Original Article

Apolipoprotein E ε4-Positive Multiple Sclerosis Patients Develop More Gray-Matter and Whole-Brain Atrophy: a 15-Year Disease History Model Based on a 4-Year Longitudinal Study

(multiple sclerosis / *APOE* / brain atrophy / gray matter / MRI / mixed-effect model)

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Abstract. Multiple sclerosis is a disease with considerable individual variation, and genetic background plays a key role in disease susceptibility and severity. The objective of the study was to evaluate the relationship between apolipoprotein E (*APOE*) genotype and the evolution of different clinical and MRI parameters. We investigated a group of 150 relapsing-remitting patients that completed 4-year follow-up. The mean age was 30.2 years, disease duration 56.8 months, and baseline Expanded Disability Status Scale (EDSS) 1.8. The changes in brain parenchymal volume (BPV), gray matter (GMV), white matter (WMV) and peripheral gray volume (PGMV) were measured by SIENA/X. T2-lesion volume was assessed by semi-automated methods. The mixed-effect model analysis was used to investigate evolution of clinical and MRI parameters in relation to the *APOE* ε4 genotype considering two different time models: 4-year follow-up and 15-year period from disease onset. We identified 36 *APOE* ε4-positive patients. Decline of GMV (P = 0.017), and BPV (P = 0.029) were significantly faster in *APOE* ε4-positive than in *APOE* ε4-negative patients in the 15-year model. In the 4-year model, a trend for faster decrease of GMV was found in *APOE* ε4-positive patients (P = 0.067). No differences in other MRI parameters or EDSS were found between the *APOE* groups. The results of the study suggest that *APOE* ε4-positive patients experience faster rate of gray matter atrophy.

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Abbreviations: ApoE – apolipoprotein E, *APOE* – apolipoprotein E gene, ASA – Avonex-Steroids-Azathioprine, BPV – brain parenchymal volume, EDSS – Expanded Disability Status Scale, GM – gray matter, GMV – gray matter volume, LV – lesion volume, MRI – magnetic resonance imaging, MS – multiple sclerosis, NBV – normalized brain volume, NGMV – normalized gray matter volume, NWMV – normalized white matter volume, PBVC – percentage brain volume change, PGMV – peripheral gray matter volume, ROI – region of interest, WMV – white matter volume.

Introduction

Multiple sclerosis (MS) is a chronic neurological disease with a highly variable clinical course. Genetic background is one of the factors that likely contribute to the heterogeneity of the disease. Among various genes, the apolipoprotein E (*APOE*) gene, which is located in the chromosome 19q13 region (Haines et al., 2002) and codes for apolipoprotein E (ApoE), has been one of the most studied in the past. ApoE is synthesized predomi-

nantly by astrocytes and delivers cholesterol and other essential lipids to neurons through members of the low-density lipoprotein receptor family. This process is significant in remodelling and repair of nerve tissue (Siest et al., 1995; Bu, 2009). ApoE also has immunomodulatory properties and plays an important role in modifying brain inflammatory responses (Barger and Harmon, 1997; Laskowitz et al., 1998; Lynch et al., 2003). In humans, the *APOE* gene exists in three common allelic forms, *APOE* ε2, ε3 and ε4, which engender six different genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4), and three corresponding protein isoforms, Apo E2, E3 and E4, are recognizable by the different location of the amino acids arginine and cysteine at specific positions. These amino acid differences among the three ApoE isoforms alter the protein's structure and influence its lipid association and receptor binding. *APOE* ε3 is the most (77 %) and ε2 the least (8 %) common allele. Frequency of the ε4 allele is approximately 15 % in general population (Bu, 2009). Frequency of the *APOE* ε4 allele appears to be higher in the northern regions of Europe and among the Caucasian population of North America and Canada than in the southern regions of Europe (Gerdes et al., 1992). It is suggested that isoform *APOE* ε4 may be associated with accelerated neurodegeneration and plays a role in the development and progression of several diseases (e.g., Alzheimer's disease (Bertram and Tanzi, 2008), atherosclerosis (Mahley, 1988), HIV progression (Burt et al., 2008), and Parkinson's disease (Martinez et al., 2005)).

The relationship between the *APOE* genotype and MS has been studied over the last two decades. The majority of studies have found no association between the *APOE* ε4 allele and the risk of developing MS, MS subgroups, age at onset, and gender (Weatherby et al., 2000; Burwick et al., 2006; Pinholt et al., 2006).

The relationship between the *APOE* ε4 allele and disease progression is still unclear. Five prospective studies investigated the evolution of clinical parameters (Expanded Disability Status Scale (EDSS) (Kurtzke, 1983), relapse rate, progression index, separation into benign and malignant subgroups, etc.) and found a positive association between *APOE* ε4 and the risk of disease progression (Hogh et al., 2000; Chapman et al., 2001; Fazekas et al., 2001; Schmidt et al., 2002; Pinholt et al., 2005). However, several other mostly retrospective studies found no association (Weatherby et al., 2000; Masterman et al., 2002; Savettieri et al., 2003). A positive association between *APOE* ε4 and cognitive impairment was detected (Savettieri et al., 2004; Koutsis et al., 2007; Shi et al., 2008).

In the last decade, it was established that measurement of whole-brain and, especially, gray-matter (GM) atrophy correlates best with long-term disability in patients with MS (Dalton et al., 2004; Fisher et al., 2008; Fisniku et al., 2008; Horakova et al., 2009). There were some magnetic resonance imaging (MRI) studies supporting the hypothesis that *APOE* ε4 is a predisposing factor to accelerated brain atrophy in patients with MS (Fazekas et al., 2000; Enzinger et al., 2003, 2004; De Stefano et al., 2004). These observations were not confirmed in other studies (Schreiber et al., 2002; Zakrzewska-Pniewska et al., 2004; Zwemmer et al., 2004). The possible explanation for these contradictory results is related to the cross-sectional character of the majority of those studies, use of non-standardized MRI metrics, and the fact that *APOE* ε4 has, at best, only a mild influence on the disease course; therefore, long-term data with more sophisticated techniques are needed to reveal any relationship.

Our study evaluates the relationship between *APOE* genotype and disease progression in a cohort of patients followed within a clinical trial protocol (Avonex-Steroids-Azathioprine (ASA) Study). The advantage of the study group is the availability of clinical and imaging data for 150 MS patients collected prospectively over four years and their use in the modelling of disease progression over 15 years. The study investigates the relationship between the presence or absence of *APOE* ε4 genotype and changes in different clinical (EDSS, relapse rate) and MRI metrics (especially total brain atrophy and GM atrophy).

Material and Methods

Patients

This study involved 150 of 181 patients from the original ASA study, who underwent *APOE* genotyping and had complete clinical and MRI data over four years. Baseline demographic and clinical characteristics of the *APOE* group did not significantly differ from the original ASA group (data not shown). The methods and results of the ASA study have been previously reported in detail (Havrdova et al., 2009).

The study inclusion criteria were: clinically definite MS (Poser et al., 1983) confirmed by MRI and presence of at least two oligoclonal bands in the cerebrospinal fluid, age 18–55 years, EDSS $\leq$ 3.5, active disease defined by two relapses in the last 12 months or three relapses in the last 24 months. EDSS was assessed every two months over the first 12 months of the study and then every three months until month 60. The study was approved by the local Ethical Committee.

APOE genotyping

APOE genotypes were determined by the restriction isotyping method according to Hixson and Vernier (1990). Genomic DNA was extracted from peripheral blood leukocytes using the standard method described by Miller (Miller et al., 1988). The extracted DNA was amplified using specific primers for ApoE: Fw 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3'; Rev 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'.

The amplification products were digested with the *Hha*I enzyme and separated by agarose gel electrophoresis. Products were visualized under UV light after the gel was stained with ethidium bromide. The *APOE* genotype frequencies in our study group were as follows:

ε2/ε2 = 0, ε2/ε3 = 18 (12 %), ε3/ε3 = 96 (64 %); ε2/ε4 = 5 (3 %), ε3/ε4 = 31 (21 %) and ε4/ε4 = 0.

Image acquisition

Brain MRI was performed in a Philips Gyroscan 1.5 Tesla unit (Best, The Netherlands). Axial images of the brain were acquired with 1.5 mm slice thickness using fast attenuated inversion recovery (FLAIR) (TR/TE 1100/14 ms, matrix size 256 × 181, flip angle 30) and axial T1 weighted 3-dimensional (3D) SPGR images (TR/TE 25/5 ms, matrix size 256 × 256) obtained with 1 mm slice thickness. All images were non-gapped. The scans were acquired on a bi-monthly basis in MS patients for the first two years and then yearly thereafter up to four years (on average 15 scans in each patient). In case of a relapse, the MRI scan was delayed for a minimum of 14 days after the last steroid treatment to avoid any transient effects of steroids on MRI results.

Image analysis

Image analysis was performed at the Buffalo Neuroimaging Analysis Center, The Jacobs Neurological Institute, Department of Neurology, State University at Buffalo, NY, USA. MRI evaluators were blinded to the patients' clinical characteristics and clinical status.

Semi-automated edge detection methods, with manual corrections for region of interest (ROI) definition, were used for the measurement of T2- lesion volume (T2-LV) (Horakova et al., 2009)

For brain extraction and tissue segmentation, we utilized the SIENA/X cross-sectional and longitudinal brain atrophy analyses methods, also described previously (Smith et al., 2002; Zivadinov et al., 2005; Horakova et al., 2008, 2009). We measured whole-brain and compartment-specific absolute volumes: brain parenchymal volume (BPV), gray matter volume (GMV), white matter volume (WMV) and peripheral gray volume (PGV). Normalized volumes of whole brain (NBV), GM (NGMV), and WM (NWMV) were also obtained via this process. To measure normalized peripheral grey volume (NPGV), a standard space mask was used to separate neocortical GM from non-neocortical volumes. The SIENA method was used to calculate the percentage brain volume change (PBVC) between all available time points.

Statistical Analysis

Summary statistics were used for data description. Data were tested for baseline differences between *APOE* ε4 +/- groups using *t*-test, Mann-Whitney test or χ^2 test, depending on the data characteristics.

Time models

In order to evaluate the disease course over time, time models were constructed for the following parameters: brain atrophy measures (BPV, GMV, WMV and PGV), T2-LV and EDSS. PBVC (besides its basic descriptive statistics) was also evaluated in the time models showing its behaviour from the same point as absolute brain volume measures. PBVC can be considered as a derivative of a function with respect to time.

Kinetics of the MRI parameters and EDSS were analysed using mixed-effect models. Two-level models were fitted. General formula of the models (for a cubic trend) is as below:

Level 1: $Y_{ij} = \beta_{0i} + \beta_{1i}T_{ij} + \beta_{2i}T_{ij}*T_{ij} + \beta_{3i}T_{ij}*T_{ij}*T_{ij} + e_{ij}$

Level 2: $\beta_{0i} = \beta_0 + \beta_4 apoE_i + b_{0i}$

$\beta_{1i} = \beta_1 + \beta_5 apoE_i + b_{1i}$

$\beta_{2i} = \beta_2 + b_{2i}$

$\beta_{3i} = \beta_3 + b_{3i}$

Where $\beta_0..\beta_5$ represent fixed effects, $b_0..b_3$ represent random effects and e_{ij} is a residual error (Verbeke and Molenberghs, 2000; Singer and Willett, 2003).

At level 1, the response variable Y was considered a function of time up to a cubic term, with random parameters for each patient. The linear, quadratic or cubic function was selected based on the model fit and parameters significance with respect to the assumed trend over time. At level 2 of the model, *APOE* ε4 variable was fitted as fixed effects (as a main effect and its interaction with time). *APOE* ε4 effects (up to a linear term) were left in the model, although non-significant because this was our focus of interest.

Normal distribution of random effects and linearity of all effects were assumed. A square root transformation was applied to T2-LV – sqrt(T2-LV) – to achieve an approximately normal distribution. Model diagnostics was based on evaluating normality and homoscedasticity of conditional residuals using graphical analysis (Nobre and da Motta Singer, 2007).

Two model types were constructed. The first one was a 4-year model, in which the time 0 for each patient was considered his/her enrolment into the trial. In this model, the kinetics of evaluated parameters represented their trend over a 4-year follow-up of the clinical trial. The second model was a hypothetical 15-year disease evolution model in which the time 0 for each patient was considered the beginning of the disease (considered as the first symptom occurrence). Mixed-effect models make this semi-longitudinal type of model possible utilizing different disease courses of the patients spread over the 15-year period overlapping one another.

All statistical analyses were performed with the SAS software version 9.1.3. (SAS, Cary, NC) The level of significance was considered 5 % ($P < 0.05$) in all cases.

Results

Baseline demographic, clinical and MRI data

Table 1 summarizes baseline demographic and clinical data of the entire group and *APOE* ε4-positive and ε4-negative subgroups. The mean age of the patients was 30.2 years, mean age at disease onset 25.5 years, mean EDSS was 1.8, and the mean disease duration was 56.8 months. There were 119 (79 %) women. Table 2 summarizes baseline MRI measures. Brain volume changes evaluated by PBVC are presented as sequential ba-

Table 1. Baseline demographic and clinical characteristics of the entire MS sample and APOE ε4 subgroups.

	Total RR MS (N = 150)	***APOE* ε4 pos (N = 36)**	***APOE* ε4 neg (N = 114)**	**P*value**
Sex M (%)	31 (21 %)	10 (28 %)	21 (18 %)	0.243
Age [y], mean ± SD	30.2 ± 7.7	30.5 ± 7.2	30.1 ± 7.9	0.664
Disease duration [m], mean ± SD	56.8 ± 41.3	60.1 ± 43.9	55.7 ± 40.6	0.619
Age at first symptom [y], mean ± SD	25.5 ± 7.2	25.6 ± 6.5	25.4 ± 7.5	0.690
EDSS, mean ± SD	1.8 ± 0.9	1.7 ± 0.9	1.9 ± 0.9	0.478
Baseline annual relapse rate, mean ± SD	1.8 (0.7)	1.8 (0.6)	1.8 (0.7)	0.825

RR MS = relapsing-remitting multiple sclerosis; M = male; SD = standard deviation; m = months; y = years; EDSS = Expanded Disability Status Scale

Table 2. Baseline MRI characteristics of the entire MS sample and APOE ε4 subgroups.

	Total RR MS (N = 150)	***APOE* ε4 pos (N = 36)**	***APOE* ε4 neg (N = 114)**	**P*value**
Normalized BPV (mean ± SD), mm^3	1502139 ± 74092	1501548 ± 73111	1503959 ± 78074	0.866
Normalized GMV (mean ± SD), mm^3	796754 ± 55247	797051 ± 53466	795837 ± 61211	0.909
Normalized WMV (mean ± SD), mm^3	704958 ± 41152	704078 ± 40297	707670 ± 44166	0.651
Normalized PGV (mean ± SD), mm^3	591982 ± 44694	593194 ± 43041	588245 ± 49913	0.566
T2-LV, (mean ± SD), mm^3	9739 ± 11566	8636 ± 9780	13199 ± 15599	0.104

RR MS = relapsing-remitting multiple sclerosis; BPV = brain parenchymal volume, GMV = gray matter volume, WMV = white matter volume, PGV = peripheral gray volume, T2-LV = T2 lesion volume

Table 3. Comparison of the evolution of PBVC in the APOE ε4⁺ and ε4⁻ subgroups.

	***APOE* ε4 neg (N = 114)**	***APOE* ε4 pos (N = 36)**	
PBVC	Mean (SD) %	Mean (SD) %	*t*-test
M 0-6	-0.30 (1.07)	-0.50 (0.79)	NS
M 0-12	-0.80 (1.17)	-0.88 (1.08)	NS
M 0-24	-1.45 (1.71)	-1.53 (1.21)	NS
M 0-36	-2.31 (2.01)	-2.50 (1.89)	NS
M 0-48	-3.22 (2.52)	-3.36 (2.87)	NS

PBVC = percentage brain volume change, M = month, NS = not significant, SD = standard deviation

sic assessment in Table 3. No significant differences between the two *APOE* ε4 groups were found.

Time course of MRI parameters and EDSS

Linear, quadratic or cubic trend of MRI course could be identified for both 4- and 15-year models (see Figs. 1–6). Generally, brain volume measures steadily decreased. The decrease was most evident in gray matter or total brain volume measures. Although various degrees of polynomial time course improved the model fit, quadratic and especially cubic term only slightly modulated the course of development of the MRI measures. Roughly, the rate of decrease was higher in the first year and in the first five years in the 4- and 15-year model, respectively, levelling out with a steady decrease subsequently. More evident decrease could be found over the long-term 15-year models. PBVC decreased in a steady linear trend. On the other hand, sqrt(T2-LV) increased, which was more evident in the 15-year model. Similarly, a steady increase of EDSS was observed and was more evident in the 15-year model (Fig. 7).

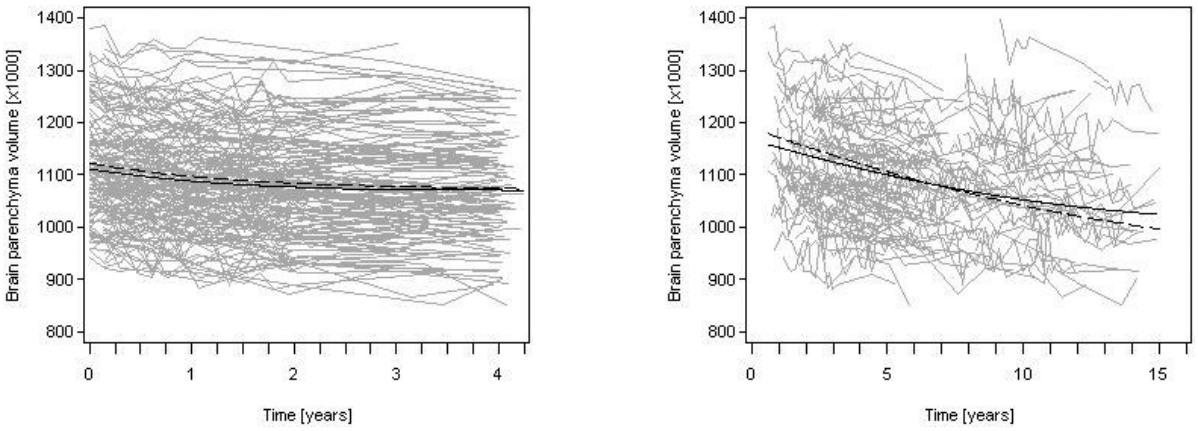

Fig. 1. Brain parenchymal volume (BPV) evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

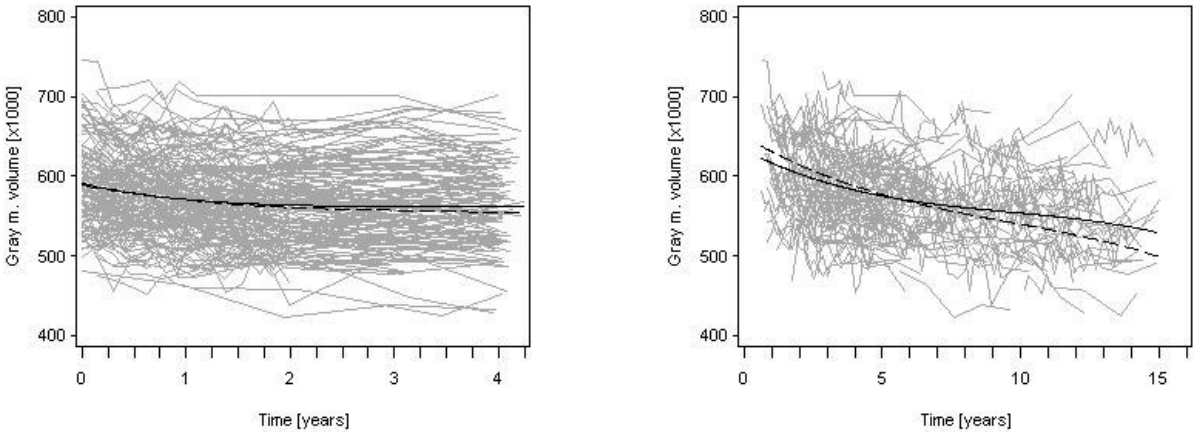

Fig. 2. Gray matter volume (GMV) evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

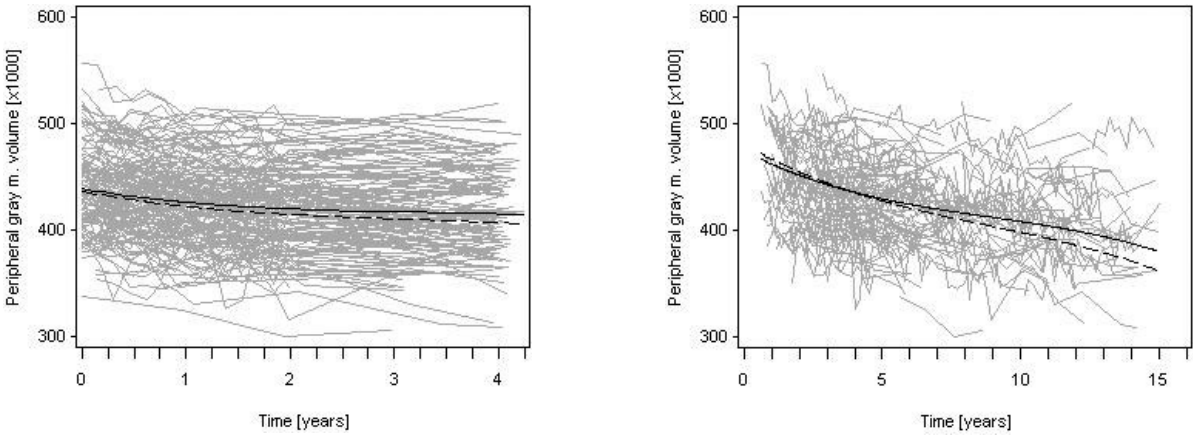

Fig. 3. Peripheral gray matter volume (PGMV) evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

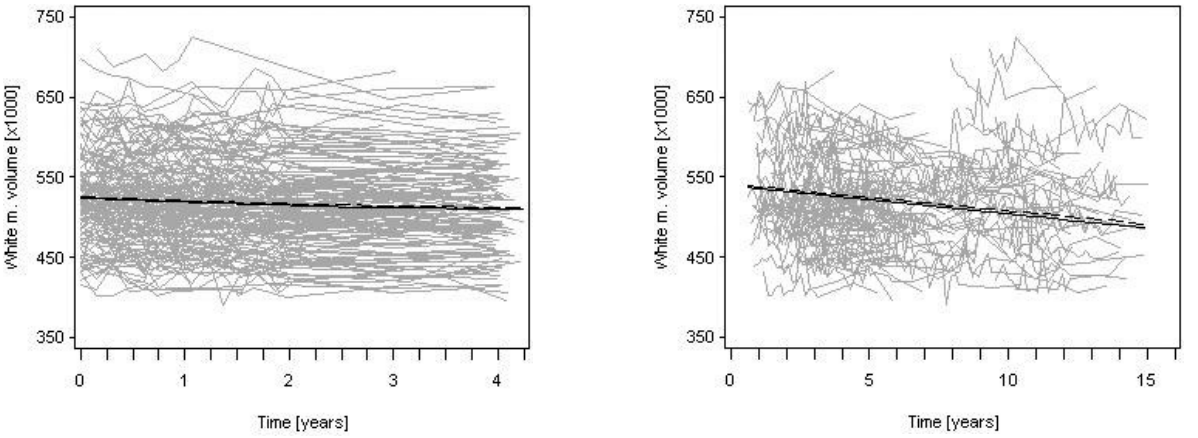

Fig. 4. White matter volume (WMV) evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

Comparison of the evolution of MRI measures and EDSS between the APOE ε4-positive and ε4-negative subgroups

No significant differences of the brain atrophy normalized measures (nBPV, nGMV, nWMV and nPGV) were identified in either 4- or 15-year models (Tables 4 and 5). Only a borderline difference of the rate of decrease was found for GMV non-normalized measure in the 4-year model (P = 0.067). However, GMV (P = 0.017) and BPV (P = 0.029) non-normalized measures decreased significantly faster in patients with APOE ε4-positive pheno-

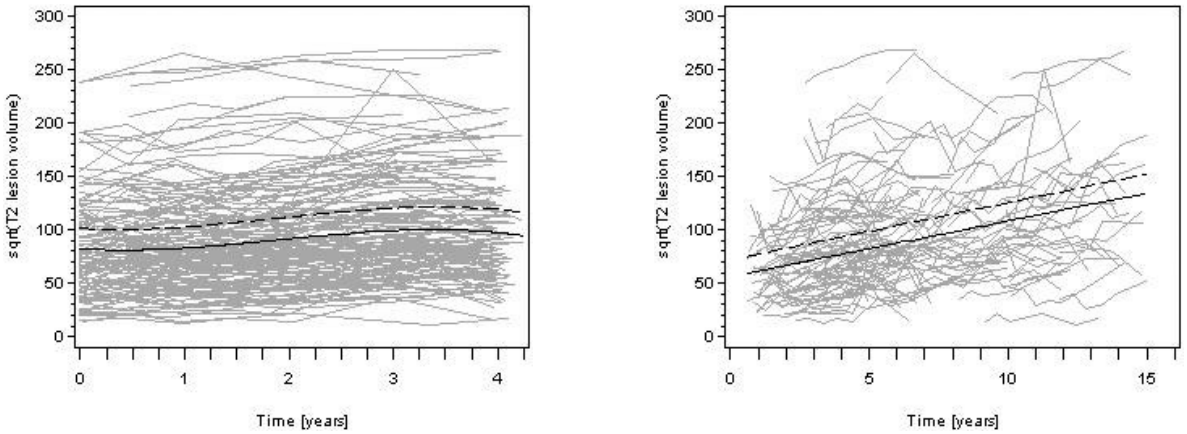

Fig. 5. T2 Lesion volume (T2-LV) evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

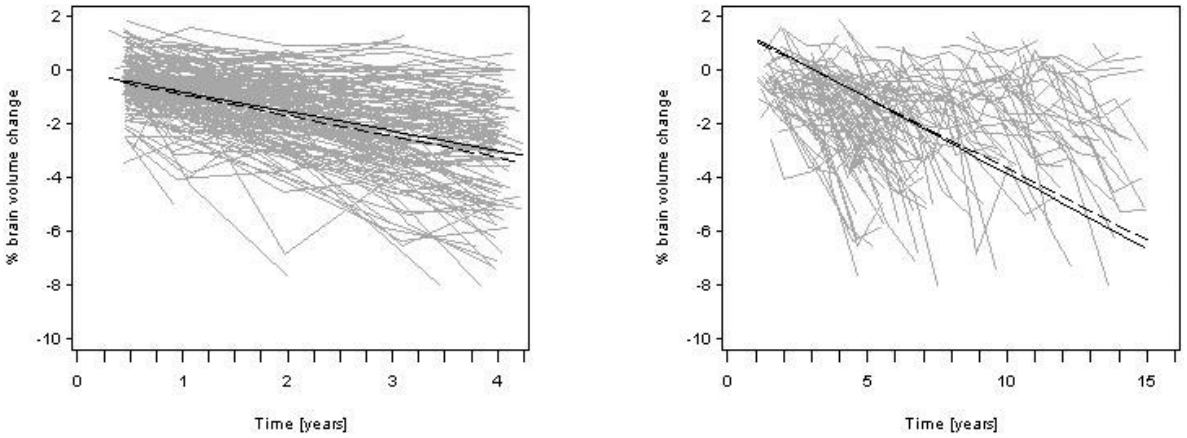

Fig. 6. Percentage brain volume change (PBVC) evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

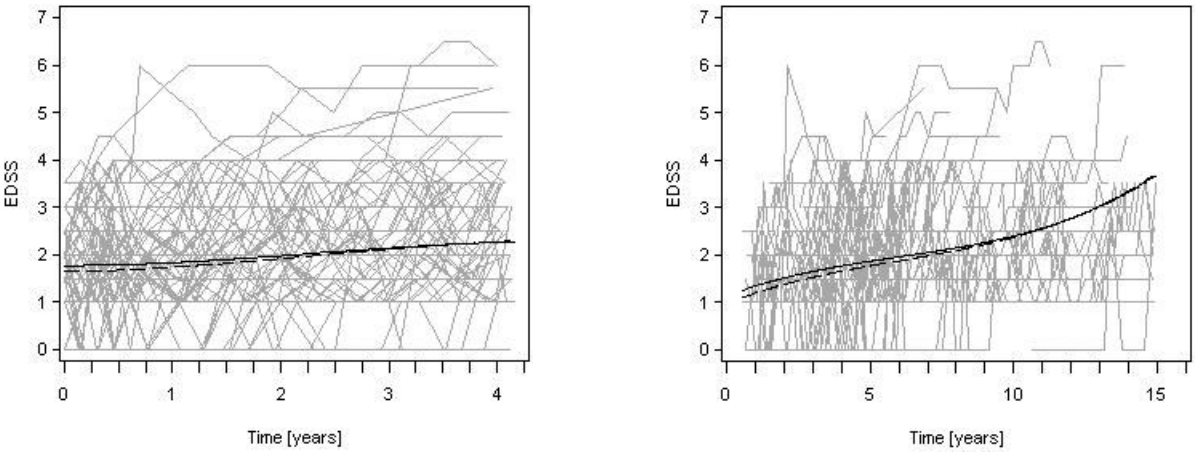

Fig. 7. EDSS score evolution in the 4-year (left) and 15-year (right) models (dashed line – *APOE* ε4 positive, solid line – *APOE* ε4 negative). Gray lines – case profiles. Black lines – mean profiles.

type in the 15-year model. This difference is indicated by apoE*time interaction term. Significantly higher sqrt (T2-LV) values (P = 0.036) were found in *APOE* ε4-positive patients in the 4-year model at baseline with no subsequent time course difference. No other significant differences of either rate of change or baseline values were found for other MRI measures. No significant differences were found for EDSS development either.

Model fit diagnostics

No major deviations from normality or homocedasticity were found for absolute MRI measures. Six outly-

Table 4. 4-year models (parameter estimates)

Fixed effects		Absolute values GMV	WMV	BPV	PGMV	nGMV	nWMV	nBPV	nPGMV	sqrt(T2-LV)	Change PBVC	Clinical EDSS
Baseline	Intercept (β_0)	589.2e3***	524.2e3***	1111.4e3***	438.7e3***	794.7e3***	704.3e3***	1500.0e3***	591.7e3***	82.39***	-74.3e-3^{NS}	1.77***
Main effect	apoE (β_4):	1.8e3^{NS}	1.6e3^{NS}	11.1e3^{NS}	-2.5e3^{NS}	1.2e3^{NS}	3.4e3^{NS}	4.2e3^{NS}	-4.5e3^{NS}	19.06*	-51.1e-3^{NS}	-0.13^{NS}
Rate of change	T (β_1):	-25.6e3***	-5.5e3***	-30.4e3***	-16.8e3***	-20.9e3***	7.7e3**	-18.5e3***	-12.4e3***	-6.13**	-730.0e-3***	<-0.01^{NS}
	T*T (β_2):	8.2e3***	0.5e3*	8.1e3**	4.6e3**	7.1e3**	-2.3e3***	6.7e3*	3.3e3*	8.27***	-	0.07*
	T*T*T(β_3):	-0.9e3*	-	-0.7e3^{++}	-0.5e3*	-1.1e3**	-	-1.2e3*	-0.6e3*	-1.45***	-	-0.01*
	apoE*T (β_5):	**-2.4e3^{+}**	**0.2e3^{NS}**	**-1.9e3^{NS}**	**-1.4e3^{NS}**	**-1.5e3^{NS}**	**1.6e3^{NS}**	**-3.0e3^{NS}**	**-0.4e3^{NS}**	**0.69^{NS}**	**-47.7e-3^{NS}**	**0.04^{NS}**
AIC (full model)		43339.0	43016.3	44227.6	42044.2	44082.9	44505.0	42789.5	43086.4	7809.8	1928.5	3724.6
AIC (unconditional linear model)		43580.0	43014.7	44435.7	42191.9	44126.1	44565.1	42799.9	43097.9	7937.7	1924.9	3978.2

*< 0.05, **< 0.01, ***< 0.001, NS not significant, $^{+}$P = 0.067, $^{++}$P = 0.066; AIC – Akaike's information criterion, effects of interest in **bold** (dynamics difference)

Table 5. 15-year models (parameter estimates)

Fixed effects		Absolute values GMV	WMV	BPV	PGMV	nGMV	nWMV	nBPV	nPGMV	sqrt(T2-LV)	Change PBVC	Clinical EDSS
Baseline	Intercept (β_0)	633.0e3***	539.2e3***	1167.5e3***	474.5e3***	838.0e3***	723.7e3***	1534.2e3***	625.7e3***	56.43***	1738.4e-3***	1.14***
Main effect	apoE (β_4):	17.5e3^{NS}	1.9e3^{NS}	23.0e3^{NS}	6.3e3^{NS}	9.3e3^{NS}	-7.5e3^{NS}	22.1e3^{NS}	-0.7e3^{NS}	15.54^{NS}	-105.9e-3^{NS}	-0.16^{NS}
Rate of change	T (β_1):	-17.8e3***	-3.5e3***	-15.4e3***	-13.4e3***	-15.2e3***	-3.1e3**	-8.3e3***	-10.6e3***	5.19***	-557.3e-3***	0.23**
	T*T (β_2):	1.5e3***	-	0.39e3***	1.1e3***	1.2e3**	-	-	0.7e3*	-	-	-0.02*
	T*T*T(β_3):	<-0.1e3**	-	-	<-0.1***	<-0.1e3**	-	-	<-0.1e3*	-	-	<0.01*
	apoE*T (β_5):	**-3.2e3***	**0.2e3^{NS}**	**-3.4e3***	**-1.6e3^{NS}**	**-1.7e3^{NS}**	**1.9e3^{NS}**	**-3.1e3^{NS}**	**-0.5e3^{NS}**	**0.18^{NS}**	**29.2e-3^{NS}**	**0.01^{NS}**
AIC (full model)		43555.7	43034.8	44429.6	42174.6	44129.7	44610.2	42807.9	43108.7	7935.3	2233.0	3966.9
AIC (unconditional linear model)		43578.3	43030.9	44442.8	42189.8	44130.5	44608.6	42806.0	43106.7	7934.6	2229.1	4030.9

*< 0.05, **< 0.01, ***< 0.001, NS not significant, effects of interest in **bold** (dynamics difference)

ing observations in PBVC measurements (change of more than -10 % from baseline) considerably worsened model diagnostic characteristics and were therefore excluded. A slight deviation from normality of conditional residuals was observed for EDSS, which is not surprising since EDSS is a score scale with a semi-continuous character of data.

Discussion

The influence of *APOE* genotype on the course of MS is still contradictory. The results from this study support the possibility that the *APOE* ε4 allele correlates with faster development of brain atrophy and, in particular, faster decline in GMV. Mixed-effect modelling over a mid- to long-term time period could reveal such a relationship, which is evident in the 15-year models.

MS is a disease with a very variable and heterogeneous course, and an individual patient-based approach in prognostication and therapy adjustment is preferred. To properly set the risk of progression for an individual patient and to judge the activity of the disease, comprehensive follow-up is needed. Among different variables that are currently available for monitoring the disease, clinical parameters (EDSS, relapse rate) and MRI variables (lesion volume, different atrophy markers) are used in both clinical and research settings to determine the prognosis. Knowledge of crucial genetic factors that can influence susceptibility and the course of the disease would be of extreme help.

Among different genes, *APOE* has been one of the most studied in the past. To date, many studies have addressed the question regarding an association between the *APOE* genotype and disease risk and disease severity, with contradictory results.

In our study, we concentrated mainly on the relationship between the *APOE* genotype and the evolution of different MRI parameters. The reason for that was the enormous number of high-quality MRI scans (on average 15 for each patient within the 4-year interval).

To fully utilize all the available data we used mixed-effect models, exploring the kinetics over time. One important benefit of this model is that we were able to utilize the variability of the disease course of each patient and construct the hypothetical 15-year long term model only with the data available from a 4-year follow-up. This model could be constructed thanks to the fact that disease duration was different in each patient and meaningfully distributed over 15 years. Actually, the variability (heterogeneity) of the time phases of the disease in the sample overlapping one another was then taken as an advantage that allowed restoring of a long-term disease history with only a short-time follow-up. As the results indicate (Figs. 1–7), changes in MRI and EDSS parameters over time were gradual and thus more evident in the long-term models. Different (linear, quadratic, cubic) time models adequately represented the course of MRI and EDSS measures. We also evaluated PBVC in time models similarly to discover the association between absolute brain measures and change measure based on automated evaluation (SIENA). The linear time course of PBVC, which is a derivative of a function with respect to time, suggests and may explain quadratic forms of some absolute brain measure courses. The fact that this finding is not as a rule may be explained by a different strategy in evaluating the disease course in

time; SIENA method and mixed-effect models. This consideration suggests that both are complementary methods only indirectly associated with each other, evaluating time course from different points of view.

The significant effect of *APOE* on the time course could be revealed only in the 15-year model and was associated with worse progression of the disease (according to MRI measures). Thus, negative results reported in other studies (Schreiber et al., 2002; Zakrzewska-Pniewska et al., 2004; Zwemmer et al., 2004) may be due to an insufficiently long period of evaluation. However, this phenomenon was identified only in non-normalized MRI measures, which suggests an interesting consideration. The normalization process is based, among others, on age adjustment. If we admit that the disease tends to last longer in older patients, which means a significant correlation, then, adjusting (normalizing) for age actually means adjusting for disease duration. Thus, it may indirectly influence (decrease) the variability introduced by the disease duration. Since we, in the 15-year models, utilize the disease duration variability, normalization of the MRI parameter could conceal the effects that are associated with the disease time course. These thoughts may explain our finding of significant effects (apoE) differences only in non-normalized MRI measures and suggest a cautious consideration of normalization in long-term disease history-based studies. Possibly biasing covariates could be included in the model to be controlled for. However, in mixed-effect models, inter-individual variability (patient differences) is solved through random effects. On the other hand, differences in demographic parameters between the analysed groups could bias the results and produce those significant effects found. We believe that this is not the case because baseline characteristics did not significantly differ. To be sure of our conclusions, we included available possibly confounding covariates (gender, age, age at 1st symptom occurrence) into the model. After controlling for these factors, the effects of apoE on atrophy progression (GMV, BPV) were still significant.

An interesting result of this study is the fastest atrophy decline in the GM compartment, which is in agreement with other studies showing that total brain atrophy is mostly driven by GM decline, and measurement of GM atrophy could be the most sensitive marker of long-term disability (Valsasina et al., 2005; Fisher et al., 2008; Fisniku et al., 2008; Horakova et al., 2009). The effect was not reflected in clinical parameter EDSS. This finding might be due to a less informative (sensitive) character of scale data as well as the mild effect of apoE distinguishable only in sensitive MRI measures (GMV, BPV). Whether the combination of *APOE* ε4 positivity with faster GM atrophy could be associated with more pronounced cognitive impairment (Koutsis et al., 2007; Shi et al., 2008) rather than with motor or sensory symptoms of MS is unclear and this hypothesis requires further investigation. Unfortunately, we did not conduct systematic neuropsychological assessment in this study.

The results of this study support the hypothesis that the carrying ability of the *APOE* ε4 allele leads to faster brain atrophy development, especially of the GM compartment. Despite this clear long-term trend, the effect is at best mild as well as difficult to prove in a short period of time. The clinical meaning of this isolated correlation is currently unclear, but in the future *APOE* ε4 carrying ability could be among parameters used in an aggregate risk score helping to stratify patients according to the severity of the disease.

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