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Genetic variation in the choline acetyltransferase (CHAT) gene may be associated with the risk of Alzheimer's disease

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Abstract

Several independent linkage studies have mapped a broad susceptibility region for Alzheimer's disease (AD) on the long arm of chromosome 10. There are several biological candidate genes in this region, including choline acetyltransferase (CHAT). A number of studies have examined the role of CHAT genetic variants with AD risk and age-at-onset (AAO), but the results are equivocal. We examined the association of three Single Nucleotide Polymorphisms (SNPs) in the CHAT gene in 1001 white sporadic late-onset AD (LOAD) cases and 708 white controls. We also examined the role of these three SNP with quantitative traits of AD including AAO, disease duration, and Mini-Mental State Examination (MMSE) score. We observed both allelic and genotypic associations of the intron 9 SNP with AD risk in the total sample (p=0.029 for genotype and p=0.028 for allele frequency differences) as well as among non-*APOE*4* carriers (p=0.007 for genotype and p=0.006 for allele frequency differences). Three-site haplotype analysis confirmed that haplotypes determined by the intron 9 SNP were associated with either risk (p=0.0009) or protective (p=0.0082) effects among non-*APOE*4* carriers. The three CHAT SNPs also showed a modest association with MMSE score. Our data suggest that genetic variation in the CHAT gene may be associated with AD risk and quantitative traits related to AD.

Keywords

Alzheimer's Disease; Genetics; CHAT; Age-at-onset; MMSE

Introduction

Late onset Alzheimer disease (LOAD) is a genetically complex heterogeneous disease. Both genetic and environmental factors have been implicated in the etiology of AD. Despite the evidence for substantial genetic effect in the etiology of LOAD, to date APOE that accounts for 20–30 % of AD risk [16,17], is the only established risk factor for LOAD. A large number of candidate genes have been evaluated for the development of LOAD but none of them are consistently proven to be associated with the disease. Linkage studies have identified several promising chromosomal regions to harbor additional AD genes, including chromosomes 12, 10, 9 and 6 [reviewed in 7]. A broad linkage peak encompassing >60 cM region between chromosome 10q21 and 10q25 that influence both AD risk and AAO has been suggested [2, 4,10,13]. There are more than 300 genes in this broad genomic region of chromosome 10 and

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thus the task of identifying the chromosome 10 candidate gene is daunting. One approach is to focus on the known biological 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 among these genes is the choline acetyltransferase (CHAT) gene. CHAT is the key enzyme responsible for the synthesis of a neurotransmitter acetylcholine (ACh). Several lines of evidence support the biological relevance of CHAT in the pathogenesis of AD [1,14,19] and thus, CHAT is both a positional and biological candidate gene in the development of AD.

Previously, some groups have examined the role of CHAT genetic variation with AD risk or AAO, but the results have been equivocal [5,8,12,15]. With the exception of one study that examined multiple SNPs in the CHAT gene [5], other studies examined only one SNP in exon 5, A120T [8,12,15], and most of these 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 and large case-control cohort to examine the role of three CHAT SNPs that previously showed suggestive associations with AD risk and quantitative trait of AD.

1. Materials and Methods

2.1. Subjects

The study sample compromised 1001 white LOAD sporadic subjects and 708 white control subjects. The LOAD (=60) cases (66.7 % female, 25.8% autopsy-confirmed) were from the University of Pittsburgh Alzheimer's Disease Research Center (ADRC). The mean age of patients was 76.57.± [SD] 5.7 years with a mean AAO of 72.29 ± 6.33 years. Clinical diagnoses of the patients were made according to the NINCDS/ADRDA criteria [11]. 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. The controls (61.5% female, mean age 75.20±5.63 years) were recruited from the same Western Pennsylvania region as the cases, and were determined to be cognitively intact following extensive clinical examination as described elsewhere [18]. For the post-hoc AAO analysis, we also included 127 early-onset (mean age 58.2 ± 6.6; mean AAO 52.8 ± 5.6 years) sporadic white AD cases (EOAD) in addition to the 1001 LOAD cases, and reanalyzed the AAO data on 1128 AD cases. This study was approved by the University of Pittsburgh Institutional Review Board.

2.2. Genotyping using Pyrosequencing

Two coding SNPS, exon 5/A120T (rs3810950) and exon S/D7N (rs1880676) and one SNP located 140 bp downstream of exon 9, 11604 G? A (rs868750) were screened by pyrosequencing (see detail in the Supplementary Material).

2.3. Statistical Analyses

Allele frequencies were calculated by the allele counting method. Goodness of fit to Hardy-Weinberg expected proportions was examined by chi-square test. The differences in genotype frequencies between cases and controls were tested by chi-square tests. The pair-wise linkage disequilibrium (LD) between markers was estimated using the D’ method [9]. Multiple logistic regression models were used to examine the association of each genetic marker with AD. The estimated odds ratios (ORs) were adjusted for the effects of significant covariates such as age, sex and APOE. The mean AAO between different genotype groups were compared using one-way analysis of variance (ANOVA) and adjusted for the effects of significant covariates. All computations were performed using R statistical 1.8.1 program [6]. The three-site haplotype frequencies were estimated using the Expectation-Maximization algorithm in the EH software program (http://linkage.rockefeller.edu/ott/eh.htm). Haplotype frequencies between AD cases

and controls were compared using z-test. Kaplan Maier survival analysis was also used to compare the AAO between genotypes.

2. Results

All three CHAT sites were in significant linkage disequilibrium with each other ($p<0.001$). The distribution of 3 CHAT SNPs is presented in Table 1. The most significant association was observed with the intron 9 SNP. The frequency of the intron 9/A allele was significantly higher in cases than controls (20.2% vs. 17.2%; p=0.028). The age-, sex- and APOE-adjusted OR for AA vs. AG+GG genotypes was 2.37(95% CI: 1.19–4.73; p=0.01). Stratification of the intron 9 SNP data by APOE genotype revealed that association was confined among non-*APOE*4* carriers were the age- and sex- adjusted OR for AA vs. AG+GG genotypes was 2.94 (95% CI, 1.33–6.51; p=0.007). This suggests that the association observed in the total sample was attributed to non-*APOE*4* carriers. The stratification of genotype data of the other two SNPs by APOE revealed significant associations among non-*APOE*4* carriers. The frequencies of the exon 5/A (23.4% vs. 28.1%; p=0.02) and exon S (23.3% vs. 27.2%; p=0.056) alleles were lower in cases than controls.

In addition to single site analysis, we also performed 3-site haplotype analysis (Table 2). Haplotype analyses were carried out on the total sample and sample stratified by APOE genotype. Four haplotypes (H1–H4) accounted for more than 99% of all genotype combination in the total sample. A suggestive association was observed with haplotypes H2 (p=0.063) and H3 (p=0.094) in the total sample, which became significant among non-*APOE*4* carriers where haplotype H2 was associated with risk (20.1 % in cases vs. 15.7 % in controls; p=0.0009) and haplotype H3 was protective (22.1 % in cases vs. 26.2 % in controls; p=0.0082). The overall haplotype distribution between cases and controls was also significant among non-*APOE*4* carriers (p=0.038).

We also examined the association of CHAT SNPs with quantitative traits related to AD namely disease duration, AAO, and MMSE scores (Table 1 in the Supplementary Material). The exon 5 SNP showed a marginal association with AAO (p=0.07) after adjusting for sex and *APOE*4*, which became significant when comparison was made between the AA and AG+GG genotypes (70.89±0.75 vs. 72.49± 0.21; p=0.05.) Kaplan-Maier survival analysis showed that the effect of exon 5 polymorphism was mainly confined to between the ages of 70 and 80 years (p=0.036) (Fig. 1 in the Supplementary Material). No significant association was found between disease duration and AD with any of the CHAT SNPs examined. The severity of cognitive impairment was assessed from the MMSE score measured at baseline and during the last examination. Although CHAT SNPs did not reveal any effect on baseline MMSE score, they showed a significant correlation with the last MMSE score. Comparisons of AA homozygous versus AG+GG were significant for exon S (p=0.03), exon 5 (p=0.02) and intron 9 (p=0.05). When we included early onset cases, the results became more significant for MMSE score (p=0.016 for exon S, 0.018 for exon 5 and 0.05 for intron 9). Logistic regression analyses to determine possible interaction between APOE and CHAT SNPs for affecting AAO and MMSE scores revealed no interaction.

3. Discussion

We have investigated the association of 3 SNPs in the CHAT gene on chromosome 10 with AD risk and quantitative traits related to AD. The selection of these SNPs was based on their prior reported associations with AD risk in at least one case-control sample. The objective of this study was to asses their associations with AD risk in a large case-control sample for a meaningful interpretation of the data. We found suggestive associations of the exon 5 and intron 9 SNPs with the risk of AD. In addition, we also found suggestive associations of all 3 SNPs

with cognitive function as measured by MMSE score. Originally, Mubumbila et al. [12] reported a significant association of the exon 5 SNP in 122 LOAD cases and 112 controls in a French-German population, but it was not confirmed in subsequent studies [3,5,15]. Recently, Kim et al. [8] reported a significant association of this SNP among *APOE*4* carriers in a Korean sample. In our large case-control sample we did not see significant association either in the total sample or among *APOE*4* carriers, but we observed an association among non-*APOE*4* carriers. However this is a provisional finding that needs to be replicated in a comparable large sample.

In our cohort, the most significant association was found with the intron 9 SNP in the total sample (p=0.028) and among non-*APOE*4* carriers (p=0.006). In a pilot study of 119 AD cases and 116 controls, Harold et al. [5] reported significantly lower frequency of the A allele of the intron 9 SNP in AD cases than controls (15% vs. 23 %; p=0.034). In two replication samples, Harold et al. [5] found similar but non-significant trend in one sample but not in the other sample. The total number of 463 AD cases and 469 controls from three samples demonstrated a modest increase in the frequency of A allele in AD cases than controls (20.7 % vs. 18.5 %; p=0.238), which is similar to what we observed in our larger cohort of 989 cases and 706 controls (20.2 % vs. 17.2 %; p=0.028). Because of the same trend observed in our study and that of Harold et al's study [5], we performed a meta analysis after pooling AD cases (n=1452) and controls (n=1175) from both studies. In the meta analysis the frequency of the A allele was significantly higher in cases than controls (20.4 % vs. 17.7 %; p=0.013). The unadjusted OR between AA and AG+GG genotypes was 1.81(95% CI: 1.13–2.89; p=0.013). In our sample the association of the intron 9 SNP was confined to non-*APOE*4* carriers. Since Harold et al. [5] did not present the APOE stratified data, we could not analyze the APOE specific risk in the meta analysis.

The biological implication of the association of intron 9 SNP with AD is not clear. For variants effecting protein structure or length or specific functions, it can be inferred that they are likely to contribute to a phenotype. The intron 9 SNP may be in linkage disequilibrium with a functional SNP 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 sequence of the CHAT gene that encompasses the polymorphic site harbor regulatory elements and thus this polymorphic site might be functional by itself. Our 3-site haplotype analysis strongly suggests that either intron 9 SNP by itself or a variant associated with intron 9 SNP in the same haplotype is associated with AD risk in an APOE-dependent fashion (Table 2). In the total sample, Haplotype H2 carrying the intron 9/A allele suggested AD risk (p=0.06) and haplotype carrying the intron 9 /G allele suggested protection against AD risk (p=0.09) and these differences became significant among non-*APOE*4* carriers (haplotype H2: p=0.0009; haplotype H3: p=0.0082). Recently a CHAT intron 10 SNP (rs2177369) was found to be associated with AD risk in a pilot sample of 202 cases and 295 controls, but not in a replication sample of 179 cases and 295 controls [2]. The intron 10 SNP is about 10 kb from the intron 9 SNP and it is likely that both SNP may be in LD, although their frequencies are different from each other.

In addition to the AD risk, we also observed a suggestive association between all three SNPs and MMSE scores at last examination. Significantly, the A allele of intron 9 SNP and G alleles of exon S and exon 5 SNPs, which were associated with AD risk in haplotype H2 combination (G-G-A) were also associated with lower MMSE scores. Although the association of the CHAT SNPs with MMSE scores is modest, the parallel association of genotypes with MMSE scores and AD risk suggest a genuine relationship between CHAT genetic variation and AD risk. Additional comprehensive studies assessing the role of full genetic variation in the CHAT gene may be helpful in resolving the role of CHAT in the etiology of AD. Our study also underscores

the important point that yet to be identified genes for LOAD have small to modest effects and they can only be detected using a large and well-powered case-control samples [7].

Supplementary Material

Materials and Methods

Genotyping and Pyrosequencing—Exon 5/A120T (rs3810950), exon S/D7N (rs1880676) and intron 9 11604 G? A (rs868750) SNPs were screened by pyrosequencing using duplex, (rs3810950 and rs868750) or simplex (rs1880676) assays. The following PCR (F, R) and sequencing (S) primers were used: rs3810950; F_ACTCACCAAGACGCCCATC (biotinylated), R_ACTGCTGGGAGTTTTTGCT, S_GGTCCCCCGTAAGAT; rs18806796 F_CCAGAGATGTGGCCGGAAT, R_CTCTTTCCCACTAGCTTCTCAAGG (biotinylated), S_CTGTGCTCAGTGCTTC; rs868750; F_GTGGCCATGCGTTCACGT, (biotinylated) R_CGGCTCTCATTCTTAGAAGGCAAC, S_ACTGGAAGTAGGGGC. All pyrosequencing chemicals were obtained from Biotage (Uppsala, Sweden), unless otherwise indicated. Pyrosequencing was carried out according to the manufacturer's instructions. Briefly, for each genotype determination, single-stranded DNA was purified from the 25-μl PCR reaction with streptavidin sepharose (Amersham Biosciences, Piscataway, NJ). The streptavidin sepharose with captured DNA were then sequentially washed with 70% ethanol followed by 0.2 M NaOH and finally with washing buffer (10 mM Tris-acetate, pH 7.6). Genotypes were determined in a 96-well reaction plate format by annealing 10 pmol of the corresponding sequence-specific primer to the single-stranded DNA. Annealing was conducted in the annealing buffer by heating the sample to 90 °C and allowing it to cool to room temperature. Sequencing was performed on the PSQ HS 96 system and results were analyzed using the PSQ HS 96 SNP software. The success rate for genotyping was 90%. 10% of the samples were regenotyped for confirmation.

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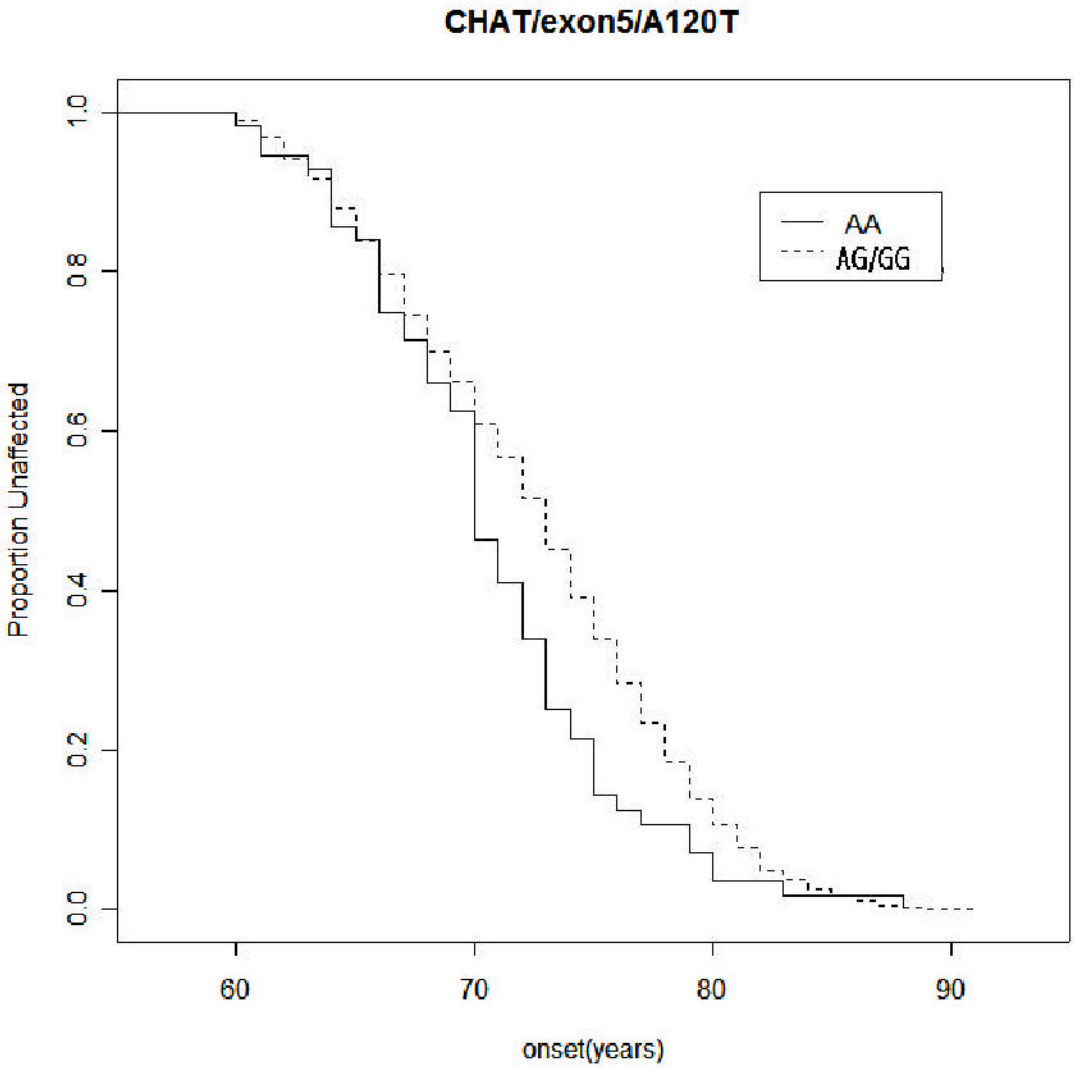

Supplement Figure 1.
Kaplan Maier Survival analysis for age-at-onset (AAO) among exon 5/A120T genotypes. The comparison of AAO is made between AA and AG+GG genotype. The AAO is plotted against proportion of unaffected by AD.

Table 1

Distribution of CHAT/ exon S, exon 5 and intron 9 polymorphisms in LOAD cases and controls

Polymorphism	n	Genotype			A/G Allele (%)	p-value Comparison	
		AA	AG	GG		Allele	Genotype
Exon S/D7N (rs1880676)		n (%)	n (%)	n (%)			
Total AD Cases	901	62 (6.2)	376 (37.6)	563 (56.2)	25.0 / 75.0	0.19	0.38
Total Controls	705	44 (6.2)	292 (41.4)	369 (52.3)	27.0 / 73.0		
*APOE*4* / AD Cases	575	41 (7.1)	219 (38.1)	315 (54.8)	26.2 / 73.8	0.99	0.98
*APOE*4* / Controls	144	10 (6.9)	56 (38.9)	78 (54.2)	26.4 / 73.6		
non-*APOE*4* / AD Cases	407	19 (4.7)	152 (37.3)	236 (58.0)	23.3 / 76.7	**0.056**	0.15
non-*APOE*4* / Controls	558	34 (6.1)	235 (42.1)	289 (51.8)	27.2 / 72.8		
Exon 5/A120T(rs3810950)							
Total AD Cases	999	60 (6.0)	377 (37.7)	562 (56.3)	24.9 / 75.1	**0.054**	0.24
Total Controls	708	49 (6.9)	296 (41.8)	363 (51.3)	27.8 / 72.2		
*APOE*4* / AD Cases	581	40 (6.9)	220 (37.9)	321 (55.2)	25.8 / 74.2	0.79	0.96
*APOE*4* / Controls	141	10 (7.1)	55 (39.0)	76 (53.9)	26.6 / 73.4		
non-*APOE*4* / AD Cases	408	18 (4.4)	155 (38.0)	235 (57.6)	23.4 / 76.6	**0.02**	**0.057**
non-*APOE*4* / Controls	566	39 (6.9)	240 (42.4)	287 (50.7)	28.1 / 71.9		
Intron 9/11604G>A(rs868750)							
Total AD Cases	989	39 (3.9)	322 (32.6)	628 (63.5)	20.2 / 79.8	**0.028**	**0.029**
Total Controls	706	13 (1.8)	217 (30.7)	476 (67.4)	17.2 / 82.8		
*APOE*4* / AD Cases	579	19 (3.3)	190 (32.8)	370 (64.9)	19.7 / 80.3	0.97	0.76
*APOE*4* / Controls	140	3 (2.1)	49 (35.0)	88 (62.9)	19.6/80.4		
non-*APOE*4* / AD Cases	401	20 (5.0)	131(32.7)	250 (62.3)	21.3 / 78.7	**0.006**	**0.007**
non-*APOE*4* / Controls	565	10 (1.8)	168 (29.7)	387 (68.5)	16.6 / 83.4		

Table 2

CHAT Haplotype distribution in the combined sample and samples stratified by APOE genotypes

Haplotype	Haplotype			Combined				non-*APOE*4* Carriers				*APOE*4*Carriers			
	Exon S	Exon 5	Intron 9	Total	AD	Control	*p′*	Total	AD	Control	*p′*	Total	AD	Control	*P′*
H1	G	G	G	0.557	0.557	0.556	0.947	0.562	0.564	0.562	0.926	0.549	0.553	0.529	0.187
H2	G	G	A	0.180	0.190	0.165	0.063	0.176	0.201	0.157	0.001	0.186	0.183	0.202	0.181
H3	A	A	G	0.245	0.234	0.260	0.094	0.245	0.221	0.262	0.008	0.244	0.241	0.257	0.295
H4	A	A	A	0.011	0.013	0.007	0.107	0.009	0.010	0.009	0.659	0.013	0.015	0.000	0.000
				1640	961	679		933	391	542		697	561	136	
				−2757.6	−1617.72	−1134.2	0.12[a]	−1546.94	−642.26	−897.27	0.038[a]	−1189	−957.23	−230.33	0.80[a]

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

Supplement Table 1

Adjusted mean quantitative traits (±SD) among genotypes

Polymorphisms	AA	AG	GG	p-value
Exon S/D7N (rs1880676)				
AAO*	71.10±0.83 (55)	72.17±0.33 (346)	72.68±0.27 (516)	0.14
Disease duration**	9.45±1.36 (12)	10.20±0.53 (69)	9.57±0.35 (124)	0.57
MMSE baseline†	22.86±0.47 (87)	21.99±0.20 (557)	21.92±0.17 (775)	0.21
MMSE last†	21.11±0.65 (67)	19.50±0.27 (488)	19.69±0.21 (687)	0.08 (0.03)[a]
Exon 5/A120T (rs3810950)				
AAO*	70.89±0.75 (54)	72.19±0.33 (350)	72.70±0.27 (520)	0.07 (0.05)[a]
Disease duration**	9.34±1.21 (13)	10.27±0.54 (68)	9.73±0.35 (120)	0.59
MMSE baseline†	22.54±0.46 (95)	21.84±0.20 (618)	21.66±0.17 (857)	0.20
MMSE last†	21.13±0.63 (71)	19.57±0.26 (496)	19.71±0.21 (693)	0.08 (0.02)[a]
Intron 9/11604G>A (rs868750)				
AAO*	72.81±0.81 (37)	72.57±0.36 (295)	72.36±0.26 (585)	0.85
Disease duration**	8.55±0.90 (15)	10.26±0.50 (58)	9.85±37 (127)	0.35
MMSE baseline†	21.02±0.90 (46)	21.80±0.22 (470)	21.98±1.16 (915)	0.36
MMSE last†	18.17±0.94 (44)	19.43±0.27 (417)	19.93±0.20 (792)	0.05 (0.05)[a]

* Adjusted for gender and *APOE*4*

** Adjusted for gender, *APOE*4* and AAO

† Adjusted for age controls, AAO cases, disease status, gender and *APOE*4*

[a] p-values in parentheses are for comparison between the AA and AG+GG genotypes

Number in parentheses indicate number of subjects in each genotype