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Association study of rs3846662 with Alzheimer's disease in a population-based cohort: the Cache County Study

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Abstract

3-Hydroxy-3-methylglutaryl coenzyme A reductase (*HMGCR*) is associated with monitoring cholesterol levels. The presence of the single nucleotide polymorphism rs3846662 introduces alternative splicing at exon 13; the exclusion of this exon leads to a reduction in total cholesterol levels. Lower cholesterol levels are linked to a reduction in Alzheimer's disease (AD) risk. The major allele of rs3846662, which encourages the splicing of exon 13, has recently been shown to act as a preventative allele for AD, especially in women. The purpose of our research was to replicate and confirm this finding. Using logistic regressions and survival curves, we found a significant association between AD and rs3846662, with a stronger association in individuals that carry the *APOE* e4 allele, supporting previously published work. The effect of rs3846662 on women is insignificant in our cohort. We confirmed that rs3846662 is associated with reduced risk for AD without gender differences; however, we failed to detect association between rs3846662 and delayed mild cognitive impairment conversion to AD for either of the *APOE* e4 allelic groups.

Keywords

HMGCR; Alzheimer's disease; APOE; cholesterol synthesis; genetics; association

1. Introduction

Alzheimer's disease (AD) is a geriatric neurodegenerative disorder characterized by extracellular senile plaques and intracellular neurofibrillary tangles (Armstrong, 2011;

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Disclosure Statement

The authors declare no conflict of interest.

Newell et al., 1999; Ridge et al., 2013). The disease is thought to be caused by the malfunctioning of systems which transport, synthesize, and break down the proteins that constitute the plaques and tangles (Adlard and Cummings, 2004; Hardy and Higgins, 1992; Ridge et al., 2013; Swerdlow and Khan, 2004). Several variants associated with AD risk are in genes involved in these mechanisms, including *CD33, CLU, PICALM* and *MS4A6A1* (Bertram et al., 2007; Lambert et al., 2013). The apolipoprotein E (*APOE*) gene in particular, which has deleterious (*APOE* e4) and protective (*APOE* e2) alleles, is significantly associated with AD (Corder et al., 1993). This gene regulates cholesterol metabolism in the central nervous system (Ridge et al., 2013).

Recent data suggests that the 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGCR*) gene may be another region associated with AD (Leduc et al., 2015). *HMGCR* is the rate-limiting step in cholesterol synthesis, and as such, is the target for low-density lipoprotein cholesterol lowering drugs known as statins (Burkhardt et al., 2008; Cano-Corres et al., 2018; Kathiresan et al., 2008; Krauss et al., 2008; Leduc et al., 2016; Medina et al., 2008). It also interacts with *ABCA1* to increase AD risk (Rodriguez-Rodriguez et al., 2009). *HMGCR* undergoes alternative splicing at exon 13 with the presence of the intronic single-nucleotide polymorphism (SNP) rs3846662 (Burkhardt et al., 2008; Medina et al., 2008). The exclusion of the 53 amino acids in exon 13 results in a catalytically inactive protein called Δ*exon13* (Burkhardt et al., 2008). When compared to the full-length isoform, cells with high levels of Δ*exon13* have a poor response to statin therapy (Medina, 2010; Medina et al., 2008; Medina and Krauss, 2009), leading to increased concentrations of cholesterol. Since functional *HMGCR* is a tetramer composed of two dimers (Istvan et al., 2000), Medina and Krauss (2009) hypothesized that the combination of Δ*exon13* with functional proteins could be among the factors that reduces its statin sensitivity. Additional research proposed that different combinations of Δ*exon13* in the tetramer could lead to different levels of enzymatic activity and statin sensitivity (Leduc et al., 2016; Medina, 2010).

The frequency of Δ*exon13* and the resulting dysfunctional protein levels are associated with the genotype of rs3846662 (Burkhardt et al., 2008; Medina, 2010; Medina et al., 2008). The major allele at this SNP for Caucasian populations is AA; the minor allele is GG (Burkhardt et al., 2008). The major A allele promotes the skipping of exon 13 and increases the amount of circulating Δ*exon13*, while the minor G allele retains exon 13 at a much higher rate (Burkhardt et al., 2008; Medina and Krauss, 2009; Simmons et al., 2011). The difference in abundance of Δ*exon13* between these two alleles is around 16 to 20 percent (Burkhardt et al., 2008; Medina et al., 2008). Heterozygotic (GA) expression of Δ*exon13* clearly falls between that of homozygotic AA and homozygotic GG (Burkhardt et al., 2008).

Leduc et al. (2015) found that the AA allele of rs3846662 acts as a protective variant and delays the onset of AD (*p*-value = 0.017). Homozygosity for the A allele is associated with a decrease in *HMGCR* activity (Krauss et al., 2008; Leduc et al., 2015; Medina et al., 2008), and as such, a corresponding decrease in cholesterol levels (Aulchenko et al., 2008). Reduction in cholesterol levels has been shown to inhibit the generation of amyloid plaques (Simons et al., 1998). Leduc et al. (2015) reported that this effect was more significant in women; however, their initial results conflicted between cohorts (Quebec founder population [QFP] cohort *p*-value = 0.003; Alzheimer's Disease Cooperative Study [ADCS] cohort *p*-

value = 0.342). Leduc et al. (2015) additionally reported that the lack of the G allele had a significant effect in *APOE* e2 non-carriers (QFP *p*-value = 0.05) and *APOE* e4 carriers (ADCS *p*-value = 0.041). In this study we have evaluated Leduc et al.'s (2015) findings in samples from the Cache County Study on Memory Health and Aging. This sample is a true population-based sample of 5092 individuals. This population is representative of the general European American population (Sharp et al., 2014). Here, we test the associations between AD and *HMGCR* that were reported by Leduc et al. (2015).

2. Materials and Methods

2.1 Samples

The Cache County Study on Memory Health and Aging began in 1994. It is a population-based study, which recruited everyone in Cache County, Utah that was age 65 or older. Over 95% of the population, 5092 subjects, enrolled in the study. AD status was determined using a variety of assessments administered periodically over twelve years. There were no cases of early-onset AD. Additional information about this dataset, such as diagnostic and screening criteria, has been previously reported (Breitner et al., 1999; Tschanz et al., 2002). The general demographics of this sample are presented in Table 1.

DNA was available for genotyping of 3473 samples, including 490 AD cases (14.1%) and 2983 controls (85.9%). Of these 1438 are male (41.4%), and 2035 are female (58.6%). The *HMGCR* allele status of these samples had 697 samples with the GG genotype (20.07%), 1708 with the GA genotype (49.18%), and 1068 with the AA genotype (30.75%). 1069 were *APOE* e4 carriers (30.78%) and 2404 were not carriers (69.22%). There are 570 *APOE* e2 carriers (16.41%), with 2903 non-carriers (83.59%). See Table 1 for a summary of genotype frequencies.

2.2 Statistical Analyses

We ran our analysis on R version 3.3.2 (Sincere Pumpkin Patch) (R Core Team, 2016). To conform with the analyses conducted by Leduc et al. (2015) we used a dominant model with respect to allele "G" for coding the genotypes of rs3846662: (a) G carriers, which accounts for both the minor allele homozygote, GG, and the heterozygotic GA genotype; and (b) G non-carriers, which is the AA genotype. We used logistic regression models to assess the association of AD with *HMGCR* status in order to allow for the inclusion of age, the number of *APOE* e4 alleles, and the number of *APOE* e2 alleles as covariates. All p-values reported from our cohort are one-tailed, in contrast to Leduc et al.'s (2015) two-tailed p-values, as we are restricted to the hypothesis that the G non-carrier status is protective. A significant outcome in the other direction is viewed as a failure to replicate, and as such, this analysis is a classic case for a one-tailed test (Bland and Altman, 1994; Kimmel, 1957; Ruxton and Neuhäuser, 2010). By restricting our analyses to a one-tailed model and the specific genetic models used by Leduc et al. (2015), statistical power to validate their findings is maximized. We first replicated the analyses of Leduc et al. (2015) and evaluated differential effects related to gender (see Tables 2 and 3).

We additionally conducted a survival analysis, to replicate and extend the results of Leduc et al. (2015) with respect to AD-free survival. Survivor curves by *HMGCR* status and gender were generated using Kaplan-Meier estimators in R. We formally compared differences in survival between allelic variants using Cox Proportional Hazards Regression Models. We applied the same model to determine if rs3846662 is associated with a delay in the conversion time from normal and mild cognitive impairment (MCI) to AD.

We conducted a post-hoc power analysis to determine the statistical power to observe an effect of *HMGCR* on AD status in our cohort. We used the number of cases and controls with the frequency of the *HMGCR* allele to calculate the probability that we could detect the odds ratio reported by Leduc et al. (2015) at the 0.05 significance level. We used the calculator provided by Skol et al. (2006) to calculate these probabilities.

3. Results

Our logistic regression analysis indicated that rs3846662 was significantly correlated with AD status (p-value = 0.049). This effect was more evident in all carriers of *APOE* e4 (p-value = 0.016), in both male (p-value = 0.022) and female (p-value = 0.022) carriers. Additionally, significance was observed in non-carriers of *APOE* e2 (p-value = 0.029). See Table 4 for a summary of the regressions that revealed significant results.

We were unable to replicate Leduc et al.'s (2015) finding that rs3846662 was significantly correlated with female AD status in the general female group (p-value = 0.129). We were able to replicate the finding that the lack of the G allele was associated with protection from AD (p-value = 0.049). See Table 5 for a direct comparison of these three regressions to the findings of Leduc et al. (2015).

We also examined the effect of rs3846662 in *APOE* allele subgroups reported by Leduc et al. (2015). We found that *APOE* e4 carriers without the G allele experience a protective effect (p-value = 0.016), as do *APOE* e2 non-carriers (p-value = 0.029), which is in concordance with the findings in Leduc et al. (2015). See Table 6 for a direct comparison of these models to the findings of Leduc et al. (2015).

The survival curves compared the onset of AD between males and their rs3846662 allele status (p-value = 0.161; Figure 1a) and the onset of AD between females and their rs3846662 allele status (p-value = 0.327; Figure 1b). We then created survival curves for four more additional comparisons: in Figure 1c, a comparison between genders (p-value = 0.059); in Figure 1d, a comparison between the allele status of rs3846662 (p-value = 0.102); in Figure 1e, a comparison between genders for G carrier rs3846662 allele status (p-value = 0.209); and in Figure 1f, a comparison between genders for G non-carrier rs3846662 allele status (p-value = 0.134). We found no difference in conversion time from MCI to AD between G non-carrier and G carrier patients with the *APOE* e4 allele (p-value = 0.663; Figure 2a) and without the *APOE* e4 allele (p-value = 0.671; Figure 2b).

Power analyses demonstrated that each experiment had adequate statistical power (Tables 2, 3, 5, and 6). The lowest power observed in the seven replication experiments was found in the male *APOE* e2 carrier group at 75 percent.

4. Discussion

We have conducted a well-powered validation study of previous reports of a protective role in AD for rs3846662. Our findings provide support for several of Leduc et al.'s (2015) reported associations, including an overall protective effect and associations within *APOE* e2 non-carriers and *APOE* e4 carriers. These replication findings are in concordance with other recent studies. Chang et al. (2016) reported that they were able to confirm that the rs3846662 G non-carrier allele was significant (p-value = 0.02) and acted as a protective variant for AD in a northern Han Chinese population.

Since the majority of statins target *HMGCR*, several studies have analyzed the effect differing levels of Δ*exon13* may have on treatment efficacy (Cano-Corres et al., 2018; Leduc et al., 2016; Medina, 2010; Medina et al., 2008; Medina and Krauss, 2009; Simmons et al., 2011). Since rs3846662 modulates levels of Δ*exon13*, the genotype of this variant could be used to predict the effectiveness of treatment. However, results have been inconclusive. A 2015 study found that only women with higher levels of Δ*exon13* had a worse response to statin therapy (Leduc et al., 2016). Other studies have suggested that individuals with an abundance of Δ*exon13* have poor response to statin treatment (Medina et al., 2008), suggesting that statin therapy might be a viable option in individuals that are G carriers, as G non-carriers produce higher amounts of Δ*exon13* (Simmons et al., 2011). However, a recent study by Cano-Corres et al. (2018) found that statin therapy was ineffective in G carrier patients despite the lower levels of the inactive protein. Discrepancy in statin response experiments may possibly be influenced by a SNP in linkage disequilibrium with rs3846662 instead, prompting the need for further analysis.

We were unable to confirm the larger effect in women that was reported previously. We also failed to detect significant association in our survival analyses. Differences in our findings and those of Leduc et al. (2015) could be due to differences in sample sizes: our sample size was over 3000 individuals with only 490 cases from a population-based cohort, while the population of Leduc et al. (2015) had 334 cases from a total of 584 individuals in a clinical case/control cohort. There were also differences in the age at onset and age at death of AD subjects in the Cache County Study. The mean age of onset for our cases was 82.5 years; the Leduc et al. (2015) population had a mean onset age of 71.7 years. The mean age of death for our cases was 89.2 years, which is ten years higher than the population of Leduc et al. (2015) at 79.2 years. The difference between the especially long-lived Cache participants and the populations reported in Leduc et al. (2015) may contribute to the divergence in our findings.

While our findings do not definitely characterize the relationship between rs3846662 and AD, they do provide support for a protective effect for non-carriers of the "G" allele, which is pronounced in *APOE* e4 carriers and *APOE* e2 non-carriers. These findings suggest that further study of the role of rs3846662 in AD risk and conversion from MCI to AD is warranted.

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Abbreviations:

AD	Alzheimer's disease
QFP	Quebec Founder Population
ADCS	Alzheimer's Disease Cooperative Study
MCI	mild cognitive impairment

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Highlights

- rs3846662 is associated with reduced risk for Alzheimer's disease
- Individuals with APOE e4 experience a greater protective effect from rs3846662
- Failed to detect gender-specific association of rs3846662, contrasting prior reports
- Failed to detect association between rs3846662 and delayed MCI conversion to AD

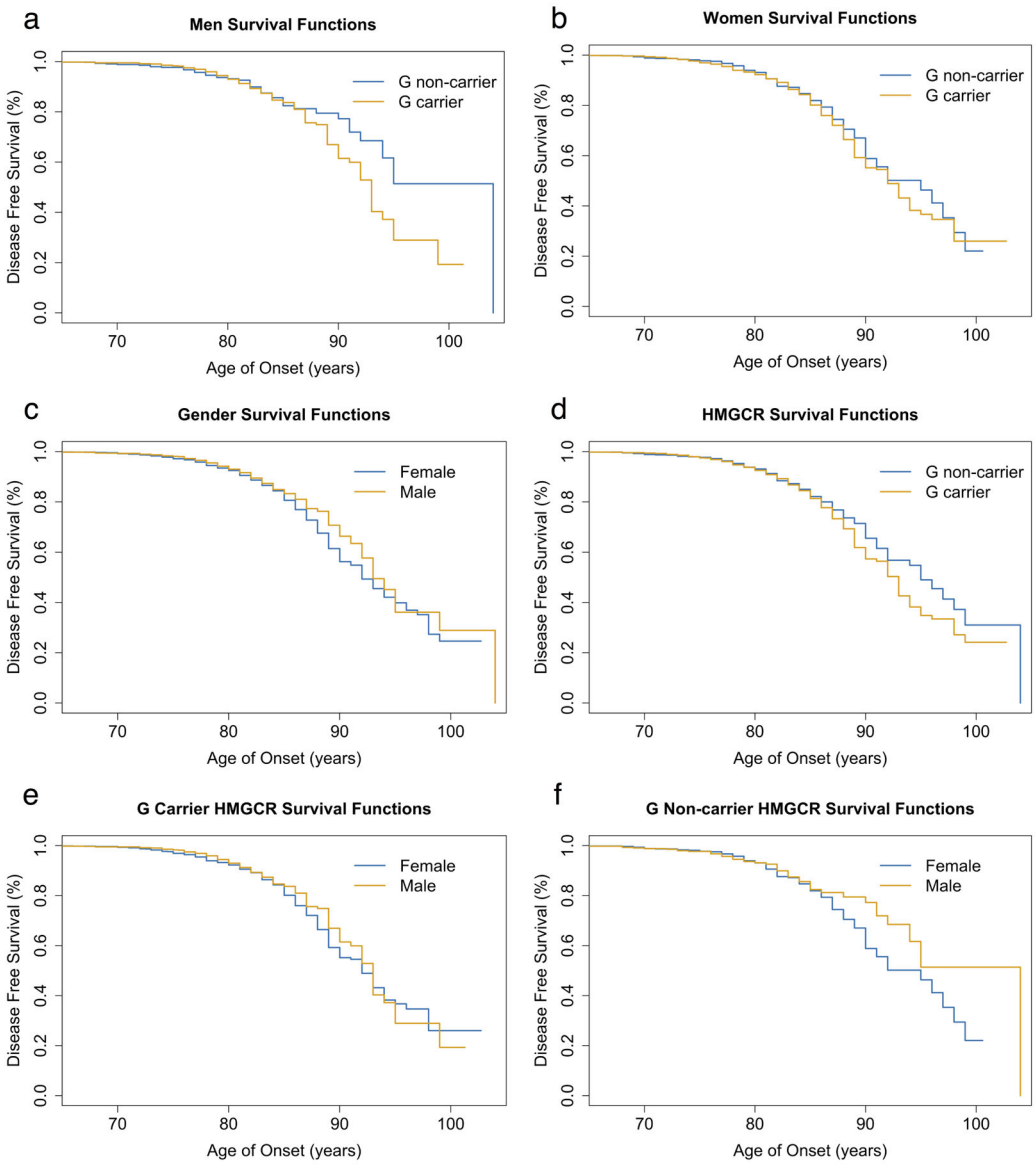

Figure 1.

Survival curves measuring effect of *HMGCR* rs3846662 intron 13 on Alzheimer's Disease (AD) free survival. (**a**) Male age of onset of AD in rs3846662 G non-carriers vs. G carriers (*p*-value = 0.161). (**b**) Female age of onset of AD in rs3846662 G non-carriers vs. G carriers (*p*-value = 0.327). (**c**) Age of onset of AD in males vs females (*p*-value = 0.059). (**d**) Age of onset of AD in rs3846662 G non-carriers vs. G carriers (*p*-value = 0.102). (**e**) Age of onset of AD in rs3846662 G carrier males vs. females (*p*-value =0.209). (**f**) Age of onset of AD in rs3846662 G non-carrier males vs. females (*p*-value = 0.134)

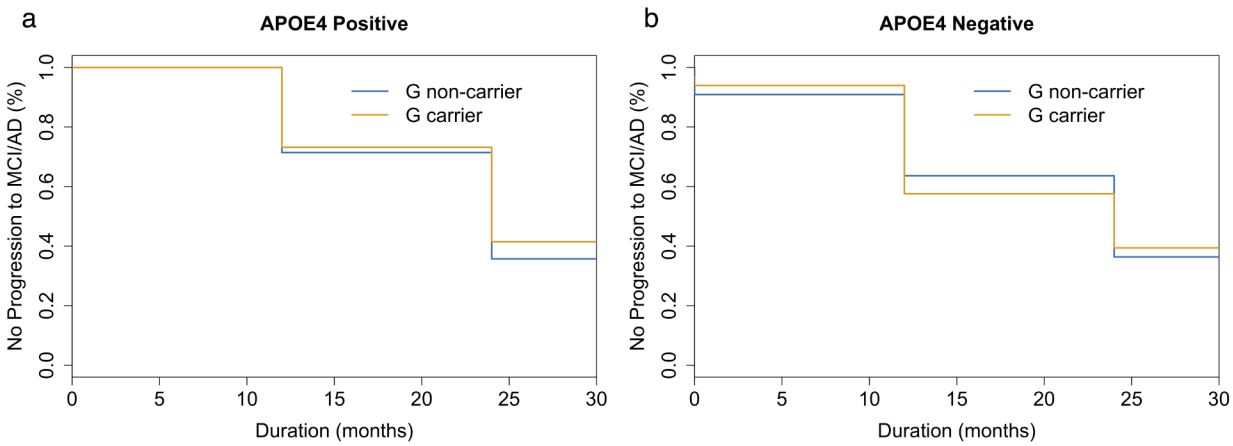

Figure 2.

Survival curves measuring the effect of *HMGCR* rs3846662 intron 13 on mild cognitive impairment (MCI) conversion to Alzheimer's Disease (AD). (**a**) *APOE* e4 carriers comparing rs3846662 G non-carriers vs. G carriers (*p*-value = 0.663). (**b**) *APOE* e4 non-carriers comparing rs3846662 G non-carriers vs. G carriers (*p*-value = 0.671)

Table 1.

General demographics for the Cache County Study population

	General	Cases	Controls
Male	1438	160	1278
Female	2035	330	1705
Mean Age of AD onset ± SD[a]	80.2 ± 6.45	82.5 ± 6.93	79.8 ± 6.29
Mean Age of Death ± SD[a]	85.5 ± 7.13	89.2 ± 6.22	84.6 ± 7.04
Mean Years of Education ± SD[a]	13.3 ± 2.88	13.1 ± 2.96	13.3 ± 2.87
APOE e2/e2 genotype frequency	27 (0.78%)	1 (0.2%)	26 (0.87%)
APOE e2/e3 genotype frequency	438 (12.61%)	33 (6.74%)	405 (13.58%)
APOE e2/e4 genotype frequency	105 (3.02%)	26 (5.31%)	79 (2.65%)
APOE e3/e3 genotype frequency	1939 (55.83%)	188 (38.37%)	1751 (58.7%)
APOE e3/e4 genotype frequency	878 (25.28%)	204 (41.63%)	674 (22.59%)
APOE e4/e4 genotype frequency	86 (2.48%)	38 (7.75%)	48 (1.61%)
HMGCR GG genotype frequency	697 (20.07%)	105 (21.43%)	592 (19.84%)
HMGCR GA genotype frequency	1708 (49.18%)	250 (51.02%)	1458 (48.88%)
HMGCR AA genotype frequency	1068 (30.75%)	105 (27.55%)	933 (31.28%)

[a]SD stands for standard deviation.

Table 2.

Odds ratios and one-sided p-values from the seven replication logistic regressions (LR)

Analysis		Cases / Controls	Intercept	*HMGCR*[a]	*APOE* e4	*APOE* e2	Gender	Power
General	LR *p*-value	490 / 2983	< 1e-16	0.049*	< 1e-16*	0.086[b]	1.685e-05*	100%
	Odds ratio		0.055	0.832	3.231	0.814	1.549	
General Male	LR *p*-value	160 / 1278	<1e-16	0.111	4.87e-10*	0.285	NA	98%
	Odds ratio		0.091	0.791	2.862	0.865	NA	
General Female	LR *p*-value	330 / 1705	<1e-16	0.129	<1e-16*	0.101	NA	100%
	Odds ratio		0.127	0.856	3.449	0.789	NA	
APOE e4−	LR *p*-value	222 / 2182	<1e-16	0.414	NA	NA	NA	99%
	Odds ratio		0.103	0.967	NA	NA	NA	
APOE e4+	LR *p*-value	268 / 801	<1e-16	0.016*	NA	NA	NA	99%
	Odds ratio		0.369	0.712	NA	NA	NA	
APOE e2−	LR *p*-value	430 / 2473	<1e-16	0.029*	NA	NA	NA	100%
	Odds ratio		0.186	0.802	NA	NA	NA	
APOE e2+	LR *p*-value	60 / 510	<1e-16	0.395	NA	NA	NA	83%
	Odds ratio		0.115	1.081	NA	NA	NA	

*indicates significance

[a]refers to rs3846662. Odds ratios in this column are relative to the G-negative genotype

[b]indicates a trend

+ and − indicate carrier and non-carrier respectively

Table 3.

Odds ratios and one-sided p-values from the eight additional logistic regressions (LR) that examined smaller gender subgroupings

Analysis		Cases / Controls	Intercept	*HMGCR*[a]	Power
Female *APOE* e4−	LR *p*-value	145 / 1252	<1e-16	0.337	96%
	Odds ratio		0.113	1.082	
Female *APOE* e4+	LR *p*-value	185 / 453	5.8e-15	0.022*	96%
	Odds ratio		0.456	0.669	
Male *APOE* e4−	LR *p*-value	77 / 930	<1e-16	0.154	85%
	Odds ratio		0.089	0.757	
Male *APOE* e4+	LR *p*-value	83 / 348	<1e-16	0.022*	81%
	Odds ratio		0.253	0.824	
Female *APOE* e2−	LR *p*-value	390 / 1407	<1e-16	0.057[b]	99%
	Odds ratio		0.113	1.082	
Female *APOE* e2+	LR *p*-value	40 / 298	<1e-16	0.334	79%
	Odds ratio		0.456	0.669	
Male *APOE* e2−	LR *p*-value	140 / 1066	<1e-16	0.137	95%
	Odds ratio		0.139	0.801	
Male *APOE* e2+	LR *p*-value	20 / 212	<1e-16	0.459	75%
	Odds ratio		0.096	0.948	

*indicates significance

[a]refers to rs3846662. Odds ratios in this column are relative to the G-negative genotype

[b]indicates a trend

+ and − indicate carrier and non-carrier respectively

Table 4.

Summary of all five logistic regressions that generated statistically significant results

	General	***APOE* e4+**	**Female *APOE* e4+**	**Male *APOE* e4+**	***APOE* e2−**
Cases / Controls	490 / 2983	268 / 801	185 / 453	83 / 348	430 / 2473
HMGCR[a] Sig (One-tailed)	0.049*	0.016*	0.022*	0.022*	0.029*
HMGCR[a] Odds Ratio[b]	0.832	0.711	0.669	0.824	0.802
Power	100%	99%	96%	81%	100%

* indicates significance.

[a] refers to rs3846662

[b] relative to the G-negative genotype

+ and − indicate carrier and non-carrier respectively.

Table 5.

Direct comparison of results with Leduc et al. (2015) findings

Cohort		Cases / Controls	*HMGCR*[a]	*APOE* e4+	*APOE* e2+	Power
Cache County[b]	Overall Effect	490 / 2983	0.049*	<1e-16*	0.086	100%
	Females	330 / 1705	0.129	<1e-16*	0.101	100%
	Males	160 / 1278	0.111	4.87e-10*	0.285	98%
QFP[c]	Overall Effect	574 / 250	0.024*	0.001*	0.001*	NA
	Females	334 / 250	0.003*	0.001*	0.001*	NA
	Males	240 / 250	0.686	0.001*	0.293	NA
ADCS[c]	Overall Effect	409 / 409	0.129	0.029*	0.118	NA
	Females	164 / 409	0.342	0.017*	0.209	NA
	Males	245 / 409	0.145	0.285	0.296	NA

*indicates significance

[a]refers to rs3846662

[b]indicates Cache County cohorts with a one-tailed significance test

[c]indicates cohorts from Leduc et al. (2015) that are two-tailed significance values

+indicates carrier.

Key: QFP = Quebec Founder Population; ADCS = Alzheimer's Disease Cooperative Study

Table 6.

Direct comparison of results with Leduc et al. (2015) findings

Cohort		Cases / Controls	*HMGCR*[a]	Power
Cache County[b]	*APOE* e4−	222 / 2182	0.414	99%
	APOE e4+	268 / 801	0.016*	99%
	APOE e2−	430 / 2473	0.029*	100%
	APOE e2+	60 / 510	0.395	83%
QFP[c]	*APOE* e4−	308 / 250	0.634	NA
	APOE e4+	262 / 250	0.183	NA
	APOE e2−	469 / 250	0.05*	NA
	APOE e2+	101 / 250	0.304	NA
ADCS[c]	*APOE* e4−	140 / 409	0.476	NA
	APOE e4+	268 / 409	0.041*	NA
	APOE e2−	392 / 409	0.156	NA
	APOE e2+	17 / 409	0.579	NA

* indicates significance

[a] refers to rs3846662

[b] indicates Cache County cohorts with a one-tailed significance test

[c] indicates cohorts from Leduc et al. (2015) that are two-tailed significance values

+ and − indicate carrier and non-carrier respectively.

Key: QFP = Quebec Founder Population; ADCS = Alzheimer's Disease Cooperative Study