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Regional Differences in Effects of *APOE* ε4 on Cognitive Impairment in Non-Demented Subjects

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Key Words
Alzheimer's disease · *APOE* ε4 allele · Europe · Gradient

Abstract
Background: The *APOE* ε4 allele is a risk factor for Alzheimer's disease (AD). *APOE* ε4 is common in non-demented subjects with cognitive impairment. In both healthy people and people with AD, its prevalence has a north-south gradient across Europe. In the present study, we investigated whether the relation between the *APOE* ε4 allele and cognitive impairment varied across Northern, Middle and Southern Europe. We also investigated whether a north-south gradient existed in subjects with subjective cognitive impairment (SCI), amnestic mild cognitive impairment (MCI) and non-amnestic MCI. ***Methods:*** Data from 16 centers across Europe were analyzed. ***Results:*** A north-south gradient in *APOE* ε4 prevalence existed in the total sample (62.7% for *APOE* ε4 carriers in the northern region, 42.1% in the middle region, and 31.5% in the southern region) and in subjects with SCI and amnestic MCI separately. Only in Middle Europe was the *APOE* ε4 allele significantly associated with poor performance on tests of delayed recall and learning, as well as with the amnestic subtype of MCI. ***Conclusion:*** The *APOE* ε4 allele frequencies in subjects with SCI and amnestic MCI have a north-south gradient. The relation between the *APOE* ε4 allele and cognition is region dependent.

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Introduction

The ε4 allele of the apolipoprotein E gene (*APOE* ε4) is one of the most important and well-replicated genetic risk factors for Alzheimer disease (AD) [1]. Studies have also linked the *APOE* ε4 allele to subjective cognitive impairment (SCI) and mild cognitive impairment (MCI) [2, 3]. MCI refers to subjects with cognitive impairment worse than what would be expected relative to their age, but where the impairment is not severe enough to fulfill the diagnostic criteria of dementia [4, 5]. Subjects with SCI have cognitive complaints, but do not show impairments on cognitive testing [3].

The prevalence of the *APOE* ε4 allele varies greatly in different populations. In Europe, a north-south gradient has been observed in healthy subjects but also in subjects with AD [6]. The frequency of the ε4 allele is highest in the north and lowest in the south [6–10] (table 1). It is not clear whether such a gradient also exists in subjects with SCI and MCI. Further, it is possible that the *APOE* ε4 allele contributes differently to cognitive impairment across geographical regions, but this has not yet been investigated.

The aim of this study was to investigate whether a north-south gradient exists for the prevalence of the *APOE* ε4 allele in subjects with SCI and MCI. Further, we investigated the association between the *APOE* ε4 allele and neuropsychological test performance, as well as with SCI and the amnestic and non-amnestic subgroups of MCI, in Northern, Middle and Southern Europe separately. Because previous research has shown that *APOE* ε4 interacts with age [11, 12] and gender [13] in cognitive decline, we also investigated the impact of these demographic factors on cognition.

Methods

Study Sample

Subjects were selected from the DESCRIPA study, a European multicenter study with the goal of developing screening guidelines and clinical criteria for patients with pre-dementia AD [14]. Inclusion criteria were: consecutive referral to a memory clinic and age 55 years or older. Exclusion criteria were: previous clinical evaluation, no symptoms, dementia at baseline according to ICD-10 (2 centers) or DSM-IV (14 centers) criteria, and cognitive impairment due to known somatic, psychiatric or neurological disorders (e.g. brain infection, epilepsy that was currently being treated, and severe depression). The study closely followed regular clinical practice or was performed as part of a research project. All subjects had consented to participation in the study, and it was approved by the local medical ethics committee in each centre.

For the present analyses, we selected subjects from 16 centers where blood for *APOE* genotyping had been collected (n = 696). Subjects whose *APOE* status was unknown (n = 128) or could not be classified into a diagnostic group because of missing data on neuropsychological tests (n = 48) were excluded. The final sample consisted of 520 subjects (59% female). The 16 centers were subdivided into three regions (according to [10]): North (n = 83): Huddinge and Malmö (Sweden), Kuopio (Finland); Middle (n = 202): Maastricht, Nijmegen and Amsterdam (The Netherlands), Paris (France), Bristol, Bath (England), Mannheim and Munich (Germany); and South (n = 235): Toulouse (France), Thessaloniki (Greece), Genoa and Brescia (Italy), and Bucharest (Romania).

Excluded subjects were compared to included subjects in terms of demographic variables (age, gender, and education), Mini-Mental State Examination (MMSE) and neuropsychological tests (delayed recall, learning, trail making test A and B, language, and visuoconstruction). This was done separately for the three regions. In the northern region, excluded subjects had better performance than those retained in the study on the MMSE [$F(d.f. = 1) = 4.588$, $p = 0.035$] and delayed recall [$F(d.f. = 1) = 5.20$, $p = 0.021$]. In the southern region, excluded subjects had more years of education than those retained in the study [$F(d.f. = 1) = 5.197$, $p = 0.023$]. There was also a significant difference in the percentage of missing data across regions ($p = 0.001$): the southern region had 36.6% missing data, the middle region had 18.8%, and the northern region had 23.5%.

Baseline Clinical Assessment

Standard procedure at all centers for baseline clinical assessment included physical examination, laboratory assessment, blood, and – where feasible – CSF sampling, as well as clinical evaluation using the MMSE [15], the Clinical Dementia Rating Scale [16] or the Global Deterioration Scale [17]. These procedures have been described in more detail elsewhere [14].

Neuropsychological Assessment

There was a battery of neuropsychological tests performed on all subjects in order to assess performance in the cognitive domains of memory, language, executive function and attention, and visuospatial function. As the DESCRIPA study followed routine clinical practice in each center, the tests used to assess each domain sometimes varied between centers. For that reason, a primary neuropsychological test for each cognitive domain was chosen in all centers that was identical or similar to tests used in other centers [14]. Raw scores were converted to age-, education-, and gender-corrected z-scores according to locally collected normative data or published normative data, and these z-scores were used for further analyses. The tests used were as follows:

Verbal Memory. Immediate or delayed recall from the Rey Auditory Verbal Learning Test [18] (8 centers), the word list of the Consortium to Establish a Registry for AD (CERAD) neuropsychological battery [19] (3 centers), the Grober-Buschke Test [20] (1 center), the Selective Reminding Test [21] (1 center), the Alzheimer's Disease Assessment Scale-cognitive subscale, 10 word list [22] (1 center), or the Hopkins Verbal Learning Test [23] (2 centers).

Language. Verbal Fluency Test, using either animals for 1 min [24] (15 centers) or 1 min for cars, fruits, and animals [25] (1 center).

Visuospatial Function. The Rey-Osterrieth Figure Copy Test [26] (10 centers), the copy figures part from the Mental Deterioration Battery [27] (1 center), the figures part from CERAD [19] (3 centers), the cube analysis test of the Visual Object and Space Per-

Table 1. North-south gradient in *APOE* ε4 frequency across Europe in population-based studies (%)

Study	Subjects	North[1]	Middle[2]	South[3]
APOE ε4 carriers				
Schiele et al. [9]	middle-aged subjects 25–65 years	33	25	20
Tiret et al. [8]	young subjects (18–26 years) with family history of myocardial infarction	29	25	21
Panza et al. [6]	middle-aged subjects	42	20	15
	centenarians	17	11	4.5
APOE ε4 allele frequency				
Corbo and Scacchi [10]	population studies	20	14	9

[1] Finland (n = 1,911) [9]; Sweden, Denmark, Northern Germany (n = 315) [8]; Finland (n = 170–207) [6]; Finland, Sweden, Norway, Iceland (n = 3,104) [10].
[2] France, Ireland (n = 2,585) [9]; Belgium, Austria, Switzerland (n = 327) [8]; France (n = 161–325) [6]; Scotland, The Netherlands, Germany, France, Hungary, Austria, Switzerland (n = 6,821) [10].
[3] Portugal, Greece, Spain (n = 1,979) [9]; Italy, Spain, Southern France (n = 311) [8], Italy (n = 67–311) [6]; Italy, Spain (n = 2,117) [10].

ception Battery [28] (1 center), or the copy figure item of the MMSE (1 center) using a score of 0 as indicative of impairment.

Executive Function. The Trail Making Test A and B [29] (15 centers) or the attention item of the MMSE (1 center) using a score of 1 or lower as indicative of impairment.

Definition of SCI and MCI

Subjects were classified centrally into three subgroups based on performance on the neuropsychological tests. The classification was made independently of other variables, including the *APOE* genotype. Impairment was defined as age-, gender- and education-corrected z-score below –1.5, unless specified otherwise. Subjects without psychometric impairment on any of the primary tests were classified as SCI (n = 130). Subjective complaints were considered to be present as these subjects had attended the memory clinic because of perceived cognitive impairments. Subjects with impairment on the learning subtask or delayed recall subtask of the word-list learning tasks were classified as amnestic MCI (n = 256). Subjects with impairment on any of the other tests other than memory (i.e. fluency, visuospatial function, attention) were classified as non-amnestic MCI (n = 134).

Genotyping

APOE genotypes were determined on genomic DNA extracted from EDTA blood using the SNaPshot technique [30].

Statistical Analysis

SPSS 16.0 for Windows was used for statistical analyses (α = 0.05). Differences between subjects with or without missing data were analyzed using ANOVA or Pearson's χ^2 test. Also, differences between diagnostic groups (SCI, amnestic MCI and non-amnestic MCI) in demographic variables (age, gender, and education) were analyzed with ANOVA or Pearson's χ^2 test. Post hoc analyses were done using Scheffé and logistic regression. Hardy-Weinberg equilibrium was tested in the *APOE* genotype, also by using Pearson's χ^2 test.

The cognitive profiles of *APOE* ε4 carriers (one or two ε4 alleles) were compared to *APOE*-ε4 -negative subjects (no *APOE* ε4 alleles). This was done with both t-tests and ANCOVA (with age and education as covariates), which provided similar results. Outliers (defined as any score $\pm$3.29 SD, $p < 0.001$ two-tailed test, of each subgroup or regional mean) were excluded, resulting in 0.3% data loss.

Differences in *APOE* ε4 genotypes (presence of at least one *APOE* ε4 allele) between diagnostic groups were analyzed with multinomial logistic regression, by using clinical subgroup (SCI as reference, non-amnestic MCI, and amnestic MCI) as dependent variables, and controlling for age, gender and education. This was done in the total sample and in each region separately. Interactions could not be included since this gave rise to singularities in the Hessian matrix.

Binary logistic regression was used for pairwise comparisons (amnestic MCI vs. SCI, amnestic MCI vs. non-amnestic MCI and non-amnestic MCI vs. SCI). Demographic variables (age, education and gender) were entered in the first step, the *APOE* ε4 variable in the second step, and interactions for the presence of *APOE* ε4 with age and gender in the third step. Because both main effects and interaction effects of age were studied, this variable was centered in order to avoid multicolinearity.

Results

The distribution of *APOE* genotypes was in Hardy-Weinberg equilibrium in the total material as well as in the diagnostic subgroups, indicating a stable relationship between the different alleles (Hardy-Weinberg equilibrium test $p > 0.05$).

Demographic characteristics (age, gender and education), MMSE scores and *APOE* genotype data in the total group and in the diagnostic categories of SCI, non-amnestic MCI and amnestic MCI in each region are shown in tables 2 and 3. In the total sample, we noted differences in

Table 2. Baseline characteristics according to diagnostic group

	Total	SCI	naMCI	aMCI	p	Post hoc
Northern Europe						
n	83	36	18	29		
Male/female	43/40	19/17	8/10	16/13	0.8	
Age, years	67.2 ± 7.0	65.6 ± 7.0	66.3 ± 5.3	69.6 ± 7.5	0.06	
Education, years	10.9 ± 3.8	11.5 ± 3.7	11.0 ± 4.0	10.0 ± 3.9	0.3	
MMSE	27.3 ± 2.6	28.4 ± 1.6	27.6 ± 2.0	25.7 ± 3.2	0.001	aMCI<SCI, naMCI
Middle Europe						
n	202	59	42	101		
Male/female	95/107	29/30	28/14	38/63	0.006	aMCI>naMCI
Age, years	69.8 ± 8.3	67.9 ± 8.6	70.3 ± 9.1	70.7 ± 7.7	0.1	
Education, years	11.3 ± 3.7	12.5 ± 4.2	11.1 ± 3.8	10.7 ± 3.3	0.01	aMCI<SCI
MMSE	27.2 ± 2.2	28.4 ± 1.5	27.6 ± 1.8	26.4 ± 2.4	0.001	aMCI<SCI, naMCI
Southern Europe						
n	235	35	74	126		
Male/female	76/159	12/23	17/57	47/79	0.2	
Age, years	70.1 ± 7.7	67.8 ± 6.5	70.2 ± 7.4	70.7 ± 8.1	0.1	
Education, years	8.9 ± 4.5	9.4 ± 4.1	7.7 ± 4.2	9.4 ± 4.7	0.03	aMCI>naMCI
MMSE	27.6 ± 2.0	28.8 ± 1.3	27.6 ± 2.1	27.2 ± 1.9	0.001	SCI>aMCI, naMCI

Where indicated, data are means ± SD.

Table 3. *APOE* allele frequency across diagnostic groups

	ε2ε3	ε2ε4	ε3ε3	ε3ε4	ε4ε4	Total ε2	Total ε3	Total ε4
Northern Europe								
SCI	3 (8)	1 (3)	9 (25)	22 (61)	1 (3)	4 (6)	43 (60)	25 (35)
naMCI	0	0	9 (50)	9 (50)	0	0	27 (75)	9 (25)
aMCI	0	3 (10)	10 (35)	12 (41)	4 (14)	3 (5)	32 (55)	23 (40)
Middle Europe								
SCI	4 (7)	2 (3)	37 (63)	14 (24)	2 (3)	6 (5)	92 (78)	20 (17)
naMCI	3 (7)	1 (2)	25 (60)	11 (26)	2 (5)	4 (5)	64 (76)	16 (19)
aMCI	10 (10)	3 (3)	38 (38)	36 (36)	14 (14)	13 (6)	122 (60)	67 (33)
Southern Europe								
SCI	3 (9)	1 (3)	25 (71)	6 (17)	0	4 (6)	59 (84)	7 (10)
naMCI	6 (8)	2 (3)	42 (57)	20 (27)	4 (5)	8 (5)	110 (74)	30 (20)
aMCI	8 (6)	0	77 (61)	36 (29)	5 (4)	8 (3)	198 (79)	46 (19)

Data are n (%) for each genotype.

age, education and gender between the regions (p = 0.001 for all differences), but absolute differences were small.

In the total sample, the prevalence of *APOE* ε4 carriers was 40.0 % in SCI, 36.6% in non-amnestic MCI and 44.5% in amnestic MCI. These differences were not statistically significant [χ^2(d.f. = 2, n = 520) = 2.430, p = 0.3]. There was a significant difference in the frequency of *APOE* ε4 carriers between regions [χ^2(d.f. = 2, n = 520) = 25.010, p = 0.001] and it showed a north-south gradient with a prevalence of 62.7% for *APOE* ε4 carriers in the northern region, 42.1% in the middle region, and 31.5% in the southern region. Such a gradient also existed for subjects with SCI (p = 0.001) and subjects with amnestic MCI (p = 0.001), but not for subjects with non-amnestic MCI (fig. 1).

The effect of *APOE* ε4 on test performance in each region is shown in table 4. In Middle Europe, the presence of the *APOE* ε4 allele was associated with worse performance on learning [F(d.f. = 1) = 5.0, p = 0.03] and delayed

Table 4. Relation between *APOE* ε4 carriers and neuropsychological test data across regions

	Northern Europe				Middle Europe				Southern Europe			
	n	no ε4	ε4 carrier	p	n	no ε4	ε4 carrier	p	n	no ε4	ε4 carrier	p
Language												
Fluency	78	–0.7 (1.2)	–0.3 (1.1)	0.1	201	–0.7 (1.0)	–0.7 (1.0)	0.9	234	–1.0 (1.0)	–1.1 (0.9)	0.5
Executive functioning												
TMT-A	81	–0.3 (1.5)	0.06 (1.0)	0.2	201	–0.6 (1.6)	–0.5 (1.2)	0.9	198	–1.3 (1.8)	–1.4 (1.7)	0.8
TMT-B	78	–0.09 (1.1)	–0.2 (1.2)	0.7	197	–0.5 (1.8)	–0.5 (1.6)	0.9	197	–1.9 (2.1)	–1.7 (2.0)	0.6
Memory												
Learning	83	–0.4 (1.3)	–0.4 (1.4)	0.9	201	–0.9 (1.2)	–1.3 (1.2)	0.03	234	–1.0 (1.2)	–1.1 (1.1)	0.7
Delayed recall	82	–0.5 (1.3)	–0.6 (1.4)	0.7	195	–0.8 (1.2)	–1.7 (1.5)	0.001	235	–1.1 (1.2)	–1.2 (1.3)	0.4
Visuospatial function												
Figure	79	0.1 (1.1)	0.6 (1.0)	0.07	179	0.04 (1.2)	0.2 (1.0)	0.5	228	0.06 (1.2)	–0.05 (1.2)	0.6

Data are Z-scores (means and SD). TMT = Trail-Making Test.

recall [F(d.f. = 1) = 20.3, p = 0.001]. In Northern Europe, there was also a tendency for *APOE* ε4 carriers to perform better on tests of visuospatial function than non-carriers [F(d.f. = 1) = 3.5, p = 0.07]. There was no significant effect of *APOE* ε4 on neuropsychological test performance in Southern Europe.

The same pattern also held for categorical data. The multinomial logistic regression showed that in the total sample, *APOE* ε4 was not associated with diagnostic group. When multinomial logistic regression was done separately for the three regions, the frequency of *APOE* ε4 carriers differed between diagnostic groups in Middle Europe only [likelihood ratio test: χ^2(d.f. = 8, n = 202) = 32.88, p = 0.001], explaining 17.2% of the variance in group membership (Nagelkerke's R^2). Pair-wise comparisons showed that *APOE* ε4 carriers were more common in amnestic MCI than in non-amnestic MCI (OR = 2.4, 95% CI = 1.1–5.2, p = 0.032) or in SCI (OR = 2.9, 95% CI 1.42–6.1, p = 0.004). In this region, age interacted with *APOE* ε4 in the comparison between non-amnestic MCI and SCI (OR = 1.1, 95% CI 1.0–1.3, p = 0.043), indicating that the likelihood for *APOE* ε4 carriers of being in the non-amnestic MCI subgroup compared to the SCI subgroup increased with age.

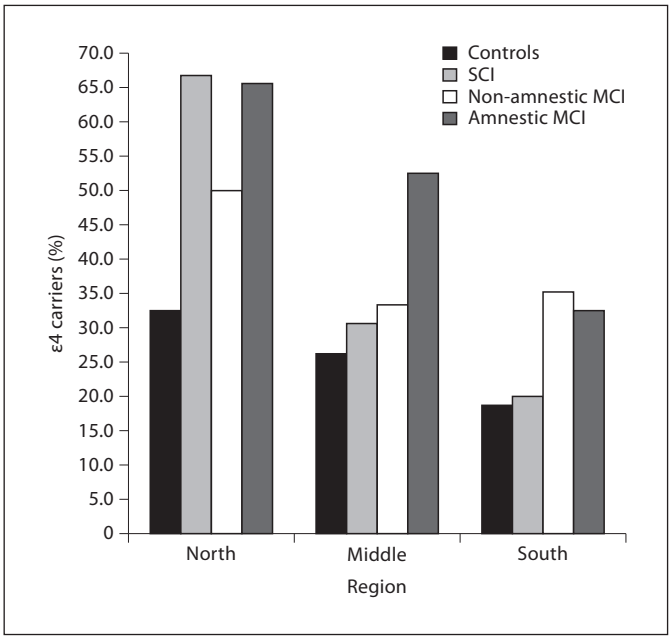

Fig. 1. *APOE* ε4 carriers (%) according to diagnosis and region (North, Middle and South Europe). Data for controls are taken from Schiele et al. [9] and used for comparison purposes only.

Discussion

The present study provides evidence for regional effects of *APOE* ε4 on cognitive function. Results show that there was an association between *APOE* ε4 and poor performance on tests of learning and delayed recall only in Middle Europe, where *APOE* ε4 also was associated with amnestic MCI. Further, *APOE* ε4 interacted with old age in this region to predict non-amnestic MCI. In the northern region, there was a trend for *APOE* ε4 carriers to be associated with improved performance on tests of visuospatial function. There was also a north-

south gradient in the regional distribution of *APOE* ε4 carriers.

The association of *APOE* ε4 with amnestic MCI and poor performance on tests of learning and delayed recall is consistent with previous studies [12, 31, 32]. However, this study shows that the association only holds for Middle Europe. Indeed, two of the previous studies investigating this association only involved subjects from this region [12, 31]. The study conducted by Farlow et al. [32] used data from the InDDEx study, which includes patients from 14 different countries in Europe and North America [33], and therefore their results cannot be interpreted in terms of regional effects.

It could be the case that the observed association between *APOE* ε4 and poor cognitive performance is related to lifestyle. *APOE* alleles have previously been found to modulate alcohol-mediated effects on LDL cholesterol [34], whilst *APOE* ε2 is thought to be associated with a stronger triacylglycerol response to high dietary sucrose intakes than seen in coronary artery disease patients with the *APOE* ε3 or ε4 alleles [35]. Both alcohol and dietary sucrose intake is likely to vary across the Middle Europe region which, when combined with suggested gender [36] and geographical associations with *APOE* variability [6], would certainly make this a complicated association, but one which is nonetheless possible and perhaps more pronounced within Middle Europe.

In the northern region, *APOE* ε4 carriers tended to be associated with better performance on visuospatial function than subjects with no *APOE* ε4. This was an unexpected result that, to our knowledge, has not been demonstrated before and therefore needs to be replicated.

A remarkable finding was the high prevalence of *APOE* ε4 in subjects with SCI. Indeed, in the northern region the prevalence of *APOE* ε4 in SCI was just as high as in amnestic MCI, and much higher than expected for the region based on control data (fig. 1). The association of SCI and neurodegeneration is disputed. Some studies indicate that SCI is not primarily linked to neurodegeneration, but rather to depression [e.g. 37] and personality [e.g. 38]. However, other studies have also found a high prevalence of *APOE* ε4 in SCI [e.g. 3, 39, 40]. A review of studies by Jonker et al. [41] concluded that SCI should not be considered as being merely a symptom of depression, but rather as an early sign of dementia. Recent studies have also found that SCI is related to AD biomarkers, such as a smaller entorhinal cortex or hippocampal volume [42, 43] or abnormal cerebrospinal fluid markers [44]. In separate studies from the DESCRIPA project, SCI was associated with abnormal EEG, consistent with latent AD pathology [45] and CSF markers suggestive of AD [46]. Together, this implies an increased risk for AD in subjects with SCI.

The non-amnestic MCI subgroup is usually thought to have a heterogeneous etiology leading to various types of dementia, such as vascular dementia, frontotemporal dementia and dementia with Lewy bodies [2]. The high prevalence in this study of *APOE* ε4 in the non-amnestic MCI subgroup implies increased risk for AD for subjects in this diagnostic group as well. Indeed, in a separate study from the DESCRIPA project, non-amnestic MCI was associated with CSF markers suggestive of AD [46].

Previous studies [11, 12] have shown that the *APOE* ε4 allele interacts with age in affecting memory – meaning that increased age strengthens the negative impact of *APOE* ε4 on memory performance. In our study, it was shown that this interaction could predict non-amnestic MCI when contrasted with SCI in Middle Europe. This meant that in elderly subjects, those with an *APOE* ε4 allele were more likely to develop impairment in domains other than memory, rather than just subjective complaints. There was no interaction found with gender.

Our study is the first to show a north-south gradient of *APOE* ε4 in MCI patients, possibly accelerating the onset of AD in these subjects. It extends findings from studies conducted in healthy controls and subjects with AD. In the analysis with the different diagnostic groups, a north-south gradient was present only for subjects with amnestic MCI and SCI. The lack of a gradient in subjects with non-amnestic MCI could be explained by the small sample size in this group, especially in the northern region. Further, it should be noted that even though the *APOE* ε4 allele was only associated with amnestic MCI in Middle Europe, the prevalence of the *APOE* ε4 allele carriers in subjects with amnestic MCI was increased in all regions relative to the control data in figure 1.

There were differences between centers in procedures, and there are for that reason a number of potential threats to the conclusions of the study that need to be addressed. First, the results could not have been influenced by differences between regions in demographic variables, because these were controlled for in all the analyses. In parallel, the results could not have been an artifact of arbitrary categorization procedures across centers (which could otherwise explain the association between *APOE* ε4 and MCI subgroup), because the same pattern was also found for continuous measures (i.e. association between *APOE* ε4 and neuropsychological tests). To investigate

other threats to the conclusions, we ran a number of post hoc analyses. First, two centers used ICD-10 for diagnosis, instead of DSM-IV as the rest. For that reason, analyses were made when excluding those centers. This did not change the results. Further, it is possible that the present results could be explained by selection bias. For that reason, differences in referral source were analyzed across regions. This showed an increasing north-south gradient in self-referral (north = 4.8%; middle = 9%; south = 33%; p = 0.001). However, after exclusion of self-referrals, results were essentially the same. Still, a specific referral pattern to a memory clinic could not be excluded. Finally, the results could also have been an artifact of the classification of centers in geographical regions (which was made according to Corbo and Scacchi [10]). One concern regarding this is that the Bucharest center was included in the region of Southern Europe, although this center is really located in Eastern Europe. However, post hoc analyses after exclusion of this centre from the southern region yielded similar results.

However, there were also two limitations to the present study that were not addressed. First, a number of subjects were excluded from analyses due to missing data on genotype or neuropsychological tests. There was a trend for these subjects to have a better performance on memory tests and higher level of education in some regions. Second, the centers did not all use the same neuropsychological tests or norms. Differences in these procedures could have confounded the results. Future studies should consider using the same neuropsychological tests and norms in all regions.

In conclusion, geographical location was strongly associated with the frequency of *APOE* ε4 across Europe and modulated the relationship between the *APOE* ε4 allele and cognition. For this reason, region-specific cutoffs may be needed to define what could be considered to be a deviation from the reference *APOE* ε4 allele frequency. Future studies should focus on interactions of lifestyle or other genetic factors with the *APOE* ε4 allele that could account for the regional differences in the relation between *APOE* genotype and cognitive impairment. Longitudinal studies are also needed to see whether the rate of decline or the conversion from different MCI subtypes to different forms of dementia also varies between regions. The high prevalence of *APOE* ε4 carriers in all diagnostic groups suggests that SCI, non-amnestic MCI and amnestic MCI all have an increased risk for AD.

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