

ORIGINAL ARTICLE

# *HMGCR* is a genetic modifier for risk, age of onset and MCI conversion to Alzheimer's disease in a three cohorts study

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Several retrospective epidemiological studies report that utilization of 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) inhibitors called statins at mid-life can reduce the risk of developing sporadic Alzheimer's disease (AD) by as much as 70%. Conversely, the administration of these inhibitors in clinically diagnosed subjects with AD confers little or no benefits over time. Here, we investigated the association between AD and *HMGCR* rs3846662, a polymorphism known to be involved in the regulation of *HMGCR* exon 13 skipping, in a founder population and in two distinct mixed North American populations of converting mild cognitively impaired (MCI) subjects (Alzheimer's disease Cooperative study (ADCS) and Alzheimer's disease Neuroimaging Initiative (ADNI) cohorts). Targeting more specifically women, the G allele negative (G<sup>-</sup>) AD subjects exhibit delayed age of onset of AD ( $P=0.017$ ) and significantly reduced risk of AD (OR: 0.521;  $P=0.0028$ ), matching the effect size reported by the apolipoprotein E type 2 variant. Stratification for *APOE4* in a large sample of MCI patients from the ADCS cohort revealed a significant protective effect of G negative carriers on AD conversion 3 years after MCI diagnosis (odds ratio (OR): 0.554;  $P=0.041$ ). Conversion rate among *APOE4* carriers with the *HMGCR*'s G negative allele was markedly reduced (from 76% to 27%) to levels similar to *APOE4* non-carriers (27.14%), which strongly indicate protection. Conversion data from the independent ADNI cohort also showed significantly reduced MCI or AD conversion among *APOE4* carriers with the protective A allele ( $P=0.005$ ). In conclusion, *HMGCR* rs3846662 acts as a potent genetic modifier for AD risk, age of onset and conversion.

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## INTRODUCTION

Alzheimer's disease (AD) is an adult-onset chronic neurodegenerative disorder that occurs predominantly later in life. It is the commonest cause of dementia and represents the fourth most common cause of death in the developed world.<sup>1</sup> The two most famous pathological features of AD are the extracellular senile plaques, primarily composed of A $\beta$  peptides and the intracellular neurofibrillary tangles, resulting from the truncation and/or hyperphosphorylation of the microtubule-stabilizing Tau proteins (reviewed in Adlard and Cummings<sup>2</sup>). In recent years, patients diagnosed with mild cognitive impairment (MCI), a transitional stage between normal ageing and AD,<sup>3,4</sup> received overwhelming attention from the AD scientific community. It is estimated from previous research that nearly 80% of amnesic MCI patients, the dominant MCI subtype with a primary memory component,<sup>5</sup> will have converted to AD within the course of 6 years<sup>6</sup> at an annual conversion rate of 10–15%.<sup>7</sup> Given the absence of curative treatment, elucidation of factors affecting conversion of MCI to AD represents one of the most challenging and urgent medical mysteries affecting our ageing population.

About 5% of all AD cases show an autosomal dominant inheritance,<sup>8</sup> whereas a greater challenge lies in discovering the causes of the more common form of AD – dubbed sporadic AD. Indeed, the concordance rate of AD among identical twins was shown to vary from 60–72%,<sup>9,10</sup> highlighting the existence of

interplay between genetic, environmental and health factors.<sup>11</sup> Apolipoprotein E (*APOE*) encodes the main lipid carrier protein in the central nervous system and is the most robustly and consistently associated gene with AD risk, with the  $\epsilon 4$  (referred to here as *APOE4*) and  $\epsilon 2$  (referred to here as *APOE2*) alleles substantially increasing and decreasing the risk level, respectively.<sup>12–17</sup> The *APOE4* allele is also known to increase the likelihood of cognitive impairments in clinically normal 50+ years old over time<sup>18</sup> and to precipitate conversion to AD among MCI patients.<sup>5</sup> Despite being present in about 50% of AD cases, the *APOE4* allele is neither necessary nor sufficient for the development of AD.<sup>12,14,15</sup> The search for the identification of additional genes contributing to AD led to the identification of 695 candidate genes<sup>19</sup> of which a surprising number are directly involved in lipid metabolism at the level of transport, synthesis, storage and internalization of lipoproteins.<sup>20</sup> These include *BIN1*,<sup>21</sup> *PICALM*,<sup>22</sup> *ABCA7*<sup>21</sup> and *CLU*.<sup>22,23</sup> Although the genetic heterogeneity of these large genome-wide association studies have an increased power to detect risk genes with smaller effect sizes, population-relevant signal will likely go undetected.<sup>24</sup> The use of isolated populations with a few founders, such as the French Canadians of Quebec,<sup>25</sup> reduces the genetic background noise and allows the detection of population-specific signals.<sup>24</sup> Moreover, targeted testing of polymorphisms known to strongly associate with altered transcript levels may be a powerful way to identify

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genetic associations with diseases that would otherwise be difficult to detect.<sup>26</sup> Here, we evaluate a functional polymorphism (rs3846662) in 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) for association with AD in an isolated population, the Quebec founder population (QFP),<sup>25</sup> and corroborate our findings in two other well-characterized cohorts: the Alzheimer's disease Cooperative study (ADCS)<sup>3</sup> and the Alzheimer's disease Neuroimaging Initiative (ADNI) cohorts.<sup>27</sup>

*HMGCR* is a strong functional AD candidate gene because it encodes the 3-hydroxy-3-methylglutaryl-CoA reductase enzyme, which serves as the rate-limiting step in cholesterol synthesis in all mammalian cells. Cholesterol requirement of most brain cells are met by two separate yet interrelated processes: synthesis by *HMGCR* and internalization of lipoproteins via the APOE/low-density lipoprotein receptor cascade,<sup>28,29</sup> which is compromised in AD. Alterations in lipid homeostasis are known to severely impair neuronal function and elicit neurodegenerative disorders such as Niemann–Pick type C disease, a fatal cholesterol storage disorder characterized by the presence of AD-like intracellular neurofibrillary tangles in the brain.<sup>30</sup> Elevated plasma cholesterol levels are known among vascular risk factors of AD.<sup>31,32</sup> Although not a universal finding, the treatment of hypercholesterolemia with *HMGCR* inhibitors (statins) in middle-aged individuals confers some level of neuroprotection against late-life development of AD.<sup>33–36</sup> In addition, statin treatment was shown to reduce the cerebrospinal fluid phospho-Tau content.<sup>37</sup> This finding is consistent with the quasi-absence of cortical intracellular neurofibrillary tangles in autopsy-confirmed cognitively intact subjects who have used statins for several years as opposed to non-users.<sup>38</sup> Furthermore, whole-genome scans of late-onset AD cases reported the presence of several linkage hot spots across the genome, including one in the vicinity of the *HMGCR* gene on chromosome 5.<sup>19,39</sup> Accordingly, two recent studies found an association between *HMGCR* gene locus and AD.<sup>40,41</sup> One of *HMGCR*'s most important co-regulator of cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA synthase,<sup>42</sup> was also shown to be significantly associated with sporadic AD in stratified populations enriched in *APOE4*-negative subjects, pointing toward a possible cholesterol metabolism dysfunction in AD subjects born without the *APOE4* allele risk factor.

Overall, studies provide evidences of an association between *HMGCR* and AD, but this association is not compelling because none of the 21 genome-wide association studies performed to date substantiated *HMGCR* as a susceptibility gene for AD (current as of 31 January 2014: <http://www.alzgene.org/largescale.asp>). Interestingly, *HMGCR* undergoes alternative splicing of exon 13, which encodes a part of the catalytic domain of the enzyme. Two independent group reported the involvement of the intron 13 single-nucleotide polymorphism (SNP) rs3846662 in the regulation of *HMGCR* exon 13 skipping<sup>43,44</sup> by altering the binding motif of a molecule that regulates *HMGCR* alternative splicing: heterogeneous nuclear ribonucleoprotein A1.<sup>45</sup> As the  $\Delta 13$  transcript could not rescue *HMGCR* activity in UT2 *HMGCR*-deficient cells upon transfection,<sup>44</sup> it has been associated with lower levels of plasma low-density lipoprotein-cholesterol<sup>46</sup> and is one of the single most informative molecular marker of low-density lipoprotein-cholesterol response to statins.<sup>43,47,48</sup> The rs3846662 represented the ideal functional polymorphism to study in our three cohorts study. The rs3846662 is even more important to study in the context of AD and MCI given our original preliminary report of the presence of multiple genetic risk factors in the *HMGCR* gene and their impacts on AD pathology in a small cohort of autopsy-confirmed cases.<sup>49</sup>

## MATERIALS AND METHODS

### QFP (cohort 1): *HMGCR* gene in controls and AD cases

*Human Subjects Demographics.* Patients demographic characteristics are summarized in Table 1. Definite diagnosis of AD was based on the histopathological confirmation of AD according to NINCDS–ADRDA criteria,<sup>50</sup> whereas controls had to be free of neurological or psychiatric diseases and, for autopsy-confirmed cases, of brain structural lesions (tangle and plaque indices reading  $< 20 \text{ mm}^3$  and  $< 10 \text{ mm}^2$ , respectively). All subjects are from the so-called QFP (French Canadians of Quebec). This population (age-matched controls:  $N=250$ /autopsy-confirmed AD cases:  $N=324$ ) descended in genetic isolation from several thousand founders who emigrated from France in the 17th century.<sup>25</sup> The demographic history of the QFP, which is characterized by population bottleneck, rapid population expansion and little admixture, makes it a valuable resource for use in genetic studies.<sup>51</sup> The population has been well characterized as having reduced genetic heterogeneity for Mendelian

**Table 1.** QFP, ADCS and ADNI demographics

	<i>AD autopsy-confirmed cases</i>		<i>MCI patients</i>		<i>AD, MCI and elderly controls</i>	
	<i>Control</i> n = 250	<i>AD</i> n = 324	<i>Non-converters</i> n = 271	<i>AD converters</i> n = 138	<i>Non-converters</i> n = 935	<i>Converters</i> n = 298
Age at death/ recruitment, mean $\pm$ s.d. (years)	75.5 $\pm$ 11.1	79.2 $\pm$ 8.3	71.56 $\pm$ 7.48	74.18 $\pm$ 6.54	75.02 $\pm$ 7.06	75.43 $\pm$ 6.56
Age at onset/conversion, mean $\pm$ s.d. (years)	—	71.7 $\pm$ 8.9	—	75.4 $\pm$ 8.43	—	77.27 $\pm$ 6.8
Sex, no. of female (%)	124 (50)	210 (65)	115 (38.7)	69 (45)	348 (39)	97 (35)
Schooling, mean $\pm$ s.d. (years)	8.1 $\pm$ 4.0	8.9 $\pm$ 4.4	15.13 $\pm$ 2.86	14.67 $\pm$ 3.09	14.92 $\pm$ 4.74	14.90 $\pm$ 5.25
<i>APOE3</i> allele frequency	0.76	0.56	0.611	0.536	0.71	0.59
<i>APOE4</i> allele frequency	0.11	0.38	0.354	0.428	0.24	0.39
<i>APOE2</i> allele frequency	0.13	0.06	0.035	0.036	0.05	0.03
<i>HMGCR</i> A Allele frequency	0.60	0.53	0.53	0.49	0.47	0.46

Abbreviations: AD, Alzheimer's disease; ADCS, Alzheimer's disease cooperative study; ADNI, Alzheimer's disease neuroimaging initiative; QFP, Quebec Founder Population.

diseases.<sup>52</sup> Age at death, age at recruitment and education are not significantly different between controls and autopsy-confirmed AD subjects. *APOE* genotypes distribution is similar to previously reported prevalence for Eastern Canadians,<sup>12</sup> with a strong and significant enrichment of the *APOE4* allele in autopsied AD cases (Table 1). All brain and blood tissues were obtained from the Douglas Hospital Brain Bank, Montreal, Canada. Post-mortem delays generally varied from 10–20 h and were matched for control and AD subjects.

**DNA Extraction.** DNA was extracted from brain tissues (AD and control cases) or blood lymphocytes (control cases) using the DNeasy tissue kit (from Qiagen, Toronto, ON, Canada) and automated DNA extraction (NA-1000; AutoGen, Holliston, MA, USA), respectively.

**Sequencing of the *HMGR* gene and mapping of rs3846662.** Complete sequencing of the coding and non-coding regions of the *HMGR* gene was performed in 30 autopsy-confirmed AD cases and 15 age-matched control subjects using the Applied Biosystem 3730xl DNA analyzer from the McGill Innovation Centre. Genotype profiling of intron 13 of *HMGR* was performed with PCR followed by pyrosequencing.<sup>53</sup> The intron 13 SNP (rs3846662) was amplified using a PCR approach, with the following primer pairs: forward biotin 5'-TTTGCCAGTTTAAAATACATCAT-3' and reverse 5'-T TGACCCAAAAGGTA-TCACATAATT-3'. Genomic DNA (250–500 ng) was amplified with 0.2  $\mu$ M of each primer, 1X PCR buffer (Qiagen kit), 0.4 mM dNTP, 1.25 mM MgCl<sub>2</sub>, 0.05% DMSO and 0.01U of Qiagen Taq polymerase. Amplification was carried out on a Biometra T professional Basic thermocycler (Biometra, Göttingen, Germany) with the following conditions for 35 cycles: 30 s at 95 °C, 30 s at 50.9 °C and 1 min at 72 °C. These 35 amplification cycles were preceded by a 3-min hot start at 95 °C and followed by a final 4-min extension at 62 °C to the last cycle. PCR products were visualized on a 1.2% agarose gel. The intron 13 SNP was subsequently determined via an established pyrosequencing protocol<sup>53</sup> with oligo-sequencing 5'-ACTCTTCTCATTGCTTAC-3'. The sequence to analyze was: C/TTATGATGAT.

#### *ADCS (cohort 2): HMGR gene in MCI subjects*

**Human Subjects Demographics:** Patients demographic characteristics are summarized in Table 1. MCI patients recruited for the purpose of the present study took part in the 3 years follow-up, double-blind ADCS<sup>3</sup> and provided written informed consent for AD-related genetic screening. Information about the study design, methods to determine MCI diagnosis as well as conversion to AD can be found in the published ADCS study.<sup>3</sup> Age at recruitment and education was equivalent between AD converters and non-converters. Consistent with previous findings,<sup>6</sup> *APOE* genotypes distribution was significantly different across groups, with a significant disproportion of *APOE4* allele found in AD converters (Table 1). **DNA Extraction and mapping of rs3846662:** DNA extraction from blood samples was performed using Qiagen kits as described in the published ADCS study.<sup>3</sup> Mapping of rs3846662 in intron 13 was performed as described for the QFP cohort.

#### *ADNI (cohort 3): HMGR gene in a mix population of AD, MCI and cognitively intact elderly*

**Human Subjects Demographics, DNA Extraction and mapping of rs3846662.** Patients demographic characteristics are summarized in Table 1. Genotyping information on elderly controls from the ADNI cohort was obtained via a genome-wide whole-brain analysis including 620 901 SNPs using the Human 610-Quad Bead Chip (Illumina, San Diego, CA, USA). Genotype profiling of the intron 13 of the *HMGR* gene was extracted from the open-access database and data from the 1233 individuals recruited during ADNI-I were compiled for further conversion-to-deteriorated cognitive level analyses. Information about the specific genome-wide association studies protocol used to obtain genotyping information can be found in a recent report by Shen and collaborators,<sup>27</sup> whereas the full clinical data set used in the preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the United States and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date, these three protocols have recruited over 1500 adults, ages 55–90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI and people with early AD. The follow-up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO. Subjects

originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see [www.adni-info.org](http://www.adni-info.org).

#### Statistical Analyses for the three cohorts

Binary logistic regressions were computed between *HMGR* polymorphism and disease status in the QFP and ADCS cohorts. Stratification by gender and *APOE* genotype was performed on these cohorts using Wald statistics. Odds ratios (ORs) for *HMGR* and *APOE* polymorphisms were also calculated across cohorts.

Wilcoxon  $X^2$ -rank tests derived from the Kaplan–Meier survival curves were used to contrast the effects of the different genetic variants of the *HMGR* and *APOE* polymorphisms on the age of onset of AD in autopsy-confirmed AD cases from the QFP cohort. Finally, MCI-to-AD conversion rate during the 3 years ADCS study and conversion rate during the 48-months ADNI study was computed as a function of the *HMGR*'s intron 13 allele using Wilcoxon rank  $X^2$ -tests derived from the Kaplan–Meier survival curves stratified by *APOE* genotype.

## RESULTS

In order to identify AD-specific SNPs within the *HMGR* gene, a complete sequencing of the coding and non-coding regions of the *HMGR* gene was performed in 30 autopsy-confirmed AD cases and 15 age-matched control subjects of the QFP. The relatively homogeneous environmental exposures and reduced genetic heterogeneity associated with this population<sup>25,51,52</sup> were likely to be advantageous for the study of AD, a disease resulting from the genetic and environmental interplay. The use of autopsy-confirmed cases allowed us to further reduce the false-positive background noise normally seen in clinical diagnosis. This preliminary study failed to reveal any disease-specific genetic mutations in all 20 exons. A rare polymorphism in exon 15 was detected but did not differ in terms of group incidence between AD and age-matched control subjects. Mapping of the introns, on the other hand, turned out to be more interesting as the rs3846662 SNP in intron 13 of the *HMGR* gene (A or G allele) was found to significantly associate with sporadic AD.

Table 1 summarizes the frequency distribution obtained in our QFP cohort that included 250 controls and 324 AD cases. The association between *HMGR*'s intron 13 G negative polymorphism (AA) and sporadic AD was found to be significant (OR=0.694;  $P=0.024$ ; Table 2). Stratification by gender revealed that this association is significant only in women (women: OR=0.521;  $P=0.0028$ ; men: OR=0.890;  $P=0.686$ ) as is the case for the well-known *APOE2* allele benefit (women: OR=0.316,  $P<0.001$ ; men: OR=0.679;  $P=0.293$ ) in the same group of subjects (Table 2).

Analyses of the impact of the *HMGR* polymorphism on age of onset in AD is summarized in Figure 1. Although we did not observe any effect of this variant on the age at death (Wilcoxon survival test:  $X^2_{1, 293} = 0.950$ ;  $P=0.330$ ), a significant effect of a double dose of intron 13 A allele (G negative genotype) on the age of onset was detected (Wilcoxon survival test:  $X^2_{1, 289} = 4.57$ ;  $P=0.024$ ). This *HMGR* protective genotype exerted a strong impact in women (Wilcoxon survival test:  $X^2_{1, 188} = 6.09$ ;  $P=0.017$ ) who exhibited a delayed age of onset of about 3.6 years. This age effect in G negative subjects was not found in men (Wilcoxon survival test:  $X^2_{1, 100} = 1.88$ ;  $P=0.170$ ). Analysis of the 'A' variant dose effect on age of onset reveals a significant association ( $P<0.03$ ) in women, particularly between the age of 60 and 80 (Supplementary Materials).

As reported previously in several independent studies,<sup>16,17</sup> a very similar protective effect was observed with the *APOE2* allele (Wilcoxon survival test:  $X^2_{1, 289} = 5.05$ ;  $P=0.019$ ), women displaying again the most significant impact on age of onset (Wilcoxon survival test:  $X^2_{1, 188} = 6.43$ ;  $P=0.013$ ; Figure 1). In sharp contrast, the *APOE4* allele in this study was strongly associated with an earlier age of onset of AD (Wilcoxon survival test:  $X^2_{1, 289} = 11.32$ ;  $P<0.001$ ), particularly so in women (Wilcoxon survival test:  $X^2_{1, 188} = 7.48$ ;  $P=0.009$ ).

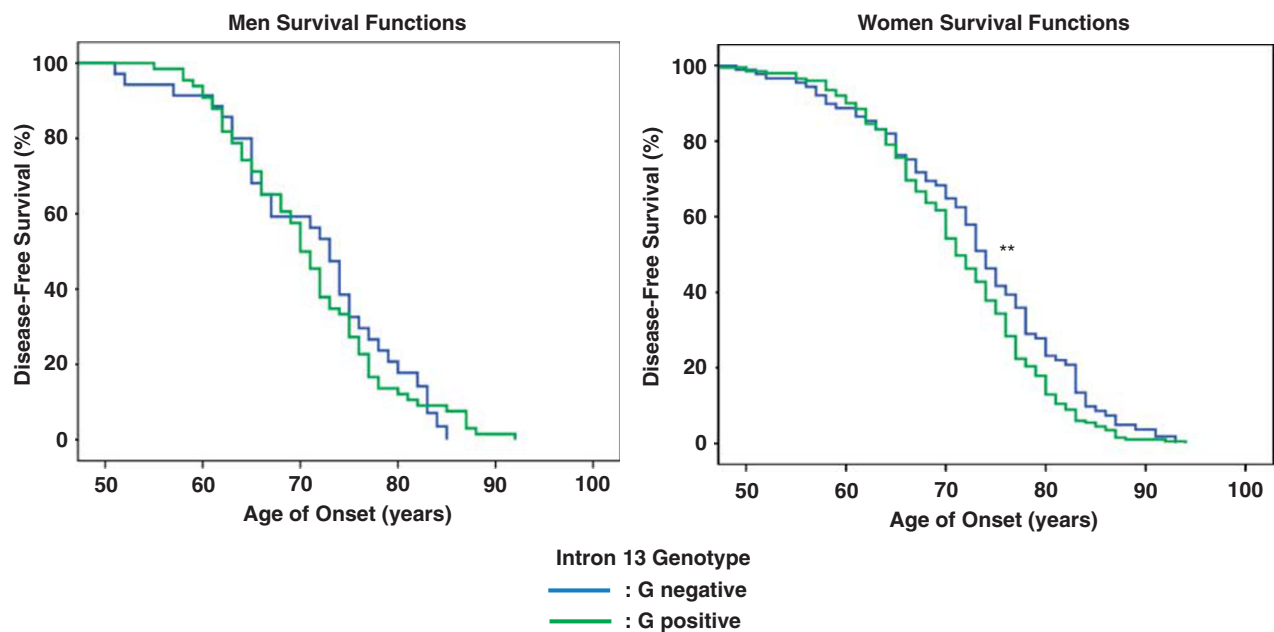
**Table 2.** Binary logistic regression between *HMGR* rs3846662 and the AD status (QFP cohort)

Allele	Overall effect			Women			Men		
	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR
<i>HMGR</i> -G-	574	0.024*	0.694	334	0.003*	0.521	240	0.686	0.890
<i>APOE4</i>	573	0.001**	6.180	333	0.001**	7.204	240	0.001**	5.253
<i>APOE2</i>	573	0.001**	0.447	333	0.001**	0.316	240	0.293	0.679

Allele	Non- <i>APOE4</i> carriers			<i>APOE4</i> carriers			Non- <i>APOE2</i> carriers			<i>APOE2</i> carriers		
	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR
<i>HMGR</i> -G-	308	0.634	0.881	262	0.183	0.713	469	0.05*	0.634	101	0.304	1.558

Abbreviations: AD, Alzheimer's disease; *HMGR*, 3-hydroxy-3-methylglutaryl-CoA reductase; *HMGR*-G-, G negative versus G positive genotype; N, sample size; OR, odds ratio. Asterisks represent significant risk for AD at the 95% confidence interval (\*) or 99% confidence interval (\*\*) level. The regression was performed in the autopsy-confirmed AD cases of the QFP cohort. Secondary analyses were performed in the same population but stratified by gender or *APOE* genotype, using Wald statistic. The odds ratios are also provided.

**Figure 1.** Age of onset in *HMGR* rs3846662 intron 13 G negative vs G positive carriers. The joint table contrasts the effects of the different genetic variants of the *HMGR* gene to those of *APOE* using a Wilcoxon  $\chi^2$ -rank test. *HMGR* G-, G negative genotype versus G positive. Asterisks represent significant association for AD at the 99% CI (\*\*) level.

In summary, studies performed in our autopsied-confirmed QFP subjects uncovered a protective association between *HMGR*'s G negative genotype, AD risk and age of onset, particularly in women. The question was then to decipher whether or not the A allele could modulate the *APOE4* risk in patients not affected by full-blown AD, such as in MCI patients.

In a follow-up study, a total of 409 MCI patients from the original Petersen *et al.*,<sup>3</sup> MCI conversion trial (ADCS) were thus genotyped for *APOE* and *HMGR* polymorphisms. Table 1 summarizes the frequency distribution obtained in MCI patients from the 3 years ADCS study that included 271 non-converters and 138 converters to AD. Conversion was determined by an expert panel from the ADCS study (refer to Petersen *et al.*<sup>3</sup> for more details). Associations between intron 13 A allele and AD conversion were not found to be significant (OR=0.726;  $P=0.129$ , Table 3). However, *APOE4* genotype stratification revealed a significant protective effect in G negative carriers on AD conversion among *APOE4*-positive MCI patients (OR=0.554;  $P=0.041$ , Table 3). Interestingly, the conversion rate among *APOE4*/*HMGR*'s

G negative subjects was markedly reduced (from 76–26.97% conversion to AD) to levels similar to *APOE4* non-carriers (27.14% conversion to AD) at 3 years' post-MCI diagnosis. This suggests that the *HMGR* gene variant can markedly attenuate *APOE4* risk, especially in the pre-dementia stages of the disease.

To extend and replicate the conversion dataset in the ADCS cohort, we decided to examine the conversion rate from normal controls/MCI/AD to MCI/AD in the ADNI cohort as a function of *HMGR*'s G allele (positive vs negative) status and *APOE* stratification. To this end, we used genotyping data from ADNI that included 1233 individuals who were followed over a period of 48 months for conversion to MCI or AD. Table 1 summarizes the frequency distribution obtained in this mix population of cognitively intact, MCI and AD subjects that included 935 non-converters and 298 converters. Consistent with the above-mentioned ADCS' findings, associations between G negative status and MCI/AD conversion were not found to be significant (OR=1.019;  $P=0.908$ ). However, the G negative polymorphism exerted a very significant protective effect specific to *APOE4*



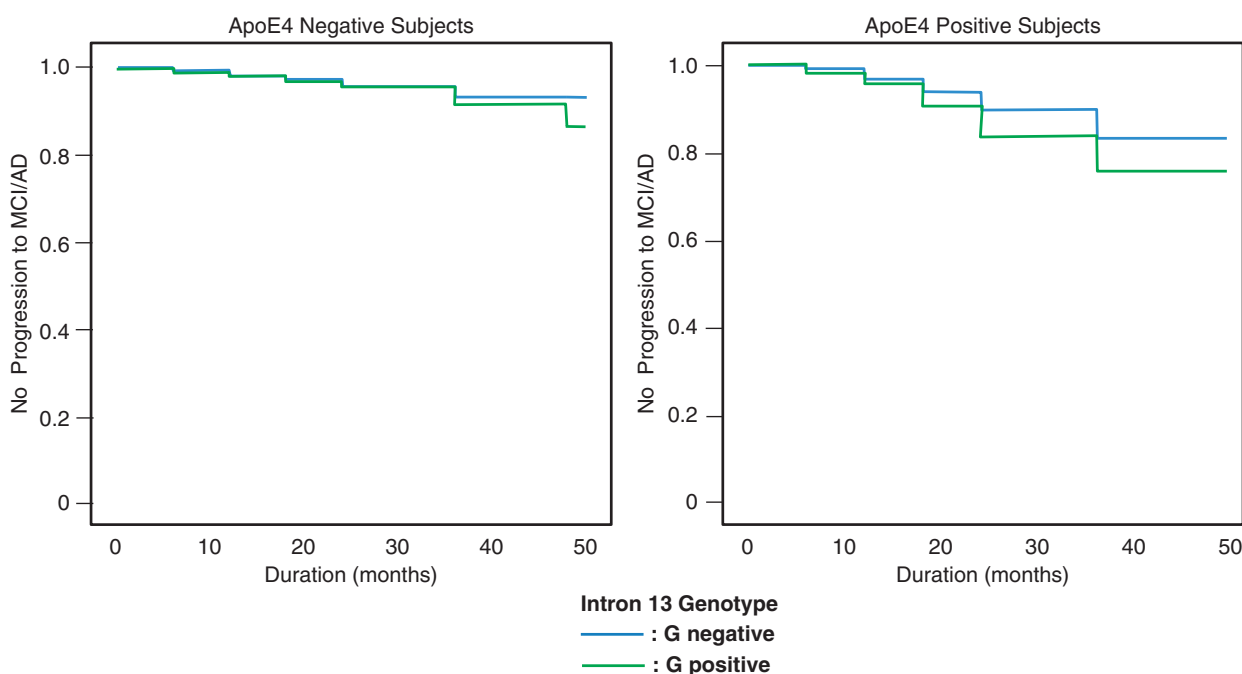
**Table 3.** Binary logistic regression between *HMGR* rs3846662 and the AD status (ADCS cohort)

Allele	Overall effect			Women			Men		
	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR
<i>HMGR</i> -G-	409	0.129	0.726	164	0.342	0.798	245	0.145	0.650
<i>APOE4</i>	408	0.029*	1.573	164	0.017*	2.24	244	0.285	1.238
<i>APOE2</i>	409	0.118	0.408	164	0.209	0.403	245	0.296	0.355

Allele	Non- <i>APOE4</i> carriers			<i>APOE4</i> carriers			Non- <i>APOE2</i> carriers			<i>APOE2</i> carriers		
	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR	N	Sig. (two-tailed)	OR
<i>HMGR</i> -G-	140	0.476	1.129	268	0.041*	0.554	392	0.156	0.742	17	0.579	0.545

Abbreviations: AD, Alzheimer's disease; ADCS, Alzheimer's disease cooperative study; *HMGR*, 3-hydroxy-3-methylglutaryl-CoA reductase; *HMGR*-G-, G negative versus G positive genotype; N, sample size; OR, odds ratio. Asterisks represent significant risk for AD at the 95% C.I. (\*) level. Regressions were computed in the MCI patients of the ADCS cohort. Secondary analyses were performed in the same cohort stratified by gender and *APOE* genotype using Wald Statistics. Odds ratios are also provided



**Figure 2.** Kaplan–Meier estimates of the rate of progression from normal to mild cognitive impairment (MCI) and Alzheimer's Disease (AD). Conversion rate, stratified by *APOE* genotype, among subjects from the Alzheimer's disease Neuroimaging Initiative (ADNI) cohort as a function of the *HMGR* intron 13 genotype (*APOE4*-positive subject,  $P=0.005$ ). AA: G negative genotype; AG/GG: G positive genotype.

carriers ( $\chi^2_{1, 756} = 7.751$ ;  $P=0.005$ ; Figure 2). Analysis of senile plaques and neurofibrillary tangles densities in a cohort of 118 autopsied AD cases derived from our eastern Canadian population isolate reveals that the reported apoE4-mediated increases in hippocampal and cortical plaques and tangles density in AD is actually prevented in G negative carriers as opposed to G positive subjects (Supplementary Materials), consistent with the protective role of G negative polymorphism on age of onset and conversion rate. As initially hypothesized, these confirmatory results clearly indicate that *APOE* and *HMGR* genes are interdependently modulating the conversion to MCI or AD among an at-risk cognitively intact or MCI population.

## DISCUSSION

The association between sporadic AD and the *HMGR*'s rs3846662 G negative status in this three cohorts study clearly identify the

*HMGR* gene as one of the most important and common protective variant ever identified for sporadic AD, second only to *APOE2*. It provides us with a novel lead explanation as for the discrepancies between retrospective and prospective studies dealing with the potential benefit of statins in sporadic AD. Indeed, several retrospective cross-sectional observational studies have shown that statins can reduce by up to 70% the risk of developing AD.<sup>33,35</sup> However, results of prospective studies have been inconsistent,<sup>54–56</sup> and recent double blind placebo-controlled clinical trials in mild-to-moderate AD using simvastatin and atorvastatin for at least 6 months failed to show disease stabilization or improvement,<sup>57,58</sup> mostly due to a marked treatment response heterogeneity. This suggests that for statins to reduce risk, it must be taken during a certain critical period and for a certain length of time, preferably years before the expected onset of the disease. Corroborating this conclusion, the protective *HMGR*'s G negative polymorphism was shown to modulate

*APOE4* risk in cognitively intact and MCI subjects and, to delay age of onset of AD by 3.6 years. These findings are consistent, at least in part, with a recent small case-control study that reported an interaction between the rs3761740 A allele in the promoter region of the *HMGCR* gene, the *APOE E4* allele and an altered risk of AD (OR = 2.41; 95% confidence interval = 0.93–6.22).<sup>59</sup> Analysis of the allelic distribution of this promoter variant and the rs3846662 SNP examined in the present study reveals a potent linkage disequilibrium (probability: 0.97) between the two variants in our population isolate from eastern Canada, which could very well explain the complementary nature of findings reported by the two research teams (data not shown).

Whether the protective effect of statins on AD risk is mediated through reduction of vascular risk factors or through the direct modulation of central nervous system cholesterol homeostasis remains to be clarified. Interestingly, Wolozin *et al.*<sup>36</sup> reported that statins neuroprotective properties sharply differed as a function of lipids solubility, an observation recently replicated in a secondary analysis of the 'preventive' Ginkgo Evaluation of Memory Study.<sup>60</sup> These studies found that the more lipid-soluble (that is, simvastatin) *HMGCR* inhibitors exhibited high protective effects as opposed to the more lipophobic and less likely to cross the blood-brain barrier statins (that is, atorvastatin), which exhibited little to no protective effect. These observations would thus favor the hypothesis that statin mediates its neuroprotective effect through direct modulation of central nervous system cholesterol homeostasis. Unfortunately, none of the statin studies examined the contribution of the genetic polymorphisms of *HMGCR* and *APOE* on the extent of the protective effect.

Combined with the results of the genome-wide screening on chromosome 5 (refs 19,39) our findings clearly point toward a potential role of the *HMGCR* in the etiopathology of AD. Our study indicates that carriers of the intron 13 rs3846662 variant display a protective effect that resemble in size and gender to what has been reported for *APOE2* in humans. The similarity between the genetic association of *APOE*, the brain's most important cholesterol transporter, and *HMGCR*, the rate-limiting step in cholesterol synthesis in the brain, is revealing to be quite interesting. On one hand, *APOE4* is a risk factor that precipitates age of onset, markedly so in women, whereas the *APOE2* variant as well as the *HMGCR*'s G negative polymorphism both delay age of onset of AD, more so, in women. Furthermore, *APOE* is perceived by lipid neurobiologists as a key extracellular lipid transport protein, whereas *HMGCR*, which is localized in the endoplasmic reticulum, is primarily an intracellular, organelle-specific protein that regulates intracellular lipid production. It is thus quite conceivable that these two proteins actually have complementary roles in the maintenance of local brain cholesterol homeostasis, particularly in presence of neurodegeneration or damage.

In conclusion, this three cohorts study provides strong evidence that *HMGCR* is a genetic modifier for risk, age of onset and MCI conversion to AD. Converging evidence have now confirmed the involvement of rs3846662 SNP in *HMGCR* exon 13 skipping in peripheral cells *in vivo*, the A allele being associated with an increased in exon 13 skipping.<sup>44</sup> As reflected by its association with decreased low-density lipoprotein-cholesterol levels,<sup>46</sup> the rs3846662 A allele is associated with decreased *HMGCR* activity. This finding is in accordance with the blunted response to statin therapy observed in G negative (or AA allele) carriers.<sup>43,47,48</sup> Given that these findings were obtained mostly in the periphery, whether the rs3846662 is as important for the central nervous system needs to be substantiated. Studies addressing if the rs3846662 modulates mRNA splicing, protein *HMGCR* levels and activity in the human brain are currently underway in autopsied human brains.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

A complete listing of ADNI investigators can be found at: [http://adni.loni.ucla.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Acknowledgement\\_List.pdf](http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf)Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)