Genotype patterns at *PICALM, CR1, BIN1, CLU,* and *APOE* genes are associated with episodic memory

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ABSTRACT

Objective: Several genome-wide association studies (GWAS) have associated variants in late-onset Alzheimer disease (LOAD) susceptibility genes; however, these single nucleotide polymorphisms (SNPs) have very modest effects, suggesting that single SNP approaches may be inadequate to identify genetic risks. An alternative approach is the use of multilocus genotype patterns (MLGPs) that combine SNPs at different susceptibility genes.

Methods: Using data from 1,365 subjects in the National Institute on Aging Late-Onset Alzheimer's Disease Family Study, we conducted a family-based association study in which we tabulated MLGPs for SNPs at *CR1*, *BIN1*, *CLU*, *PICALM*, and *APOE*. We used generalized estimating equations to model episodic memory as the dependent endophenotype of LOAD and the MLGPs as predictors while adjusting for sex, age, and education.

Results: Several genotype patterns influenced episodic memory performance. A pattern that included *PICALM* and *CLU* was the strongest genotypic profile for lower memory performance ($\beta = -0.32$, SE = 0.19, $p = 0.021$). The effect was stronger after addition of *APOE* ($p = 0.016$). Two additional patterns involving *PICALM*, *CR1*, and *APOE* and another pattern involving *PICALM*, *BIN1*, and *APOE* were also associated with significantly poorer memory performance ($\beta = -0.44$, SE = 0.09, $p = 0.009$ and $\beta = -0.29$, SE = 0.07, $p = 0.012$) even after exclusion of patients with LOAD. We also identified genotype pattern involving variants in *PICALM*, *CLU*, and *APOE* as a predictor of better memory performance ($\beta = 0.26$, SE = 0.10, $p = 0.010$).

Conclusions: MLGPs provide an alternative analytical approach to predict an individual's genetic risk for episodic memory performance, a surrogate indicator of LOAD. Identifying genotypic patterns contributing to the decline of an individual's cognitive performance may be a critical step along the road to preclinical detection of Alzheimer disease. ***Neurology*® 2012;78:1464–1471**

GLOSSARY

AD = Alzheimer disease; **GWAS** = genome-wide association; **LOAD** = late-onset Alzheimer disease; **MLGP** = multilocus genotype pattern; **NIA-LOAD** = National Institute on Aging Late-Onset Alzheimer's Disease; **SNP** = single nucleotide polymorphism.

Recent genome-wide association (GWAS) studies have associated susceptibility variants with late-onset Alzheimer disease (LOAD). However, the reported effect sizes are small, suggesting that additional genes contributing to the overall risk remain to be identified.

Most analytic approaches use single marker association analysis; however, empirical evidence from model organisms[1] and human studies[2] suggests that interactions among loci broadly contribute to complex traits.[3] Because of the large number of single nucleotide polymorphisms

Supplemental data at www.neurology.org

Supplemental Data

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(SNPs) implicated in GWAS, many of the multilocus methods previously proposed are not suitable, and two-step approaches[4,5] are more appropriate.

We used a multilocus analysis approach to evaluate the association between LOAD genetic variants and episodic memory. We studied the effects of SNPs at different LOAD risk genes[6–8]: the complement component (3b/4b) receptor 1 (*CR1*), phosphatidylinositol-binding clathrin assembly protein (*PICALM*), clusterin (*CLU*), bridging integrator 1 (*BIN1*), and *APOE*. We followed a two-step multilocus approach proposed previously[9] and epistatic interaction effects, so we could identify disease-associated SNPs contributing to disease through their interaction effects rather than by the single locus direct effects.

We used episodic memory as a quantitative endophenotype. The list of valuable LOAD endophenotypes is increasing, from age at onset to biomarkers such as CSF β-amyloid or CSF tau levels[10,11] and cognitive,[12] neuroimaging,[13] and neuropathologic traits.[14]

We aimed to investigate whether multilocus genotype patterns (MLGPs), based on variants consistently associated with LOAD susceptibility loci, influence an individual's cognitive performance. We postulate that MLGPs may be a powerful method to identify individuals at higher risk of cognitive decline.

METHODS

Study participants. The study participants were from National Institute on Aging Late-Onset Alzheimer's Disease (NIA-LOAD) Family Study as well as the National Cell Repository for Alzheimer's Disease and included patients with LOAD and healthy control subjects without dementia. A detailed description of the sample was described elsewhere.[15] For the present analyses, all subjects had completed a standardized cognitive battery and clinical examination for clinical diagnoses. To minimize the risk of population stratification, the analysis was restricted to European subjects. The final dataset consisted of 1,365 subjects, including 337 subjects with LOAD (301 familial and 36 nonfamilial after a failed attempt to recruit additional family members) and 1,028 unaffected subjects (502 familial and 526 from a control series).

Clinical evaluation. Clinical classification of LOAD was based on the guidelines of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association.[16] These require a history of cognitive decline and impairment in at least 2 cognitive domains, one of which must be memory to meet Alzheimer disease (AD) criteria.[16] In a subset of persons who could not be examined directly, clinical classification was based on a detailed review of medical records. The age at onset for LOAD was the age at which the family first reported cognitive complaints. For unaffected subjects, we used their age at the time of their examination confirming the absence of dementia.

Cognitive assessment: episodic memory factor. Cognitive function was measured with a battery described previously.[17] Two measures of episodic memory were included: immediate and delayed recall of Story A from the Wechsler Memory Scale–Revised.[18] Using results from a previous factor analysis, the 2 tests were summarized as a composite domain, episodic memory. Raw scores on both tests were converted to *z* scores using the mean and SD derived from the subset of unaffected subjects from the control series. Episodic memory was computed as the average of the standardized individual cognitive tests as described previously.[19] As *z* scores, the composite measure will quantify how the observed episodic memory values deviated above or below the average value.

Standard protocol approvals, registrations, and patient consents. Informed consent for the study was obtained for all participants. Recruitment for the NIA-LOAD study was approved by the relevant institutional review boards of the participating centers. The study was conducted according to the principles expressed in the Declaration of Helsinki.

GWAS candidate genes selected for multilocus approach. The analysis includes the genetic variants identified by the largest GWAS for LOAD to date, specifically *CLU* (rs11136000), *CR1* (rs6656401), *PICALM* (rs3851179), and *BIN1* (rs744773). In addition, based on its major role as a genetic risk factor for LOAD and also because of its association with cognitive performance,[12] we included the *APOE* gene. The genomic characteristics of the SNPs are shown in table e-1 on the *Neurology*® Web site at www.neurology.org. Genotypes at the *CR1* locus were imputed in a sample of 1,877 unrelated individuals using European HapMap samples y as a reference panel.

Genome-wide genotyping. Direct genotyping of the selected loci was done at the Center for Inherited Disease Research (http://www.cidr.jhmi.edu) using the Illumina Infinium II assay protocol with hybridization to Illumina Human610Quadv1_B BeadChips (Illumina, San Diego, CA). Genotyping of *APOE* polymorphisms (based on SNPs rs7412 and rs429358) for all samples was performed at Prevention Genetics (http://www.preventiongenetics.com). All of the SNPs used in the present analysis have successfully passed the standard quality assessment as described previously.[15]

Statistical analysis. ***Episodic memory performance.*** The effects of age and education on episodic performance in unrelated individuals were assessed using Pearson correlation tests as implemented in SPSS software (PASW Statistics 18 Inc., Chicago, IL). Individuals who did not complete cognitive testing were not included in the analysis (deceased individuals or patients with AD for whom the severity of dementia prevented them from being tested).

Single marker SNP association analysis with dichotomous LOAD phenotype. For each of the SNPs, genetic association with the presence or absence of LOAD was evaluated using a logistic regression analysis. Age, sex, education, and family membership were modeled as covariates.

Single marker SNP association analysis with continuous memory endophenotype. Individual SNPs associations with memory performance were assessed using generalized estimating equations to adjust for the relatedness of the participants by

treating family membership as a cluster. All multivariate analyses were adjusted for age, sex, and education.

MLGPs quantitative association analysis. MLGPs were constructed using the SNP genotypes at the different genes, *CR1* (rs6656401), *PICALM* (rs3851179), *BIN1* (rs744773), *CLU* (rs11136000), and *APOE*. For a given dataset, the number of potential genotype patterns is 3m, m being the number of SNPs. Thus, a genotype pattern at 2 different loci leads to a maximum number of 9 genotype patterns. Because some patterns may not occur in the dataset, we established a frequency threshold >1% to define common MLGPs. Genotypes at the *APOE* locus were recoded into 3 categories after exclusion of heterozygous individuals ϵ2ϵ4: 1) having at least one copy of ϵ2, 2) homozygotes ϵ3ϵ3, and 3) having at least one copy of ϵ4 allele.

The episodic memory factor was used as the dependent variable, the specific MLGP as a predictor variable, and age, sex, and education as covariates. Because the episodic memory distribution in LOAD does not fit a normal distribution, analyses were repeated using cognitively normal individuals only. Statistical analysis were performed using SPSS software (PASW Statistics 18, Inc.) and PLINK.[20] Analyses were performed initially in unaffected subjects and subsequently using all subjects in the cohort.

Power estimation for family data. To perform power calculations in the familial fraction of the NIA-LOAD cohort, we used PBAT software.[21] Power was computed by modeling a family design consisting of 764 extended pedigrees with 3 offspring per family and one of the parents available. We assumed an additive mode of inheritance, 3 different values of disease allele frequency (0.05, 0.10, and 0.15), and 5 different heritability values (0.010, 0.0125, 0.015, 0.0175, and 0.02). The analysis indicated that the maximum power of 0.945 to detect associations between SNPs and episodic memory performance occurs when we considered a trait heritability of 0.02 and a disease allele frequency of 15%. However, statistical power is still high when both heritability and disease allele frequency are lower (power = 0.81 for heritability of 0.015 and disease allele frequency of 10%).

Experiment-wise significance. We initially performed a linear regression analysis modeling episodic memory as the dependent variable and sex, age, and education as predictor variables. The residuals of the model were used as quantitative trait for randomization analysis. Subsequently, we assessed the experiment-wise significance level associated with the identified MLGPs by performing random permutations (20,000) of unrelated individuals as implemented in the Randompat program (http://linkage.rockefeller.edu/ott/randompat.html).[4,9]

RESULTS

Demographics. Patients with LOAD were, on average, 4 years older than unaffected participants (76.2 [SD = 7.5] vs 71.8 [SD = 9.7]) and had, on average, 2 fewer years of education (13.1 [SD = 3.0] vs 14.9 [SD = 3.0]). No differences in sex were found. Demographic characteristics of the participants are detailed in table 1.

Episodic memory score. Among the unaffected participants, better memory performance was associated with younger age ($r = -0.18$, $p < 0.001$) and more education ($r = 0.26$, $p < 0.001$). Compared with unaffected participants, episodic memory performance was significantly lower among patients with LOAD (mean = −0.16, SD = 0.98).

Table 1 Characteristics of the study participants

Characteristic	Value
AD cases, n (%)	337 (20.3)
Familial AD cases, n (%)	301 (89.3)
Nonfamilial AD cases, n (%)	36 (10.7)
Unaffected subjects, n (%)	1028 (61.9)
Familial unaffected subjects, n (%)	502 (48.8)
Population controls, n (%)	526 (51.2)
Proportion of women among AD cases, %	59.6[a]
Proportion of women among unaffected subjects, %	60.2
Age of onset of AD, y, average (SD)	76.2 (7.5)[a]
Age of unaffected subjects, y, average (SD)	71.8 (9.7)
Education of patients with AD, y, mean (SD)	13.1 (3.0)[a]
Education of unaffected subjects, y, mean (SD)	14.9 (3.0)
Patients with AD with at least 1 copy of *APOE*-ϵ4, n (%)	226 (68)[a]
Unaffected subjects with at least 1 copy of *APOE*-ϵ4, n (%)	349 (35)

Abbreviation: AD = Alzheimer disease.
[a] Significant difference between patients with AD and control subjects for that variable.

Single SNP association with LOAD phenotype. We found significant associations with *APOE*_ϵ4 (experiment-wise significant p value of 10−13), *APOE*_ϵ2 (nominally significant p value of 0.004), and the SNP marker at the *PICALM* gene (nominally significant p value of 0.039). Results of the single SNP test of association with the LOAD phenotype are summarized in table 2.

Single SNP and MLGP association with episodic memory. Single marker SNP tests of association with episodic memory performance after exclusion of patients with LOAD from the analysis were evaluated. Tested individually, none of the SNPs were associated with episodic memory performance (table 3). After evaluation of genotype patterns for each of the SNPs and the *APOE* locus (defined as 1 or 2 copies of ϵ4 allele), genotype pattern *PICALM*-GG_ϵ4 resulted in the smallest point-wise p value ($\beta = -0.23$, SE = 0.09, $p = 0.007$). Two additional patterns at *CR1* and *BIN1*, *CR1*-GG_ϵ4 ($\beta = -0.28$, SE = 0.12, $p = 0.015$), and *BIN1*-TT_ϵ4 ($\beta = -0.18$, SE = 0.08, $p = 0.02$), also showed a significant association with lower episodic memory.

We selected the MLGP with the strongest effect (*PICALM*-GG_*APOE*-ϵ4) and tabulated the genotype patterns resulting from the addition of each of the other loci (*CR1*, *BIN1*, and *CLU*) in the unaf-

Table 2 Single SNP association with LOAD phenotype

	n	*B*	SE	*p* Value
***CR1* (rs6656401)**				
AA	27	−1.26	0.83	0.128
AG	203	0.01	0.43	0.985
GG	418	0.27	0.41	0.514
***BIN1* (rs744373)**				
GG	120	−0.16	0.25	0.528
GT	523	0.25	0.16	0.118
TT	640	−0.18	0.16	0.246
***CLU* (rs111360000)**				
CC	462	−0.30	0.16	0.062
CT	630	0.24	0.16	0.132
TT	190	0.10	0.23	0.650
***PICALM* (rs3851179)**				
AA	184	0.51	0.25	0.039[a]
AG	577	−0.06	0.16	0.688
GG	521	−0.17	0.16	0.301
APOE				
Any $\epsilon 2$	118	−1.05	0.37	0.004[a]
Any $\epsilon 4$	530	−1.31	0.18	1×10^{-13}[b]

Abbreviations: LOAD = late-onset Alzheimer disease; SNP = single nucleotide polymorphism.
[a] Nominally significant difference between patients with AD and control subjects.
[b] Experiment-wise significant difference between patients with AD and control subjects.

fected subjects (table 4). The most significant lowering of episodic memory performance corresponded to genotype patterns *PICALM*-GG and *CLU*-TT ($\beta = -0.32$, SE = 0.14, $p = 0.021$), which also showed an even greater effect on episodic performance after inclusion of the $\epsilon 4$ allele at the *APOE* locus ($\beta = -0.47$, SE = 0.19, $p = 0.016$) (figure). Two other genotypic profiles comprising *CR1* and *BIN1* genes were also identified after exclusion of patients with LOAD from analysis.

Table 3 Single SNP and 2-locus MLGP with *APOE* locus test of association with episodic memory performance in unaffected subjects

	PICALM	*CR1*	*BIN1*	*CLU*
Without *APOE* locus	NS	NS	NS	NS
With *APOE* locus				
Genotype	GG_$\epsilon 4$	GG_$\epsilon 4$	TT_$\epsilon 4$	CC_$\epsilon 4$
Npatt	136	70	168	121
β	−0.23	−0.28	−0.18	−0.19
***p* Value**	0.007	0.015	0.02	0.052

Abbreviations: LOAD = late-onset Alzheimer disease; MLGP = multilocus genotype pattern; Npatt = number of unaffected subjects having that particular genotype combination vs having any other; NS = not significant.

The genotype pattern *PICALM*-GG, *CR1*-GG showed no association with memory performance ($\beta = -0.07$, SE = 0.09, $p = 0.445$); however, it became significantly lower ($\beta = -0.44$, SE = 0.17, $p = 0.009$) after the *APOE* locus was included, suggesting that the association is most likely due to the effect of the *APOE* locus (figure). For the genotype pattern *PICALM*-GG, *BIN1*-TT, we observed a trend toward an association ($\beta = -0.14$, SE = 0.07, $p = 0.052$) that increased ($\beta = -0.29$, SE = 0.11, $p = 0.012$) after inclusion of *APOE*-$\epsilon 4$ (figure). None of the genotypic profiles identified were associated with episodic memory performance when tabulated using homozygous genotype *APOE*-$\epsilon 3\epsilon 3$ (data not shown). However, the few homozygous individuals may have limited power.

When we considered all subjects in the dataset, 2 of the patterns, *PICALM*-GG, *CR1*-GG, *APOE*-$\epsilon 4$ and *PICALM*-GG, *BIN1*-TT, *APOE*-$\epsilon 4$, also resulted in a significant association with lower memory performance ($\beta = -0.23$, SE = 0.12, $p = 0.021$ and $\beta = -0.23$, SE = 0.08, $p = 0.005$, respectively). The genotypic profile *PICALM*-GG, *CLU*-TT, *APOE*-$\epsilon 4$, with the strongest effect in the unaffected subjects, did not reach significance when all subjects were considered ($\beta = -0.21$, SE = 0.15, $p = 0.182$). Finally, we also identified a genotype profile comprising the same loci (*PICALM* and *CLU*), that influenced better episodic performance. The genotype pattern *PICALM*-AG and *CLU*-CC showed a trend of association with better performance of episodic memory ($\beta = 0.15$, SE = 0.08, $p = 0.057$) that reached nominal significance when the homozygous genotype $\epsilon 3\epsilon 3$ at *APOE* was included as part of the genotype pattern ($\beta = 0.26$, SE = 0.10, $p = 0.010$) and no significant association when $\epsilon 4$ allele is considered ($\beta = -0.15$, SE = 0.14, $p = 0.305$). None of the other MLGPs identified appeared to influence episodic memory performance (data not shown). The figure represents the estimated means of the episodic memory scores for the *APOE*-$\epsilon 4$ genotype and for each of the reported genotype patterns in the unaffected subjects.

Experiment-wise significance levels. Permutation results showed that for a frequency threshold of 0.01 (that is, MLGPs with frequencies <0.01 were pooled into a single class of rare patterns), patterns with 3 different variants, such as the identified *PICALM*-GG_*CLU*-TT_*APOE*-$\epsilon 4$, had an associated experiment-wise significance level of 0.038.

Table 4 MLGPs associated with episodic memory performance after exclusion of patients with LOAD

	Npatt[a]	%patt	β	SE	p Values[b]
PICALM-GG_*CR1*-GG_*APOE*-ε4	50	7.6	−0.44	0.09	0.009[c]
PICALM-GG_BIN1-TT_*APOE*-ε4	67	7.1	−0.29	0.07	0.012[c]
PICALM-GG_*CLU*-TT_*APOE*-ε4	13	1.4	−0.47	0.14	0.016

Abbreviations: LOAD = late-onset Alzheimer disease; MLGP = multilocus genotype pattern.
[a] Npatt indicates the number of unaffected subjects having that particular genotype combination vs having any other. %patt indicates the frequency of the pattern.
[b] Significance values correspond to nominal *p* values.
[c] Also significant when all subjects are considered.

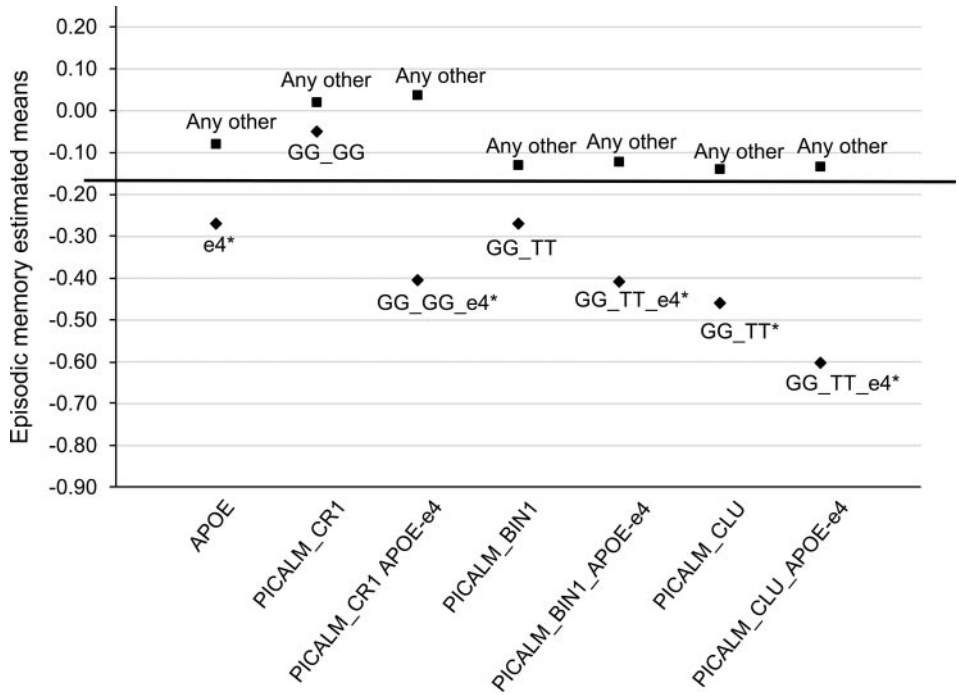
DISCUSSION In the present study we used a multilocus approach to investigate whether genotypic risk profiles based on recently identified LOAD variants influence cognitive performance. The genotype patterns, *PICALM*-rs3851179-GG and *CLU*-rs11136000-TT, had the strongest effect on episodic memory performance in unaffected subjects that increased after addition of the *APOE* locus. A different genotype combination at these same loci, *PICALM*-AG, *CLU*-CC, and *APOE*, also appeared to be a significant predictor of better episodic performance. In addition, genotypic profiles comprising different susceptibility genes such as *CR1* and *BIN1* also showed association with lower scores on the episodic memory tests in study subjects with and without LOAD.

Previous studies[22] have shown that decline in a composite measure of episodic memory based on immediate and delayed recall of Logical Memory Story A is related to the clinical and pathologic phenotypes of the disease and to its most robust genetic marker. Episodic memory was associated with dementia (declining during a mean of 5.8 years in subjects with LOAD), with inheritance of a copy of the ε4 allele among persons with and without dementia, and with a postmortem measure of level of plaques and tangles. Moreover, when investigating the measure's heritability,[17] we found that the measure had moderately high heritability in families with multiple affected members. Overall, these data support the idea that these tests provide a psychometrically sound measure of episodic memory impairment in LOAD.

We were able to confirm *APOE*-ε3 and *APOE*-ε2 as significant risk factors and the *PICALM* homozygous genotype AA at SNP marker rs3851179 as having a protective effect. We did not find an association with any of the other loci, but this is probably due to the small sample size.

Two of the identified genotypic profiles were associated with significantly lower episodic memory

Figure Episodic memory estimated means for the associated multilocus genotype patterns in unaffected individuals



Any other corresponds to any other genotypic combination for the same loci, the black line marks the value of episodic memory in unaffected individuals (average = −0.16, SD = 0.98), and an asterisk marks genotype pattern comparisons with statistically significance differences in the average score of episodic memory (the β coefficients and *p* values are shown in table 4).

scores even among unaffected participants. These results may reinforce the hypothesis of a presymptomatic LOAD state, in which cognitive performance could be an endophenotypic marker of disease, detectable before the individual experiences frank dementia. Alternatively, the genotype pattern with the strongest effect on memory performance in the unaffected subject group was no longer significant after all subjects were included in the analysis, suggesting that the influence of the MLGP on memory performance may be restricted to individual differences rather than to the LOAD phenotype. Similar results have also been reported for the role of *APOE* not only as genetic risk factor for AD but also as a risk factor for nonpathologic cognitive aging.[23]

Previous work on the genetic risk factors of memory performance has been mainly focused on the association between *APOE*-ϵ4 and the rate of cognitive decline (in both cognitively normal subjects and subjects with AD) and has produced some conflicting results. Although some investigations have reported slower cognitive decline among ϵ4 carriers with LOAD[24,25] and healthy subjects,[26] several different studies have documented that possession of *APOE*-ϵ4 relates to a faster rate of cognitive decline.[12,27] Beside the *APOE* gene, a GWAS from the Framingham community–based AD cohort has also found that the strongest gene-phenotype association was between *SORL1*, implicated in amyloid precursor protein processing and risk of LOAD[28] and abstract reasoning.[29]

The derived genotypic risk profile based on several associated genetic variants might provide a useful genetic risk prediction for cognitive endophenotypes. Identifying genes conferring risk for LOAD that also influence cognitive performance would provide important confirmation of the role of these genetic variants and suggest a mechanism of action.

In our approach, the effect of MLGPs encompasses both main and interaction effects among the SNPs, which is particularly valuable when individual main or interaction effects are very small. The effect of interactions between different loci on complex human traits has been extensively discussed in the literature[30] and shown to be problematic. It has even been suggested that there is "an overemphasis on seeking statistical interaction effects."[31] Although we acknowledge that it may be difficult to disentangle the various effects, leading to a pattern effect when the effects are small, it still provides valuable information regarding the individual's genetic risk associated with cognitive deterioration. Individuals carrying the risk MLGP, *PICALM*-GG_*CLU*-TT_*APOE*-ϵ4, have an average episodic memory score of −0.60 compared with an average score of −0.13 for any other MLGP; that is, there is almost a 50% decline in the cognitive score (there is a decrease of 32% for *PICALM*-GG_*CLU*-TT and only 19% for *APOE*-ϵ4). In terms of effect size, there is an approximately 15% increase in effect size (from −0.32 to −0.47) when *APOE*-ϵ4 is included, compared with that for other MLGPs, i.e., *PICALM*-GG_*CR1*-GG, with a more substantial increase in effect size, approximately 37% (from −0.07 to −0.44), suggesting that there is a much more stronger APOE interaction effect. For clinical purposes, identifying individuals at higher risk to experience a stronger cognitive decline will be a powerful approach to identify prodromal stages to LOAD.

There are potential limitations of our study. As in any other statistical approach, the small sample size (because some MLGPs may not occur in the dataset) will limit the power of the study. To reduce too much imbalance due to sample size fluctuation, we combined rare patterns into a single class and we established a frequency threshold >1%, to define common MLGPs that will be considered for analysis purposes. Randomization analyses showed that the MLGP identified reached experiment-wise significance, further suggesting that the specific set of 3 genetic variants identified seems to predispose to disease. In a progressive disorder such as LOAD, measuring memory performance at a single point in time is an imperfect indicator of the rate of memory decline, and, to that end, the NIA-LOAD Family Study is currently collecting longitudinal cognitive data. Cognitive decline is a complex multifactorial process, and therefore the possibility of many other risk factors influencing the outcome cannot be ruled out. Among the reported risk factors is alcohol consumption,[32] mentally stimulating activities,[33] physical inactivity,[34] and smoking and vascular risk factors.[35,36] It has also been proposed that environmental risk factors such as diet[37] might be also associated with the cognitive decline that eventually leads to LOAD.

AUTHOR CONTRIBUTIONS

Dr. Barral was primarily responsible for conducting the statistical analysis, interpretation of data, and drafting the manuscript. Dr. Bird, Dr. Goate, Dr. Farlow, Dr. Diaz-Arrastia, Dr. Bennett, Dr. Graff-Radford, Dr. Boeve, Dr. Sweet, Dr. Wilson, and Dr. Foroud were responsible of the acquisition of the data and obtaining grant funding. Dr. Stern and Dr. Ott assisted in the analysis and interpretation of the data. Dr. Mayeux conceptualized the study, supervised it, assisted in the interpretation of data and drafting the manuscript, and revised the edited manuscript.

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DISCLOSURE

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