Monophasic demyelination reduces brain growth in children

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Editorial, page 1691

Supplemental data
at Neurology.org

ABSTRACT

Objective: To investigate how monophasic acquired demyelinating syndromes (ADS) affect age-expected brain growth over time.

Methods: We analyzed 83 pediatric patients imaged serially from initial demyelinating attack: 18 with acute disseminated encephalomyelitis (ADEM) and 65 with other monophasic ADS presentations (monoADS). We further subdivided the monoADS group by the presence ($n = 33$; monoADSlesion) or absence ($n = 32$; monoADSnolesion) of T2 lesions involving the brain at onset. We used normative data to compare brain volumes and calculate age- and sex-specific z scores, and used mixed-effect models to investigate their relationship with time from demyelinating illness.

Results: Children with monophasic demyelination (ADEM, non-ADEM with brain lesions, and those without brain involvement) demonstrated reduced age-expected brain growth on serial images, driven by reduced age-expected white matter growth. Cortical gray matter volumes were not reduced at onset but demonstrated reduced age-expected growth afterwards in all groups. Brain volumes differed from age- and sex-expected values to the greatest extent in children with ADEM. All patient groups failed to recover age-expected brain growth trajectories.

Conclusions: Brain volume, and more importantly age-expected brain growth, is negatively affected by acquired demyelination, even in the absence of chronicity, implicating factors other than active inflammation as operative in this process. *Neurology*® 2017;88:1744-1750

GLOSSARY

ADEM = acute disseminated encephalomyelitis; **ADS** = acquired demyelinating syndrome; **GM** = gray matter; **monoADS** = monophasic acquired demyelinating syndrome; **MS** = multiple sclerosis; **NC** = normally developing control; **NIHPD** = NIH pediatric database; **NMO** = neuromyelitis optica; **WM** = white matter.

Acquired demyelinating syndromes (ADS) of the CNS during childhood or adolescence are associated with inflammatory lesions, visualized as bright foci in T2-weighted images. While relapsing-remitting multiple sclerosis (MS) in children is characterized by reduced whole brain volume, reduced gray matter (GM) and thalamic volumes,^{1,2} and ongoing loss of brain volume (atrophy) during adolescence,³ the general assumption has been that the negative effect of pediatric-onset MS on age-expected brain growth is mediated by chronic and active CNS inflammation, demyelination, and possible neurodegeneration. It is not known whether monophasic demyelination leads to impaired brain volume over time. Of interest, a recent cross-sectional study⁴ of 24 pediatric patients with monophasic transverse myelitis demonstrated trends towards reduced fine motor dexterity (as might be expected from spinal cord impairment), despite the absence of demyelination in the brain, suggesting that visible brain inflammation may not be required for there to be a more global CNS effect in patients with acquired demyelination.

To better understand the effect of monophasic demyelination, we evaluated serial brain images obtained from children with a single episode of demyelination (monophasic ADS

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[monoADS]) and compared these to a normative dataset. We specifically tested the following hypotheses: (1) monoADS occurs as an acute process, without chronic subclinical inflammation, and thus baseline brain volume will be normal (or even slightly increased owing to acute lesional edema); (2) monoADS has the potential to injure the CNS, leading to subsequent reduced cerebral tissue volume as a consequence of the acute event. This transient insult will not impede subsequent capacity for age-expected brain growth, and may even be associated with subsequent catch-up growth if children possess enhanced reparative capacity; (3) monoADS localized solely to the optic nerves or spinal cord will have no effect on brain volume and will be associated with normal age-expected brain volume at onset and normal brain growth afterwards.

METHODS Participants. The study cohort consisted of 83 children presenting with incident CNS demyelination (M/F = 46/37, age range at first scan 3.8–16.5 years). Each child was scanned clinically at onset, and then once demyelination was diagnosed, was offered standardized research MRI protocol scans on average 2 weeks after the initial episode of demyelination (baseline) and then prospectively at 3, 6, and 12 months and annually thereafter (up to 8 years), as part of the Canadian Paediatric Demyelinating Disease Study, as detailed previously.⁵

As described previously,⁶ patients were evaluated prospectively. MS and neuromyelitis optica (NMO) spectrum disorder were diagnosed using established criteria,^{7,8} and these patients were excluded from the present analysis. Serum NMO immunoglobulin G testing (performed on 63 of the 83 included participants) was negative. Participants who experienced no further clinical events and whose serial MRI scans failed to demonstrate accrual of new lesions were defined as monoADS. MonoADS patients were further subdivided based on their initial phenotype as acute disseminated encephalomyelitis (ADEM group; defined by international consortium guidelines⁹), non-ADEM monoADS with T2 bright brain lesions on the clinical scan acquired at time of ADS (monoADSlesion group), and non-ADEM monoADS involving the optic nerves or spinal cord alone (monoADSnolesion group), where “nolesion” refers to the absence of T2 bright lesions in the brain.

To determine age-normative brain volumes and age-expected brain growth, a total of 874 scans from 339 children from the publicly available NIH-funded 1.5T MRI Study of Normal Brain Development¹⁰ were analyzed (the NIH pediatric database [NIHPD] group; M/F = 160/179; range 4.9–19.8 years).

To permit evaluation of any scanner effects that might confound our analysis and to ensure comparability with the NIH normative data, 24 healthy, normally developing controls (the NC group) were recruited (M/F = 5/19; range 11.2–22.0 years) and were scanned on the same scanner as the patient group on 2 occasions at intervals of 12–24 months. All NC participants had a negative history for neurologic, medical, or psychiatric illness, learning disability, major head injury, or alcohol or illicit drug abuse.

Statistical analysis. We performed descriptive statistics on the demographic and clinical characteristics. We used a χ^2 test to compare the M/F ratios among the 5 groups and among the 3 patient groups. Other characteristics were compared between groups by using a Kruskal-Wallis rank sum test and adjusted pairwise comparisons.¹¹

MRI protocol. MRI of the patients and NC were obtained using a single 1.5T scanner using a standardized research MRI protocol detailed in appendix e-1 at Neurology.org.

Serial image processing. All data from patient, NC, and NIHPD groups were submitted to the same processing steps detailed in appendix e-1: preprocessing, spatial normalization, volume calculation (brain, cortical GM, deep GM, supratentorial white matter [WM], as well as internal structures including thalamus), and SIENAX¹² scaling factor computation.

Longitudinal growth analysis. Normal growth models. According to previous published studies,^{2,13} mixed effect models were used to evaluate normal growth curves for the NIHPD group. From these models, it is possible to define the expected mean volume and SD at a specific age and for a specific sex and therefore to compute z score for each time point of each patient.

Using the computed z scores, the following mixed-effect model describing a linear relationship between the volume z scores and the disease duration was built:

$$Z_{ij} = \beta_{0k} + \gamma_{0i} + (\beta_{1k} + \gamma_{1i})\text{DiseaseDuration}_{ij} + E_{ij}$$

where Z_{ij} is the value of the volume z score for time point j of participant i , k is the group of the patient, $\text{DiseaseDuration}_{ij}$ is the time since first demyelinating event for time point j of participant i , β_{0k} is the model intercept for the group k , β_{1k} is the model slope for the group k , and γ_{0i} and γ_{1i} are the coefficients specific for participant i .

More details about the z score computation and the mixed-effect models are given in appendix e-2.

Brain volumes and growth. Given concerns regarding possible acute edema at baseline and given that not all children were able to undergo a research study during their acute illness, we evaluated baseline MRI data in several ways: (1) for children with research scans at baseline, we compared baseline brain volumes to the NIHPD data and established age- and sex-specific z score values; (2) we utilized the serial MRI data to backwards project an estimated baseline brain volume for all participants (discussed further below); and (3) we compared the true baseline volume to the estimated baseline volume as determined in (4) for those children with research baseline scans.

We then evaluated brain growth trajectories to determine which of 4 possible hypothetical patterns emerged: (1) pattern 1: patient and control curves diverge over time (i.e., progressively negative effect over time); (2) pattern 2: trajectories run parallel following ADS (i.e., initial effect leading to reduced volumes, followed by subsequent age-expected growth curves) (i.e., volumes remain reduced, but rate of growth is not affected); (3) pattern 3: trajectories of patients increasingly approach normal growth curves over time (i.e., recovery from initial effect with compensatory catch up growth); or (4) pattern 4: trajectories are normal for age (no effect on brain volume or growth).

We built mixed-effect models describing a linear relationship between the volume z scores and the time from ADS presentation. The models are described in detail in appendix e-2.

RESULTS Demographics. Table 1 lists demographic characteristics and table 2 lists clinical and MRI

Table 1 Demographic features and summary of acquired data

	MonoADSlesion	MonoADSnolesion	ADEM	NC	NIHPD controls	Statistical analysis
Participants (F/M)	33 (14/19)	32 (16/16)	18 (7/11)	24 (19/5)	339 (178/161)	$p = 0.048$ (5 groups) ^a ; $p = 0.71$ (3 patient groups) ^a
Age at first available research scan, y						
Mean $\pm$ SD	11.4 $\pm$ 3.3	11.4 $\pm$ 3.3	7.8 $\pm$ 3.5	16.4 $\pm$ 2.3	11.0 $\pm$ 3.7	ADEM < all groups ^b ; NC > all groups ^b
Range	5.7-16.4	5.7-16.5	3.8-14.5	11.2-22.0	4.9-19.8	
Age at onset, y						
Mean $\pm$ SD	10.9 $\pm$ 3.1	11.1 $\pm$ 3.4	6.8 $\pm$ 3.8	—	—	ADEM < all patient groups ^b
Range	5.4-15.9	4.1-16.0	2.3-13.6			
Follow-up time, y	3.6 $\pm$ 2.0	4.2 $\pm$ 2.1	3.3 $\pm$ 2.1	1.33 $\pm$ 0.3	3.2 $\pm$ 1.0	NC < all groups ^b ; NIHPD < monoADSnolesion ^b
No. of MRI scans per participant, mean $\pm$ SD	5.4 $\pm$ 2.1	5.9 $\pm$ 2.1	4.7 $\pm$ 2.3	2 $\pm$ 0	2.6 $\pm$ 0.5	NC < all patient groups ^b ; NIHPD < all patient groups ^b

Abbreviations: ADEM = acute disseminated encephalomyelitis; ADS = acquired demyelinating syndrome; monoADSlesion = non-ADEM monophasic ADS with T2 brain lesions at onset; monoADSnolesion = non-ADEM monophasic ADS without T2 brain lesions at onset; NC = normal controls acquired on the same scanner as the patients; NIHPD = normal controls from the NIH pediatric database.

Only the group differences with corrected p value <1% are given (detailed results in table A2, appendix e-3).

^aTest of equal proportions for male/female ratio (detailed results in table A3-1, appendix e-3).

^bKruskal-Wallis rank sum test with post hoc multiple comparison test.

features of the 83 patients (18 children with ADEM and 65 patients with monophasic ADS not meeting criteria for ADEM, 33 of whom had brain lesions at baseline [monoADSlesion group] and 32 of whom did not [monoADSnolesion]), 24 NC, and 339 controls from the NIHPD database.

As shown in table 2, lesion volumes, as measured from the acute clinical scans, were highest in the children with ADEM as compared to monofocal lesion volumes.

Longitudinal measurements. When comparing the z scores computed from brain volume measured from parenchymal segmentation of the baseline research MRI (available for 51 of the 83 participants) vs the

z scores computed from the brain volume estimated from the mixed effects model, the z scores with measured and estimated volumes are similar (figure e-1, Pearson correlation coefficient = 0.99, $p < 0.001$), even in the case where patients were treated with steroid before the scan (figure e-2). The z scores computed from measured brain volumes at baseline (51 participants) are significantly smaller than 0 (Wilcoxon test, $p < 0.001$).

Table 3 summarizes the mixed effect model coefficients for the model describing a linear relationship between the z scores and time since ADS event. Figure 1 represents the estimated mean z score trajectories. The brain z score intercepts were slightly negative and significantly different from zero for the

Table 2 Clinical and MRI features

Patient group	Age at onset, y, median (IQR)	Female (%)	Treated with corticosteroids at any time prior to most recent MRI evaluated for current analysis, n (%)	Discrete courses of steroids prior to most recent MRI evaluated for current analysis, median (IQR)	EDSS at last follow-up, median (IQR)	T2 lesion volume at onset, mL, mean $\pm$ SD	Other key features
Non-ADEM monophasic with T2 lesions at onset (n = 33) ^a	11.61 (8.02-12.90)	14 (42)	29 (88)	1 (1-1)	1 (0-1.5)	7.1 $\pm$ 17.4	12 (36.4%) with ON; 12 (36.4%) with TM; 9 (27.2%) with polyfocal clinical features; 25 NMO IgG negative (25 tested)
Non-ADEM monophasic No T2 lesions at onset (n = 32) ^a	11.61 (8.93-13.42)	16 (50)	28 (88)	1 (1-1)	1 (1-2)	NA	15 (46.9%) with isolated ON; 15 (46.9%) with isolated TM; 2 (6.2%) with polyfocal clinical features; 25 NMO IgG negative (25 tested)
ADEM (n = 18) ^a	5.87 (3.28-10.26)	7 (39)	18 (100)	1 (1-1)	1 (0-1)	25.5 $\pm$ 30.8	13 NMO IgG negative (13 tested)

Abbreviations: ADEM = acute disseminated encephalomyelitis; EDSS = Expanded Disability Status Scale; IgG = immunoglobulin G; IQR = interquartile range; NMO = neuromyelitis optica; ON = optic neuritis; TM = transverse myelitis.

^aNo patient experienced a second clinical attack and none of the patients demonstrated accrual of clinically silent new lesions.

Table 3 Mixed-effect models predicting z scores by disease duration

	MonoADSlesion				MonoADSnolesion				ADEM			
	Coefficient	SE	z Value	Pr(> z)	Coefficient	SE	z Value	Pr(> z)	Coefficient	SE	z Value	Pr(> z)
Brain volume												
Intercept (β_0)	-0.552 ^a	0.191	-2.88	0.012	-0.531 ^a	0.194	-2.74	0.018	-0.405	0.259	-1.56	0.31
Slope (β_1)	-0.045 ^a	0.008	-5.62	<1e-04	-0.035 ^a	0.007	-4.47	2.36e-05	-0.071 ^a	0.012	-5.94	<1e-05
WM volume												
Intercept (β_0)	-0.715 ^a	0.079	-9.03	<1e-08	-0.685 ^a	0.080	-8.52	<1e-08	-0.899 ^a	0.107	-8.37	<1e-04
Slope (β_1)	-0.021 ^a	0.004	-5.35	2.62e-07	-0.011 ^a	0.004	-2.84	0.0134	-0.032 ^a	0.005	-5.56	<1e-04
Cortical GM volume												
Intercept (β_0)	0.221	0.160	1.38	0.415	0.157	0.162	0.97	0.693	0.538	0.216	2.48	0.038
Slope (β_1)	-0.024 ^a	0.006	-4.13	1.07e-04	-0.026 ^a	0.006	-4.59	<1e-04	-0.033 ^a	0.009	-3.80	4.33e-04
Normalized thalamus												
Intercept (β_0)	-0.234	0.179	-1.31	0.468	0.391	0.181	2.15	0.091	-0.431	0.244	-1.77	0.212
Slope (β_1)	-0.019	0.013	-1.45	0.376	-0.018	0.012	-1.41	0.401	-0.073 ^a	0.019	-3.78	4.61e-04
SIENAX scaling factor												
Intercept (β_0)	0.273	0.218	1.25	0.503	0.141	0.221	0.64	0.890	-0.283	0.297	-0.95	0.709
Slope (β_1)	0.035	0.014	2.57	0.029	0.051 ^a	0.012	4.18	8.73e-05	0.089 ^a	0.020	4.40	3.20e-05

Abbreviations: ADEM = acute disseminated encephalomyelitis; ADS = acquired demyelinating syndrome; GM = gray matter; monoADSlesion = non-ADEM monophasic ADS with T2 brain lesions at onset; monoADSnolesion = non-ADEM monophasic ADS without T2 brain lesions at onset; WM = white matter. The details of these models are given in appendix e-2. The results of the normality tests for each age/sex subgroup are given in appendix e-3, table A3-3. The graphs of these linear functions are given in figure 1.

^a Coefficient significantly different from 0 (p value <0.05 corrected for multiple comparisons).

monoADSlesion and monoADSnolesion patient groups (see first row of table 3), demonstrating that the estimated baseline volumes were slightly smaller compared to NC, but were not significantly different between the patient groups (post hoc analysis of between-patient groups). The brain z score slopes were also negative and significantly different from zero (see second row of table 3, first row of figure 1) for the 3 patient groups, consistent with a progressively negative effect over time (pattern 1 described in Methods). The brain z score slope was steepest for the ADEM patients, moderate for the monoADSlesion group, and less markedly deviant from normal for the monoADSnolesion group. The brain z score slope was significantly steeper for the ADEM group compared to the monoADSnolesion group (post hoc analysis, $p < 0.05$). The SIENAX scaling factor z score intercepts (see ninth row, table 3) are not significantly different from zero, showing that the skulls are not smaller at baseline. However, the scaling factor slopes (see last row, table 3) are positive (indicating smaller skull size) and significantly different from zero for the monoADSlesion and ADEM groups, indicating progressively more fall-off from normal head growth.

The WM seems to be the most reduced brain component at baseline, as shown by the intercepts (that represent an estimation of the z scores at baseline), which are significantly smaller than zero for all

groups when compared to the normal growth reference (see third row table 3, second row figure 1). In all patient groups, the cortical GM volume is not significantly different compared to NIHPD healthy controls (z score intercept) at the time of the demyelinating event (fifth row, table 3; third row, figure 1). Neither WM nor cortical GM follows age-expected growth trajectories over time (see fourth and sixth rows, table 3). Patients with ADEM show the most significant deviation from age-expected trajectories. The WM z score slope of the monoADSnolesion group is significantly smaller than the slope of the other patient groups ($p < 0.05$, post hoc analysis), indicating that WM growth is less affected in children who did not have brain lesions as part of their monophasic demyelinating illness.

Unlike our previously published results in pediatric MS,^{1,2} the normalized thalamus (i.e., thalamus volume divided by the brain volume) of monophasic participants does not appear to be reduced at the time of the presentation (see seventh row, table 3; fourth row, figure 1) or over time (see eighth row, table 3), meaning that the thalamus is not preferentially affected compared to the whole brain.

To exclude any scanner-related influence on our data, we confirmed that there were no significant differences between intercept or slope coefficients in the adjusted models between the NC and NIHPD groups.

Figure 1 Estimated mean z score models for each patient group

From left to right: Monophasic acute disseminated encephalomyelitis (ADS) with T2 lesions involving the brain at onset (monoADSlesion), monophasic ADS with no T2 lesions involving the brain at onset (monoADSnolesion), and acute disseminated encephalomyelitis (ADEM) groups, and for each structure (from top to bottom: brain volume, white matter [WM] volume, cortical gray matter [GM] volume, and normalized thalamus volume). Individual measured and fitted z scores are plotted in light and dark gray, respectively. The model coefficients are given in table 3. Overall we see that for brain volume (row 1) the 2 monoADS groups are pattern 2 (patients < controls) and all 3 groups are pattern 1 (patients diverge from controls over time). For supratentorial WM (row 2), all 3 groups are a combination of pattern 2 (patients < controls) and pattern 1 (patients diverge from controls). For cortical GM (row 3) all 3 groups are pattern 1 (patients diverge from controls), but the GM volume is not significantly different between patients and controls. Only the ADEM group shows statistically significant pattern 1 for normalized thalamus (row 4). Finally, the SIENAX scaling (row 5) shows that monoADSnolesion and ADEM follow pattern 1 (with patients having smaller heads over time, compared to controls).

Effects of age at onset. When added as a potential covariate in the mixed effects model, no significant interactions were found between age at onset and any of the structure volumes for any of the groups tested (results not shown).

DISCUSSION The goal of this longitudinal study was to examine the effect of monophasic forms of ADS on brain growth in the maturing CNS. We have previously demonstrated a negative effect of relapsing

CNS demyelination on brain volume.^{1–3} To our knowledge, the present study is the first to evaluate the effect of monophasic demyelination on brain growth in children.

We demonstrate that monophasic demyelination negatively affects brain growth, not only in children with ADEM, a presentation associated typically with prominent multifocal involvement of the brain (and often optic nerves and spinal cord), but also in more CNS-regionally restricted presentations, and even in

children with demyelination involving only the optic nerves or spinal cord. The reduction in age-expected brain growth was most markedly driven by lack of age-expected WM growth, consistent with a greater insult to WM during acute demyelination. While cortical GM volumes in the patient groups appeared similar to age-expected measures at onset, subsequent growth did not reach age-expected values, indicating that monophasic acquired demyelination affects more than WM. The potential clinical effect of impaired age-expected brain growth will require longitudinal cognitive evaluation in future work.

Our analyses also indicate that children with acquired demyelination may have smaller than expected brain volumes at baseline. This finding was present both when considering patients whose first research scan occurred at onset and when based on model-based extrapolation. We utilized a brain modeling approach to estimate baseline brain volumes given the inherent challenges of brain measures during acute illness. Specifically, brain volume analyses at the time of acute demyelination are subject to lesional edema (which may be particularly prominent in children with ADEM), and thus may not reflect the brain volume as it would have been prior to the acute illness. While we have found statistically significant differences in modeled brain volume presentation with respect to the normal control cohort, it is important to note that these differences are small and must be reproduced in a larger cohort. However, if our findings are replicated, and with the consideration that the absolute difference in brain size from the normative population is small, it is possible that children who experience acquired CNS demyelination have an increased vulnerability (or less resilience) to the negative effect of an immunologic attack on brain growth.

Nearly all patients were treated with corticosteroids at the time of their demyelinating event (75 of 83 patients) and none received corticosteroids at any subsequent time points. We were not able to determine the precise corticosteroid dose (in mg/kg/total course), although all study sites were provided a suggested treatment algorithm that advocated 30 mg/kg/d of IV methylprednisolone (maximum 1,000 mg/d) for 3–5 days followed by a 10- to 14-day oral prednisone taper. We do not believe that exposure to corticosteroids, rather than ADS, explains our findings given data that brain volume reduction following corticosteroid treatment in adults with relapsing-remitting MS is transient.¹⁴

In addition to the potential clinical ramifications of impaired brain growth in children with monophasic demyelination, our findings also have potential importance for understanding the effect of chronic disorders, such as MS. We demonstrate that even

a single demyelinating attack has a negative effect on brain growth. While chronically active CNS inflammation may have a greater effect, the fact that even monophasic demyelination impairs brain health leads to important considerations. For example, reduced brain volumes and progressive loss of brain volume may occur as a consequence of even the first MS attack, even in patients diagnosed promptly (as per 2010 McDonald criteria at the first attack⁷) and even if therapy is provided immediately after diagnosis. If so, then analysis of brain volumes as a metric of treatment efficacy must consider that some aspects of brain volume loss (or failure of brain growth) are preordained by the initial inflammatory phase experienced prior to treatment initiation. Mitigating a negative effect on brain growth, both in monophasic demyelination and in MS, may also require approaches that are distinct from anti-inflammatory strategies.

ADEM had a particularly negative effect on brain growth. The higher lesion volumes at baseline that characterized ADEM might have had a more deleterious effect as compared to the much smaller monofocal lesion volumes measured in the other monoADS patients (table 2). Another possibility is that the younger age at onset of ADEM occurs concurrent with a greater normative growth rate, and thus might have a greater effect. However, we did not find a significant effect of age at onset.

We utilized *z* score measures to compare the brain volume of each patient with an age- and sex-matched healthy population, in units of SD. In this way, not only the continual development of the brain was taken into account but also the normal intersubject developmental variability. This method enabled the comparison of patients with different ages of onset, i.e., with different stages of brain maturation at first measurement. Although the average age at onset of the patients varied between groups, the particular model chosen allowed a comparative analysis of the effect of disease duration on the brain structures independent of the age at onset.

We built mixed-effect models describing a linear relationship between structure volume *z* scores and time from ADS event. The intercept coefficient estimates what was happening at the time of the first event, while the slope reflects change in volume over time. With more data, it would be interesting to fit more complex models such as Gompertz curves for growth combined with an exponential decay to model atrophy.

A potential caveat of our study is that patients in our prospective cohort diagnosed with monophasic ADS at time of this analysis could, in principle, accrue new lesions or experience subsequent relapses in the future, changing their diagnosis to MS. While

possible, this is unlikely given these patients did not have lesion distributions consistent with the 2010 McDonald criteria and have not developed new lesions over time despite a median period of observation of 4 years. Only ongoing observation will ensure that no children currently classified as monophasic develop evidence of relapsing disease.

We demonstrate that even monophasic acquired demyelination has a negative effect on the developing CNS in children. Our findings implicate a vulnerability rather than resiliency of the immature CNS to inflammatory insults. The clinical effect of failure of age-expected brain growth in monophasic demyelination requires evaluation of cognition and quality of life: components being addressed in our ongoing work. Furthermore, and of potential relevance to adult-onset MS as well, is the observation that chronic inflammation and active relapses are not required for there to be a negative effect on brain volumes.

AUTHOR CONTRIBUTIONS

B.B., B.A.-B., D.L.C., and D.L.A. were particularly involved in the conceptual design of the project. B.A.-B. did the longitudinal MRI analysis and the statistical analysis. K.W. and V.S.F. contributed to the longitudinal MRI analysis. D.L.C. contributed to the longitudinal MRI analysis and provided input on the statistical analysis. S.N. was responsible for the MRI protocol. L.H.V. was responsible for producing T2 lesion scores and G.L. was responsible for producing T2 lesion volumes at baseline. B.B., A.B.-O., A.M.R., A.Y., and D.L.A. were principal investigators for the Canadian Pediatric Demyelinating Disease Study. All authors contributed to the interpretation of data and to the writing and reviewing of the submitted manuscript and have seen and approved the final version.

ACKNOWLEDGMENT

The authors thank the participating children and their families of the Canadian Pediatric Acquired Demyelinating Disease Study and the NIHPD Study for their cooperation and commitment to research; and the coordinators of the Canadian Pediatric Acquired Demyelinating Disease Study Julia O'Mahony and Stephanie Grover at the Hospital for Sick Children and Rozie Arnaoutelis at the McConnell Brain Imaging Centre (Montreal, Canada).

STUDY FUNDING

The Canadian Pediatric Acquired Demyelinating Disease Study is supported by the Canadian Multiple Sclerosis Scientific Research Foundation.

DISCLOSURE

B. Aubert-Broche, K. Weier, and G. Longoni report no disclosures relevant to the manuscript. V. Fonov reports personal fees from NeuroRx Research outside the submitted work. A. Bar-Or reports grants from MS Society of Canada Research Foundation during the conduct of the study and personal fees from Biogen Idec, Diogenix, Genentech, GlaxoSmithKline, Guthy-Jackson/GGF, Merck/EMD Serono, Medimmune, Mitsubishi Pharma, Novartis, Cellgene-Receptos, Roche, Sanofi-Genzyme, and Teva Neuroscience outside the submitted work. R. Marrie reports support from Sanofi-Aventis outside the submitted work. E. Yeh reports fees from ACI for clinical trial relapse adjudication. S. Narayanan has received personal compensation from NeuroRx Research for consulting services and a speaker's honorarium from Novartis Canada. D. Arnold reports grants from MS Foundation (Canada) during the conduct of the study and personal fees from Acorda, grants and personal fees from Biogen, personal fees from Hoffman LaRoche, personal fees from MedImmune, personal fees from

Mitsubishi Tanabe Pharma Corporation, grants and personal fees from Novartis, personal fees from Receptos, and personal fees from Sanofi-Aventis outside the submitted work. L. Verhey reports grants from Multiple Sclerosis Scientific Research Foundation during the conduct of the study. B. Banwell reports personal fees from Novartis outside the submitted work and grants (but not for salary) from the Canadian Multiple Sclerosis Scientific Research Foundation, MS Society of Canada, CIHR, and NMSS. D. Collins reports grants from Multiple Sclerosis Society of Canada during the conduct of the study, support from True Positive Medical Devices, and personal fees from NeuroRx Inc. outside the submitted work. Go to Neurology.org for full disclosures.

Received June 10, 2016. Accepted in final form January 23, 2017.

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