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Association and heterogeneity at the *GAPDH* locus in Alzheimer's disease

M. Allen[1,2], **C. Cox**[1], **O. Belbin**[1], **L. Ma**[1], **G. D. Bisceglio**[1], **S. L. Wilcox**[1], **C. C. Howell**[1], **T. A. Hunter**[1], **O. Culley**[1], **L. P. Walker**[1], **M. M. Carrasquillo, Ph.D.**[1], **D. W. Dickson, M.D.**[1], **R. C. Petersen, M.D., Ph.D**[3], **N. R. Graff-Radford**[4], **S. G. Younkin**[1,#], and **N. Ertekin-Taner**[1,4,#]

[1]Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL 32224, USA

[2]Human Genetics Unit, Institute of Genetics and Molecular Medicine, Medical Research Council, Edinburgh, United Kingdom

[3]Department of Neurology and the Mayo Alzheimer Disease Research Center, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

[4]Department of Neurology, Mayo Clinic College of Medicine, Jacksonville, FL 32224, USA

Abstract

Glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) and its paralogues were implicated in late-onset Alzheimer's disease (LOAD), although the strength and direction of association have not been consistent. We genotyped three previously reported SNPs (rs3741916-*GAPDH* 5'UTR, rs2029721-*pGAPD* and rs4806173-*GAPDHS*) in three case-control series (2112 cases and 3808 controls). *R*s3741916 showed the strongest LOAD association (p=0.003). The minor allele of rs3741916 showed a protective effect in our combined series (OR=0.87, 95% confidence interval (CI)=0.79–0.96). This is consistent with results from the two published follow-up studies and in

[#]To whom correspondence should be address: taner.nilufer@mayo.edu or younkin.steven@mayo.edu, (Phone: ++1-904-953-6424, FAX: ++1-904-953-7370).

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opposite direction of the original report. Meta-analysis of the published series with ours suggests presence of heterogeneity (Breslow-Day p<0.0001). Meta-analysis of only the follow-up series including ours revealed a significant protective effect for the minor allele of rs3741916 (OR=0.85, 95% CI=0.76–0.96, p=0.009). Our results support the presence of LOAD variants and heterogeneity at the *GAPDH* locus. The most promising rs3741916 variant is unlikely to be functional given opposing effects in different series. Identification of functional variant(s) in this region likely awaits deep sequencing.

Search Terms

Alzheimer's disease; Association studies in genetics; Case control studies

Introduction

Late Onset Alzheimer's disease (LOAD) is a complex disease with an estimated 80% genetic component(Gatz, et al., 2006). Until recently only the *APOE4* allele showed consistent, reproducible association with LOAD (reviewed in(Ertekin-Taner, 2007)). The large LOAD GWAS published in the past two years have identified five novel LOAD genes with genome-wide significance(Carrasquillo, et al., 2009,Harold, et al., 2009,Lambert, et al., 2009,Reiman, et al., 2007). Three (*CLU, PICALM, CR1*) of these genes achieved genome-wide significance in the first stage of the two largest LOAD GWAS to date(Harold, et al., 2009,Lambert, et al., 2009). The two remaining genes (*PCDH11X* and *GAB2*) reached this level of significance in the combined Stage 1 and 2 analyses(Carrasquillo, et al., 2009,Reiman, et al., 2007). An additional 500+ LOAD candidate genes and alleles have been published(Bertram, et al., 2007) but most failed to show consistent replication. One such example is the *GAPDH* locus on chromosome 12p. GAPDH encodes an glyceraldehyde 3-phosphate dehydrogenase, most commonly known for its role in glycolysis, but which has also been recently implicated in neuronal apoptosis and transcriptional activation(Colell, et al., 2007,Nakajima, et al., 2009).. *GAPDH* is located on chromosome 12p proximal to a LOAD linkage peak described in multiple studies(12–15), making it both a positional and functional candidate LOAD gene.

In 2004, Li *et al.*(Li, et al., 2004) reported replicable association of multiple SNPs at the *GAPDH* locus and its paralogues, *GAPDHS* (on 19q) and *pGAPD* (on 12q), with LOAD in up to four Caucasian case-control series. This study focused on rs3741916 in the 5'UTR of *GAPDH*, rs4806173 in intron 1 of *GAPDHS* and rs2029721, a missense mutation, in *pGAPD*. All three SNPs were also analyzed in follow-up studies by Lin et al. (9), who analyzed a Caucasian case-control and a Caucasian family-based series. GAPDH SNP rs3741916 was also assessed by Lee et all (10), who analyzed one Caucasian and one Carribbean-Hispanic family-based series in addition to a Caucasian case-control series.

In the initial study of Li et al. (8), the minor allele of rs3741916 was significantly associated with increased risk of LOAD but in both follow up studies (9, 10) it was significantly associated with decreased risk These results suggest that the association of rs3741916 with LOAD may be influenced by genetic and/or environmental factors that vary among the populations studied. Significant series to series heterogeneity of this sort, with increased risk in some studies and decreased risk in others, is relatively common in genetic association studies of LOAD and other genetically complex diseases. Given the multiple, independent LOAD associations reported for SNPs in *GAPDH* and its paralogues, it seemed likely to us that variants in these genes could have a complicated effect on LOAD pathogenesis. To investigate this possibility further, we genotyped the three SNPs that were previously reported (8–10) to show significant association (rs3741916, rs2029721, and rs4806173) in

three additional case-control series with a combined total of 5920 subjects (2112 cases and 3808 controls). We then analyzed these SNPs using models identical to those employed in the previous studies to assess the same stratified sets of subjects that were analyzed in those studies. To characterize the association at these loci more fully, we assessed an additional 22 SNPs in these genes.

Materials and Methods

Patient samples

Two independent clinically diagnosed series of late-onset AD (LOAD) cases (age of diagnosis > 60) and elderly controls (age at evaluation >60) were collected at Mayo Clinic Jacksonville (JS series; 882 cases and 986 controls) and Mayo Clinic Rochester (RS series; 640 cases and 2460 controls), in addition to an autopsy confirmed series of elderly AD cases maintained at the Brain Bank at Mayo Clinic Jacksonville (AUT; 590 cases and 362 controls, age at death >60). These three series combined have 2112 cases and 3808 controls; details of which can be found in Table 1.

All subjects from the JS and RS series were diagnosed by a Mayo Clinic neurologist. The neurologist confirmed a Clinical Dementia Rating score of 0 for all subjects enrolled as controls; cases had diagnoses of possible or probable AD made according to NINCDS-ADRDA criteria(McKhann G, et al., 1984). In the autopsy-confirmed series all brains were evaluated by the neuropathologist, Dr. Dennis Dickson where diagnosis of definite AD was also made according to NINCDS-ADRDA criteria. This study was approved by the appropriate institutional review board and appropriate informed consent was obtained from all participants. These series have previously been used in our studies including the recent Mayo Clinic late-onset AD genome-wide association study(Carrasquillo, et al., 2009).

SNP selection

Three SNPs reported in the initial study (rs3741916, rs2029721, rs4806173)(Li, et al., 2004) were genotyped in our complete case-control series with ages at diagnosis/evaluation/death above 60 years. An additional 22 SNPs were successfully assessed in the 60–78 age group. These SNPs were selected according to the criteria outlined in the Supplementary Text.

Genotyping

Three platforms were used for genotyping; Taqman, Sequenom and Illumina. The details of genotyping methods are in the Supplementary Text.

Statistical analysis

Single SNP association analysis—Each SNP was assessed individually for association with LOAD by multivariate logistic regression analysis using an allelic dosage model, adjusted for the following covariates: presence of an *APOE4* allele (0,1), age at diagnosis/evaluation/death and gender.

In order to accurately replicate the tests from the published studies we analyzed the 3 key SNPs using the “best model” described by each prior study(Li, et al., 2004)-(Lee, et al., 2008). Furthermore we tested each of these three SNPs for difference in effect based on the following covariate strata: *APOE4* allele +/−, Age > vs. ≤ 78 years and male vs. female gender. Breslow-Day tests for each of these strata did not identify significant differences in effect for each of the 3 key SNPs (Breslow-Day p-value > 0.3).

Meta-Analysis

To perform meta-analysis of all published rs3741916 allelic associations and our series, allelic counts were calculated from the reported allelic frequency and sample size information, when available(Lee, et al., 2008,Li, et al., 2004). Breslow-Day test for non-compatibility was used to test for series heterogeneity. Test statistics are reported for each series and pooled test statistics are reported using the random effects model (DerSimonian-Laird).

Linkage Disequilibrium Analysis

Analysis (solid spine of LD) by HaploView(Barrett, et al., 2005) of the genotypes for all 25 SNPs (3 key SNPs initially genotyped and 22 subsequent SNPs) in our 60–78 year age group was used to identify LD blocks in *GAPDH* (Figures 2 a–b) and *GAPDHS* (Figure A1–2). To compare the LD surrounding the rs3741916 SNP with other Caucasian subjects, we analyzed downloaded HapMap data according to genome build 36 and assessed it in Haploview. Only a subset of our genotyped *GAPDH* SNPs were available in HapMap. Figure 2c depicts the LD plot of this subset in our series and figure 2d is that in the HapMap Caucasian (CEU) series.

Results

Replication analysis of SNPs previously reported by others

The demographics of the three case-control series that we analyzed are summarized in Table 1. Table 2 compares the results from previous studies of rs3741916, rs2029721, and rs4806173 with the results we obtained for each case-control series and for the three series combined.

***GAPDH* SNP rs3741916—**In the original study of rs3741916, Li *et al.*(8) showed strongest association in the *APOE4*- group. Using an allelic association model to analyze the *APOE4*- group in their combined series (Table 2*A*), they found that the minor allele of rs3741916 was associated with (p=0.008) *increased* risk of LOAD (OR=1.27, 95%CI=1.06–1.53). When we used the same model to analyze the *APOE4*- subjects in our AUT series (Table 2*A*), we found that the minor allele of rs3741916 was associated with (p=0.042) *decreased* risk of LOAD (OR=0.73, 95%CI = 0.54–0.99). There was, however, no evidence of association when we analyzed the *APOE4*- group in our JS, RS, or combined series (Table 2*A*).

In a follow up study of rs3741916, *Lin et al*. found strongest evidence of association in their young age group with onset below the series mean. Using logistic regression under a dominant model to analyze the young age group (Table 2*B*), they found that the minor allele of rs3741916 was associated with (p=0.002) *decreased* risk of LOAD (OR=0.39, 95%CI=0.21–0.70). When we used the same model to analyze the young age group with age at diagnosis/evaluation below our mean of 78 years, none of our series showed significant association. (Table 2*B*).

In another follow-up study, *Lee et al*. analyzed their Northern European case control series using an allelic association model. This analysis (Table 2*C*) showed that the minor allele of rs3741916 was associated with (p=0.027) *decreased* risk of LOAD. When we analyzed our combined series in the same way (Table 2*C*), we also found that the minor allele of rs3741916 was associated with ($p=8\times10^{-4}$) *decreased* risk of LOAD (OR=0.86, 95%CI=0.79–0.94). Moreover, each of our 3 series trended toward decreased risk with ORs ranging from 0.81–0.91 and p values ranging from 0.053–0.212.

To evaluate rs3741916 for series to series heterogeneity based on *APOE4*, age and gender, we stratified our combined series by *APOE4* (+ vs. −), age (> vs ≤ 78 years) and gender (male vs. female) and did not find any significant difference between the groups (Breslow-Day test $P > 0.32$).

***pGAPD* SNP rs2029721—**In the initial study of rs2029721, Li *et al.*(8) showed strongest association in the older age group with onset above the series mean. Using an allelic association model to analyze this group (Table 2*D*), they found that the minor allele of rs2029721 was associated with (p=0.018) decreased risk of LOAD (OR=0.80, 95%CI=0.68–0.97). In follow-up, using the same model to analyze their older group, *Lin et al*.(Lin, et al., 2006) also found that the minor allele was associated with (p=0.004) decreased risk of LOAD (Table 2*D*). When they analyzed their entire series, Lin et al. also found that the minor allele of rs2029721 was associated with significantly decreased risk (data not shown). Using the same allelic association model, we did not identify any significant association of this SNP with LOAD in the older age group of our individual or combined series (Table 2*D*). Analysis of all subjects in each series or in the combined series also yielded no significant association (data not shown).

GAPDHS SNP rs4806173—In the initial study of rs4806173, Li *et al*.(8) showed strongest association in the young age group with onset below the series mean. Using an allelic association model to analyze this group (Table 2*E*), they found that the minor allele of rs4806173 was associated with (p=0.0003) decreased risk of LOAD (OR=0.66, 95%CI=0.55–0.80). When we used the same model to analyze the young age group in our individual or combined series, we did not observe any significant association with the minor allele of this SNP (Table 2*E*).

Logistic regression using an additive model with covariates

To explore a conventional additive model while controlling for covariates, we analyzed the three key SNPs by logistic regression with covariates age at diagnosis/evaluation/death, gender and presence of an *APOE4* allele in our 3 series, individually and combined. We also analyzed each of these series after stratifying by mean age at diagnosis/evaluation/death (≥ or ≤ 78 years), gender and presence or absence of an *APOE4* allele.

As expected from our replication analyses, *GAPDH* SNP rs3741916 was the only SNP that showed significant association in any of our series (Table 3). Using the additive model with covariates, rs3741916 showed association in the combined series (p=0.003), where the minor allele was associated with decreased risk of LOAD (OR= 0.87, 95%CI=0.79–0.96). Each of the 3 series had ORs associated with reduced risk of LOAD, and the AUT series achieved significance (p=0.047). When corrected for the 3 original SNPs tested in this study, the rs3741916 association in our combined series would still be significant (p=3×0.003=0.009). However, when the stringent Bonferroni correction is applied for all 25 SNPs tested in this study, this overall association becomes marginal (p=25×0.003=0.075).

The other 2 SNPs did not reach significance in the combined series, under this model, although rs2029721 was significantly risky in the JS series (OR=1.39, 95%CI=1.07–1.81) and also had a risky trend in the combined series (OR=1.14, 95%CI=0.98–1.31). The stratum that yielded the most significant results in the combined group using the additive logistic regression approach is shown in Table 3.

Meta-analysis

We performed a meta-analysis of *GAPDH* SNP rs3741916 (Figure 1) because it had the strongest evidence of association in our series and showed association with AD in all

previously published series. Allele counts for rs3741916 were calculated for the previously reported case control series where allele frequencies and series sizes were available. In Figure 1*a*, the "Wash", "UCSD", "Linkage" and "UK" series are from the initial publication (Li, et al., 2004) and the "NE" series is from Lee *et al*. There was insufficient data to calculate the allele counts for the Lin *et al*. study (Lin, et al., 2006). As shown Figure 1*a*, three of the four series from the initial study are opposite in direction to all of the follow-up studies, leading to significant series-to-series heterogeneity for rs3741916 (Breslow-Day p value < 0.0001). When the first, exploratory series (WashU) from the initial study (Li *et al*) was removed from the meta analysis (Figure 1*b*), the pooled OR estimate for the minor allele of rs3741916 was 0.95 (95%CI=0.81–1.10); and there was still evidence for significant series-to-series heterogeneity for rs3741916 (Breslow-Day p value = 0.0003).

When the four series from the inital study (Li *et al*) were removed and the four follow-up series (NE, JS, RS, and AUT) were analyzed, meta-analysis yielded a pooled OR estimate of 0.85 (95%CI=0.76–0.96) for the minor allele G of rs3741916 (random effects p=0.0094) (Figure 1.*c*). We expect that addition of the Lin *et al* series to the meta-analysis would further improve this estimate, since that series reported significant association in the same direction as the four other follow-up series.

We assessed LD amongst our 12 *GAPDH* SNPs (Figures 2a–b). We also downloaded HapMap data of this region and determined that 8 of our 12 SNPs had data in the Caucasian HapMap subjects (CEU), where we assessed LD (Figure 2d) in comparison to that in our study population (Figure 2c). We determined that the extent of LD between rs3741916 and its surrounding SNPs was slightly different between these two datasets, which may be one potential source of heterogeneity, though the extent of heterogeneity in LD between this SNP and rarer functional variants in this region may not be possible to fully appreciate based on the LD plots for these available SNP.

Discussion

GAPDH is an excellent LOAD candidate gene given the genetic linkage(Mayeux, et al., 2002,Myers, et al., 2002,Rogaeva E, et al., 1998,Scott WK, et al., 2000) and association(Lee, et al., 2008,Li, et al., 2004,Lin, et al., 2006) findings reported at this locus and functional evidence for its role in neurodegeneration(Colell, et al., 2007,Nakajima, et al., 2009). Three previous studies analyzed SNPs at the *GAPDH* locus and/or its paralogues in a total of 6 case-control and 3 family-based series. Using models identical to those employed in the previous studies, we evaluated three SNPs previously reported to show significant association, rs3741916, rs2029721, and rs4806173. We also analyzed 22 additional SNPs (Supplementary Text) in *GAPDH* and *GAPDHS*.

The only SNP that showed significant association in our study was rs3741916. This SNP yielded nominally significant associations with LOAD in each of the three previous studies, but the minor allele was associated with increased risk of LOAD in the initial study and with decreased risk in the two Caucasian follow-up studies. In our combined series, rs3741916 was significantly associated with decreased risk of LOAD and trended toward association with decreased risk in each of the 3 individual series. Meta-analysis of our 3 case-control series and a series from a published follow-up study with sufficient data for analysis(Lee, et al., 2008) showed significant association with decreased risk and no evidence of heterogeneity. However, meta-analysis that included the initial series as well as these follow-up series showed highly significant heterogeneity. Thus, in the studies performed to date, the minor allele of rs3741916 was associated with decreased risk of LOAD in the majority of the series. There is, however, marked heterogeneity because in 3 series from the

original study(Lee, et al., 2008,Li, et al., 2004,Lin, et al., 2006), the minor allele was associated with significantly increased risk.

Heterogeneity for candidate LOAD loci is a common problem(Newton-Cheh and Hirschhorn, 2005). Small sample size can lead to false positive results in initial studies as well as false negative results in underpowered follow-up studies. Although small sample size can explain lack of replication, it is unlikely to account for results like those for rs3741916 where there is significant association with *increased* risk in some studies and *decreased* risk in others. Lin *et al.*(Lin, et al., 2007) investigated the reasons for this "flip-flop" of the *GAPDH* rs3741916 locus in their series(9) vs. the initial report(8) and concluded that differences in the correlation of this SNP with *APOE* could account for the different effects in the two series. Specifically, in subjects of younger age at onset, the minor G allele of rs3741916 was inversely correlated with *APOE4* in the follow-up Lin et al. study(Lin, et al., 2006), offering an explanation as to why this allele associated with reduced risk of LOAD in their study. The authors observed that the strongest association in the initial study was obtained in those subjects who lacked *APOE4*, where the minor allele of rs3741916 was associated with increased risk. Thus, the differential correlation between *APOE4* and the rs3741916 G allele might account for the opposite effects of this *GAPDH* SNP allele in the two studies. The authors also concluded that differences in the ages-of-onset of the study subjects might contribute to the "flip-flop" that was observed.

In our series, we did not see a difference in the association of rs3741916 with LOAD when neither our *APOE*4+ and – subjects nor our old and young subjects were analyzed separately. Furthermore, in analyses where we controlled for age, gender and presence of *APOE4* allele, we still found that the minor allele of rs3741916 was associated with decreased risk of LOAD. This does not invalidate the hypothesis previously suggested(11) to account for the difference between the studies by Li *et al.*(8) and Lin et al.(9), but it does suggest that there may be heterogeneity unrelated to *APOE4* or age that accounts for the opposite effects of rs3741916 in different series.

It is possible that multiple alleles with weak effects and/or environmental factors have an important influence on rs3741916/LOAD association and that these factors vary enough among series that there is significantly increased risk in some series and significantly decreased risk in others. Another possible explanation is that the major and/or minor alleles of rs3741916 are in LD with rarer functional SNP(s) that have relatively strong effect(s) on AD risk. If LD and/or functional allele frequency varied substantially from series to series, the minor allele of rs3741916 could be associated with increased risk of LOAD in some series and decreased risk in others.

Our results support a role for the chromosome 12p locus in LOAD, where our most significant association is reported for rs3741916 in the 5'UTR of *GAPDH* using all subjects from all 3 series. More work is needed to determine if this association is the result of *GAPDH* variants or those at other loci which are in LD with the *GAPDH* variants. It may be that multiple variants in multiple genes within this chromosome 12p region contribute to the findings reported at this locus. This possibility may explain the heterogeneity observed for rs3741916 and likely requires deep sequencing to uncover the true functional variants accounting for the association with LOAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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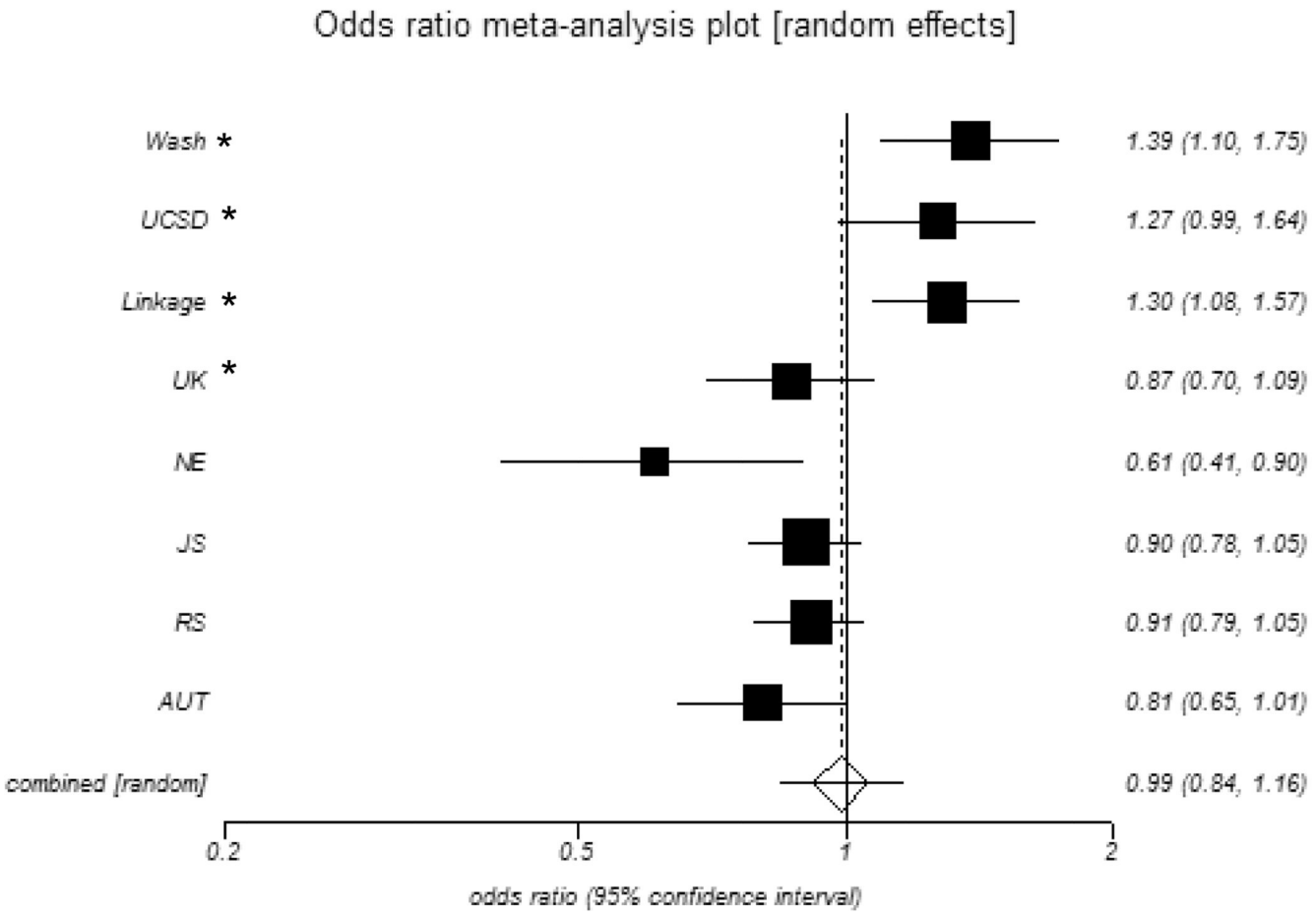

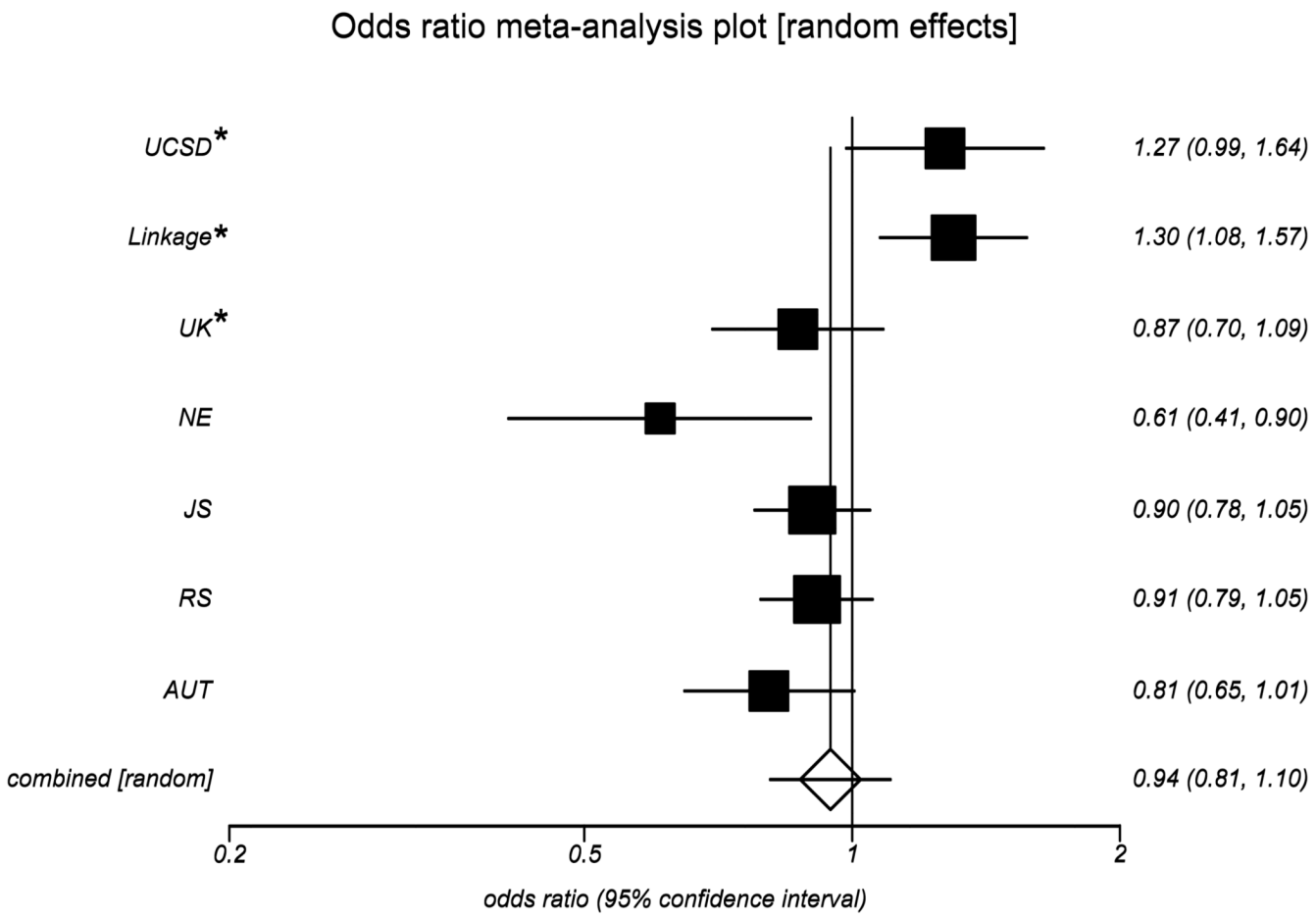

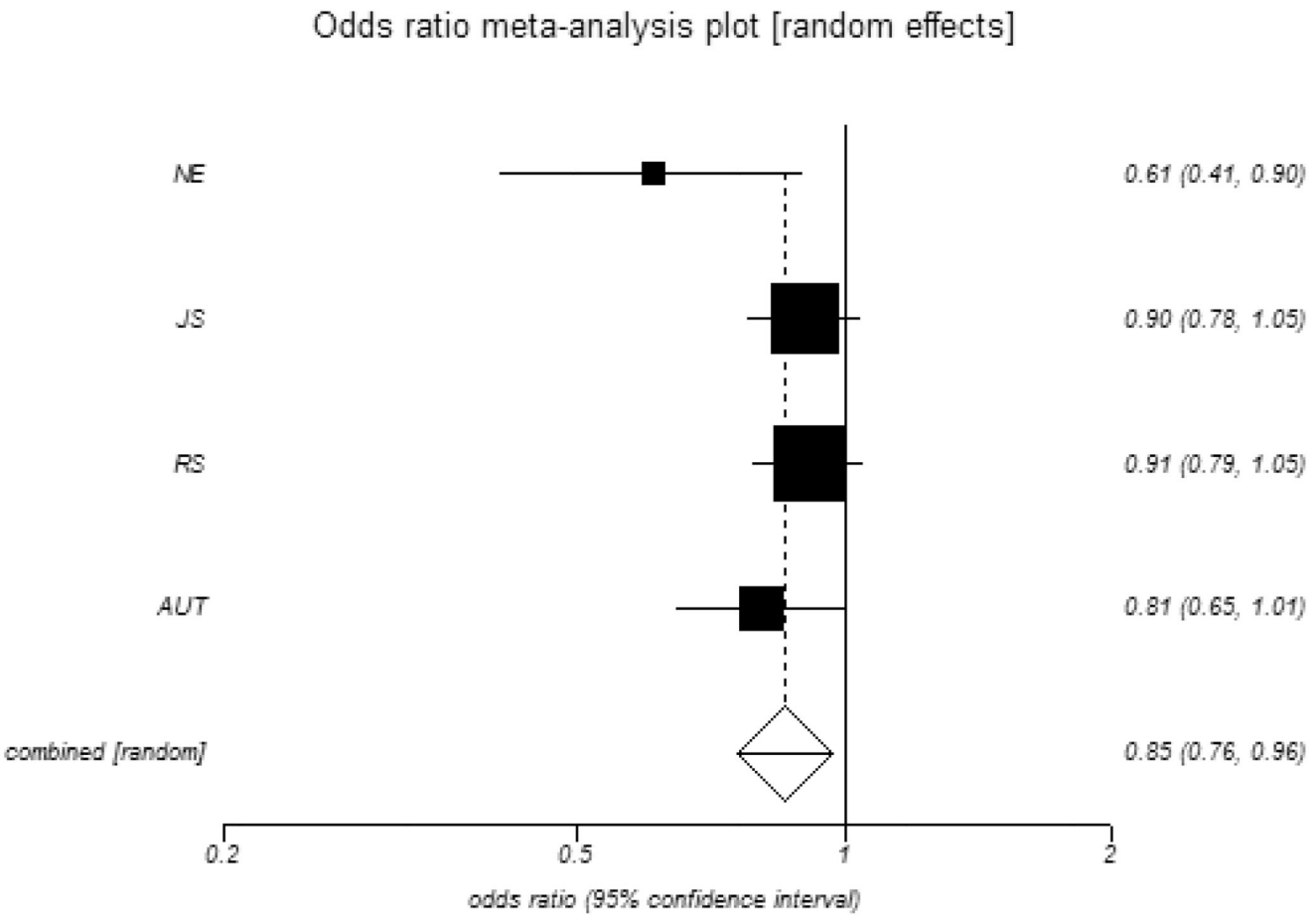

Figure 1.

Figure 1 a. Meta-Analysis of all series with reported counts and frequencies. Breslow-Day p-value <0.0001. * indicates series reported in the original study (*Li et al*).

Figure 1 b. Meta-Analysis of all series with reported counts and frequencies except first series from the original study. Breslow-Day p-value =0.0003. * indicates series reported in the original study (*Li et al*).

Figure 1.c Meta-Analysis of all follow-up series. Breslow Day p-value = 0.1967, Combined series p-value = 0.0094

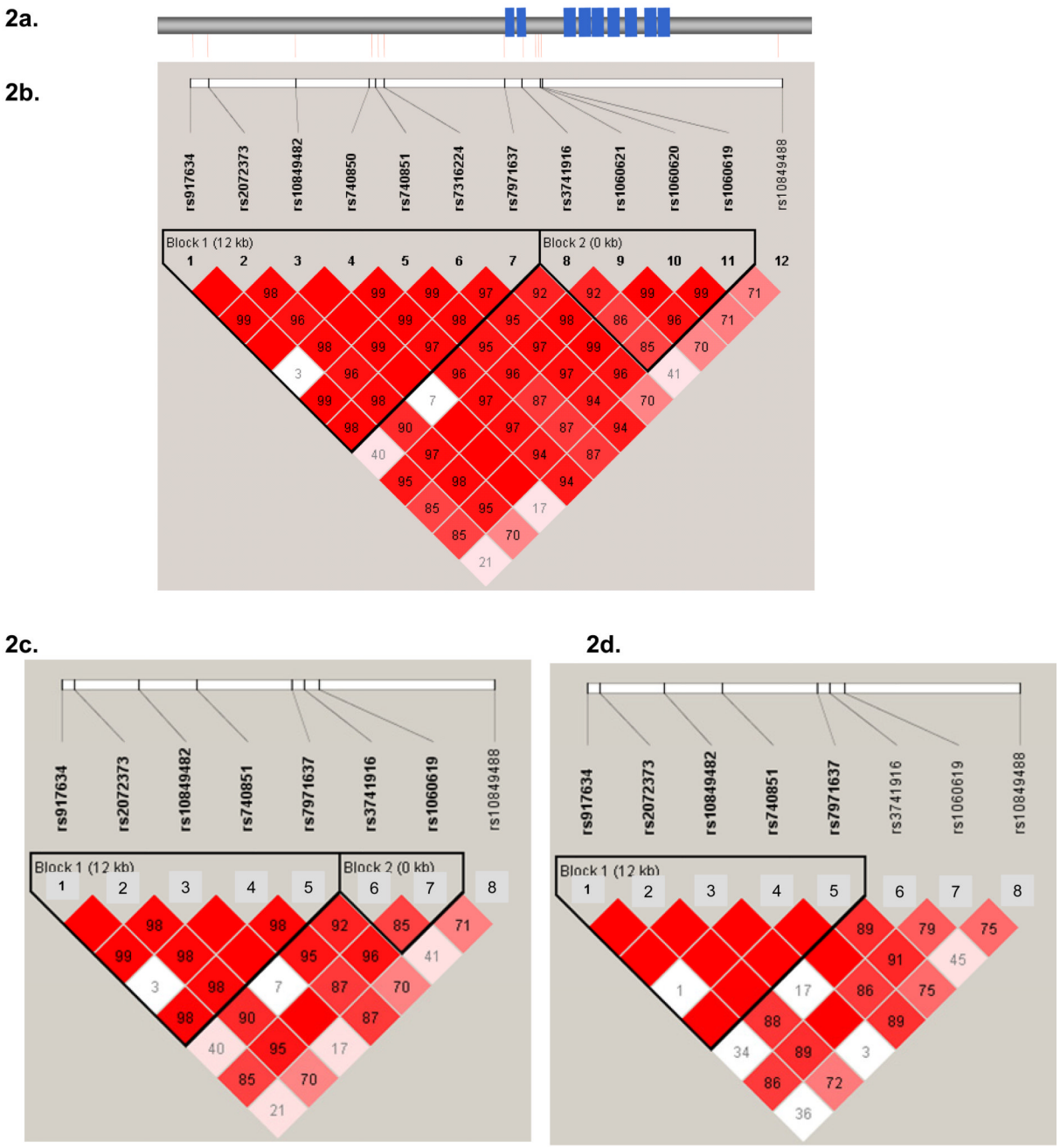

Figure 2.

Figure 2a–d: Linkage disequilibrium in the combined Mayo Clinic series at the *GAPDH* locus for all genotyped SNPs (a–b), the subset of HapMap SNPs in our series (c) and HapMap Caucasian subjects (d). SNP = Single nucleotide Polymorphism. 2a: Exons are represented with blue boxes and SNPs are represented with red lines. 2b–d: LD was estimated and haplotype blocks were defined using the “Solid Spine” method implemented in HAPLOVIEW.

Darker shades of red indicate increasing strength of LD (D’). 2d. HapMap SNP data is based on their Caucasian (CEU) subjects and genome build 36 downloaded from the HapMap website.

Table 1

Demographic details of three “Mayo Clinic case-control series” and the subset strata used for analysis models.

Series	Strata	Cs N	Cn N	Total N	Mean Age	Males (%)	ApoE4+ (%)
JS	All Ages	882	986	1868	77.1	745 (40%)	837 (45%)
	>78	452	418	870	71.9	347 (40%)	353 (41%)
	<78	430	568	998	83.1	398 (40%)	484 (49%)
	ApoE4−	321	710	1031	77.7	421 (41%)	na
	ApoE4+	561	276	837	76.4	324 (39%)	na
RS	All Ages	640	2460	3100	78.7	1363 (44%)	916 (30%)
	>78	383	1223	1606	83.4	642 (40%)	467 (29%)
	<78	233	1211	1444	73.4	721 (50%)	449 (31%)
	ApoE4−	278	1856	2134	78.9	963 (45%)	na
	ApoE4+	338	578	916	78.1	400 (44%)	na
AUT	All Ages	590	362	952	79.1	452 (47%)	437 (46%)
	>78	379	130	509	85.7	200 (39%)	257 (51%)
	<78	211	232	443	71.5	252 (57%)	180 (41%)
	ApoE4−	233	282	515	78.5	254 (49%)	na
	ApoE4+	357	80	437	79.7	198 (45%)	na

Cs = LOAD Case, Cn = LOAD Control. JS = Jacksonville Series, RS = Rochester Series, AUT = Autopsy confirmed series. <78 Age of Diagnosis/Examination/Death is less than 78 years, >78 Age of Diagnosis/Examination/Death is greater than 78 years.

Table 2

Replication analysis of previous reports

SNP (Locus)	Chr	Base Position	Strata	Series	Cs N (MAF)	Cn N (MAF)	Allelic OR (95%CI)	Allelic p-value	Logistic Regression (Dominant Model) OR (95%CI)	Logistic Regression (Dominant Model) p-value
A rs3741916 (GAPDH) aka rs1136666	12	6,514,252	ApoE4- (Li *et al*, 2004)	**Li *et al* W/UC/ UK**	(0.297)	(0.247)	**1.27(1.06:1.53)**	**0.008**	nr	nr
				JS	311 (0.25)	702 (0.27)	0.92 (0.74: 1.15)	0.478	0.95 (0.75: 1.28)	0.874
				RS	276 (0.28)	1838 (0.28)	1.00 (0.82: 1.23)	0.960	1.12 (0.86: 1.45)	0.406
				AUT	274 (0.22)	232 (0.28)	**0.73 (0.54: 0.99)**	**0.042**	**0.67 (0.46: 0.97)**	**0.034**
				JS/RS/AUT	819 (0.25)	2814 (0.28)	0.88 (0.78: 1.00)	0.051	0.92 (0.79: 1.08)	0.313
B rs3741916 (GAPDH) aka rs1136666	12	6,514,252	AAE/D* < mean (Lin *et al*, 2006)	**Lin *et al* C-C series**	nr	nr	nr	nr	**0.39 (0.21:0.70)**	**0.002**
				JS	417 (0.27)	563 (0.28)	0.95 (0.78: 1.17)	0.683	0.98 (0.74: 1.29)	0.870
				RS	229 (0.28)	1200 (0.29)	0.93 (0.74: 1.16)	0.537	0.95 (0.70: 1.30)	0.765
				AUT	209 (0.24)	225 (0.27)	0.83 (0.60: 1.13)	0.243	0.85 (0.56: 1.28)	0.435
				JS/RS/AUT	855 (0.26)	1988 (0.29)	0.89 (0.79: 1.02)	0.089	0.92 (0.77: 1.10)	0.349
C rs3741916 (GAPDH) aka rs1136666	12	6,514,252	All (Lee *et al*, 2008)	**Lee *et al* C-C series**	(0.14)	(0.21)	nr	**0.027**	nr	0.054
				JS	859 (0.26)	974 (0.28)	0.90 (0.78: 1.05)	0.178	0.92 (0.75: 1.12)	0.399
				RS	611 (0.27)	2411 (0.28)	0.91 (0.79: 1.05)	0.212	0.95 (0.78: 1.14)	0.554
				AUT	585 (0.23)	354 (0.27)	0.81 (0.65: 1.00)	0.053	0.77 (0.57: 1.04)	0.086
				JS/RS/AUT	2055 (0.25)	3739 (0.28)	**0.86 (0.79: 0.94)**	**8.0E-04**	**0.88 (0.78: 0.99)**	**0.033**
D rs2029721 (pGAPD)	12	61,435,611	AAE/D* > mean(Li *et al*, 2004 & Lin *et al* 2006)	**Li *et al* W/UC/ UK**	(0.308)	(0.352)	0.80 (0.68:0.97)	**0.018**	nr	nr
				Lin *et al* C-C series	nr	nr	nr	**0.004**	0.55 (0.32:0.96)	**0.036**
				JS	320 (0.39)	309 (0.36)	1.13 (0.89: 1.42)	0.322	1.16 (0.82: 1.63)	0.404
				RS	336 (0.36)	719 (0.38)	0.94 (0.78: 1.14)	0.530	0.96 (0.73: 1.27)	0.766
				AUT	369 (0.36)	130 (0.34)	0.95 (0.70: 1.31)	0.759	1.31 (0.84: 2.05)	0.237
				JS/RS/AUT	1025 (0.37)	1158 (0.37)	1.00 (0.88:1.13	0.999	1.05 (0.87: 1.26)	0.642

SNP (Locus)	Chr	Base Position	Strata	Series	Cs N (MAF)	Cn N (MAF)	Allelic OR (95%CI)	Allelic p-value	Logistic Regression (Dominant Model) OR (95%CI)	Logistic Regression (Dominant Model) p-value
				Li *et al* W/UC/ UK	(0.326)	(0.42)	**0.66 (0.55:0.80)**	**3.0E-04**	nr	nr
E rs4806173 (GAPDHS)	19	40,716,765	AAE/D* < mean (Li *et al*, 2004)	JS	270 (0.39)	330 (0.40)	0.97 (0.76: 1.23)	0.812	0.95 (0.65: 1.38)	0.785
				RS	220 (0.39)	644 (0.38)	1.05 (0.83: 1.32)	0.691	1.00 (0.70: 1.41)	0.977
				AUT	209 (0.38)	229 (0.36)	1.09 (0.82: 1.45)	0.575	1.16 (0.77: 1.75)	0.490
				JS/RS/AUT	699 (0.39)	1203 (0.38)	1.03 (0.89: 1.18)	0.729	1.00 (0.81: 1.23)	0.960

Chr = Chromosome, Cs = AD case, Cn = Control subject. N = number of subjects in series that have genotype data. MAF = minor allele frequency. OR = Odds Ratio, CI = Confidence Interval.

Allelic association tested using chi-squared test with no covariates. Logistic regression uses Age*, Gender and presence of an APOE4 allele as covariates, *Age and AAE/D refers to Age at Diagnosis, Examination or Death. The mean age differs between the different publications. The mean age is 78 in our combined series. Nominally significant p-values (<0.05) are highlighted in bold, nr = Not reported. .Panels A and E: Li et al., 2004 models of analysis. Panel B: Lin et al., 2006 model. Panel C: Lee et al., 2008 model. Panel D: Li et al., 2004 and Lin et al., 2006 models.

Table 3

The results of logistic regression analysis under an additive model in the Mayo Clinic Series.

							Logistic Regression Additive Model	
SNP (Locus)	**Chr**	**Base Position**	**Strata**	**Series**	**Cs N (MAF)**	**Cn N(MAF)**	**OR (95%CI)**	**p-value**
rs3741916 (GAPDH) aka rs1136666	12	6,514,252	**All**	JS	859 (0.26)	974 (0.28)	0.90 (0.77: 1.05)	0.159
				RS	611 (0.27)	2411 (0.28)	0.93 (0.81: 1.08)	0.361
				AUT	585 (0.23)	354 (0.27)	**0.78 (0.61: 1.00)**	**0.047**
				JS/RS/AUT	2055 (0.25)	3739 (0.28)	**0.87 (0.79: 0.96)**	**0.003**
rs2029721 (pGAPD)	12	61,435,611	**AAE/D ≤78**	JS	590 (0.40)	637 (0.36)	**1.39 (1.07: 1.81)**	**0.013**
				RS	556 (0.37)	1356 (0.38)	0.94 (0.74: 1.21)	0.638
				AUT	574 (0.37)	354 (0.36)	1.20 (0.90: 1.60)	0.225
				JS/RS/AUT	1720 (0.38)	2347 (0.37)	1.14 (0.98: 1.31)	0.090
rs4806173 (GAPDHS)	19	40,716,765	**All**	JS	591 (0.39)	642 (0.38)	1.03 (0.87: 1.23)	0.732
				RS	556 (0.39)	1372 (0.38)	0.99 (0.86: 1.15)	0.923
				AUT	586 (0.38)	358 (0.38)	0.87 (0.70: 1.09)	0.222
				JS/RS/AUT	1733 (0.38)	2372 (0.38)	0.98 (0.89: 1.08)	0.688

Chr = Chromosome Cs = AD case, Cn = Control subject. N = number of subjects in series that have genotype data. MAF = minor allele frequency. OR = Odds Ratio, CI = Confidence Interval. Logistic regression includes Age*, Gender and presence of an ApoE4 allele included as covariates, *Age and AAE/D refers to Age at Diagnosis, Examination or Death. Nominally significant p-values (<0.05) are highlighted in bold.