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Is *APOE* ε4 associated with cognitive performance in early MS?

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Abstract

Objective
To assess the impact of *APOE* polymorphisms on cognitive performance in patients newly diagnosed with clinically isolated syndrome (CIS) or relapsing-remitting MS (RRMS).

Methods
This multicenter cohort study included 552 untreated patients recently diagnosed with CIS or RRMS according to the 2005 revised McDonald criteria. The single nucleotide polymorphisms rs429358 (ε4) and rs7412 (ε2) of the *APOE* haplotype were assessed by allelic discrimination assays. Cognitive performance was evaluated using the 3-second paced auditory serial addition test and the Multiple Sclerosis Inventory Cognition (MUSIC). Sum scores were calculated to approximate the overall cognitive performance and memory-centered cognitive functions. The impact of the *APOE* carrier status on cognitive performance was assessed using multiple linear regression models, also including demographic, clinical, MRI, and lifestyle factors.

Results
APOE ε4 homozygosity was associated with lower overall cognitive performance, whereas no relevant association was observed for *APOE* ε4 heterozygosity or *APOE* ε2 carrier status. Furthermore, higher disability levels, MRI lesion load, and depressive symptoms were associated with lower cognitive performance. Patients consuming alcohol had higher test scores than patients not consuming alcohol. Female sex, lower disability, and alcohol consumption were associated with better performance in the memory-centered subtests of MUSIC, whereas no relevant association was observed for *APOE* carrier status.

Conclusion
Along with parameters of a higher disease burden, *APOE* ε4 homozygosity was identified as a potential predictor of cognitive performance in this large cohort of patients with CIS and early RRMS.

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German Competence Network of Multiple Sclerosis coinvestigators are listed in the appendix 2 at the end of the article.

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Glossary

AD = Alzheimer disease; **BDI-II** = Beck Depression Inventory II; **BMI** = body mass index; **CIS** = clinically isolated syndrome; **EDSS** = Expanded Disability Status Scale; **FSMC** = Fatigue Scale for Motor and Cognitive Function; **HWE** = Hardy-Weinberg equilibrium; **MCI** = mild cognitive impairment; **MUSIC** = Multiple Sclerosis Inventory Cognition; **PASAT 3** = 3-second paced auditory serial addition test; **RRMS** = relapsing-remitting MS; **SNP** = single nucleotide polymorphism.

MS is a chronic neuroinflammatory disease, which mostly affects young adults. Apart from physical impairment, decline of cognitive functions is one of its most disabling aspects. A meta-analysis of data acquired by genome-wide association studies identified a total of 234 significant associations and a further 416 variants potentially associated with MS.[1] However, so far, little is known about the contribution of genetic risk factors to the development of cognitive impairment in MS.

The *APOE* gene locus has been discussed as a possible mediator of cognitive impairment as it is associated with the evolution of dementias like Alzheimer disease (AD).[2] It may encode 3 different isoforms of apolipoprotein E (APOE2, APOE3, and APOE4), which are defined by the haplotype combination of common single nucleotide polymorphisms (SNPs) at 2 nearby loci on the *APOE* gene. The SNPs are labeled rs429358 (base exchange from cytosine to thymine [C>T] leading to the haplotype *APOE* ε4) and rs7412 (base exchange C>T resulting in the haplotype *APOE* ε2). The *APOE* ε4 haplotype leads to an amino acid exchange from cysteine to arginine at position 112 of the APOE protein resulting in the isoform APOE4, whereas the haplotype *APOE* ε2 leads to an amino acid exchange from arginine to cysteine at position 158 of the APOE protein resulting in the isoform APOE2. *APOE* ε3 is the common variant.[3] *APOE* ε4 is associated with faster memory decline over the adult life course[4] and is a major risk factor for AD, with an 8- to 12-fold increase in *APOE* ε4 homozygotes.[3] Although it was shown in a sufficiently powered study that *APOE* variants have no effect on MS susceptibility,[5] reports on the influence of *APOE* variants on cognitive performance in patients with MS have been contradictory.[6,7] Therefore, this study aims to assess the potential impact of *APOE* polymorphisms on parameters of cognitive function in a large multicenter, prospectively collected German data set of untreated patients with clinically isolated syndrome (CIS) and early relapsing-remitting MS (RRMS). As a number of demographic, clinical, MRI, and lifestyle risk factors have been shown to enhance cognitive decline in MS and to adversely influence disease progression,[8–10] these were also included in the analyses.

Methods

Standard protocol approvals, registrations, and patient consents

This multicenter prospective longitudinal observational cohort study (German National MS Cohort) was approved by the ethics committee of Ruhr-University Bochum (registration no. 3714-10) and consecutively all local committees of the participating centers (22 centers in Germany). All patients provided written informed consent.

The German National MS cohort and clinical data

A total of 552 participants from the German National MS cohort, a multicenter, prospective, and observational study, were included. This study was approved by the ethics committee of Ruhr-University Bochum (registration no. 3714-10) as described previously.[11] All participants were aged at least 18 years, untreated regarding disease-modifying therapies, and diagnosed with either CIS with first symptoms within the previous 6 months and fulfilling at least 3 Barkhof criteria[12] or RRMS according to the 2005 revised McDonald criteria[13] with first symptoms not more than 3 years before study enrollment. For inclusion, patients must not have received a steroid pulse due to a relapse in the 4 weeks before study enrollment. All participants provided written informed consent.

Assessments included clinical, demographic, MRI, and lifestyle variables and screening tests for cognitive function and blood sampling.[11] At the point of study enrollment, patients were asked to assess their current drinking and smoking habits via questionnaire. In response to the question "Do you currently drink alcohol?", patients could select from 3 categories: (0) no, (1) occasionally, and (2) regularly. Based on this, we dichotomized the participants into current no alcohol consumers (category 0) and current alcohol consumers (categories 1 and 2). Similarly, in response to the question "Do you currently smoke?", patients could select from 6 categories: (0) no, (1) occasionally, but not on a daily basis, (2) up to 5 cigarettes daily, (3) 6–10 cigarettes daily, (4) 11–20 cigarettes daily, and (5) >20 cigarettes daily. We dichotomized the participants into current nonsmokers (category 0) and current smokers (all other categories). Body weight and height were physically measured on site at the time of study enrollment. Body mass index (BMI) was then calculated as BMI = weight (kg)/height $(m)^2$. School-level education was categorized according to the highest school leaving qualification (level 1: lower-level secondary school [German Hauptschule]; level 2: higher-level secondary school; level 3: higher education entrance qualification [German Abitur]). Depressive symptoms were assessed by the 21-item Beck Depression Inventory II (BDI-II),[14] and severity of fatigue was evaluated by the Fatigue Scale for Motor and Cognitive Functions (FSMC).[15]

Cognitive assessment

Cognitive assessment included the 3-second paced auditory serial addition test (PASAT 3) and the Multiple Sclerosis Inventory Cognition (MUSIC) cognitive screening tests.

The PASAT 3 is a measure of cognitive function that assesses auditory information processing speed, working memory, divided attention, and calculation ability. PASAT 3 data were extracted from the Multiple Sclerosis Functional Composite.[16] Individual PASAT 3 test scores were z-standardized, stratified for age and education based on normative data from a German sample of n = 241 healthy controls.[17,18]

MUSIC is a brief multiple-domain cognitive screening test, which is widely used in German-speaking countries and was developed for the rapid assessment of the most frequently impaired cognitive domains in patients with MS. It consists of 5 cognitive subtests. In the subtests (1) and (2), the patient is asked to remember as many words as possible out of 2 consecutive word lists, each consisting of 10 different words to evaluate working memory. In subtest (3), the patient is given 2 alternating word categories, for which they are asked to find as many associated terms as possible within 1 minute. This subtest was designed to test verbal fluency. Subtest (4) is a modified Stroop Task and assesses susceptibility to interference. In subtest (5), the patient is asked to recall the terms of the first given list of words to asses memory consolidation.[19] Individual test scores were z-standardized based on normative data from n = 158 German-speaking healthy young adults.[17,19] All tests were taken for the first time at study enrollment so that results were not expected to be biased by learning effects.

Biosamples and genotyping

The SNPs rs429358 (ε4) and rs7412 (ε2) in *APOE* and the Y chromosome marker rs2032598 (for sampling and handling control) were analyzed using allelic discrimination assays based on TaqMan chemistry according to the manufacturer's protocol (Applied Biosystems, Inc.). Genotyping was performed on 96-well plates with approximately 5% controls run in duplicates across plates. Genotyping efficiency was ≥99.6% for all SNPs. Deviation of the genotypes from Hardy-Weinberg equilibrium (HWE) as a potential marker for genotyping quality was assessed using the Pearson χ^2 test. The genotype distribution of rs429358 and rs7412 did not deviate from HWE.

MRI analysis

MRI scans of all patients with CIS and MS included a T1-weighted sequence, a fluid-attenuated inversion recovery sequence, and contrast-enhanced T1-weighted images and were analyzed by a neuroradiologist with regard to lesion number, size, and location and to contrast-enhancing lesions. The neuroradiologist was blinded to clinical data.

Statistical analysis

Statistical analyses were performed using SPSS 23.0 software (IBM Corp.). Continuous variables are described by their median and interquartile range, and categorical variables by numbers and percentages.

A variety of general sociodemographic factors known to influence cognitive status and previously discussed disease-specific risk factors for cognitive impairment in MS[20] were assessed. The list of the potential predictors and their baseline characteristics are summarized in table 1. Age and education were not included in the further analyses as cognitive test results had already been corrected for age and education by z-standardization.

To approximate the overall cognitive performance of each patient, we calculated an unweighted mean z-score:

Mean z-score = (z-score of PASAT 3 + z-score of MUSIC total test score)/2.

In addition, the results of the memory-centered MUSIC subtests 1, 2, and 5 were added up to form a memory-centered sum score:

Memory-centered sum score = (z-score of verbal learning list A + z-score of verbal learning list B + z-score of verbal recall)/3.

To extract those factors, which contributed most to cognitive performance in our cohort, variables were preselected by performing univariate linear regression analyses of each potential predictor with the cognitive outcome parameter under investigation. Variables with *p* values of regression coefficients <0.1 were subsequently selected for inclusion to a multiple linear regression model for the respective outcome. Dichotomous variables were dummy coded.

All our analyses are exploratory. Hence, *p* values are only given for descriptive reasons. However, we consider an association as statistically relevant in case of $p < 0.05$.

Data availability

The raw data used in preparation of the figures and tables will be shared in anonymized format on request of a qualified investigator to the corresponding author for purposes of replicating procedures and results.

Results

Characteristics of the 552 patients included in this study are reported in table 1. Of note, 25.2% of the patients were carriers of the *APOE* ε4 allele. Ten of these (1.8%) were homozygotes, which is in line with the reported prevalence in healthy control populations of Caucasians[21] and with the prevalence in a larger German cohort of patients with MS.[22]

Table 1 Patient characteristics

	Number (%)	Median (IQR)
Demographic characteristics		
Sex		
Female	395 (71.6)	
Male	157 (28.4)	
Age (y)		32 (27–42)
Education (school leaving level)		
Level 1	60 (10.9)	
Level 2	251 (45.5)	
Level 3	241 (43.7)	
Ethnic origin of grandparents		
Only German	522 (94.6)	
One other than German	30 (5.4)	
Clinical characteristics		
Diagnosis		
CIS	244 (44.2)	
RRMS	308 (55.8)	
Disease duration (mo)		4 (2–9)
EDSS score		1.5 (1.0–2.0)
Occurrence of relapse within 30 days		
Relapse	76 (13.8)	
No relapse	468 (84.8)	
No information	8 (1.4)	
BMI		24.1 (21.6–27.7)
BDI-II		5.0 (2.0–9.0)
Fatigue score (FSMC)		15.00 (11.00–25.75)
Current smoking		
Smokers	172 (31.2)	
Nonsmokers	380 (68.8)	
Alcohol consumption		
Occasional or regular drinking	426 (77.2)	
No drinking	126 (22.8)	
MRI characteristics		
Lesion number		9 (6–9)
Lesion localization		
Periventricular (yes/no)	528 (95.7)/24 (4.3)	
Juxtacortical (yes/no)	416 (75.4)/136 (24.6)	
Infratentorial (yes/no)	319 (57.8)/233 (42.2)	
Presence of CEL		
Yes	197 8 (35.7)	
No	340 (61.6)	
No information	15 (2.7)	
Black holes		
Yes	243 (44.0)	
No	172 (31.2)	
No information	137 (24.8)	
Visible atrophy		
Yes	46 (8.3)	
No	396 (71.7)	
No information	110 (19.9)	
Genetic characteristics		
***APOE* ε4 carriers**		
Homozygotes	ε4/ε4	10 (1.8)
Heterozygotes	ε4/ε3 or ε4/ε2	129 (23.4)
***APOE* ε4 noncarriers**	ε3/ε3 or ε2/ε3 or ε2/ε2	413 (74.8)

Abbreviations: BDI-II = Beck Depression Inventory II; BMI = body mass index; CEL = contrast-enhancing lesion; CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; FSMC = Fatigue Scale for Motor and Cognitive Function; IQR: interquartile range; RRMS = relapsing-remitting MS.
The table summarizes the assumed predictors of cognitive performance and their baseline characteristics in our cohort of 552 patients with CIS and early RRMS. Age and education were used for the z-standardization and were therefore not included in the regression models. Continuous variables are described by their median and interquartile range, and categorical variables by numbers and percentages.

Ethnicity of our cohort was homogeneous. Of note, 94.6% of the patients had grandparents of German origin only. The others (5.4%) had 1 grandparent with origin other than German. There were no patients with more than 1 grandparent with origin other than German enrolled in this study.

After preselection as described above, the parameters Expanded Disability Status Scale (EDSS) score, BMI, BDI-II, FSMC, alcohol consumption, smoking, MRI lesion number, and *APOE* ε4 carrier status were included in a multiple linear regression model for the prediction of the overall cognitive performance evaluated by the mean score of the z-standardized PASAT 3 and MUSIC test scores. It was found that *APOE* ε4 homozygosity, higher disability level measured by the EDSS, higher MRI lesion number, and higher BDI-II scores were associated with lower performance in cognitive testing, whereas patients who consumed alcohol scored higher compared with patients who did not consume alcohol (table 2). The R^2 of the overall model was 0.133 (adjusted R^2 = 0.118). *APOE* ε4 heterozygosity was not associated with the overall cognitive performance.

To evaluate whether the effect of *APOE* ε4 carrier status was more pronounced in memory-mediated cognitive domains resembling its effects in AD, a sum score of the memory-centered subparts of the MUSIC test was investigated in a second multiple linear regression model. However, we found no relevant association of *APOE* ε4 homo- or heterozygosity with the memory-centered sum score in the univariate regression analysis. Therefore, *APOE* ε4 carrier status was not included in the multiple regression model for the memory-centered sum score. We found that male sex and higher EDSS scores were associated with worse performance in these subtests. In line with the results of the mean score of overall cognitive performance, alcohol consumption was associated with better test results again (table 3). The R^2 of the overall model was 0.109 (adjusted R^2 = 0.094).

The findings concerning the effect of alcohol consumption are limited by the fact that they were no longer detectable after variation of dichotomization into nondrinkers and occassional drinkers vs regular drinkers.

We observed no association of the *APOE* ε2 carrier status with any of the cognitive outcome parameters in the univariate regression analyses. Therefore, *APOE* ε2 carrier status was not included in any of the multiple linear regression models.

Discussion

Cognitive impairment is one of the most difficult challenges for young adults faced with a diagnosis of MS because neuropsychological symptoms may already be experienced early on[23,24] and are among the main reasons for unemployment and reduced quality of life.[25] We here assessed the putative role of *APOE* polymorphisms on the cognitive outcome parameters PASAT 3 and MUSIC test scores in patients with CIS and early RRMS of a homogenous cohort in terms of origin, short disease duration, and treatment-naive state.

Neither *APOE* ε2 carrier status nor *APOE* ε4 heterozygosity showed an influence on the evaluated cognitive outcome parameters. However, we observed a relevant association of *APOE* ε4 homozygosity with a lower overall cognitive performance.

In AD, *APOE* ε4 carriers have a higher risk of developing AD and show decreased APOE plasma levels compared with *APOE* ε2 and *APOE* ε3 carriers. Among *APOE* ε4 carriers, lower plasma levels are associated with an even greater risk of developing AD. Therefore, it was suggested that a decrease of

Table 2 Regression coefficients for mean cognitive test scores

	CI						
	β	**SE**	**Standardized β**	**t Value**	**95% lower**	**95% upper**	***p* Value**
Intercept	0.920	0.341		2.698	0.250	1.590	0.007
Genetic characteristics							
***APOE* ε4 homozygosity**	**−0.922**	**0.394**	**−0.095**	**−2.340**	**−1.697**	**−0.148**	**0.020**
***APOE* ε4 heterozygosity**	0.128	0.124	0.043	1.035	−0.115	0.372	0.301
Clinical characteristics							
Disability (EDSS score)	**−0.180**	**0.057**	**−0.135**	**−3.143**	**−0.293**	**−0.068**	**0.002**
BMI	−0.010	0.010	−0.041	−0.987	−0.031	0.010	0.324
Depressive symptoms (BDI-II)	**−0.021**	**0.010**	**−0.116**	**−2.024**	**−0.040**	**−0.001**	**0.043**
Fatigue score (FSMC)	−0.005	0.004	−0.065	−1.121	−0.013	0.003	0.263
Alcohol consumption	**0.493**	**0.126**	**0.160**	**3.929**	**0.247**	**0.740**	**<0.001**
Smoking	−0.221	0.115	−0.079	−1.932	−0.446	0.004	0.054
MRI characteristics							
Lesion number	**−0.056**	**0.022**	**−0.103**	**−2.514**	**−0.099**	**−0.012**	**0.012**

Abbreviations: β = regression coefficient; BDI-II = Beck Depression Inventory II; BMI = body mass index; EDSS = Expanded Disability Status Scale; FSMC = Fatigue Scale for Motor and Cognitive Function; MUSIC = Multiple Sclerosis Inventory Cognition; SE = standard error.
As a proxy for overall cognitive performance, the mean of the z-standardized PASAT 3 and MUSIC total scores was calculated. The following parameters were selected for inclusion in the multiple linear regression model: EDSS score, BMI, BDI-II FSMC, alcohol consumption, smoking, MRI lesion number, and *APOE* ε4 carrier status (n = 545). *APOE* ε4 homozygosity, higher disability level measured by the EDSS, higher MRI lesion number, and higher BDI-II scores were associated with lower performance in cognitive testing, whereas patients who consumed alcohol scored higher compared with patients who did not consume alcohol. Parameters, which were found to be relevant predictors (defined by $p < 0.05$) of mean cognitive test scores, are written in bold.

Table 3 Regression coefficients of the memory-centered MUSIC test subparts

	CI						
	B	SE	Standardized β	t Value	95% lower	95% upper	*p* Value
Intercept	0.443	0.301		1.469	−0.149	1.035	0.142
Demographic characteristics							
Sex (female vs male)	**0.370**	**0.088**	**0.176**	**4.178**	**0.196**	**0.543**	**<0.001**
Clinical characteristics							
Disease duration (mo)	−0.006	0.005	−0.052	−1.249	−0.016	0.004	0.212
Disability (EDSS score)	**−0.123**	**0.043**	**−0.127**	**−2.882**	**−0.206**	**−0.039**	**0.004**
BMI	−0.014	0.008	−0.079	−1.867	−0.029	0.001	0.062
Depressive symptoms (BDI-II)	−0.008	0.007	−0.064	−1.108	−0.023	0.006	0.268
Fatigue score (FSMC)	−0.002	0.003	−0.046	−0.775	−0.008	0.004	0.439
Alcohol consumption	**0.236**	**0.093**	**0.106**	**2.536**	**0.053**	**0.418**	**0.012**
Smoking	−0.156	0.085	−0.077	−1.842	−0.322	0.010	0.066
MRI characteristics							
Lesion number	−0.025	0.016	−0.064	−1.536	−0.057	0.007	0.125

Abbreviations: BDI-II = Beck Depression Inventory II; BMI = body mass index; EDSS = Expanded Disability Status Scale; FSMC = Fatigue Scale for Motor and Cognitive Function; SE = standard error.
A sum score of the memory-centered subparts of the MUSIC test (Wordlist A and B and verbal recall) was calculated to evaluate the potential effect of *APOE* ε4 carrier status on memory function. However, *APOE* ε4 carrier status did not show any association with this sum score in the preselection process and was therefore not included in the regression model. The following parameters were selected for inclusion in the multiple linear regression model: sex, disease duration, EDSS score, BMI, BDI-II, FSMC, alcohol consumption, smoking, and MRI lesion number (n = 538). Female sex, lower disability level measured by the EDSS, and alcohol consumption were associated with better performance in the memory-centered subtests of MUSIC. Parameters, which were found to be relevant predictors (defined by $p < 0.05$) of mean cognitive test scores, are written in bold.

APOE protein function mediates the evolution of AD and that the risk increases dose dependently.[3] As APOE is thought to be involved in repairing neuronal injury, synapse formation, and scavenging of toxins,[26,27] we hypothesized that impaired repair mechanisms in MS lesions could mediate more pronounced neurodegenerative processes in *APOE* ε4 carriers compared with noncarriers.

Our current finding that cognitive performance was impaired in homozygous *APOE* ε4 carriers only might indicate that a dose-dependent decrease of APOE function mediates cognitive decline in MS, as it does in AD, and that a prolonged follow-up would reveal more pronounced effects of APOE later in the course of MS. Supporting this, previous studies in smaller cohorts of patients with MS with mean disease durations of 8.3[28] and 13 years[26] reported an association of *APOE* ε4 with dysfunction in some cognitive domains including verbal fluency and memory.

To evaluate whether the observed negative impact of *APOE* ε4 homozygosity on cognitive performance was mainly caused by impaired memory functions, resembling the assumed *APOE* ε4-mediated effects in AD,[29] additional analysis of a memory-centered sum score was performed. However, we found no relevant association of *APOE* ε4 homo- or heterozygosity with this memory-centered sum score. This might indicate an AD-independent *APOE* ε4-mediated effect on cognitive performance in patients with MS. This hypothesis is also supported by the young median age of our cohort as *APOE* ε4-dependent progression of formerly cognitive unimpaired people to mild cognitive impairment (MCI) and AD was found to be most pronounced in older people aged 70–75 years.[30] Furthermore, a recent study using PET imaging biomarkers of AD even suggested that some aspects of MS pathobiology retard the accumulation of β-amyloid, which is one of the main pathologic correlates of AD.[31] Nevertheless, we cannot exclude the possibility that patients with *APOE* ε4 homozygosity performed worse in the cognitive tests because of an *APOE* ε4-mediated increased risk of developing MCI or AD.

In an effort to correct for potential confounders, we included a range of parameters known or assumed to influence cognitive performance in patients with MS in our analyses. Apart from the putative impact of *APOE* ε4 homozygosity, we observed a relevant association of markers of the disease burden with the overall cognitive performance. Patients with a higher disability level as assessed by the EDSS and with a higher number of T2 lesions in MRI performed worse in cognitive testing, which is in line with previous reports.[17,23,32] Higher scores of depressive symptoms in BDI-II were also associated with an impaired performance. Depression is known to be associated with reduced attention and processing speed in patients with MS.[33] As PASAT 3 and MUSIC tests both include an assessment of these cognitive domains, our current finding seems plausible. Surprisingly, we observed a positive influence of alcohol consumption on the cognitive outcome, shedding light on recent reports associating alcohol consumption with lower neurologic disability in MS,[34] and a reduced risk of developing MS.[35] However, this finding has to be interpreted with care as it may be attributed to the dichotomization of drinking habits and as the questionnaires used in this study were not laid out for accurate quantification of alcohol consumption (e.g., units/month).

Two additional limitations of this study have to be addressed. First, the observed effect of *APOE* ε4 is based on a very low number of *APOE* ε4 homozygotes, which makes our findings sensitive to potential confounders, not accounted for. As the estimated prevalence of *APOE* ε4 homozygosity in Caucasian MS populations is only 1.8%, an even larger cohort than ours would be needed to improve the statistical power. Second, the tests used to assess cognitive performance in this study pose another potential limitation of our observations. PASAT 3 and MUSIC are both screening tests for cognitive performance, which offer the advantage that they may be incorporated into routine diagnostics comparatively easily. However, they lack the sensitivity and reliability to detect MS-specific cognitive impairment of extended test batteries, like for instance the Symbol Digit Modalities Test.[36]

Besides markers of disease burden, depression, and lifestyle habits, this study identified *APOE* ε4 homozygosity as a potential predictor of cognitive performance in this cohort of patients with CIS and early RRMS. This indicates a role of *APOE* as a genetic risk factor for cognitive impairment in MS and might even suggest an *APOE* ε4 effect unrelated to concomitant AD. Therefore, future work confirming the current findings in young homozygous *APOE* ε4 patients in a larger and independent MS cohort would be valuable.

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Publication history

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Sinah Engel	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Design and conceptualization of the study; acquisition and analysis of data; and drafting a significant proportion of the manuscript
Christiane Graetz, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Design and conceptualization of the study and acquisition of data
Anke Salmen, MD	Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, Germany	Design and conceptualization of the study and revision of the manuscript
Muthuraman Muthuraman, PhD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Analysis of data
Gerrit Toenges, MSc	Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany	Analysis of data
Björn Ambrosius, PhD	Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, Germany	Revision of the manuscript
Antonios Bayas, MD	Department of Neurology, Klinikum Augsburg, Germany	Revision of the manuscript
Achim Berthele, MD	Department of Neurology, Klinikum rechts der Isar, Technical University of Munich, Germany	Revision of the manuscript

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Appendix 1 *(continued)*

Name	Location	Contribution
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Bernhard Hemmer, MD	Department of Neurology, Klinikum rechts der Isar, Technical University of Munich, Germany	Design and conceptualization of the study and revision of the manuscript
Heinz Wiendl, MD	Clinic of Neurology, University Hospital Münster, Westphalian-Wilhelms-University Münster, Germany	Design and conceptualization of the study and revision of the manuscript
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Frauke Zipp, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Design and conceptualization of the study and revision of the manuscript
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Felix Luessi, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Design and conceptualization of the study; acquisition and analysis of data; and drafting a significant proportion of the manuscript

Appendix 2 Coinvestigators

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