Age at onset and seizure frequency affect white matter diffusion coefficient in patients with mesial temporal lobe epilepsy

Szilvia A. Nagy[a,b], Réka Horváth[c], Gábor Perlaki[a,d], Gergely Orsi[a,d], Péter Barsi[e], Flóra John[c], Andrea Horváth[a,f], Norbert Kovács[c,d], Péter Bogner[g], Hajnalka Ábrahám[h,i], Beáta Bóné[c], Csilla Gyimesi[c], Tamás Dóczi[a,d,f], József Janszky[c,d,*]

The first two authors contributed equally to this work.

[a]Pécs Diagnostics Center, H-7623 Pécs, Rét Street 2., Hungary

[b]MTA - PTE Neurobiology of Stress Research Group, H-7624 Pécs, Ifjúság Street 20., Hungary

[c]Department of Neurology, University of Pécs, H-7623 Pécs, Rét Street 2., Hungary

[d]MTA-PTE Clinical Neuroscience MR Research Group, H-7623 Pécs, Rét Street 2., Hungary

[e]MR Research Centre, Semmelweis University, H-1083 Budapest, Balassa Street 6., Hungary

[f]Department of Neurosurgery, University of Pécs, H-7623 Pécs, Rét Street 2., Hungary

[g]Department of Radiology, University of Pécs, H-7624 Pécs, Ifjúság Street 13., Hungary

[h]Department of Medical Biology, University of Pécs, H-7624 Pécs, Szigeti Street 12., Hungary

[i]Central Electron Microscopic Laboratory, University of Pécs, H-7624 Pécs, Honvéd Street 1., Hungary

E-mail addresses: szilvia.anett.nagy@gmail.com (S. A. Nagy), horvath.reka@pte.hu (R. Horváth), petzinger.gabor@gmail.com (G. Perlaki), gergo.orsi@gmail.com (G. Orsi), pbarsi@mrkk.sote.hu (P. Barsi), john.flora04@gmail.com (F. John), andrhorvath@gmail.com (A. Horváth), kovacs.norbert@pte.hu (N. Kovács), bogner.peter@pte.hu (P. Bogner), hajnalka.abraham@aok.pte.hu (H. Ábrahám), bone.beata@pte.hu (B. Bóné), gyimesi.csilla@pte.hu (Cs. Gyimesi), doczi.tamas@pte.hu (T. Dóczi)

[*]Address correspondence to Professor József Janszky, Department of Neurology, University of Pécs, H-7623 Pécs, Rét Street 2., Hungary, Phone: 003672535900, Fax: 003672535911, E-mail: janszky.jozsef@pte.hu

Summary

In mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS), structural abnormalities are present not only in the hippocampus, but also in the white matter with ipsilateral predominance. Although the timing of epilepsy onset is commonly associated with clinical and semiological dissimilarities, limited data exist regarding white matter diffusion changes with respect to age at epilepsy onset. The aim of this study was to investigate diffusion changes in the white matter of unilateral MTLE-HS patients with respect to clinical parameters and to compare them to an age- and sex matched healthy control group. Apparent diffusion coefficients (ADCs) were derived using mono-exponential approaches from 22 (11 early and 11 late age at onset) unilateral MTLE-HS patients and 22 age- and sex matched control subjects after acquiring diffusion-weighted images on a 3T MRI system. Data were analyzed using two-tailed t-tests and multiple linear regression models. In the early onset MTLE-HS group ADC was significantly elevated in the ipsilateral hemispheric ($p=0.04$) and temporal lobe white matter ($p=0.01$) compared to controls. These differences were not detectable in late onset MTLE-HS patients. ADC of the early onset MTLE-HS group was negatively related to age at epilepsy onset in the ipsilateral hemispheric white matter ($p=0.03$) and the uncinate fasciculus ($p=0.03$), while in late onset patients ADC was no longer dependent on age at epilepsy onset itself, but rather on the seizure frequency in the ipsilateral uncinate fasciculus ($p=0.03$). Such diffusivity pattern has been associated with chronic white matter degeneration, reflecting myelin loss and higher extracellular volume which are more pronounced in the fronto-temporal regions and also depends on clinical features. In the early onset group, the timing of epilepsy seems to be the major cause of white matter abnormalities while in late onset disease it has a secondary role in provoking diffusion changes.

Keywords: Mesial temporal lobe epilepsy with hippocampal sclerosis, age at epilepsy onset, diffusion-weighted imaging, apparent diffusion coefficient

1. Introduction

Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) is the most common focal epilepsy syndrome in adults [1-3]. It is well known that structural abnormalities associated with MTLE-HS involve not only the hippocampus and the structures of the limbic system [4-7], but also the bilateral white matter (WM) with ipsilateral predominance [8-17]. It might be hypothesized that neuronal dysfunction/loss in the sclerotic hippocampus causes damage to WM structures connected to the epileptic foci due to the excitotoxic effects of the spreading epileptogenic activity which then induces microstructural abnormalities far away from the seizure focus [8, 18]. Over the last years, diffusion-weighted imaging (DWI) was established as a useful tool to measure microstructural changes of the brain tissue in a variety of neurological diseases [19, 20], including epilepsy [21]. Most of these studies focus on specific individual WM tracts and make inferences about microstructural integrity of axons and myelin sheaths through the analysis of diffusivity perpendicular and parallel to the tracts. In our previous study we found that the age at epilepsy onset (AAO) may distinguish different clinical subgroups in MTLE-HS [22]. The AAO is commonly associated with clinical and semiological dissimilarities [22, 23]. Although the age at first non-provoked seizure can have a decisive effect on brain tissues in MTLE-HS, limited data exist regarding WM diffusion changes with respect to the epilepsy-related clinical parameters such as AAO and chronic seizure activity. To shed light on these issues, we investigated diffusion changes in the WM of MTLE-HS patients with respect to clinical parameters and compared them to an age- and sex matched healthy control group. We hypothesized that chronic WM damage occurs in both the

early and the late onset disease groups, but the extent of WM damage is different according to the timing of epilepsy onset.

2. Materials and methods

The experimental protocol was approved by the local Ethical Committee and performed in accordance with the ethical standards described in the Declaration of Helsinki (1964). Participants were informed about the procedure and they signed an informed consent prior to the examination.

2.1. Subjects

Inclusion criteria for patients:

(1) Detailed presurgical evaluation or under the care of outpatient clinic

(2) Head magnetic resonance imaging (MRI) with epilepsy specific protocol

(3) Unilateral hippocampal sclerosis

Twenty-two patients (14 female) with temporal lobe epilepsy accompanied by unilateral hippocampal sclerosis were collected in the study (14 right-sided, 8 left-sided). All patients in the study presented initially to the tertiary epilepsy center and underwent a 32- to 64 -channel surface non-invasive electroencephalogram (EEG) monitoring. The electrodes were placed according to 10–10 system; the number of electrodes and their placement varied individually corresponding to the suspected epileptogenic region and side. FP1, F3, C3, P3, O1, F7, FT7, T7, TP7, P7, FT9, TP9, homologous right-sided electrodes, PZ, CZ, FZ were always used. Thirteen patients underwent an adult presurgical evaluation protocol including continuous video-EEG monitoring, neuropsychological testing and high resolution head MRI with epilepsy specific protocol. The remaining nine patients were examined with long term EEG and they also had high resolution head MRI with epilepsy specific protocol. Clinical MR images were analyzed by a trained neuroradiologist (P. Ba.) with 23 years of experience in

epilepsy imaging. Neither high resolution head MRI with epilepsy specific protocol, nor presurgical evaluation using continuous video-EEG monitoring showed the existence of bitemporal epilepsy. Patients with neurodegenerative disorders, intracranial tumors, cognitive deficits and those who underwent previous brain surgery were excluded from the study. The following clinical data were collected for each MTLE-HS patient before the MRI examination: age, sex, seizure frequency, AAO, disease duration, level of education, history of febrile seizure, occurrence of generalized tonic-clonic seizure within the last 5 years, current and previous antiepileptic drugs, head trauma prior to AAO, history of brain infections such as encephalitis/meningitis and possible perinatal complication. Seizure frequency was determined from the seizure diary completed by the patient and defined as the mean frequency of complex partial seizures per month during at least the last 12 months prior to enrollment. In order to examine the effect of AAO on WM diffusion, patients were divided into two groups according to the median AAO: one group of early onset patients (N=11; 9F) with AAO≤16 years and the other group of late onset subjects (N=11; 5 F) with AAO>16 years. All patients were taking antiepileptic medication at the time of study. 6 patients (1 early onset MTLE-HS patient) were treated with a single drug (carbamazepine [CBZ], levetiracetam [LEV], oxcarbazepine [OXCBZ] or valproate [VPA], whereas sixteen patients (10 early onset MTLE-HS patient) received more than one drug. Lamotrigine (LTG) and clobazam were used most frequently as adjunct drugs in these cases. CBZ/OXCBZ or sodium channel blockers (LTG, CBZ, OXCBZ and Lacosamide) as a group or drug polytherapy showed no significant effect on diffusion in the ipsilateral hemispheric, temporal lobe WM or in the uncinate fasciculus. Therefore these variables were not considered as confounding factors in the final statistical models. Clinical and demographic details for MTLE-HS patients are summarized in Table 1.

22 healthy control subjects (14 female; age range 19-66 years; mean age 43.5±14.4 years) were also examined and matched for age and sex to the early (N=11, 9 female age range 19-56 years; mean age 39.4±13.9 years) and the late (N=11, 5 female age range 21-66 years; mean age 47.6±14.2 years) onset patient groups. Control volunteers were not included if they had alcohol or drug abuse, psychiatric illness, traumatic brain injury or history of significant medical or neurological conditions that would be associated with remarkable changes in the brain such as seizures, stroke or migraine headache [24].

2.2. Magnetic Resonance Imaging

All the measurements were carried out using a 3T Magnetom TIM Trio whole-body MRI scanner (Siemens AG, Erlangen, Germany).

In all patients at least two MRI were performed:

a) an MRI with epilepsy specific protocol which is part of our standard clinical practice and of inclusion criteria for study purpose to screen HS. Beyond the routine axial, coronal T2-weighted measurements, the epilepsy specific protocol also contained the 2D FLAIR imaging sequence with fat suppression and T1-weighted high-resolution images (voxel size: 0.98x0.98x0.98) to detect subtle structural alterations in the mesial temporal lobe. Routine DWI was carried out to localize the epileptogenic foci.

b) An MRI with research protocol was also obtained for all patients between January 2013 and June 2015 at least 48 hours after the last ictus. For tissue segmentation and registration a T1-weighted 3D magnetization-prepared rapid acquisition with gradient echo (MPRAGE) sequence was utilized using the following parameters: TR/TE/TI = 2530/3.4/1100 ms; flip angle = 7 degrees; 176 sagittal slices; slice thickness = 1 mm; field of view = 256x256 mm^2; matrix size = 256 x 256; bandwidth = 200 Hz/pixel. To investigate diffusion signal changes, a diffusion-weighted 2D spin-echo echo-planar imaging sequence using a 3-scan trace mode

was performed (TR/TE = 4800/128 ms; slice thickness = 3.5 mm; interslice gap= 1 mm; field of view = 188x250 mm^2; matrix size = 144x192; bandwidth = 1302 Hz/pixel; number of averages = 5; b-values = 0, 500, 1000 s/mm^2). Diffusion weighting was applied in the (1.0 1.0 -0.5), (1.0 -0.5 1.0) and (-0.5 1.0 1.0) directions. Trace image was formed for each b-value (except b = 0 s/mm^2) by calculating the geometric mean (I_{xyz}) of the signals in the three orthogonal spatial directions (I_x, I_y and I_z) (E.q.1):

$$I_{xyz} = \sqrt[3]{I_x + I_y + I_z} = I_0 \times exp^{-b(ADC_{xx}+ADC_{yy}+ADC_{zz})/3} = I_0 \times exp^{-b\times ADC}, \quad \text{E.q.1}$$

where I_0 is the signal intensity without diffusion weighting, b is the b-value, ADC_{xx}, ADC_{yy}, ADC_{zz} are the apparent diffusion coefficients measured along the three diffusion-sensitizing directions and the ADC is the average of them (E.q.2):

$$\text{ADC} = \frac{ADC_{xx}+ADC_{yy}+ADC_{zz}}{3} \quad \text{E.q.2}$$

2.3. Image processing

2.3.1. Preprocessing

Diffusion data were first corrected for eddy current distortion and simple head motion using a 12 degrees of freedom affine registration to the volume without diffusion-weighting [25]. The Brain Extraction Tool, provided by FMRIB Software Library, was applied on both MPRAGE and DWI data to eliminate non-brain tissues [26].

2.3.2. Regions of interest analysis

Subcortical hemispheric WM was segmented by FMRIB's Automated Segmentation Tool to evaluate global white matter deviations [27]. In order to examine regional subcortical WM abnormalities, frontal, temporal, parietal and occipital WM lobes were segmented using Freesurfer 5.3 image analysis suite (https://surfer.nmr.mgh.harvard.edu/) [28]. Error correction was performed when necessary, based on the recommended reconstruction

workflow (https://surfer.nmr.mgh.harvard.edu/fswiki/RecommendedReconstruction). To evaluate diffusion changes in specific WM bundles, three regions of the limbic tracts (left and right Hippocampal Cingulum, Fornix cres with Stria terminalis, Uncinate fasciculus) and one region of association tract (left and right Sagittal Stratum including inferior longitudinal fasciculus and inferior fronto-occipital fasciculus) were created as Regions of Interest (ROIs) using ICBM-DTI-WM atlas with reference to the MRI Atlas of Human White Matter 2nd edition [29]. These regions were chosen because they present the location where functional abnormalities have been consistently observed in patients with MTLE-HS [30, 31]. Image registrations were performed using FMRIB's Linear- and Non-Linear Image Registration Tools (FLIRT and FNIRT) [32, 33]. For tract based analysis, MPRAGE images were spatially registered into MNI152 T1-weighted 1mm standard space image using a two-step process:

1) Brain-extracted MPRAGE was linearly registered to standard space with 12 degrees-of-freedom,

2) after registration was refined by FNIRT, an inverse transformation was applied to warp the ICBM-DTI-WM atlas labels into the MPRAGE space.

In order to derive WM labels, the brain-extracted b0 image of each subject was linearly registered to that subject's brain-extracted MPRAGE image using a 6 degrees-of-freedom linear fit. Finally, the inverse of the spatial transformation from diffusion space to MPRAGE space was applied to align the segmented brain masks to diffusion space, where diffusion analyses were performed.

The resulting masks were eroded by using a 2D kernel of 3x3x1 voxels to avoid partial volume effects and to minimize possible impacts of misregistration. ROIs were defined as the eroded WM masks and obtained separately for both left and right sides of the structures (Figure 1).

2.4. Diffusion analysis

Diffusion data processing was carried out using Matlab® software's curve fitting toolbox and a self-written program code (The MathWorks, Inc., Natick, MA). ADC was measured within each ROI by calculating and mono-exponentially fitting the mean signal intensity for the b0, b500 and b1000 images using the equation of E.q.1.

2.5. Statistical evaluation

Data analyses were performed using SPSS® statistical software version 20.0 (IBM Corp., Armonk, NY). Differences were estimated by independent two-tailed t-tests for Gaussian values. Before using parametric t-tests, the homogeneity of variance was inspected by Levene's test, while the normality of data was examined by Shapiro-Wilk statistics for independent samples. Welch's correction was applied for parametric data demonstrating unequal variances. Multiple linear regression models were constructed to determine whether ADC was related to predictors, such as AAO, seizure frequency, age, sex in patients while age and sex in the control group. The assumptions of multiple linear regressions were satisfied, as judged by testing for linearity, normality assumptions of the residues, outliers, independence of errors, homoscedasticity and multi-collinearity [34]. Results were considered significant at $p \leq 0.05$ for all statistical tests.

3. Results

ADC values measured in patients were presented as the side ipsilateral and contralateral to the seizure focus and compared to the corresponding side of healthy controls.

3.1.Differences between groups

Except for the ipsilateral hemispheric, temporal lobe WM and the uncinate fasciculus, none of the other regions showed ADC difference between patients and controls. There was a significant ADC difference of the ipsilateral hemispheric WM between the whole patient and control groups (Figure 2a). Here, patients in the early onset MTLE-HS group showed higher ADC compared to their matched controls, but it disappeared in the late onset group (Table 2). In the ipsilateral temporal lobe WM, ADC was only tendentiously higher ($p=0.056$) in the whole patient group compared to controls (Figure 2b). Conversely, ADC was significantly elevated ($p=0.01$) in patients with early onset MTLE-HS (8.21 ± 0.30) compared to controls (7.88 ± 0.29), but this association was missing in late onset patients (Table 2).

In the ipsilateral uncinate fasciculus, a significant ADC difference was observed ($p=0.02$) between the whole patient (7.93 ± 0.50) and control groups (7.61 ± 0.36), however neither the early nor the late onset patient groups showed significant ADC elevation compared to controls (Table 2).

3.2.Differences within groups

Patients with early onset disease had higher ADC ($p=0.04$) in the ipsilateral side of temporal lobe WM (8.21 ± 0.30) compared to the contralateral side (7.97 ± 0.20) but this difference was not present in the late onset group. Besides the temporal lobe WM, data derived from patients were not significantly different between the ipsi- and the contralateral sides.

Multiple linear regression analysis indicated significant effects of AAO, age and seizure frequency on ADC, while no sex differences were observed in patients. There were no significant effects of either age or sex on ADC in controls.

In the whole patient group, ADC showed negative relationship with AAO in the ipsilateral side of the hemispheric, temporal lobe WM and the uncinate fasciculus while ADC was

positively related to age in the ipsilateral hemispheric WM and the uncinate fasciculus (corrected p and r values in Table 3.).

In early onset patients, ADC was negatively related to AAO in the ipsilateral hemispheric WM (Table 3.) and the uncinate fasciculus (Table 3, Figure 3a). In the ipsilateral temporal lobe WM, ADC tended to decrease as a function of AAO, but no significant correlation was found between them (Table 3).

In late onset patients, AAO had no contribution to ADC, but seizure frequency revealed a significantly positive influence on diffusion in the ipsilateral uncinate fasciculus (Table 3, Figure 3b).

4. Discussion

The aim of this study was to evaluate diffusion changes in hemispheric, lobar WM and selected bundles in patients with MTLE-HS and to examine their relation to clinical parameters. Three major findings emerged:

(1) The ipsilateral hemispheric WM and the uncinate fasciculus showed ADC differences between patients and controls.

(2) In the early onset MTLE-HS group, ADC was significantly elevated not only in the ipsilateral hemispheric WM but also in the ipsilateral temporal lobe compared to matched controls. These differences were not detectable in the late onset MTLE-HS group.

(3) AAO was of statistical significance as demonstrated by the negative relationship with ADC in the ipsilateral hemispheric, temporal lobe WM and the uncinate fasciculus. In the early onset group, ADC was negatively related to AAO in the ipsilateral hemispheric WM and

the uncinate fasciculus. In the late onset group, seizure frequency had positive influence on ADC in the ipsilateral uncinate fasciculus.

A number of structural and DWI studies examined the presence and the extent of WM abnormalities in temporal lobe epilepsy. Quantitative MR volumetric data showed that temporal lobe epilepsy with unilateral hippocampal sclerosis is associated with significant reduction in the cerebral WM volume in temporal and extratemporal regions [8]. The causality between the clinical markers (e. g. recurrent seizure-related factors, underlying pathology) and the temporal/extratemporal WM volumetric abnormalities was found to be controversial [9]. Seidenberg et al. found a greater reduction in the ipsilateral WM volume relative to the contralateral side [8]. This volume asymmetry was associated with both the duration of epilepsy and the AAO. According to others, WM differences in temporal lobe epilepsy patients were detectable compared to healthy controls but they were more pronounced in the early onset group [16, 23]. Additionally, the earlier AAO rather than the duration of the disease was associated with reduced cerebral WM volume, and it was not related to initial precipitating injury, occurrence/number of generalized seizures and number of antiepileptic drugs [9]. Beyond the volumetric studies there have been numerous reports based on diffusion imaging in adult temporal lobe epilepsy patients in which bilateral WM alterations can usually be detected in the limbic and non-limbic areas with ipsilateral predominance [10-16]. These changes are more extensive in patients with mesiotemporal sclerosis than in the non-lesional group [11, 18]. Although the underlying cause is not clear for such alterations, several possible theories exist. According to one hypothesis, these changes may be the result of neuronal loss in epileptic foci causing secondary axonal degeneration far away from the seizure focus. There are important fronto-temporal tracts or other pathways connecting the two temporal lobes or hemispheres (e. g. uncinate fasciculi, arcuate fasciculi, corpus callosum) which are commonly associated with diffusion alterations.

These are frequently involved in seizure propagation from the temporal lobe and might explain the role of recurring epileptic activity with abnormal neuronal firing on WM abnormalities. Findings of WM diffusion alterations have also been complemented by gray matter disruption. These studies have demonstrated bilateral extensive cortical gray matter volume loss in MTLE-HS [35] which is greater in patients with left sided disease [36]. The extant of gray matter disruption is closely related to the start of the disease [37] and also more severe in regions that are anatomically and functionally connected to the hippocampus [38, 39]. An extensive literature has shown disruptions in inter-regional fiber diffusivity in the mesiotemporal and the fronto-temporal networks [30, 40] and in the speech dominant hemisphere [41] suggesting decreased fiber arrangement and altered myelin membranes. These findings support the hypothesis that gray matter volume loss and WM damage may be related to each other and probably due to the excitotoxic effects of spreading epileptogenic activity in brain regions that are directly or indirectly connected to the hippocampus.

Our results revealed that ADC derived from the ipsilateral hemispheric WM of MTLE-HS patients was higher compared to the corresponding side of controls. These are consistent with previous findings suggesting that WM abnormalities are most pronounced in the cerebral hemisphere ipsilateral to the seizure focus [16, 30, 42]. Additionally, ADC of early onset MTLE-HS patients was elevated not only in the ipsilateral hemispheric but also in the ipsilateral temporal lobe WM compared to controls, however this differences were not detectable in late onset MTLE-HS group. According to animal and human studies, the impairment of WM is related to age at recurrent seizure onset and being more severe when seizures appeared at younger ages [9, 43]. This could be explained either by a primary pathology or by different neurodevelopmental processes precipitating epilepsy. However, it cannot be ruled out, that the adverse effect of the recurrent seizures in the developing, immature brain might lead to WM impairment in early onset epilepsy. Indeed, myelination

and the consequent cerebral volume increase is a long lasting event, and occurs in the hippocampus and in the cerebral white matter beyond the age of 16 (the age limit of the early onset group in our study) [44].

In addition, we found that, ADC of early onset group was negatively related to AAO in the ipsilateral hemispheric WM and the uncinate fasciculus. But in the late onset group the seizure frequency had a positive influence on ADC in the ipsilateral uncinate fasciculus. It could be assumed that different pathophysiological mechanisms (such as seizure-related injury as well as epileptogenesis) and etiological factors do not equally participate in WM degeneration. In agreement with others, our results revealed that the uncinate fasciculus was impaired, particularly ipsilateral to the epileptogenic focus [15]. This impairment was related to the timing of epilepsy in the early onset MTLE-HS group while in late onset disease it was probably coming from chronic seizure activity.

The current study has some limitations. (1) We should emphasize that one of the limitations is the low number of patients and controls involved. ADC in the ipsilateral temporal lobe of early onset patients tended to decrease as a function of AAO but no significant correlation was found between them. Probably the effect of AAO averaged out leaving no difference in diffusion due to the relatively small number of observations and large number of predictors. (2) In this study, 13 of the 22 patients underwent an adult presurgical evaluation protocol, therefore the possibility of subtle bilateral disease is not ruled out. (3) In order to perform powerful statistical analysis, patients were divided into two groups according to the median AAO as early and late onset MTLE-HS groups. Although, no significant differences were observed in clinical and demographic data (e.g. seizure frequency, level of education), the comparison of white matter diffusivity between the early and the late onset groups may be problematic due to the atypical average AAO of late onset patients. (4) Beside the timing of seizure onset, disease duration also plays an important role on MTLE-HS; however previous

reports support the hypothesis that the age at initial precipitating injury might play the major role in the development of mesial temporal sclerosis [38]. (5) The frequency of seizures may change over years in MTLE-HS patients, which can also have dominant influence on white matter integrity. To shed light on this relationship, a longitudinal study is needed using a larger patient population. (6) Since subtle abnormalities may remain hidden with hemispheric and lobar approaches, particularly when they occur in small fractions of the brain, we performed diffusion analysis also in the tracts connected to the temporal lobe. In spite of this, diffusion tensor imaging would have been beneficial to investigate tract based WM deviation.

5. Conclusion

Taken together, ADC was higher in MTLE-HS, especially in the cerebral hemisphere ipsilateral to the seizure onset. Such diffusivity pattern has been associated with chronic WM degeneration, reflecting myelin and axonal loss, gliosis and higher extracellular volume which are more pronounced in the fronto-temporal regions and also depends on clinical features like AAO and chronic seizure activity. In the early onset MTLE-HS group, the timing of epilepsy seems to be the major origin of WM abnormalities while it should be assumed as a secondary effect provoking diffusion changes in the late onset MTLE-HS patients. Our results showed that, the combination of clinical data and novel MR methods has led to valuable insights into epilepsy-associated changes in the white matter and has increased our understanding of the pathophysiology of MTLE-HS.

Acknowledgements

The authors would like to thank the participants who took part in this study. The local Diagnostic Center is gratefully acknowledged for the MR imaging and technical devices. This work was supported by the grant SROP-4.2.2/A-11/1/KONV-2012-0017.

Disclosure

S.A.N. was an employee of the grant SROP-4.2.2/A-11/1/KONV-2012-0017 between 2013 and 2014. She is an assistant research fellow at Hungarian Brain Research Program “B” (KTIA_NAP_13-2-2014-0019). The remaining authors were not paid for their participation in this particular study but they were supported by grants for other projects: R.H, J.J and H.Á. were supported by the Hungarian Brain Research Program “ A” (KTIA-NAP-13-a-II/9 and (KTIA-NAP-13-a-II/11). G.O. and N.K. were supported by the Bolyai Scholarship of the Hungarian Academy of Science.

References

[1] Wieser HG, Epilepsy ICoNo. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. Epilepsia 2004;45:695-714.

[2] Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 2010;51:676-85.

[3] Engel J, Jr. Surgery for seizures. N Engl J Med 1996;334:647-52.

[4] Baldwin GN, Tsuruda JS, Maravilla KR, Hamill GS, Hayes CE. The fornix in patients with seizures caused by unilateral hippocampal sclerosis: detection of unilateral volume loss on MR images. AJR Am J Roentgenol 1994;162:1185-9.

[5] Mamourian AC, Brown DB. Asymmetric mamillary bodies: MR identification. AJNR Am J Neuroradiol 1993;14:1332-5; discussion 1336-42.

[6] Van Paesschen W, Connelly A, Johnson CL, Duncan JS. The amygdala and intractable temporal lobe epilepsy: a quantitative magnetic resonance imaging study. Neurology 1996;47:1021-31.

[7] Cendes F, Leproux F, Melanson D, Ethier R, Evans A, Peters T, et al. MRI of amygdala and hippocampus in temporal lobe epilepsy. J Comput Assist Tomogr 1993;17:206-10.

[8] Seidenberg M, Kelly KG, Parrish J, Geary E, Dow C, Rutecki P, et al. Ipsilateral and contralateral MRI volumetric abnormalities in chronic unilateral temporal lobe epilepsy and their clinical correlates. Epilepsia 2005;46:420-30.

[9] Hermann B, Seidenberg M, Bell B, Rutecki P, Sheth R, Ruggles K, et al. The neurodevelopmental impact of childhood-onset temporal lobe epilepsy on brain structure and function. Epilepsia 2002;43:1062-71.

[10] Concha L, Beaulieu C, Gross DW. Bilateral limbic diffusion abnormalities in unilateral temporal lobe epilepsy. Ann Neurol 2005;57:188-96.

[11] Concha L, Beaulieu C, Collins DL, Gross DW. White-matter diffusion abnormalities in temporal-lobe epilepsy with and without mesial temporal sclerosis. J Neurol Neurosurg Psychiatry 2009;80:312-9.

[12] Focke NK, Yogarajah M, Bonelli SB, Bartlett PA, Symms MR, Duncan JS. Voxel-based diffusion tensor imaging in patients with mesial temporal lobe epilepsy and hippocampal sclerosis. Neuroimage 2008;40:728-37.

[13] Gross DW, Concha L, Beaulieu C. Extratemporal white matter abnormalities in mesial temporal lobe epilepsy demonstrated with diffusion tensor imaging. Epilepsia 2006;47:1360-3.

[14] Kim H, Piao Z, Liu P, Bingaman W, Diehl B. Secondary white matter degeneration of the corpus callosum in patients with intractable temporal lobe epilepsy: a diffusion tensor imaging study. Epilepsy Res 2008;81:136-42.

[15] Lin JJ, Riley JD, Juranek J, Cramer SC. Vulnerability of the frontal-temporal connections in temporal lobe epilepsy. Epilepsy Res 2008;82:162-70.

[16] Riley JD, Franklin DL, Choi V, Kim RC, Binder DK, Cramer SC, et al. Altered white matter integrity in temporal lobe epilepsy: association with cognitive and clinical profiles. Epilepsia 2010;51:536-45.

[17] Flugel D, Cercignani M, Symms MR, O'Toole A, Thompson PJ, Koepp MJ, et al. Diffusion tensor imaging findings and their correlation with neuropsychological deficits in patients with temporal lobe epilepsy and interictal psychosis. Epilepsia 2006;47:941-4.

[18] Scanlon C, Mueller SG, Cheong I, Hartig M, Weiner MW, Laxer KD. Grey and white matter abnormalities in temporal lobe epilepsy with and without mesial temporal sclerosis. J Neurol 2013;260:2320-9.

[19] Schaefer PW, Grant PE, Gonzalez RG. Diffusion-weighted MR imaging of the brain. Radiology 2000;217:331-45.

[20] Nagy SA, Aradi M, Orsi G, Perlaki G, Kamson DO, Mike A, et al. Bi-exponential diffusion signal decay in normal appearing white matter of multiple sclerosis. Magn Reson Imaging 2013;31:286-95.

[21] Hugg JW, Butterworth EJ, Kuzniecky RI. Diffusion mapping applied to mesial temporal lobe epilepsy: preliminary observations. Neurology 1999;53:173-6.

[22] Janszky J, Janszky I, Ebner A. Age at onset in mesial temporal lobe epilepsy with a history of febrile seizures. Neurology 2004;63:1296-8.

[23] Villanueva V, Serratosa JM. Temporal lobe epilepsy: clinical semiology and age at onset. Epileptic Disord 2005;7:83-90.

[24] Orsi G, Aradi M, Nagy SA, Perlaki G, Trauninger A, Bogner P, et al. Differentiating white matter lesions in multiple sclerosis and migraine using monoexponential and biexponential diffusion measurements. J Magn Reson Imaging 2015;41:676-83.

[25] Rohde GK, Barnett AS, Basser PJ, Marenco S, Pierpaoli C. Comprehensive approach for correction of motion and distortion in diffusion-weighted MRI. Magn Reson Med 2004;51:103-14.

[26] Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17:143-55.

[27] Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 2001;20:45-57.

[28] Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron 2002;33:341-55.

[29] Oishi K, Faria A. MRI atlas of human white matter. 2nd ed. London: Academic; 2011.

[30] Ahmadi ME, Hagler DJ, Jr., McDonald CR, Tecoma ES, Iragui VJ, Dale AM, et al. Side matters: diffusion tensor imaging tractography in left and right temporal lobe epilepsy. AJNR Am J Neuroradiol 2009;30:1740-7.

[31] Diehl B, Busch RM, Duncan JS, Piao Z, Tkach J, Luders HO. Abnormalities in diffusion tensor imaging of the uncinate fasciculus relate to reduced memory in temporal lobe epilepsy. Epilepsia 2008;49:1409-18.

[32] Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Med Image Anal 2001;5:143-56.

[33] Andersson J, Jenkinson M, Smith S. Non-linear registration aka Spatial normalisation. FMRIB Centre, Oxford, United Kingdom Technial Report TR07JA2 2007.

[34] Chan YH. Biostatistics 201: linear regression analysis. Singapore Med J 2004;45:55-61.

[35] Keller SS, Mackay CE, Barrick TR, Wieshmann UC, Howard MA, Roberts N. Voxel-based morphometric comparison of hippocampal and extrahippocampal abnormalities in patients with left and right hippocampal atrophy. Neuroimage 2002;16:23-31.

[36] Bonilha L, Rorden C, Halford JJ, Eckert M, Appenzeller S, Cendes F, et al. Asymmetrical extra-hippocampal grey matter loss related to hippocampal atrophy in patients with medial temporal lobe epilepsy. J Neurol Neurosurg Psychiatry 2007;78:286-94.

[37] Kemmotsu N, Girard HM, Bernhardt BC, Bonilha L, Lin JJ, Tecoma ES, et al. MRI analysis in temporal lobe epilepsy: cortical thinning and white matter disruptions are related to side of seizure onset. Epilepsia 2011;52:2257-66.

[38] McDonald CR, Hagler DJ, Jr., Ahmadi ME, Tecoma E, Iragui V, Gharapetian L, et al. Regional neocortical thinning in mesial temporal lobe epilepsy. Epilepsia 2008;49:794-803.

[39] Bernasconi N, Natsume J, Bernasconi A. Progression in temporal lobe epilepsy: differential atrophy in mesial temporal structures. Neurology 2005;65:223-8.

[40] Rodrigo S, Oppenheim C, Chassoux F, Golestani N, Cointepas Y, Poupon C, et al. Uncinate fasciculus fiber tracking in mesial temporal lobe epilepsy. Initial findings. Eur Radiol 2007;17:1663-8.

[41] Powell HW, Parker GJ, Alexander DC, Symms MR, Boulby PA, Wheeler-Kingshott CA, et al. Abnormalities of language networks in temporal lobe epilepsy. Neuroimage 2007;36:209-21.

[42] Otte WM, van Eijsden P, Sander JW, Duncan JS, Dijkhuizen RM, Braun KP. A meta-analysis of white matter changes in temporal lobe epilepsy as studied with diffusion tensor imaging. Epilepsia 2012;53:659-67.

[43] Sayin U, Hutchinson E, Meyerand ME, Sutula T. Age-dependent long-term structural and functional effects of early-life seizures: evidence for a hippocampal critical period influencing plasticity in adulthood. Neuroscience 2015;288:120-34.

[44] Suzuki M, Hagino H, Nohara S, Zhou SY, Kawasaki Y, Takahashi T, et al. Male-specific volume expansion of the human hippocampus during adolescence. Cereb Cortex 2005;15:187-93.

Figure captions

Figure 1

Representations of the regions of interest analysis. Regional averages of ADC values were extracted from diffusion-weighted images using eroded masks of the left and right hemispheric WM (A); temporal, occipital lobes WM (B); frontal, parietal lobes WM (C); Uncinate Fasciculus (UNC); Fornix cres with Stria terminalis (FX with ST); Hippocampal Cingulum (HCg); Sagittal Stratum (SS) include inferior longitudinal fasciculus and inferior fronto-occipital fasciculus (D).

Images are presented in radiological convention.

Figure 2

DWI images overlaid with color-coded ipsilateral hemispheric (upper left of A) and temporal lobe WM (upper right of B) ADC values in a representative MTLE-HS patient and a control subject. The box- and whiskers plots show the distribution of ADC values in the ipsilateral hemispheric (lower left of A) and temporal lobe WM (lower right of B) of MTLE-HS patients (N=22) and control subjects (N=22). The whiskers in the plot represent the minimum and the maximum ADC values, the cross depicts the mean while the band inside the box shows the median ADC.

MTLE-HS: Mesial temporal lobe epilepsy with hippocampal sclerosis; ADC: Apparent diffusion coefficient.

Figure 3

Partial regression plots demonstrating the influence of AAO in early onset disease (A) and seizure frequency in late onset patients (B) on ADC in the ipsilateral uncinate fasciculus. The lines indicate the slope as fitted by linear regression and the 95% confidence intervals. Linear correlation coefficients (r) are also presented.

MTLE-HS: Mesial temporal lobe epilepsy with hippocampal sclerosis; ADC: Apparent diffusion coefficient; AAO: Age at epilepsy onset.

Table 1. Clinical and demographic details for MTLE-HS patients.

Investigated data	MTLE-HS patients		
	Early onset group (N=11)	***Late onset group (N=11)***	***Total (N=22)***
age (years)[a]	39.3±13.8 (19-61)	47.4±13.7 (20-63)	43.3±14.1 (19-63)
seizure frequency[a, ‡]	4.7±5.4 (0-20)	3.2±5.3 (0-18)	4.0±5.2 (0-20)
AAO (years)[a]	7.6±5.5 (1-16)	38.3±13.5 (17-57)	23.0±18.6 (1-57)
level of education (years)[a]	12.7±3.5 (8-17)	11.8±2.9 (8-16)	12.3±3.1 (8-17)
history of febrile seizure	5	2	7
occurrence of GTCS	3	5	8
head trauma prior to AAO	0	2	2
mild encephalitis/meningitis	3	0	3
perinatal complication	1	0	1

MTLE-HS: Mesial temporal lobe epilepsy with hippocampal sclerosis; AAO: age at epilepsy onset; GTCS: generalized tonic-clonic seizure within the last 5 years.

[a] Values given as: mean ± standard deviation (range).

[‡] Seizure frequency was defined as the mean frequency of complex partial seizures per month during at least the last 12 months prior to enrollment.

Table 2. Diffusion differences in the ipsilateral WM areas.

	Groups	Hemispheric WM	Temporal lobe WM	Uncinate fasciculus
MTLE-HS vs. Control	**Whole**	p=0.01	p=0.056	p=0.02
	Early onset	p=0.04	p=0.01	ns*
	Late onset	ns	ns	ns

MTLE-HS: Mesial temporal lobe epilepsy with hippocampal sclerosis; WM: white matter; ns: not significant.

*Independent two-tailed t-test with Welch correction for unequal variances.

Table 3. Multiple linear regression analysis of the ipsilateral WM structures in patients[†].

Groups	Clinical variables	Hemispheric WM	Temporal lobe WM	Uncinate fasciculus
Whole MTLE-HS	AAO	p=0.02 (-0.54)	p=0.001 (-0.70)	p=0.006 (-0.61)
	Age	p=0.009 (+0.58)	ns	p=0.03 (+0.51)
	seizure frequency	ns	ns	ns
	Sex	ns	ns	ns
Early onset MTLE-HS	AAO	p=0.03 (-0.76)	ns	p=0.03 (-0.76)
	Age	ns	ns	ns
	seizure frequency	ns	ns	ns
	Sex	ns	ns	ns
Late onset MTLE-HS	AAO	ns	ns	ns
	Age	ns	ns	ns
	seizure frequency	ns	ns	p=0.03 (+0.75)
	Sex	ns	ns	ns

[†]Results are presented as corrected p values and linear correlation coefficients (r).

MTLE-HS: Mesial temporal lobe epilepsy with hippocampal sclerosis; WM: white matter; AAO: Age at epilepsy onset; ns: not significant.

Figure1

Click here to download high resolution image

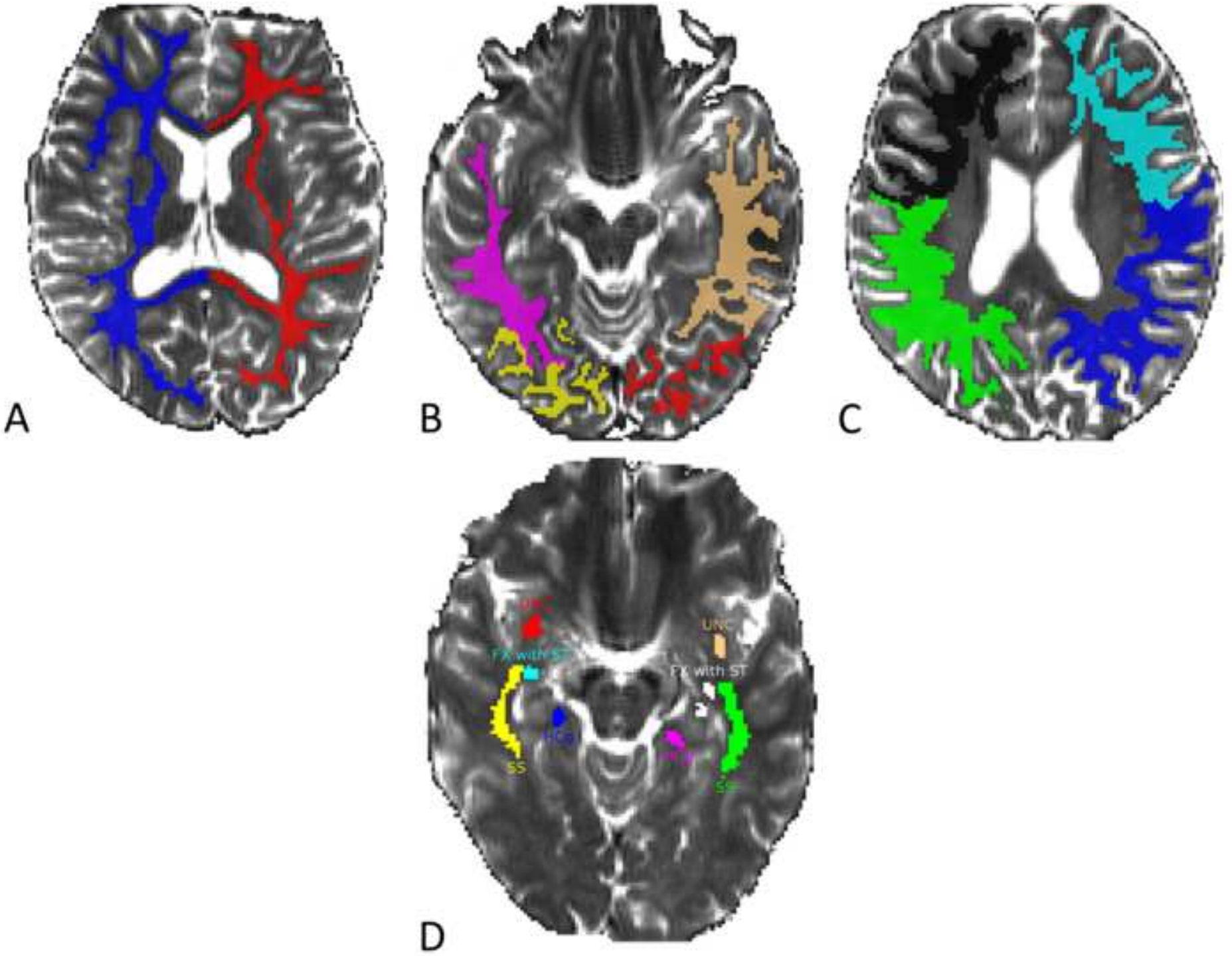

Figure2

Click here to download high resolution image

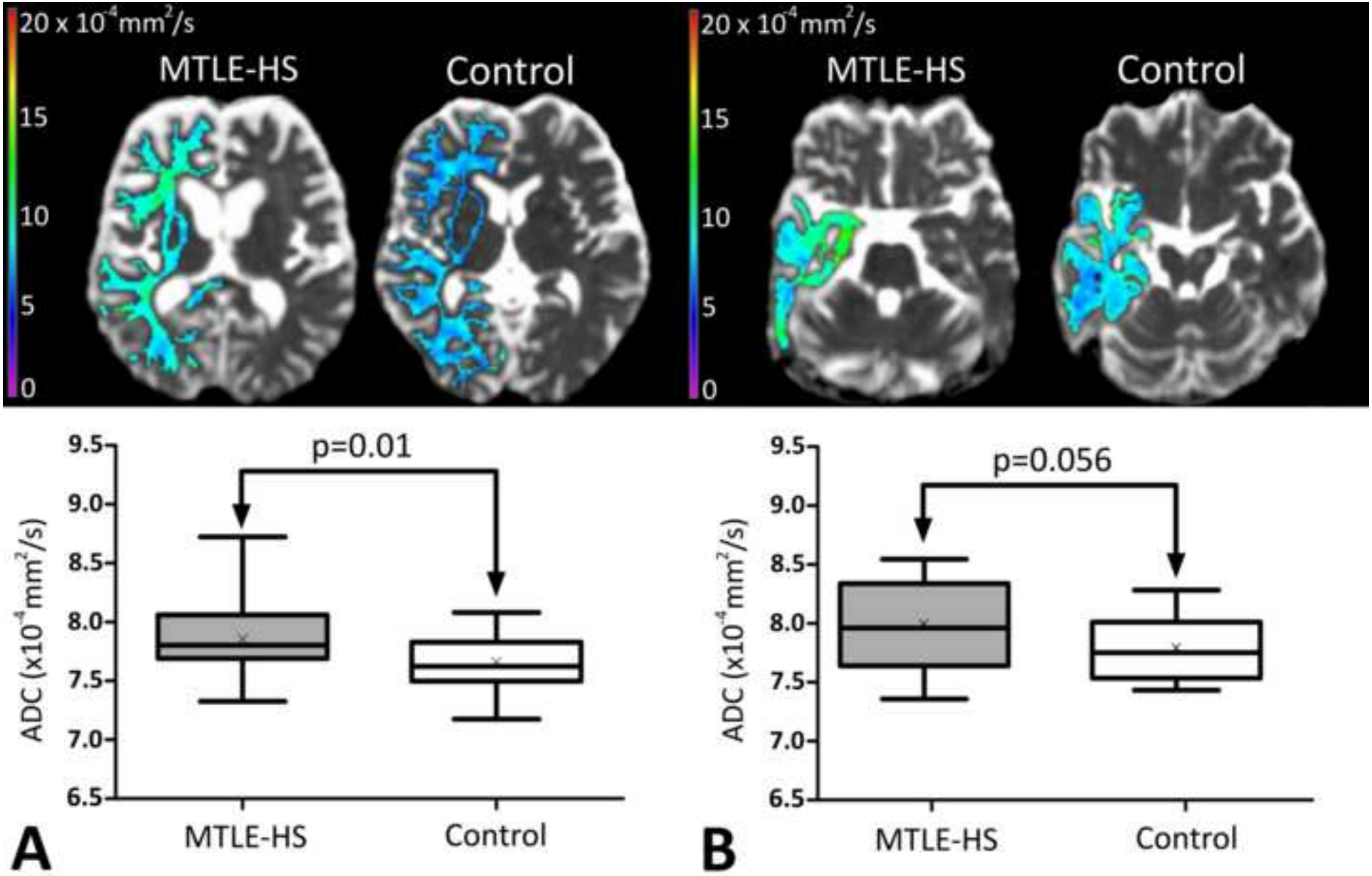

Figure3

Click here to download high resolution image