1 Introduction

Autism spectrum disorder (ASD) is a range of conditions classified as neurodevelopmental disorders in the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders [1]. Individuals diagnosed with autism spectrum disorder are characterized by deficits in social communication and social interaction, as well as restricted, repetitive patterns of behavior, interests and/or activities.

Although language deficits are not observed in all ASD individuals, Barger et al. have suggested that language regression occurs as an initial symptom at an early age in toddlers (around 2 years old) with ASD [2]. However, most studies focused on children above 2 years of age during the past two decades, and alterations of the cerebral language area in ASD patients at an early age (<2 years old) remain largely unknown. Early diagnosis would minimize the impairments of social communication development in children with early and appropriate intervention. Dawson et al. demonstrated the importance of early detection of and early intervention in autism [3].

The underlying pathogenesis of ASD remains poorly understood. Most evidence supports the view that alterations of brain structural and functional connectivity, as well as alterations of white matter tracts, may contribute to the underlying pathogenesis of ASD patients [4–8]. Diffusion tensor imaging (DTI) provides an unprecedented and quantitative capability to probe tissue microstructures noninvasively using small water molecules as a ubiquitous marker.

Arcuate fasciculus (AF) is a white matter tract related to language and can be visualized by 3-dimensional (3D) tractography using DTI. The quantitative evaluation of this tract using diverse DTI parameters may provide an indicator for early diagnosis of ASD in critical periods during childhood. Furthermore, social developmental skills can be attained through the early delivered therapeutic intervention.

In this study, we examined the variations of the language-related white matter tract, arcuate fasciculus...
(AF), between ASD toddlers and the typically developing (TD) control group using DTI tractography.

2 Materials and Methods

2.1 Subjects

The study was conducted from September 2015 to November 2016 at Jining No.1 People’s Hospital (Jining, Shangdong Province, China). Participants were 29 toddlers (15 diagnosed with ASD and 14 TD individuals) with age, gender and handedness matched. Toddlers with ASD who presented language regression were recruited through the local autism organization. The diagnosis of ASD was assessed by two experienced neuropsychologists and a clinical psychologist using the Autism Behavior Checklist (ABC). All of them had a score of at least 30 on the ABC tests. In addition, all those toddlers had a history of regression, as measured by the operational definition, which was previously described by Richler et al [9]. Based on retrospective parental reports, those toddlers had spontaneously used at least three meaningful words on daily basis for at least 1 month (during the first 1–2 years after birth), and subsequently stopped using all words for at least 1 month. The patients with such an abrupt or gradual loss of previously acquired language skills had an age range from 1.42 to 3.25 years old.

The assessment of language function was based on Expressive Language (25 items) and Receptive Language (19 items) subtests of the Chinese Version of the Psychoeducational Profile (C-PEP). The mean IQs of the ADS and TD children were obtained using the Wechsler Intelligence Scale for Children (WISC). The age-equivalent scores assessed with the C-PEP and WISC are presented in Table 1. TD children had a score of 90 or above on percentile ranks of expressive language (EL) and receptive language (RL) as an inclusion criteria. Participants were excluded if they had neurodevelopmental disorder, history of head trauma, or other severe medical problems.

The study protocol was approved by the Institutional Review Board of the Jining No.1 People’s Hospital. Signed informed consent was obtained from parents of the participating children.

2.2 MRI protocols

All magnetic resonance imaging (MRI) scans were acquired with a 3T Siemens TIM Trio scanner (Siemens AG, Munich, Germany). T₁-weighted images were acquired using a 3-dimensional magnetization-prepared rapid acquisition gradient-echo (T₁3D MP-RAGE) sequence covering the whole brain with TR = 1900 ms, TE = 2.5 ms, FOV = 250 mm, slices = 176, slice thickness = 1.0 mm, and bandwidth = 170. For DTI acquisition, a single-shot echo planar (SE-EPI) sequence was applied with TR = 5500 ms, TE = 92 ms, FOV = 260 mm, matrix = 128 x 128, 20 diffusion encoding directions, slice thickness = 3.0 mm, and variable b-values between 0 and 1000 s/mm². Note that each subject was sedated using chloral hydrate (0.5 g in 10 ml) during MRI scanning with parental consent.

2.3 Data analysis

After T₁3D MP-RAGE acquisition, we excluded one toddler diagnosed with ASD who had gray matter heterotopias. We also excluded one toddler with leukomalacia from the control group. In addition, one TD toddler was excluded due to failure in fusing the images between the two acquisitions. T₁3D MP-RAGE also assisted with the precise localization of tractography for DTI using image fusion techniques (Fig. 1 and Fig. 2A).

For tractography, post-processing was performed using Neuro 3D in the Siemens Syngo Workstation, which automatically detects artifacts and corrects eddy current deformations. Tractography of the AF was performed by two raters who were blinded to the participants 1-3 [10, 11]. To access the fiber tracking of AF separately (Fig. 2), a seed region of interest (ROI) was first defined in the middle of the posterior limb of the internal capsule (Fig. 1A) by tracking the AF on the directionally encoded color maps, and then a target ROI was defined at the splenium of the corpus callosum (Fig. 1B).

Subsequently, fractional anisotropy (FA), average fiber length (AFL), tract volume (TV) and number of voxels (NV) were calculated to evaluate the tract properties. Given that language function is hemispherically lateralized and which is related to handedness, we only analyzed parameters of the left AF of these right-handed individuals [12].

Statistical analysis was performed using IBM SPSS v22 statistical software (IBM SPSS, Chicago, IL). Independent sample t-tests and Pearson Chi-squared test were used to compare age, gender, IQ and C-PEP scores respectively. Intergroup differences in the properties of AF were analyzed with analysis of variance (ANOVA). In addition, analysis of covariance (ANCOVA) was applied to compare the AF measurements between the two groups while controlling for age. Results were considered significant when P<0.05.
3 Results

As shown in Table 1, there were no significant differences in age, gender or performance intelligence quotient (PIQ) between the two groups. However, ASD group had lower scores than TD group in verbal intelligence quotient (VIQ), EL and PL. The ANOVA revealed that the ASD group had a significantly lower FA ($F = 6.726$, $P < 0.05$), as well as significantly higher TV and NV compared to the TD group, as shown in Table 2. Covarying for age enhanced the significance of TV and NV. ANOVA for AFL did not reveal any significant differences between the two groups.
4 Discussion

A recent meta-analysis revealed that the prevalence rate for regression in ASD was approximately 32% Confidence Interval (CI) [30–35] (language regression, 24.9%; language/social regression, 38.1%), and the average age at which regression occurred was about 2 years of age; 95% CI [1.7 –1.9] [2]. However, there is no consensus regarding the formal diagnosis of regression, and not all ASD patients suffer with language deficits. Thus, language regression occurring in the early age of ASD can often be overlooked and toddlers with ASD may miss early detection and early intervention [3,13–17]. We examined the variations of the language-related white matter tract, arcuate fasciculus (AF), between ASD toddlers and typically developing (TD) group.

The mechanisms of developmental regression in ASD remain largely unknown. Thomas et al. reported that regression is triggered by overaggressive synaptic pruning[18]. Similar to the Weak Central Coherence Theory devised by Frith, the symptoms of ASD have been hypothesized to be caused by alterations in brain connectivity[19].

White matter (WM) tracts form the structural foundation for brain connectivity by linking discrete grey matter regions into integrated neural circuits [20, 21]. In other words, disruption of the WM tracts that mediate connectivity within neural networks could play an important pathogenic role contributing to abnormal brain maturation in ASD.

The AF is a WM tract of great importance to language. This WM tract connects the frontal expressive language area (Broca area) with the posterior temporoparietal receptive language areas (Wernicke area) [22]. We quantitatively evaluated the properties of AF using multi-parametric DTI \textit{in vivo}. DTI is a non-invasive technique capable of delineating WM tracts and providing indirect quantitative measures of WM integrity by measuring water diffusion in the underlying tissue microstructure.

Previous DTI studies in ASD generally report higher diffusivity and lower FA in multiple brain areas [23–25], such as the corpus callosum [26–28], superior longitudinal fasciculus [28–30], internal capsule [31] and frontoparietal white matter [31, 32]. Our results demonstrated toddlers with ASD exhibit significantly lower FA in the left AF. Although the nature of AF abnormalities remains to

| Characteristics | ASD (n=14) | TD (n=12) | p value |
|-----------------|-----------|-----------|---------|
| Gender (male/female) | 10/4 | 8/4 | 0.56 |
| Age (mean) | 2.39±0.32 | 2.41±0.77 | 0.93 |
| Age (range) | 1.42–3.25 | 1.25–3.83 | |
| Gender (male/female) | 30.50±10.39 | 94.00±3.10 | <0.001 |
| Language (range) | 33.79±15.34 | 93.83±3.10 | <0.001 |
| VIQ | 81.21±18.96 | 104.33±17.53 | 0.004 |
| PIQ | 95.00±14.06 | 103.25±13.16 | 0.14 |

\textbf{Table 1.} Participant characteristics for autism spectrum disorder (ASD) toddlers and typically developing (TD) controls

| Characteristics | ASD (n=14) | TD (n=12) | ANOVA | ANOVAa |
|-----------------|-----------|-----------|-------|--------|
| Mean | SD | Mean | SD | F | p value | F | p value |
| Range | Range | Range | Range |
| FA | 376.11 | 18.79 | 401.01 | 29.72 | 6.726 | 0.016 | 6.835 | 0.016 |
| 365.25–386.95 | 382.12–419.89 |
| AFL | 87.52 | 10.77 | 78.61 | 15.91 | 2.867 | 0.100 | 3.299 | 0.082 |
| 81.30–93.74 | 68.50–88.72 |
| TV | 5206.69 | 2172.40 | 2997.90 | 1775.38 | 7.876 | 0.010 | 9.512 | 0.005 |
| 3950.38–6460.99 | 1870.37–4126.42 |
| NV | 322.71 | 134.57 | 186.33 | 110.33 | 7.810 | 0.010 | 9.451 | 0.005 |
| 245.01–400.42 | 116.23–256.44 |

\textbf{Table 2.} FA, AFL, TV, and NV of AF comparison between ASD toddlers and typically developing controls

\textbf{Abbreviations:} C-PEP: Chinese Version of the Psychoeducational Profile; EL: expressive language; RL: receptive language; VIQ: Verbal Intelligence Quotient; PIQ: Performance Intelligence Quotient.
be elucidated, we speculate that the leading causes of the lower FA values are associated with the reduced myelination in nerve pathways, decreased axonal density (a decrease in the number of axons in the AF with an increase in intra-axonal space), impaired axonal integrity and organization, a decrease in the organization of fibers, and/or an increase in tortuosity.

A longitudinal DTI study [33] and several post-mortem studies [34–36] found an early overabundance of thin axons (representing short-range connections) secondary to suboptimal refinement which can be related to higher TV and NV. In addition, a review by Travers et al. (containing 10 ROI studies and 26 VBA studies investigating AF) indicated that the results of these studies have been inconsistent [37]. Although most studies suggest that FA is decreasing, there are still some exceptions reported. For example, Lee et al. did not find any significant group FA difference in AF [38]. Additionally, Solso et al. found that there was an increased FA in frontal tracts in ASD toddlers [39]. The discrepancies between these studies may be related to the complexity of fiber geometry and crossing in AF, or differing participant characteristics such as language ability and/or age.

To minimize the effects of these issues, we tracked the AF by using the ROI technique for DTI and assessed it separately to eliminate the interference of the crossing fibers along this WM tract. In addition, it is generally known that WM develops dynamically and changes across the lifespan, with myelination continuing from childhood in adolescence. To reduce the influence of developmental trajectories of the brains, our study limited participants to those aged 2–4 years.

Regarding study limitations, our study lacked internationally recognized diagnosis criteria for regression. Furthermore, our findings were also mixed depending on the measurements by parents and clinicians; for example, the disappearance of words that the child had previously established is measured by their parents. In addition, other cognitive functions were not taken into consideration and were not well controlled. Thus, future studies with larger and more diverse samples are needed to verify these findings. The influences of the toddlers with language retardation may be considered another limitation in our study.

Future research should investigate the underlying mechanisms for AF microstructural alterations associated with delayed language development and ASD. Notwithstanding these limitations, our study adds to the few existing studies on the phenomenon of regression during the early ages of children with ASD.

5 Conclusion

The AF of ASD toddlers had significantly lower fractional anisotropy as well as significantly higher tract volume and number of voxels when compared to typically developing controls. Since AF is associated with language, our results suggested that language deficits occur at an early age in ASD patients.

Ethics approval and consent to participate: The protocol was approved by the Institutional Review Board of the Jining No.1 People’s Hospital. All subjects agreed to participate in this study. Written informed consent was obtained from their legal guardians.

Availability of data and materials: The dataset supporting the results of this article are included within the article. Additional clinical data are available from the Jining Rehabilitation Center for Autism and neuroimaging data are available from the corresponding author.

Competing interests: The authors declare that they have no competing interests.

Authors’ contributions: GBW, CQZ, and LZ were responsible for the study’s design. LZ contributed to the acquisition of neuroimaging sequences, conducted the clinical data collection, and wrote the first draft of the manuscript. LZ, XLQ, NZ analyzed the neuroimaging data. ZL, KOL were responsible for statistical analysis. GBW, CHZ assisted in revising the overall manuscript. All authors read and approved the final manuscript.

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