	CC(51)	CG(277)	GG(415)	*p*
rs1859849	-4.57 ± 6.41	-4.22 ± 4.97	-4.63 ± 5.15	0.076
SNP9 *BCR*	CC(72)	CT(331)	TT(337)	*p*
rs9608099	-4.35 ± 5.35	-4.45 ± 4.82	-4.51 ± 5.49	0.837
SNP10 *MYH13*	CC(294)	CT(341)	TT(108)	*P*
rs2074877	-4.44 ± 4.97	-4.76 ± 5.41	-3.69 ± 4.94	0.121
SNP11 *EBF3*	CC(21)	CT(189)	TT(534)	*p*
rs11016976	-3.31 ± 4.44	-4.47 ± 5.22	-4.51 ± 5.19	0.552
SNP12 *AGC1*	AA(105)	AC(332)	CC(304)	*p*
rs2882676	-5.23 ± 5.78	-4.27 ± 5.09	-4.42 ± 5.06	0.457
SNP13 *CTSS*	AA(635)	AG(106)	GG(3)	*p*
	-4.51 ± 5.18	-4.12 ± 5.06	-7.28 ± 8.86	0.811
SNP14 *FAM63A*	AA(3)	AT(85)	TT(656)	
	-7.28 ± 8.86	-4.32 ± 5.30	-4.47 ± 5.15	0.748
SNP15 *TRAK2*	CC(83)	CT(330)	TT(331)	*p*
rs13022344	-4.04 ± 5.19	-4.49 ± 5.14	-4.55 ± 5.23	0.819
SNP 16	AA(203)	AG(321)	GG(194)	*p*
rs6907175	-4.63 ± 5.50	-3.96 ± 4.99	-5.02 ± 5.11	0.314
SNP17	CC(17)	CT(180)	TT(5.17)	*p*
rs501120	-4.11 ± 5.24	-4.51 ± 5.22	-4.43 ± 5.17	0.814
SNP18	AA(91)	CA(304)	CC(348)	*p*
rs2943634	-5.21 ± 5.03	-4.27 ± 4.92	-4.40 ± 5.41	0.264
SNP19 SMAD3	CC(59)	TC(287)	TT(403)	*p*
rs17228212	-4.13 ± 5.19	-4.69 ± 5.37	-4.32 ± 5.04	0.442
SNP20	CC(188)	GC(375)	GG(186)	*p*
rs1333049	-3.94 ± 5.03	-4.43 ± 5.06	-5.01 ± 5.52	0.354
SNP21 *MTHRD1L*	AA(67)	AG(295)	GG(386)	*p*
rs6922269	-4.43 ± 5.58	-4.07 ± 4.63	-4.73 ± 5.49	0.209
SNP22 *TCF7L2*	CC(71)	CT(300)	TT(378)	*p*
rs7901695	-4.11 ± 5.89	-4.63 ± 5.29	-4.37 ± 4.94	0.615

3.11 ANALYSIS OF VARIANCE OF 22 SNPS FOR DISEASE DURATION

APOE-adjusted mean value of disease duration among AD cases for each SNP is given in Table 3.10. Analysis of variance (ANOVA) was used in order to determine if the mean of a variable is significantly different between genotypes. The ANOVA calculation for disease duration showed a statically significant difference between genotypes for SNP13 *CTSS*

(p=0.0006) and SNP14 *FAM63A* (p=0.0014). In both SNPs the heterozygotes had a shorter duration of disease. Both of these SNPs had no homozygotes for the minor allele, therefore we are not able to fully investigate a dosage or dominant model. The number of genotypes analyzed is small compared to the whole population, due to the fact that we only have disease duration for those patients that were confirmed with autopsy and we have their date at onset and date at death.

Table 3.10. Analysis of variance of 22 SNPs for disease duration (age at death - age at onset), taking into account *APOE* status, gender and AAO

	Disease duration according to Genotypes			*p-value*
SNP1 GALP	CC(41)	CG(29)	GG(10)	*p*
rs3745833	9.84 ± 3.46	8.88 ± 4.54	8.62 ± 3.05	0.479
SNP2 PCK1	AA(64)	AG(15)	GG(1)	*p*
rs8192708	9.71 ± 3.71	7.88 ± 4.23	7.21 ± NA	0.221
SNP3 TNK1	AA(15)	AT(44)	TT(21)	*p*
rs1554948	9.61 ± 3.13	9.19 ± 3.67	9.46 ± 4.69	0.923
SNP4 SERPINA13	AA(21)	AT(29)	TT(30)	*p*
rs11622883	10.29 ± 4.23	8.69 ± 3.11	9.29 ± 4.15	0.344
SNP5 PGBD1	AA(0)	AG(7)	GG(73)	*p*
rs3800324	NA	6.93 ± 2.50	9.57 ± 3.87	0.084
SNP6 LAMIN AC	CC(0)	CT(11)	TT(69)	*p*
rs505058	NA	8.03 ± 3.21	9.55 ± 3.90	0.234
SNP7 UBD	CC(20)	CG(43)	GG(17)	*p*
rs444013	9.46 ± 2.39	9.30 ± 4.48	9.29 ± 3.61	0.987
SNP8	CC(6)	CG(27)	GG(47)	*p*
rs1859849	7.81 ± 3.44	10.51 ± 4.18	8.86 ± 3.57	0.118
SNP9 BCR	CC(6)	CT(34)	TT(39)	*p*
rs9608099	11.30 ± 6.29	9.81 ± 3.76	8.58 ± 3.40	0.149
SNP10 MYH13	CC(25)	CT(43)	TT(12)	*p*
rs2074877	8.87 ± 4.75	9.36 ± 3.38	12.22 ± 3.35	0.604
SNP11 EBF3	CC(3)	CT(21)	TT(56)	*p*
rs11016976	10.73 ± 8.55	8.71 ± 3.01	9.50 ± 3.86	0.604
SNP12 AGC1	AA(8)	AC(41)	CC(31)	*p*
rs2882676	7.86 ± 3.85	9.47 ± 3.80	9.54 ± 3.91	0.533
SNP13 CTSS	AA(69)	AG(11)	GG(0)	*p*
	9.80 ± 3.81	6.44 ± 2.61	NA	0.006
SNP14 FAM63A	AA(0)	AT(8)	TT(72)	
	NA	5.37 ± 2.03	9.78 ± 3.74	0.0014
SNP15 TRAK2	CC(6)	CT(40)	TT(34)	*p*
rs13022344	11.79 ± 2.17	9.74 ± 4.20	8.42 ± 3.38	0.093

Table 3.10 continued:

SNP16 rs6907175	AA(15)	AG(22)	GG(17)	*p*
	9.58 ± 4.21	10.00 ± 2.43	11.27 ± 4.15	0.395
SNP17 rs501120	CC(0)	CT(19)	TT(38)	*P*
	NA	8.97 ± 4.17	10.96 ± 3.38	0.053
SNP18 rs2943634	AA(11)	CA(21)	CC(25)	*p*
	10.74 ± 4.49	9.94 ± 3.57	10.40 ± 3.67	0.840
SNP19 SMAD3 rs17228212	CC(1)	TC(22)	TT(34)	*p*
	5.27 ± NA	10.76 ± 4.16	10.14 ± 3.45	0.346
SNP20 rs1333049	CC(15)	GC(24)	GG(18)	*p*
	10.39 ± 4.70	10.43 ± 3.64	10.04 ± 3.16	0.943
SNP21 MTHRD1L rs6922269	AA(5)	AG(18)	GG(34)	*p*
	9.75 ± 5.88	11.04 ± 4.21	9.98 ± 3.17	0.600
SNP22 TCF7L2 rs7901695	CC(4)	CT(21)	TT(32)	*p*
	11.00 ± 5.27	11.30 ± 4.05	9.55 ± 3.28	0.227

3.12 POWER CALCULATION AT α 0.05 FOR EXAMINED SNPS

80% power at α 0.05 for 1,000 cases and 1,000 controls to detect a risk or protective allele reflected by odds ratios is given in Table 3.11. As indicated in the table our sample size had sufficient power to detect small differences. For 12 SNPs power was sufficient to detect an OR of 1.2 or lower. For the other 10 SNPs, power was sufficient to detect ORs between 1.22 and 1.42.

Table 3.11. 80% Power Calculation at α 0.05 for 22 SNPs

SNP	> Odds Ratio for risk allele	> Odds Ratio for protective allele
SNP 1 *GALP* rs3745833	1.1553	0.8684
SNP 2 *PCK1* rs8192708	1.2608	0.8087
SNP 3 *TNK1* rs1554948	1.1452	0.8721
SNP 4 *SERPINA13* rs11622883	1.1452	0.8727
SNP 5 *PGBD1* rs3800324	1.4134	0.6614
SNP 6 *LAMIN AC* rs505058	1.3145	0.7325
SNP 7 *UBD* rs444013	1.1453	0.8732
SNP 8 rs1859849	1.1729	0.8464
SNP 9 *BCR* rs9608099	1.1634	0.8547

Table 3.11 continued:

SNP 10 *MYH13* rs2074877	1.1544	0.8689
SNP 11 *EBF3* rs11016976	1.2178	0.8083
SNP 12 *AGC1* rs2882676	1.1517	0.8654
SNP 13 *CTSS*	1.2769	0.8007
SNP 14 *FAM63A*	1.2583	0.7758
SNP 15 *TRAK2* rs13022344	1.1562	0.8613
SNP 16 rs6907175	1.1475	0.8696
SNP 17 rs501120	1.4198	0.6680
SNP 18 rs2943634	1.2967	0.7625
SNP 19 *SMAD3* rs17228212	1.3059	0.7541
SNP 20 rs1333049	1.2851	0.7767
SNP 21 *MTHRD1L* rs6922269	1.3235	0.7398
SNP 22 *TCF7L2* rs7901695	1.3181	0.7440

We used the risk alleles identified in the paper and coded our population in order to determine how many risk alleles they carried total (assuming a dosage model) and how many loci contained a risk allele (assuming a dominant model). We carried out a linear regression for each model adjusting for age, sex and *APOE* status. The analysis testing a dosage model was not statistically significant with a p value of 0.80420. The analysis testing a dominant model was not statistically significant with a p value of 0.63398.

4.0 DISCUSSION

Alzheimer's disease is the most common form of dementia in the elderly, and has a significant effect on public health in the United States. Although AD has been described for more than 100 years, no effective treatment has been described. Late onset AD is a complex disorder in which gene-gene as well as gene-environment interactions are involved in the etiology of the disease. Although the exact etiology of AD has not been identified first-degree relatives of individuals with AD have been found to have an increased risk of developing the disease. A risk of 15-19% was seen in first-degree relatives of probands with AD as compared to a risk of 5% in the general population (a 3-4 fold increase in risk) (Liddell et al., 2001). Despite the evidence of substantial genetic effect on LOAD, to date the only known genetic risk factor is *APOE*.

In a genome wide association study examining 17,343 putative functional SNPs located in 11221 unique genes, 19 SNPs were found to have a statistically significant association with AD (Grupe et al., 2007). Three of these SNPs included the *APOE* polymorphism and two linked polymorphisms in the *TOMM40* and *APOC2* genes. Our goal was to replicate the 16 new associations in a large case-control sample of white Americans. Additionally we examined six positional and/or biological candidates as risk factors for AD (SNPs 17-22). The secondary goals of the study were to examine the association of 22 SNPs with quantitative traits linked to AD, including AAO, MMSE score and disease duration.

We did not observe a statistically significant association between the SNPs and the risk of AD in our primary analysis. Our data suggest that the association in the 16 previously identified SNPs, if it exists, is not statistically significant in our study population. Furthermore, the additional six SNPs in positional and biological candidate regions for AD also showed no association. Our sample size had sufficient power to detect relatively small effect sizes. We found some significant associations when data were stratified. Considering we performed multiple testing, however, these associations could be due to chance.

Genome wide association studies allow us to detect new genes for a disease not previously implicated based on the known biology of the disease. These studies may shed light on novel genes and SNPs that affect risk for complex diseases. Grupe et al. (2007) examined the whole genome for association of putative functional variants, instead of examining variants that are known biological and/or positional candidates. Many possible associations have been found without the immediate knowledge of their biological link to the disease. This information may help us understand the disease biology in greater detail in the future (Drazen et al., 2007).

There are many difficulties when using whole genome wide association studies to identify candidate genes for complex diseases (Baron, 2001). It may be difficult to differentiate between genes with true association and those that are more common due to ethnic variation. Our study was ethnically homogenous because patients were of Caucasian decent, and from a single geographical location. Similarly, the population from Grupe et al. (2007) was also of Caucasian descent. However, the category "Caucasian" is broad, and individuals may still vary widely in genetic history and therefore significant genetic variation may be present.

Alzheimer's disease has several possible confounding factors, such as how the condition is diagnosed. Clinical diagnoses are made based on physical exam and MMSE scores. There are numerous types of dementia, and AD can only be differentiated from these other conditions with an autopsy and pathological examination of the brain tissue. Although only ~4.5% of our patients had a diagnosis of AD confirmed by autopsy, our Alzheimer's Disease Research Center has found a high correlation (>95%) between clinically diagnosed AD cases and those confirmed at autopsy.

In complex diseases genes do not generally act in a simple additive manner, but through complex networks with gene-gene and gene-environment interactions (Colhoun et al., 2003). According to Calhoun et al. (2003) "if the effect of genotype on disease risk is entirely restricted to people exposed to the environmental factor, and prevalence of this exposure varies twofold between populations, a twofold variation in average effect size of genotype on disease risk would exist". Environmental factors can confound studies that are investigating genetic risk, due to the fact that they are numerous and hard to control for.

It is possible that linkage disequilibrium played a large role in why no associations were found. It is possible that in the original studies the SNPs found to have a significant association were in LD with the causal risk allele. It is also possible that our population did not have this same LD, with the risk allele at the same SNP. This LD could be with another SNP on the same gene or could not exist at all with our specific population (Colhoun et al., 2003).

In 2007 it has been estimated that 5.1 million Americans have AD, 4.9 million of these individuals are age 65 or older (Alzheimer's association, 2007). Advances in medicine have lead to more individuals living into their 80s and 90s. The risk of AD increases with age, therefore the number of persons with the disease is expected to grow as individuals continue to live longer.

In 2005, Medicare spent $91 billion on AD and other dementias, projected to increase to $160 and $189 billion in 2010 and 2015 (Alzheimer's Association, 2007). State and federally funded Medicaid spent $21 billion in 2005 on Alzheimer's and other dementias. This is projected to increase to $24 and $27 billion in 2010 and 2015 respectively (Alzheimer's Association, 2007). Finding genes associated with AD will help us target those at risk. Preventative measures could be taken in order to delay the onset of the disease, and lessen the financial burden on the health care system. Even small reduction in risks for common diseases may have major public health significance (Merikangas et al., 2004).

There are different strategies used to replicate the data from a whole genome wide study. An exact replication strategy attempts to replicate the results from a past study exactly, looking specifically at the SNPs identified as possible risk alleles. This type of strategy is balanced in terms of cost and the ability to replicate a maximum number of loci (Clarke et al., 2007). Our study design was an exact strategy. This design is focused and may have missed any other SNPs in these genes with an association with the risk of AD.

Another possible strategy includes the exact replication of risk SNPs as well as surrounding SNPs in the same gene, this has been called a local replication strategy. This study design is more costly but may have the ability to pick up associations missed by the exact strategy. This design may have the ability identify associations that may be masked under the exact replication strategy. If there are other SNPs on the gene of interest in LD with a casual allele this study may have the ability to find them. However, this procedure can also reveal different loci than those initially identified, making interpretation difficult and usually requiring another validation study (Clarke et al., 2007).

We must study these genes in much greater depth in order to examine all SNPs, with potential biological or positional significance. However, even when all SNPs are studied, confounding factors such as the make up of the population and environment still act to affect the outcome of the disease.

From a genetic counseling standpoint it is important to explore possible patient reactions to genetic information that may be found from similar studies. AD has substantial physical, psychological and social burdens for individuals with AD and their families. Surveys and data from Cupples et al. (2004) found that substantial portions of relatives of those with AD were interested in more fully understanding their risk.

Current recommendations for early onset families with a clear autosomal dominant pattern (or a possible de novo mutation) state that individuals should have the opportunity to test for possible mutations in *APP* (research basis) *PSEN1* and *PSEN2* and should also be counseled appropriately regarding recurrence risks. A positive *PSEN1, PSEN2 or APP* mutation confirms the etiology of an individual's AD, but, a negative result does not rule out other genetic causes. Individuals may choose not to have testing due to the psychological impact it may have, especially because there are currently no treatments for AD (Goldman et al. 2004). One study looked at the psychological effect of testing on nonsymptomatic individuals (Molinuevo et al., 2005). This study found that test results did lead to lifestyle changes. One carrier decided along with their partner not to have any more children, while some individuals found to be negative sometimes decided to have more children. One mutation carrier made some lifestyle changes including spending more time with his family and less time at work. Over the two years of follow up neither carriers nor noncarriers made any employment, marital status or financial changes. All but one positive individual's anxiety levels returned to normal within the two year

follow up period. This one individual was showing symptoms of disease and, in addition, had a general anxiety disorder. Overall it was found that when testing is made available and optional it has the ability to benefit nonsymptomatic at risk individuals.

Recommendations for late onset AD are not as clear. Various groups have agreed that there is no role for *APOE* genotyping in prediction or risk assessment in relatives of those with LOAD (Liddell et al., 2001). This is due to the fact that the *APOE*4* allele is a risk factor and not a predictor of disease, it is questionable whether this information would be beneficial to patients. If an individual is found to carry the risk allele there is greater than a 50% chance that they will not develop the disease (Liddell et al., 2001). Individuals have the ability to modify possible environmental risk factors of the disease such as head trauma, tobacco and alcohol usage, vascular disease and cholesterol levels, however, the only known risk factor to AD is age. There is also no current treatment for AD, therefore it could be argued that genetic testing could do more harm than good.

A test for APOE will be offered in the near future by Smart Genetics in Philadelphia (Couzin, 2008). Previously, *APOE* testing has only been clinically available to those with dementia. The REVEAL study evaluated behavioral and psychological impact of *APOE* disclosure to first degree relatives of those with AD. The study split participants into two groups; one group received risk analysis based solely on their family history, the other group received risk analysis based on their family history and *APOE* status (Cupples et al., 2004). Researchers hypothesized that individuals who receive a positive *APOE*4* test will make health behavior changes while those that receive a negative test or no *APOE* testing will not (Chao et al., 2008). The study confirmed the hypothesis, showing that individuals who received a positive *APOE*4* genotype result were more likely to make health behavior changes even after they were

explicitly informed that they are not proven to prevent AD. Fewer individuals who received risk information solely based on family history and those that received a negative *APOE*4* genotype result participated in these changes. These results suggest that individuals may be more motivated to make health behavior changes based on genetic testing and family history, as opposed to family history alone, even in the absence of proven risk-reduction strategies. Further research on the genetics of LOAD will allow us to counsel patients of late onset families more accurately about their risk.

Future research may elucidate risk alleles for late onset AD. Even if the risk to an individual is slight these findings may help us more fully understand the biology behind AD, and may lead to more effective treatments in the future.

BIBLIOGRAPHY

Agnati LF, Genedani S, Rasio G, Galantucci M, Saltini S, Filaferro M, Franco R, Mora R, Ferre S, Fuxe K. 2005. Studies on homocysteine plasma levels in Alzheimer's patients. Relevance for neurodegeneration. Journal of Neural Transmission 12:163-169

Alzheimer's Association: Alzheimer's Facts and Figures, 2007
http://www.alz.org/alzheimers_disease_alzheimer_statistics.asp

Alzheimer Disease & Frontotemporal Dementia Mutation Database, Oct. 2007
www.molgen.ua.ac.be/ADMutations

Ashley-Koch AE, Shao Y, Rimmler JB, Gaskell PC, Welsh-Bomer KA, Jackson CE, Scott WK, Haines JL, Pericak-Vance MA. 2005. An autosomal genomic screen for dementia in an extended Amish family. Nueroscience Letters 379:199-204

Auld DS, Kornecook TJ, Bastiannetto S, Quirion R. 2002. Alzheimer's disease and the basal forebrain cholinergic system: relations to β-amyloid peptides, cognition, and treatment strategies. Progress in Neurobiology 68:209-245

Bachman DL,Wolf PA, Linn RT, Knoefel JE, Cobb JL, Belanger AJ, White LR, D'Agostino RB. 1993. Incidence of dementia and probable Alzheimer's disease in a general population: the Framingham study. Neurology 43:515–19

Baron M. 2001. The search for complex disease genes: fault by linkage or fault by association? Molecular Psychiatry 6:143-149

Blacker D, Bertram L, Saunders AJ, Moscarillo TH, Albert MS, Winer H, Perry RT, Colins JS, Harrell LE, Go RCP, Beaty T, Fallin MD, Avramopoulos D, Chase GA, Folstein MF, Mcinnis MG, Bassett SS, Doheny KJ, Pugh EW, Tanzi RE. 2003. Results of a high-resolution genome screen of 437 Alzheimer's disease families. Human Molecular Genetics 12:23-32

Chanock SJ, Manolio T, Boehnke M, Boerwinkel E, Hunter DJ, Thomas G, Hirschhorn JN, Abecais G, Altshuler D, Bailey-Wilson JE, Brooks LD, Cardon LR, Daly M, Donnelly P, Fraumeni JF, Friemer NB, Gerhard DS, Gunter C, Guttmacher AE, Guyer MS, Harris EL, Hoh J, Hoover R, Kong CA, Merikangas KR, Morton CC, Palmer LJ, Phimister EG, Rice JP, Roberts J, Rotimi C, Tucker MA, Vogan KJ, Wacholder S, Wijsman EM, Winn

DM, Collins FS. 2007. Replicating genotype-phenotype associations, Nature 447:655-660

Chao S, Roberts JS, Marteau TM, Silliman R, Cupples AL, Green RC. 2008. Health Behavior Changes After Genetic Risk Assessment for Alzheimer Disease: The REVEAL study. Alzheimers Disease and Associated Disorders 22:94-97

Chen JX, Yan SD. 2007. Amyloid-β-Induced Mitochondrial Dysfunction. Journal of Alzheimer's Disease 12:177-184

Citron M, Teplow DB, Dennis J S. 1995. Generation of Amyloid β Protein from its precursor Is Sequence Specific. Neuron 14:661-670

Clarke GM, Carter KW, Palmer LJ, Morris AP, Cardon LR. 2007. Fine Mapping versus Replication in Whole-Genome Association Studies. The American Journal of Human Genetics 81:995-1005

Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, Zismann VL, Beach TG, Leung D, Bryden L, Halperin RF, Marlowe L, Kaleem M, Walker DG, Ravid R, Heward CB, Rogers J, Papassotiropoulos A, Reiman EM, Hardy J, Stephan DA. 2007. A high-Density Whole-Genome Association Study Reveals that APOE Is the Major Susceptibility Gene for Sporadic Late-Onset Alzheimer's Disease. Journal of clinical Psychiatry 68:613-618

Colhoun H M, McKeigue P M, Smith G D. 2003. Problems of reporting genetic associations with complex outcomes. Lancet 361:865-372

Couzin, Jennifer. 2008. Once Shunned, Test for Alzheimer's Risk Headed to Market. Science 319:1022-1023

Cupples IA, Farrer LA, Sadovnick AD, Relkin N, Whitehouse P, Green RC. 2004. Estimating risk curves for first-degree relatives of patients with Alzheimer's disease: the REVEAL study. Genetics in Medicine 6:192-196

dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP/

Dorszewska J, Florczak J, Rozycka A, Kempisty B, Jarosqewska-Kolecka J, Chojnacka K, Trzeciak WH, Kozubski W. 2007. Oxidative DNA damage and level of thiols as related to polymorphisms of MTHFR, MTR, MTHFD1 in Alzheimer's and Parkinson's disease. Acta Neurobiologiae Experimentalis 67:113-129

Drazen JM, Phimister EG. 2007. Publishing Genomewide Association Studies. The New England Journal of Medicine 357:496

Fassbender K., Simons M., Bergmann C., Stroick M., Lutjohann D., Keller P., Runz H., Kuhl S., Bertsch T., Von Bergmann K., Hennerici M, Beyreuther K, Hartmann T. 2001. Simvastatin strongly reduces levels of Alzheimer's disease beta-amyloid peptides

Abeta42 and Abeta40 in vitro and in vivo. Proceedings of the National Academy of Sciences USA 98:5856-5861

Filley C.M., Rollins Y.D., Anderson C.A., Arciniegas D.B., Howard K.L., Murrell J.R., Boyer P.J., Kleinschmidt-DeMasters B.K., Ghetti B. 2007. The Genetics of Very Early Onset Alzheimer Disease. Cognitive and Behavioral Neurology 20:149-156

Fratiglioni L, Viitanen M, Von Strauss E, Tontodonati V, Herlitz A, Winblad B. 2007. Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen project, Stockholm. Neurology 48:132–8

Goldman JS, Craig HE. 2004. Early-Onset Alzheimer Disease: When Is Genetic Testing Appropriate? Alzheimer Disease and Associated Disorders 18:65-67

Grant SFA, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, BAgger Y, christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K. 2005. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nature Genetics 38:320-323

Grupe A, Abraham R, Li Y, Rowland C, Hollingworth P, Morgan A, Jehu L, Segurado R, Stone D, Schadt E, Karnoub M, Nowotny P, TAcey Kristinak, Catanese J, Sninsky J, Frayne C, Rubinsztein D, Gill M, Lawlor B, Lovestone S, Holmans P, O'Donovan M, Morris JC, Thal L, goate A, Owen MJ, Williams J. 2007. Evidence for novel susceptibility genes for late-onset Alzheimer's disease from a genome-wide association study of putative functional variants. Human Molecular Genetics 16(8):65-73

Guskiewicz KM, Marshall SW, Bailes J, McCrea M, Cantu RC, Randolph C, Jordan BD. 2005. Association Between Recurrent Concussion and Late-Life Cognitive Impariment In Retired Professional Football Players. Neurosurgery 57:719-726

Gussekloo J, Heeren T, Izaks G, Ligthart GJ, Rolljmans HG. 2005. A community based study of the incidence of dementia in subjects aged 85 years and over. Journal of Neurology, Neurosurgery, and Psychiatry 59:507–10

Guo Z, Cupples LA, Kurz A, Auerbach SH, Volicer L, Chui H, GReeen RC, Sadovnick AD, Duara R, DeCarli C, Johnson K, Go RC, Growdon JH, Haines JL, Kukull WA, Farrer LA. 2000. Head injury and the risk of AD in the MIRAGE study. Neurology 54:1316-23

Haines JL, Bailey R, Grubber JM. 2001. A genomic search for Alzheimer's disease genes. In: Alzheimer's disease: Advances in Etiology, Pathogenesis and Therapeutics (eds. K. Iqbal, S. S. Sisodia and B. Winblad), pp. 33-43. Chichster: John Wiley & Sons

Harold D, Peirce T, Moskvina V, Myers A, Jones S, Hollingworth P, Moore P, Lovestone S, Powell J, Foy C, Archer N, Walter S, Edmonson A, McIroy S, Craig D, Passmore PA,

Goate A, Hardy MO, Williams J, Liddell M, Owen MJ, Jones L. 2003. Sequence variation in the CHAT locus shows no association with late-onset Alzheimer's disease. Human Genetics 113:258-267

Hartmann T, Kuchenbecker J, Grimm MO. 2007. Alzheimer's disease: the lipid connection. Journal of Neurochemistry. 103:159-170

Ho PI, Ortiz D, Rogers E, Shea TB. 2002. Multiple aspects of homocysteine neurotoxicity: glutamate exicitotoxicity, kinase hyperactivation and DNA damage. Journal of Neuroscience Research 70:694-702

Holmans P, Hamshere M, Hollingworth P, Rice F, Tunstall N. 2005. Genome Screen for Loci Influencing Age at Onset and Rate of Decline in Late Onset Alzheimer's Disease. American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) 135B:24-32

Ioannidis JP. 2005. Why Most Published Research Findings Are False. PloS Medicine 2:e124

Ioannidis JP. 2007. Limitations are not properly acknowledged in the scientific literature. Journal of clinical Epidemiology 60:324-329

Jordan BD, Relkin NR, RAvdin LD. 1997. Apolipoprotein E epsilon4 associated with chronic traumatic brain injury in boxing. The Journal of The American Medical Association 278:2143

Kalaria RN. 2003. Comparison between Alzheimer's disease and vascular dementia: Implications for treatment. Neurological Research 25:661-664

Kamboh, Ilyas. 2004. Molecular Genetics of Late-Onset Alzheimer's disease. Annals of Human Genetics 68:381-404

Katzman, R. 1993. Education and the prevalence of dementia and Alzheimer's disease. Neurology, 43, 13-20

Kehoe P, Wavrant-DeVrieze F, Crook R. 1999. A full genome scan for late-onset Alzheimer's disease. Human Molecular Genetics 8:237-245

Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen Aulikki. 2001. Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study. British Medical Journal 322:1447-1451

Letenneur L, Gilleron V, Commenges D, Hlemer C, Orgogozo JM, Dartigues JF. 1999. Are sex and educational level independent predictors of dementia and Alzheimer's disease? Incidence data form the PAQID project. Journal of Neurology, Neurosurgery, and Psychiatry 66:177-183

Letenneur L, Larrieu S, Barberger-Gateau Pascale. 2004. Alcohol and tobacco consumption as

risk factors of dementia: a review of epidemiological studies. Biomedicine & Pharmacotherapy 58:95-99

Li Y, Scott WK, Hedges DJ, Zhang F, Gaskell PC, Nance MA, Watts RL, Hubble JP, Koller WC, Pahwa R, Stern MB, Hiner BC, Jankovic J, Allen FH, Goetz CG, Mastaglia F, Stajich JM, Gibson LTM, Saunders AM, Scot BL, Small GW, Nicodemus KK< Reed AD, Schmechel DE, Welsh-Bohmer KA, Conneally PM, Roses AD, Gilbert JR, Vance JM, Hanies JL, ericak-Vance MA. 2002 Age at onset in two common neurodegenerative diseases is genetically controlled. American Journal of Human Genetics 70, 985-993

Liddell MB, Lovestone S, Owen MJ. 2001. Genetic risk of Alzheimer's disease: advising relatives. British Journal of Psychiatry 178:7-11

Maccioni R B, Munoz J P, Barbeito L. 2001. The Molecular Bases of Alzheimer's Disease and Other Neurodegenerative Disorders. Archives of Medical Research 32:367-381

Mejia S, Giraldo M, Pineda D, Ardila A, Lopera F. 2003. Nongenetic Factors as Modifiers of the Age of Onset of Familial Alzheimer's Disease. International Psychogeriatrics 15:337-349.

Meyers A, Wavrant De-Vrieze F, Holmans P, Hamshere M, Crook R, Compton D, Marshall H, Meyer D, Shears S, Booth J, Ramic D, Knowles H, Morris JC, Williams N, Norton N, Abraham R, Kehoe P, Williams H, Rudrasingham V, Rice F, Giles P, Tunstall N, Jones L, Lovestone S, Willimas J, Owen MJ, Hardy J, Goate A. 2002. Full genome screen for Alzheimer disease: stage II analysis. American Journal of Medical Genetics (Neuropsychiatr Genet) 114:235-244

Merikangas K R, Risch N. 2003. Genomic Priorities and Public Health. Science 302:599-601

Molinuevo JL, Pintor L, Peri JM, Lleo A, Oliva R, Marcos T, Blesa R. 2005. Emotional reactions to predictive testing in Alzheimer's disease and other inherited dementias. American Journal of Alzheimers Disease and Other Dementias 20:233-238

Moritz, D. J., & Petitti, D. B. 1993. Association of education with reported age of onset and severity of Alzheimer's disease at presentation: Implications for the use of clinical samples. American Journal of Epidemiology 15:456-462

Online Mendelian Inheritance of Man (OMIM), 2007 http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=104311

Panza F., D'Introno A., Colacicco A.M., Basile A.M., Capurso C., Kehoe P.G., Capurso A., Solfrizzi V. 2004. Vascular risk and genetics of sporadic late-onset Alzheimer's disease. Journal of Neural transmission 111:69-89

Pericak-Vance MA, Bass MP, Yamaoka LH, Gaskell PC, Scott WK, Terwedow HA, Menold MM, conneally PM, Small GW, Vance JM, Suanders AM, Roses AD, Haines JL. 1997. Complete genomic screen in late-onset familial Alzheimer disease. Evidence for a new

locus on chromosome 12. The Journal of the American Medical Association 278:1237-1241

Pericak-Vance MA, Grubber J, Bailey LR. 2000. Identification of novel genes in late-onset Alzheimer's disease. Experimental Gerontology 35:1343-1352

Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Dixon RJ, Meitinger T, Braund P, Wichmann HE, Barrett JH, Konig IR, Stevens SE, Szymczak S, Tregouet DA, Iles MM, Pahlke F, Pollard H, Lieb W, Cambien F, Fischer M, Ouwehand W, Path FRC, Blankenberg S, Balmforth AJ, Baessler A, Ball SG, Strom TM, Braenne I, Geiger C, Deloukas P, Tobin MD, Ziegler A, Thompson JF, Schunkert H. 2007. Genomewide Association Analysis of Coronary Artery Disease. The New England Journal of Medicine 357:443-53.

Scott WK, Hauser ER, Schmechel DE, Welsh-Bohmer AW. 2003. Ordered-Subsets Linkage Analysis Detects Novel Alzheimer Disease Loci on Chromosomes 2q34 and 15q22. American Journal of Human Genetics 73:1041-1051

Sima A F, Li Z. 2006. Diabetes and Alzheimer's Disease-Is There a Connection? The Review of Diabetic Studies 3:161-168

Nussbaum RL, McInnes RR, Willard HF. 2004. Thompson & Thompson Genetics In Medicine: sixth edition. Elsevier Science

Ueberham U, Ueberham E, Gruschka H, Arendt T. 2006. Altered subcellular location of phosphorylated Smads in Alzheimer's disease. European Journal of neuroscience 24:2327-2334

Welch HG, Walsh JS, Larson EB. 1992. The cost of institutional care in Alzheimer's disease: nursing home and hospital use in a prospective cohort. Journal of American Geriatric Society 40:221-4