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Lower myelin-water content of the frontal lobe in childhood absence epilepsy

Gerhard S. Drenthen1,2,3 | Eric L. A. Fonseca Wald1,4,5 | Walter H. Backes1,2 | Mariette H. J. A. Debeij - Van Hall5 | Jos G. M. Hendriksen4,5 | Albert P. Aldenkamp1,3,5 | R. Jeroen Vermeulen1,4 | Sylvia Klinkenberg1,4 | Jacobus F. A. Jansen1,2,3

Abstract

Objective: The frontal lobe in childhood absence epilepsy (CAE) might be affected due to the suggested involvement of the frontal lobe during absence seizures and reports on attentional deficits. Previously, subtle white matter abnormalities have been reported in CAE. However, the impact of one of the most characteristic components of the white matter, the myelin content, remains underdetermined. Therefore, this study investigated whether the myelin content in frontal areas is adversely affected in CAE compared to controls.

Methods: Seventeen children with childhood absence epilepsy (mean age ± standard deviation [SD], 9.2 ± 2.1 years) and 15 age- and sex-matched controls (mean age ± SD, 9.8 ± 1.8 years) underwent neuropsychological assessment and a magnetic resonance imaging (MRI) examination. T2 relaxometry scans were used to distinguish myelin-water from tissue water and to determine the myelin-water fraction (MWF) in the frontal, temporal, parietal, occipital, and insular lobes. A linear regression model including age and sex as covariates was used to investigate group differences. Furthermore, the relationship of MWF with cognitive performance and epilepsy characteristics was determined.

Results: The frontal lobe revealed a significantly lower myelin-water content in children with CAE compared to controls over the developmental age range of 6-12 years (5.7 ± 1.0% vs 6.6 ± 1.1%, P = 0.02). This association was not found for any of the other four lobes (P > 0.10). No significant relation was found between myelin-water content and cognitive performance or epilepsy characteristics.

Significance: The lower frontal myelin-water content of children with CAE in comparison with healthy controls probably reflects an altered neurodevelopmental aspect in CAE, of which the underlying mechanisms still need to be unraveled.

Key words

myelin-water fraction, neurodevelopment, white matter
## INTRODUCTION

Childhood absence epilepsy (CAE) is characterized by seizures of brief losses of awareness occurring multiple times per day in otherwise normally developing school-aged children. Ictal activity on an electroencephalography (EEG) shows approximately 3 Hz generalized spike-and-wave discharges (GSWDs). The generation or propagation of these GSWDs was previously suggested to have a frontal involvement, although the precise identification of brain regions involved is an ongoing debate.\(^1\)\(^,\)\(^2\) Magnetic resonance imaging (MRI) of the brain is normally omitted from the standard clinical workup, as specific structural brain abnormalities are not regarded to be part of this generalized epilepsy syndrome. Nevertheless, cognitive\(^3\) and neuroimaging\(^4\)\(^–\)\(^7\) studies in children with absence epilepsy have raised concerns about the true neurocognitive impact of this allegedly benign syndrome. A higher incidence of academic difficulties and neurocognitive deficits, mainly attentional deficits, for which the frontal lobe plays an important role, has been observed in CAE.\(^8\)\(^–\)\(^16\) Furthermore, a study employing diffusion-weighted MRI reported altered structural connectivity in children with CAE, which was suggested to imply abnormal myelination.\(^17\) However, although diffusion-weighted MRI can provide markers sensitive to myelin content, these markers are not specific to the myelin content only.\(^18\)

The process of myelination is most active during the first 2 years of life but continues through adulthood.\(^19\) Myelin is wrapped around the axons causing efficient action potential conduction and increased conductance velocity in axons, which is essential for information transmission through neural circuits.\(^20\) Abnormal and exceptional synchronization of neural activity (eg, epileptic seizures) may be related to the myelination process and thus neurodevelopment in children with CAE. Currently, quantitative data on in vivo myelin content and its change during brain development are lacking in CAE imaging studies.

The myelin content can be quantified through MRI of myelin-water. Myelin-water quantification is based on the quantification of water between the bilayers of the myelin sheath using T2 relaxometry. Because water is an important component of myelin, making up to 40% of the total myelin volume, myelin-water imaging can be regarded as a specific marker of myelin content.\(^21\) Water trapped between the bilayers of the myelin has a faster T2 relaxation rate compared to more freely moving water in the intracellular and extracellular spaces. Myelin content can be quantified using the fraction of myelin-water signal to the total water signal, the so-called myelin-water fraction (MWF).

In this study, myelination was investigated in children with CAE and controls, by applying MWF imaging. Due to the frontal involvement during seizures in children with CAE, we hypothesize altered myelin content in frontal areas.

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### Key Points
- The frontal lobe might be affected in childhood absence epilepsy due to reports on attentional deficits and its suggested involvement in absence seizures
- Frontal myelin-water content is significantly lower in children with childhood absence epilepsy compared to healthy peers
- The myelin-water content in the other lobes did not differ significantly between the groups. Probably reflecting a neurodevelopmental aspect in CAE as the maturation of the frontal lobe is still ongoing

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The primary aim of the current study is to assess differences in myelin-water content between children with CAE and controls, and the secondary aim is to explore the relationship of myelin-water content with the duration of CAE and neurocognitive performance.

## METHODS

### 2.1 | Inclusion

Children with CAE were prospectively recruited via pediatricians and pediatric neurologists within the adherence areas of Kempenhaeghe Epilepsy Centre, a tertiary epilepsy center. Children with a clinical diagnosis of CAE were included based on the following criteria: (a) primarily presenting with daily occurring episodes of brief loss of consciousness in an otherwise normal child and an EEG showing ictal 3 Hz (2.5–4.5 Hz) generalized rhythmic spike-and-wave complexes with a discharge duration of at least 3 seconds on a present or former EEG (in accordance with International League Against Epilepsy [ILAE] statements for CAE\(^22\)\(^,\)\(^23\)); (b) early absence epilepsy, defined as a confirmed diagnosis or seizure onset within 2 years; And (c) 6 to 12 years of age. Healthy age- and sex-matched controls were recruited via advertisements. To be included, controls needed to follow regular school without major problems. All caregivers and children age 12 years old gave written permission prior to inclusion in the study. This research protocol was approved by the medical ethics committee aZM/UM NL55455.068.15/METC152055 and is listed at clinicaltrials.gov under NCT02954107.

### 2.2 | Clinical and neuropsychological assessment

Clinical characteristics including age at onset, seizure semiology, duration of epilepsy, drug history, and EEG reports
were collected in an online data management system (Castor Electronic Data Capture, CIWIT bv). All children underwent a multidisciplinary assessment, which included a medical assessment, a 24 hour video-EEG, and a neuropsychological assessment. Six subtests of the Wechsler Intelligence Scale for Children third edition (WISC-III) (similarities, vocabulary, picture completion, coding, block design, and symbol search) were used to assess general intelligence and processing speed.24,25 The Bourdon-Vos, a paper and pencil cancellation test, was used to test for sustained visual attention and vigilance.26 Based on age-dependent normative data, the WISC-III and Bourdon-Vos were expressed as standard scores.

2.3 | MRI Acquisition

All subjects were scanned on a 3.0T unit (Philips Achieva) using a 32-element phased-array coil. To minimize moving during the MRI, children were carefully prepared beforehand using a combination of video and written information. In addition, parents were instructed to practice lying still with the child at home and to be present with them in the magnet room during the scanning. First, for anatomic reference and segmentation, T1-weighted three-dimensional (3D) turbo field echo images were acquired (repetition time [TR] = 8.36 msec, echo time [TE] = 3.84 msec, flip angle [FA] = 8 degrees, cubic voxel size = 1 mm). For MWF estimation, whole cerebrum 2D multislice gradient and spin echo (GRASE) images were acquired (TR = 3000 msec, 32 echoes with 9.3 msec echo spacing, range 9.3-297.6 msec, echo-planar imaging (EPI) factor = 3, Turbo factor = 32, 24 slices, 1 mm slice gap, field of view = 230 × 180 × 119 mm, matrix 152 × 120, voxel size = 1.5 × 1.5 × 4 mm) with parallel acquisition (sensitivity encoding, SENSE = 2), scan duration = 5:45 minutes.27

3 | ANALYSIS

3.1 | Preprocessing

For each subject, the T1 images were registered to the first echo image of the GRASE scans using the statistical parametric mapping (SPM12) toolkit.28 A singular value decomposition filter was used to reduce noise in the multiecho data,29 and a Gaussian kernel of 2.4 mm full width at half maximum was used to spatially smooth the GRASE images. Furthermore, all images were visually checked for motion artifacts. Some slices with severe motion artifacts were removed from subsequent analyses.

3.2 | ROIs

The white matter was parcellated into five lobes: the frontal, temporal, parietal, occipital, and insular lobes (Figure 1). First, the white matter was parcellated using the T1-weighted images employing Freesurfer (version 5.1)30 based on the 68 cortical regions in the Desikan-Killiany atlas31 by assigning each white matter voxel to the most proximal cortical region. Finally, the ROIs were eroded slightly to cope with potential partial-volume errors occurring during the co-registration. The volumes of the segmented white matter lobes are shown in Table 1.

3.3 | Multiexponential analysis

Multiexponential analysis of the multiecho signal decay data was performed using the nonnegative least squares (NNLS) algorithm.32 To solve the NNLS, a basis set of 120 logarithmically spaced relaxation functions (T2 range 15 to 2000 msec) was used. The algorithm was regularized using an additional minimal energy constraint that allows an increased misfit between 2% and 2.5% (1.020 ≤ χreg/χmin ≤ 1.025).
The extended phase graph (EPG) algorithm along with the Fourier transform of the slice-selective excitation pulse was used to account for possible stimulated echoes caused by B$_1$ inhomogeneities, and imperfect slice profiles due to slice-selective excitation.

The NNLS results in a T2 spectrum, indicating which T2 components are present in the signal. The water trapped between the bilayers of the myelin is known to decay with a relatively fast T2 time of 15 to 40 msec. Therefore, the MWF was calculated as the ratio of myelin-water–associated T2 components (15–40 msec) to all T2 components (15–2000 msec). Because the MWF distribution in a region of interest (ROI) is (right-)skewed, we applied the median to represent the MWF in the ROIs.

### 3.4 Statistical analysis

Differences between children with CAE and controls in age, general intelligence, processing speed, and the speed and accuracy of the Bourdon–Vos test were assessed using the Student’s $t$ test for independent samples. Differences in sex and handedness were assessed with the chi-square test of independence.

To investigate whether lobar myelin-water content differed between groups a multivariate analysis of covariance (MANCOVA) was performed, where the MWF of the five lobes were used as dependent variables. Because myelin varies with age and was shown previously to differ between males and females, age and sex are added as covariates in the MANCOVA model. Subsequently, post hoc linear multivariable regression analysis was performed for each lobe to assess differences between groups, correcting for age and sex.

The impact of the duration of CAE and the duration of antiepileptic drug (AED) use on the frontal myelin-water content was evaluated using a linear multivariable regression model for the CAE group, correcting for age and sex. Furthermore, to explore the relation of frontal myelin-water content with neurocognitive performance, a linear multivariable regression model was used for both groups combined, correcting for age and sex. This multivariable regression model was used for each of the neurocognitive performance variables, general intelligence, and processing speed from the WISC-III, as well as the speed and accuracy of the Bourdon–Vos.

Statistical significance was inferred when $P < 0.05$.

### 4 RESULTS

#### 4.1 Subject characteristics

The population consisted of 17 children with CAE and 15 controls. The subject characteristics are shown in Table 2. The two groups did not differ significantly regarding age, sex, and handedness. All included children were following regular education, except one child included in the CAE group. Nine were taking ethosuximide (range 14.7-27.2 mg/
kg), three were taking valproic acid (range 13.3–30.8 mg/kg), two were taking ethosuximide + valproic acid (1.31.8 mg/kg and 22.7 mg/kg, respectively; 2. 25.7 mg/kg and 14.12 mg/kg, respectively) and one was taking lamotrigine + clobazam (1.4 + 0.42 mg/kg). At the time of the MRI, two children were still drug-naive, whereas 15 were receiving AED treatment. Processing speed index was significantly lower for children with CAE compared to controls, although both groups were still within normal clinical range. Mean performance on the WISC subtests and Bourdon-Vos did not differ significantly between groups. A trend toward worse performance in CAE was present in the WISC subtests and speed for sustained attention in the Bourdon-Vos compared to controls.

4.2 | Myelin content

A T1-weighted scan with the corresponding MWF map of a child with CAE and a control is shown in Figure 2. The MANCOVA revealed that the lobar myelin-water content is significantly different between children with CAE and controls, while controlling for effects of age and sex (P < 0.05). Post hoc regression analysis showed a significantly lower frontal myelin-water content in children with CAE compared to controls. For any of the other four lobes, no significant differences were found (Table 3). A scatterplot of the frontal MWF values as a function of age is shown in Figure 3. Least-square lines for CAE (solid) and controls (dashed) are added for visualization, qualitatively revealing that the frontal myelin-water content increases with age.

Moreover, the difference between children with CAE and controls does not vary over age.

The deepest white matter regions (insular lobe) show the highest myelin-water content, while the myelin-water content in the frontal and temporal lobes is lower compared to the parietal and occipital lobes. These findings are consistent with the known patterns of myelin development, moving from posterior to anterior areas, and outward from the central to the peripheral regions.

Using regression, significance relations were not found between frontal myelin-water content and the duration of CAE (β = 0.13, P = 0.65) or the duration of AED use (β = −0.13, P = 0.54). In the whole sample, no significant relations were found between frontal myelin-water content and general intelligence (β = 0.22, P = 0.19), the processing speed index (β = 0.22, P = 0.18), and the speed.

### TABLE 3 MWF values in the five lobes of children with CAE and controls

<table>
<thead>
<tr>
<th>MWF Lobes</th>
<th>CAE (%)</th>
<th>Controls (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal</td>
<td>5.7 ± 1.0</td>
<td>6.6 ± 1.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Parietal</td>
<td>7.4 ± 1.2</td>
<td>7.5 ± 1.3</td>
<td>0.30</td>
</tr>
<tr>
<td>Occipital</td>
<td>7.2 ± 1.2</td>
<td>7.9 ± 1.1</td>
<td>0.51</td>
</tr>
<tr>
<td>Temporal</td>
<td>4.6 ± 0.8</td>
<td>5.0 ± 0.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Insular</td>
<td>8.6 ± 1.7</td>
<td>9.4 ± 1.7</td>
<td>0.28</td>
</tr>
</tbody>
</table>

*Note: The raw MWF values are shown, and that the effects of age and sex are not included. The P-value denotes the significance level of the group difference obtained with the regression model. Bold values denote statistical significance. Abbreviations: CAE, childhood absence epilepsy; MWF, myelin-water fraction.*

FIGURE 2 An axial slice of a T1-weighted image and myelin-water fraction map for a 10-year-old boy with childhood absence epilepsy (CAE) and a 10-year-old male healthy control (HC), positioned through the splenium and genu of the corpus callosum.

FIGURE 3 Frontal myelin-water fraction as a function of age. Least-square lines for childhood absence epilepsy (solid) and controls (dashed) are added for visualization, qualitatively revealing that the frontal myelin-water content increases with age. Abbreviations: CAE, childhood absence epilepsy; HC, healthy control; MWF, myelin-water fraction; y, years.
as a neurodevelopmental aspect of the disorder because the in this study, the observed abnormality can be interpreted definite association for the duration of epilepsy was found during the vulnerable years of childhood). Although no or after epilepsy onset (ie, related to epileptiform activity lated to a genetic component), or might be acquired during or topographic deviations.40 Previously, Spader et al41 showed the feasibility of identifying in vivo myelin abnormalities using MRI and reported a reduced MWF in three children with epilepsy. However, it has not been previously employed in a group with generalized epilepsy. The main function of myelin is accelerating information processing due to the propagation of electrical signals (ie, action potentials) using saltatory conduction. However, the precise mechanisms are yet to be completely understood. The observed lower frontal myelin-water content might occur in an early stage of the neurodevelopment (ie, related to a genetic component), or might be acquired during or after epilepsy onset (ie, related to epileptiform activity during the vulnerable years of childhood). Although no definite association for the duration of epilepsy was found in this study, the observed abnormality can be interpreted as a neurodevelopmental aspect of the disorder because the maturation of the frontal lobe is still ongoing.42 Human and animal studies have suggested frontal involvement during the generation or propagation of generalized GSWDs, although the exact brain regions involved are of ongoing debate.1,2 EEG–functional MRI (fMRI) studies have found different patterns of activation2 and animal studies point to the somatosensory cortex in the generation of GSWDs. On the other hand, deactivation of frontal areas during GSWDs43 and/or differences in resting state networks involved in attention have also been reported5 and might be related to changes in myelin content as well. From this relatively small cross-sectional sample we cannot affirm whether the reduced myelin-water content predates the onset of absence epilepsy, or whether the difference in myelin-water content worsens or improves with a longer follow-up. These aspects need to be elucidated in longitudinal studies.

We observed a lower performance for processing speed index and a trend toward lower general intelligence and speed for sustained attention in children with CAE. This is in agreement with previous studies on neurocognition in CAE.9,12,44 Although no definite association between neurocognition and myelin-water was found in the current study, it has been suggested that myelin may be important in optimizing the timing of information through neural circuits.20 Evidence also suggests that myelin may be influenced by seizures, as has been shown in preclinical studies where epilepsy induced rats show a reduced myelin content.45–47 Moreover, histopathological studies in focal epilepsy have shown abnormalities in white matter myelination, axonal integrity, and cellular composition,48,49 although limited data exist in generalized epilepsy as it is more difficult to collect tissue samples. Therefore, the used methods and findings of this study are promising for epilepsy research, as it may help to better understand the relationship between epileptiform activity and neurocognitive co-morbidities.

5 | DISCUSSION

In the current study, the cerebral myelin-water content of children with early onset CAE was investigated and compared with age- and sex-matched controls. A lower myelin-water content was found in the frontal lobe of children with CAE compared to controls, whereas myelin-water content did not differ in any of the other lobes. Neurocognitive performance and sustained attention in our cohort of children with CAE did not relate significantly to the myelin-water content, although the results were indicative of a better performance for increased myelin-water content.

Previously, CAE studies using diffusion-weighted MRI reported lower fractional anisotropy (FA) values in the genu, an important part of the corpus callosum in the frontal lobe.6,39 However, although diffusion-weighted MRI measures are valuable in white matter research, they fail to provide more specific information on the myelin content and cannot discriminate between axonal damage, alterations in myelin content, or topographic deviations.40 Previously, Spader et al41 showed the feasibility of identifying in vivo myelin abnormalities using MRI and reported a reduced MWF in three children with epilepsy. However, it has not been previously employed in a group with generalized epilepsy.

The main function of myelin is accelerating information processing due to the propagation of electrical signals (ie, action potentials) using saltatory conduction. However, the precise mechanisms are yet to be completely understood. The observed lower frontal myelin-water content might occur in an early stage of the neurodevelopment (ie, related to a genetic component), or might be acquired during or after epilepsy onset (ie, related to epileptiform activity during the vulnerable years of childhood). Although no definite association for the duration of epilepsy was found in this study, the observed abnormality can be interpreted as a neurodevelopmental aspect of the disorder because the maturation of the frontal lobe is still ongoing.42 Human and animal studies have suggested frontal involvement during the generation or propagation of generalized GSWDs, although the exact brain regions involved are of ongoing debate.1,2 EEG–functional MRI (fMRI) studies have found different patterns of activation2 and animal studies point to the somatosensory cortex in the generation of GSWDs. On the other hand, deactivation of frontal areas during GSWDs43 and/or differences in resting state networks involved in attention have also been reported5 and might be related to changes in myelin content as well. From this relatively small cross-sectional sample we cannot affirm whether the reduced myelin-water content predates the onset of absence epilepsy, or whether the difference in myelin-water content worsens or improves with a longer follow-up. These aspects need to be elucidated in longitudinal studies.

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5.1 | Study considerations

Our study has several important strengths. The study used well-defined inclusion criteria for children with CAE in agreement with current ILAE standards. Moreover, the age- and sex-matched controls enabled us to make reliable group-level comparisons. Furthermore, to minimize the influence of subject motion, which is an inherent problem when scanning young children, we employed a 2D multislice acquisition instead of the more commonly applied 3D acquisition.27 Although 2D imaging does not prevent subject motion artifacts completely, it does allow for removal of corrupted slices, whereas subject motion in 3D acquisition affects the entire volume. In this study, less than 4% of the total slices were corrupted and subsequently removed. The main limitation of this study is the sample size, which limits the power for more in-depth correlation analysis with clinical/epilepsy variables, for which we did not find a relation. Furthermore, it cannot be excluded that the use of AEDs has had a bearing on the results of this study, as ethosuximide and valproic acid have been associated with neurocognitive side effects.9,50 Nonetheless, because current guidelines prescribe the use of these AEDs, the results in this study reflect children with CAE at this point in time. Furthermore, no relation was found between the duration of AED use and the frontal myelin-water content in this study, indicating that our results are not driven by medication use. Future studies, preferably, larger populations followed over time, are necessary to elucidate the relationship between AED use and the myelination process.
5.2 | Concluding remark

This study, for the first time, found a lower myelin-water content in the frontal lobe in children with CAE compared with age- and sex-matched controls, probably implicating an altered neurodevelopmental aspect in CAE. Whether the altered myelin-water content predates the onset of absence epilepsy, or whether the myelin-water content worsens over time cannot be concluded from this study. Therefore, more myelin-specific studies with longitudinal designs are warranted to further investigate the relation of (frontal) myelin content and CAE.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Jacobus F. A. Jansen https://orcid.org/0000-0002-5271-8060

REFERENCES


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