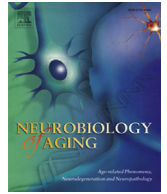




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Association study of rs3846662 with Alzheimer's disease in a population-based cohort: the Cache County Study

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ABSTRACT

3-Hydroxy-3-methylglutaryl coenzyme A reductase 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 who 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.

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1. Introduction

Alzheimer's disease (AD) is a geriatric neurodegenerative disorder characterized by extracellular senile plaques and intracellular neurofibrillary tangles (Armstrong, 2011; 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 (Arlard 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 suggest that the 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGR*) gene may be another region

associated with AD (Leduc et al., 2015). *HMGR* 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). *HMGR* 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 with 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. Because functional *HMGR* is a tetramer composed of 2 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).

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Table 1
General demographics for the Cache County Study population

Demographic	General	Cases	Controls
Male	1438	160	1278
Female	2035	330	1705
Mean age of AD onset \pm SD	80.2 \pm 6.45	82.5 \pm 6.93	79.8 \pm 6.29
Mean age of death \pm SD	85.5 \pm 7.13	89.2 \pm 6.22	84.6 \pm 7.04
Mean years of education \pm SD	13.3 \pm 2.88	13.1 \pm 2.96	13.3 \pm 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%)
HMGR GG genotype frequency	697 (20.07%)	105 (21.43%)	592 (19.84%)
HMGR GA genotype frequency	1708 (49.18%)	250 (51.02%)	1458 (48.88%)
HMGR AA genotype frequency	1068 (30.75%)	105 (27.55%)	933 (31.28%)

Key: AD, Alzheimer's disease; APOE, Apolipoprotein E; SD, standard deviation.

The frequency of *Δxon13* 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 *Δxon13*, whereas 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 *Δxon13* between these 2 alleles is around 16 to 20 percent (Burkhardt et al., 2008; Medina et al., 2008). Heterozygotic (GA) expression of *Δxon13* 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 HMGR 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 cohort p -value = 0.003; Alzheimer's Disease Cooperative Study 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 noncarriers (Quebec founder

population p -value = 0.05) and APOE e4 carriers (Alzheimer's Disease Cooperative Study p -value = 0.041). In this study, we have evaluated Leduc et al. (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 HMGR 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, aged 65 years 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 data set, 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 HMGR 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 noncarriers (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: (1) G carriers, which accounts for both the minor allele homozygote, GG, and the heterozygotic GA genotype; and (2) G noncarriers, which is the AA genotype. We used logistic regression models to assess the association of AD with HMGR status to allow for the inclusion of age,

Table 2
Odds ratios and one-sided p -values from the 7 replication logistic regressions

Analysis		Cases/controls	Intercept	HMGR ^b	APOE e4	APOE e2	Gender	Power
General	LR p -value	490/2983	<1e-16	0.049 ^a	<1e-16 ^a	0.086 ^c	1.685e-05 ^a	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 ^a	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 ^a	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 ^a	NA	NA	NA	99%
	Odds ratio		0.369	0.712	NA	NA	NA	
APOE e2–	LR p -value	430/2473	<1e-16	0.029 ^a	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	

+ and – indicate carrier and noncarrier, respectively.

Key: APOE, Apolipoprotein E; LR, logistic regression.

^a Indicates significance.

^b Refers to rs3846662. Odds ratios in this column are relative to the G-negative genotype.

^c Indicates a trend.

Table 3

Odds ratios and one-sided *p*-values from the 8 additional logistic regressions that examined smaller gender subgroupings

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

+ and – indicate carrier and noncarrier, respectively.

Key: APOE, Apolipoprotein E; LR, logistic regression.

^a Indicates significance.

^b Refers to rs3846662. Odds ratios in this column are relative to the G-negative genotype.

^c Indicates a trend.

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. (2015) two-tailed *p*-values as we are restricted to the hypothesis that the G noncarrier 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; Kimmell, 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 HMGCRC 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 HMGCRC on AD status in our cohort. We used the number of cases and controls with the frequency of the HMGCRC allele to calculate the probability that we

Table 4

Summary of all 5 logistic regressions that generated statistically significant results

Category	General	APOE e4+	Female APOE e4+	Male APOE e4+	APOE e2-
Cases/controls	490/2983	268/801	185/453	83/348	430/2473
HMGCRC ^b Sig (one-tailed)	0.049 ^a	0.016 ^a	0.022 ^a	0.022 ^a	0.029 ^a
HMGCRC ^b odds ratio ^c	0.832	0.711	0.669	0.824	0.802
Power	100%	99%	96%	81%	100%

+ and – indicate carrier and noncarrier, respectively.

Key: APOE, Apolipoprotein E.

^a Indicates significance.

^b Refers to rs3846662.

^c Relative to the G-negative genotype.

Table 5

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

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

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

^a Indicates significance.

^b Refers to rs3846662.

^c Indicates Cache County cohorts with a one-tailed significance test.

^d Indicates cohorts from the study by Leduc et al. (2015) that are two-tailed significance values.

^e Indicates carrier.

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. In addition, significance was observed in noncarriers 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. (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 3 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*-

Table 6

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

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

+ and – indicate carrier and noncarrier, respectively.

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

^a Indicates significance.

^b Refers to rs3846662.

^c Indicates Cache County cohorts with a one-tailed significance test.

^d Indicates cohorts from the study by Leduc et al. (2015) that are two-tailed significance values.

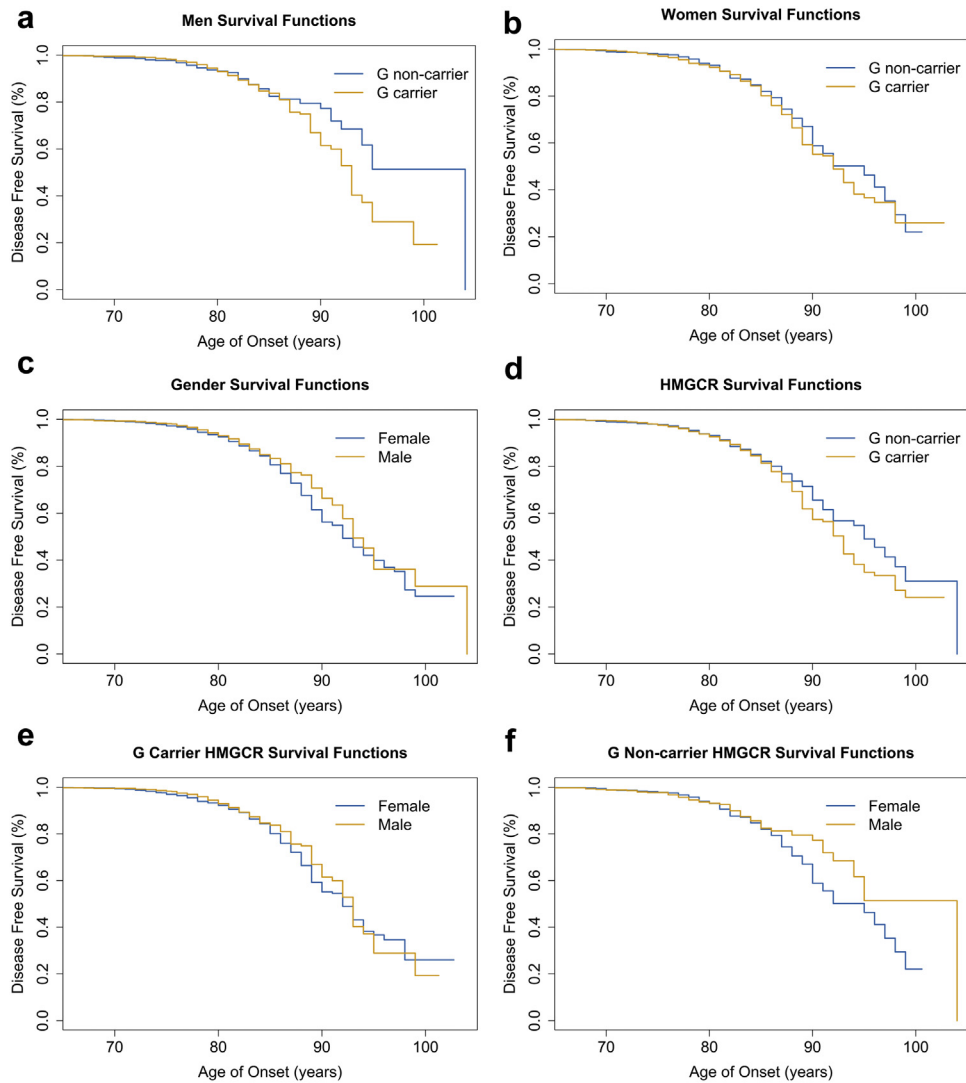


Fig. 1. Survival curves measuring effect of *HMGCRC* rs3846662 intron 13 on Alzheimer's disease (AD)-free survival. (A) Male age of onset of AD in rs3846662G noncarriers versus G carriers (p -value = 0.161). (B) Female age of onset of AD in rs3846662G noncarriers versus G carriers (p -value = 0.327). (C) Age of onset of AD in males versus females (p -value = 0.059). (D) Age of onset of AD in rs3846662G noncarriers versus G carriers (p -value = 0.102). (E) Age of onset of AD in rs3846662G carrier males versus females (p -value = 0.209). (F) Age of onset of AD in rs3846662G noncarrier males versus females (p -value = 0.134).

value = 0.016), as do *APOE* e2 noncarriers (p -value = 0.029), which is in concordance with the findings in the study by [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; [Fig. 1A](#)) and the onset of AD between females and their rs3846662 allele status (p -value = 0.327; [Fig. 1B](#)). We then created survival curves for 4 more

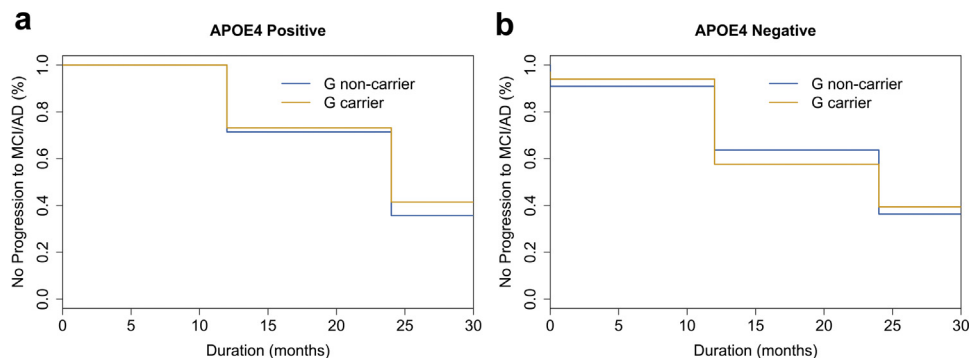


Fig. 2. Survival curves measuring the effect of *HMGCRC* rs3846662 intron 13 on mild cognitive impairment (MCI) conversion to Alzheimer's disease (AD). (A) Apolipoprotein E (*APOE*) e4 carriers comparing rs3846662G non-carriers versus G carriers (p -value = 0.663). (B) *APOE* e4 noncarriers comparing rs3846662G noncarriers versus G carriers (p -value = 0.671).

additional comparisons; in Fig. 1C and A comparison between genders (p -value = 0.059); in Fig. 1D and A comparison between the allele status of rs3846662 (p -value = 0.102); in Fig. 1E and A comparison between genders for G carrier rs3846662 allele status (p -value = 0.209); and in Fig. 1F and A comparison between genders for G noncarrier rs3846662 allele status (p -value = 0.134). We found no difference in conversion time from MCI to AD between G noncarrier and G carrier patients with the *APOE* e4 allele (p -value = 0.663; Fig. 2A) and without the *APOE* e4 allele (p -value = 0.671; Fig. 2B).

Power analyses demonstrated that each experiment had adequate statistical power (Tables 2, 3, 5 and 6). The lowest power observed in the 7 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. (2015) reported associations, including an overall protective effect and associations within *APOE* e2 noncarriers 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 rs3846662G noncarrier allele was significant (p -value = 0.02) and acted as a protective variant for AD in a northern Han Chinese population.

Because most 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). Because rs3846662 modulates levels of *Delta*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 *Delta*exon13 had a worse response to statin therapy (Leduc et al., 2016). Other studies have suggested that individuals with an abundance of *Delta*exon13 have poor response to statin treatment (Medina et al., 2008), suggesting that statin therapy might be a viable option in individuals who are G carriers as G noncarriers produce higher amounts of *Delta*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, whereas 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.

Although our findings do not definitely characterize the relationship between rs3846662 and AD, they do provide support for a protective effect for noncarriers of the “G” allele, which is pronounced in *APOE* e4 carriers and *APOE* e2 noncarriers. These

findings suggest that further study of the role of rs3846662 in AD risk and conversion from MCI to AD is warranted.

Disclosure

The authors declare no conflict of interest.

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