INVESTIGATION OF THE RELATIONSHIP OF CANDIDATE GENES ON CHROMOSOME 10 WITH THE RISK AND AGE-AT-ONSET OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), a common debilitating neurodegenerative disease, is a major public health problem in the United States because it affects almost 50% of the people after age 85. Although AD has been described since 1907, no cure or universally accepted effective treatment has been devised. Studying factors that play a role in risk and pathogenesis of LOAD may lead to development of new therapies and give an insight to the etiology of disease. Both genetic and environmental factors have been implicated in the etiology of AD. Twin studies have shown heredity to be a major causal factor in the development of LOAD. Despite the evidence for substantial genetic effect in the etiology of LOAD, the *APOE*4* allele in the APOE gene is the only established risk factor in the development of LOAD. However, as the *APOE*4* allele is neither necessary nor sufficient for the development of AD, this emphasizes the involvement of other genetic and/or environmental factors, which alone or in combination with APOE*4 can modify the risk of AD. Recently, linkage studies in multiplex families with LOAD have provided evidence for the existence of additional putative genes for AD on several chromosomes, including chromosome 10. A broad linkage peak encompassing >50 cM region between chromosome 10q21 and 10q25 has been implicated that influence both AD risk and age-at-onset (AAO). There are more than 300 genes in this broad genomic region of chromosome 10 and thus task for identifying the chromosome 10 gene is daunting. One approach is to focus on

the known candidate genes in the region. There are number of promising candidate genes in this region that are involved in either in the production, processing or clearance of Aβ peptide and include choline acetyltransferase (CHAT), plasminogen activator, urinary (PLAU), insulin-degrading enzyme (IDE), hematopoietically expressed homeobox (HHEX), glutathione S-transferase, omega-1 (GSTO1), glutathione S-transferase, omega-2 (GSTO2), and protease, serine, 11 (PRSS11). In this study, we have examined the role of these genes under the linkage peak on chromosome 10 to assess their role with AD risk and AAO. Association studies for 14 markers were performed in a large case-control cohort comprising 1012 white LOAD subjects and 771 white control subjects. No significant associations were observed with any of the polymorphism examined in the IDE, HHEX, GSTO1, GSTO2 and PRSS11 genes. Of the 3 CHAT SNPs examined, we detected both allelic and genotypic association of the intron 9 polymorphism with AD risk. The rare AA genotype appears to confer a modest risk for the development of AD in a recessive fashion (OR: 2.37; p=0.007). In addition, we observed APOE-dependent effect of the CHAT exon 5 polymorphism with AD risk (OR=0.76; p=0.046). Although these associations are modest, they suggest the presence of putative functional variants in the CHAT gene or nearby genes. In the PLAU gene, we examined 3 tagSNPs and found a modest protective effect with one SNP in the 3' UTR (OR=0.71; p=0.02), which was confined to APOE*4 carriers (OR=0.58; p=0.02). In our analysis of the association of the candidate genes with AAO, suggestive association were observed only with the PLAU 3' UTR (p=0.10) and intron 9 (p=0.04) polymorphisms.

In summary, our data on a large number of AD cases and controls suggest that genetic variation in two positional candidate genes on chromosome 10 (PLAU and CHAT) may affect the risk and AAO of LOAD.

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Finally, I dedicate this dissertation to my parents Mehmet and Fatma Ozturk, who have encouraged me throughout all my life, instructing me in overcoming numerous obstacles and impediments. I cannot thank them enough for their unconditional love, and support.

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1. Background and Significance

Alzheimer's disease (AD), the most common form of dementia among the elderly, is a progressive, degenerative disorder of the brain with a loss of memory and cognition. A defining characteristic of AD is the deposition of amyloid fibrils and neurofibrillary tangles in the brain of afflicted individuals; biochemically, they are mainly composed of β-amyloid protein (Aβ) and phosphorylated tau proteins, respectively (Burns et al.1970; Selkoe, 2000). The precise biochemical pathways leading to AD are presently unknown. Alzheimer's disease is an age-dependent disorder. The prevalence of AD increases exponentially with age estimates showing that it increases from 10% at age 65 years to nearly 50% after age 85 years. A person with AD lives an average of 8 years, but some patients live 20 years or more following the onset of symptoms.

1.1. Signs, Symptoms and Cause

Alzheimer's disease is a progressive, neurodegenerative disease of the brain. Alzheimer disease is characterized by more than just memory loss; it also results in other cognitive and behavioral symptoms that progressively impair function in activities of daily living. The cognitive symptoms include memory loss, disorientation, confusion, problems with reasoning and thinking. Behavioral symptoms include agitation, anxiety, delusions, depression, hallucinations, insomnia, and wandering.

Alzheimer disease is a genetically complex and heterogeneous disorder, as both genetic and environmental factors play a role in development of AD. Mutations in three genes are involved in early onset AD: β-amyloid protein precursor (APP) gene on chromosome 21 (Goate et al.1991), the presenilin 1 (PS1) gene on chromosome 14 (Sherrington et al 1995), and presenilin 2 (PS2) gene on chromosome 1 (Levy-Lahad et al. 1995; Rogaeva et al. 1995). However, these mutations account for only a small percentage of all AD patients young and old. Genetic variation in the APOE gene has been identified to be a strong risk factorfor LOAD that explains nearly 20-30 % of risk (Slooter et al. 1998, Seshadri et al.1995). However, as APOE genetic variation is neither necessary nor required for AD, additional genetic and /or environmental factors are thought to be involved in the etiology of LOAD. Many risk factors are under investigation for association with AD. For example, certain genes make some families vulnerable, head injuries may increase risk, and high blood pressure is a new suspect (Kalaria, 2001; Mark, 2001). The established risk for AD is age: cases double with every 5 years as patients age between 65 and 85.

1.2. Environmental factors

No specific environmental toxin has been found to be associated with AD. While depression, smoking, traumatic head injury and cardiovascular-related disorders such as hypertension, myocardial infarct, hypercholesterolemia and stroke have been associated with AD, it remains unclear whether these are true antecedents or simply comorbidities (Mayeux et al. 2003). Variation in socioeconomic factors such as literacy, educational achievement and the type of early home environment have been associated with AD (Stern et al. 1994; Friedland et al. 2001), but the mechanism by which these factors are related to disease remain uncertain. The use of

estrogen, anti-inflammatory drugs, the consumption of wine and devoting time to complex physical and mental activities have been related to decreased risk (Tang et al. 1996; Yaffe et al. 2000, Orgogozo et al.1997; Laurin et al. 2001; In't Veld et al. 2001).

1.3. Apolipoprotein E

Apolipopretein E (APOE), a 34 kDa molecular weight protein, is product of a single gene on chromosome 19. APOE is a plasma lipoprotein that is one of the principal constituents of chylomicrons and very low density lipoproteins (VLDL), playing a vital role in cholesterol transport. Its primary site of biosynthesis is the liver, but the second major site of synthesis is the brain. In the central nervous system, APOE is produced by astrocytes and microglia and has an essential role in transporting lipids from astrocytes to injured neurons for repair (Poirier et al. 1994). APOE is a protein with roles in lipid metabolism and tissue repair. Like APP, the synthesis of APOE is up-regulated after the nervous system has been damaged (Horsburgh et al. 1996).

The APOE gene is polymorphic with the existing of three alleles that code for E2, E3, E4 isoforms that differ from each other by one amino acid only: while E3 has cysteine at position 112 and arginine at position 158, E2 has Cys at both position and E4 has Arg at both positions. APOE is associated with both risk and AAO of AD (Meyer et al. 1998). Family studies led to the identification of APOE as a "susceptibility" gene because possession of a single *APOE*4* allele, present in approximately 25% of the Caucasian population, is associated with a two- to three-fold increase in disease risk, while having two copies is associated with a five-fold rise. Unlike the early onset mutations, the *APOE*4* allele is not fully penetrant, and the population attributable risk has been estimated at 20%-30% (Slooter et al.1998; Seshadri et al. 1995). More

significantly, there is dose-dependent relationship between the number of *APOE*4* alleles and the AAO of AD such that E4/E4 subjects have an earlier onset than heterozygous E4 subjects (Corder et al. 1993, Meyer et al.1998). Subjects with an *APOE*2* allele, on the other hand, have a later onset (Corder et al. 1994) However, this association is weaker with advanced age at onset, and the putative protective role of the *APOE*2* allele is not clear.

In addition to the *APOE E2/E3/E4* coding sequence polymorphism, several polymorphisms have been discovered in the 5'-promoter of the APOE gene, (Lambert et al. 1998; Town et al.1998) as well as a missense mutation at codon 28 (Kamboh et al. 1999). The promoter polymorphisms are believed to affect the risk of AD independently of *APOE*4*, due to altering the transcriptional activity of APOE. However, several other studies have been unable to replicate these findings (Song et al. 1998; Schellenberg et al. 1992). One of the reasons for this inconsistency seems to be due to use of relatively small sample sizes in published studies. This becomes an important issue for those markers that have small effects on the disease risk. It appears that the effect of the APOE promoter polymorphism is small relative to established impact of APOE the E2/E3/E4 polymorphism.

2. Alzheimer's Disease Gene in Chromosome 10

Genome-wide linkage studies on AD show that this is a genetically complex disorder involving several genes in addition to APOE. Linkage studies have identified several promising chromosomal regions that may harbor additional AD genes, including chromosome 10 (figure 2.1).

Initially, linkage to chromosome 10 was reported by Kehoe et al. (1999) in a genome-wide screen of LOAD sibling pairs. On follow-up, linkage to the same region on chromosome 10 with a maximal lod score of 3.83 at 81 cM close to D10S1225 was confirmed (Myers et al. 2000). Linkage to the same region was replicated in a genome-wide screen of LOAD sibling pairs by the same group (Myers et al 2002). They performed a two-stage screen, first genotyping markers at 20 cM intervals in 292 affected sibling pairs using 237 markers, then examining regions with a LOD score ≥ 1 in an additional 91 markers in the 16 regions.

There is evidence that a quantitative trait locus for high amyloid β42 (Aβ42) levels maps to the same chromosomal region (Ertekin-Taner et al. 2000). It has been shown that the processing of APP is a critical step in the pathogenesis of AD. Most mutations in the known genes involved in autosomal dominant inherited forms of AD (*APP*, *PS1*, and *PS2*) cause an increase in Aβ42 levels in patients (Scheuner et al. 1996) by affecting the processing of APP. Ertekin-Taner et al. (2000) collected families with increased plasma Aβ42 levels and screened them for quantitative trait loci (QTL) controlling Aβ levels. They hypothesized that risk loci for high plasma Aβ would also be risk loci for AD and thus focused on the regions Kehoe et al. (1999) had found in their first study. Indeed, linkage to Aβ42 levels was observed near D10S1225 on chromosome 10 with a maximal lod score of 3.93 at 81 cM. These results provide strong evidence for a novel LOAD locus on chromosome 10 that acts to increase Aβ.

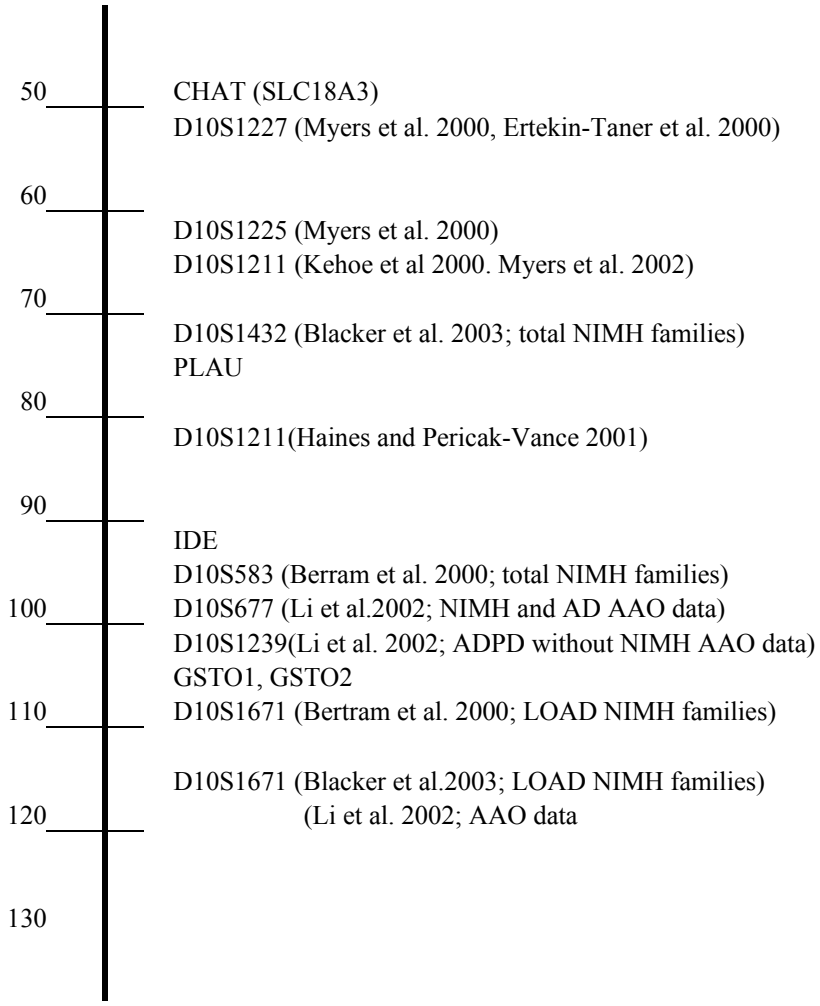

Figure 2.1 Map position of reported linkage results for the risk and AAO genes for AD on chromosome 10q

On the other hand, Bertram et al. (2003) identified a putative susceptibility locus about 30 cM distal to D10S1225 at 94 cM near D10S583. Bertram et al. (2000) genotyped six markers surrounding the insulin degrading enzyme (IDE) gene, since IDE is thought to be a good candidate gene for LOAD due to its putative role in the degradation of Aβ (Vekrellis et al. 2000). They also genotyped marker D10S1225, a marker within same linkage peak implicated by others (Kehoe et al. 1999; Myers et al. 2000; Ertekin-Taner et al. 2000). They found a maximum Z-score for the likelihood ratio of 2.1 at D10S1710 in the subset of their sample, which had AAO ≥ 65. This marker is located 9 cM away from IDE and 42 cM away reported by Myers et al (2000).

Using different set of markers in the same sample, the same group observed MLS of 0.9 at 115.8 cM, which lies about 9 cM distal to their early reported peak (Blacker et al. 2003). In order to gain a better understanding of the broad signal on chromosome 10, additional analyses of the chromosome 10 data were performed in GENEFINDER, which estimates the location and a 95% confidence interval for an unobserved trait locus using multipoint marker information to approximate identity by descent (IBD) sharing in affected sib pairs, yielded strong signals with overlapping confidence intervals across a very broad region from 62 to 131 cM in the late-onset stratum (with *Z*-scores ranging from 2.5 (p=0.006) to 3.1 (p=0.001)), while the early/mixed stratum had a weaker but somewhat narrower signal from 13 to 37 cM (*Z*-score 1.6 (p=0.054)). Thus, it is still unclear whether there are one or two underlying genes located on the long arm of chromosome 10.

Recently, Li and colleagues (2002) have analyzed their data using AAO as a quantitative trait, and reported a peak around 125 cM, which is closer to the linkage region identified by

Bertram et al. (2000) (between 115 and 127 cM), but different from other locations (Bertram et al. 2000; Ertekin-Taner et al. 2000; Myers et al. 2000). Li et al.(2002) propose three possible explanations for the previously reported inconsistent localizations of risk genes on chromosome 10; i) the same gene may affect both risk of AD and onset in a subset of families, ii) different genes located within this region may affect risk and onset, or iii) It is the effect of AAO rather than risk detected by statistical methods.

2.1. Candidate Genes on Chromosome 10

There are several candidate genes under the linkage peaks on chromosome 10. Glutathione S-transferases (GSTs) are a superfamily of enzymes with broad range of substrates and catalytic activities. Utilization of glutathione in reactions contributes to the transformation of a wide range of exogenous and endogenous compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. (OMIM 60548) GSTO1 and GSTO2 are located next to each other on chromosome 10q24.3 and composed of six exons (Fig.2.2).

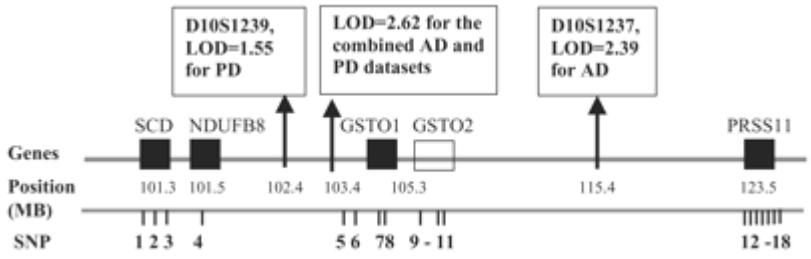

Figure 2.2 The locations of candidate genes used by Li et al. (2003) to examine their association with AD. The solid black boxes indicate the genes significantly down regulated in the microarray study. The short vertical lines represent the SNPs genotyped in the AD dataset.

GSTO2 has 64% amino acid identity with GSTO1. GSTO1 is expressed in a wide range of tissues, specifically neural glial cells (Board et al. 2000). Li et al. (2003) have reported the association of GSTO1/ Ala 104 Asp polymorphism as well as GSTO2/C → T polymorphism with AAO for both AD and PD, but not the risk of disease. Although the functions of GSTO1 and GSTO2 are not well understood, study by Laliberte et al. (2003) suggests that they may be involved in the activation of IL-1ß.

Serine protease 11 (PRSS11), is a 480-amino acid protein. The gene is encoded by 10 exons and 9 intervening introns. It is located on chromosome 10q25.3-q26.2 (Zumbrunn and Trueb, 1997), which is about 18 Kb downstream of the GSTO1/GSTO2 (fig2.2). Li et al. (2003) demonstrated a significant association of a PRSS11/T→C with AAO of AD.

Insulin-degrading enzyme (IDE) plays a role in degradation of number of peptides, including insulin and beta-amyloid. Since deposition of Aβ is one of the hallmarks in the pathology of AD, the role of IDE in the clearance of Aβ makes it a good candidate gene for AD. Animal studies supported the viability of this putative mode of action, in that IDE deficiency has been shown to promote significant net increases in Aβ deposition in mouse brain (Farris et al. 2003; Miller et al.2003). IDE is located on chromosome 10q23–q24, a region in which a susceptibility locus for AD has been mapped. Two linkage studies reported distinct linkage peaks close to the IDE region (Bertram et al. 2000; Li et al. 2002). Despite the linkage data, several case control studies (Abraham et al. 2001; Boussaha et al. 2002; Edland et al. 2003; Sakai et al. 2004) did not provide any convincing evidence of association for IDE and AD. However, in a comprehensive study by Prince et al. (2003), significant associations with the severity of disease, as measured by quantitative traits such as Mini-Mental Examination Scores

(MMSE), protein tau levels in cerebral spinal fluid, the AAO, and degree of brain pathology was found. Prince et al. (2003) concluded that the effect of IDE might be in the severity of the disease rather than on disease risk. A recent study by Ertekin-Taner et al. (2004) replicated the results of Prince et al. (2003) in case-control series and also found significant association of IDE haplotypes with plasma Aβ42. The only possible association with AD risk reported by Edland et al. (2003) among non-*APOE*4* carriers had too small a sample size for meaningful interpretation. Although IDE is an excellent candidate functionally and positionally as a gene involved in AD, association of IDE with AD risk is yet to be established.

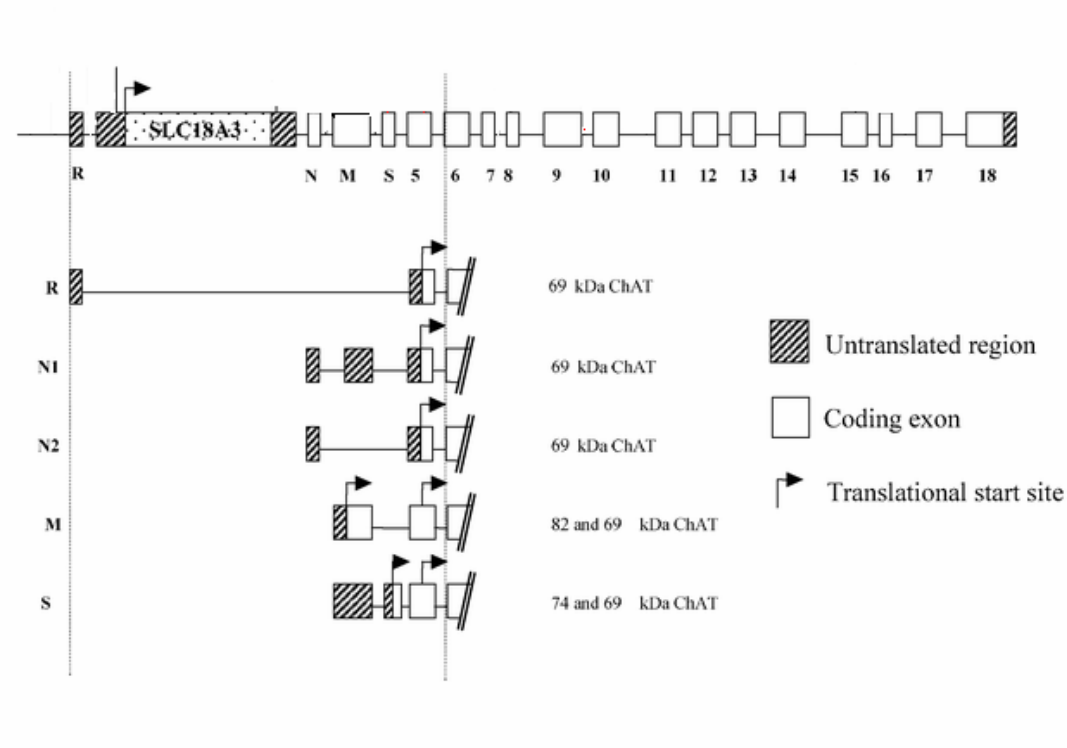

Figure 2.3 Genomic structure of the CHAT gene (Harold et al. 2003)

The gene for choline acetyltransferase (CHAT), an enzyme responsible for acetylcholine biosyntheses in cholinergic neurons, is located under the linkage peak on chromosome 10 (Bertram et al. 2000). In humans, CHAT may be produced in 82- and 69-kDa forms and encodes

5 splice variants: M, N1, N2, R, and S. While human M and S type mRNAs have the capability of generating both large and small form of CHAT proteins, R and N-type CHAT mRNAs generate only small form of CHAT protein. The first intron of CHAT harbors SLC18A3 or vesicular acetylcholine transporter (VACHT) proteins (Fig 2.3). A single regulatory locus controls the transcription of both genes and they are referred to as the cholinergic gene locus.

CHAT is essential for cholinergic neurotransmission (Nachmansohn et al. 1943). One of the characteristic features of AD is the selective degeneration of cholinergic neurons among different populations of nerve cells. Reduced CHAT activity has been shown to cause damage to cholinergic neurons in neurodegenerative diseases such as AD (Perry et al.1978; Whitehouse et al 1982; Oda 1999). Additionally, the concentration of CHAT is markedly reduced in AD brains compared to controls. (Oda 1999; Bartus et al. 1982). It has been shown that degree of reduction in the cerebral CHAT activity and acetylcholine (ACh) synthesis is strongly correlated with the severity of dementia (Wilcock et al. 1982), suggesting that cellular loss and dysfunction of cholinergic neurons result in the development of dementia in AD. Furhermore, Lin et al (1999) have demonstrated that cognitive deficits induced by cholinergic lesions correlate with APP protein levels in the cerebral cortex and that cholinergic muscarinic receptors participate in the regulation of APP metabolism. Additionally, Aβ has been shown to decrease both choline uptake and ACh release in rat biopsy (Kar et al. 1998). These findings suggest that cholinergic neurotransmission may be a specific target for Aβ. These data support the role of CHAT in the development of AD. An original study reported by Mubumbila et al. (2002) found a significant association of a CHAT missense mutation with AD, but subsequent studies failed to confirm this association (Schwartz et al. 2003; Harold et al. 2003).

Plasminogen Activator, Urinary (PLAU) converts plasminogen to plasmin. Plasmin is involved in the processing of amyloid precursor protein, one of the hallmarks of AD (Finckh et al. 2003). PLAU is located on chromosome 10q22.2 between two regions showing linkage to LOAD. Studies by Finckh et al. (2003) demonstrated that a Pro141Leu polymorphism in the PLAU gene was associated with the risk of AD. However, another group examined this polymorphism along with 7 others spanning the entire gene and found no association with any of the polymorphism examined (Myers et al. 2004).

The complex set of genetic and environmental factors that contribute to common diseases like LOAD produce heterogeneity that makes it difficult to replicate genetic association studies, and thus additional functional and genetic association studies are needed to confirm or to refute the original associations. In this study, we propose to assess the reported associations in biological and positional candidate gene in a large and independent case control cohort from the University of Pittsburgh ADRC.

3. Specific Aims

The objective of this study was to examine the association of multiple polymorphisms in 7 positional and biological candidate genes on chromosome 10 with the risk and AAO of AD.

Hypothesis1: Chromosome 10 genes affect the risk of AD independent of the APOE E2/E3/E4 polymorphism.

Hypothesis2: Chromosome 10 genes affect AAO of AD independent of the APOE E2/E3/E4 polymorphism.

Hypothesis3: Chromosome 10 harbors independent liability genes for AD risk and AAO and their effects are independent of the APOE E2/E3/E4 polymorphism.

Hypothesis4: The effects of the chromosome 10 genes on AD risk and AAO are dependent on the APOE E2/E3/E4 polymorphism.

4. Research Design and Methods

This project is a case-control study designed to test the association of genetic variations within candidate genes with the risk and AAO of AD. The subjects in this study are all Caucasian Americans (Table 4.1). All patients are sporadic, late onset (≥60 years) AD cases from the University of Pittsburgh Alzheimer Disease Research Center (ADRC). The controls are recruited from three different sources: 1) University of Pittsburgh ADRC, 2) Monongahela Valley Independent Elders Survey (MoVIES) and 3) White Matter Hyperintensity Study.

4.1. Subjects

4.1.1. Patients

A total of 1012 white patients (63.21% female) from the University of Pittsburgh ADRC was used for this study (Table 4.1). The mean age is 76.57 ± 5.7 years with a mean age at onset of 72.29 ± 6.33 years. Among the patients, 280 (25.2%) are autopsy confirmed AD cases. Clinical diagnoses of the patients were made according to the NINCDS/ADRDA criteria (McKhann et al. 1984). The ADRC follows a standard evaluation protocol, which includes medical history, general medical and neurological examinations, a psychiatric interview, neuropsychological testing and a MRI scan.

4.1.2. Controls

A total of 771 controls was used from three resources at the University of Pittsburgh.

There are 159 (57.2% female) white controls from the ADRC cohort with a mean age of 73.36 ± 3.71 years. Control subjects are considered normal after undergoing clinical and neuropathological examinations as followed by the ADRC evaluation protocol.

Four hundred twenty six subjects (66.4% female) with a mean age of 77.46 ± 4.18 years that have a MMSE score above 25 on test number 5 were obtained from the Monongahela Valley Independent Elders Survey (MoVIES) cohort. MoVIES is a prospective community study of cognitive impairment and dementia in the rural mid-Monongahela Valley region of southwestern Pennsylvania (Ganguli et al. 1991). Subjects have not undergone the standard ADRC clinical evaluation, but have had clinical and neuropsychological assessment and are known to be high functioning and non-demented. There are 120 (50% female) subjects available from the White Matter Hyperintensity Study (WHM) study with a mean age of 73.7 ± 3.3 years. Although the subjects did not undergo ADRC clinical diagnostic protocol, they have been assessed as non-demented using standard cognitive tests. WMH is a study of normal aging served by University of Pittsburgh Western Psychiatric Institute and Clinic.

To examine the comparability between the cases and controls we performed the comparison between the two groups in terms of age, sex, education, MMSE score, and family history (Table 4.1). The cases and controls found to be similar for age education and family history of dementia. As expected, the cases and controls are significantly different from each other in their MMSE scores (Table 4.1).

Table 4.1 Composition of the Case-Control Cohort

	Control (771)	Cases (1012)	p value
Age±SD(year)	76.10±4.98	77.26±6.08	0.22
Female	63.21%	66.9 %	0.35
Education±SD(year)	13.07±3.04	12.04±3.10	0.15
Familly History of Dementia	38.29%	40.96%	0.51
MMSE score±SD	28.04±1.51	17.01±6.20	

4.2. DNA Isolation and PCR

Genomic DNA has been isolated from buffy coat or brain biopsy tissue using the QIAamp kit (QIAGEN). Briefly, 1 μg of genomic DNA is amplified in a 50 μl reaction mixture consisting of 5 μl of 10 X PCR buffer (100 mM Tris-HCl, pH= 8.3, 500 mM KCl), 1.0-2.5 mM $MgCl_2$, 1.25mM of each dNTP (Pharmacia), 0.2 μM of each primer, and 1.25 units of *Taq* DNA polymerase (Display Biosystems) or 9:1 mixture of Amplitaq Gold (Perkin Elmer) and Pfu polymerase (Strategene). Genotyping was performed using standard polymerase chain reaction (PCR) procedures. After initial denaturation of 95 °C, the reaction mixture is subjected to 45 cycles of denaturation of 30 sec at 95 °C, annealing for 30 sec at a particular temperature, and a extension for 30 sec at 72 °C. This was followed by a final extension step for 10 minutes at 72 °C (table 4.2). The correct size and purity of amplified product is verified by running 5 μl of PCR product on a 2 % agarose gel.

The polymorphisms were screened by using restriction enzyme analysis or pyrosequencing methods. Restriction endonucleases were chosen (Mac DNAsis Program)

whenever the genetic variant creates or abolishes a restriction site. Pyrosequencing is method for performing genotyping through sequencing by synthesis and capable of analysis of genetic diversities such as bi-, tri- and tetra-allelic polymorphisms, multiple SNPs, mutation, and insertion/deletions. PCR products are converted to single stranded templates onto which a sequencing primer is annealed in 96 well plates. Analysis begins with dispensation of the substrate-enzyme reagents into the wells containing test samples. Light is produced when a nucleotide forms a base pair with its compliment. The light and base are registered by a charge coupled device (CCD) camera, and is seen as a peak. If the next nucleotide added to the template is not complimentary to the nucleotide base, no light is detected on the CCD. The height of the peaks is proportional to the number of bases that have been integrated. Software used with the PSQ-96MA automatically analyzes the quantitative data. The data can be evaluated by the user on computer as well. The software allows for multiplex genotyping of up to three polymorphisms in a single well.

Table 4.2 PCR condition for markers on chromosome 10

SNP ID	SNP	Fragment (bp)	Conditions	Primer sequence 5'->3'	Additional information
CHAT-rs868750 CHAT-rs868750 CHAT-rs868750	G>A	156	56 °C	R:GTGGCCATGCGTTCACGT F:CGGCTCTCATTCTTAGAAGGCAAC seqR:ACTGGAAGTAGGGGC	biotin
CHAT-rs3810950 CHAT-rs3810950 CHAT-rs3810950	A>G	64	56 °C	F:ACTCACCAAGACGCCCATC R:ACTGCTGGGAGTTTTTGCT seqF:GGTCCCCCGTAAGAT	biotin
CHAT-rs1880676 CHAT-rs1880676 CHAT-rs1880676	A>G	63	56 °C	F:CCAGAGATGTGGCCGGAAT R:CTCTTTCCCACTAGCTTCTCAAGG seqR:CTGTGCTCAGTGCTTC	biotin
PLAU-rs2227562 PLAU-rs2227562 PLAU-rs2227562	G>A	94	56 °C	R:AGCTTCCCAGAAACCTTGTTACCA F:CCCCTCAGGGAAGACTCAA seqF:TGGCCATAGCACAAGAGA	biotin
PLAU-rs2227571 PLAU-rs2227571 PLAU-rs2227571	T>C	73	56 °C	F:AGTGTTTTGACCTGAAAATGAGC R:CCTCAGTGCTCCCACCTCTA seqR:CTGCAGTCTTCCCTTGA	biotin
PLAU-rs4065 PLAU-rs4065 PLAU-rs4065	T>C	77	56 °C	F:GGAAGATAGGCTCTGCACAGATG R:ATGCCTGAGGGTAAAGCTATTGT seqF:GTGCCACCCACCAGG	biotin
IDE7-rs2251101 IDE7-rs2251101 IDE7-rs2251101	T>C	130	60 °C	F:AGATCGCACGACTGCACTGTAG R:TGAGTCCCTCCATGTATCATGAAT seq:GGGGGACCTGCTG	biotin
IDE8-rs551266 IDE8-rs551266 IDE8-rs551266	T>C	90	55 °C	F:ACACTGCTAGGTACACGGCAAA R:TGAATCCAACTGCTAGATAAATTATAAGTAGG seq:AATGCTCAATAAATGAGAGA	biotin
IDE14-rs1832196 IDE14-rs1832196 IDE14-rs1832196	C>T	123	57 °C	F:ATGTGACCATTTTGGGTTAGTG R:CCATTTTGCCTAGGCTAGTCTCAA seq:TTGGCACGCTGTTG	biotin
HHEX23-rs1544210 HHEX23-rs1544210 HHEX23-rs1544210	G>A	79	60 °C	F:GGCCTGGGATTTTACTGTACTATCA R:TTCCTGCATTTTGATTTTCTTCTTG seq:GCTACTGTTTTCCTGCA	biotin
IGS6-rs967878 IGS6-rs967878 IGS6-rs967878	G>T	110	60 °C	F:TGGTATCCGTGGGTTACAGACA R:CCTGCTCCTGGGTTCTCCTTTCATC seq:ATCCAGGACTTGCTGA	biotin
GSTO1-rs4925 GSTO1-rs4925 GSTO1-rs4925	C>A	107	60 °C	F:TGTCTAGGTGCCATCCTTGGT R:TCCTCTAGCTTGGTAAATTCTTTACGA seq:AATTCTTCTTTTAGGCCA	biotin
GSTO2-rs2297235 GSTO2-rs2297235 GSTO2-rs2297235	G>A	68	58 °C	F:ACTCTCGGGCTTCCAAATCTG R:GCGATCTGGAGCAGGAGCTA seq:TGTCTCCCCAGGTTAA	biotin
PRSS11-rs2293871 PRSS11-rs2293871 PRSS11-rs2293871	T>C	69	58 °C	F:CATGTAAAGTCAGACCAGGAGGAA R:TGCAACACAAAGGGAAACACA seq:CCAGGAGGAATGGAA	biotin

4.3. Statistical Analysis

Allele frequencies for each polymorphic site were calculated by allele counting method. Goodness of fit to Hardy-Weinberg equilibrium was examined by chi square test. Differences in genotype frequencies between patients and controls were tested by χ^2 test. Comparison of allele frequencies between patients and controls was performed by Z test. Linkage disequilibrium between different polymorphic sites was tested as a function of the difference between observed and expected genotype frequencies. Odd ratios (ORs) and corresponding 95% confidence intervals (CIs) were assessed for genotypes that significantly differed between cases and controls. Variables such as age (onset for cases, age for controls), sex and APOE carrier status were included as covariates in the model. All analyses were performed using R 1.2.1 (Gentleman and Ihaka, 1996) program. Haplotype frequencies were estimated by EH program (Xie and Ott 1993). The EH program implements expectation-maximization algorithm to estimate haplotype frequencies in unrelated individuals and applicable to case-control comparison. The mean AAO between different genotype groups were compared using one way analysis of variance (ANOVA) and adjusted for the effects of significant covariates such as sex and APOE.

5. Results

5.1. Association of the APOE E2/E3/E4 Polymorphism with AD (Tables 6.1-6.3)

The overall APOE genotype distribution differed significantly between cases and controls ($p<0.0001$) (Table 5.1). The frequency of *APOE*4* allele was 34.0 % in cases compared to 10. 6 % in controls. The age and sex adjusted odds ratio (OR) for developing AD with one (genotype 24, 34, 44) and two copies of *APOE*4* (genotype 4/4) compared with no copies of *APOE*4* (genotype 22, 23, 33) was 6.50 (95% CI: 5.20-8.15; p=0.0001) and 14.30 (95% CI: 6.79-26.23; p=0.0001), respectively (Table 5.2). These results are consistent with previous reports demonstrating a dose-dependent effect of *APOE*4 on* AD risk.

Additionally, AAO was analyzed as a quantitative trait. The mean AAO ± (SD) was 72.36 ± 8.19 years for AD. APOE showed strong association with AAO in AD *APOE*4* carriers had significantly lower AAO than non-*APOE*4* carriers (p=0.0001) (Table 5.3).

Table 5.1 Distribution of APOE E2/E3/E4 Polymorphism

APOE	AD Cases(1012)		Controls(771)		
	n	(%)	n	(%)	
33	374	0.37	502	0.65	
34	478	0.47	122	0.16	
23	37	0.06	112	0.15	
24	25	0.16	17	0.02	
44	92	0.76	12	0.02	
22	3	0.03	3	0.00	
3	0.63		0.81		p< 0.001
4	0.34		0.11		p< 0.001
2	0.03		0.09		p< 0.001

Table 5.2 Sex Adjusted Age at Onset (Mean ± S.E.) Among APOE Genotype

APOE	Adjusted Age at Onset
22	79.396±2.91
23	72.137± 0.93
24	73.373±1.04
33	73.701±0.32
34	71.13±0.27
44	71.953±1.31
	p-value over APOE < 0.0001

Table 5.3 Sex Adjusted Age at Onset Among APOE*4 and non-APOE4*4 Carriers

APOE	Adjusted Age of Onset
Non-E4 Carrier	73.608±0.31
E4 Carrier	71.278±0.26
	p-value over APOE < 0.0001

Table 5.4 Distribution of the CHAT/exon S/ D7N (rs1880676) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	62	6.19	44	6.24	
AG	376	37.56	292	41.42	
GG	563	56.24	369	52.34	
Total	1001		705		
Allele frequency					p value
A	0.250		0.270		
G	0.750		0.730		0.19
Age-sex and APOE adjusted OR					
		AA vs. AG+GG			0.98(0.65-1.47;p=0.90)

Table 5.5 Distribution of the CHAT/exon S/ D7N (rs1880676) Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	*AA*	*AG*	*GG*	*A*	*G*
AD Cases	575	41	219	315	0.262	0.738
	%	7.13	38.09	54.78		
Controls	144	10	56	78	0.264	0.736
	%	6.94	38.89	54.17		
						p=0.99
*non-APOE*4*						
AD cases	407	19	152	236	0.233	0.767
	%	4.67	37.35	57.99		
Controls	558	34	235	289	0.272	0.728
	%	6.09	42.11	51.79		
						p=0.06

Age- and sex- adjusted OR

	AA vs. AG+GG
*APOE*4*	0.97(0.47,2.01;p=0.94)
*non- APOE*4*	1.18(0.65,2.16;p=0.58)

Table 5.6 Sex and APOE Adjusted Age-at-Onset (Mean ± SE) Among CHAT/exonS/ D7N (rs1880676) Genotype (late onset)

	Genotype		
	AA	AG	GG
Mean AAO	70.94±0.75	72.48±0.34	72.41±0.27
sample size	57	340	495
	p value= 0.25		

Table 5.7 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among CHAT/exonS/ D7N (rs1880676) Genotype (late and early onset)

	Genotype		
	AA	AG	GG
Mean AAO	69.20±0.88	70.33±0.44	70.17±0.37
sample size	62	379	569
	p value= 0.85		

Table 5.8 Summary of AAO Analysis CHAT/exonS/ D7N (rs1880676)

Effect	AAO (late onset) ANOVA p-value	AAO (late and early) ANOVA p-value
N	917	1037
CHATrs1880676 Genotype	0.11	0.78
APOE	< 0.0001	< 0.0001
Sex	0.08	0.01
CHATrs1880676 Genotype* APOE	0.42	0.62

Table 5.9 Distribution of the CHAT/exon 5/A120T (rs3810950) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	60	6.01	49	6.92	
AG	377	37.74	296	41.81	
GG	562	56.26	363	51.27	
Total	999		708		
Allele frequency					p value
A	0.249		0.278		
G	0.751		0.722		0.06
Age, sex and APOE adjusted OR					
		AA+AG vs. GG			0.98(0.79,1.22;p=0.10)

Table 5.10 Distribution of the CHAT/exon 5/A120T (rs3810950) Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	*AA*	*AG*	*GG*	*A*	*G*
AD Cases	581	40	220	321	0.258	0.742
	%	6.88	37.87	55.25		
Controls	141	10	55	76	0.266	0.734
	%	7.09	39.01	53.90		
						p=0.79
*non-APOE*4*						
AD Cases	408	18	155	235	0.234	0.766
	%	4.41	37.99	57.60		
Controls	566	39	240	287	0.281	0.719
	%	6.89	42.40	50.71		
						p=0.02

Age and sex adjusted OR

	AA+AG vs. GG
*APOE*4*	1.0(0.69,1.46;p=0.99)
*non- APOE*4*	0.76(0.58,0.99;p=0.046)

Table 5.11 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the CHAT/exon 5/A120T (rs3810950) Genotype (late onset)

	Genotype		
	AA	AG	GG
Mean AAO	70.53±0.78	72.23±0.33	72.72±0.27
sample size	54	350	520
		p value= 0.08	

Table 5.12 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the CHAT/exon 5/A120T (rs3810950) Genotype (late and early onset)

	Genotype		
	AA	AG	GG
Mean AAO	69.29±0.95	70.13±0.45	70.39±0.36
sample size	58	390	591
		p value= 0.62	

Table 5.13 Summary of AAO Analysis the CHAT/exon 5/A120T (rs3810950)

Effect	AAO (Late Onset) ANOVA p-value	AAO (Early and Late) ANOVA p-value
N	924	1039
CHATrs3810950 Genotype	0.035	0.62
APOE	< 0.0001	< 0.0001
Sex	0.18	0.01
CHATrs3810950 Genotype * APOE	0.31	0.52

Table 5.14 Distribution of the CHAT/intron9/11604 G→A (rs868750) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	39	3.94	13	1.84	
GA	322	32.56	217	30.74	
GG	628	63.50	476	67.42	
Total	989		706		
Allele frequency					p value
A	0.202		0.172		
G	0.798		0.828		0.03
Age, sex and APOE adjusted OR					
		AA vs. GA+GG		2.37(1.19-4.73;p=0.007)	

Table 5.15 Distribution of the CHAT/intron9/11604 G→A (rs868750) Polymorphism Among APOE*4 and non-APOE*4 Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	*AA*	*GA*	*GG*	*A*	*G*
AD Cases	579	19	190	370	0.197	0.803
	%	3.28	32.82	63.90		
Controls	140	3	49	88	0.196	0.804
	%	2.14	35.00	62.86		
						p=0.97
*non-APOE*4*						
AD Cases	401	20	131	250	0.213	0.787
	%	4.99	32.67	62.34		
Controls	565	10	168	387	0.166	0.834
	%	1.77	29.73	68.50		
						p=0.006

Age and sex adjusted OR	AA vs. GA+GG
*APOE*4*	1.33(0.38,4.59;p=0.65)
*non- APOE*4*	2.94(1.33,6.51;p=0.007)

Table 5.16 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the CHAT/intron9/11604 G→A (rs868750) Genotype (late onset)

	Genotype		
	AA	GA	GG
Mean AAO	73.56±0.99	72.71±0.37	71.95±0.26
sample size	31	285	565
		p-value= 0.77	

Table 5.17 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the CHAT/intron9/11604 G→A (rs868750) Genotype (late and early onset)

	Genotype		
	AA	GA	GG
Mean AAO	71.62±1.13	70.27±0.37	69.94±0.25
sample size	35	292	573
		p-value= 0.89	

Table 5.18 Summary of AAO Analysis CHAT/intron9/11604 G→A (rs868750)

Effect	AAO (late onset) ANOVA p-value	AAO (late and early onset) ANOVA p-value
N	917	1033
CHATrs8687650 Genotype	0.79	0.90
APOE	< 0.0001	< 0.0001
Sex	0.18	0.18
CHATrs8687650 Genotype * APOE	0.48	0.17

Table 5.19 Estimated 3-site CHAT Haplotype Distribution in AD cases and Controls Using the EH Method

Haplotype								
rs1880676	rs3810950	rs8687650	Total	AD	C	Z	p value	Effect
A	A	A	0.0052	0.0030	0.0081	1.859	0.0630	
A	A	G	0.0000	0.0001	0.0000	0.405	0.6856	
A	G	A	0.5566	0.5570	0.5558	0.067	0.9465	
A	G	G	0.1796	0.1901	0.1650	1.860	0.0629	
G	A	A	0.2448	0.2343	0.2600	1.677	0.0936	
G	A	G	0.0107	0.0128	0.0073	1.613	0.1068	
G	G	A	0.0029	0.0022	0.0037	0.765	0.4443	
G	G	G	0.0002	0.0004	0.0000	0.878	0.3799	
			1640	961	679			
		Ln(L)	2757.64	1617.72	1134.21	0.12[a]		

[a] Calculated from the T5 statistic: $2[\ln(L)_{case} + \ln(L)_{control} - \ln(L)_{case+control}]$; df=7

5.2. Distribution of CHAT/exon S/D7N (rs1880676) Polymorphism in AD Cases and Controls (Tables 5.4- 5.5)

The overall distribution pattern of genotype and allele frequencies of the CHAT/exon S/ D7N/A→G transition was similar between cases and controls (Table 5.4). The age-, sex-, and APOE-adjusted OR of developing AD was 0.98(95% CI: 0.65-1.47; p=0.90). There was no significant difference in CHAT genotype or allele distribution among either *APOE*4* carriers or non-*APOE4* carriers (table 5.5).

5.3. Distribution of CHAT/ exon 5/A120T (rs3810950) Polymorphism in AD Cases and Controls (Table 5.9-5.10)

There was no significant difference in genotype (p=0.10) or allele (0.06) distribution among AD cases and controls for CHAT/exon 5/A→G transition (Table 5.9). The OR for developing AD for individuals carrying A allele (AA+AG vs. GG) was 0.98(95% CI: 0.79, 1.22; p=0.10) after adjusting for effects of age, sex and APOE. However, when the sample was stratified by APOE status, the A allele of CHAT/A120T was associated with protective effect among non-APOE*4 carriers in both genotype (p= 0.046) or allele (p=0.02) distribution. The age and sex adjusted OR was among non-APOE*4 carriers with A allele was 0.76(0.58, 0.99; p=0.046) (Table 5.10). This indicates that exon 5/A allele has a modest protective effect against developing AD.

5.4. Distribution of CHAT/intron 9, 11604 G→A (rs868750) Polymorphism in AD Cases and Controls (Table 5.14-5.15)

The CHAT/intron 9 polymorphism showed a significant difference in both allele (p=0.03) and genotype (p=0.007) frequencies between cases and controls. The frequencies of the AA genotype was significantly higher in AD patients compared to controls (p=0.007). The age, sex and APOE adjusted OR for (AA vs. AG+GG) was 2.37(95% CI: 1.19-4.73; p=0.007) (Table 5.14). However, stratification of data by APOE genotype revealed that association was confined among non-*APOE*4* carriers only; genotype (p=0.007) and allele (p=0.006). The age, sex and APOE adjusted OR among the non-*APOE*4* carriers (AA vs. AG+GG) was 2.94(95% CI, 1.33, 6.51; p=0.007) and among *APOE*4* carriers, it was 1.33(95% CI: 0.38, 4.59; p=0.65) (Table 5.15). This suggests that the association observed in total sample was attributed to non-*APOE*4* carriers. It should be noted that the controls have slight deviation from HWE (p=0.04) Repeating genotypes in the control samples revealed the same results, suggesting the deviation from HWE is not attributable to the genotyping error. When we checked the HWE within *APOE*4* carriers (p=0.20) and non-*APOE*4* carriers (p=0.09) they were in HWE.

5.5. Three-site CHAT Haplotype Distribution in AD Cases and Controls (Table 5.19)

The 3-site CHAT haplotype distribution in AD cases and controls is presented in table 5.19. We observed three major haplotypes (haplotypes AGA, AGG, GAA), which accounted for 98% of all possible marker combinations in AD cases, and 97% in the control sample. No evidence for association with AD risk was provided by individual haplotype tests. Likewise, the examination

of the distribution differences in overall haplotype showed no significant association between case and control subjects (p=0.12).

5.6. Sex- and APOE-Adjusted Age-At-Onset Among CHAT Genotypes (5.6-5.8, 5.11- 5.13, 5.7.16-5.18)

AAO was analyzed as a quantitative trait. Mean AAO was 72.29 ±0.201 in LOAD and 70.05±0.263 in all AD patients. Genotypic association study for AAO effect in AD revealed no association for CHAT gene: exon S, exon 5, and intron 9 polymorphisms p=0.25, 0.08, 0.77, respectively, after adjusting for the effects of gender and APOE (Table 7.10). Logistic regression analysis to determine possible interaction between APOE and CHAT: exon S, exon 5, and intron 9 polymorphism revealed no interaction (p=0.62, 0.52, 0.17, respectively) (Tables: 5.8, 5.13, 5.18).

Table 5.20 Distribution of the PLAU/intron5/G→A (rs2227562) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	20	2.48	21	3.17	
GA	187	23.17	166	25.04	
GG	600	74.35	476	71.79	
Total	807		663		
Allele frequency					p value
A	0.141		0.157		
G	0.859		0.843		0.23
Age, sex and APOE adjusted OR					
		AA vs. GA+GG			0.80(0.40-1.62;p=0.54)

Table 5.21 Distribution of the PLAU/intron5/G→A (rs2227562) Polymorphism Among APOE*4 and non-APOE*4 Carriers

		Genotype Frequency			*Allele*	*Frequency*
*APOE*4*	*n*	*AA*	*GA*	*GG*	*A*	*G*
AD Cases	469	14	110	345	0.147	0.853
	%	2.99	23.45	73.56		
Controls	129	2	36	91	0.155	0.845
	%	1.55	27.91	70.54		
						p=0.75
*non-APOE*4*						
AD Cases	330	6	75	249	0.132	0.868
	%	1.82	22.73	75.45		
Controls	531	18	130	383	0.156	0.844
	%	3.39	24.48	72.13		
						p=0.16

Age and sex adjusted OR

	AA vs. GA+GG
*APOE*4*	1.96(0.44,8.81;p=0.38)
*non- APOE*4*	0.53(0.20,1.37;p=0.19)

Table 5.22 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PLAU/intron5/G→A (rs2227562) (late onset)

	Genotype		
	TT	CT	CC
Mean AAO	71.62±1.13	70.27±0.37	69.94±0.25
sample size	35	292	573
		p-value= 0.89	

Table 5.23 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PLAU/intron5/G→A (rs2227562) Genotype (early and late onset)

	Genotype		
	AA	GA	GG
Mean AAO	70.56±1.77	70.30±0.57	70.00±0.35
sample size	19	185	610
		p value= 0.96	

Table 5.24 Summary of AAO Analysis PLAU/intron5/G→A (rs2227562)

Effect	AAO (late onset)	AAO (early + late)
	ANOVA p-value	ANOVA p value
N	741	846
PLAU rs3855 Genotype	0.81	0.98
APOE	< 0.0001	< 0.0001
Sex	0.05	0.01
PLAU rs3855 Genotype * APOE	0.79	0.82

Table 5.25 Distribution of the PLAU/intron9/T→C (rs2227571) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
CC	184	18.40	147	21.09	
CT	485	48.50	328	47.06	
TT	331	33.10	222	31.85	
Total	1000		697		
Allele frequency					
					p value
C	0.427		0.446		
T	0.574		0.554		0.27
Age, sex and APOE adjusted OR					
		CC vs. CT+TT			0.84(0.64-1.10;p=0.21)

Table 5.26 Distribution of the PLAU/intron9/T→C (rs2227571) Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	*CC*	*CT*	*TT*	*C*	*T*
AD Cases	575	104	286	185	0.430	0.570
	%	18.09	49.74	32.17		
Controls	143	33	69	41	0.472	0.528
	%	23.08	48.25	28.67		
						p=0.20
*non-APOE*4*						
AD Cases	407	77	190	140	0.423	0.577
	%	18.92	46.68	34.40		
Controls	551	113	258	180	0.439	0.561
	%	20.51	46.82	32.67		
						p=0.63

Age and sex adjusted OR

	CC vs. CT+TT
*APOE*4*	0.71(0.45,1.11;p=0.14)
*non- APOE*4*	0.93(0.66,1.30;p=0.66)

Table 5.27 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PLAU/intron9/T→C (rs2227571) (late onset)

	Genotype		
	CC	CT	TT
Mean AAO	72.96±0.46	72.27±0.30	72.23±0.35
sample size	163	433	297
	p value= 0.043 (0.01)*		

*p value for (CC+CT vs. TT)

Table 5.28 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PLAU/intron9/T→C (rs2227571) (early and late onset)

	Genotype		
	CC	CT	TT
Mean AAO	70.20±0.63	70.43±0.39	69.77±0.48
sample size	189	488	335
	p value= 0.42		

Table 5.29 Summary of AAO Analysis PLAU/intron9/T→C (rs2227571)

Effect	AAO (late onset) ANOVA p value	AAO (early and late) ANOVA p value
N	917	1038
PLAUrs5634 Genotype	0.043*	0.42
APOE	< 0.0001	< 0.0001
Sex	0.10	0.02
PLAUrs5634 Genotype * APOE	0.44	0.88

Table 5.30 Distribution of the PLAU/3'UTR/T→C (rs4065) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
CC	149	15.07	131	18.99	
TC	476	48.13	320	46.38	
TT	364	36.80	239	34.64	
Total	989		690		
Allele frequency					p value
C	0.391		0.422		
T	0.609		0.578		0.07
Age, sex and APOE adjusted OR					
		CC vs. TC+TT			0.71(0.53-0.95;p=0.02)

Table 5.31 Distribution of the PLAU/3'UTR/T→C (rs4065) Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	CC	TC	TT	*C*	*T*
AD Cases	572	87	279	206	0.396	0.604
	%	15.21	48.78	36.01		
Controls	139	32	64	43	0.460	0.540
	%	23.02	46.04	30.94		
						p=0.04
*non-APOE*4*						
AD Cases	399	59	189	151	0.385	0.615
	%	14.79	47.37	37.84		
Controls	548	98	256	194	0.412	0.588
	%	17.88	46.72	35.40		
						p=0.19

Age and sex adjusted OR

	CC vs. TC+TT
*APOE*4*	0.58(0.36,0.92;p=0.02)
*non- APOE*4*	0.82(0.57,1.18;p=0.28)

Table 5.32 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PLAU/3'UTR/T→C (rs4065) Polymorphism (late onset)

	Genotype		
	CC	CT	TT
Mean AAO	70.97±0.48	72.33±0.31	72.14±0.34
sample size	131	423	328
	p value= 0.10 (0.036)*		

*p value for (CC+CT vs. TT)

Table 5.33 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PLAU/3'UTR/T→C (rs4065) Polymorphism (early and late onset)

	Genotype		
	CC	CT	TT
Mean AAO	69.85±0.71	70.56±0.39	69.74±0.46
sample size	155	474	371
	p value= 0.51		

Table 5.34 Summary of AAO Analysis PLAU/3'UTR/T→C (rs4065)

Effect	AAO (late onset) ANOVA p value	AAO (early and late) ANOVA p value
N	906	1026
PLAU/3'UTR Polymorphism	0.12	0.62
APOE	< 0.0001	< 0.0001
Sex	0.08	0.01
PLAU/3'UTR Polymorphism * APOE	0.48	0.41

Table 5.35 PLAU Haplotype Distribution in AD Cases and Controls

Haplotype								
rs3855	rs5634	rs7338	Total	AD	C	Z	p′	Effect
A	C	C	0.000	0.000	0.000			
A	C	T	0.002	0.003	0.002	0.613	0.5400	
A	T	C	0.145	0.138	0.153	1.104	0.2694	
A	T	T	0.256	0.251	0.263	0.740	0.4595	
G	C	C	0.003	0.003	0.003	0.292	0.7704	
G	C	T	0.564	0.577	0.551	1.357	0.1747	
G	T	C	0.002	0.002	0.003	0.876	0.3813	
G	T	T	0.028	0.027	0.025	0.398	0.6909	
			1399	774	625			
		ln(L)	-2472.36	-1346.76	-1123.88	0.84[a]		

[a] Calculated from the T5 statistic: $2[\ln(L)_{case} + \ln(L)_{control} - \ln(L)_{case+control}]$; df=7

5.7. Distribution of the PLAU/intron5/G→A (rs2227562) Genotype and Allele Frequencies in AD Patients and Controls (Tables 5.20-5.21)

The genotype and allele distribution of G→A at intron 5 in PLAU gene were similar between cases and controls (Table 5.20). The OR for AA genotype was 0.80(95% CI: 0.40-1.62; p=0.54) after adjusting for the effect of age, sex and APOE. There was no difference in genotype or allele frequencies among *APOE*4* carriers (p=0.20) and non-*APOE*4* carriers (p=0.63) (Table 5.21).

5.8. Distribution of the PLAU/intron9/T→C (rs2227571) Genotype and Allele Frequencies in AD Patients and Controls (Tables 5.25-5.26)

The overall genotype frequency was comparable between cases and controls (p=0.21). The OR for developing AD was 0.84(95% CI: 0.64-1.10; p=0.21) for intron 9 C carriers (TT vs. CT+CC) compared to non-C carriers after adjusting for age, sex and APOE (Table 5.25). When the data were stratified by APOE status, the results did not change (Table 5.26). The age-, sex-, and APOE adjusted OR for C allele carriers was 0.71(95% CI: 0.45, 1.11; p=0.14) for *APOE*4* carriers and 0.93(95% CI: 0.66, 1.30; p=0.66) for non-*APOE*4* carriers (Table 5.26).

5.9. Distribution of the PLAU/3'UTR/T→C (rs4065) Genotype and Allele Frequencies in AD Patients and Controls (Tables 5.30-5.31)

There was a borderline difference in allele distribution between cases and controls for the PLAU 3' UTR polymorphism in the total sample (39.1 % vs. 42.2 %; p=0.07; Table 5.30). The age, sex, and APOE adjusted OR between the CC and other genotypes 0.71(95% CI 0.53-0.95; p=0.02). Further stratification of data by APOE status showed that the association between PLAU and AD was confined to *APOE*4* carriers only (p=0.02). The age-, sex- adjusted OR among APOE*4 carriers was 0.58(95% CI: 0.36, 0.92; p=0.02; Table 5.31). This suggests that the effect seen in the total sample was from *APOE*4* carriers.

5.10. Three-site PLAU Haplotype Distribution in AD Cases and Controls (Table 5.35)

Table 5.35 demonstrates the frequencies for the estimated three-site haplotypes among AD cases and control subjects. There were three major haplotypes: ATC, ATT, and GCT, which represents for 96% of all possible marker combinations in AD and control sample. Haplotype ACC was not present in either AD cases or controls samples. The remaining haplotypes were less than 1% with the exception of GTT haplotype, which is less than 3 %. Individual haplotype tests reveal no evidence of association with AD risk. Likewise, the examination of the distribution differences in overall haplotype showed no significant association between PLAU haplotypes using case and control subjects (p=0.84).

5.11. Sex- and APOE-Adjusted Age-at-Onset Among PLAU Genotypes (5.22-5.23, 5.27-5.28, 5.32-5.33)

The mean age at onset was 72.29 ±0.201 years for LOAD (range between 60-91 years) and 70.05±0.263 years in the total AD sample (range between 33-91 years). There was a significant difference in AAO among the intron 9 genotypes (p=0.04) after adjusting for sex and age. When we grouped the data, C carriers (CC+CT) vs. TT, the difference in AAO between genotypes became more significant (p=0.01) (Table 5.27). As LOAD is complex and heterogeneous disorder, we look for possible interaction between APOE and 3 PLAU polymorphisms. Logistic regression analysis of APOE and 3 PLAU: intron 5, intron 9, and 3' UTR polymorphisms revealed no interaction (p= 0.79, 0.44, and 0.48, respectively) (Tables: 5.24, 5.29, and 5.34).
9 polymorphism revealed no interaction (p=0.62, 0.52, and 0.17, respectively) (Tables: 5.8, 5.13, 5.18).

Table 5.36 Distribution of the IGS6/G→T (rs967878) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
TT	161	17.69	109	14.65	
GT	458	50.33	374	50.27	
GG	291	31.98	261	35.08	
Total	910		744		
Allele frequency					p value
T	0.429		0.398		
G	0.571		0.602		0.07
Age, sex and APOE adjusted OR					
		TT vs. GT+GG		1.29 (0.96-1.32;p=0.09)	

Table 5.37 Distribution of the IGS6/G→T (rs967878) Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	TT	GT	GG	*A*	*C*
AD Cases	534	94	263	177	0.422	0.578
	%	17.60	49.25	33.15		
Controls	142	22	79	41	0.433	0.567
	%	15.49	55.63	28.87		
						p=0.90
*non-APOE*4*						
AD Cases	371	67	191	113	0.438	0.562
	%	18.06	51.48	30.46		
Controls	599	87	293	219	0.390	0.610
	%	14.52	48.91	36.56		
						p=0.04

Age and sex adjusted OR

	TT vs. GT+GG
*APOE*4*	1.20(0.72,2.00;p=0.49)
*non- APOE*4*	1.34(0.93,1.92;p=0.12)

Table 5.38 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IGS6/G→T (rs967878) Polymorphism (late onset)

	Genotype		
	CC	CT	TT
Mean AAO	72.79±0.51	72.54±0.31	72.11±0.38
sample size	149	421	261
		p-value= 0.52	

Table 5.39 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IGS6/G→T (rs967878) Polymorphism (early and late onset)

	Genotype		
	CC	CT	TT
Mean AAO	70.20±0.72	70.36±0.39	70.05±0.55
sample size	154	459	284
		p-value= 0.33	

Table 5.40 Summary of AAO Analysis IGS6/G→T (rs967878)

Effect	AAO (late onset) ANOVA p value	AAO (early and late onset) ANOVA p value
N	845	943
IGS6 Genotype	0.52	0.33
APOE	< 0.0001	< 0.0001
Sex	0.14	0.03
IGS6 Genotype * APOE	0.97	0.44

Table 5.41 Distribution of the IDE7/T→C (rs2251101) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
CC	102	10.56	68	9.29	
CT	384	39.75	291	39.75	
TT	480	49.69	373	50.96	
Total	966		732		
Allele frequency					p value
C	0.304		0.292		
T	0.696		0.708		0.45
Age, sex and APOE adjusted OR					
		CC vs. CT+TT			1.20(0.83-1.71;p=0.33)

Table 5.42 Distribution of the IDE7/T→C (rs2251101) Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	CC	CT	TT	*C*	*T*
AD Cases	559	59	221	279	0.303	0.697
	%	10.55	39.53	49.91		
Controls	149	17	63	69	0.326	0.674
	%	11.41	42.28	46.31		
						p=0.39
*non-APOE*4*						
AD Cases	390	43	158	189	0.313	0.687
	%	11.03	40.51	48.46		
Controls	580	51	226	303	0.283	0.717
	%	8.79	38.97	52.24		
						p=0.16

Age and sex adjusted OR	CC vs. CT+TT
*APOE*4*	0.92(0.52,1.64;p=0.78)
*non- APOE*4*	1.40(0.90,2.19;p=0.14)

Table 5.43 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IDE7/T→C (rs2251101) Polymorphism (late onset)

	Genotype		
	CC	CT	TT
Mean AAO	72.65±0.60	72.05±0.32	72.54±0.30
sample size	97	356	446
		p-value= 0.63	

Table 5.44 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IDE7/T→C (rs2251101) Polymorphism (early and late Onset)

	Genotype		
	CC	CT	TT
Mean AAO	70.12±0.94	70.19±0.40	70.25±0.40
sample size	101	406	503
		p-value= 0.38	

Table 5.45 Summary of AAO Analysis IDE7/T→C (rs2251101)

Effect	AAO (late onset) ANOVA p-value	AAO (early and late) ANOVA p value
N	896	1037
IDE7 Genotype	0.63	0.38
APOE	< 0.0001	< 0.0001
Sex	0.16	0.03
IDE7 Genotype * APOE	0.79	0.89

Table 5.46 Distribution of the IDE8/T→C (rs551266) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
CC	27	3.10	16	2.37	
TC	269	30.88	189	28.04	
TT	575	66.02	469	69.58	
Total	871		674		
Allele frequency					p value
C	0.185		0.164		
T	0.815		0.836		0.13
Age, sex and APOE adjusted OR					
		CC v. TC+TT			1.38(0.68-2.80;p=0.37)

Table 5.47 Distribution of the IDE8/T→C (rs551266) Polymorphism Among APOE*4 and non-APOE*4 Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	CC	TC	TT	*C*	*T*
AD Cases	509	18	165	326	0.197	0.803
	%	3.54	32.42	64.05		
Controls	137	3	46	88	0.190	0.810
	%	2.19	33.58	64.23		
						p=0.79
*non-APOE*4*						
AD Cases	354	9	103	242	0.171	0.829
	%	2.54	29.10	68.36		
Controls	534	13	143	378	0.158	0.842
	%	2.43	26.78	70.79		
						p=0.26

Age and sex adjusted OR

	CC vs. TC+TT
*APOE*4*	1.72(0.50,5.97;p=0.39)
*non- APOE*4*	1.23(0.50,3.05;p=0.65)

Table 5.48 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IDE8/T→C (rs551266) Polymorphism (late onset)

	Genotype		
	CC	CT	TT
Mean AAO	73.15±1.02	72.16±0.39	72.83±0.27
sample size	26	233	516
		p-value= 0.35	

Table 5.49 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IDE8/T→C (rs551266) Polymorphism (early and late onset)

	Genotype		
	CC	CT	TT
Mean AAO	68.620±2.19	70.22±0.51	70.61±0.35
sample size	26	259	580
		p-value= 0.78	

Table 5.50 Summary of AAO Analysis IDE8/T→C (rs551266)

Effect	AAO (late onset) ANOVA p value	AAO (early and late) ANOVA p value
N	818	913
IDE8 Genotype	0.35	0.78
APOE	< 0.0001	< 0.0001
Sex	0.18	0.14
IDE8 Genotype * APOE	0.71	0.97

Table 5.51 Distribution of the IDE14/C→T (rs1832196) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
TT	18	1.78	12	1.72	
TC	244	24.11	164	23.50	
CC	750	74.11	522	74.79	
Total	1012		698		
Allele frequency					p value
T	0.138		0.135		
C	0.862		0.865		0.8
Age, sex and APOE adjusted OR					
		TT vs. TC+CC			1.24(0.55-2.82;p=0.60)

Table 5.52 Distribution of the IDE14/C→T (rs1832196) Polymorphism Among APOE*4 and non-APOE*4 Carriers

		IDE 14 C→T Genotype			*Allele Frequency*	
*APOE*4*	*n*	*TT*	*TC*	*CC*	*T*	*C*
AD Cases	594	11	138	445	0.135	0.865
	%	1.85	23.23	74.92		
Controls	143	1	37	105	0.136	0.864
	%	0.70	25.87	73.43		
						p=0.97
*non-APOE*4*						
AD Cases	413	7	106	300	0.145	0.855
	%	1.69	25.67	72.64		
Controls	554	11	127	416	0.134	0.866
	%	1.99	22.92	75.09		
						p=0.49

Age and sex adjusted OR

	TT vs. TC+CC
*APOE*4*	2.96(0.38,23.27;p=0.30)
*non- APOE*4*	0.96(0.35,2.60;p=0.94)

Table 5.53 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IDE14/C→T (rs1832196) Polymorphism (late onset)

	Genotype		
	TT	TC	CC
Mean AAO	72.85±1.91	72.51±0.38	71.31±0.24
sample size	16	220	665
		p-value= 0.55	

Table 5.54 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the IDE14/C→T (rs1832196) Polymorphism (early and late onset)

	Genotype		
	TT	TC	CC
Mean AAO	71.11±2.32	70.21±0.53	70.09±0.32
sample size	35	292	573
		p-value= 0.62	

Table 5.55 Summary of AAO Analysis IDE14/C→T (rs1832196)

Effect	AAO (late onset) ANOVA p-value	AAO (early and late) ANOVA p-value
N	938	1060
IDE14 Genotype	0.55	0.62
APOE	< 0.0001	< 0.0001
Sex	0.04	0.01
IDE14 Genotype * APOE	0.9	0.32

Table 5.56 Distribution of the HHEX/G→A (rs1544210) Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	218	24.44	189	24.51	
GA	440	49.33	380	49.29	
GG	234	26.23	202	26.20	
Total	892		771		
Allele frequency					p value
A	0.491		0.492		
G	0.509		0.508		0.95
Age, sex and APOE adjusted OR					
		AA vs. GA+GG			0.90(0.70-1.16;p=0.43)

Table 5.57 Distribution of the HHEX/G→A (rs1544210) Polymorphism Among APOE*4 and non-APOE*4 Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	AA	GA	GG	*A*	*G*
AD Cases	516	136	255	125	0.511	0.489
	%	26.36	49.42	24.22		
Controls	151	36	77	38	0.493	0.507
	%	23.84	50.99	25.17		
						p=0.54
*non-APOE*4*						
AD Cases	368	77	184	107	0.459	0.541
	%	20.92	50.00	29.08		
Controls	617	152	302	163	0.491	0.509
	%	24.64	48.95	26.42		
						p=0.17

Age and sex adjusted OR

	AA vs. AG+GG
*APOE*4*	1.10(0.72-1.69;p=0.65)
*non- APOE*4*	0.81(0.59,1.11;p=0.19)

Table 5.58 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the HHEX/G→A (rs1544210) Polymorphism (late onset)

	Genotype		
	AA	GA	GG
Mean AAO	71.48±0.48	72.68±0.30	73.04±0.43
sample size	190	396	206
		p-value= 0.07	

Table 5.59 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the HHEX G→A (rs1544210) Polymorphism (early and late onset)

	Genotype		
	AA	GA	GG
Mean AAO	68.55±0.67	70.93±0.38	70.45±0.59
sample size	211	444	239
		p-value= 0.83	

Table 5.60 Summary of AAO Analysis HHEX G→A (rs1544210)

Effect	AAO (late onset) ANOVA p value	AAO (early and late) ANOVA p value
N	836	944
HHEX Genotype	0.07	0.83
APOE	< 0.0001	< 0.0001
Sex	0.15	0.01
HHEX Genotype * APOE	0.63	0.72

Table 5.61 Estimated 5-site IDE-HHEX-IGS6 Haplotype Distribution in AD Cases and Control Using the EH Method

H ID	IDE7	IDE8	IEE14	HHEX	IGS6	Total	AD	C	*Z*	*p′*	Effect
H1	A	C	C	C	A	0	0	0			
H2	A	C	C	C	C	0	0	0			
H3	A	C	C	T	A	0.000003	0	0.001833	1.493	0.1354	
H4	A	C	C	T	C	0.002128	0.00238	0.000043	1.792	0.0731	
H5	A	C	T	C	A	0	0	0			
H6	A	C	T	C	C	0	0	0			
H7	A	C	T	T	A	0	0	0			
H8	A	C	T	T	C	0	0	0			
H9	A	T	C	C	A	0.17118	0.167966	0.176602	0.585	0.5584	
H10	A	T	C	C	C	0.160786	0.160038	0.163385	0.233	0.8160	
H11	A	T	C	T	A	0.070288	0.062132	0.075689	1.366	0.1719	
H12	A	T	C	T	C	0.178983	0.180278	0.180764	0.032	0.9742	
H13	A	T	T	C	A	0.079571	0.090121	0.066259	2.291	0.0220	Risk
H14	A	T	T	C	C	0.008564	0.000151	0.016027	4.387	< 0.0001	Protective
H15	A	T	T	T	A	0.016993	0.014391	0.020738	1.229	0.2191	
H16	G	T	T	T	C	0.023624	0.02591	0.021066	0.822	0.4108	
H17	G	C	C	C	A	0.000772	0.001207	0.000001	1.311	0.1899	
H18	G	C	C	C	C	0.006245	0.00665	0.006094	0.179	0.8577	
H19	G	C	C	T	A	0.023394	0.024547	0.02311	0.242	0.8091	
H20	G	C	C	T	C	0.139246	0.146317	0.12857	1.323	0.1858	
H21	G	C	T	C	A	0.000601	0.001168	0	1.291	0.1966	
H22	G	C	T	C	C	0.000005	0	0			
H23	G	C	T	T	A	0.000002	0	0.000001	0.035	0.9722	
H24	G	C	T	T	C	0.002983	0.002163	0.005092	1.228	0.2193	
H25	G	T	C	C	A	0.049163	0.052149	0.044332	0.937	0.3486	
H26	G	T	C	C	C	0.040567	0.04085	0.040557	0.038	0.9697	
H27	G	T	C	T	A	0.002048	0.004973	0.000001	2.669	0.0076	Risk
H28	G	T	C	T	C	0.021107	0.014469	0.027224	2.261	0.0237	Protective
H29	G	T	T	C	A	0	0	0			
H30	G	T	T	C	C	0.001108	0.002141	0.000746	0.960	0.3371	
H31	G	T	T	T	A	0	0	0			
H32	G	T	T	T	C	0.000639	0	0.001866	1.507	0.1319	
					n	1320	713	607			
					ln(*L*)	-4886.3900	-2621.02	-2251.19		0.60	

5.12. Distribution of the IGS6/G→T (rs967878) Genotype and Allele Frequencies in AD Patients and Controls (5.36-5.37)

The overall genotype distribution of the IGS6 G→T polymorphism was similar between cases and controls (p=0.09) (Table 5.36). The allele frequency of the A allele was similar in both cases and controls as well (p=0.07). The age-, sex-, and APOE adjusted OR for IGS6/TT genotype vs. TG+GG genotypes was 1.29(95% CI: 0.96-1.32; p=0.09) (Table 5.36). When stratifying the data by APOE status, there was no statistically significant difference in genotype or allele distribution among *APOE*4* carriers (Table 5.37). Among non-*APOE*4* carriers, despite the fact that genotype frequency was not different between cases and controls (p=0.12) (Table 5.37), there was marginally significant differences in allele distribution between cases and controls (p=0.04). The OR for TT genotype vs. TG+ GG was 1.20 (95% CI: 0.72, 2.00; p=0.49) and 1.34 (95% CI: 0.93, 1.92; p=0.12) for *APOE*4* carriers and non-*APOE*4* carriers, respectively.

5.13. Distribution of the IDE7/T→C (rs2251101) Genotype and Allele Frequencies in AD Patients and Controls (5.41-5.42)

The overall genotype and allele frequencies were similar among cases and controls for T→C transition in the IDE gene (Table 5.41). The OR for CC genotype vs. TC+TT (T allele carriers) was 1.38 (95% CI: 0.68-2.80; p=0.37) after adjusting for sex, age and APOE. Stratification of data by APOE did not reveal any significant association between cases and controls. The age-, and sex adjusted OR for CC genotype vs. TC+TT genotype was 0.92 (95% CI: 0.52, 1.64; p=0.78) among *APOE*4* carriers and 1.40(95% CI: 0.90, 2.19; p=0.14) among non-*APOE*4* carriers (Table 5.42).

5.14. Distribution of the IDE8/T→C (rs551266) Genotype and Allele Frequencies in AD Patients and Controls (5.46-5.47)

The genotype (p=0.37) or allele (p=0.13) distribution of T→C transition in IDE gene did not reveal any significant association with AD risk (Table 7.46). The age-, sex and APOE adjusted OR for CC genotype vs. TC+TT was 1.38 (95% CI: 0.68-2.80; p=0.37). We further stratified the data by APOE status. The OR for 1.72 (95% CI: 0.50, 5.97; p=0.39) was among *APOE*4* carriers, and 1.23 (95% CI: 0.50, 3.05; p=0.65) among non-*APOE*4* carriers after adjusting for age and sex (Table 5.47).

5.15. Distribution of the IDE14/C→T (rs1832196) Genotype and Allele Frequencies in AD Patients and Controls (5.51-5.52)

There was no difference in genotype or allele distribution between cases and controls for C→T (rs1832196) transition in IDE gene (Table 5.51). Upon stratification of data by APOE, there were no significant difference in genotype and allele distribution among *APOE*4* carriers and non-*APOE*4* carriers. After adjusting for age and sex effect, the OR for TT genotype vs. TC+CC was 2.96 (95% CI: 0.38, 23.27; p=0.30) among *APOE*4* carriers and 0.96 (95% CI: 0.35, 2.60; p=0.94) among non *APOE*4* carriers. The trends for the TT genotype difference between *APOE*4* carriers and non-*APOE*4* carriers were in apposite direction; the TT genotype increased the risk for *APOE*4* carriers, conversely it has a protective role among non- *APOE*4* carriers.

5.16. Distribution of the HHEX G→A (rs1544210) Genotype and Allele Frequencies in AD Patients and Controls (5.56-5.57)

The overall genotype and allele frequencies were similar among cases and controls for the HHEX/G→A polymorphisms (rs1832196) (Table 5.56). The age-, sex-, and APOE adjusted OR for AA genotype vs. GA+GG was 0.90 (95% CI: 0.70-1.16; p=0.43). Stratification of data by APOE status revealed that the AA genotype revealed a higher frequency in control subjects among *APOE*4* carriers (20.92 vs. 24.64), but it was not statistically significant (Table 5.57)

5.17. Five-site IDE region Haplotype Distribution in AD Cases and Controls (Table 5.61)

Haplotype analysis comprising of the IDE, HHEX genes and IGS6 marker was performed. There were six haplotypes (H9 ATCCA, H10 ATCCC, H11 ATCTA, H12 ATCTC, H13 ATTCA and H20 GCCTC) with a frequency of > 10%. These most frequent haplotypes accounted for 78 % of all haplotypes in the whole samples. Additional five haplotypes range from 1% to 5 % (H16, H19, H25, H26, and H28). The remaining haplotypes were either not present or occurred at frequency with less than 1%. The frequencies for these haplotypes among patients and controls and haplotype effects are presented in Table 5.61. AD patients had a higher frequency of the H13/ATTCA (p<0.02), and H27/GTCTA (p=0.007) haplotypes compared to controls. Conversely, the H14/ATTCC (p=0.001) and H28/GTCTA (p=0.023) haplotypes were associated with a decreased risk of LOAD. In order to determine the source of effects of the haplotypes H13, H14, H27, and H28, we conducted two -site haplotype analyses for the HHEX and IGS markers and three-site haplotype analysis for IDE SNPs. We found no difference among the

distribution of haplotypes between LOAD cases and controls in any of the haplotype groups. Comparing AD patients with controls, no significant association was observed concerning the other haplotypes. The overall effect of all haplotypes was not significant.

5.18. Sex- and APOE-Adjusted Age-At-Onset Among IDE, HHEX, IGS6 Genotypes (Tables 5.38, 5.43, 5.48, 5.53, 5.58)

No association of IDE, HHEX, IGS6 markers with AAO was demonstrated. Adjusting for the effects of gender and APOE did not change the results (Tables 5.38, 5.43, 5.48, 5.53, 5.58). Logistic regression analysis to determine possible interaction between the APOE and polymorphism in these genes revealed no interaction (Tables 5.40, 5.45, 5.50, 5.55, 5.60).

Table 5.62 Distribution of the GSTO1/Ala140Asp Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	99	10.02	83	12.46	
AC	440	44.53	288	43.24	
CC	449	45.45	295	44.29	
Total	988		666		
Allele frequency					
					p value
A	0.323		0.341		
C	0.677		0.659		0.231
Age, sex and APOE adjusted OR					
	AA vs. AC+CC		1.34(0.929,1.926;p=0.12)		

Table 5.63 Distribution of the GSTO1/Ala140Asp Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		GSTO1 Genotype			*Allele Frequency*	
*APOE*4*	*n*	*AA*	*AC*	*CC*	*A*	*C*
AD Cases	581	60	257	264	0.324	0.676
	%	10.33	44.23	45.44		
Controls	137	21	60	56	0.372	0.628
	%	15.33	43.80	40.88		
*non-APOE*4*						
AD Cases	402	39	182	181	0.323	0.677
	%	9.70	45.27	45.02		
Controls	529	62	228	239	0.333	0.667
	%	11.72	43.10	45.18		

Age and sex adjusted OR

	AA vs. AC+CC
*APOE*4*	1.31(0.82,2.07;P=0.25)
*non- APOE*4*	1.37(0.84,2.28;P=0.203)

Table 5.64 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the GSTO1/Ala140Asp Polymorphism (late onset)

	Genotype		
	AA	AC	CC
Mean AAO	73.37+/-0.69	72.14+/-0.293	72.48+/-0.31
sample size	94	398	397
		p value= 0.373	

Table 5.65 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the GSTO1/Ala140Asp Polymorphism (early and late onset)

	Genotype		
	AA	AC	CC
Mean AAO	70.00+/-0.95	70.059+/-0.33	70.189+/-0.41
sample size	108	448	450
		p value= 0.703	

Table 5.66 Summary of AAO Analysis GSTO1/Ala140Asp Polymorphism

Effect	AAO (late onset) ANOVA p value	AAO (early and late) ANOVA p value
N	917	1037
GSTO1 Genotype	0.462	0.790
APOE	< 0.0001	< 0.011
Sex	0.111	0.111
GSTO1 Genotype * APOE	0.386	0.505

Table 5.67 Distribution of the GSTO2/-183A→G Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
AA	485	49.04	355	48.50	
AG	419	42.37	308	42.08	
GG	85	8.59	69	9.43	
Total	989		732		
Allele frequency					p value
A	0.702		0.695		
C	0.298		0.305		0.93
Age, sex and APOE adjusted OR					
		GG vs. AG+AA			0.92(0.66-1.29;p=0.63)

Table 5.68 Distribution of the GSTO2/-183A→G Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	*AA*	*AG*	*GG*	*A*	*C*
AD Cases	580	276	249	55	0.691	0.309
	%	47.59	42.93	9.48		
Controls	148	68	60	20	0.662	0.338
	%	45.95	40.54	13.51		
						p=0.34
*non-APOE*4*						
AD Cases	404	205	169	30	0.717	0.283
	%	50.74	41.83	7.43		
Controls	583	287	247	49	0.704	0.296
	%	49.23	42.37	8.40		
						p=0.34

Age and sex adjusted OR

	GG vs. AG+AA
*APOE*4*	0.72(0.42,1.26;p=0.25)
*non- APOE*4*	0.80(0.49-1.31;p=0.38)

Table 5.69 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the GSTO2/-183A→G Polymorphism (late onset)

	Genotype		
	AA	AG	GG
Mean AAO	72.46±0.29	72.30±0.30	73.25±0.73
sample size	432	378	78
		p-value= 0.42	

Table 5.70 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the GSTO2/-183A→G Polymorphism (early and late onset)

	Genotype		
	AA	AG	GG
Mean AAO	70.34±0.37	70.05±0.43	70.25±1.05
sample size	488	429	88
		p-value= 0.28	

Table 5.71 Summary of AAO Analysis Among the GSTO2/-183A→G Polymorphism

Effect	AAO (late onset) ANOVA p value	AAO (early and late) ANOVA p value
N	918	1038
GSTO2 Genotype	0.61	0.31
APOE	< 0.0001	< 0.0001
Sex	0.127	0.02
GSTO2 Genotype * APOE	0.25	0.92

Table 5.72 Distribution of PRSS11/IVS8-36T→C Genotype and Allele Frequencies in AD Patients and Controls

	AD Cases		*Controls*		
Genotype	n	%	n	%	
TT	34	3.43	32	4.78	
CT	317	32.02	205	30.64	
CC	639	64.55	432	64.57	
Total	990		669		
Allele frequency					
T	0.194		0.201		
C	0.806		0.799		p=0.62
Age, sex and APOE adjusted OR					
	TT vs. CT+CC		1.249 (0.719,2.168;p=0.43)		

Table 5.73 Distribution of the PRSS11/IVS8-36T→C Polymorphism Among *APOE*4* and non-*APOE*4* Carriers

		Genotype Frequency			*Allele Frequency*	
*APOE*4*	*n*	*TT*	*CT*	*CC*	*T*	*C*
AD Cases	581	18	182	381	0.188	0.812
	%	3.10	31.33	65.58		
Controls	148	5	46	97	0.189	0.811
	%	3.38	31.08	65.54		
						p=0.97
*non-APOE*4*						
AD Cases	404	16	133	255	0.204	0.796
	%	3.96	32.92	63.12		
Controls	586	30	182	374	0.206	0.794
	%	5.12	31.06	63.82		
						p=0.91

Age and sex adjusted OR

	TT vs. CT+CC
*APOE*4*	1.20 (0.43,3.35;P=0.725)
*non- APOE*4*	1.25 (0.644,2.415;P=0.513)

Table 5.74 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PRSS11/IVS8-T→C Polymorphism (late onset)

	Genotype		
	TT	TC	CC
Mean AAO	71.582±1.16	72.718±0.365	72.351±0.251
sample size	29	283	577
	p value= 0.995		

Table 5.75 Sex and APOE adjusted Age-at-Onset (Mean ± SE) Among the PRSS11/IVS8-T→C Polymorphism (early and late onset)

	Genotype		
	TT	TC	CC
Mean AAO	70.705±1.662	70.291±0.483	70.072±0.339
sample size	30	327	648
	p value= 0.488		

Table 5.76 Summary of AAO Analysis Among the PRSS11/IVS8-T→C

Effect	AAO (late onset)	Age at Onset (early and late)
	ANOVA p value	ANOVA p value
N	919	1037
PRSS11 Genotype	0.981	0.497
APOE	< 0.0001	< 0.0001
Sex	0.109	0.008
PRSS11 Genotype * APOE	0.191	0.051
	0.993	

Table 5.77 Estimated 3-site GSTO1, GSTO2, PRSS11 Haplotype Distribution in AD Cases and Controls EH Methods

	SNPs				Haplotype frequency (%)			
Haplotype	GSTO1	GSTO2	PRSS11		Total	AD	C	p-value
	(Ala140Asp)	(-183A→G)	(IVS8-36T→C)		(n=1711)	(n=987)	(n=724)	
1	A	A	C		2.8	2.3	3.5	**0.04**
2	A	A	T		0.9	0.8	0.9	0.89
3	A	G	C		23.3	23.5	23.1	0.80
4	A	G	T		6.3	5.7	7.0	0.14
5	C	A	C		53.6	54.3	52.6	0.31
6	C	A	T		12.7	12.8	12.5	0.80
7	C	G	C		0.5	0.5	0.4	0.57
8	C	G	T		0.0	0.0	0.0	0.51
				ln(L)	-3432.42	-1932.47	-1496.78	
				χ^2	2287.52	1339.88	951.45	0.50[a]

[a] Calculated from the T5 statistic: $2[\ln(L)_{case} + \ln(L)_{control} - \ln(L)_{case+control}]$; df=7

5.19. Distribution of the GSTO1/Ala140Asp Polymorphism (5.62-5.64)

There was no significant difference in genotype or allele distribution between cases and controls for GSTO1/A→C (Asp→Ala) polymorphism at exon 4 (Table 5.62) The age, sex, and APOE adjusted OR for developing AD between AA and AC+CC genotypes was 1.34 (95% CI: 0.93-1.93; p=0.119). Stratification of data by *APOE*4* status showed no significant difference in either genotype or allele frequencies among *APOE*4* carriers or non- *APOE*4* carriers (Table 5.64). AAO was analyzed as a quantitative trait among GSTO1 genotypes in AD cases. However, no significant association was observed, even after adjusting for the effects of gender and APOE. (p=0.37) (Table 5.65).

5.20. Distribution of the GSTO2/-183→G Polymorphism (Tables 5.67-5.68)

The genotype (p=0.37) or allele (p=0.13) distribution of A→G polymorphism in the GSTO2 gene did not reveal any significant association with AD risk (Table 5.67). The age-, sex and APOE adjusted OR for GG genotype vs. AG+AA was 0.92 (95% CI: 0.66-1.29; p=0.63). We further stratified the data by APOE status. The OR was 0.72(95% CI: 0.42, 1.26; p=0.25) among *APOE*4* carriers, and 0.80(95% CI: 0.49-1.31; p=0.38) among non-*APOE*4* carriers after adjusting for age and sex (Table 7.68). The GSTO2 A→G polymorphism was not associated with AAO either (Table 5.69).

5.21. Distribution of the PRSS11/IVS8-36T→C Polymorphism (Tables 5.72-5.73)

The overall distribution of the PRSS11/T→C polymorphism was similar between cases and controls (p=0.43) (Table 5.72). The OR for T carriers (CT+TT vs. CC) was 1.25 (95%CI: 0.72-2.17; p=0.43). After adjusting for effect of age, sex and APOE, there was no significant effect of the PRSS11 polymorphism on AD risk (Table 5.73). Association analysis between PRSS11 and AAO did not find any significant association among cases AD (p=0.99) (Table 5.64).

5.22. Estimated 3-site GSTO1, GSTO2, PRSS11 Haplotype Distribution (Tables 5.77)

Estimated haplotype frequencies for the three markers are presented in Table 5.77. Three (haplotypes 3, 5, 6) of the 8 possible haplotypes accounted for 90% of the total chromosomes examined. Haplotype 8 was absent in the subjects examined. A marginal statistically significant difference was observed for the frequency of haplotype 1 between LOAD cases and controls (0.023 vs. 0.035; p=0.04). In order to determine the source of the haplotype 1 effect, we conducted two-site haplotype analyses and found the effect to be coming from the GSTO1 and GSTO2 polymorphisms (p=0.06). The overall haplotype frequencies were not statistically different between LOAD cases and controls (p=0.50).

6. Discussion

Alzheimer's disease (AD), a progressive, neurodegenerative disease, is a significant public health problem in the United States. It is the most common form of dementia among the elderly. Although, it has been described since 1907 (Alzheimer 1907), no cure or universally accepted effective treatment has been established. Studying factors that play a role in risk and pathogenesis of LOAD may lead to development of new therapies and give an insight to the etiology of LOAD. Both genetic and environmental factors have been implicated in the etiology of AD. Heredity has been shown to be major causal factor in LOAD (Bergem et al. 1997). In twin studies the role of genetic factors has been estimated to be as much as 74% (Bergem et al. 1997; Daw et al. 2000). Despite the evidence for substantial genetic effect in the etiology of LOAD, to date APOE which accounts for 20-30 % of AD risk (Slooter et al. 1998, Seshadri et al.1995) is the only established risk factor in the development of LOAD. A large number of candidate genes have been evaluated for development of LOAD. However, except for APOE none of them are consistently proven to be associated with LOAD. Complex heterogeneous nature of LOAD makes it difficult to find new risk factors in the development of AD. Gene-gene interaction, gene-environment interaction can explain some of the replication failures. It might make it difficult to detect mild to moderate effects. Evidence for the existence of additional candidate genes for AAO of LOAD is provided by Daw and colleagues (2000), who estimated the presence of four to seven additional loci with at least one locus making a contribution several times greater than that of APOE. In addition to this, linkage studies have identified several promising chromosomal regions to harbor additional AD genes, including chromosome 10

(reviewed in Kamboh 2004). A broad linkage peak encompassing >60 cM region between chromosome 10q21 and 10q25 has been implicated that influence both AD risk and AAO (Bertram et al. 2000; Ertekin-Taner et al. 2000; Myers et al., 2000; Li et al. 2003). There are more than 300 genes in this broad genomic region of chromosome 10 and thus the task of identifying the chromosome 10 candidate gene locus is daunting. One approach is to focus on the known candidate genes in the region. There are number of promising candidate genes in this region that are involved in either in the production, processing or clearance of Aβ peptide and include PLAU, IDE, HHEX, GSTO1, GSTO2, and PRSS11. Previously, some groups have examined the role of these genes with AD risk or AAO, but the results have been equivocal. However, most of the published studies used relatively small number of cases and controls and thus fall short of providing conclusive results. The objective of this study was to use a well powered case-control cohort to examine the role of selected positional and biological candidate genes on chromosome 10. Below, we provide the summary of our findings in relation to the published data.

6.1. Choline Acetyltransferase (CHAT)

A number of factors render CHAT a reasonable candidate gene for AD. CHAT is both a positional and functional candidate gene for AD. CHAT is mapped near the linked region on chromosome 10q11 at 50.2 cM. One of the characteristic features of AD is the degeneration and dysfunction of the cholinergic system (Bowen et al. 1976; Davies and Maloney 1976; Perry et al. 1977; Whitehouse et al. 1982) that is believed to contribute significantly to the cognitive symptoms associated with the disease. CHAT and the vesicular acetylcholine transporter

(VACHT) proteins are specifically required for cholinergic neurotransmission: CHAT catalyses the biosynthesis of the neurotransmitter acetylcholine (ACh) and VACHT it is responsible for the translocation of cytoplasmic ACh into synaptic vesicles. CHAT appears to play an important role in the neurodegeneration process, as its activity has been shown to be reduced by 50%–90% in AD patients compared with age-matched controls (Perry et al. 1978; Davies 1979) and it is correlated with the severity of dementia (Wilcock et al. 1982; Bierer et al. 1995). The relatively few effective drug treatments that slow the progressive cognitive deterioration in AD have generally targeted enhancement of the cholinergic system via cholinesterase inhibitors, such as donepezil and galanthamine, which indirectly increase the synaptic concentration of ACh (Nordberg and Svensson 1998) Thus, both CHAT and VACHT are obvious functional candidates for involvement in AD. Since the VACHT gene is located within the first intron of the CHAT gene (Berse et al. 1999) examining genetic variation in one gene may also reflect the variation in the other gene. For this reason, we restricted our focus only on the CHAT gene.

We have investigated the association of 3 polymorphisms in the CHAT gene on chromosome 10 with the risk of AD. Two of these polymorphisms are located in the coding region: exon 5/A120T (rs3810950) and exon S/D7N (rs1880676); the third one is 140 bp downstream of exon 9, 11604 G→A (rs868750). We found suggestive association with the exon 5 and intron 9 SNPs.

Our initial analysis of the CHAT/A120T polymorphism showed no association with AD. However, when the sample was stratified by APOE status, the A allele of CHAT/A120T was associated with protective effect among non APOE*4 carriers. Since we observed a modest association after multiple comparisons, these results should be interpreted with caution. Previous studies have produced variable results for the association of the CHAT/A120 T polymorphism

with AD. Originally, Mubumbila et al. (2002) reported a significant association of the CHAT/A120T polymorphism in 122 LOAD cases and 112 controls in a French-German population (OR=3.7; p=0.0005). However, it was not confirmed by subsequent studies (Schwarz et al. 2003, Harold et al. 2003), including ours. While the frequency of the A allele among controls in all studies was 24-28%, Mubumbila et al. (2002) observed a relatively high frequency of this allele in AD cases (48%), which was not the case in other studies (23-28%). Of the three published studies, only Schwarz et al. (2003) examined the distribution of the CHAT/A120T polymorphism by APOE genotype, but they did not observe any significant association. However, these sample sizes were too small for meaningful interpretation of the data.

The only comprehensive study that examined multiple SNPs in the CHAT gene comes from Harold's group (Harold et al. 2003). In their study, they identified 17 SNPs, including the three SNPs examined in our study. Their study comprised 3 independent samples, namely UK1, UK2 Caucasian groups, and UK3, a Northern Irish Caucasian group. Initially, they analyzed all SNPs in the UK1 sample and found suggestive association with the intron 9 (p=0.03) and exon S (p=0.09) SNPs. However, they could not replicate these findings in the UK2 and UK3 samples. This is possibly due to the fact that the sample sizes in each of the three groups were small (AD cases range 119-209; controls range 136-222). We examined these two SNPs in our larger case-control cohort to further asses their contribution with the risk of AD. Our study reveals both allelic (p=0.03) and genotypic (p=0.007) association of the intron 9 polymorphism with AD. The AA genotype was associated with significant AD risk (OR: 2.37; p=0.007). Stratification of data by APOE genotype revealed that association was confined among non-APOE*4 carriers only (OR: 2.94; p=0.007). Thus, our data support the initial finding in the UK1 sample that the A allele of the intron 9 polymorphism may confer significant risk in the development AD.

However, the effect seems small as this was not observed in the UK2 and UK3 samples. To date our study is the largest study that examined the association of AD with CHAT polymorphisms.

The biological implication of intron 9 association with AD is not clear. For many variants such as premature stop codons, missense mutations, mutations in consensus nucleotides of splice sites, it can easily be inferred that they are likely to contribute to a phenotype. This polymorphism may be in linkage disequilibrium with a functional polymorphism in this gene. However, we can not exclude the possibility of linkage with another marker elsewhere in the 10q11 region that is involved in the development of AD. It is also possible that the intron 9 region of the CHAT gene that encompasses the polymorphic site harbor regulatory elements and thus this polymorphic site might be functional by itself.

We also evaluated the distributions 3-site haplotypes in cases and controls. However, haplotype frequencies of the CHAT exon S and intron 9 gene polymorphisms were similar in patient and control subjects.

In addition to the association with AD risk, we also examined the association between these SNPs and AAO. We found no correlation between the three CHAT polymorphisms examined and AAO. These result are in accordance with the findings of Schwarz et al. (2003); the only other study in addition to ours that also examined the association of the exon 5 polymorphism with AAO.

In summary, we have investigated the impact of the three CHAT polymorphisms with AD risk and AAO. We detected both allelic and genotypic association of the intron 9 polymorphism with AD risk. The rare AA genotype appears to confer a modest risk for the development of AD in a recessive fashion. In addition, we observed APOE-dependent effect of the exon 5 polymorphism with AD risk. Although these associations are marginal, they suggest

the presence of putative functional variant in the CHAT gene or nearby genes. Moreover our results suggest that the exon 5 polymorphism has a potential role in earlier development of LOAD.

6.2. Plasminogen Activitory, Urinary(PLAU)

PLAU converts plasminogen to plasmin. Plasmin is involved in the processing of amyloid precursor protein, one of the hallmarks of AD. (Finckh et al. 2003) PLAU is located on chromosome 10q22.2 between two regions showing linkage to LOAD (Bertram et al., 2000); Ertekin-Taner et al. (2000); Myers et al. (2000). PLAU seems to play a significant role in the prevention of neurodegeneration process, as reduced levels of plasmin has been shown in the brain tissues of AD patients. This suggests that plasmin downregulation may cause amyloid plaque deposition in sporadic AD (Ledesma et al., 2000). Moreover, increased levels of Aβ have been observed in PLAU knockout mice (Tucker et al. 2000). Tucker et al. (2000) demonstrated that plasmin pathway is induced by aggregated Aβ, which can lead to degradation of Aβ and inhibition of its action. The same group suggested that urokinase-type plasminogen activator (uPA), PLAU's protein product, in combination with plasminogen, prevents Aβ toxicity, deposition and fibril formation. These evidences suggest that PLAU could play an important role in the pathogenesis of AD.

Previously two studies have examined the role of PLAU genetic variation with AD risk. Finckh et al. (2003) screened 347 cases and 291 controls for the PLAU/Pro141Leu polymorphism and found significant association (OR= 1.89; p= 0.0004). More recently, Myers et al. (2004) examined the role of 8 polymorphisms spanning the entire PLAU gene with AD risk, including Pro141/Leu. With the exception of the 3' UTR polymorphism that yielded a

suggestive association (p=0.15), Myers et al. (2004) did not find significant association with the remaining markers. Although, the study performed by Myers et al. (2004) covered the entire variation coding region and surrounding intron-exon junctions, and one SNP in the 5' UTR, it did not cover SNPs in the intronic and promoter region.

As a part of Seattle SNP discovery panel, the entire PLAU gene has been screened in 24 blacks and 23 Caucasians (http://pga.gs.washington.edu/data/plau_/). Among Caucasian 37, SNPs have been identified, but only 15 exist at a frequency of 5% or above. Based on their strong LD pattern (r^2=0.80), the 15 SNPs are placed into 3 bins and only one tagSNP from each bin is required for genotyping purposes. (Carson et al. 2004).

We chose these 3 tagSNP in this study that are located in intron 5, intron 9, and 3' UTR. The intron 9 and 3' UTR SNPs were also used by Myers et al. (2004) in their association study. In our study, only the 3' UTR polymorphism revealed a significant association (OR=0.71; p=0.02), which was confined to *APOE*4* carriers (OR=0.58; p=0.02). Individuals with the CC genotype appear to be protective against the risk of AD. The frequency of the CC genotype was 19 % in total controls and 15 % in total cases. However, among *APOE*4* carriers, its frequency was 23% in controls and only 15 % in cases. As noted above, among the 8 SNPs examined by Myers et al., (2004) only the 3' UTR revealed a suggestive association (p=0.15). The lack of significant results in Myers et al. (2004) study could be due to power issue, because their sample size was smaller (291 cases and 290 controls) than ours (989 cases and 690 controls). It is possible that the 3' UTR SNP by itself is functional, as these functionally independent instability-regulating sites have been found in the 3' region of uPA mRNA (Nanbu et al. 1996). The other explanation is that the 3'UTR SNP may be in LD with a functional SNP in the 3' UTR of PLAU, as by itself it has only modest effect on AD risk. Although we used the tagSNPs in

this study, if a functional SNP is not associated with a tagSNP it can easily go undetected. Since the putative functional SNP is not tightly linked with the tagSNP, it may explain the modest association observed with the 3' UTR tagSNP. In view of suggestive association seen in two studies with the PLAU/3'UTR SNP, it β be important in the future studies to screen all common and less common variants in the PLAU gene in association studies. The use of common tagSNPs (>5% minor allele frequency) in association studies assumes the common disease-common variant hypotheses (Lohmneller et al. 2003). However, recent data indicate that multiple rare variants in a gene can also explain the association with common diseases (Cohen et al. 2004).

In addition to risk, we also evaluated the role of 3 PLAU tagSNPs with AAO of AD. Suggestive association were observed with the 3' UTR (p=0.10) and intron 9 (p=0.04) polymorphisms. Interestingly the CC genotype of the 3' UTR polymorphism that was associated with AD risk had the lowest mean of AAO than the other 2 genotypes (70.97±0.48 vs. 72.33±0.31, 72.14±0.34). To our knowledge, it is the first study that examined the role of PLAU SNPs with AAO.

In summary, we have examined 3 tagSNPs in the PLAU gene and our data suggest a modest association of tagSNP with both AD risk and AAO.

6.3. Insulin-Degrading Enzyme (IDE)

In the brain, IDE has been reported to be expressed predominantly in neurons (Bernstein et al. 1999; Vekrellis et al. 2000; Cook et al. 2003). A hallmark of AD is the formation of Aβ deposits

in the brain of AD patients. That contributes to brain cell dysfunction, loss of neuron mass, and ultimately brain cell death. Studies demonstrate that Aβ is degraded by IDE (Kurochkin and Goto 1994; Qiu et al. 1998). IDE appears to play an important role in the neurodegeneration as increased Aβ levels has been shown in the brain of IDE deficient mice (Farris et al. 2003). In an in vitro study, it has been shown that recombinant rat insulin hydrolyzes Aβ peptides to products that are non neurotoxic and these new products did not deposit in amyloid plaques, suggesting a possible role of insulin in the treatment of AD (Mukherjee et al. 2001). The level of IDE is reduced 50 % in the brain of *APOE*4*-positive patients, compared to that of controls and APOE*4-negative patients (Cook et al. 2003). This indicates that reduced IDE expression is associated with a significant risk factor for AD and IDE affects Aβ metabolism by interacting with APOE. Furthermore, Hyperinsulinemia was shown to be associated with a significant decline in memory (Luchsinger et al. 2004)

A number of studies have examined the role of IDE genetic variants with AD risk, but results are not consistent. The first study that examined the IDE gene looked at the common genetic variation (>5%) in this gene covering all the coding and 5' region in relation to AD risk and found no significant association (Abraham et al. 2001). Later, same data further stratified by APOE status that yielded a possible association among non-*APOE*4* carriers (Edland et al. 2003). Studies of IDE variants in different ethnic groups yielded variable results. While IDE was associated with AD risk in an *APOE*4* dependent fashion in Han Chinese (Bian et al. 2004), no association has been found in a Japanese population in selected SNPs studied (Sakai et al 2004). Boussaha et al. (2002) with the largest cases (n=240) among all the aforementioned groups genotyped two intronic SNPs in French population and found no significant association. An additional study examined a large 276-kb region that harbors IDE gene as well in relation to

AD risk and quantitative traits associated with AD risk (Prince et al. 2003). While no association was observed with AD risk, several significant associations were found with the severity of disease as measured by quantitative traits, including Mini-Mental State Examination (MMSE) scores, microtubule-associated protein tau levels in cerebral spinal fluid, the AAO, and the degree of brain pathology. It should be emphasized, however, that as opposed to the AAO, which is static over time and provides meaningful genetic association data, the initial association of genotype and severity of disease may be difficult to interpret unless it is confirmed in independent studies. Recently, Ertekin-Taner et al. (2004) analyzed the same IDE markers previously shown to be associated with quantitative traits (Prince et al. 2003) and two additional markers, and found significant associations with Aβ42 levels and LOAD. However, the role of Aβ42 as a surrogate AD marker has some limitations; elevated levels of Aβ42 have been reported in only approximately 10 % of sporadic AD patients (Tomaoka et al. 1996). This suggest that markers associated with Aβ42 levels might represent subset AD group associated with elevated Aβ serum levels thus, additional studies in independent samples could be important in clarifying the role of IDE genetic variation in relation to quantitative traits associated with AD.

In our study we examined 5 SNPs extending locally around IDE in a large Caucasian case-control cohort. Three of these SNPs; IDE7, IDE14, and HHEX23 were haplotype tagging markers that captured the common polymorphisms (>5%) in the 276.5-kb genomic region harboring the IDE, kynesin like 1 (KNSLI), and HHEX genes (Prince et al. 2003). In addition, we examined two SNPs IGS6 and IDE8. IGS6 is outside of this haplotype block, and in a preliminary analysis (Prince et al. 2003), it has been shown to be associated with AD risk among non-*APOE*4* carriers. IDE8 has been shown to be associated with AD risk in two independent

studies (Prince et al. 2003; Edland et al. 2003). To increase the likelihood of finding an association in this region of the chromosome 10, we tested the same SNPs that were shown by others groups to be associated with AD and quantitative traits (Ertekin-Taner et al. 2004; Prince et al. 2003). In our study, neither variant was associated with LOAD or AAO when replication was sought in 1012 LOAD cases and 771 controls. Likewise, there was no difference among the cases and controls after stratifying the data by APOE status. Haplotype analysis of all typed SNPs indicated a protective effect associated with two of the haplotypes: H14/ATTCC ($p=0.0001$), and H28/GTCTC ($p=0.024$) and risk with two additional haplotypes: H13/ATTCA ($p=0.02$) and H27/GTCTA ($p=0.007$). However, these were rare haplotypes and the overall effect of all haplotypes was not significant. We conclude that analysis of our study provides no compelling evidence that common variation in the IDE gene contributes to AD susceptibility or AAO in Caucasians.

Several factors may explain the lack of association in our sample and some of previously reported positive but modest associations. First, most of the previous studies used relatively small sample sizes that are not sufficient to make a meaningful interpretation. Second, discrepancies can arise due to differences in the population studied Third, different study design between populations can lead to different outcomes: such as family-based population (Ertekin-Taner et al. 2004) or sporadic AD population (Abraham 2001; Edland 2003; Boussaha et al. 2002; Bian et al. 2004; Sakai et al. 2004). Fourth, different outcomes were studied; including AD risk, AAO or intermediate quantitative traits, MMSE scores, microtubule-associated protein tau levels in cerebral spinal fluid, and the degree of brain pathology. Fifth, different markers were studied in different investigations. We have investigated the same SNPs studied by Ertekin-Taner et al. (2004) and Prince et al. (2003), but we were unable to replicate the

association of IDE common variants with risk and AAO of AD despite using large case-control samples.

In conclusion, the results of this study do not support an association of the IDE gene with AD susceptibility or AAO. However, in our study we did not evaluate intermediate quantitative trait for AD such as Aβ levels and it is possible that the effect of IDE on AD comes through modulating this traits.

6.4. Glutathione S-transferase omega 1 and 2 (GSTO1 and GSTO2) protease serine 11 (PRSS11)

Using differential gene expression approach in brains from AD patients and controls, Li et al. (2003) found that four of the 52 differentially expressed genes (stearoyl-COA desaturase; NADH ubiquinone oxidoreductase 1β complex 8, PRSS11 and GSTO1) were located under the linkage peak for AAO on chromosome 10. Thus they targeted these genes for association analysis using a large family-based sample. Two of the four differentially expressed genes, PRSS11 and GSTO1, and a linked member of the GST omega class, GSTO2, showed significant association with AAO in AD. In order to confirm this association, we evaluated the role of reported GSTO1, GSTO2 and PRSS11 polymorphisms with AAO in 990 LOAD patients. We also assessed if these polymorphisms were associated with AD risk in a large case-control cohort. Despite using large AD cases, we were unable to confirm the association of these polymorphisms with AAO.
Li et al. (2003) obtained maximum p-values of 0.007 for GSTO1, 0.005 for GSTO2 and 0.028 for PRSS11 for their association with AAO of AD. Similar p-values for GSTO1 and GSTO2 were interpreted to be due to strong LD between the two makers. However, GSTO1/GSTO2 markers were not in LD with the PRSS11 marker. Based on the LD patterns and strength of

association results, Li et al. (2003) concluded that GSTO1/GSTO2 rather than the PRSS11 region may harbor the susceptibility alleles for AAO despite the fact the later showed the most significant difference in the gene expression profiling experiment. In addition to the association with AAO in AD, Li et al. (2003) also found significant association of GSTO1/GSTO2 polymorphisms with AAO in Parkinson disease (p=0.026, 0.042, respectively), but not with PRSS11 (p=0.06).

Although GSTO1/GSTO2 are compelling biological (Laliberte et al. 2003) and positional candidate genes and the expression and association data of Li et al. (2003) provide further credence to their possible involvement in the etiology of AD, we were unable to confirm their genetic association in our sample. Our failure to confirm the reported association with AAO is unlikely to be attributed to low power because our sample had >80% power to detect a mean AAO difference of 1.1 years between genotypes. Although Li et al. (2003) did not indicate mean AAO difference between GSTO genotypes, this difference was highly significant (p = 0.007 - 0.005) and should have been easily detected in our large sample. In our AD sample the mean AAO ± SD was 70.58 ± 8.82 years (range 33-91), while in the published family AD sample the mean AAO was 72 ± 8 years (range 40-97). Although Li et al. (2003) did not specify the number of early-onset cases (<60 years), from their mean AAO of 72 years, it appears that the bulk of their sample comprised LOAD cases, similar to what we used in this investigation. The different outcomes in two studies may be due to difference in study design, as we used sporadic AD cases whereas Li et al. (2003) used familial cases. It is possible that the effect of these genes is stronger in familial AD due to shared common genetic background than in sporadic AD, thus making it difficult to readily detect it in sporadic AD cases. To address this issue, we performed a post-hoc analysis on 126 EOAD cases (range 33 – 59 years), as 34% of EOAD cases had a

family history of dementia. However, there was no significant association with AAO in this small sample. Additional studies on familial and sporadic AD samples are needed to clarify the published association of GSTO1/GSTO2 with AAO. On the other hand, our data suggest that the GSTO1 and GSTO2 genes might modestly affect the risk of AD as revealed by haplotype analyses. Similar to our findings, recently Lee et al. (Lee et al.2004) reported no association of GSTO1 polymorphism with AAO in Caribbean Hispanics but found a modest association with AD risk.

In this study we tested the hypothesis that the reported association of GSTO1 and GSTO2 SNPs is real and thus we did not examine additional polymorphisms in these genes. Since GSTO1 and GSTO2 are potential candidate genes for AD, additional SNPs in these genes need to be analyzed to further explore their roles in the etiology of AD.

6.5. Summary

Several independent linkage studies have implicated a broad interval of > 50 Mb on chromosome 10. It is not clear whether this linkage findings indicate the existence of multiple genes or represent chance variation of the location because estimates of location of genes based on linkage studies can vary 30 cM or more for complex disorder (Roberts et al. 1999). Similar discrepancies in linkage results were found on chromosome 19 that eventually resulted in identification of APOE gene. Thus, it seems likely that at least one AD risk gene exist on chromosome 10. In this study, association studies for 14 markers on chromosome 10 were performed in a large case-control cohort comprising 1012 white LOAD subjects and 771 white

control subjects. No significant associations were observed with any of the polymorphisms examined in the HHEX, GSTO1, GSTO2, and PRSS11 genes.

Of the 3 CHAT SNPs examined, we detected both allelic and genotypic association of the intron 9 polymorphism with AD risk. The rare AA genotype appears to confer a modest risk for the development of AD in a recessive fashion (OR: 2.37; p=0.007). In addition, we observed APOE-dependent effect of the CHAT exon 5 polymorphism with AD risk (OR=0.76; p=0.046). Although these associations are modest, they suggest the presence of putative functional variants in the CHAT gene or nearby genes.

In the PLAU gene we have examined 3 tagSNPs and found a modest protective effect with one SNP in the 3' UTR (OR=0.71; p=0.02), which was confined to *APOE*4* carriers (OR=0.58; p=0.02). In our analysis of the association of the candidate genes with AAO, suggestive association were observed only with the PLAU 3' UTR (p=0.10) and intron 9 (p=0.04) polymorphisms.

In conclusion, our data on large number of AD cases and control suggest that genetic variation in two positional candidate genes (PLAU and CHAT) may affect the risk and AAO of LOAD and variation.

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