

The *CALHM1* P86L Polymorphism is a Genetic Modifier of Age at Onset in Alzheimer's Disease: a Meta-Analysis Study

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Abstract. The only established genetic determinant of non-Mendelian forms of Alzheimer's disease (AD) is the $\epsilon 4$ allele of the apolipoprotein E gene (*APOE*). Recently, it has been reported that the P86L polymorphism of the calcium homeostasis modulator 1 gene (*CALHM1*) is associated with the risk of developing AD. In order to independently assess this association, we performed a meta-analysis of 7,873 AD cases and 13,274 controls of Caucasian origin (from a total of 24 centers in Belgium, Finland, France, Italy, Spain, Sweden, the UK, and the USA). Our results indicate that the *CALHM1* P86L polymorphism is likely not a genetic determinant of AD but may modulate age of onset by interacting with the effect of the $\epsilon 4$ allele of the *APOE* gene.

Keywords: Age at onset, Alzheimer's disease, apolipoprotein E, *CALHM1*, polymorphism

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INTRODUCTION

Although Alzheimer's disease (AD) is the most common cause of dementia in the elderly, its etiology is still not fully understood. The characterization of causative factors is thus important for better defining the pathophysiological processes involved. Hereditary, early-onset forms of AD have been linked to disease-causing mutations in three different genes: the amyloid- β protein precursor (*A β PP*) gene on chromosome 21, the presenilin 1 (*PSEN1*) gene on chromosome 14, and the presenilin 2 (*PSEN2*) gene on chromosome 1 [1]. However, the known mutations in these three genes account for less than 1% of all AD cases [2]. Most forms of AD develop after the age of 65 and are considered to be sporadic because they lack an obvious familial aggregation. The term "sporadic" has, however, been gradually replaced by the concept of non-Mendelian (i.e., genetically complex) transmission. Although the importance of the genetic component of these non-Mendelian forms has long been debated, there is now a large body of evidence suggesting that genetic variation plays the major role in determining risk for this form of AD as well. This evidence is largely based on twin studies which have shown that the heritability of AD in general is high (between 60 and 80%) [3]. This latter study has also shown that age at onset (AAO) is significantly more consistent for pairs of monozygotic twins than for dizygotic twins, indicating that genetic variants also explain a substantial proportion of AAO variation across AD cases [3]. While these observations highlight the importance of genetic factors in the risk for developing AD, at present, only the ϵ 4 allele of the apolipoprotein E (*APOE*) gene has been unequivocally identified as a major determinant for the non-Mendelian forms of AD [4–6]. In addition, currently more than two dozen loci show significant risk effects in meta-analyses synthesizing the available data from all published studies in the field. (<http://www.alzgene.org>) [7].

We recently reported that the gene coding for the newly characterized calcium homeostasis modulator 1 (*CALHM1*) channel may be a potential genetic risk factor for non-Mendelian forms of AD. The less common allele (L) of a non-synonymous polymorphism (P86L or rs2986017) within this gene was found to be associated with an increased risk for developing AD. Further it was shown that the underlying amino-acid substitution from proline to leucine leads to a loss of Ca^{2+} permeability, modulation of *A β PP* metabolism and, ultimately, to an increase in *A β* peptide secretion [8]. However, although *CALHM1*'s biological

properties make it a plausible AD risk factor [8,9], most of the currently published follow-up studies in Caucasian populations were unable to confirm the association between the P86L polymorphism and the risk of developing AD [10–14], with the exception of one report [15]. Despite this contradictory data using affection status as phenotype, three studies, in addition to the original report, showed association between an earlier AAO and homozygosity of the L allele and a marker in the *CALHM1* vicinity [11,15,16].

In this study, we assessed the question whether or not *CALHM1* is a genetic susceptibility factor for non-Mendelian AD, we genotyped a total of 9,662 individuals (2,249 cases and 7,413 controls) not previously tested for *CALHM1* and performed a meta-analysis synthesizing these data with previously published genotypes in a total sample of 7,873 AD cases and 13,274 controls of Caucasian origin.

MATERIALS AND METHODS

Case-control samples were obtained from centers in Belgium (1 study) [12,17], Finland (1 study) [10], France (3 studies) [8,18], Italy (10 studies) [14,17], Spain (4 studies) [15,17], Sweden (1 studies) [10], the UK (1 study) [9], and the USA (3 studies) [8,11,13]. The main characteristics of the different populations in each country are described in Supplementary Table 3 (available online: Supplementary data available online: <http://www.j-alz.com/issues/22/vol22-1.html#supplementarydata04>). Clinical diagnoses of probable AD were all established according to the DSM-III-R and NINCDS-ADRDA criteria [19]. Controls were defined as subjects not meeting the DMS-III-R dementia criteria and with intact cognitive functions (mini mental status examination score > 25). Written informed consent to participation was provided by all subjects or, in cases of substantial cognitive impairment, a caregiver, legal guardian or other proxy. The study protocols for all populations were reviewed and approved by the appropriate institutional review boards in each country. Depending on the center, a broad range panel of technologies were used to genotype the rs2986017 SNP [8, 10–15].

Univariate analysis was performed using Pearson's χ^2 test. Review Manager software release 5.0 (<http://www.cc-ims.net/RevMan/>) was used to estimate the overall effect (random effect odds ratio). For multivariate analysis, SAS software release 9.1 was used (SAS Institute, Cary, NC) and inter-population homo-

Table 1
Association between the CALHM1 P86L polymorphism and age at onset (in years \pm SD) for all AD cases and for ϵ 4 or non- ϵ 4 AD cases

	Whole		ϵ 3 bearers		Non ϵ 4 bearers	
	n	age at onset	n	age at onset	n	age at onset
GG	3658	73.0 \pm 8.9	1969	72.0 \pm 7.9	1673	74.2 \pm 9.8
AG	2761	73.1 \pm 8.9	1473	71.9 \pm 8.3	1277	74.4 \pm 9.5
AA	588	71.8 \pm 8.9	316	70.2 \pm 8.2	271	73.6 \pm 9.3
p^1		0.004		2×10^{-4}		0.78
Δ (AA versus AG+GG) ²		-1.2		-1.8		-0.7
p^3		8×10^{-4}		4×10^{-5}		0.54

¹mixed model adjusted for gender and using center as a random variable

² Δ , the difference in AAO between LL and PL + PP carriers (in years).

³the difference in AAO between LL and PL + PP carriers, using a mixed model adjusted for gender and with center as a random variable.

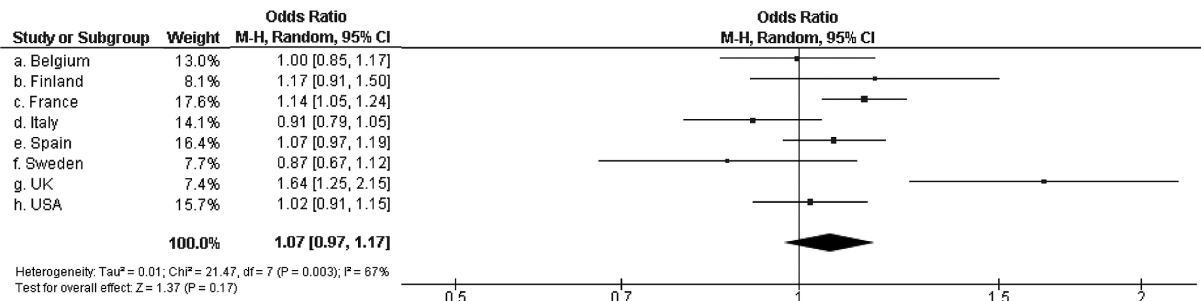


Fig. 1. Association between the P86L L allele and the risk of developing AD in the different case-control studies, according to the country of origin.

genity between was tested using Breslow-Day computation [20]. The association of the P86L polymorphism with the risk of developing AD was assessed by a multiple logistic regression model adjusted for age, gender, *APOE* status and center or country (see Supplementary Table 3 for description of AAO per country). The association between the P86L polymorphism and AAO was assessed using a mixed model adjusted for gender and using the center as a random variable. Similar results were obtained when using the country as a random variable (data not shown). The presence or absence of an interaction between *APOE* status and the P86L polymorphism was systematically assessed in all logistic regression or mixed models.

RESULTS

Upon combining all available case-control genotype data for the P86L SNP in allele-based effects meta-analyses, we observed that the population-specific ORs showed significant evidence for heterogeneity across datasets ($p = 0.003$). We thus calculated the summary OR using a random-effects model, where the

overall P86L association appeared to be not significant (OR = 1.07; 95% confidence interval (CI) [0.97–1.17]; $p = 0.17$; Fig. 1). Upon exclusion of the five initial case-control datasets (all part of the initial, positive study) [8], the heterogeneity across population-specific ORs was substantially reduced ($p = 0.29$), but neither meta-analysis showed significant results (OR = 1.01; 95% CI [0.95–1.08]; $p = 0.76$).

As we had access to subject-level genotype and phenotype data for all samples, we also tested for association between P86L and AD risk by pooling data across studies and adjusting for age, gender, *APOE* ϵ 4 status, and center using an additive logistic regression model. This model is equivalent to the allelic association approach when the conditions for Hardy-Weinberg equilibrium are met [21], which was true for the combined sample (Supplementary Table 1). In this model, the L allele of the P86L polymorphism was weakly associated with AD (OR = 1.09; 95% CI [1.03–1.15]; $p = 0.002$). However, this association was mainly driven by the initial case-control datasets of the original report, and was no longer significant after exclusion of these samples (OR = 1.02; 95% CI [0.95–1.08], adjusted for age, gender, *APOE* status, and center; $p = 0.66$).

Table 2
Association between the *APOE* $\epsilon 4$ allele alone and in combination with the P86L polymorphism with age at onset (in years \pm SD)

<i>APOE</i>	n	Age at onset ¹	<i>APOE</i>	rs2986017	n	Age at onset ²
$\epsilon 4 - / \epsilon 4 -$	3223	74.2 \pm 9.6	$\epsilon 4 - / \epsilon 4 -$	AG+GG	2952	74.3 \pm 9.7
				AA	271	73.6 \pm 9.3
$\epsilon 4 - / \epsilon 4 +$	3027	72.5 \pm 8.1	$\epsilon 4 - / \epsilon 4 +$	AG+GG	2774	72.6 \pm 8.1
				AA	253	70.9 \pm 8.3
$\epsilon 4 + / \epsilon 4 +$	736	68.4 \pm 7.5	$\epsilon 4 + / \epsilon 4 +$	AG+GG	671	69.0 \pm 7.5
				AA	65	67.2 \pm 7.0

¹ $p = 1.1 \times 10^{-31}$ (mixed model adjusted for gender and using center as a random variable).

² $p = 2.6 \times 10^{-31}$ (mixed model adjusted for gender and using center as a random variable).

Finally, we assessed the association of the P86L polymorphism with AAO using a mixed model with center of origin as a random variable. As previously reported [8,11,15], patients bearing the LL genotype displayed an earlier AAO than carriers of the LP and PP genotype (71.8 \pm 8.9 vs. 73.0 \pm 8.9 years of age, respectively; $p = 8 \times 10^{-4}$; Table 1 and supplementary Table 2). This association was still observed after exclusion of the initial samples (73.2 \pm 8.2 vs. 74.3 \pm 8.2 years of age, respectively; $p=0.001$). Following the detection of an interaction between the P86L, *APOE* $\epsilon 2/\epsilon 3/\epsilon 4$ polymorphisms and AAO ($p = 0.04$), we stratified the data according to *APOE* status and observed that the association of the LL genotype with AAO was the strongest in $\epsilon 4$ carriers (70.2 \pm 8.5 vs. 72.0 \pm 8.2 years; $p = 4 \times 10^{-5}$ (Table 1 and Supplementary Table 2). Again, this association was still observed after exclusion of the initial samples (71.9 \pm 7.4 vs. 73.2 \pm 7.5 years of age, respectively; $p = 0.002$).

When taking into account the well characterized *APOE* $\epsilon 4$ allele dose effect on AAO, we observed that the P86L LL genotype was systematically associated with a decrease in AAO in $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ carriers (Table 2). Comparison of likelihood ratio between a mixed model including only *APOE* genotype and a mixed model including both *APOE* and CALHM1 genotypes indicated that addition of the CALHM1 P86L polymorphism was more informative to explain the AAO variability than the *APOE* $\epsilon 4$ allele alone ($p = 1 \times 10^{-10}$).

DISCUSSION

Using both novel and previously published genotype data, we performed meta-analyses of 7,873 AD cases and 13,274 controls from 24 centers assessing the potential association between the P86L polymorphism in CALHM1 and risk for AD, but were unable to replicate the initial findings. The discrepancy of risk effects

between the independent follow-up data and the data first published by Dreses-Werringloer et al. [8], may indicate a false-positive finding in the initial report, a situation commonly observed in genetically complex diseases and referred to as “proteus phenomenon” or to as the “winner’s curse phenomenon” [22]. In addition to chance variation and technical artifacts, this may be caused by population substructure across cases and controls included in the affected association studies. Indeed, this type of difference can lead to spurious associations between diseases and genetic markers [23–26], particularly when low increases in risk are involved [27]. This observation may be particularly relevant for the P86L L allele, since its frequency appears to be highly variable (even ranging from 20 to 31% for Caucasian populations) and its association with AD risk was categorized as moderate in the initial report [8].

However, even though our meta-analysis results rather unequivocally refute the initial findings suggesting that CALHM1 is a genetic risk factor for AD, the present work suggests that the CALHM1 P86L polymorphism could modulate AAO and more specifically the *APOE* $\epsilon 4$ allele’s dose effect on this phenotype. Interestingly, several studies have shown that AAO in AD is highly heritable [28,29], and (in addition to the strong association of the $\epsilon 4$ allele with AAO) it has been suggested that genes such as GTS1 or GTS2 may have a specific effects on AAO without necessarily modifying the risk for developing AD [30–32], although these findings have not been independently replicated to date. In this context, it is worth noting that AAO data are difficult to acquire reliably reducing the power of such analyses. Although the large overall sample size analyzed in the present study should help to decrease the likelihood of a false-positive outcome, additional genetic studies will be required to further characterize the association between the P86L polymorphism and AAO in $\epsilon 4$ -carriers. However, it appeared that the association of the P86L polymorphism with AAO was still

observed after exclusion of the initial samples, this supporting a real impact of *CALHM1* on disease progression. It is also worth noting that factors affecting AAO tends to be spuriously associated with disease susceptibility (and the younger the cases the stronger this artifact association may be) and this confounding effect may explain in part positive results in cross-sectional studies [33].

Furthermore, it would be of particular interest to extend the association analyses to non-Caucasian populations, such as those of South-East Asian (for which conflicting results have already been reported [34–36]), or African descent. However, since the P86L L allele frequency is lower in Asian populations than Caucasian populations, particularly large sample sizes will be needed to detect significant risk or AAO effects.

Given that the P86L L allele has been associated with an increase in A β production *in vitro* [8], confirmation of this association with AAO may indicate that a variation in A β production can modulate AD progression without increasing the AD risk. Interestingly, biological evidence suggests that both the *APOE* gene and the genetic determinants characterized in two recent genome-wide association studies (GWASs) in AD may be primarily involved in A β peptide clearance [17,37]. Combination of these genetic results and physiopathological data may thus indicate that whereas familial, early-onset forms of AD are mainly linked to genes that are involved in A β overproduction, genetic variants of *APOE* and the GWAS-defined loci may influence susceptibility to late-onset forms of the disease via a role in A β clearance [38]. In this context, we could hypothesize that the moderate over-production of A β peptides associated with the P86L L allele only modifies the AD process when there is a failure in A β clearance – a failure that is likely to be particularly exacerbated in $\epsilon 4$ carriers.

In conclusion, the present meta-analysis does not support the notion that *CALHM1* is a genetic risk factor for AD. However, we found a significant association between the P86L L-allele and earlier onset for AD, particularly in carriers of the *APOE* $\epsilon 4$ -allele. Therefore, further studies are warranted aimed at investigating whether or not genetic variation at *CALHM1* may modify some of the pathophysiological processes involving Ca²⁺ homeostasis and leading to AD [39–41], in particular in carriers of the *APOE* $\epsilon 4$ allele.

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