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Brain Diffusion Tensor Imaging and Volumetric Analysis: Grey and White Matter Changes in Preschool Children with Autism Spectrum Disorder

Anna Pichiecchio[1*], Tiziana Carigi[2], Niels Bergsland[3,4], Sara Gianfelice[5,6], Fulvia Palesi[7], Matteo Alessio Chiappedi[5], Giorgio Rossi[2], Stefano Bastianello[1,6] and Umberto Balottin[5,6]

[1]*Neuroradiology Department, C Mondino National Neurological Institute, Pavia, Italy*

[2]*Circolo Hospital and Macchi Foundation, Varese, Italy*

[3]*MR Research Laboratory, IRCCS, Don Gnocchi Foundation ONLUS, Milan, Italy*

[4]*Buffalo Neuroimaging Analysis Center, Department of Neurology, University at Buffalo SUNY, Buffalo, USA*

[5]*Child Neuropsychiatry Unit, C Mondino National Neurological Institute, Pavia, Italy*

[6]*University of Pavia, Italy*

[7]*Department of Physics, University of Pavia, Pavia, Italy*

[*]**Corresponding author:** Pichiecchio A, Neuroradiology Department, C Mondino National Neurological Institute, via Mondino 2, 27100 Pavia, Italy, Tel: 0039-0382380303-241; Fax: 0039-0382380313; E-mail: anna.pichiecchio@mondino.it

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Abstract

We aimed to investigate whether DTI metrics and volumetric analysis could detect regional abnormalities in young Autism Spectrum Disorder (ASD) children. A sample of 22 pre-school children affected by ASD and a group of 10 age-matched controls underwent a conventional and advanced MRI protocol, including DTI and 3D T1-weighted imaging. Volumetric analysis revealed no significant differences between the ASD children and the controls, while the DTI results suggested an early involvement of both the cerebellum and the supratentorial grey matter (GM) in young ASD children, with fractional anisotropy (FA) emerging the most sensitive parameter.GM-FA changes correlated with Autism Diagnostic Observation Schedule-Generic (ADOS-G) and the Autism Diagnostic Interview-Revised (ADI-R). Further investigation in a larger sample is warranted in order to confirm a potential primary role of GM versus WM changes in the complex aetiopathogenesis of ASD.

Keywords: DTI; Brain MRI; Autism spectrum disorders; Early childhood; Volumetric analysis

Abbreviations

MRI: Magnetic Resonance Imaging; DTI: Diffusion Tensor Imaging; ASD: Autism Spectrum Disorder; FA Fractional Anisotropy; MD: Mean Diffusivity; ADOS-G: Autism Diagnostic Observation Schedule – Generic; ADI-R: Autism Diagnostic Interview – Revised; BT: Brain Tissue; WM: White Matter; GM: Grey Matter; DSM-5: Statistical Manual Of Mental Disorders-Fifth Edition; BDNF: Brain-Derived Neurotrophic Factor; T1W: T1-Weighted; 3D: Three-Dimensional; FFE: Fast Field Echo; TR: Repetition Time; TE: Echo Time; FOV: Field Of View; AC-PC: Anterior Commissure - Posterior Commissure; SE-EPI: Shot-Echo Planar Imaging; Rois: Regions Of Interest; FAST, FSL: FMRIB's Automated Segmentation Tool; Ad: Axial Diffusivity; Rd: Radial Diffusivity; CSF: Cerebrospinal Fluid

Introduction

Autism spectrum disorder (ASD) is a complex and heterogeneous neurodevelopmental disorder with a broad range of severity and clinical variability. It is characterised mainly by early onset of impairment in social interaction and communication and the presence of restricted and repetitive patterns of interests and behaviours [1]. Promising biomarkers for autism have recently been identified, such as abnormal tryptophan metabolism and imbalances in blood concentrations of neurotrophic factors such as the brain-derived neurotrophic factor protein [2-4]. Despite these advances, the diagnostic criteria for autism are still based on clinical observation and standardised scales. Attempts to understand autism, considering its heterogeneity and variable developmental course, have led to the hypothesis that ASD is a form of "developmental disconnection syndrome" due to a failure in the development of normal connections in the brain, rather than to destruction of previously connected regions [5,6]. Therefore, research of possible neural biomarkers of ASD has recently seen a shift from localised to more network-based approaches. Many of the latter have suggested that there is decreased communication between brain regions in people with ASD, both at a molecular level and at a functional connectivity level [7].

Advanced magnetic resonance imaging (MRI) techniques, providing a non-invasive means of examining both the functional connectivity and the macro- and microstructure of the ASD brain, are therefore playing an increasingly prominent role in autism research. Despite the wide variability of current research data, partially explained by differences in clinical and demographic characteristics of the samples studied and in the MRI parameter analysis methods used, a common observation is the peculiar pattern of brain growth in autistic children, characterised by macrostructural expansion in the first year of life followed by abnormally slow growth [8,9]. However, it is still unclear which brain regions are mainly involved in this maldevelopment process and whether the alterations involve mainly grey matter (GM) or white matter (WM) tissue [10]. Several volumetric MRI studies of ASD patients have demonstrated both GM and WM abnormalities in a number of brain regions [11-16]. Likewise,

most diffusion tensor imaging (DTI) studies of the microstructural organisation of the brain have supported the theory of under connectivity in ASD, and shown reduced fractional anisotropy (FA) values in several brain regions [7].

To date, only a few region-specific DTI studies have been performed in ASD in early childhood [17-21]. Most of them [17-19,21] showed increased FA in several WM pathways, while Williams et al. found no FA differences and Walker et al. detected decreased FA in posterior regions. To our knowledge, multimodal investigation combining both volumetric and DTI techniques in order to further explore GM and WM integrity has been performed only once in young children with ASD [21].

This study was conducted to investigate GM and WM integrity in young ASD children using DTI metrics and volumetric analysis. Our aim was two-fold: first, to examine possible brain region differences in GM and WM microstructure integrity between ASD children and typically developing controls; second, to correlate regional DTI parameters with clinical scales evaluating ASD in patients with autistic disorders.

Materials and Method

Participants

A sample of 22 children with ASD (mean age 44.5 ± 10.6 months) and 10 age range-matched controls (mean age 52.4 ± 15.8 months) were selected from children consecutively referred to our Child and Adolescent Neuropsychiatry Unit. In all cases, written informed consent was obtained from the child's parent/guardian prior to any imaging.

The diagnosis of ASD was based on parental information, clinical history and expert evaluation performed according to the Diagnostic and Statistical Manual of Mental Disorders – fourth Edition, Text Revision (DSM-IV TR) diagnostic criteria and was supported by administration of the Autism Diagnostic Observation Schedule – Generic (ADOS-G) and the Autism Diagnostic Interview-Revised (ADI-R). Children with neurological causes of autistic symptoms or associated medical conditions were excluded.

The controls were selected among children referred to our centre to undergo conventional MRI, which gave normal findings. None was suffering from any major medical, neurological or developmental problems, as determined by clinical examination. Requirements for eligibility were no history of seizures, head injuries or psychotropic medication use, no personal or family history of autism, no clinical evidence of neurological dysfunction, and a normal MRI scan.

Imaging data acquisition

Conventional MRI with T2-weighted (T2W) and T1-weighted (T1W) images and DTI sequences were performed on a Philips Intera 1.5T scanner (Philips Gyroscan, Koninklijke, The Netherlands). All subjects were sedated for scanning using halogen vapour (sevoflurane) with parental consent. During scanning, the patient's head was gently restrained by foam cushions.

High-resolution MRI images for volumetric analysis were obtained with a T1W three-dimensional (3D) Fast Field Echo (FFE) sequence with the following parameters: TR=25 s, TE=4.6 ms, flip angle=30; field of view (FOV) =250 mm; acquisition matrix=256 × 256, number of slices=170, slice thickness=1.6 mm, voxel=1 × 1 × 0.8. Image orientation was parallel to the anterior commissure - posterior commissure (AC-PC) plane. DTI data were acquired with single-shot echo planar imaging (SE-EPI) sequences with diffusion gradients applied in 15 non-collinear directions and with a b-value of 800 s/mm^2. The sequence parameters for DTI were: TR=7290 ms, TE=68 ms, FOV=200 mm, acquisition matrix=80 × 80, number of slices=60, 2.5 mm isotropic voxel, nºaverages=2. Routine clinical MRI scans (T1W, T2W and fluid-attenuated inversion recovery) were performed to further reveal incidental pathological abnormalities. The total scanning time was 30minutes.

Volumetric analysis

High-resolution 3D T1W images were segmented into WM, GM and cerebrospinal fluid (CSF) with FMRIB's Automated Segmentation Tool (FAST, FSL) [22]. Absolute and relative volumes, the latter expressed as the ratio between the absolute volume (mm^3) and the intracranial volume (mm^3), were calculated for WM, GM and whole-brain tissue (BT), considered as the sum of WM and GM.

Cortical reconstruction and volumetric segmentation were performed using the FreeSurfer image analysis suite (version 5.1, http://surfer.nmr.mgh.harvard.edu/) [23]. Briefly, the high-resolution 3D T1W images were first registered to the Talairach space and intensity normalised [24]. Next, the skull was automatically removed from the image using a hybrid watershed/surface deformation/graph cuts procedure [25,26]. With the aid of a probabilistic atlas, each voxel in the brain was labelled as one of the following: cerebral WM, cerebral cortex, ventricle, cerebellum WM, cerebellum cortex, subcortical GM (including the amygdala and hippocampus), brainstem, and CSF. For cortical parcellation, WM segmentation was performed, followed by tessellation to identify the GM and WM boundary as well as the pial surface. Automated topology correction [27,28] and surface deformation following intensity gradients were used to optimally place the GM/WM and GM/CSF borders [29]. At all processing steps, corrections by manual intervention were made as necessary.

Estimated lobar volumes and masks for both WM and GM were created by taking the sum of the individual 'Desikan-Killiany' regions of interest (ROIs) from FreeSurfer. The following ROIs were combined for the frontal lobe: superior frontal, rostral middle frontal, caudal middle frontal, pars opercularis, pars triangularis, pars orbitalis, lateral orbitofrontal, medial orbitofrontal, precentral, paracentral, and frontal pole; for the parietal lobe: superior parietal, inferior parietal, supramarginal, postcentral, and precuneus; for the temporal lobe: superior temporal, middle temporal, inferior temporal, fusiform, transverse temporal, entorhinal, temporal pole, and parahippocampal; for the occipital lobe: lateral occipital, lingual, cuneus, and pericalcarine.

DTI analysis

Diffusion tensor images were corrected for eddy current distortions using the FSL software package (version 4.1, http://fsl.fmrib.ox.ac.uk/fsl/). Skull removal was performed on the b0 image using the Brain Extraction Tool. Next, the Diffusion Toolkit (version 0.6.2.1, http://www.trackvis.org) was used to estimate the diffusion tensor. For each voxel in the brain, the computed tensor was diagonalised to obtain its eigenvalues in order to compute the FA, mean diffusivity (MD), axial diffusivity (aD), and radial diffusivity (rD) metrics.

For each subject, the FA image was affine registered to the high-resolution 3D T1W image with the FLIRT tool [30]. The resulting

transformation matrix was used to bring the MD, aD, and rD images into the high-resolution space as well. FA and MD histograms were obtained for WM, GM and BT. For each histogram, mean, standard deviation, peak height and position, skewness and kurtosis were calculated.

Region-specific DTI analysis

DTI metrics were obtained for the following regions in both the left and right hemispheres: amygdala, hippocampus, cerebellum, frontal lobe, parietal lobe, temporal lobe and occipital lobe. For the lobes and cerebellum, separate metrics for GM and WM were calculated. Data were also obtained for the corpus callosum.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18.0.2 (SPSS Inc., Chicago, IL). Considering the small sample size, we considered it more appropriate to use a non-parametric Mann-Whitney test for between-group comparisons. Due to the exploratory nature of the study, a nominal p-value of 0.05 was considered significant. Spearman's rho (high rho ≥ 0.70; medium rho 0.30-0.60; low rho<0.30) was used to perform correlation analysis between DTI measures and mean ADOS-G and ADI-R scores.

Results

The ASD and control groups did not significantly differ in sex (p=0.167), age (p=0.147) or head circumference (p=0.183).

Volumetric and DTI analysis

Global volume analysis (BT, GM and WM) and region-specific volumetric analysis showed no significant differences between the ASD patients and controls. FA histogram analysis showed significant differences only in the GM parameters (Table 1), while no significant differences in the parameters FA-WM, MD-WM and MD-GM were found between the ASD patients and the controls.

The region-specific DTI analysis demonstrated lower FA values in almost all the analysed regions in the ASD group, but these values reached significance only in the GM (all values) and in the WM of the occipital lobes and cerebellum (Table 1). Conversely, a significant decrease in MD was found in the ASD group only in the right cerebellum (in both the WM and GM, respectively p=0.016 and p=0.041). The analysis of the aD and rD metrics showed a significant decrease in aD in the cerebellum, in both WM (p=0.026, left; p=0.004, right) and GM (p=0.018, left; p=0.001, right), in the parietal lobe GM (p=0.033, left; p=0.007, right) and in the left occipital lobe GM (p=0.018), in ASD patients compared to the controls, while rD showed no significant differences between the groups.

		ASD		Controls	Group Comparison
	Mean	**SD**	**mean**	**SD**	**p**
Fractional anisotropy					
Frontal lobe					
White matter					
Left	0.314	0.017	0.314	0.018	0.881
Right	0.307	0.19	0.309	0.019	0.983
Gray matter					
Left	0.16	0.019	0.179	0.015	0.006
Right	0.157	0.016	0.177	0.015	0.001
Parietal lobe					
White matter					
Left	0.302	0.021	0.307	0.017	0.881
Right	0.303	0.021	0.306	0.016	0.781
Gray matter					
Left	0.154	0.02	0.183	0.028	0.01
Right	0.161	0.021	0.195	0.025	0.003
Temporal lobe					
White matter					
Left	0.299	0.017	0.307	0.015	0.334
Right	0.285	0.019	0.286	0.019	0.848
Gray matter					
Left	0.165	0.017	0.182	0.013	0.008
Right	0.163	0.02	0.185	0.018	0.009
Occipital lobe					
White matter					
Left	0.238	0.019	0.257	0.02	0.029
Right	0.246	0.02	0.263	0.016	0.041
Gray matter					
Left	0.143	0.018	0.171	0.02	0.001
Right	0.155	0.024	0.189	0.018	0.001
Cerebellum					
White matter					
Left	0.362	0.025	0.397	0.021	0.001
Right	0.367	0.033	0.41	0.036	0.003
Gray matter					
Left	0.224	0.032	0.262	0.025	0.004
Right	0.241	0.045	0.297	0.044	0.004
Corpus callosum					
	0.535	0.397	0.538	0.025	0.881
Amygdala					
Left	0.229	0.03	0.26	0.054	0.174
Right	0.219	0.03	0.254	0.048	0.037
Hippocampus					

Left	0.206	0.032	0.238	0.039	0.033
Right	0.206	0.033	0.237	0.036	0.016

Table 1: Group comparison of FA in hemispheric lobes, cerebellum, corpus callosum, amygdala and hippocampus.

Clinical-MRI correlation analysis

The correlation analysis between ADOS-G and ADI-R, on the one hand, and relative volumes of BT, WM and GM, on the other, showed a strong negative relationship only between the Communication sub-scale of ADI-R and WM relative volume (rho-0.719). Specifically, the lower the WM volume was, the higher the impairment in communication skills. Region-specific volumetric measures showed a medium-high-degree relationship between the clinical scales and the parietal lobe and cerebellum (Table 2). After reviewing the between-groups significance of the results of the DTI metrics, a correlation of FA-GM and FA-WM volumes with clinical scales was performed and showed many high-magnitude relationships between FA-GM values and ADOS-G (Language and Communication and Social Interaction scales) and ADI-R (Social Interaction scale) scores (Table 3).

		ADOS-T	ADOS-L	ADOS-R	ADI-R (A)	ADI-R (B)	ADI-R (C)
Frontal lobe							
WM	Left	0.147	-0.112	-0.104	-0.192	-0.111	0.014
	Right	0.203	-0.112	0.009	-0.078	0.129	0.193
GM	Left	0.138	-0.019	0.08	-0.247	0.051	-0.037
	Right	-0.009	-0.159	-0.094	-0.243	0.18	0.096
Parietal lobe							
WM	Left	0.028	-0.28	-0.17	-0.114	-0.12	0.055
	Right	0.083	-0.383	-0.08	-0.096	0.277	-0.248
GM	Left	0.604	0.262	0.387	-0.018	0	-0.188
	Right	0.203	-0.112	0.042	-0.252	0.244	-0.193
Temporal lobe							
WM	Left	0.535	0.047	0.245	0.233	0.051	0.266
	Right	0.433	-0.122	0.16	0.165	-0.106	-0.161
GM	Left	0.097	0.243	0.194	-0.037	-0.502	-0.128
	Right	0.521	0.486	0.418	0.064	-0.332	-0.037
Occipital lobe							
WM	Left	0.235	-0.29	-0.076	-0.023	0.392	0.211
	Right	0.23	-0.29	-0.052	-0.078	0.387	0.119
GM	Left	0.323	-0.206	0.024	-0.178	0.336	-0.248
	Right	0.286	-0.262	0.014	-0.05	0.364	0.069
Cerebellum							
WM	Left	-0.461	-0.739	-0.666	-0.233	0.24	-0.385
	Right	-0.023	-0.43	-0.439	0.023	-0.18	-0.11
GM	Left	-0.161	-0.57	-0.599	-0.641	-0.083	-0.606
	Right	-0.309	0.767	-0.774	-0.627	0.12	-0.546
Amygdala							
Left		-0.392	0.009	0.061	-0.124	-0.009	0.041
Right		0.143	0.168	0.236	0.183	0.092	0.463
Hippocampus							

Left	0.111	-0.243	-0.132	-0.435	0.221	-0.491
Right	0.387	0.065	0.109	-0.142	-0.166	-0.229
Corpus callosum	0.548	0.131	0.146	0.357	-0.198	0.298

Table 2: Correlation between region-specific MRI volumetric measures and clinical scales.

		ADOS-T	ADOS-L	ADOS-R	ADI-R (A)	ADI-R(B)	ADI-R (C)
Frontal lobe							
WM	Left	0.465	0.168	0.222	-0.073	0.143	-0.179
	Right	-0.028	0.383	0.198	-0.302	-0.078	-0.037
GM	Left	-0.461	-0.757	-0.793	-0.746	0.171	-0.541
	Right	-0.392	-0.701	-0.703	-0.641	0.24	-0.376
Parietal lobe							
WM	Left	0.553	0.514	0.519	0.041	0.134	0.083
	Right	0.235	0.514	0.302	-0.133	-0.346	0.05
GM	Left	-0.24	-0.608	-0.637	-0.627	0.341	-0.33
	Right	-0.323	-0.589	-0.656	-0.644	0.212	-0.284
Temporal lobe							
WM	Left	-0.065	-0.131	-0.198	-0.687	0.074	-0.56
	Right	-0.249	-0.019	-0.212	-0.659	0.078	-0.298
GM	Left	-0.525	-0.739	-0.826	-0.618	0.055	-0.477
	Right	-0.553	-0.767	-0.817	-0.696	0.06	-0.422
Occipital lobe							
WM	Left	0.111	0.112	0.123	-0.435	0.092	-0.362
	Right	-0.134	-0.065	-0.109	-0.526	-0.12	-0.284
GM	Left	-0.512	-0.505	-0.661	-0.801	0.037	-0.486
	Right	-0.516	-0.608	-0.717	-0.792	0.051	-0.436
Cerebellum							
WM	Left	0.06	-0,215	-0.363	-0.389	-0.157	-0.248
	Right	-0.184	-0.449	-0.543	-0.352	0.097	-0.069
GM	Left	-0.618	-0.823	-0.878	-0.627	0.065	-0.358
	Right	-0.489	-0.711	-0.765	-0.467	-0.046	-0.252
Amygdala							
Left		-0.558	-0.636	-0.812	-0.705	-0.009	-0.532
Right		-0.562	-0.561	-0.755	-0.677	-0.138	-0.528
Hippocampus							
Left		-0.452	-0.683	-0.699	-0.563	0.088	-0.243

Right	-0.558	-0.692	-0.765	-0.481	-0.111	-0.284
Corpus callosum	0.313	-0.028	0.146	0.096	0.525	0.069

Table 3: Correlation between MRI FA values and clinical scales.

Discussion

This study was conducted to investigate the ability of DTI metrics and volumetric analysis to detect regional abnormalities in a cohort of 22 ASD children (44.5 ± 10.6 months) compared with 10 age-matched controls. Our investigation of differences at tissue microstructure level revealed significant FA reductions in all the examined GM regions (namely frontal, parietal, temporal and occipital lobes, hippocampi, amygdala and cerebellum) and a significant decrease in WM-FA values in the occipital lobes and cerebellum. Considering that neither total nor region-specific volumes showed group differences, it is feasible that volumetric differences did not account for the differences in WM and GM integrity detected using DTI.

The presence, in autism, of diffuse alterations of microstructural integrity throughout the GM and WM has been already suggested by Groen WB et al. [31]. According to studies on abnormal brain growth in ASD, early overgrowth is followed by reduced WM growth and atypical loss of GM volume, the latter a consequence of altered likely more intense and precocious, axonal and neuronal pruning. This GM volume loss is thought to be reflected in a reduction in GM-FA [32]. GM growth abnormalities in ASD may also account for local changes in the layered architecture of the cortex and for its reduced processing efficiency in this condition, and may also contribute to abnormalities in the developmental trajectories of WM tracts [33,34]. Although the available data is limited, we suggest that the GM results we obtained might be related to the young age of our cohort; this would, in turn, suggest that GM changes occur before WM ones, as previously reported by [35].

Of note, differences in GM-FA were also found in regions known to be involved in social cognition (emotional and social functioning) such as the amygdala and hippocampi, as well in the cerebellum, which has been shown to have a role in high cognitive functions (mental imagery, anticipatory planning, aspects of attention, affective behaviour, visuospatial organisation, control of sensory data acquisitions) [36].

Unlike the authors of previous studies on DTI and ASD, we found a significant decrease in WM-FA only in the cerebellum and occipital lobes. The former finding further underlines the involvement of the cerebellum in autism, while the latter is probably related to the age range of our patients and likely linked to the well-known posterior-anterior gradient of brain myelination [37]. Moreover, in agreement with whose cohort had a similar age range to ours, we did not find any brain region-specific FA increase [17,18,21,38]. Finally, no significant variation in rD values was found in our ASD patients, in line with what was previously reported in two DTI studies of young children of a similar age to our subjects, whereas a significant aD decrease was found in regions showing a significant FA reduction [17,18]. FA diffusivity changes could be taken as an index of various microstructural alterations, e.g. abnormal myelination, axon size and density, path geometry and the presence of crossing of fibre pathways. However, the finding of decreases in both aD and FA in the same areas suggests that WM aberrations in autism may arise primarily from structural changes due to a decline in axonal integrity rather than changes in myelination [18,34]. The fact that these results and ours contradict those of previous papers is probably due to the wide heterogeneity both of the ASD clinical phenotypes studied and the methods of MRI analysis used [19].

Many studies in the literature investigated correlations between DTI measures and ASD symptoms and some of them showed a relationship between DTI metrics and severity of symptoms. To date, however, no consistent pattern has emerged [7]. This is, again, probably because of difficulties in data comparability due to sample heterogeneity and differences in the clinical scales used or brain regions investigated. In our study, we found a number of relationships, mainly between GM-FA changes and both the Language and the Communication and Social Interaction scales on ADOS-G and the Social Interaction scale on ADI-R. The trend of all these relationships was negative, meaning that a decrease in FA reflects greater symptom severity. This is in agreement with previous literature data and corroborates the hypothesis of autism as a "distributed" disorder of multiple networks rather than a disorder associated with a localised deficit [39-42].

There are several limitations to our study that should be taken into account. First, the sample size is relatively small, which might limit the extent to which the findings can be generalised. Second, it has the inherent limitations of a cross-sectional design and a group difference approach. The latter is based on the assumption that only areas showing differences between controls and autism patients are associated with autism-specific behaviours, whereas it is possible that other brain regions are also involved in generating the autism phenotype [43]. Finally, the analysis of the GM and WM metrics referred to entire lobes. In future studies, the analysis of specific WM tracts and cortical regions may reveal more focal changes. Furthermore, the possible roles of IQ and other behavioural parameters should be investigated.

Conclusion

Volumetric analysis revealed no significant differences between ASD children and controls, while our DTI results suggested an early involvement of both the cerebellum and the supratentorial GM in young ASD children, with FA emerging as the most sensitive parameter, also related to the severity of the symptoms. Further investigation of a larger sample is warranted in order to confirm a potential primary role of GM versus WM changes in the complex aetiopathogenesis of ASD.

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