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AJNR Am J Neuroradiol published online 5 April 2012
http://www.ajnr.org/content/early/2012/04/05/ajnr.A3037

Published April 5, 2012 as 10.3174/ajnr.A3037

Elevated Mean Diffusivity in the Left Hemisphere Superior Longitudinal Fasciculus in Autism Spectrum Disorders Increases with More Profound Language Impairment

ORIGINAL RESEARCH

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BACKGROUND AND PURPOSE: Language impairments are observed in a subset of individuals with ASD. To examine microstructural brain white matter features associated with language ability in ASD, we measured the DTI parameters of language-related white matter tracts (SLF) as well as non-language-related white matter tracts (CST) in children with ASD/+LI and ASD/−LI) and in TD.

MATERIALS AND METHODS: Eighteen children with ASD/−LI (age range, 6.7–17.5 years), 17 with ASD/+LI (age range, 6.8–14.8 years), and 25 TD (age range, 6.5–18 years) were evaluated with DTI and tractography. Primary DTI parameters considered for analysis were MD and FA.

RESULTS: There was a main effect of diagnostic group on age-corrected MD ($P < .05$) with ASD/+LI significantly elevated compared with TD. This was most pronounced for left hemisphere SLF fiber tracts and for the temporal portion of the SLF. There was significant negative correlation between left hemisphere SLF MD values and the clinical assessment of language ability. There was no main effect of diagnostic group or diagnostic group X hemisphere interaction for FA. Although there was a main effect of diagnostic group on values of MD in the CST, this did not survive hemispheric subanalysis.

CONCLUSIONS: Abnormal DTI parameters (specifically significantly elevated MD values in ASD) of the SLF appear to be associated with language impairment in ASD. These elevations are particularly pronounced in the left cerebral hemisphere, in the temporal portion of the SLF, and in children with clinical language impairment.

ABBREVIATIONS: ADHD = attention deficit/hyperactivity disorder; AF = arcuate fasciculus; ASD = autism spectrum disorder; CELF-4 = *Clinical Evaluation for Language Fundamentals*. 4th ed; CST = corticospinal tracts; FA = fractional anisotropy; IQ = intelligence quotient; λ_1 = axial diffusivity; 1/2 ($\lambda_2 + \lambda_3$) = radial diffusivity; +LI = with language impairment; −LI = without language impairment; MD = mean diffusivity; SLF = superior longitudinal fasciculus; tSLF = temporal lobe component of the SLF; TD = typically developing children and adolescents

PEDIATRICS

ORIGINAL RESEARCH

Autism is a pervasive neurodevelopmental disorder characterized by impairment in verbal and nonverbal communication skills, social interaction, and repetitive or stereotypic behaviors.[1,2] The degree of impairment and indeed the predominant nature of the phenotype in autism present as a spectrum; as such, a specific subgroup of patients with autism with clinical manifestation of language impairment can be identified. Neuropathology studies, further corroborated by imaging studies, have shown larger volumes of the brain in ASD, more prominently for the white matter than gray matter,[3] with special attention to the frontal "outer radial white matter." Findings suggest that at early ages, there is an abnormal growth spurt resulting in marked greater volumes of the brain in patients with ASD compared with TD, as much as 20%, which then subsides with aging.[4] The abnormality in the development of frontal white matter, specifically left motor and premotor white matter, was shown to correlate positively with motor disability.[5] Furthermore, the more peripheral U fibers for the frontal lobes[6] are thought to be involved. All these findings suggest that a white matter maturation anomaly may be a hallmark of ASD.

A study by Fletcher et al[7] pointed to diffusivity differences in the AF in a small sample of boys with ASD. The present study sought to extend this finding, evaluating DTI parameters of the SLF, which, because it is considered to be related to language function, might differentially impact children with ASD/+LI and ASD/−LI, compared with TD. To evaluate whether potential abnormalities would be specific to language tracts or rather reflect a more general phenomenon in the brain white matter of patients with autism, we extended the evaluation to the tSLF, which more closely reproduces the AF, and also compared it against non-language-related CST.

Specifically, the hypothesis of this study was that microstructural diffusion properties of white matter tracts involved in language processing would be impaired in patients with

Received November 5, 2011; accepted after revision December 21.

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This study was supported in part by a National Institutes of Health grant R01DC008871 (T.P.L.R.) and a grant from the Nancy Lurie Marks Family and Autism Speaks.

Paper previously presented in part at: Annual Meeting of the International Society for Magnetic Resonance in Medicine, May 1–7, 2010; Stockholm, Sweden; and Annual Meeting of the American Society of Neuroradiology and Neuroradiology Education and Research Foundation Symposium, May 15–20, 2010; Boston, Massachusetts.

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EBM2 indicates Evidence-Based Medicine Level 2 as designated by peer reviewers.

http://dx.doi.org/10.3174/ajnr.A3037

ASD compared with age-matched controls and that the degree of abnormality would be associated with clinical language impairment.

Materials and Methods

Subjects

Eighty-four subjects were enrolled in the study. Subjects with ASD were recruited through the Regional Autism Center at the Children's Hospital of Philadelphia. All had a prior diagnosis of ASD that was confirmed by an experienced neuropsychologist by using a combination of tests including the Autism Diagnostic Observation Schedule and Social Communication Questionnaire. Participants were excluded if they had known genetic syndromes or neurologic impairments (eg, cerebral palsy, epilepsy). Twenty-four subjects were excluded from analysis (6 ASD/−LI, 7 ASD/+LI, and 11 TD) due to excessive motion artifacts on imaging precluding full DTI acquisition or tractography, with 1 patient with ASD/+LI excluded for the finding of a radiologically abnormal corpus callosum. The final population included 18 ASD/−LI (mean age, 11.3 years; range, 6.7–17.5 years), 17 ASD/+LI (mean age, 9.6 years; range, 6.8–13.9 years), and 25 TD (mean age, 11.4 years; range, 6.5–18 years). Although there were no significant differences in age between diagnostic groups ($P > .2$), all analyses were conducted with age as a covariate (given the tendency toward younger participants in the ASD/+LI group).

We recorded medication use: Children in the TD group generally had little pertinent medication use (1 acne medication, 1 fluoride supplement, 1 diphenhydramine [Benadryl, not taken the day of scanning], 1 albuterol). The ASD group had a higher use of either ADHD medications or antidepressants. Ten were taking medications often prescribed for ADHD-like symptoms: 1 methylphenidate (Daytrana); 2 methylphenidate (Metadat); 1 dextroamphetamine sulfate (Dexedrine); 1 dexmethylphenidate (Focalin); 1 atomoxetine (Strattera); 1 racemic amphetamine aspartate monohydrate, racemic amphetamine sulfate, dextroamphetamine saccharide, and dextroamphetamine sulfate (Adderall); and 3 methylphenidate (Concerta). Of these, 2 were taking atypical antipsychotics (aripiprazole [Abilify], risperidone [Risperidal]) as well as their ADHD medications. Two were taking antidepressant medication (sertraline hydrochloride [Zoloft], fluoxetine [Prozac]). One participant was taking melatonin, perhaps to mitigate sleep disturbances.

Subanalysis of the effects of medication are beyond the scope of this study, given the small number taking medications and their variety. Additionally, a parental report of ADHD-like symptoms was taken, though formal testing was not performed. Six of the ASD participants were reported as having comorbid (or prior diagnosis of) ADHD. This group was too small for subanalysis and was retained to reflect the clinically presenting heterogeneity of ASD.

All subjects underwent a battery of cognitive and clinical neuropsychological assessments, which included the CELF-4 to assess language function.[8] The CELF-4 comprises tests of both receptive (comprehension/understanding of spoken language) and expressive (spoken) language. The Core Language Index has a mean standard score of 100 and an SD of 15. It represents a composite summary of the most reliable and diagnostically sensitive norm-referenced CELF-4 subtests of language performance at each age. Specific tasks in the Core Language Index depend on the child's age and include tasks such as sentence repetition, following directions, sentence formulation, word structure knowledge, identification and expression of semantic similarities, and definition of words. Clinically significant language impairment was defined as a score lower than 85 on the Core Language Index of the CELF-4. TD were required to score above 85 on the CELF-4 Core Language Index for inclusion.

In addition to assessment of language function via the CELF-4, quantitative assessment of full-scale IQ was made by using the Wechsler Intelligent Scale for Children-IV and assessment of social functioning was made by using the Social Responsiveness Scale. Global cognitive delay was excluded by requiring scores at or above the fifth percentile (standardized score > 75) on the Perceptual Reasoning Index of the Wechsler Intelligent Scale for Children-IV.

Imaging

DTI acquisition parameters were as follows: isotropic 2-mm-thick contiguous whole brain acquisition performed on a 3T magnet (Verio; Siemens, Erlangen, Germany); FOV, 25.6 cm; matrix, 128 × 128; TR/TE, 14,000/70 ms; a scheme with 80 sections and 30 diffusion-encoding gradient directions; 1 $b = 0$ (0 s/mm^2), b max = 1000 s/mm^2; generalized autocalibrating partially parallel acquisition (GRAPPA) with an acceleration factor of 2.0. Scanning time was approximately 7 minutes. Children were prepared for scanning with standard pediatric desensitization techniques, including mock scanning. We diligently attended to patient comfort and compliance. No sedation was used.

Postprocessing was performed by using DTIStudio (Johns Hopkins University, Baltimore, Maryland).[9] Tractography of the right and left SLF, tSLF, and CST was performed by 3 raters blinded to the clinical data and following previous tractography protocol guidelines,[10] with an FA threshold of 0.25 and an angle cutoff of 70°. For tracking of the SLF, briefly, as previously described,[10] the coronal plane was chosen at the level of the middle of the posterior limb of the internal capsule on the axial plane, and an extensive region of interest was drawn, including the entire frontal area, except for the cingulum and insula. A second region of interest was drawn on a coronal section selected at the splenium of the corpus callosum and fibers were reconstructed if they passed through both ROI1 and ROI2.

Fibers from the posterior limb of the internal capsule, superior fronto-occipital fasciculus, and inferior fronto-occipital fasciculus, as well as fibers within the occipital region and cingulum, were excluded by using the logical "NOT" operation. Fibers along the external capsule were not excluded. For the tSLF, the first region-of-interest placement followed the same steps, and a second region of interest, by using the logical "AND" operation, was placed, including the fibers of the SLF seen on the axial section at the anterior commissure level. For the corticospinal tract, the first region of interest included the entire unilateral cerebral peduncle, by using the logical "OR" operation, at the level of the decussation of the superior cerebellar peduncles. A second region of interest with a logical "AND" operation was placed on the precentral gyrus on the axial section right after the bifurcation to the motor and sensory cortex could be identified. Crossing fibers and cerebellar fibers were excluded with the logical "NOT" operation. DTI parameters considered for analysis were MD and FA, evaluated in 3D over the defined fiber course of the SLF, tSLF, and CST. For secondary analysis, the individual eigenvalues of the diffusion tensor model, λ_1, λ_2, and λ_3 were evaluated over the same tract-based VOIs.

As an inter-rater intraclass correlation coefficient of >0.9 was obtained for each of the left and right hemisphere measures of MD and FA, these measurement results were averaged across raters. Similarly, eigenvalue analyses were made on the basis of the averaged values derived by the 3 raters.

Statistical evaluation comprised an age-covaried (3 × 2) general

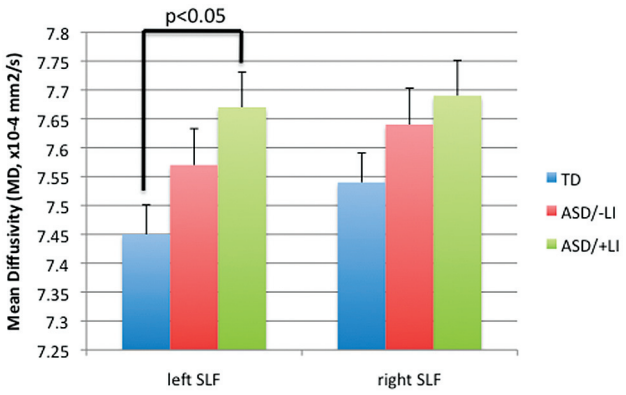

Fig 1. Left and right hemisphere MD of SLF tracts show elevation of ASD/−LI and particularly ASD/+LI versus TD controls. Differences are more pronounced in left hemisphere compared with right hemisphere tracts.

linear model with diagnostic group (TD versus ASD/−LI versus ASD/+LI) and hemisphere as factors. General linear model analyses were run separately for both MD and FA parameters. Simple-effects post hoc *t* tests were performed where indicated, and the Bonferroni correction for multiple comparisons was applied.

Finally, a hierarchic regression model considering both age and the CELF-4 Core Language Index was built and tested separately for each left and right hemisphere MD and FA. All statistical analyses were performed by using the general linear model and linear regression functions in the Statistical Package for the Social Sciences, Version 19.0 (SPSS, Chicago, Illinois).

Results

Mean and SD CELF-4 Core Language Index scores for the 3 groups were as follows: TD, 107 ± 10; ASD/−LI, 102 ± 12; ASD/+LI, 69 ± 11. There was a highly significant main effect of group ($P < .001$). Post hoc simple-effects analysis revealed significant differences in the CELF-4 Core Language Index between ASD/+LI and TD ($P < .001$) and between ASD/+LI and ASD/−LI ($P < .001$), but not between ASD/−LI and TD ($P = .145$).

As expected there was a significant effect of diagnostic group on the Social Responsiveness Scale (TD, 44 ± 2; ASD/−LI, 80 ± 2; ASD/+LI, 72 ± 3; $P < .001$) with post hoc *t* tests showing significant differences between TD and ASD/−LI as well as TD and ASD/+LI. There was also a weak effect of group on full-scale IQ ($P = .033$) (TD, 103 ± 20; ASD/−LI, 108 ± 10; ASD/+LI, 91 ± 15), with post hoc tests not showing a significant difference between TD and ASD/−LI ($P \sim 1.0$) or TD and ASD/+LI ($P = .176$), but resolving ASD/−LI versus ASD/+LI ($P = .032$). This distinction may be inevitable on the basis of recruitment of IQ-matched children with ASD (who are subsequently divided according to CELF-4 scores, which may be associated with general cognitive function in the full-scale IQ).

Evaluating the entire extent of the SLF, we found a main effect ($P < .01$) of diagnostic group on age-corrected MD, with values projected to a sample mean age of 10.9 years: ASD/+LI, $7.676 \pm 0.045 \times 10^{-4}$mm^2/s versus TD, $7.497 \pm 0.036 \times 10^{-4}$mm^2/s; and ASD/−LI, $7.605 \pm 0.043 \times 10^{-4}$mm^2/s) (Fig 1 and Table). Simple-effects analyses showed that SLF MD in ASD/+LI is significantly elevated compared with TD ($P < .01$). There was neither a main effect of hemisphere nor a significant interaction between diagnostic group and hemisphere on SLF MD. Despite the lack of significant interaction, an a priori hypothesis about hemispheric asymmetry in language processing warranted separate age-corrected ANOVA analyses for each hemisphere. When considering the left hemisphere SLF, again we found a significant effect of diagnostic group on MD ($P = .035$), with simple-effect analyses again showing significantly elevated SLF MD in ASD/+LI compared with TD. In contrast, the analysis of right hemisphere SLF MD showed no significant main effect of diagnostic group ($P = .339$).

There was no main effect of diagnostic group on age-corrected SLF FA (TD, 0.523 ± 0.003; ASD/−LI, 0.519 ± 0.004; ASD/+LI, 0.518 ± 0.004; $P > .05$), though there was a significant effect of hemisphere (with left hemisphere values being 0.526 ± 0.003 compared with right hemisphere values of 0.514 ± 0.003, $P < .01$). There was no significant diagnostic group X hemisphere interaction for FA.

When analyses were repeated for the tSLF, the above effects were replicated with more powerful significance: Namely, for the left hemisphere tSLF MD, there was a significant main effect of diagnostic group ($P = .006$), with simple-effects post hoc *t* tests showing elevated MD in ASD/+LI versus TD (7.774×10^{-4}mm^2/s versus 7.458×10^{-4}mm^2/s, $P = .003$), while in the right hemisphere, there was no significant main effect of diagnostic group ($P = .781$).

In contrast, when considering the CST, not typically associated with language function, while there was indeed an overall main effect of diagnostic group ($P = .01$) with age-corrected MD values of 7.386×10^{-4} mm^2/s (TD) versus 7.451×10^{-4} mm^2/s (ASD/−LI) versus 7.550×10^{-4} mm^2/s (ASD/+LI), analysis by hemisphere revealed no significant effect of diagnostic group in the left hemisphere tracts ($P = .119$) and only a weakly significant effect in the right hemisphere ($P = .047$), which did not yield any significant pair-wise differences on simple-effects post hoc *t* tests. There was no significant main effect of hemisphere or group X hemisphere interactions for either MD or FA.

When we considered the entire length of the SLF, secondary analysis of the individual eigenvalues of the left hemisphere tract diffusion tensor revealed a significant effect on λ_1 ($P < .01$) with ASD/+LI values significantly elevated ($12.57 \pm .08 \times 10^{-4}$mm^2/s) versus TD ($12.22 \pm .07 \times 10^{-4}$mm^2/s). There were no significant effects of diagnostic group on λ_2, λ_3 ($0.5 \times [\lambda_2 + \lambda_3]$), $P = .133$, though these values were also tending to increase. There was no significant main effect of diagnostic group for any of the 3 right hemisphere SLF diffusion tensor eigenvalues.

In each hemisphere, to examine associations between CELF-4 scores and SLF, we performed hierarchic regression, in which CELF-4 was entered first, group scores second, and their interaction last. In the left hemisphere, CELF-4 scores predicted significant variance in SLF MD, $F(1,58) = 14.23$, $P < 0.001$, with a significant negative correlation indicating that a 0.007×10^{-4}mm^2/s increase in MD is associated with a 1-point decrease in the CELF-4 score (Fig 2). The diagnostic group X CELF-4 interaction term was not significant, indicating no group differences in the nature of associations between CELF-4 scores and SLF MD. Examination of the correlations between CELF-4 and SLF MD for each group indicated that

Diffusion parameters (MD and FA) of the SLF, tSLF, and CST[a]

Tract		TD		ASD/−LI		ASD/+LI	
		LH	RH	LH	RH	LH	RH
SLF	MD $\times 10^{-4}$ mm^2/s	7.45 ± 0.05[b]	7.54 ± 0.05	7.57 ± 0.06 P = .36 vs TD	7.64 ± 0.06 P = .74 vs TD	7.67+/-0.06[b] P = .81 vs ASD/-LI P = .028 vs TD	7.69+/-0.06 P = 1 vs ASD/-LI P = .33 vs TD
tSLF	MD	7.46 ± 0.05[b]	7.60 ± 0.06	7.57 ± 0.06 P = .60 vs TD	7.66 ± 0.07 P = 1 vs TD	7.75+/-0.07[b] P = .12 vs ASD/-LI P = .003 vs TD	7.72+/-0.07 P = 1 vs ASD/-LI P = .69 vs TD
CST	MD	7.39 ± 0.05	7.38 ± 0.05	7.51 ± 0.05 P = .26 vs TD	7.40 ± 0.06 P = 1 vs TD	7.52+/-0.06 P = .24 vs ASD/-LI P = 1.0 vs TD	7.58+/-0.06 P = .12 vs ASD/-LI P = .06 vs TD
SLF	FA	.529 ± 0.005	.517 ± 0.004	.523 ± 0.006 P = 1.0 vs TD	.516 ± 0.005 P = 1 vs TD	.526+/-0.006 P = 1.0 vs ASD/-LI P = 1.0 vs TD	.509+/-0.005 P = 1.0 vs ASD/-LI P = .96 vs TD
tSLF	FA	.542 ± 0.005	.527 ± 0.005	.544 ± 0.006 P = 1.0 vs TD	.523 ± 0.006 P = 1 vs TD	.538+/-0.006 P = 1.0 vs ASD/-LI P = 1.0 vs TD	.517+/-0.007 P = 1.0 vs ASD/-LI P = .50 vs TD
CST	FA	.615 ± 0.004	.623 ± 0.007	.613 ± 0.005 P = 1.0 vs TD	.609 ± 0.008 P = .52 vs TD	.607+/-0.005 P = 1.0 vs ASD/-LI P = .88 vs TD	.611+/-0.008 P = 1.0 vs ASD/-LI P = .63 vs TD

Note:—LH indicates left hemisphere; RH, right hemisphere.
[a] All are marginal mean measures ± SEM and are projected to a sample mean age of 10.9 years.
[b] Statistically significant difference.

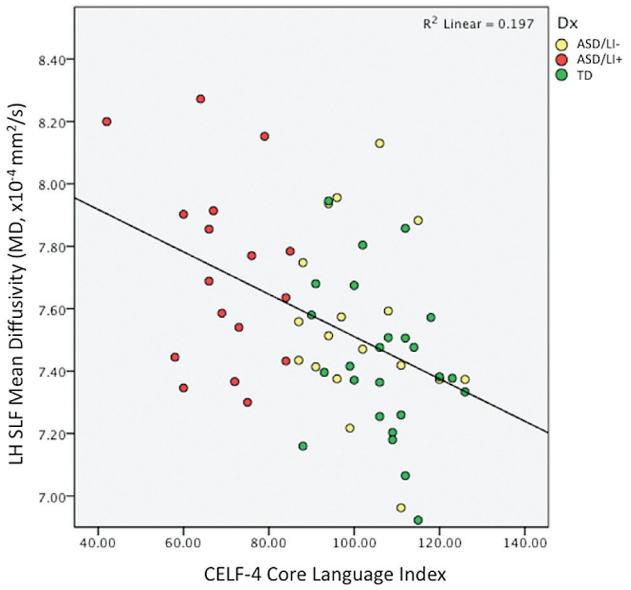

Fig 2. Clinical implications of LH SLF MD—a significant negative correlation (R^2 = 0.2, P < .05) relates diffusivity with a clinical language assessment, suggesting a 0.007 × 10^{-4} mm^2/s decrease in MD per 1-point increase in the CELF-4 Core Language Index. Color coding represents diagnostic group membership: Red indicates ASD/+LI; yellow, ASD/−LI; green, TD.

the associations, though in the expected direction (ie, lower CELF-4 scores associated with larger SLF MD), were nonsignificant in each group (TD, P = .18; ASD/+LI, P = .25; ASD/−LI, P = .49; though pooled ASD, P < .05). In the right hemisphere, entered first, CELF-4 scores predicted significant variance in SLF MD, $F(1,58) = 4.93$, $P < .05$, similar to the left hemisphere analysis, but less pronounced.

To examine the association between age and SLF MD, we performed hierarchic regression, in which age was entered first, diagnostic group second, and their interaction last. In the left hemisphere, entered first, age predicted significant variance in SLF MD, $F(1,58) = 13.93$, $P < .001$. Entered second, the main effect of diagnostic group remained significant, $F(2,56) = 3.79$, $P < .05$, indicating that the left hemisphere diagnostic group effects were not due to age. The diagnostic group X age interaction term was not significant, indicating no group differences in associations between age and SLF MD. Examination of the correlations between age and SLF MD for each group indicated that the associations were significant in the ASD/−LI group ($P < .05$). Although in the expected direction (ie, lower SLF MD in older subjects), the associations were not significant in the ASD/+LI and TD groups (TD, P = .15; ASD/+LI, P = .20).

Discussion

The main findings of this study are the following:

1) Elevated MD was observed in the SLF of children with ASD and concomitant language impairment. This was particularly evident in the left hemisphere and in the temporal portion of the SLF.
2) A significant negative correlation was found between the SLF MD and clinical assessment of language function, as indexed by the Core Language Index of the CELF-4. Associations tended to be stronger in the left hemisphere (where a CELF-4 age model accounted for >40% of the observed variance in MD) than in the right hemisphere (where the model accounted for 26%, though both were significant). These associations suggest that abnormal white matter microstructure has functional consequences and suggest that SLF is a core element of the human language network.

Overall, it appears that diffusion properties and, in particular, the MD of cerebral white matter are atypical in ASD, with MD being elevated somewhat generally but especially in the left hemisphere, language-related tracts of children with ASD, and especially in those subjects with concomitant clinical language impairment.

DTI uses the water molecule diffusion pattern as a probe to

infer the microstructural architecture of the white matter, and as such, it provides one with the capability of identifying different fiber bundles, along which diverse parameters can be evaluated to yield quantitative information[11] about white matter integrity.

Evaluation of the brain microstructural environment revealed by DTI has been used not only for early diagnosis of pathologic conditions, such as in early diagnosis of cerebral infarction,[12,13] but also for better understanding of pathophysiologic mechanisms and, furthermore, to correlate normal and abnormal anatomy and physiology to brain function.[14,15] Regarding language function, early reports established association of lesions in specific cortical anatomic locations in the left frontal lobe with clinically recognized symptoms, defined as aphasia.[16] With time, subcortical pathways, such as the SLF, particularly its subdivision, the AF,[17-19] were also shown to be involved in language function.[20]

Identification of language tracts by DTI has been pursued for a long time, and the imaging technique gained high acceptance in the literature. As a method, DTI has even contributed to better understanding of language pathways and was used as a means to revisit the peri-Sylvian network,[21,22] increasing understanding of the complexity, both anatomically as well as functionally, of the language pathways; expanding the concept of language pathways, including 2 basic receptive and executive centers, known as the Wernicke and Broca areas; and introducing an associative area named "Geshwind's area." Also, the classic description of the AF as a tract connecting the frontal and temporal lobes, named the "direct long pathway," was supplemented by an indirect pathway, which includes a posterior lateral segment connecting the temporal and parietal lobes as well as an anterior lateral segment connecting the frontal and parietal lobes.

Asymmetry of the SLF and AF has been described in the literature,[23] though other reports have not found this asymmetry,[6,10] possibly related to different parameters being assessed in the different studies. In terms of language, loss of the leftward asymmetry in the number of fibers in the AF has been used as a predictor of aphasia at discharge in patients with left middle cerebral artery infarct. Using this measure, the study obtained a sensitivity of 0.83 and specificity of 0.86.[24] Kumar et al[25] have found, among other findings, increased length of the right AF in patients with ASD, associated with lower FA and higher MD. The language tracts evaluated in this study were obtained following a previously reported protocol.[10] The tSLF was found to be very similar to the AF reported on other studies.

There has been a considerable number of recent publications implicating abnormal diffusion tensor properties in the white matter of children with ASD.[6,7,25-32] Lee et al[33] also demonstrated an increase in MD in the bilateral white matter of the superior temporal gyrus and right temporal stem in patients with high-functioning autism compared with age-matched TD. The authors also found an overall reduction in λ, λ_2, λ_3 and attributed the findings along these pathways, critically involved in language and social cognition, to microstructural disorganization. Even though increases in λ_2 and λ_3 have been attributed to abnormalities of myelin, the authors believed that changes in axonal attenuation, organization, and gliosis could as well lead to increase in λ_2 and λ_3. Sundaram et al[6] also found an increase in MD with associated reduction in FA in short association fibers in the frontal lobes of patients with ASD compared with TD; MD was also increased in the long fibers of the frontal lobes in ASD. Likewise, despite descriptions of increased white matter in autism, if one examined the number of fibers, no significant difference was found between ASD and TD. Increased MD and decreased FA were also seen in patients with autism along a smaller-than-normal corpus callosum,[34] more pronounced in a group with lower, however normal, performance IQ measures. Most relevantly, Fletcher et al[7] showed elevated MD in the AF in a pilot study ($n = 10$) by using an automated fiber selection, similar to the findings in our larger sample with a manual fiber-drawing approach. Most interesting, the implication suggested for language function is borne out in our study.

Our results corroborate the concept of the correlation of anatomic structure quantitative evaluation and clinical and behavioral function, establishing that a decrease of 0.007×10^{-4} mm^2/s was associated with a 1-point improvement in CELF-4. Although analyses indicated possible associations within each group between CELF-4 scores and SLF MD, larger n studies are needed to determine whether associations between CELF-4 and SLF MD hold in each group. Given an estimated correlation of 0.35, power analyses indicate that a sample size (n) of ~60 per group is needed (power = 0.80, $\alpha = .05$).

Of note, our findings implicate MD as a sensitive marker of ASD, but there were no significant effects on FA. Close examination of the eigenvalues of the diffusion tensor may account for this differential sensitivity: Although only λ_1 showed a significant increase in the ASD group, both λ_2 and λ_3 also showed nonsignificant tendencies to increase in ASD, supporting an overall increase in MD but a diminished change in FA (because all 3 eigenvalues were found to increase and FA relates to the SD across the 3 eigenvalues). While the biologic underpinning of the diffusion observation must, at this time, remain speculative, 1 possible etiology that would lead to elevated MD would be an incomplete or insufficient pruning of white matter branches (part of the normal developmental process), preventing axons from bundling tightly and allowing water to diffuse more readily. This, however, might be expected to elevate the λ_2 and λ_3 more than the λ_1, and such elevations (though observed) were not statistically dominant.

Patients with ASD/−LI presented with MD values between ASD/+LI and TD, reflecting a spectrum of abnormalities that could culminate in a clinical expression of language impairment. Present results point to increased MD along the left SLF and especially the left tSLF. Abnormal MD could result in reduced functional connectivity, a hypothesis consistent with previous fMRI findings of atypical right lateralization of the letter fluency task to the right inferior frontal lobe[35] and consistent with the theory of long-range underconnectivity in autism.[36] While connectivity inferences are beyond the scope of this analysis, it is perhaps worth considering a high value of MD as indicative of immature or abnormal WM development; therefore, high MD may well not be directly equivalent to hyperconnectivity and may, in fact, be associated with underdeveloped connectivity. The less-significant quantitative differences seen in the CST reinforce the idea that this observation is somewhat composite—comprising a general tendency to-

ward elevated MD, as well as a tract-specific element, that might relate more specifically to individual symptom profile (eg, clinical language impairment). Most interesting, the weak difference in IQ between groups might speculatively account for the weaker difference between groups more generally in white matter (eg, CST), whereas the highly significant difference in language function may be reflected in the more significant difference in MD observed in the SLF. However, simple regression analyses of MD in either SLF or CST showed no significant association with full-scale IQ.

One caveat regarding this evaluation, however, was that handedness was not considered as an exclusion criterion for our sample, because in our population, 1/25 subjects of the TD group, 3/18 of the ASD/−LI, and 1/17 of the ASD/+LI were left-handed. One subject of the ASD/−LI group was ambidextrous. Additionally, because of the heterogeneity and small samples, we did not stratify by medication usage.

Conclusions

Increase in MD was found in the children with ASD and, especially, ASD/+LI compared with TD, in the tracts of the left hemisphere related to language function, especially, the tSLF. This appears to reflect a change in the microstructure of the white matter subserving language functions in patients with ASD/+LI.

Acknowledgments

We acknowledge Ralph Magee, Peter Lam, Rachel Golembski, and Jamie Rundio for technical assistance. The authors gratefully acknowledge the contributions of all faculty, staff, and students of the University of Pennsylvania/Children's Hospital of PhiladelphiaCenter for Autism Research. Dr Roberts thanks the Oberkircher Family for the Oberkircher Family Endowed Chair in Pediatric Radiology.

Disclosures: Timothy Roberts—*UNRELATED*: *Board Membership*: Prism Clinical Imaging, *Comments*: No overlap, only stock options (no fees), *Stock/Stock Options*: Prism Clinical Imaging, *Comments*: no overlap.

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