White matter development in children with benign childhood epilepsy with centro-temporal spikes

Carolina Ciumas,1,* Mani Saignavongs,1,* Faustine Ilski,1,2 Vania Herbillon,2 Agathe Laurent,1,2 Amelie Lothe,1 Rolf A. Heckemann,3,4 Julitta de Bellescize,2 Eleni Panagiotakaki,2 Salem Hannoun,5 Dominique Sappey Marinier,5,6 Alexandra Montavont,1,2,7 Karine Ostrowsky-Coste,2 Nathalie Bedoin8,9 and Philippe Ryvlin1,2,7

1 INSERM U1028, CNRS UMR5292, Centre de Recherche en Neurosciences de Lyon, Translational and Integrative Group in Epilepsy Research (TIGER), Université Lyon-1, Lyon, France
2 Department of Epilepsy, Sleep and Pediatric Neurophysiology, Hospices Civils de Lyon, Lyon, France
3 The Neurodis Foundation, Lyon, France
4 Institute for Neuroscience and Physiology, University of Gothenburg, Sweden
5 CREATIS-CNRS UMR 5220 & INSERM U1044, Lyon, France
6 CERMEP – Imagerie du Vivant, Lyon, France
7 Department of Functional Neurology and Epileptology, Hospices Civils de Lyon, Lyon, France
8 Laboratoire Dynamique du Langage, CNRS UMR 5596, Lyon, France
9 Université Lyon 2, Lyon, France

*These authors equally contributed to this work.

Correspondence to: Carolina Ciumas, MD, PhD, TIGER, Translational and Integrative Group in Epilepsy Research, INSERM U1028, CNRS UMR5292, Centre de Recherche en Neurosciences de Lyon, Centre Hospitalier Le Vinatier, Bâtiment 452, 95 Boulevard Pinel, 69500 Bron, France
E-mail: carolina.ciumas@inserm.fr

Benign childhood epilepsy with centro-temporal spikes (BCECTS) is a unique form of non-lesional age-dependent epilepsy with rare seizures, focal electroencephalographic abnormalities affecting the same well delineated cortical region in most patients, and frequent mild to moderate cognitive dysfunctions. In this condition, it is hypothesized that interictal electroencephalographic discharges might interfere with local brain maturation, resulting in altered cognition. Diffusion tensor imaging allows testing of this hypothesis by investigating the white matter microstructure, and has previously proved sensitive to epilepsy-related alterations of fractional anisotropy and diffusivity. However, no diffusion tensor imaging study has yet been performed with a focus on BCECTS. We investigated 25 children suffering from BCECTS and 25 age-matched control subjects using diffusion tensor imaging, 3D-T1 magnetic resonance imaging, and a battery of neuropsychological tests including Conner’s scale and Wechsler Intelligence Scale for Children (fourth revision). Electroencephalography was also performed in all patients within 2 months of the magnetic resonance imaging assessment. Parametric maps of fractional anisotropy, mean-, radial-, and axial diffusivity were extracted from diffusion tensor imaging data. Patients were compared with control subjects using voxel-based statistics and family-wise error correction for multiple comparisons. Each patient was also compared to control subjects. Fractional anisotropy and diffusivity images were correlated to neuropsychological and clinical variables. Group analysis showed significantly reduced fractional anisotropy and increased diffusivity in patients compared with control subjects, predominantly over the left pre- and postcentral gyri and ipsilateral to the electroencephalographic focus. At the individual level, regions of significant differences were observed in 10 patients (40%) for anisotropy (eight reduced fractional anisotropy, one increased fractional anisotropy,
Introduction

There is increasing evidence that childhood epilepsies are associated with abnormal developmental trajectory of the brain (Saito et al., 2008; Kanemura and Aihara, 2009; Kanemura et al., 2011; Tosun et al., 2011), particularly of its white matter (Hutchinson et al., 2010; Tosun et al., 2011; Widjaja et al., 2012). Diffusion tensor imaging (DTI) enables investigations into this issue, and was successfully applied to the study of brain development in healthy children (Neil et al., 2002), as well as in autism (Alexander et al., 2007; Ben Bashat et al., 2007; Langen et al., 2012) and attention deficit hyperactivity disorder (Davenport et al., 2010). In temporal lobe epilepsy, DTI studies have shown large white matter abnormalities in regions supposedly participating in the epileptogenic network, either unilaterally (Arfanakis et al., 2002; Thivard et al., 2005) or bilaterally (Concha et al., 2005). Two recent DTI studies also reported white matter changes in a mixed population of children with new onset generalized and partial epilepsies (Hutchinson et al., 2010; Widjaja et al., 2012). One of these series reported decreased fractional anisotropy and increased radial diffusivity in the posterior callosum and cingulum (Hutchinson et al., 2010), whereas the other reported elevated radial diffusivity in the bilateral posterior cingulum, increased axial diffusivity in the left middle frontal, reduced axial diffusivity in the left temporal, right parietal and right supramarginals white matter in partial epilepsy (Widjaja et al., 2012). To date no DTI study has concentrated on patients with localization-related idiopathic epilepsy, and more specifically benign childhood epilepsy with centro-temporal spikes (BCECTS).

BCECTS is often viewed as a developmental disease, characterized by an age-dependent onset, typically between 3 and 13 years, a male to female predominance, a genetic predisposition and recovery during adolescence (ILAE, 1989; Panayiotopoulos, 2005). EEG typically shows focal medium to high-voltage spikes or spike and waves over the centro-temporal region, usually unilaterally that may shift from side to side over time or bilaterally.

Although considered benign, BCECTS is often associated with cognitive disturbances thought to reflect the interference between the epileptic focus and brain development. Both the age dependence and cognitive abnormalities observed in BCECTS strongly suggest the possibility of altered maturation of brain white matter. To test this hypothesis, we undertook a DTI study in a homogenous group of children with BCECTS and age-matched control subjects. We also correlated the presence of DTI abnormalities with several clinical and cognitive variables.

Materials and methods

Participants

We included 25 patients with BCECTS aged 6–13 [mean age ± standard deviation (SD) 9.6 ± 1.9 years] and 25 healthy volunteers aged 6–16 (mean age ± SD, 10.0 ± 3.0 years), all attending regular schools. There were no significant differences between patients and control subjects in age P = 0.60 or gender (BCECTS = 72% males; control subjects = 56% males; P = 0.34). Our selection criteria included: (i) diagnosed with BCECTS according to the current diagnostic criteria (ILAE, 1989); (ii) no anti-epileptic drug for > 24 months; (iii) no other neurological disease; and (iv) normal MRI if available before inclusion. Patients with no previously performed MRI who fulfilled all other criteria were included in the protocol, but not in data analysis if T1-weighted and DTI magnetic resonance images demonstrated significant abnormalities at visual inspection.

Patients were recruited through our paediatric epilepsy outpatient clinic and diagnosed on the basis of all available clinical and EEG data by a paediatric epileptologist (J.D.B., E.P., K.O-C). Twenty patients were right-handed, three left-handed, two ambidextrous; 19 control subjects were right-handed, five left-handed and one ambidextrous. The mean duration of epilepsy from onset to time of scanning was 20.7 months (SD = 20, min = 4, max = 84).

At the time of inclusion in the study, EEG spike foci were left-sided in 14 patients, right-sided in two and bilateral in nine, including five patients with left-side predominance. Fifteen patients had no anti-epileptic drug, eight had one anti-epileptic drug, one had two anti-epileptic drugs, and one had three anti-epileptic drugs. Three patients...
were treated with methylphenidate for co-morbid attention deficit hyperactivity disorder, including one with no anti-epileptic drug treatment (Table 1). Written consent to participate in the study was obtained from the parents and from the children who could write. The study was reviewed and approved by the local ethical committee.

Neuropsychological assessment

All subjects were individually evaluated on the same day as the magnetic resonance examination. A comprehensive standardized neuropsychological test battery was used, including a developmental screening test providing measure of verbal and non-verbal intelligence, verbal-auditory memory, visual processing speed, visual-spatial attention, cognitive flexibility and inhibition. Some of the tests were performed using a computer presentation, while others used printed material. Adjusted norms were commercially available for all tests.

The Conners’ Parent Rating Scale (Conners, 1998, 1999; Conners et al., 1998) was used to assess symptoms of attention deficit hyperactivity disorder and other psychopathologies and behavioural problems. We included the six following subscales: hyperactivity/impulsivity, psychosomatic, learning problems, indices of attention deficit hyperactivity disorder, anxiety, and conduct disorder. Individual raw scores were converted into T-scores. The Wechsler Intelligence Scale for Children (WISC-IV) and its four subscales (Wechsler, 1976; Cooper, 1995; Weiss, 2006) were used to assess: (i) verbal comprehension index; (ii) perceptual reasoning index; (iii) working memory index; and (iv) processing speed index.

Magnetic resonance imaging

Data acquisition

MRI scans were performed without any sedation, and always in the morning. Magnetic resonance images were acquired using a Siemens Sonata whole-body 1.5 T MRI scanner with a Siemens Sonata body coil. Three-dimensional T1 sequence imaging parameters were: sagittal orientation, 160 slices, echo time = 3.55 ms, repetition time = 2400 ms, inversion time = 1000 ms, field of view (in-plane) = 230 mm, flip angle = 8°, slice thickness = 1.2 mm, voxel size = 1.2 x 1.2 x 1.2 mm³, total scan time was 7.42 min. DTI images were acquired using the following parameters: echo time = 86 ms, repetition time = 6900 ms, field of view (in-plane) = 240 mm, voxel size = 2.5 x 2.5 x 2.5 mm³, 52 gradient images with four B0 volumes (1 B0) with no diffusion sensitization (i.e. T2-weighted images) and 48 diffusion-weighted images (b-value: 10 000 s/mm²) in 48 directions. The reconstruction matrix was 96 x 96, voxel size 2.5 x 2.5 x 2.5 mm³, and total scan time was 6.13 min.

Both DTI and T1-weighted data were visually inspected (C.C, Ph.R) to detect artefacts arising from subject motion or scanner malfunction, and confirm the lack of visually detectable abnormality.
Image analysis

DTI is used to investigate the microstructural features of white matter, and relies on the principle that water molecules diffuse mostly along tissue boundaries rather than across them (Beaulieu, 2002; Chua et al., 2008). This anisotropy of diffusion is increased in regions of high axonal integrity and strong myelination, and decreased in regions where white matter is less well organized (Basser, 1995). Fractional anisotropy and mean diffusivity are two quantitative indices of diffusion reflecting the integrity of the brain tissue (Basser and Pierpaoli, 1996; Pierpaoli et al., 1996). Two other DTI-derived indices were considered: axial diffusivity (largest eigenvalue corresponding to the diffusion of water in the direction parallel to the fibre bundles) and radial diffusivity (average of the two smallest eigenvalues (L2 and L3), which measures water diffusion perpendicular to the axonal wall). Decreased fractional anisotropy and increased mean-, axial- and radial diffusivity values suggest alterations of white matter.

DTI and 3D T1 data were preprocessed using statistical parametric mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), Matlab 2009 (The MathWorks) and FSL diffusion toolbox 4.1.9 (http://www.fmrib.ox.ac.uk/fsl/) (Smith et al., 2004).

Distortions in the DTI raw images, induced by the eddy currents and head motion, were corrected by affine co-registration to B0 image using FSL’s ‘eddy current correction’ (Jenkinson and Smith, 2001). Non-brain tissues were then removed using the Brain Extraction Tool (http://fsl.fmrib.ox.ac.uk/fsl/bet2/) and a brain mask was generated to further define the space in which diffusion parameters were calculated (Smith, 2002). A diffusion tensor model was computed at each voxel providing maps of fractional anisotropy, mean- and axial-diffusivity using DTIFIT (http://www.fmrib.ox.ac.uk/fsl/fdt/fdt_dtifit.html).

Radial diffusivity maps were calculated by averaging the second and third eigenvalues (L2 and L3) maps using fslmaths. To create a customized template, 80 images from the control group were normalized to the T2 template in SPM8, and estimated parameters were applied to corresponding diffusion maps (fractional anisotropy, mean-, axial- and radial diffusivity). The resulting maps, converted into MNI space, were averaged to form an initial template, and smoothed to an 8 x 8 x 8 mm full-width at half-maximum Gaussian kernel (SPM routine template creation procedure). These study-specific templates for the T2 template in SPM8, and estimated parameters were applied to the T1 images. Briefly, each image was bias corrected, optimally normalized using rigid-body transformation (with translation and rotation only) and segmented using the ‘unified segmentation’ approach (Ashburner and Friston, 2000). The resulting modulated grey and white matter images were smoothed with an 8-mm full-width at half-maximum kernel. The total intracranial volume was automatically calculated through VBM8 toolbox and corresponded to the sum of the three tissue fractions: grey and white matter, and CSF. This approach has been considered accurate for estimation of total intracranial volume (Pengas et al., 2009).

Statistical analysis

Statistical parametric mapping analysis of diffusion tensor imaging data

Group analysis

Statistical analysis was performed using the general linear model as implemented in SPM8. Between-group analyses were processed by contrasting diffusion maps and adding both gender and age as covariates of no interest, as these measures might influence the structural brain development (Good et al., 2001). The statistical maps were thresholded at a level of P = 0.05 and corrected for multiple comparisons using the conservative SPM family-wise error (FWE), with cluster size ≥ 5 voxels. We used an inclusive mask to restrict analyses to voxels of white matter only. Stereotactic coordinates of significant clusters are reported in MNI space.

Individual analysis

An individual analysis was conducted by comparing voxel-wise diffusion values of each patient to those of the control group, using a two-sample t-test (Group 1, one patient; Group 2, all control subjects). Age and gender were used as covariates of no interest. We used a less conservative statistical threshold than that used for the group analysis, with a cut-off of P < 0.001 at the voxel level, and a correction for multiple comparisons at the cluster level (P < 0.05). According to the number of voxels tested within the explicit mask of interest (white matter), significant clusters needed to include more than 150 voxels.

Statistical parametric mapping analysis of brain volume

Group analysis

We tested whether differences between groups’ global and regional grey and white matter could account for diffusion findings. The global values of the grey matter, white matter and CSF volumes were obtained from non-normalized segmented images as well as regional volumetric differences between groups and assessed on a voxel-by-voxel basis. Voxel-based morphometry analysis was computed using an analysis of covariance with age, gender and the total amount of grey and white matter as confounders (ANCOVA).

Neuropsychological data analysis

Neuropsychological scores were compared between patients with BCECTS and control subjects using an unpaired t-test. Statistical significance was set to P < 0.005 to account for Bonferroni correction and the number of test performed (n = 10).

Clinical correlations

Multiple regression analyses (SPM8) were conducted to examine the relationship between diffusion maps and age (in patients and control subjects), duration of disease, age of onset, total number of seizures reported in seizure diary since onset of epilepsy, time since last seizure, presence of anti-epileptic drug treatment at the time of the study, and all cognitive scores. These analyses were conducted using masks corresponding to the significant clusters resulting from the SPM group analysis of the DTI data.
Results

Voxel-based analysis of diffusion tensor imaging

Patients showed significant differences as compared to control subjects on all four DTI parametric images, using the most conservative family-wise error corrected $P$-value < 0.05 (Table 2 and Fig. 1). Specifically, patients demonstrated: (i) decreased fractional anisotropy in the left pre- and postcentral gyri; (ii) increased mean diffusivity over the left pre- and postcentral gyri, left middle frontal gyrus, left inferior parietal lobule, left and right cuneus, right middle and medial frontal gyri; (iii) increased axial diffusivity over the left precentral gyrus, left superior parietal and paracentral lobule and right anterior cingulate gyrus; and (iv) increased radial diffusivity in the left pre- and postcentral gyri, left medial frontal gyrus, left inferior parietal lobule, left precuneus and right anterior and posterior cingulate gyri. No increases in fractional anisotropy or decreases in mean-, axial-, and radial diffusivity were detected in patients.

Single subject statistical parametric mapping analysis

Comparisons of each of the 25 patients with the control group also showed significant abnormalities in patients at the individual level, with decreased fractional anisotropy in nine (36%), increased mean diffusivity in 13 (52%), increased axial diffusivity in 11 (44%) and increased radial diffusivity in 13 (52%) (Supplementary Table 1). Although the abnormal pattern of decreased anisotropy and increased diffusivity varied among patients, clusters were mostly located in the frontal and parietal lobes, always predominating ipsilateral to, and often localized in the same brain region as, the main EEG focus. Apart from the corpus callosum, which was affected in almost all patients showing significant clusters, the main abnormal area was the operculomotor region. Increased diffusivity was generally more extensive than decreased fractional anisotropy, and accounted for all abnormalities discordant with the side of the EEG focus (Supplementary Table 1 and Supplementary Fig. 1A–D).

A few abnormalities were observed in individual patients in the opposite direction as the pattern described above, i.e. increased fractional anisotropy and decreased diffusivity. Increased fractional anisotropy was detected in two patients, one of whom also showed significantly decreased fractional anisotropy over the region of his EEG focus. Decreased diffusivity was found in four patients, mostly affecting the cuneus in two, left parietal lobule in one and the frontal lobes in the other.

Comparisons between each of the healthy children and the rest of the control group showed significant clusters at the individual level, with decreased fractional anisotropy in three control subjects.

Table 2 Group comparisons

<table>
<thead>
<tr>
<th>Left/Right</th>
<th>Area of white matter</th>
<th>P-value</th>
<th>K</th>
<th>Z-score</th>
<th>x, y, z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractional anisotropy maps, control subjects versus BCECTS</td>
<td>Left</td>
<td>Precentral gyrus</td>
<td>0.003</td>
<td>28</td>
<td>4.90</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Postcentral gyrus</td>
<td>0.012</td>
<td>11</td>
<td>4.66</td>
</tr>
<tr>
<td>Mean diffusivity maps, BCECTS versus control subjects</td>
<td>Left</td>
<td>Postcentral gyrus</td>
<td>0.000</td>
<td>1179</td>
<td>5.85</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Postcentral gyrus</td>
<td>0.006</td>
<td>63</td>
<td>5.09</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Cuneus</td>
<td>0.004</td>
<td>91</td>
<td>5.04</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Medial frontal gyrus</td>
<td>0.008</td>
<td>54</td>
<td>4.96</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Cuneus</td>
<td>0.007</td>
<td>56</td>
<td>4.82</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Middle frontal gyrus</td>
<td>0.011</td>
<td>41</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Inferior parietal lobule</td>
<td>0.018</td>
<td>23</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Cuneus</td>
<td>0.033</td>
<td>6</td>
<td>4.39</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Middle frontal gyrus</td>
<td>0.031</td>
<td>7</td>
<td>4.37</td>
</tr>
<tr>
<td>Axial diffusivity maps, BCECTS versus control subjects</td>
<td>Left</td>
<td>Precentral gyrus</td>
<td>0.000</td>
<td>188</td>
<td>5.94</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Superior parietal lobule</td>
<td>0.023</td>
<td>9</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Cingulate gyrus</td>
<td>0.010</td>
<td>26</td>
<td>4.62</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Paracentral lobule</td>
<td>0.010</td>
<td>26</td>
<td>4.57</td>
</tr>
<tr>
<td>Radial diffusivity maps, BCECTS versus control subjects</td>
<td>Left</td>
<td>Postcentral gyrus</td>
<td>0.000</td>
<td>362</td>
<td>5.66</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Precentral gyrus</td>
<td>0.002</td>
<td>66</td>
<td>5.40</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Inferior parietal lobule</td>
<td>0.004</td>
<td>51</td>
<td>5.18</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Medial frontal gyrus</td>
<td>0.001</td>
<td>82</td>
<td>4.91</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Precuneus</td>
<td>0.025</td>
<td>7</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>Precentral gyrus</td>
<td>0.010</td>
<td>25</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Posterior cingulate gyrus</td>
<td>0.013</td>
<td>18</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>Cingulate gyrus</td>
<td>0.015</td>
<td>16</td>
<td>4.52</td>
</tr>
</tbody>
</table>

FWE $P = 0.05$, $k = 5$; $x$, $y$, $z$ = coordinates for the local maxima of the cluster.
(12%), increased mean-, radial- and axial diffusivity also in three control subjects (12%), and a combination of increased fractional anisotropy and decreased diffusivity in one subject (4%). Clusters were smaller than those detected in patients and were located in the right posterior cingulate, left internal capsule, left cuneus and left inferior parietal lobule.

Volumetric measurements

There were no significant differences in the global brain volume (total grey matter, total white matter, and total intracranial volume) between patients and control subjects (Supplementary Table 2). Similarly, SPM analysis of covariance showed no significant group difference for grey or white matter in any region.

Results of neuropsychological assessment

Patients with BCECTS showed significantly lower performance than control subjects at the Conner’s scale for the following scores: hyperactivity/impulsivity (BCECTS 60.1 ± 12 versus control subjects 43.1 ± 8.1; \( P = 0.0036 \)) and indices of attention deficit hyperactivity disorder (BCECTS 59.7 ± 10 versus control subjects 42.7 ± 7.3; \( P = 0.00043 \)). A non-significant trend towards abnormal scores for conduct disorder (mean ± SD, BCECTS 52.6 ± 13.7 versus control subjects 45.1 ± 6.9; \( P = 0.0092 \)) and learning problems (BCECTS 56.7 ± 10.7 versus control subjects 44.2 ± 9.5; \( P = 0.0054 \)) was also observed.

Patients with BCECTS showed significantly lower performance than control subjects on the following WISC-IV subscales (Table 3): verbal comprehension index (mean ± SD, BCECTS 95.3 ± 18.9 versus control subjects 115.5 ± 14.6, \( P = 0.0039 \)) and processing speed index (BCECTS 86.2 ± 18.2 versus control subjects 113.8 ± 14.2, \( P = 0.0004 \)), with a non-significant trend for working memory index (BCECTS 89.9 ± 15.9 versus control subjects 103.2 ± 14.3, \( P = 0.028 \)). No differences between groups were observed for the perceptual reasoning index subscale and for the individual memory tests.

Some individual patients also showed scores below two standard deviations from the normal range: one patient on verbal
Table 3 Neuropsychological testing results

<table>
<thead>
<tr>
<th></th>
<th>Controls, mean ± SD</th>
<th>BCECTS, mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The Conners’ Parent Rating Scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactivity/impulsivity</td>
<td>43.1 ± 8.1</td>
<td>60.1 ± 12</td>
<td>0.0036*</td>
</tr>
<tr>
<td>Psychosomatic</td>
<td>50.1 ± 12.8</td>
<td>66.3 ± 14.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Learning Problems</td>
<td>44.2 ± 9.5</td>
<td>56.7 ± 10.7</td>
<td>0.0054</td>
</tr>
<tr>
<td>Indices of ADHD</td>
<td>42.7 ± 7.3</td>
<td>59.7 ± 10</td>
<td>0.004*</td>
</tr>
<tr>
<td>Anxiety</td>
<td>51.6 ± 9.5</td>
<td>55.1 ± 10.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Conduct Disorder</td>
<td>45.1 ± 6.9</td>
<td>52.6 ± 13.7</td>
<td>0.0092</td>
</tr>
<tr>
<td><strong>The Wechsler Intelligence Scale for Children</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal comprehension index</td>
<td>115.5 ± 14.6</td>
<td>95.3 ± 18.9</td>
<td>0.0039*</td>
</tr>
<tr>
<td>Perceptual reasoning index</td>
<td>106.3 ± 11.4</td>
<td>99.4 ± 13.8</td>
<td>0.16</td>
</tr>
<tr>
<td>Working memory index</td>
<td>103.2 ± 14.3</td>
<td>89.9 ± 15.9</td>
<td>0.028</td>
</tr>
<tr>
<td>Processing speed index</td>
<td>113.8 ± 14.2</td>
<td>86.2 ± 18.2</td>
<td>0.0004*</td>
</tr>
</tbody>
</table>

ADHD = attention deficit hyperactivity disorder; *P < 0.005 after Bonferroni correction.

Table 4 Correlation with neuropsychological variables in BCECTS

<table>
<thead>
<tr>
<th>Left/right</th>
<th>Area of white matter</th>
<th>P-value</th>
<th>k</th>
<th>Z-score</th>
<th>x, y, z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative correlation with anxiety score, Conner’s scale and fractional anisotropy maps</td>
<td>Precentral gyrus</td>
<td>0.038</td>
<td>8</td>
<td>2.75</td>
<td>−50, −6, 22</td>
</tr>
<tr>
<td>Negative correlation with learning score, Conner’s scale and fractional anisotropy maps</td>
<td>Precentral gyrus</td>
<td>0.022</td>
<td>7</td>
<td>2.82</td>
<td>−46, −28, 34</td>
</tr>
<tr>
<td>Positive correlation with processing speed index, fractional anisotropy maps, duration of epilepsy fractional anisotropy</td>
<td>Precentral gyrus</td>
<td>0.044</td>
<td>6</td>
<td>3.22</td>
<td>28, −18, 52</td>
</tr>
</tbody>
</table>

FWE P < 0.05, k = 5; x, y, z = coordinates for the local maxima of the cluster.

comprehension index (4%), two on working memory index (8%) and four on processing speed index (16%) subscales. In addition, four patients with BCECTS (16%) had a delay in oral or written language, six (24%) had an attention disorder and one (4%) suffered from dyspraxia.

Correlation analysis

Neuropsychological tests

Fractional anisotropy in the postcentral gyrus negatively correlated with anxiety and learning scores of the Conner’s scale (Table 4), whereas it positively correlated with the processing speed index (WISC-IV) in the precentral gyrus. Both correlations indicated greater fractional anisotropy abnormalities in patients with lower cognitive performance. Diffusivity maps showed no correlation with the neuropsychological tests.

Clinical variables

Fractional anisotropy correlated positively with age of onset and negatively with duration of epilepsy, indicating more abnormalities in patients with long duration of epilepsy Fig. 2. The correlation with age of onset was observed in the left and right precentral gyri, left parietal superior lobule and right medial frontal and cingulate gyri, whereas the correlation with duration of epilepsy was noted in the left pre- and postcentral gyri, right precentral gyrus and parietal lobe white matter. Mean diffusivity also positively correlated with duration of epilepsy in the right cuneus (Fig. 2, Supplementary Table 3), again indicating more abnormalities in patients with longer disease duration. All diffusivity parameters, but not fractional anisotropy, positively correlated with the number of anti-epileptic drugs, primarily over the cuneus and fusiform gyri (Supplementary Table 3). No correlations were observed in the opposite directions.

Post hoc analyses were performed to further investigate the impact of duration of epilepsy on anisotropy, diffusivity, and cognitive performance. We separated patients with BCECTS into those whose duration of epilepsy was >12 months (Subgroup 1, n = 12, mean age ± SD 9.9 ± 1.9 years, four females) and those whose duration of epilepsy was ≤12 months (Subgroup 2, n = 13, mean age ± SD 9.2 ± 2.0 years, three females) (Table 1). There was no significant difference between these two subgroups for age (P = 0.33) or gender (P = 0.59). Subgroup 1 showed significantly reduced fractional anisotropy and increased diffusivity values as compared to control subjects over the left pre- and postcentral gyri, as well as in many other brain regions, primarily left-sided (Supplementary Table 4). Conversely, Subgroup 2 did not show any significant difference compared to control subjects. Significant differences were also observed when directly comparing the two subgroups. Subgroup 1 demonstrated lower fractional anisotropy than Subgroup 2 in the cingulate gyrus, anterior corpus callosum, and precuneus, all bilaterally, as well as over the left inferior frontal and left transverse gyri, the right superior and medial frontal
gyri, and the right parahippocampal and fusiform gyri. Subgroup 1 also showed greater diffusivity than Subgroup 2 in the frontal lobes. These abnormalities were more diffuse for mean diffusivity than for axial- and radial diffusivity. Accordingly, all individual patients showing significant fractional anisotropy abnormalities, and the majority of those with significant mean-, axial-, or radial diffusivity abnormalities, belonged to Subgroup 1 (Supplementary Table 4).

Neuropsychological tests also proved to distinguish the two subgroups, with only Subgroup 1 showing significantly worse performance than control subjects on the processing speed index subscale of the WISC-IV (mean ± SD, BCECTS 79.3 ± 16.2 versus control subjects 110.9 ± 14.9; \( P = 0.0026 \)) whereas for conduct disorder score of the Conner’s scale (BCECTS 61.3 ± 17.2 versus control subjects 43.2 ± 5, \( P = 0.048 \)), and the two following subscales of the WISC-IV: verbal comprehension index (BCECTS 91.1 ± 17.9 versus control subjects 117.9 ± 12.2, \( P = 0.008 \)) and perceptual reasoning index (BCECTS 91.7 ± 7.5 versus control subjects 106.6 ± 10.1; \( P = 0.01 \)) there was an apparent trend towards significance.

Discussion

The present study found alterations in the microstructure of the white matter in brain regions affected by the epileptic focus in children with BCECTS, both at the group and individual levels. Abnormalities in fractional anisotropy and diffusivity predominated over the left pre- and postcentral gyri, with changes in diffusivity extending to adjacent brain regions. These alterations in the microstructure of the white matter were more marked in patients with longer duration of epilepsy and in those with worse cognitive performance, and were neither confounded by age nor by grey and white matter volumes. Although such cross-sectional findings cannot demonstrate causality, co-localization of EEG foci and DTI changes in BCECTS is consistent with the hypothesis that the two disorders might be directly related to each other, though one cannot exclude a third common causal factor. The greater DTI abnormalities observed with longer duration of epilepsy also supports the view that chronic interictal epileptiform discharges might lead to microstructural alterations of the white matter, rather than the opposite, an issue that will be later addressed by the ongoing longitudinal follow-up of our study population.

Normal brain development

It has been shown that fractional anisotropy and mean diffusivity follow exponential age trajectories (Schmithorst et al., 2002; Taki et al., 2012). Mature white matter tracts demonstrate high anisotropy values that have been linked to myelinization and brain maturation (Schneider et al., 2004). Fractional anisotropy increases from childhood to adulthood, reaches a peak between 20–42 years and then decreases during late adulthood at a rate slower than the initial increase. Mean diffusivity follows an opposite dynamic, initially decreasing, reaching a minimum between 18–43 years, and then increasing at a slower rate (Lebel et al., 2012). Furthermore, there is a difference in maturation between males and females with, for example, the left superior longitudinal fasciculus showing a slower rate of maturation in girls than in boys (Taki et al., 2012). This should be noted as we had more male subjects in the patient group (72%) than in the control group (56%). Though not statistically significant, this difference was partly taken into account by including gender as a covariate in all group- and individual analyses. Furthermore, the differences observed in healthy subjects point to higher fractional anisotropy and lower mean diffusivity values in males than females (Lebel et al., 2012). Given a greater proportion of males included in our patient group compared with the control group, the expected...
physiological gender differences in fractional anisotropy and mean diffusivity bias our main finding toward the null (i.e. decreased fractional anisotropy and increased mean diffusivity in patients). Finally, these physiological differences are primarily observed over the cingulum and superior longitudinal fasciculus (Lebel et al., 2012), and not within the pre- and postcentral regions where we detected our main abnormalities. Overall, it is unlikely that the non-significant gender difference between our patients’ and control populations have impacted our findings.

**Diffusion changes in epilepsy**

Diffusion parameters might change over short periods of time in patients with epilepsy as a consequence of seizures that are associated with an immediate decrease in diffusion, followed by an increase after 72 h (Diehl et al., 2001; Salmenera et al., 2006; Yu and Tan, 2008). However, most DTI studies in epilepsy have concentrated on interictal findings, when diffusion is typically increased (Thivard et al., 2006). This is particularly relevant for BCECTS where the occurrence of seizures is very rare, from one to six over the entire duration of the disease in 70% of cases (Rosser, 2007). Intercital DTI studies in children with epilepsy have mostly been performed in temporal lobe epilepsy, showing reduction of fractional anisotropy in the hippocampus (Kimiwada et al., 2006) and in the white matter tracts ipsilateral to left temporal foci (Govindan et al., 2008), or increased mean-, axial- and radial diffusivity in the temporal and cingulate white matter (Lee et al., 2004; Nilsson et al., 2008). Similarly, the coefficient of diffusion (ACD), which is often used interchangeably with mean diffusivity, increased interictally in the epileptogenic hippocampus of adult patients with temporal lobe epilepsy (Kantarci et al., 2002; Duzel et al., 2004). Abnormalities of fractional anisotropy and mean diffusivity have also been reported in malformations of cortical development, as well as within normal appearing epileptic tissue (Eriksson et al., 2001; Rugg-Gunn et al., 2001). A few studies have addressed the issue of white matter integrity in children with new onset epilepsy in relation to cognitive development (Hutchinson et al., 2010; Widjaja et al., 2012). These studies included populations of mixed epilepsy syndromes, including only a minority of BCECTS (four in Hutchinson et al. (2010) and none mentioned in Widjaja et al. (2012)), and reported decreased fractional anisotropy and increased radial diffusivity in the posterior corpus callosum (Hutchinson et al., 2010), increased radial diffusivity in the cingulum (Hutchinson et al., 2010; Widjaja et al., 2012), and increased axial diffusivity in the left middle frontal region (Widjaja et al., 2012). Although many of our patients demonstrated abnormalities in the corpus callosum, it is interesting to note that this region failed to show significant difference at the group level, a finding that might reflect the variation in the portion of the corpus callosum affected in each patient.

Our data in BCECTS showed localized regions of white matter alteration in brain regions that have not been reported in previous studies. Specifically, the white matter in the left pre- and postcentral gyri demonstrated abnormalities in all four diffusion maps consistent with the usual location of the epileptic foci delineated in BCECTS (Baumgartner et al., 1996; Pataria et al., 2008). Accordingly, functional MRI of epileptic spikes in BCECTS patients have shown increased BOLD responses in the face area around the central sulcus (Archer et al., 2003). The left side predominance of DTI abnormalities reported in our study appears likely to reflect the fact that the majority of our patients had a left-sided EEG focus. Indeed, diffusion changes ipsilateral to the epileptic focus have been observed in other epilepsy syndromes (Kimiwada et al., 2006; Govindan et al., 2008; Meng et al., 2010). The group findings observed in our BCECTS population were strengthened by similar observations at the individual level in many patients. The interindividual variability did not seem to reflect the potential impact of seizures on diffusion, given that there was no correlation between DTI changes and seizure frequency, and MRI was performed >2 days after the last seizure in all patients, and >1 month in 88% of patients. All of our patients had a typical EEG pattern for BCECTS and underwent an EEG investigation within the 2 months preceding the MRI examination. However, EEG abnormalities in BCECTS might shift from one side to the other according to a dynamic that might not have been captured by available EEGs, possibly accounting for the few discords observed between the lateralization of DTI and EEG abnormalities at the individual level.

In principle, alterations of the white matter microstructure milieu might result from small vessel alterations, loss of axonal structure, or gliosis, all of which reduce directional diffusion (Niquet et al., 1995; Oberheim et al., 2008). In non-lesional epilepsy, decreased fractional anisotropy is usually considered a reflection of the disruption of axonal integrity, and more specifically, of altered density or organization of fibres. However, it may also reflect myelin abnormalities. Increased diffusivity (mean, axial and radial) is usually thought to result from decreased tissue density, reflecting cell loss of both neurons and glia, or, as described in developmental studies, a delayed maturation. This latter hypothesis seems most likely in BCECTS, which age-dependent onset and remission has long suggested an underlying abnormality of brain maturation (Panayiotopoulos et al., 2008).

The possibility that brain regions generating centro-temporal spikes also suffer delayed maturation in BCECTS raises speculative hypotheses on the relation between these two abnormalities. One hypothesis would be that both EEG and DTI findings derive from another common factor, such as a genetic predisposition to cortical hyperexcitability and delayed maturation. The genetic background of BCECTS is a complex issue however, with some twin data suggesting that the typical form of this idiopathic syndrome is not heavily dependent on traditional genetic factors (Vadamudi et al., 2004), whereas atypical forms of BCECTS with language impairment are more likely to share a genetic origin. Indeed, recent reports have disclosed various genes, such as SRPX2, GRIN2A and ELPL4, which mutation of could lead to such conditions (Rudolf et al., 2009; Lesca et al., 2013). Another possibility would be that delayed brain maturation, regardless of its mechanisms, would be responsible for the development of cortical hyperexcitability and BCECTS electroclinical features. Conversely, the centro-temporal spike focus could interfere with the biological process underlying brain maturation, or produce irreversible alterations of neuronal connectivity in the developing brain (Holmes and Ben-Ari, 2001). In fact, all of the three above hypotheses could be combined. The observation that duration of epilepsy...
was the main factor associated with interindividual DTI differences in our BCECTS population does not necessarily help clarify this issue, but is consistent with the view that delayed maturation might partly result from prolonged interictal EEG abnormalities. Indeed, all patients showing significantly decreased fractional anisotropy suffered from BCECTS for >1 year. Furthermore, the longer the duration of the disease, the greater the reduction in fractional anisotropy and increase in diffusivity around the left central sulcus. Weaker associations were also found with age of onset, most likely reflecting the role of epilepsy duration, given that these two clinical variables strongly correlated with each other. Alternatively, one might hypothesize that younger children might be more sensitive to the deleterious impact of BCECTS on brain maturation. Interestingly, adults, children and adolescents with temporal lobe epilepsy also demonstrate fractional anisotropy and axial diffusivity abnormalities that correlate with duration of epilepsy, suggesting a causal role of the epileptic activity in promoting DTI changes across epilepsy syndromes and age (Meng et al., 2010; Keller et al., 2012). In these series, however, duration of epilepsy might have been confounded by duration of treatment, which is unlikely to be the case in our study. Indeed, changes in fractional anisotropy did not correlate with the number of anti-epileptic drug treatments, whereas diffusivity maps only showed modest correlation with this parameter over small sized clusters.

Despite its benign seizure outcome, BCECTS is often associated with mild neuropsychological and/or learning disability such as low academic achievement in reading, numeracy and/or spelling ability, drawing, visuo-spatial skills, attention, visuo-spatial memory (Baglietto et al., 2001; Pinton et al., 2006; Goldberg-Stern et al., 2010; Bedoin et al., 2012), cognitive flexibility, picture naming, fluency, visuo-perceptual skills and visuo-motor coordination (Baglietto et al., 2001; Brancati et al., 2012). Patients also exhibit difficulties in phonologic awareness that affects literacy, with subsequent memory problems and poor academic performance (Northcott et al., 2005, 2007). Similar to these findings, our patients had more learning difficulties at school and exhibited deficient scores on neuropsychological scales testing attention hyperactivity disorder, visuo-motor coordination, written and spoken language, verbal reasoning, working memory and processing speed. Some of these abnormalities were more pronounced in patients with longer duration of epilepsy, including those detected with the Conductor Disorders score of Conner’s Scale, and the verbal comprehension index, perceptual reasoning index and processing speed index subscales of the WISC-IV. Most importantly, fractional anisotropy abnormalities correlated with the processing speed index of WISC-IV and the anxiety and learning scores of the Conner’s scale. This association between DTI abnormalities and cognitive disturbances in BCECTS raises the same unsolved issue as that discussed above regarding the respective role and impact on cognition of an underlying genetic predisposition, interictal EEG abnormalities and delayed maturation. Indeed, the cross-sectional design of our study does not allow for a conclusion on causality between these various abnormalities. The ongoing 5-year longitudinal follow-up of our cohorts of patients with BCECTS and control children should help partly address this issue in the future. Specifically, the dynamic of cognitive, EEG and DTI findings over time will provide insights into their potential relationship. For example, we might observe that children recruited in our study at the onset of their epilepsy and who did not demonstrate delayed maturation at that stage, will later develop such abnormalities, supporting the view that interictal EEG discharges have an impact on brain maturation. This view would also be supported by the observation that brain maturation over the central region may improve or normalize once the EEG focus has faded out. Conversely, DTI abnormalities might prove stable over time, suggesting a predisposing condition that would promote the development of the more severe forms of BCECTS associated with cognitive dysfunction. The possibility that such a predisposing condition may be genetically driven might also be investigated in the future since most of our patients underwent blood sampling for this purpose.

The detection of DTI abnormalities at the individual level in BCECTS patients raises the possibility of using such information as a clinically useful biomarker. This would first require demonstrating that this biomarker helps in assessing prognosis or decision making regarding treatment, which is currently not the case. Moreover, to detect DTI abnormalities at the individual level, one needs to compare patients’ magnetic resonance images to a normal database of age-matched children, ideally acquired on the same scan. This represents a strong limitation to the development of DTI for clinical purpose. Quantification of intra-individual DTI changes over time, which is currently underway, might offer a solution to this problem.

**Acknowledgements**

We thank all subjects that took part in this research, as well as their parents for the time spent during all evaluations. We are indebted to many healthy volunteers families and teaching personnel from school “Sainte Marie Lyon les Maristes”. In addition we would like to thank Véronique Laplane for her invaluable help with control subjects recruitment. We would also like to thank the medical staff at CEMERPE and nursing and secretary staff from the Department of Epilepsy, Sleep and Pediatric Neurophysiology for their collaboration and assistance and Danielle Ibarrola for technical support.

**Funding**

The study was supported by a grant from PICRI (Partenariats Institution-Citoyens pour la Recherche et l’Innovation) Région Ile-de-France, Epilepsie France, Fondation Le Roch-Les Mousquetaires and Fondation IDEE. Dr Ciumas received financial support from Fondation pour la Recherche Médicale. Dr Laurent received financial support from PICRI. Dr Amélie Lothe and Faustine Ilski received financial support from Fondation Le Roch-Les Mousquetaires.

**Supplementary material**

Supplementary material is available at *Brain* online.
References


Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed 1995; 8: 333–44.


