Universidade do Minho
Escola de Psicologia

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White matter microstructure in adolescents diagnosed with highly functioning autism spectrum disorder: A Tract-Based Spatial Statistics analysis

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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

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(Pedro Miguel Castro Azevedo)

Microestrutura da substância branca em adolescentes diagnosticados com Perturbação do Espectro do Autismo de alto funcionamento: uma análise de *Tract-Based Spatial Statistics*

Resumo

A Perturbação do Espectro do Autismo (PEA), apesar de ser uma perturbação que afeta cerca de 1% da população mundial, tem a sua origem e funcionamento interno envolvidos em mistério, e o seu diagnóstico depende de critérios comportamentais e de interação social. Utilizando Diffusion Tensor Imaging (DTI), um método promissor de ressonância magnética (MRI) capaz de reconstruir imagens *in vivo* de tecidos biológicos, como os axônios neurais, e Tract-Based Spatial Statistics (TBSS), realizamos uma análise dos tratos da substância branca (TSB) para procurar possíveis alterações da mielina na PEA. A nossa amostra foi composta por 6 participantes, adolescentes, com PEA de alto funcionamento, e com 6 controles, com desenvolvimento típico, da mesma idade. Apesar de a literatura apontar para a existência de alterações nos TSB, possivelmente causadas por anomalias de crescimento da mielina, não foram encontradas diferenças estatisticamente significativas neste estudo. Nós ponderamos o efeito de vários fatores, incluindo o tamanho da amostra, variáveis individuais (especificamente idade), metodologia estatística ou/e viés de publicação, como causas para a discrepância dos resultados não significativos. No entanto, esta análise contribuiu para uma maior compreensão das possíveis alterações nos TSB no TEA e deixou sugestões para futuras pesquisas neste campo.

Palavras-chave: perturbação do espectro do autismo, diffusion tensor imaging, mielina, tratos da substância branca, tract-based spatial statistics.

White matter microstructure in adolescents diagnosed with highly functioning autism spectrum disorder: A Tract-Based Spatial Statistics analysis

Summary

Autism spectrum disorder (ASD), despite being a disorder affecting around 1% of the global population, has its origin and inner workings shrouded in mystery, and its diagnosis relies on behaviour and social interaction criteria. Using diffusion tensor imaging (DTI), a promising magnetic resonance imaging (MRI) method capable of reconstructing images of *in vivo* biological tissues, such as the neural axons, and tract-based spatial statistics (TBSS), we carried out an analysis on the white matter tracts (WMT) to search for possible myelin alterations in ASD. Our sample was composed by 6 high functioning late teenage ASD participants with 6 age-matched typically developing controls. Despite the literature pointing to the existence of WMT alterations possibly caused by myelin growth anomalies, no statistically significant differences were found in this study. We weighted the effect of various factors, including the sample size, individual variables (specifically age), statistical methodology, or/and publication bias as causes for the discrepant non-significant results. Nonetheless, this analysis contributed to the further understanding of possible WMT alterations in ASD and left suggestions for future research in this field.

Key-words: autism spectrum disorder*, diffusion tensor imaging, myelin, white matter tracts,* tract-based spatial statistics*.*

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Introduction

Autism spectrum disorder is currently defined as a spectrum composed primarily of two core features, as reported in the Diagnostic and Statistical Manual of Mental Disorders (5th ed.; DSM–5; American Psychiatric Association, 2013), “Persistent deficits in social communication and social interaction across multiple contexts” and “Restricted, repetitive patterns of behaviour, interests, or activities” with the existence of subtypes such as Asperger’s disorder and pervasive developmental disorder not otherwise specified. Due to the fact that there are no reliable biomarkers, the diagnosis of ASD must be made based on these behavioural symptoms, which usually manifest themselves in the early developmental period. In a recently updated systematic review (Zeidan et al., 2022), commissioned by the World Health Organization (WHO), it was estimated that the global prevalence of ASD was about 1%, with sample sizes of the studies considered ranging from 465 to 50 million participants, and the prevalence was found ranging from 1.09/10,000 to 436/10,000, with a median prevalence of 100/10,000, based on the different populations of the studies. The median male-to-female ASD prevalence ratio was 4.2.

Due to the relatively high prevalence of this disorder, the scientific community developed an effort to try to discover the origin and the underlying mechanisms of ASD, with various studies being conducted in distinct, although interconnected fields of research, including genetics, animal models, neurobiology, neuroimmunopathogenesis, and neurosience. The results of these varied works point to abnormal development of the ASD brain and possible disruptions of the neuronal connectivity, with each field arriving at these conclusions using distinct methods that we are going to describe with more detail.

One of the first studies in the field that tried to quantify developmental abnormalities in cerebral and cerebellar volume in ASD was conducted by Courchesne et al., in 2001 using a longitudinal varied sample of 60 autistic and 52 typically developing boys, ages from 2 to 16 years old. This study has a peculiarity rarely seen on ASD studies since, as of the 15 ASD boys with ages comprised between 2- and 5-year-olds, they had access to the neonatal head circumferences from clinical records of 14 of them, that indicated a typical overall brain volume at birth. However, by ages 2 to 4 years, 90% of the boys with ASD had a brain volume larger than the normal average, including more cerebral (18%) and cerebellar (39%) white matter, and more cerebral cortical grey matter (12%). This was opposed to volumes of older

ASD children and adolescents that did not have such enlarged grey and white matter volumes, the contrary in fact, leading to the conclusion of abnormal regulation of brain growth in ASD resulting in early overgrowth followed by abnormally slowed growth.

This was demonstrated in a post-mortem human brain tissue study conducted by Zikopoulos and Barbas (2010) into single axons and their ultrastructure in the white matter. The authors found various alterations in the structure and connectivity in the brain, including a decrease in myelin thickness, an overexpression of growth-associated proteins), with a decrease in axons that communicate over long distances and an excessive number of thin axons that link neighbouring areas. These alterations led them to conclude that "this connectivity bias may help explain why individuals with autism do not adequately shift attention when necessary, and engage in repetitive and inflexible behavior" (p. 12)

Catani et al., (2015) also found similar patterns of brain growth abnormalities in a sample composed of 61 adult males ASD and 61 neurotypical controls. Their study found evidence of abnormal connectivity of the frontal lobes, for example in the anterior portions of the corpus callosum connecting the left and right frontal lobes, and various regional differences and microstructural alterations in brain anatomy, further correlated with specific aspects of ASD symptoms (eg."ASD subjects with severely impaired reciprocal conversation in childhood had a significantly lower number of streamlines in the anterior segment of the arcuate fasciculus compared to ASD subjects with moderate symptoms" (p.6)), that led to the conclusion that "male adults with ASD have regional differences in brain anatomy, (..). We also found that ASD was associated with specific structural abnormalities of white matter fibres, compatible with the concept of autism being associated with atypical developmental connectivity of the frontal lobes." (p.12).

A more recent review by Galvez-Contreras et al. (2020) also explored the alterations in the abnormal development of brain regions in the autistic population and possible disruptions in the neuronal connectivity associated with neural alterations. Their findings led them to conclude that "patients with ASD show an aberrant growth pattern in several white-matter regions that varies throughout life. During the first 15 years of life, axon tracts in the arcuate fasciculus, occipitofrontal fasciculus, and external capsule show a significant hypomyelination, whereas the fusiform gyrus and hippocampal area show a substantial increase in their myelin volume (…). In both cases, these alterations tend to normalize throughout development.

Intriguingly, other brain regions such as cerebellum, cingulum, and internal capsule remain hypomyelinated" (p.8).

An attempt to utilize these, and other studies, that suggested that brain volume overgrowth is a possible factor in the emergence and severity of autistic social deficits was conducted by Hazlett et al., in 2017 with promising results. Utilizing a magnetic resonance imaging (MRI) deep-learning algorithm that used surface area information (specifically the growth rate of the total brain volume of 6–12-month-old high-risk children) they were able to predict the diagnosis of autism at 24 months, with a positive predictive value of 81% and a sensitivity of 88%. Their analysis of the data correlated the hyperexpansion of cortical surface areas to subsequent brain overgrowth, which, in turn, was linked to the emergence of social deficits. Although they didn't conclude if these were the causes or the consequences of the emergence of autism, they provided, what themselves called, "a proof of principle that early prodromal detection using a brain biomarker may be possible" (p.4).

Different efforts to discover such a brain biomarker, or other helpful evidence that could lead to an earlier ASD diagnostic, had already been researched in a series of studies published from 2011 to 2016 in the area of genetics, with positive, but mixed, results that deserve a mention.

Firstly, Ozonoff et al., (2011) published a longitudinal study of infants at risk for ASD by following 664 infants with an older biological sibling with an ASD diagnostic. These infants were monitored from the earliest age possible until they reached the 36 months mark. At this age mark, they were, if not before, evaluated and classified as having or not ASD. Of these 664 infants, 132 (18.7%) were consequently diagnosed with ASD. The significant predictors of the diagnosis were the gender and the existence of one or more older siblings diagnosed with ASD, with a threefold increase in risk for male subjects and an additional twofold increase if more than one sibling was autistic.

In 2014 Sandin et al., utilized a population-based cohort spanning 24 years, including 2 049 973 Swedish children, 37 570 of which twin pairs, 2 642 064 full sibling pairs, 432 281 maternal and 445 531 paternal half-sibling pairs, and 5 799 875 cousin pairs with whom they tried to measure the relative recurrence risk (RRR) of autism in a participant with a sibling or cousin who has an ASD diagnostic vs no diagnostic. The results reported a probability of an ASD diagnosis of 59.2% for monozygotic twins, 12.9% for dizygotic twins, 8.6% for maternal half-siblings, and 6.8% for paternal half-siblings, and 2.6% for cousins expressing a marked

genetic predisposition for autism, leading to the conclusion that “the individual risk of ASD and autistic disorder increased with increasing genetic relatedness. Heritability of ASD and autistic disorder were estimated to be approximately 50%”.

Tick et al., (2016) conducted a systematic review and meta-analysis that identified all twin studies on ASD to the date in order to explores the ethology of the spectrum through these lenses. Of the thirteen eligible studies gathered, only seven meet the Systematic Recruitment criterion. The meta-analysis of these studies concluded that the correlations for monozygotic twins was almost perfect, .98, while dizygotic twins had a significantly lower correlation of .53, thus remarking that “we demonstrated that the ethology of ASD in a combined sample is more consistent with strong genetic influences” (p. 9).

While this is strong evidence of the genetic component of autism, the values varied significantly between studies and posed limited practical benefits to the inner workings of this spectrum, as no specific gene or genome was pointed out as a possible origin of autism. Yet researchers were able to use these findings to support a new avenue of research, utilizing animal modelling.

One such animal model study was conducted by Khanbabaei et al. (2019), where they investigated the myelin development in a model of idiopathic ASD, the BTBR mice. Using MRI they found increased volume in the frontal fibber tracts of the BTBR mice´s brain in the postnatal period that was consequently reduced over time, (similar to the findings in young patients of Courchesne et al. (2001). High levels of myelin basic protein were also found in the young mice; however, the myelin pattern was unaltered in adult BTBR mice.

In a most recent study by Phan et al. (2020), brain transcriptional changes were analysed in five mouse models of Pitt–Hopkins syndrome (PTHS), a syndromic form of ASD caused by mutations in the TCF4 gene. Analyses of differentially expressed genes of the PTHS mouse models showed oligodendrocyte (a type of neuroglia whose main functions are to provide support and insulation to axons) dysregulation, namely cell-autonomous reductions in the oligodendrocyte numbers and myelination. This study also compared the mouse model differentially expressed genes with human idiopathic ASD post-mortem brain RNA-sequencing data, where significant enrichment of overlapping of the differentially expressed genes and common myelination-associated pathways were found, the implication being that disruptions in oligodendrocyte biology are a cellular mechanism in ASD pathology.

One last study in animal models worth mentioning was led by van Tilborg et al. (2017). These authors attempted to create autism-like behaviour in a rat model by combining fetal inflammation and postnatal hypoxia to cause myelin deficits and diffuse white matter injuries. While they only achieved success when both conditions were present (fetal inflammation and postnatal hypoxia) this led to the observation of "signs of autism-like behavior, i.e., repetitive self-grooming and a reduction in social play behavior" (p.10-11) and, similarly to Phan et al. (2020), delayed cortical myelination and long-term changes in the oligodendrocyte maturation. Interestingly they observed that "social play behavior was affected in a more pronounced manner in male rats, compared with females (i.e., males showed attenuated pinning and pouncing, females showed reduced pouncing). This observation seems to reflect the clinical situation, where male gender is an important predictor for ASD in the preterm population" (p.12). This is an important study to keep in mind and correlate to the previous genetic studies because, while the latter predicted ASD in families, the former presents a possible explanation for the emergence in non-ASD-related environments.

While these studies were all conducted on animal models, where experimental conditions can be manipulated and autism-like behavior mimicked, such manipulations cannot happen on human subjects, yet there exists an area of research able to analyse gene expression, cortical myelination, and oligodendrocyte maturation, neuroimmunopathogenesis, whose findings led directly and indirectly to the same conclusions on role of myelin in autism in humans.

A laboratory evaluation of brain autoantibodies and virus serology conducted by Singh (2001) in approximately 250 children with ASD and 150 controls focused on studying the autoantibodies to three major constituents of myelin sheath, myelin basic protein (MBP), galactocerebrosides (GC) and 2, 3-cyclic nucleotide 3'-phosphohydrolase (CNP). This analysis led to the detection of antibodies to myelin basic protein (MBP) in 84% of autistic children (n = 223), compared to less than 1% in normal children (n = 60). There weren't found any statistically significant different between groups regarding the anti-CNP anti-GC antibodies. Even so they concluded "that autism is quite likely a neuro-immune dysfunction syndrome (NIDS) or neuro-immune biology syndrome (NIBS)" (p.10)

These findings were corroborated by Mostafa et al., in their 2008 study of Serum Anti-Myelin–Associated Glycoprotein Antibodies in Egyptian Autistic Children, which considered both the neuroimmunopathogenesis and the genetic hypothesis. They found anti-myelin–

associated glycoproteins in 62.5% of autistic children vs 9.4% of controls. Additionally, family history was taken into account with 50% of the autistic children having a first- or a second-degree relative with an autoimmune disease. Anti-myelin–associated glycoproteins levels were also significantly higher in these children compared to those without it.

Therefore, the evidence converges and points toward myelin being, if not "the" crucial factor, at least "a" crucial factor in the origin of autism. MRI had already been employed in reaching a link between autism and abnormalities in myelin development, as seen by the work of Lewis et al. (2014) and Ecker et al. (2015) with the former analysing the network efficiency of the ASD brain and the latter reviewing these similar studies. The results and conclusions are also similar, finding significantly decreased local and global efficiency over temporal, parietal, and occipital lobes in infants diagnosed with ASD and an atypical trajectory of brain maturation, which gives rise to differences in neuroanatomy, functioning, and connectivity of both local and global aspects of network structure, ending their papers with a call for future exploration of these alterations using "four-dimensional multimodal methods to accurately estimate and analyse changes in connection lengths, connection strengths, network organization and behavior" (Lewis et al., 2014, p. 10), such as Diffusion Tensor Imaging (DTI).

DTI is a Magnetic Resonance Imaging technique that utilizes the physical principles of water diffusion and applies them to access the mobility of water molecules in human tissues, for example, within the brain´s white matter. Le Bihan et al. (2001), and Hagmann et al., (2006) both gave an overview of this technique and its use to demonstrate subtle abnormalities in various diseases (including strokes, multiple sclerosis, dyslexia, and schizophrenia). Their approach to explain its basic principles start with a glass of water. In the glass, the motion of the water molecules is completely random and limited only by the boundaries of its container, the same happens in neuronal axons, where the myelin sheaths surrounding them limit the diffusivity of water molecules. In statistical terms, this fact is called displacement distribution. When this displacement is homogenous, it's called isotropy. When a specific orientation is preferred, this is called anisotropy. From the application of one single pulsed gradient spin-echo sequence in one gradient direction, it is possible to calculate the effect of the isotropy or anisotropy of the white matter tracts (by verifying the water molecules movement within the myelin sheets, taking into account not only the myelin density but also the packaging of the fibber, meaning, the number of axons going in a preferred direction). By computing the normal movement in typical developing controls and in an experimental group it is

(theoretically) possible to compare the degree of myelination of the individual axons and the density of cellular packing that modulate the anisotropy.

The derived measures from this computing are the fractional anisotropy (FA, that describes the degree of anisotropy of the diffusion process), mean diffusivity (MD, the measure of the total diffusion within a voxel), radial diffusivity (RD, that reflects the diffusivity perpendicularly to the axonal fibres), and axial diffusivity (AD, that reflects the diffusivity parallel to axonal fibres).

Before introducing the articles with findings regarding DTI and myelin it is important to mention the work of Alexander et al. (2007), who explored the relationships between DTI measures and white matter pathologic features (ischemia, myelination, axonal damage, inflammation, and edema). In summary, they found that FA is highly sensitive to microstructural changes, but not very specific to the type of changes and is thus a non-specific biomarker of neuropathology and microstructural architecture and recommend the use of multiple diffusion tensor measures (e.g., AD, MD, and RD) to better characterize the tissue microstructure.

Deoni et al. (2014) specifically focused on understanding the myelin alteration in ASD through the analysis of white-matter and myelin differences in young adults. These authors found "for the first time that adults with ASD have highly significant (widespread) differences in myelin content (as measured by the myelin water fraction) compared to age- and IQ-matched controls; and that these myelin content differences in some brain regions are related to clinical symptoms and autistic traits." (p.8).

Barnea-Goraly et al. (2004), investigated white matter structure in seven male children and adolescents with autism and nine age-, gender-, and IQ-matched control subjects. They observed reduced FA values in white matter in the following areas: adjacent to the ventromedial prefrontal cortices; the anterior cingulate gyri; the temporoparietal junctions; adjacent to the superior temporal sulcus (bilaterally); the temporal lobes approaching the amygdala (bilaterally); occipitotemporal tracts; and the corpus callosum.

Other DTI studies that utilized fractional anisotropy as their central measure reached similar conclusions such as Brito et al. (2009), that compared a sample with ASD (9.53 ± 1.83 years), and a neurotypical control sample, (9.57 ± 1.36 years) with the FA values suggesting impairment of white matter microstructure, possibly associated with reduced connectivity in the corpus callosum, internal capsule, and superior and middle cerebellar peduncles.

Jou et al. (2011), in a mean age sample of 13.5 ± 4.0 years, reported that total brain volume and total white matter volume were significantly higher in the ASD group vs neurotypicals. Moreover, while there were no volumes of interest (VOIs) where FA was significantly higher in the ASD group, there existed VOIs with significantly lower FA in the ASD group, namely in the corpus callosum/cingulum and temporal lobes involving the inferior longitudinal fasciculus/inferior frontal-occipital fasciculus and superior longitudinal fasciculus in ASDs.

Inversely, Andrews et al. (2019), in a study with preschool-aged children (i.e., < 30–40 months), found significantly increased measures of FA were in several WM tracts including regions of the genu, body, and splenium of the corpus callosum, inferior frontal-occipital fasciculi, inferior and superior longitudinal fasciculi, middle and superior cerebellar peduncles, and corticospinal tract. Interestingly, in these tracts, females with ASD showed increased AD compared to controls, while males with ASD showed decreased AD compared to matching controls.

Considering the existence of these contradictory findings, reviews and meta-analysis such as the one conducted in 2012 by Travers et al., may shed light on the subject. This work encompassed 48 studies with the theme "Diffusion Tensor Imaging in Autism Spectrum Disorder" that consistently demonstrated reduced FA and increased MD in ASD. Furthermore, they pointed out that children (>4 years of age) and young adults with ASD tend to have decreased FA in WM tracts spanning many regions of the brain (most consistently in the corpus callosum, cingulum, and WM tracts connecting aspects of the temporal lobe) that are often accompanied by an increase in both MD and RD. In contrast with this general pattern of findings, there were 8 studies (16,66%) that found no significant group difference or increased FA in ASD, and 7 studies (14,58%) that found an increased or a mixture of increased and decreased FA across different WM areas in persons with ASD, making a 31,25% of studies going against the reigning hypothesis. They forwarded one important issue that could have impacted these findings, the fact that DTI measurements are highly sensitive to several nonbiologic factors such as scanner, coils, pulse sequences, parameters, signal-to-noise, and that these limitations of DTI should be addressed.

A last word of warning before proceeding with this paper´s analysis comes from a more recent systematic review conducted by Sousa et al. (2017), on diffusion tensor imaging studies on the developmental trajectory of the prefrontal cortex. This review found, from a sample of

27 studies, that while FA, MD, RD and AD have been observed as undergoing linear age-related changes, some periods of steadiness may also occur during development. Interestingly, the highest FA and lowest MD and RD were found in young adults. And again, a reminder that "DTI measures are not intrinsic properties of the tissues but instead, assumptions driven by a conglomerate of knowledge derived from several fields (mathematics, physics, engineering, computer science, and neurosciences) that might limit the associations between DTI data and biological variables" (Sousa et al., 2017, p. 11).

Hypothesis

With all this body of literature supporting us, we also aim to explore the white matter tracts in an autistic population. Specifically, in a population of high functioning teenagers/young adults. Since most of the studies found worked with populations of children, with some adult findings as well, we will assume the generally linear development of white matter tracts and associated measures and guide our work hypothesis with the findings of Sousa et al. (2017), and their report of high FA and low MD and RD measures in this age sample. We will consequently search for differences in these three measures and additionally AD, in order to obtain the clearest possible image of the white matter tracts and associated myelin properties in ASD. Since there are some discrepancies in the literature regarding the direction of the measures, we will respect the strength of the majority and predict that we will find a decrease in FA and AD, and an increase in MD and RD.

Method

Participants

Our sample was composed of 6 individuals with ASD (4♂, 2♀) and 6 typically developing individuals (6♂), ages 15-19 years old (Table 1).

Table 1.

Participant demographics

	ASD (n = 6)	**TD (*n* = 6)**
Age (Years)	16.66 (1.86)	17.16 (1.72)
Sex	4♂, 2♀	6♂, 0♀

The ASD participants enrolled in this study were professionally diagnosed following the criteria established by the Diagnostic and Statistical Manual of Mental Disorders in its revised fourth version (DSM-IV-TR) or fifth version (DSM-5) with “high-functioning autism”. Participants were contacted via several ASD associations. The participants' diagnosis was confirmed by qualified clinicians who were part of the research team using the Autism Diagnostic Interview Revised, ADI-R (Rutter et al., 2006) and the Autism Diagnostic Observation Scale, ADOS (Lord et al., 2008). The control group was recruited using a convenience strategy.

All participants were required to be ambulatory, have no contraindications for MRI, no suspected vision or hearing problems, or other known genetic disorders or neurological conditions. All participants (or their parents in the case of minors) voluntarily agreed to participate and gave the written informed consent, obtained in accordance with the Declaration of Helsinki.

Image acquisition

All MRI scanning was performed at the Grupo de Medicina Xenómica, Universidade de Santiago de Compostela (USC) during the period of the evening, with all the participants awoken and in an alert state. The diffusion-weighted images were acquired between July 2017 and January 2020, using a 3 Tesla Philips Achieva MRI Scanner. DWI scans were performed using a spin echo–echo planar imaging (SE-EPI) sequence: TR = 9312.163 ms, TE = 93.408 ms, FoV = 240 mm x 240 mm, acquisition matrix = 128 x 128, 70 2mm axial slices with no gap, 34 non-collinear gradient directions with b = 700 s/mm 2, one b = 0 s/mm 2 acquisition.

Diffusion-weighted image pre-processing and Tensor Fitting

The diffusion data were pre-processed using the FMRIB Diffusion Toolbox (FDT) provided with the FMRIB Software Library (FSL v6.0.3) (fsl.fmrib.ox.ac.uk), created by the Analysis Group, FMRIB, Oxford, UK.

Pre-processing steps included: (1) transforming the raw DICOM images collected from the machine into NIfTI format (utilized by FSL); (2) performing a DWI visual quality control using the tool FSLEyes; (3) correcting for motion artifacts and eddy current distortions using FSL's eddy tool; (4) applying affine transformations to register each volume and rotate the gradient vectors; (5) removing nonbrain structures by extracting the first b0 volume of each subject and skull stripping. A mask was created and applied to the remaining volumes; (6) using the DTIFIT tool (included in the FDT toolbox) to perform tensor fitting (i. e. fit each voxel with a diffusion tensor that can be defined by its three principal Eigen vectors (iλ1, λ2, λ3)) and use the derived tensor maps to calculate the corresponding scalar maps of fractional anisotropy, mean diffusivity, radial diffusivity, and axial diffusivity (Table 2).

Table 2.

Scalar Maps Formulas

	Formula
Fractional Anisotropy	$\sqrt{\frac{(\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_1-\lambda_3)^2}{2(\lambda_1^2+\lambda_2^2+\lambda_3^2)}}$
Mean Diffusivity	$\frac{(\lambda 1\ +\ \lambda 2\ +\ \lambda 3)}{3}$
Radial Diffusivity	$\frac{(\lambda 2\ +\ \lambda 3)}{3}$
Axial Diffusivity	$\lambda 1$

Diffusion tensor modelling and tract-based spatial statistics

Whole-brain voxel-wise statistical analysis of FA, MD, RD, and AD maps was conducted using tract-based spatial statistics (TBSS) procedures (Smith et al., 2006), also part of the FSL library.

The first step involved non-linearly registering each subject's FA template to each other to find a study-specific template most representative of the sample (i.e., requiring the least warping to align all images, in this case, C_04), into a 1-mm. 1- mm. 1-mm standard space. Secondly, all FA images were then transformed into the Montreal Neurological Institute (MNI) 152 standard space by combining the non-linear transform to the target, with the affine transformation of the target which was subsequently averaged, and the resulting image skeletonized thus producing a mean FA image of all participants. Thirdly, this white matter "skeleton" was thresholded to include FA values at the recommended value of 0.2 to remove from the skeleton regions encompassing other tissues, such as grey matter or cerebrospinal fluid (CSF). The resulting final white matter skeleton was used as a binary mask on which individual measures of FA, MD, RD, and AD were separately projected and subsequently exported for voxel-wise statistical analysis.

Statistical analyses

The statistical analyses of this study were performed using the non-parametric permutation methods of the "randomize" tool from FSL (Winkler et al., 2014). A two-sample t-test was fitted to the data and the Threshold-Free Cluster Enhancement (TFCE), using the 924 unique permutations for each contrast in the skeletonized maps, was calculated to search for between-groups differences. Family-wise error (FWE) correction, $p \leq 0.05$, was applied to control for multiple comparisons

Results

No statistically significant differences were observed between groups for the measures of FA (Figure 1), MD (Figure 2), RD (Figure 3), or AD (Figure 4).

In the figures, the green colour is used to identify the WMT where the calculated scalar maps are consistent between participants. If there were any statistically significant differences, they would be highlighted in red colour (with its intensity, from almost orange to dark red, signalling the increasing differences).

Figure 2

Fractional Anisotropy

Figure 3

Mean Diffusivity

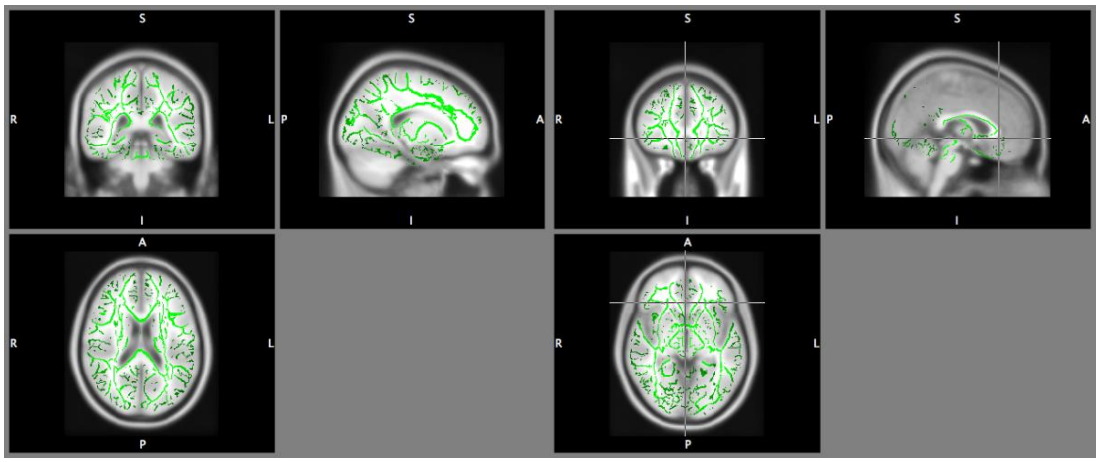


Figure 4

Radial Diffusivity

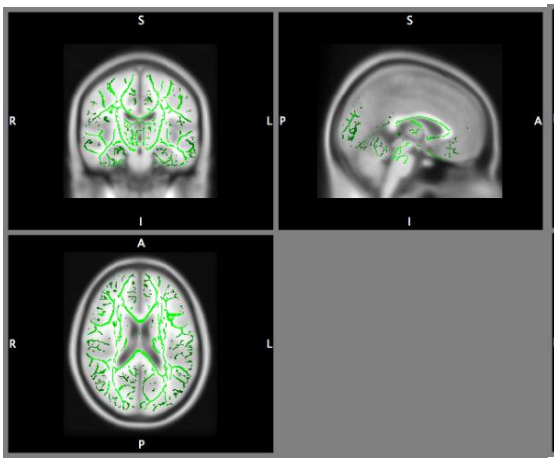
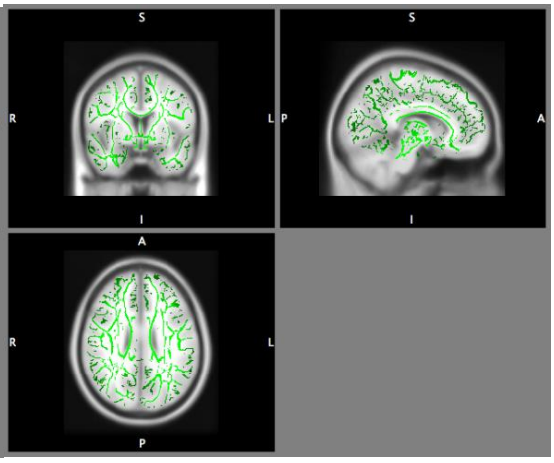


Figure 5

Axial Diffusivity



Discussion

In our work we tried to verify the reported existence of myelin alterations in with matter tracts in ASD using a DTI analysis and TBSS. According to the literature we expected to find differences between conditions, with an increase in FA and AD, and a decrease in MD and RD, in the ASD brain. However, no statistically significant differences weren´t found.

The first factor that might have contributed for the lack of statistically significant differences is the sample size of the study. With only 12 participants, the sample size is admittedly very small. In statistics, small samples have the drawback of reducing the power of the study and increasing the margin of error, which can overshadow statistically significant differences and, consequently, affect the relevance of the conclusions. This coupled with our very conservative threshold might have been a decisive factor in the lack of statistically significant differences.

Our population´s age could also have contributed for this outcome. While most of the studies included either of infants and children (<10) or full adults (>21), our sample was composed by teenagers (15 < x < 19). Based on the evidence gathered from the review by Galvez-Contreras et al. (2020) and the reported stabilization of alterations in the abnormal development of brain regions in the autistic population after the 15 years old threshold, our results should have been similar to those reported by Catani et al. (2016) and Deoni et al.

(2014), both with reports of statistically significantly lower fractional anisotropy. The discrepancy here evidenced could be due in part to the peak of scalar map measures (such as FA) reported by Sousa et al. (2017) that could overshadow the underlying effects. Further studies in this particular age sample could shed better light in this issue.

One possible explanation for the lack of differences in our work is the study of a sample with high-functioning autism, a population in which there may be fewer dissimilarities when compared with the neurotypical population. However, there is previous evidence of similar alterations on the scalar maps of the white matter tracts in high functioning autism (Fletcher et al., 2010; Groen, 2011 (this one in a similar age sample); Mueller et al., 2013; Thomas et al., 2011; Vissers et al., 2012). This leads to the conclusion that the "high functioning" specific diagnostic probably had no effect on the findings when compared to other ASD samples.

Another point to touch upon is the fact that, working backward, the evidence that pointed towards the significance differences of the FA, AD, MD, and RD measures between participants were the various studies that reported such findings (Barnea-Goraly et al., 2004; Ecker et al., 2007; Lewis et al., 2014;Travers et al., 2012), while the only non-significant studies mentioned in the literature were the 8 studies (16,66%) included in the review by Travers et al. (2012) (to which the author offered no commentary).

A comparison between these studies included in the review by Travers et al. (2012) and our analysis revealed only one potential similarity. Of the 8 studies that reported non-significant results, 4 employed the same TBSS procedure as this paper, furthermore, considering that 10 studies in total employed TBSS, there was a 40% non-significance with this procedure. While there is a small possibility that the TBSS method has some intrinsic quality that could account for the lack of significance (or rather its use, as previously stated in the usage of conservative thresholds in small samples), this is not a likely explanation, as there were other non-significant studies that didn't employ TBSS (and studies that employed TBSS, inclusively in both small and large samples, and still reported significant results).

One final point to consider in the reduced existence of non-significant studies. Even the review by Travers et al. (2012), in a universe of 47 studies, found only 8 non-significant studies and 7 that went against the established predispositions. This similar number of non-significant and opposed studies might be and indicator of a publication bias against non-significant results that warrants more research.

We could envision a series of follow-up studies with the intention of tackling the shortcomings of this analysis and advancing the study of the developmental relationship of white matter tracts and myelin alterations in autism spectrum disorder such as:

(i) A study on the impact that different sample sizes would have on the ability of DTI to compute statistical differences (with a study specifically using TBSS and different thresholds of particular interest).

(ii) Utilize various different statistical methodologies on the same sample in other to ascertain its congruency regarding the statistical significance of the results (while also being mindful of the fact that the likelihood of finding false positives will also increase with each statistical analysis performed).

(iii) Expanding the literature on late teenager autistic population (preferably using bigger sample sizes while maintaining the best possible age- and sex- sample matching).

(iv) Investigate the possibility of publication bias against non-significant results (taking measures to ensure that studies are able to be published regardless of existence of results, thus increasing the quantity and quality of the body of literature available).

Conclusion

While the literature pointed towards a defined hypothesis (existence of differences between groups, with decreased in FA and AD measures, and increased MD and RD), it resulted in non-statistically significance, the cause of which could not be pinpointed to one specific reason. Possible factors contributing to this outcome includes the sample size, individual variables (specifically age), statistical methodology, or/and publication bias. Aiming to tackle the shortcomings of this analysis we left some promising suggestions for future research on the field.

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