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ORIGINAL ARTICLE

Associations of cerebrovascular metabolism genotypes with neuropsychiatric symptoms and age at onset of Alzheimer's disease dementia

Fabricio F. de Oliveira,[1] Elizabeth S. Chen,[2] Marilia C. Smith,[2] Paulo H. Bertolucci[1]

[1]*Departamento de Neurologia e Neurocirurgia, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.* [2]*Departamento de Morfologia e Genética, EPM, UNIFESP, São Paulo, SP, Brazil.*

Objective: To study associations of cerebrovascular metabolism genotypes and haplotypes with age at Alzheimer's disease dementia (AD) onset and with neuropsychiatric symptoms according to each dementia stage.
Methods: Consecutive outpatients with late-onset AD were assessed for age at dementia onset and Neuropsychiatric Inventory scores according to Clinical Dementia Rating scores, apolipoprotein E gene (*APOE*) haplotypes, angiotensin-converting enzyme gene (*ACE*) variants rs1800764 and rs4291, low-density lipoprotein cholesterol receptor gene (*LDLR*) variants rs11669576 and rs5930, cholesteryl ester transfer protein gene (*CETP*) variants I422V and *Taq*IB, and liver X receptor beta gene (*NR1H2*) polymorphism rs2695121.
Results: Considering 201 patients, only *APOE*-ε4 carriers had earlier dementia onset in multiple correlations, as well as less apathy, more delusions, and more aberrant motor behavior. Both *ACE* polymorphisms were associated with less intense frontally mediated behaviors. Regarding *LDLR* variants, carriers of the A allele of rs11669576 had less anxiety and more aberrant motor behavior, whereas carriers of the A allele of rs5930 had less delusions, less anxiety, more apathy, and more irritability. *CETP* variants that included G alleles of I422V and *Taq*IB were mostly associated with less intense frontally mediated behaviors, while severely impaired carriers of the T allele of rs2695121 had more anxiety and more aberrant motor behavior.
Conclusion: Though only *APOE* haplotypes affected AD onset, cerebrovascular metabolism genotypes were associated with differences in several neuropsychiatric manifestations of AD.

Keywords: Alzheimer disease; dementia; cerebrovascular disorders; genetics; neuropsychiatry

Introduction

Cerebrovascular risk factors play an important role in the pathogenesis of Alzheimer's disease dementia (AD). When present in midlife, pooled cerebrovascular risk factors synergistically increase AD risk and lower the age at AD onset.[1] As a result, these patients usually present with a combination of AD and vascular neuropathological profiles.[2] Conversely, we have previously shown that higher cerebrovascular risk in late life leads to cognitive and functional stabilization or even improvement for patients with AD, possibly related to enhanced cerebral perfusion,[3] though some studies have shown that cerebrovascular risk factors may lead to faster cognitive decline.[2]

The astrocyte-secreted apolipoprotein E is a cholesterol-binding lipoprotein that transports cholesterol through cell membranes[4] and is involved in cholinergic dysfunction,[5] atherogenesis, and amyloidogenesis,[6] besides being a high-affinity ligand for the low-density lipoprotein receptor (LDLR) within the central nervous system.[7] The apolipoprotein E gene (*APOE*) is a moderately penetrant gene that is neither a prerequisite nor a sufficient agent for development of AD, despite the fact that *APOE*-ε4 is the most important genetic risk factor for incidence[8] and earlier onset of late-onset AD,[1] while also affecting behavioral performance[2] and decreasing prospective physical activity in such patients.[3] *APOE*-ε4 carriers are also more prone to decreased risk of dementia associated with physical activity,[9] while neuropsychiatric assessment findings may correlate with specific biomarkers.[10]

Several other genetic variants involved in cerebrovascular metabolism have been associated with AD. The two functional variants of the angiotensin-converting enzyme gene (*ACE*) with the most significant effects for higher activity and boosted serum levels of the angiotensin-converting enzyme (ACE) are the promoter polymorphisms rs1800764 and rs4291, affecting risk and age at onset of the amnestic phenotype of AD,[11] as well as cognitive decline and incidence of early-onset hypertension.[12] The LDLR mediates increased astrocytic expression of *APOE* induced by amyloid-β,[4] whereas storage and release of cholesterol depend on the expression of the low-density lipoprotein receptor gene (*LDLR*), which resides within a

Correspondence: Fabricio Ferreira de Oliveira, Departamento de Neurologia e Neurocirurgia, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Botucatu 740, CEP 04023-900, São Paulo, SP, Brazil.
E-mail: fabricioferreiradeoliveira@hotmail.com
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region linked to AD in 19p13.3; rs11669576[7] and rs5930[13] are two of the most important genetic variants of the epidermal growth factor precursor homology domain of *LDLR* to be associated with disrupted cholesterol metabolism and variability in the risk of AD. Cholesterol governs synaptogenesis and myelin biosynthesis,[14] while the cholesteryl ester transfer protein (CETP) is associated with reverse cholesterol transport (from tissues to the liver)[5]; protective cholesteryl ester transfer protein gene (*CETP*) variants lead to lower serum CETP levels and healthier lipid profiles,[15] though not all studies have shown genetically mediated lifetime cognitive effects.[16] Nevertheless, the A allele of rs708272 (*Taq*IB) is associated with lower serum CETP activity and lower coronary heart disease risk,[17] while the G allele of rs5882 (I422V) has been associated with lower serum CETP levels and greater white matter integrity in young adults,[14] as well as with preserved cognitive function in longevity[18] and less medial temporal lobe atrophy in *APOE*-ε4 carriers with AD.[19] Moreover, the nuclear liver X receptor β (LXR-β) isoform is also expressed in the brain,[4] acting as a regulator of cholesterol homeostasis, controlling amyloidogenesis, and modulating inhibition of angiotensin II, while several variants of the LXR-β gene (*NR1H2*) close to *APOE* in chromosome 19 have also been linked with variable risk of AD.[20]

We aimed to examine associations of genetic variants of *APOE*, *ACE*, *LDLR*, *CETP*, and intron 2 of *NR1H2* with age at onset of late-onset AD and with neuropsychiatric symptoms, stratified by stage of dementia.

Methods

Participants

In this cross-sectional study, consecutive outpatients with late-onset AD according to U.S. National Institute on Aging-Alzheimer's Association criteria[21] were recruited from the Setor de Neurologia do Comportamento, Hospital São Paulo, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil, from October 2010 to February 2013. All outpatients with this diagnosis who were followed during the study period were recruited. Late-onset AD was defined as AD with onset of dementia syndrome after age 60 years.[1] All patients underwent magnetic resonance imaging (MRI) to evaluate either medial parietal or medial, basal, or lateral temporal atrophy or, in cases of claustrophobia or pacemaker use, a computed tomography (CT) scan to exclude vascular lesions. Patients with history of stroke were not included in the study.

A detailed self-report and/or proxy report was conducted for assessment of age at AD onset, years of schooling, gender, and history of antipsychotic and antidepressant therapy. Information on age at dementia onset was obtained through a review of medical records and confirmed after an interview with the caregiver (preferably a family member), who was required to visit the patient frequently. The time of onset of subjective memory complaints or mild cognitive impairment was not taken into account; rather, only the time of dementia onset (when functional decline actually began) was considered. Use of antipsychotics or antidepressants was quantified in patients who had been receiving them for at least 3 months before the evaluation, to ensure that proper clinical response resulting from stability of drug levels in the central nervous system had been achieved, as well as because follow-up assessments at the study facility are usually conducted in 3-month intervals.

Neuropsychiatric assessment

A thorough neuropsychiatric evaluation was conducted, with particular attention to caregiver-reported Clinical Dementia Rating (CDR)[22] and the 10-item version of the Neuropsychiatric Inventory, which does not take into account night-time behavior disturbances or appetite and eating abnormalities.[23] Effects of body mass index on age at AD onset[1] and cognitive and functional change[3] have been previously assessed in our population, and associations of sleep duration and sleep satisfaction with neuropsychiatric symptoms and *APOE* haplotypes have been described elsewhere.[24] Ratings for the final score of each item in the Neuropsychiatric Inventory took into account the frequency and severity of each patient's behaviors, while the total score was the sum of the subscale scores. All functional and behavioral assessments were conducted on weekday mornings, by the same examiner (FFO).

Genotyping procedures

Blood samples were collected from all patients into tubes containing 0.1% ethylenediaminetetraacetic acid (EDTA). Genomic DNA was extracted from these samples using a standard salting-out procedure for determination of *APOE* haplotypes (polymorphisms rs7412 and rs429358), *ACE* genotypes and haplotypes (polymorphisms rs1800764 and rs4291), *LDLR* genotypes and haplotypes (polymorphisms rs11669576 and rs5930), *CETP* genotypes and haplotypes (polymorphisms rs5882 and rs708272), and genotypes of rs2695121 (intron 2 of the *NR1H2* gene). All single nucleotide polymorphisms (SNPs) were assessed by real-time polymerase chain reactions using TaqMan® SNP Genotyping Assays on the Applied Biosystems® 7500 Fast Real-Time PCR System (Applied Biosystems®, Carlsbad, USA). Standard manufacturer protocols were followed. Genotyping procedures were carried out only after clinical data had been collected from all patients, thus ensuring observer blinding to genetic data.

Outcome measures

Primarily, we sought to determine associations of genetic variants of *APOE*, *ACE*, *LDLR*, *CETP*, and intron 2 of the LXR-β gene *NR1H2* with age at AD onset and neuropsychiatric symptoms, stratified by dementia stage. Secondarily, we also investigated combined associations of gender and schooling with age at AD onset, as well as associations of gender, schooling, age at dementia onset, antipsychotic therapy, and antidepressant therapy with each neuropsychiatric symptom according to CDR scores.[22] Finally, frequencies and scores for all behavioral symptoms were compared according to stage of dementia.

Table 1 Demographic and clinical profile (n=201)

Assessed factors	n (%)	Mean ± SD	Range
Gender			
Female	141 (70.1)	-	-
Male	60 (29.9)	-	-
Age at dementia onset (years)	-	73.53±6.3	60.0-88.0
Schooling (years)	-	4.22±3.7	0-15
Neuropsychiatric Inventory score (0-120 points)	-	22.11±16.3	0-87
Antipsychotic therapy (mg/day)			
Olanzapine	1 (0.5)	10.00±0.0	10-10
Quetiapine	36 (17.9)	70.83±74.5	25-400
Risperidone	10 (5.0)	1.90±0.9	1.0-4.0
Antidepressant therapy (mg/day)*			
Amitriptyline	1 (0.5)	25.00±0.0	25-25
Bupropion	8 (4.0)	150.00±0.0	150-150
Citalopram	18 (9.0)	23.33±7.7	20-40
Clomipramine	1 (0.5)	25.00±0.0	25-25
Escitalopram	3 (1.5)	10.00±0.0	10-10
Fluoxetine	11 (5.5)	27.27±10.1	20-40
Mirtazapine	2 (1.0)	30.00±0.0	30-30
Nortriptyline	1 (0.5)	10.00±0.0	10-10
Paroxetine	8 (4.0)	27.50±14.9	20-60
Sertraline	25 (12.4)	60.00±20.4	50-100
Trazodone	9 (4.5)	61.11±22.0	50-100
Venlafaxine	3 (1.5)	175.00±114.6	75-300

* Overall, 85 patients (42.3%) used antidepressants during the evaluation, five of whom were on two antidepressants at the same time: one took bupropion 150 mg/day + trazodone 50 mg/day, one took citalopram 20 mg/day + mirtazapine 30 mg/day, and three took sertraline 50 mg/day + trazodone 50 mg/day.
SD = standard deviation.

Statistical analysis

Associations of *APOE* haplotypes, *ACE* genotypes and haplotypes of rs1800764 and rs4291, *LDLR* genotypes and haplotypes of rs11669576 and rs5930, *CETP* genotypes and haplotypes of rs5882 and rs708272, and genotypes of rs2695121 with age at AD onset were investigated independently by way of the Mann-Whitney *U* test and further assessed in combination in a multiple linear regression model that included male gender and schooling as covariates. Multiple linear regression models including a copy of each minor allele, each copy of *APOE*-ε4, male gender, years of schooling, age at dementia onset, antipsychotic therapy, and antidepressant therapy were employed at each dementia stage to investigate associations of these factors with each behavioral symptom and with Neuropsychiatric Inventory total scores. Each haplotype that was represented in at least three patients was associated with each behavioral symptom and with Neuropsychiatric Inventory total scores at each dementia stage by the Mann-Whitney *U* test. The threshold of significance was set at $p < 0.05$.

Ethical considerations

This study is part of a larger research project (1067/10) approved by the Ethics Committee of Hospital São Paulo, UNIFESP, in August 2010 (CAAE 0540.0.174.000-10). All invited patients and their legal representatives agreed to participate in the study and provided written informed consent before the evaluation.

Results

At the end of the recruitment period, 217 patients had been included. Of these, 16 (7.4%) were excluded due to missing genetic information, resulting in a final sample of 201 patients.

Table 1 shows demographic and clinical results for the final sample. More than two-thirds of patients were female, 47 patients (23.4%) received antipsychotic treatment, and 85 (42.3%) were on at least one antidepressant.

Table 2 reports genotypes and haplotypes for the final sample. Overall, 108 patients (53.7%) were *APOE*-ε4 carriers, while 93 (46.3%) were *APOE*-ε4 non-carriers. All assessed genetic variants were in Hardy-Weinberg equilibrium.

Regarding independent haplotypes that could affect age at dementia onset, we found that *APOE*-ε4/ε4 carriers had earlier dementia onset by 4.31 years (p = 0.001), rs5882-AA/rs708272-AG carriers had later dementia onset by 1.77 year (p = 0.048), rs5882-AG/rs708272-AA carriers had earlier dementia onset by 4.55 years (p = 0.014), and rs11669576-GG/rs5930-GG carriers had later dementia onset by 2.14 years (p = 0.021). The only isolated genotype that affected age at AD onset was rs5882-AA: carriers had later dementia onset by 1.76 year (p = 0.018), but this analysis did not survive multiple correlations. According to Table 3, the only variable to affect age at AD onset in a multiple linear regression model was the *APOE* haplotype: each copy of *APOE*-ε4 led to earlier dementia onset by 1.728 year (p = 0.012).

Tables 4, 5, and 6 show the results of multiple linear regression analyses regarding factors that may affect

Table 2 Genetic analysis findings (n=201)

Genotypes and haplotypes	n (%)	p-value*
APOE haplotypes		
ε4/ε4	23 (11.4)	-
ε4/ε3	78 (38.8)	-
ε4/ε2	7 (3.5)	-
ε3/ε3	83 (41.3)	-
ε3/ε2	10 (5.0)	-
ε2/ε2	0 (0.0)	-
rs1800764 genotypes		
CC	53 (26.4)	0.291
CT	93 (46.3)	
TT	55 (27.3)	
rs4291 genotypes		
AA	93 (46.3)	0.245
AT	82 (40.8)	
TT	26 (12.9)	
ACE haplotypes		
rs1800764 CC/rs4291 AA	7 (3.5)	-
rs1800764 CC/rs4291 AT	20 (10.0)	-
rs1800764 CC/rs4291 TT	26 (12.9)	-
rs1800764 CT/rs4291 AA	31 (15.4)	-
rs1800764 CT/rs4291 AT	62 (30.9)	-
rs1800764 CT/rs4291 TT	0 (0.0)	-
rs1800764 TT/rs4291 AA	55 (27.3)	-
rs1800764 TT/rs4291 AT	0 (0.0)	-
rs1800764 TT/rs4291 TT	0 (0.0)	-
rs11669576 genotypes (*LDLR*8)		
AA	2 (1.0)	0.425
AG	27 (13.4)	
GG	172 (85.6)	
rs5930 genotypes (*LDLR*10)		
AA	23 (11.4)	0.593
AG	95 (47.3)	
GG	83 (41.3)	
LDLR haplotypes		
rs11669576 AA/rs5930 AA	0 (0.0)	-
rs11669576 AA/rs5930 AG	0 (0.0)	-
rs11669576 AA/rs5930 GG	2 (1.0)	-
rs11669576 AG/rs5930 AA	0 (0.0)	-
rs11669576 AG/rs5930 AG	10 (5.0)	-
rs11669576 AG/rs5930 GG	17 (8.5)	-
rs11669576 GG/rs5930 AA	23 (11.4)	-
rs11669576 GG/rs5930 AG	85 (42.3)	-
rs11669576 GG/rs5930 GG	64 (31.8)	-
rs2695121 genotypes (*NR1H2* gene)		
CC	76 (37.8)	0.691
CT	93 (46.3)	
TT	32 (15.9)	
rs5882 genotypes (*CETP* gene)		
AA	75 (37.3)	0.412
AG	100 (49.8)	
GG	26 (12.9)	
rs708272 genotypes (*CETP* gene)		
AA	21 (10.5)	0.058
AG	106 (52.7)	
GG	74 (36.8)	
CETP haplotypes		
rs5882 AA/rs708272 AA	3 (1.5)	-
rs5882 AA/rs708272 AG	38 (18.9)	-
rs5882 AA/rs708272 GG	34 (16.9)	-
rs5882 AG/rs708272 AA	11 (5.5)	-
rs5882 AG/rs708272 AG	56 (27.8)	-
rs5882 AG/rs708272 GG	33 (16.4)	-
rs5882 GG/rs708272 AA	7 (3.5)	-
rs5882 GG/rs708272 AG	12 (6.0)	-
rs5882 GG/rs708272 GG	7 (3.5)	-

ACE = angiotensin-converting enzyme gene; *APOE* = apolipoprotein E gene; *CETP* = cholesteryl ester transfer protein gene; *LDLR* = low-density lipoprotein cholesterol receptor gene; *NR1H2* = liver X receptor beta gene.
* Hardy-Weinberg equilibrium (chi-square test).

Table 3 Multiple linear regression for age at AD onset (n=201)

		95%CI		
Effects	β	Lower	Upper	p-value
Constant	75.099	72.513	77.686	< **0.001**
Male gender	-1.473	-3.419	0.472	0.137
Years of schooling	0.134	-0.113	0.381	0.285
Copies of *APOE*-ε4	-1.728	-3.071	-0.385	**0.012**
Copies of rs1800764-C	0.608	-1.119	2.335	0.488
Copies of rs4291-T	0.310	-1.502	2.122	0.736
Copies of rs11669576-A	-1.177	-3.562	1.207	0.331
Copies of rs5930-A	-1.251	-2.601	0.099	0.069
Copies of rs2695121-T	0.745	-0.516	2.006	0.246
Copies of rs5882-G	-0.668	-2.039	0.703	0.338
Copies of rs708272-A	-0.530	-1.963	0.902	0.466

F-ratio = 2.044; multiple R = 0.312; adjusted squared multiple R = 0.050; p = 0.031.
95%CI = 95% confidence interval; AD = Alzheimer's disease dementia; *APOE* = apolipoprotein E gene.
Results in bold are significant.

each behavioral symptom and Neuropsychiatric Inventory total score for patients who were mildly impaired (CDR = 1.0), moderately impaired (CDR = 2.0), and severely impaired (CDR = 3.0), respectively. Overall, Neuropsychiatric Inventory total scores increased according to dementia severity. For mildly impaired patients (n=78), anxiety was the most frequent and highest-scored symptom, while euphoria was the least frequent and hallucinations were the lowest-scored symptom. For moderately impaired patients (n=92), apathy was the most frequent and the highest-scored symptom, while euphoria was the least frequent and hallucinations were the lowest-scored symptom. For severely impaired patients (n=31), apathy was the most frequent and highest-scored symptom, while euphoria was the least frequent and lowest-scored symptom.

Considering associations of independent haplotypes with behavioral scores in mildly impaired patients, rs1800764-CC/rs4291-AT carriers (n=10) had more hallucinations (1.40±2.6 versus 0.15±0.4, p=0.031), rs1800764-CT/rs4291-AA carriers (n=11) had more disinhibition (1.00±1.3 versus 0.39±1.3, p=0.021) and more irritability (3.00±4.1 versus 1.12±3.0, p=0.022), rs1800764-TT/rs4291-AA carriers (n=19) had more agitation (3.32±3.1 versus 1.69±3.1, p=0.017), rs11669576-AG/rs5930-AG carriers (n=5) had more apathy (7.20±3.6 versus 3.19±3.7, p=0.026), rs11669576-AG/rs5930-GG carriers (n=8) had less apathy (1.00±2.1 versus 3.73±3.8, p=0.033), rs5882-AA/rs708272-AG carriers (n=14) had lower Neuropsychiatric Inventory total scores (9.86±6.7 versus 18.66±11.3, p=0.007) and less irritability (0.57±0.8 versus 2.80±3.1, p=0.008), rs5882-AA/rs708272-GG carriers (n=13) had higher Neuropsychiatric Inventory total scores (24.38±9.4 versus 15.62±11.0, p=0.007) and more apathy (5.31±3.4 versus 3.08±3.8, p=0.041) and more dysphoria (3.38±3.8 versus 1.34±2.2, p=0.036), rs5882-AG/rs708272-GG carriers (n=12) had less anxiety (1.42±2.7 versus 4.39±4.5, p=0.025), and rs5882-GG/rs708272-AG carriers (n=5) had more delusions (3.40±5.1 versus 0.58±1.7, p=0.027).

Table 4 Multiple linear regression analysis of each neuropsychiatric symptom in mildly impaired patients (CDR = 1.0) (n=78)

	NPI total score (F-ratio = 1.655, p = 0.093)		Delusions (F-ratio = 1.717, p = 0.079)		Hallucinations (F-ratio = 1.741, p = 0.073)		Agitation (F-ratio = 1.245, p = 0.270)		Dysphoria (F-ratio = 1.351, p = 0.209)		Anxiety (F-ratio = 1.006, p = 0.456)		Euphoria (F-ratio = 0.860, p = 0.597)		Apathy (F-ratio = 0.789, p = 0.669)		Disinhibition (F-ratio = 1.422, p = 0.174)		Irritability (F-ratio = 1.317, p = 0.227)		Aberrant motor behavior (F-ratio = 0.831, p = 0.627)	
Effects	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Constant	9.04	0.601	-0.47	0.884	-0.07	0.963	0.39	0.939	0.62	0.881	7.50	0.295	1.86	0.538	1.32	0.833	0.94	0.645	0.34	0.941	-3.39	0.520
Male gender	1.88	0.502	0.56	0.293	-0.11	0.684	-0.30	0.713	-0.94	0.163	-0.13	0.908	-0.31	0.521	0.55	0.586	-0.24	0.463	1.93	**0.013**	0.88	0.304
Years of schooling	-0.26	0.389	-0.08	0.140	0.04	0.145	-0.10	0.277	-0.05	0.530	0.09	0.483	0.01	0.983	-0.10	0.382	-0.01	0.845	-0.08	0.314	0.02	0.817
Age at dementia onset*	0.06	0.792	0.01	0.814	-0.01	0.935	0.05	0.475	0.01	0.793	-0.07	0.458	-0.02	0.646	0.03	0.743	-0.01	0.861	0.01	0.798	0.04	0.568
Copies of *APOE*-ε4	-1.37	0.490	0.03	0.930	0.02	0.907	0.42	0.467	0.02	0.973	-0.89	0.279	-0.40	0.248	-0.06	0.932	0.21	0.365	-0.45	0.403	-0.28	0.646
Copies of rs1800764-C	0.72	0.768	0.41	0.369	0.51	**0.028**	-1.15	0.109	-0.09	0.870	-0.01	0.997	0.33	0.428	-1.09	0.220	0.38	0.191	-0.01	0.981	1.43	0.056
Copies of rs4291-T	-0.72	0.779	0.10	0.831	-0.17	0.472	-0.37	0.617	0.61	0.317	0.05	0.959	-0.65	0.147	0.99	0.286	-0.23	0.440	0.02	0.979	-1.07	0.171
Copies of rs11669576-A	1.69	0.599	0.52	0.390	0.07	0.815	0.03	0.970	0.14	0.859	-1.18	0.373	0.14	0.808	1.72	0.143	0.08	0.837	0.06	0.945	0.11	0.906
Copies of rs5930-A	0.64	0.752	-0.77	**0.046**	-0.37	0.051	0.30	0.615	-0.40	0.404	0.04	0.958	0.68	0.059	1.12	0.132	-0.30	0.212	-0.20	0.714	0.56	0.367
Copies of rs2695121-T	-0.74	0.683	-0.15	0.658	-0.31	0.070	-0.41	0.443	0.12	0.785	0.55	0.462	-0.23	0.468	-0.33	0.612	-0.17	0.418	-0.17	0.726	0.37	0.503
Copies of rs5882-G	1.15	0.551	0.81	**0.028**	0.16	0.383	-0.01	0.979	-0.20	0.672	-0.59	0.458	-0.03	0.927	0.51	0.472	-0.36	0.117	0.31	0.560	0.57	0.336
Copies of rs708272-A	-0.30	0.890	0.39	0.339	0.23	0.247	-0.25	0.690	-0.16	0.757	1.15	0.200	-0.13	0.732	-1.30	0.100	0.11	0.668	0.49	0.400	-0.83	0.208
Antipsychotic therapy†	13.35	**0.009**	-0.47	0.613	-0.45	0.330	2.74	0.062	1.75	0.142	2.92	0.156	0.12	0.887	0.97	0.588	1.69	**0.005**	2.39	0.078	1.67	0.269
Antidepressant therapy†	7.38	**0.008**	-0.40	0.434	0.33	0.194	0.55	0.489	1.38	**0.035**	1.93	0.087	0.70	0.139	1.25	0.207	0.11	0.739	0.98	0.182	0.55	0.504
Symptom frequency (%)	-		23.1		17.9		50.0		52.6		67.9		16.7		60.3		20.5		65.4		26.9	
Scores (mean ± SD)	17.08±11.1		0.76±2.1		0.31±1.0		2.09±3.2		1.68±2.6		3.94±4.4		0.60±1.8		3.45±3.8		0.47±1.3		2.40±2.9		1.38±3.2	

APOE = apolipoprotein E gene; CDR = Clinical Dementia Rating; NPI = 10-item Neuropsychiatric Inventory; SD = standard deviation.
* Age in years. † Pharmacological therapy = yes or no.
Results in bold are significant.

Table 5 Multiple linear regression analysis of each neuropsychiatric symptom in moderately impaired patients (CDR = 2.0) (n=92)

	NPI total score (F-ratio = 0.713, p = 0.745)		Delusions (F-ratio = 1.205, p = 0.292)		Hallucinations (F-ratio = 1.413, p = 0.172)		Agitation (F-ratio = 1.291, p = 0.237)		Dysphoria (F-ratio = 2.150, p = 0.020)		Anxiety (F-ratio = 1.305, p = 0.228)		Euphoria (F-ratio = 0.928, p = 0.528)		Apathy (F-ratio = 1.818, p = 0.055)		Disinhibition (F-ratio = 1.015, p = 0.446)		Irritability (F-ratio = 0.649, p = 0.805)		Aberrant motor behavior (F-ratio = 1.223, p = 0.279)	
Effects	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Constant	40.10	0.100	0.04	0.993	10.25	**0.002**	-1.91	0.705	5.69	0.225	11.40	**0.044**	10.47	**0.013**	17.23	**0.009**	-5.16	0.238	-3.09	0.534	-4.83	0.411
Male gender	1.45	0.726	-1.18	0.829	-0.47	0.391	1.10	0.203	-0.65	0.419	0.21	0.823	0.10	0.890	2.64	**0.019**	-0.47	0.532	-0.43	0.614	-0.40	0.689
Years of schooling	-0.61	0.285	-0.11	0.337	-0.09	0.234	0.07	0.570	-0.32	**0.005**	-0.22	0.096	-0.08	0.388	-0.19	0.208	0.08	0.460	0.01	0.962	0.26	0.064
Age at dementia onset*	-0.21	0.472	0.03	0.618	-0.12	**0.004**	0.04	0.527	0.01	0.889	-0.08	0.248	-0.10	**0.043**	-0.17	**0.033**	0.05	0.319	0.06	0.360	0.07	0.316
Copies of *APOE*-ε4	1.65	0.570	0.45	0.440	0.07	0.850	-0.01	0.997	0.50	0.378	0.55	0.410	0.28	0.571	-1.58	**0.044**	0.84	0.112	0.44	0.466	0.10	0.882
Copies of rs1800764-C	-4.79	0.234	-0.27	0.739	-0.64	0.225	-0.87	0.296	-1.82	**0.021**	-0.17	0.857	-1.05	0.129	-0.04	0.971	-0.15	0.838	-0.65	0.430	0.87	0.371
Copies of rs4291-T	5.23	0.186	0.41	0.609	0.57	0.273	0.51	0.534	1.45	0.060	0.43	0.636	1.13	0.096	1.07	0.311	0.64	0.369	0.14	0.861	-1.11	0.246
Copies of rs11669576-A	-1.48	0.769	-1.47	0.152	-0.98	0.143	-0.58	0.583	1.25	0.203	-2.34	**0.047**	-0.62	0.468	-0.26	0.849	0.51	0.574	0.28	0.790	2.74	**0.027**
Copies of rs5930-A	0.78	0.786	0.90	0.123	0.23	0.542	0.61	0.306	-1.01	0.072	-1.61	**0.017**	-0.55	0.262	0.28	0.714	0.59	0.250	1.14	0.055	0.18	0.790
Copies of rs2695121-T	-1.86	0.476	-0.75	0.155	0.10	0.766	-0.19	0.730	0.23	0.647	-0.74	0.218	-0.39	0.376	0.04	0.956	0.15	0.745	0.39	0.468	-0.69	0.274
Copies of rs5882-G	-1.59	0.594	0.46	0.447	-0.03	0.937	-0.01	0.986	-1.33	**0.024**	-0.38	0.581	-0.37	0.465	0.10	0.896	0.01	0.980	-0.36	0.553	0.31	0.664
Copies of rs708272-A	-0.71	0.799	-0.53	0.356	0.24	0.519	-0.01	0.985	-0.37	0.493	-0.50	0.441	-0.31	0.523	0.06	0.940	0.21	0.673	0.49	0.392	0.00	0.999
Antipsychotic therapy†	7.11	0.060	1.70	**0.028**	0.67	0.178	1.64	**0.038**	-1.30	0.074	0.93	0.285	0.84	0.192	0.89	0.374	0.71	0.291	0.79	0.307	0.25	0.780
Antidepressant therapy†	2.52	0.498	-0.63	0.402	-0.69	0.161	1.88	**0.017**	-0.20	0.781	-0.79	0.358	-0.42	0.503	0.24	0.805	1.26	0.063	0.50	0.513	1.37	0.130
Symptom frequency (%)	-		45.7		39.1		63.0		60.9		46.7		27.2		68.5		30.4		56.5		37.0	
Scores (mean ± SD)	22.67±15.7		2.00±3.3		1.08±2.2		2.55±3.4		2.35±3.4		2.46±3.8		1.22±2.7		4.82±4.5		1.34±2.9		2.45±3.2		2.42±4.0	

APOE = apolipoprotein E gene; CDR = Clinical Dementia Rating; NPI = 10-item Neuropsychiatric Inventory; SD = standard deviation.
* Age in years. † Pharmacological therapy = yes or no.
Results in bold are significant.

Table 6 Multiple linear regression analysis of each neuropsychiatric symptom in severely impaired patients (CDR = 3.0) (n=31)

Effects	NPI total score (F-ratio = 2.801, p = 0.024)		Delusions (F-ratio = 2.837, p = 0.023)		Hallucinations (F-ratio = 1.184, p = 0.366)		Agitation (F-ratio = 1.205, p = 0.353)		Dysphoria (F-ratio = 1.747, p = 0.140)		Anxiety (F-ratio = 1.830, p = 0.121)		Euphoria (F-ratio = 0.753, p = 0.694)		Apathy (F-ratio = 1.592, p = 0.182)		Disinhibition (F-ratio = 1.550, p = 0.196)		Irritability (F-ratio = 1.225, p = 0.342)		Aberrant motor behavior (F-ratio = 2.339, p = 0.051)	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Constant	-106.0	0.056	-4.02	0.628	-21.57	0.092	-9.04	0.456	-6.52	0.550	-21.73	0.080	-9.91	0.372	-19.71	0.109	-27.17	**0.018**	-12.11	0.333	25.82	**0.040**
Male gender	20.44	**0.043**	5.52	**0.002**	2.38	0.292	-0.46	0.832	0.17	0.930	3.56	0.109	0.84	0.673	2.05	0.345	1.92	0.318	2.41	0.287	2.06	0.340
Years of schooling	-5.40	**0.003**	-0.03	0.901	-0.51	0.171	-0.76	**0.044**	-0.46	0.167	-0.70	0.057	-0.66	0.054	-0.28	0.431	-0.53	0.103	-0.83	**0.034**	-0.64	0.078
Age at dementia onset*	1.45	**0.041**	-0.03	0.784	0.28	0.086	0.14	0.366	0.19	0.189	0.26	0.099	0.11	0.443	-0.36	**0.027**	0.39	**0.009**	0.13	0.409	-0.37	**0.022**
Copies of *APOE*-ε4	11.00	0.116	2.98	**0.011**	0.45	0.777	1.23	0.431	-0.26	0.854	2.49	0.115	-0.03	0.984	-1.99	0.200	0.89	0.511	1.94	0.230	3.31	**0.040**
Copies of rs1800764-C	-7.00	0.279	0.88	0.386	-1.19	0.428	-2.04	0.173	0.08	0.949	-0.09	0.948	-0.51	0.702	-1.34	0.354	-2.18	0.100	-1.58	0.297	0.96	0.502
Copies of rs4291-T	-6.43	0.471	-2.52	0.084	-0.03	0.988	1.09	0.592	-3.83	**0.049**	-1.82	0.368	1.26	0.498	0.84	0.675	0.31	0.861	2.22	0.294	-3.95	0.060
Copies of rs11669576-A	7.53	0.638	-2.62	0.305	0.51	0.891	-1.31	0.720	4.08	0.227	1.82	0.615	0.74	0.825	5.73	0.125	0.86	0.789	-0.45	0.904	-1.82	0.615
Copies of rs5930-A	17.15	**0.003**	1.55	0.064	1.34	0.265	1.79	0.133	1.84	0.092	2.01	0.090	0.37	0.724	3.13	**0.012**	1.60	0.126	2.68	**0.034**	0.83	0.465
Copies of rs2695121-T	9.50	0.244	0.31	0.806	-0.09	0.963	1.05	0.567	-1.63	0.331	3.86	**0.044**	2.83	0.103	-1.52	0.402	-1.10	0.492	1.21	0.521	4.58	**0.019**
Copies of rs5882-G	2.61	0.680	0.38	0.706	2.52	0.102	2.07	0.165	-1.56	0.242	-3.08	**0.043**	0.84	0.528	1.09	0.449	1.51	0.241	1.41	0.349	-2.55	0.086
Copies of rs708272-A	16.69	**0.036**	4.18	**0.002**	1.41	0.420	0.07	0.967	0.18	0.906	4.66	**0.012**	1.41	0.369	-3.21	0.068	0.28	0.850	1.17	0.504	6.54	**0.001**
Antipsychotic therapy†	4.37	0.588	1.58	0.223	1.26	0.504	-0.48	0.794	0.16	0.924	0.81	0.656	1.16	0.494	-0.95	0.602	-0.52	0.748	0.81	0.670	0.54	0.764
Antidepressant therapy†	23.78	**0.025**	1.64	0.296	2.13	0.359	2.14	0.348	0.74	0.716	3.66	0.112	2.85	0.177	3.99	0.085	2.91	0.151	4.25	0.079	-0.54	0.806
Symptom frequency (%)	-		58.1		64.5		74.2		71.0		45.2		38.7		87.1		48.4		67.7		45.2	
Scores (mean ± SD)	33.10±22.4		2.29±3.6		2.94±4.1		3.16±4.0		3.55±4.0		3.26±4.4		1.87±3.3		6.03±4.2		2.61±3.7		3.65±4.1		3.74±4.8	

APOE = apolipoprotein E gene; CDR = Clinical Dementia Rating; NPI = 10-item Neuropsychiatric Inventory; SD = standard deviation.
* Age in years. † Pharmacological therapy = yes or no.
Results in bold are significant.

Considering associations of independent haplotypes with behavioral scores in moderately impaired patients, rs1800764-CC/rs4291-AT carriers (n=8) had less agitation (0.37±0.7 versus 2.76±3.5, p=0.018), rs11669576-AG/rs5930-GG carriers (n=7) had less apathy (2.00±4.5 versus 5.05±4.5, p=0.049), rs11669576-GG/rs5930-AA carriers (n=8) had less anxiety (0.12±0.4 versus 2.68±3.9, p=0.033), rs11669576-GG/rs5930-AG carriers (n=42) had less irritability (3.26±3.7 versus 1.76±2.6, p=0.034), rs11669576-GG/rs5930-GG carriers (n=30) had less irritability (1.37±2.4 versus 2.97±3.4, p=0.006), rs5882-AG/rs708272-AA carriers (n=8) had more agitation (4.75±2.9 versus 2.35±3.4, p=0.008) and more hallucinations (3.00±3.8 versus 0.89±1.9, p=0.018) and more disinhibition (4.00±5.3 versus 1.08±2.5, p=0.029), and rs5882-AG/rs708272-AG carriers (n=23) had less agitation (1.39±2.9 versus 2.94±3.5, p=0.012).

Considering associations of independent haplotypes with behavioral scores in severely impaired patients, rs1800764-CT/rs4291-AA carriers (n=3) had more aberrant motor behavior (9.33±2.3 versus 3.14±4.6, p=0.033), rs1800764-CT/rs4291-AT carriers (n=11) had less disinhibition (1.27±2.9 versus 3.35±4.0, p=0.038), rs1800764-TT/rs4291-AA carriers (n=12) had more disinhibition (4.25±4.5 versus 1.58±2.8, p=0.029), rs5882-AA/rs708272-AG carriers (n=5) had more anxiety (8.00±5.7 versus 2.35±3.6, p=0.023), rs5882-AG/rs708272-AG carriers (n=11) had more irritability (6.09±4.1 versus 2.30±3.6, p=0.028), and rs5882-AG/rs708272-GG carriers (n=8) had less delusions (0.25±0.5 versus 3.00±3.9, p=0.017).

Discussion

With the exceptions of anxiety and irritability, a trend was found for all other behavioral symptoms to be intensified according to functional decline; likewise, most significant correlations were found in later dementia stages. Anxiety is usually inversely correlated with apathy,[25] whereas overall neuropsychiatric symptoms tend to correlate better with functional independence than with cognitive decline.[25,26] Antipsychotic therapy was associated with higher Neuropsychiatric Inventory total scores for mildly impaired patients, particularly regarding disinhibition, and with more delusions and more agitation for moderately impaired patients, probably representing the need for psychotropic treatment when more behavioral symptoms are present, while agitation does not usually improve with atypical antipsychotics in the long term.[26] Likewise, antidepressant therapy was associated with higher Neuropsychiatric Inventory total scores for mildly impaired and severely impaired patients (particularly regarding dysphoria), and with more agitation for moderately impaired patients, also representing the expected need for such therapy according to symptoms of depression.

Male gender was associated with more irritability in mildly impaired patients, more apathy in moderately impaired patients, and higher Neuropsychiatric Inventory total scores, particularly regarding delusions, in severely impaired patients. Gender differences have been reported in other studies, particularly regarding greater anxiety and dysphoria in women.[6]

Schooling did not correlate with dementia onset in this sample. Nevertheless, more years of education were associated with less dysphoria in moderately impaired patients and with lower Neuropsychiatric Inventory total scores in severely impaired patients, particularly regarding agitation and irritability. Though an earlier study showed that educational attainment and AD duration did not affect behavior,[25] patients were not stratified by dementia stage, and some associations may have been missed. Education enhances cognitive reserve and may affect the way the brain copes with the burden of AD neuropathology, thus modulating behavior.

Later dementia onset was associated with fewer hallucinations, less euphoria, and less apathy for moderately impaired patients. Considering that hallucinations are strong predictors of faster functional decline,[26] they may also be more frequent when dementia starts earlier. A high prevalence of hallucinations and extrapyramidal signs might predict the presence of Lewy bodies in patients with AD.[27] In severely impaired patients, later dementia onset was associated with higher Neuropsychiatric Inventory total scores, particularly regarding disinhibition, but also with less apathy and less aberrant motor behavior, a complex link that could be partly due to *APOE*-ε4 non-carriers having later AD onset.

On analysis of multiple correlations, only *APOE* haplotypes affected the age at dementia onset in this sample, confirming the essential role of genetically mediated neurodegeneration on AD onset.[1] Furthermore, moderately impaired *APOE*-ε4 carriers had less apathy, whereas severely impaired *APOE*-ε4 carriers had more delusions and more aberrant motor behavior. Whereas some cross-sectional studies have not found associations of *APOE* haplotypes with specific neuropsychiatric symptoms[25] or sleep satisfaction,[24] other studies have shown dose-dependent associations of copies of *APOE*-ε4 with delusions,[28] aberrant motor behavior,[6] anxiety,[29] and agitation.[30] Mechanisms involve atrophy of the prefrontal cortex,[29] temporal lobe hypoperfusion,[28] cholinergic imbalance,[6] and tau-mediated disruption of frontal serotonergic and dopaminergic networks.[30] *APOE* haplotypes have also been associated with variable frequency of hallucinations.[6,28] The incidence of delusions and hallucinations tends to be dissociated, considering that in AD delusions are usually more common than hallucinations, while in Lewy body dementia syndromes, hallucinations are more common than delusions.[31] Though *APOE*-ε4 carrier status seems to predispose to late-onset depression,[3] particularly in women,[32] we did not find correlations with dysphoria in our sample. Nevertheless, one study showed that women have earlier AD onset when they are *APOE*-ε4 carriers with a history of depression,[33] confirming that gender differences might affect dementia onset.

Considering *ACE* genotypes, carriers of the C allele of rs1800764 had more hallucinations when mildly impaired and less dysphoria when moderately impaired, whereas carriers of the T allele of rs4291 had less dysphoria when severely impaired. Regarding represented haplotypes in independent analyses, presence of the C allele of rs1800764 was associated with less agitation, while presence of the A allele of rs4291 was associated with more agitation, more disinhibition, more irritability, and worse aberrant motor behavior. To the best of our knowledge, these are all previously unreported associations, while only the T allele of rs4291 was reported as a susceptibility factor for unipolar major depression and hypothalamic-hypophyseal-adrenocortical axis hyperactivity in an earlier study of subjects without dementia.[34] Given that angiotensin-converting enzyme degrades amyloid-β[35] and is overexpressed in a compensatory manner in the hippocampus, frontal cortex, and caudate nucleus of both hemispheres of patients with AD,[12] suppression of frontally mediated behaviors could occur by boosted enzyme levels and activity. Functional studies are needed to confirm these associations.

Considering *LDLR* genotypes, carriers of the A allele of rs11669576 had less anxiety and more aberrant motor behavior when moderately impaired, whereas carriers of the A allele of rs5930 had less delusions when mildly impaired, less anxiety when moderately impaired, and higher Neuropsychiatric Inventory total scores when severely impaired, particularly regarding apathy and irritability. Regarding represented haplotypes in independent analyses, presence of the G allele of rs5930 was associated with less apathy in mildly and moderately impaired patients. Still, carriers of rs11669576-GG/rs5930-GG had later dementia onset. The A allele of rs11669576 has been associated with variable risk of AD, either increasing risk when in combination with copies of *APOE*-ε4[7] or decreasing risk - only in women - when in combination with other *LDLR* polymorphisms,[36] while the A allele of rs5930 was associated with lower risk of AD when combined with other *LDLR* polymorphisms in an earlier study.[13] Although neuropsychiatric symptoms have not previously been studied in association with variants of *LDLR*, genotypes associated with less AD neuropathology[36] could lead to later dementia onset and milder behavioral burden in earlier stages.

Considering *CETP* genotypes, carriers of the G allele of rs5882 had more delusions when mildly impaired, less dysphoria when moderately impaired, and less anxiety when severely impaired, whereas carriers of the A allele of rs708272 had higher Neuropsychiatric Inventory total scores when severely impaired, particularly regarding delusions, anxiety, and aberrant motor behavior. Regarding represented haplotypes in independent analyses, mildly impaired carriers of rs5882-AA/rs708272-GG had higher Neuropsychiatric Inventory total scores, particularly regarding apathy and dysphoria, whereas moderately impaired carriers of rs5882-AG/rs708272-AA had more agitation, more hallucinations, and more disinhibition. Carriers of rs5882-AG/rs708272-AG had less agitation when moderately impaired and more irritability when severely impaired. Carriers of rs5882-AA had later dementia onset, but this finding did not survive multiple correlations; conversely, carriers of rs5882-AG/rs708272-AA had earlier dementia onset on independent analysis. Though associations of *CETP* genotypes with behavioral symptoms have not been reported before, the AA genotype of rs708272 was associated with increased risk of AD for *APOE*-ε4 carriers in one meta-analysis,[37] while the GG genotype of rs5882 has been associated either with slower cognitive decline and lower risk of AD,[15] with faster

cognitive decline and higher risk of AD,[38] or with increased risk of AD only for *APOE*-ε4 non-carriers.[39] The G allele of rs708272 and the G allele of rs5882 seemed to play protective roles in our sample, possibly by improving myelin biosynthesis in the brain.

Severely impaired carriers of the T allele of rs2695121 had more anxiety and more aberrant motor behavior. Among all polymorphisms of the LXR-β gene (*NR1H2*), the T allele of rs2695121 is the most significant for risk of AD,[20] though it had not been previously associated with behavioral symptoms. Polymorphisms in cellular cholesterol efflux-related genes may affect the neuropsychiatric profile of patients with AD, as increased cholesterol levels induce amyloidogenesis.

An important limitation of this study is that it was conducted in a single center with no randomization. We tried to mitigate this drawback by ensuring that examiners were blinded to genetic data during the evaluations. In addition, the genotype and haplotype frequencies found were similar to those reported in most previous studies, confirming that our outpatient sample was probably representative of our population as a whole. The cross-sectional design of this study precludes deeper assumptions regarding causal relations. Even though *APOE* haplotypes were the only genetic factors to consistently affect age at AD onset, variants involved in cerebrovascular metabolism were associated with several behavioral symptoms, leading to the conclusion that cerebrovascular risk plays an important genetically mediated role in neuropsychiatric manifestations of AD.

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Disclosure

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