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Cortical and subcortical brain alterations in Juvenile Absence Epilepsy

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ABSTRACT

Despite the common assumption that genetic generalized epilepsies are characterized by a macroscopically normal brain on magnetic resonance imaging, subtle structural brain alterations have been detected by advanced neuroimaging techniques in Childhood Absence Epilepsy syndrome. We applied quantitative structural MRI analysis to a group of adolescents and adults with Juvenile Absence Epilepsy (JAE) in order to investigate micro-structural brain changes using different brain measures. We examined grey matter volumes, cortical thickness, surface areas, and subcortical volumes in 24 patients with JAE compared to 24 healthy controls; whole-brain voxel-based morphometry (VBM) and Freesurfer analyses were used. When compared to healthy controls, patients revealed both grey matter volume and surface area reduction in bilateral frontal regions, anterior cingulate, and right mesial-temporal lobe. Correlation analysis with disease duration showed that longer disease was correlated with reduced surface area in right pre- and post-central gyrus. A possible effect of valproate treatment on brain structures was excluded. Our results indicate that subtle structural brain changes are detectable in JAE and are mainly located in anterior nodes of regions known to be crucial for awareness, attention and memory.

1. Introduction

Childhood Absence Epilepsy (CAE) and Juvenile Absence Epilepsy (JAE) are two sub-syndromes of Genetic Generalized Epilepsy (GGE; formerly known as idiopathic generalized epilepsies) whose hallmark seizures are represented by absences (Fisher et al., 2005). CAE is a childhood epilepsy syndrome occurring in 10–17% of all childhood onset epilepsy, making it the most common paediatric epileptic syndrome. JAE usually begins between 10 and 17 years of age, but, at lower age limit, there is a great deal of overlap with CAE. By definition, GGE patients have no abnormalities on visual inspection of brain magnetic resonance imaging (MRI), but recently focal structural and functional abnormalities are emerging from studies using advanced MRI techniques. Volumetric studies using voxel-based morphometry (VBM) have shown both regional cortical grey matter (GM) and thalamic volumes alterations in GGE compared to controls: thalamic volume reduction was the most consistent finding across all the studies, while both increased and decreased cortical volumes were found (Seneviratne et al., 2014). Among all GGEs, only few studies have analysed structural MRI specifically in absence epilepsy patients separately from other sub-syndromes, and studies mainly focused to investigate populations with childhood onset absence seizures. Chan et al. (2006) compared CAE with controls and found areas of GM decrease in both thalami and in the subcallosal gyrus together with white matter reduction in extranuclear subcortical areas and basal forebrain (Chan et al., 2006). Pardoe et al. (2008), found a consistent reduction in thalami volume in CAE both within and across three different sites of MRI acquisition (Pardoe et al., 2008). Caplan et al. (2009) demonstrated that children with CAE have smaller grey matter volumes in orbito-frontal gyrus and in bilateral temporal lobe compared to children without epilepsy (Caplan et al., 2009). Finally, in a study analysing both CAE and JAE, an increased GM volume in superior mesio-frontal regions was reported in patients compared to controls (Betting et al., 2006). In conclusion, despite some variability in the location and direction of volumetric changes, there is an emerging body of evidence suggesting that subtle alterations in brain structure occur in absence epilepsy syndromes. This heterogeneity could be due to several factors, including mixed patients groups, different neuroimaging techniques, different sample size, and publication bias reporting only positive results. In addition, if we consider recent evidence suggesting that GGE syndromes are neurodevelopmental disorders with different patterns of prospective grey and white matter volume changes across childhood and adolescence (Hermann et al., 2006; Tosun et al., 2011a; Tosun et al., 2011b), one would expect distinct structural imaging patterns in CAE and JAE patients. Given the lack of specific structural neuroimaging studies on juvenile onset of Absence Epilepsy, we focused our study on JAE. We analysed cortical and subcortical brain changes in patients compared to healthy controls by means of different methods/measures: VBM, cortical thickness and surface, and subcortical volumes. We also correlated MRI abnormalities with the clinical

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features of epilepsy and we analysed the potential effect of drug treatment with sodium valproate on the brain structure (Pardoe et al., 2013).

2. Methods

2.1. Subjects

Twenty-four patients with JAE were recruited. Diagnosis of JAE was based on electroclinical criteria according to the International League Against Epilepsy (ILAE) classification (Berg et al., 2010). Demographic data and clinical information such as age of seizure onset, duration of epilepsy, antiepileptic drugs (AED) prescription and response to treatment, were collected. For group comparison, 24 volunteers were recruited to serve as healthy controls (HC). Healthy subjects had no history of neurological diseases or family history of epilepsy. All subjects, both patients and controls, had normal MRI at visual inspection. The human ethic committee of the University of Modena and Reggio Emilia approved this study and written informed consent was obtained from all the patients recruited and their parents if underage.

2.2. MRI acquisition

Three-dimensional (3D) T1-weighted MRI images were acquired using a 3 Tesla Philips Intera MRI scanner (Best, The Netherlands). A SPGR pulse sequence (echo time (TE) = 4.6, repetition time (TR) = 9.9 ms) was used. One hundred seventy contiguous sagittal slices were acquired (voxel size = 1 × 1 × 1 mm) and the field of view was 240 mm with a matrix size of 256 × 256 × 170. A T2-weighted axial scan was also acquired to allow visual determination of vascular burden or tissue abnormalities.

2.3. Statistical analysis of demographical and clinical variables

Demographical and clinical characteristics of subjects were analysed using Stata11 software and parametric and non-parametric statistics were used as appropriate. Independent samples *t*-tests were used to compare age and years of education between patients and controls; chi-square was used to compare the two groups according to gender.

2.4. Voxel-based morphometry analysis

VBM was performed using VBM8 (http://dbm.neuro.uni-jena.de/vbm/) a toolbox of SPM8 (http://www.fil.ion.ucl.ac.uk/spm/); default settings were applied. Structural images were bias-corrected, tissue-classified, and normalized to standard template using high-dimensional DARTEL normalization. Grey-matter volume was calculated modulating the normalized segmented images with a non-linear only warping resulting in an analysis of relative differences in regional grey-matter volumes corrected for individual brain size. To check the quality of the segmentation and normalization procedures, the normalized, bias-corrected images were visually inspected and covariance between normalized images was calculated to check homogeneity of variance and to identify potential outliers. Finally the normalized, segmented, and modulated images were smoothed with a 8 mm FWHM isotropic Gaussian kernel. To identify GM differences between JAE and controls, we performed a *t*-test comparison between the two groups and a double statistical threshold was used (voxel-wise $p < 0.001$ and cluster size ≥686 voxels, as determined by AlphaSim with 10,000 Monte Carlo simulations) to obtain an overall alpha level of <0.05 (see details on the procedure at http://afni.nimh.nih.gov/afni/doc/manual/AlphaSim) (Forman et al., 1995). In addition, a correlation analysis with GM volume and disease duration was performed in the patients' group and a statistical threshold of $p < 0.001$ with a cluster size ≥808 voxels was accepted. Finally, we performed a *t*-test comparison between patients taking valproate (either alone or in polytherapy, VPA+) versus patients not taking valproate or drugs-free (VPA−) at the time of MRI. In this model a double statistical threshold of $p < 0.001$ with a cluster size ≥785 voxels was used. In all analyses, age, gender, and education were entered as covariates of no interest in order to control results for the potential effect of these variables.

2.5. Cortical and subcortical Freesurfer analyses

Scans were analysed using standardized image toolbox (Freesurfer, version 5.0) (Fischl, 2012), quality assurance (outlier detection based on inter quartile of 1.5 standard deviations along with visual inspection of segmentations), and statistical methods. Briefly, the pipeline involves removal of non-brain tissue, automated Talaraich transformation, segmentation of white matter and grey matter, tessellation of grey/white matter boundary, automated correction of topology defects, surface deformation to form the grey/white matter boundary and grey/cerebrospinal fluid boundary, and parcellation of cerebral cortex. Cortical thickness estimates were calculated as the distance between the grey/white matter border and the pial surface at each vertex; surface area was derived by taking the sum of the area of the vertices in each parcellation. Labels were constructed and values were extracted based on automatic algorithm (Desikan et al., 2006; Fischl et al., 2004). According to previous literature (Jalbrzikowski et al., 2013), we combined caudal and rostral regions of the middle frontal cortex and anterior cingulate respectively to make a unique label; the same approach was used to combine pars orbitalis, pars triangularis, and pars opercularis to create the inferior frontal cortex label. In conclusion, we calculated cortical thickness and surface area measures from 30 regions for hemisphere. Subcortical volumes were calculated with FreeSurfer's automated procedure for volumetric measures. Each voxel in the normalized brain volume was assigned to one label using a probabilistic atlas obtained from a manually labeled training set (Fischl et al., 2002). The labels we used for the analysis were the putamen, caudate nucleus, globus pallidus, nucleus accumbens, thalamus, amygdala, hippocampus, and the ventricular system.

After visual inspection and quality control, one patient was removed from analyses because of poor segmentation. Statistical analyses were performed using SPSS software (IBM, Chicago, IL). All neuroanatomical measures were examined for normality using Shapiro-Wilk test and transformed appropriately if they violated assumptions of normality. To compare cortical and subcortical measures between patients and controls, we conducted a univariate ANCOVA with each neuroanatomical value as the dependent variable, group diagnosis as fixed factor, and age, gender, education, and intracranial volume as covariates. The same approach was uses to compare patients taking valproate (VPA+) versus patients not taking valproate (VPA−) at the time of MRI. False discovery rate (FDR) was used to correct for multiple comparisons and a threshold of $q < 0.05$ estimated using SPSS command, according to Bejamini and Hochberg methods (Bejamini and Hochberg, 1995), was considered statically significant. Finally, we performed a correlation analysis in the patient group between both cortical and subcortical neuroanatomical measures and disease duration calculated in years; for this analysis, we regressed out the effect of education, age, total brain volume, and gender and we considered significant results with a $p < 0.05$, corrected for multiple comparisons.

3. Results

3.1. Demographical and clinical characteristics

JAE patients (n = 24) had a mean age of 26.33 years (±11.9; range 16–52); 19 were female. Mean age of onset was 13.8 years (range between 10 and 24 years of age); mean duration of epilepsy was 12.9 years. Control subjects (n = 24) had a mean age of 30.6 years (±5.4; range 19–38); 14 were female. Patients and controls only differed in years of education ($p = 0.000$), with controls showing higher level of education (17.2 years ± 1.38) compared to patients