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Diffusion Tensor Imaging Provides Evidence of Possible Axonal Overconnectivity in Frontal Lobes in Autism Spectrum Disorder Toddlers

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ABSTRACT

BACKGROUND: Theories of brain abnormality in autism spectrum disorder (ASD) have focused on underconnectivity as an explanation for social, language, and behavioral deficits but are based mainly on studies of older autistic children and adults.

METHODS: In 94 ASD and typical toddlers ages 1 to 4 years, we examined the microstructure (indexed by fractional anisotropy) and volume of axon pathways using in vivo diffusion tensor imaging of fronto-frontal, fronto-temporal, fronto-striatal, and fronto-amygdala axon pathways, as well as posterior contrast tracts. Differences between ASD and typical toddlers in the nature of the relationship of age to these measures were tested.

RESULTS: Frontal tracts in ASD toddlers displayed abnormal age-related changes with greater fractional anisotropy and volume than normal at younger ages but an overall slower than typical apparent rate of continued development across the span of years. Posterior cortical contrast tracts had few significant abnormalities.

CONCLUSIONS: Frontal fiber tracts displayed deviant early development and age-related changes that could underlie impaired brain functioning and impact social and communication behaviors in ASD.

Keywords: Autism, Development, DTI, Frontal tracts, Tract FA, Tract volume

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The first warning signs of autism spectrum disorder (ASD) involve abnormal social, communication, language, and emotional behavior (1–3). In typically developing infants and toddlers, these higher order abilities depend on the normal growth of long-distance connections and widely distributed neural networks in the brain, especially fiber tracts between frontal and temporal cortices, the amygdala, and the striatum (4). Across the early years, tracts normally display robust changes in microstructure, such as increases in axon size, myelination, and overall volume, and diffusion tensor imaging (DTI) measures of tract microstructure, such as fractional anisotropy (FA) and volume, and index these robust changes from fetal life through childhood (5), although DTI studies of normal development between ages 1 to 4 years remain rare. Whether these critical frontal, temporal, and limbic fiber tracts display pathologic development by the ages when autistic symptoms first begin has been studied little.

Almost 40 years ago, Damasio and Maurer (6) proposed that autism was due to frontal-temporal-limbic disconnection and dysfunction. The theory supposed ASD involved reduced numbers of cortical neurons and underdevelopment of axons and frontolimbic fiber tracts. This early speculation has been further refined and elaborated and is a prevalent theory today: namely, that disconnection or underconnectivity between different brain regions underlies ASD (7,8), despite structural evidence that cerebral white and gray matter in ASD at young ages may be increased (9–11). Reduced functional connectivity in functional magnetic resonance imaging (fMRI)-based studies (8,12) and reduced fiber tract FA in the great majority of DTI-based studies of older children (13–15), adolescents, and adults with ASD (16,17) do seem to support the original and prevalent view of structural/axonal, as well as functional, underconnectivity.

While abnormally reduced FA is one of the most consistent types of biological findings in ASD, few tracts have been consistently reported as abnormal, study sample sizes are typically small, volumes of specific tracts are seldom measured, and few studies have mapped and measured specific tracts (as opposed to measuring voxels within regions of interest) (17). Importantly, even though ASD is a disorder of very early development (18–20), nearly all DTI studies have been on older children, adolescents, and adults (17).

New fMRI and DTI data from the 1- to 4-year-old ASD brain do not fit neatly into the structural/functional disconnection model. Instead, new studies suggest a complex view of age-related changes in pathologic circuitry across early development in ASD. In toddlers with ASD, one fMRI study found reduced left-right synchronous functional activity (21), but another found hyperconnectivity of fronto-temporal-cerebellar activity (JH Manning, unpublished data, February 28, 2014).

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In contrast to numerous DTI studies of ASD adults and older ASD children that report decreased FA (e.g., decreased FA in fronto-frontal short-range fibers) (16,17,22), the three DTI studies of ASD infants and young children report the opposite: increased FA (23–25), including increased FA in frontal, but not posterior, regions of interest (23). In the only longitudinal DTI study of young ASD toddlers, Wolff *et al.* (24) found increased FA at 6 months of age but nonsignificant tendencies for decreased FA at 24 months in several fiber tracts including the left uncinate fasciculus, left inferior longitudinal fasciculus, and body of the corpus callosum; the comparison subjects were unaffected younger siblings of ASD children. In that study, increased tract FA at young ages in ASD was interpreted as indicating more compact, dense tracts. Whether this unexpected age-related change in FA in some tracts is general to ASD or specific to the ASD versus siblings at-risk for ASD comparison in Wolff *et al.* (24) remains untested and is an important question, especially in light of studies showing that behavior and neurobiology of unaffected siblings of children with ASD lies somewhere between that of ASD groups and typically developing infants and young children (26–28). Nonetheless, Ben Bashat *et al.* (23) and Wolff *et al.* (24) raise novel questions and possibilities about how fiber tracts develop in early life in ASD as compared with typical toddlers.

Postmortem data from ASD children, genetic findings (e.g., *CHD8, PTEN, EIF4A, WDFY3, KCTD13-CUL3-RHOA*) (29–37), and ASD animal models (31,28,39) point to disruption of cell cycle in fetal development, excess neuron proliferation, and brain overgrowth and call into question the anatomical underconnectivity hypothesis. For instance, in young ASD children with heavier than normal brain weight, prefrontal cortex has a 67% excess of neurons (40). This neuron excess predicts greater, not reduced, axon numbers in prefrontal tracts at young ages in ASD. Theoretical models predict doubling neuron numbers could quadruple axon numbers (41). Such postmortem data suggest that, at young ages, there should be an increase in prefrontal axon numbers, which could increase frontal tract volumes in ASD. If ASD does involve an excess of axons in prefrontal tracts at young ages, then ASD might be better modeled as a disorder of early overconnectivity, not underconnectivity, of prefrontal axons. Additionally, these frontal tracts might also display deviant growth trajectories across the first years of life, because, at young ages, genes and gene networks underlying cell differentiation and growth are downregulated (42), and by later childhood and adulthood in ASD, cell size in the cortex is reduced (40,43–44).

To test this general hypothesis of abnormal density, volume, and/or growth of frontal tracts, we DTI imaged 94 ASD and typically developing (TD) 1- to 4-year-olds and used probabilistic atlas-based mapping of multiple frontal fiber tracts; because ASD often displays an anterior to posterior gradient of neural pathology and dysfunction, posterior tracts served as a priori contrast tracts. Validation of this type of tractography methodology comes from a study of showing high correlations between anatomically dissected frontal tracts and DTI tractography-based measures of frontal tracts (45). We also examined the correlation between outcome ASD social and communication symptom severity and FA and volume.

The corpus callosum was measured because decreased FA in the callosum in older children, adolescents, and adults with ASD is the most consistently reported DTI abnormality in the literature (17); its measurement provides a strong test of the nature of early callosal development in ASD relative to a large literature on ASD at older ages.

METHODS AND MATERIALS

Subjects

Participants included 94 toddlers: 61 ASD and 33 TD toddlers ranging in age between 12 and 48 months (Table 1). A subsample of ASD (n = 14) and TD (n = 13) toddlers had a second DTI scan at a follow-up assessment that took place approximately 1 year after the initial scan. An additional 12 participants (7 ASD, 5 TD) were scanned but not included in analyses (Supplement). This project was reviewed and approved by the Human Subjects Protection Review Board at University of California San Diego. Informed consent was obtained from parents or guardians of toddlers.

Table 1. Diagnostic and Clinical Characteristics of ASD and TD Participants

Clinical Measurement	ASD (n = 61)	TD (n = 33)	p Value
Sex (M/F)	48/13	20/13	.103[a]
Age in Months	30.2 (8.4); 12–48	25.9 (11.1); 13–46	.056[b]
Mullen Subscale Scores			
Receptive language (earliest)	29.8 (10.8); 20–62	52.7 (8.8); 39–72	≤.001[c]
Receptive language (recent)	33.0 (11.6); 20–58	55.7 (8.3); 42–72	≤.001[c]
Expressive language (earliest)	32.3 (10.0); 20–62	55.3 (10.0); 38–75	≤.001[c]
Expressive language (recent)	33.8 (12.0); 20–60	58.1 (9.9); 41–80	≤.001[c]
ADOS Communication and Social Total Score (recent)	14.2 (3.9); 7-20	1.7 (1.5); 0–5	≤.001[b]
ADOS Restricted and Repetitive Behavior Score (recent)	3.6 (1.4); 1-6	.2 (.5); 0–2	≤.001[b]

Values for age and Mullen Early Scales of Learning scores are presented as mean (SD) and range.

ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; F, female; M, male; Mullen, Mullen Early Scales of Learning; TD, typically developing.

[a]Pearson's chi-squared test.

[b]Welch's t test.

[c]Accelerated failure time model.

Subject Recruitment

Toddlers were recruited through community referral and a population-based screening method, the 1-Year Well-Baby Check-Up Approach, by the University of California San Diego Autism Center of Excellence (46) (Supplement). Because clinical signs of risk for ASD in general pediatric settings naturally begin at diverse ages from infancy to 3 years of age, these recruitment strategies enabled us to detect individuals at the age when they first showed clinical risk signs, namely at any age from 12 months to 3 years.

Diagnostic and Developmental Evaluations

Toddlers were clinically diagnosed, evaluated, and tracked every 6 months until they reached at least 3 years of age, when a final diagnosis was made (Table 1; Supplement). Evaluations included the Autism Diagnostic Observation Schedule (47); Vineland-II Adaptive Behavior Scales, Second Edition (48); and Mullen Scales of Early Learning (49).

Image Acquisition and Processing

Natural sleep magnetic resonance imaging scanning (50–52) was used to maximize success rates and to ensure that findings generalized to all toddlers from both study groups. Imaging was performed on a GE 1.5T scanner (General Electric High-Definition 1.5T twin-speed EXCITE scanner, Buckinghamshire, United Kingdom) and T1-weighted three-dimensional structural scans and 51-direction diffusion-weighted sequences were collected (Supplement). Images were inspected to exclude data with scanner artifacts or head motion; processing was via a published automated method (53), and conventional DTI methods calculated FA and the principal orientation of diffusion at each voxel (54,55).

Fiber Tracts and Primary and Secondary Outcome Measures of Interest

Primary outcome measures of interest were FA and volume of forceps minor, inferior frontal-superior frontal tract (IFSF), uncinate tract, frontal projection of the superior corticostriatal tract (fSCS), and the arcuate fasciculus division of the superior longitudinal fasciculus (Figure 1; Supplemental Table S1) (45). FA and volume measures were obtained independent of each other as described below and elsewhere (56).

Contrast tracts were forceps major of the corpus callosum, parietal projection of the superior corticostriatal tract, parietal portion of the superior longitudinal fasciculus, cingulate portion of the cingulum, parahippocampal portion of the cingulum, inferior occipital-frontal fasciculus, and inferior longitudinal fasciculus (Figure 1). FA and volume were obtained from each.

The same measures were also obtained from the corpus callosum as a whole (including the forceps minor, body of the callosum, and forceps major).

Automated, Atlas-Based Tract Identification

A probabilistic atlas by Hagler *et al.* (53) containing information about the locations and orientations of fiber tracts was used to estimate the a posteriori probability that a voxel belonged to one of the primary or contrast fiber tracts of interest. During the course of this study, we additionally developed an infant/toddler DTI atlas from 23 of the study subjects using exactly the same approach as Hagler *et al.* (53) and found very similar performance (Supplemental Figure S1). Results reported herein are from the validated and published Hagler *et al.* (53) atlas, but our pediatric probabilistic atlas is available upon request (Supplement). Voxels containing primarily gray matter or cerebral spinal fluid—identified using FreeSurfer's automated brain segmentation—were excluded from analyses (FreeSurfer 5.2; http://surfer.nmr.mgh.harvard.edu) (57). Average FA values were calculated for each fiber tract, weighted by the fiber probability at each voxel. Fiber tract volumes were calculated as the number of voxels with fiber probability greater than .15, the value that provided optimal correspondence in volume between atlas-derived tracts and manually traced fiber tracts. No FA threshold was used in the determination of tract volume. For bilateral tracts, FA and volume were averaged across left and right sides. For each individual, tract FA and volume were calculated separately and derived in an unbiased, consistent manner.

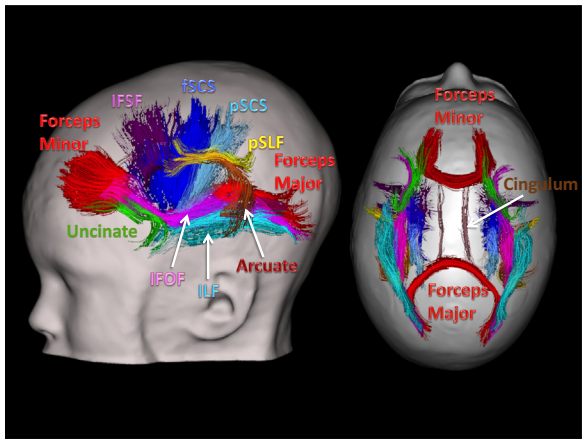

Figure 1. Atlas-based tract reconstructions for a representative single subject showing all primary and contrast tracts examined in the study. fSCS, frontal projection of the superior corticostriatal tract; IFOF, inferior frontal occipital fasciculus; IFSF, inferior frontal superior frontal tract; ILF, inferior longitudinal fasciculus; pSCS, parietal projection of the superior corticostriatal tract; pSLF, parietal portion of the superior longitudinal fasciculus.

Statistical Analysis Methods

Gender and age were compared between ASD and TD subjects with Pearson's test and Welch's two-sample *t* test, respectively. Clinical variables that had censored values were compared between groups with a parametric survival model (accelerated failure time model), assuming an underlying normal distribution.

For each of the primary tracts (forceps minor, IFSF, uncinate, fSCS, and arcuate), FA, volume, and age-related changes were compared between ASD and TD. The design of our study allowed us to make use of both cross-sectional and longitudinal data in estimating group age-related changes.

Linear mixed effects models were used with group, age, gender, and a group × age interaction as independent variables and tract volume or FA as dependent variables. A random intercept was included to account for repeated

measures. We included a measure of the coefficient of determination (R^2) (58) to assess model fit and to examine how well DTI measures at early ages predict the same measures at later ages in the context of the overall model. To account for multiple testing of the age × group effect, we used the Benjamini-Hochberg (59) correction for multiple comparisons and report unadjusted *p* values but only interpret those findings with significant q value for the tracts included in the primary analysis.

Post hoc analyses were carried out to better understand observed differences in age-related changes in FA and volume in ASD relative to TD groups. First, for those tracts with significant group × age interactions for FA, we conducted the same analysis for three other diffusion measures, namely the apparent diffusion coefficient, the longitudinal diffusion coefficient, and the transverse diffusion coefficient. Second, for both FA and volume, we examined how measures with significant group × age interactions related to clinical severity measured at the age of final diagnosis. Measures of clinical severity at older ages are expected to be more stable (60) compared with evaluations at a younger age. Given the nature of the group × age interaction, we examined the clinical relationships separately in two subgroups of ASD children: the youngest subgroup (ages 12 to 28 months, $n = 25$) and the oldest subgroup (ages 37 to 48 months, $n = 25$). We calculated the Pearson's correlation between Autism Diagnostic Observation Schedule communication and social total score and both FA and volume of the primary tracts that showed significant age × group interactions.

Analyses of the effect of group and group × age interaction were undertaken for volume and FA of contrast tracts (listed above) and the corpus callosum. We did not employ correction for multiple tests among the contrast tracts to be more lenient in allowing for discovery of group differences in the nature of the association with age in these nonfrontal tracts.

RESULTS

Frontal Tract Differences Between TD and ASD Toddlers

Fractional Anisotropy and Volume of Frontal Tracts. After Benjamini-Hochberg correction for multiple (10) comparisons, mixed effects models showed significant ASD versus TD group × age effects for FA and volume for multiple frontal tracts. For FA, significant effects were in forceps minor, IFSF, uncinate, and arcuate tracts ($p = .019$, .039, .001, and .04, respectively, for all $q < .05$; Supplemental Table S2). For volume, significant effects were in forceps minor, IFSF, fSCS, and uncinate ($p = .024$, .02, .031, and .038, respectively, for all $q < .05$; Supplemental Table S4).

As shown in Figure 2, there was a slower apparent rate of change with age in both FA and volume in ASD as compared with TD subjects: very young ASD toddlers began with higher tract FA and volume than TD toddlers, but by 3 to 4 years of age, this effect for FA and volume had disappeared in ASD. Comparable group × age effects for FA and volume in each tract were present in male and female subjects (Supplemental Figures S2 and S3). Coefficients of determination in frontal tracts were very high, ranging from .63 to .81 for partial R^2 for FA (Supplemental Table S2) and from .37 to .66 for R^2 for volume (Supplemental Table S4), indicating very high ability of the model to predict a second FA measurement or volume measurement given group, age, sex, and the first observed measurement. Post hoc analyses of hemisphere effects were nonsignificant and similar effects were seen in each hemisphere for FA (Supplemental Table S6) and volume (Supplemental Table S7).

Significant main effects of group were seen for FA in all five frontal tracts ($p = .001$ to .032; Supplemental Table S2) and for volume in forceps minor, IFSF, and fSCS ($p = .005$, .009, and .011, respectively; Supplemental Table S4) with higher FA and volume at early ages but not at all ages, as indicated by the significant group × age effects (Figure 2). This main effect should be interpreted, therefore, in light of the significant group × age interactions, which indicate that higher FA in the ASD group is not observed at older ages.

We followed up these results with a post hoc analysis of apparent diffusion coefficient, longitudinal diffusion coefficient, and transverse diffusion coefficient within the four significant tracts (Supplemental Table S3). There were no significant group × age effects for these other tract-based diffusion measures.

Clinical Relationships With Frontal Tract FA and Volume. Within the frontal tracts that showed significant group × age interactions, we examined whether severity of communication and social deficits at the age of final diagnosis were related to FA or volume among a subgroup of ASD children who were younger (12 to 28 months, $n = 25$) and older (37 to 48 months, $n = 25$) at the time of DTI scanning. Among the younger group, all of the observed correlations were positive for both FA (range of *r*s: .23 to .41) and volume (range of *r*s: .07 to .30) such that children with the largest FA and volume when scanned before 28 months of age tended to be those with the greatest severity at their final evaluation. Correlations were significant for arcuate FA ($r_{23} = .41$, $p = .04$) and at trend level for FA of the forceps minor ($r_{23} = .37$, $p = .07$) and uncinate ($r_{23} = .36$, $p = .08$). In contrast, among the subgroup of ASD children who were older than 37 months when scanned, all observed correlations were negative, although none were significant at $p < .05$ in this subsample. There was a trend-level correlation ($r_{23} = -.34$, $p = .096$) observed for the volume of the IFSF. Thus, the general pattern observed was that higher FA and volume in the first 2 years of life predicted greater autism severity at the age of final diagnosis, but the brain-behavior correlation was somewhat reversed among older children, in whom lower FA and volume tended to relate to greater severity.

Posterior Comparison Tracts in TD and ASD Toddlers

Mixed effects models showed no significant group × age (or group) effects for FA in any contrast tract or for volume in most contrast tracts, the exceptions being group × age effects for volume for inferior longitudinal fasciculus (uncorrected $p = .028$) and inferior occipital-frontal fasciculus (uncorrected $p = .012$; Supplemental Table S5). Findings were generally consistent across left and right hemispheres for bilateral tracts (Supplemental Tables S8 and S9). The frontal and contrast

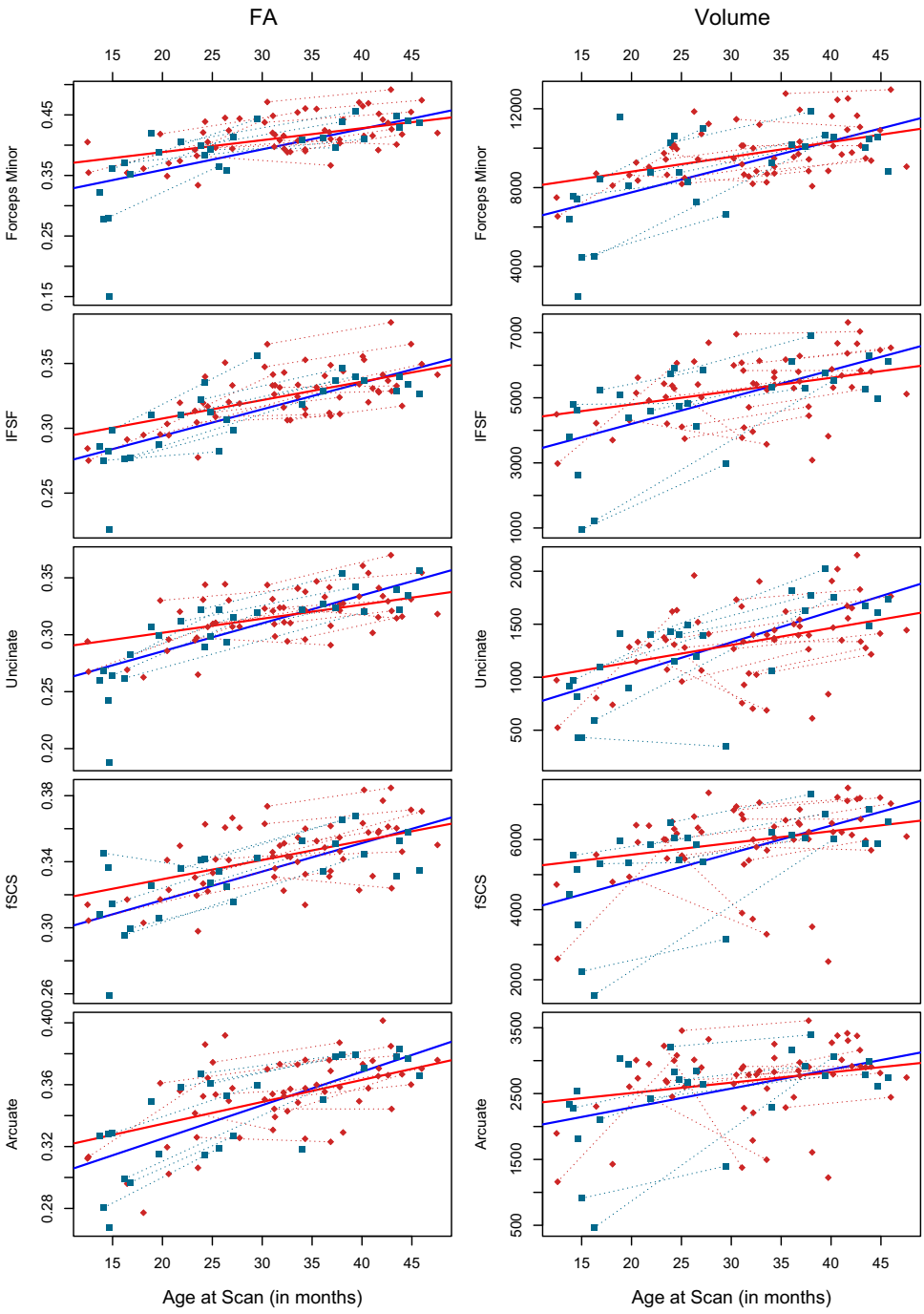

Figure 2. Fractional anisotropy (FA) (left panel) and volume (right panel) measures for forceps minor, inferior frontal superior frontal tract (IFSF), uncinate, frontal projection of the superior corticostriatal tract (fSCS), and arcuate fasciculus portion of the superior longitudinal fasciculus by age for male participants (for female participants, see Supplemental Figures S1 and S2). Blue squares and red diamonds represent typically developing and autism spectrum disorder subjects, respectively. The solid blue and red lines in each plot represent model fits for typically developing and autism spectrum disorder subjects; dotted lines connect longitudinal measures for individual participants.

tracts with significant group × age effects for FA or volume are summarized in Figure 3.

Corpus Callosum

There were significant group × age (and group) effects for FA and volume for the corpus callosum (FA: $p = .036$; volume: $p = .036$; Supplemental Table S5), likely driven by significantly greater forceps minor FA (Supplemental Table S2) and volume (Supplemental Table S4) in ASD as compared with TD in early ages, as well as significantly flatter slope of apparent age-related change in ASD in this frontal portion of the callosum (Figure 2). Since group differences were not seen for the posterior forceps major (Supplemental Table S5), the group results for the whole callosum may be relatively uninfluenced by the posterior portion.

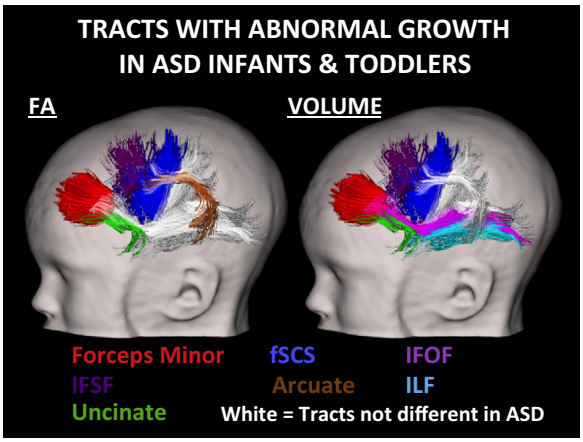

Figure 3. Atlas-based tract reconstructions for a representative single subject showing all tracts with abnormal age-related trajectories for fractional anisotropy (FA) (left) and/or volume (right). fSCS, frontal projection of the superior corticostriatal tract; IFOF, inferior frontal occipital fasciculus; IFSF, inferior frontal superior frontal tract; ILF, inferior longitudinal fasciculus.

DISCUSSION

Abnormalities of multiple frontal axon pathways were present at the age of first clinical signs of ASD. Abnormal FA and volume were found for intrafrontal and interfrontal pathways involved in higher order social, language communication, speech, cognition, and behavior control. These abnormalities may impede integration of neural activity within and between different subregions in left and right frontal lobes. They were also found for frontal projections to important subcortical and cortical structures involved in social, emotional, language, and behavioral control functions (61–70). The nature of the ASD versus TD group difference varied by age, such that younger ASD infants had abnormally higher FA and volume, whereas this was no longer the case in older ASD children who tended to have slightly lower FA and volume, suggesting an altered white-matter developmental trajectory in ASD. Wolff *et al.* (24) reported a similar abnormal trajectory for several of the fiber tracts studied here. We did not observe group differences in age-related change in other diffusion measures, perhaps due to lower reliability of these indices. The relationship between FA and volume measures and severity of social and communication symptoms seemed to differ depending on age at the DTI measurement: higher FA and volume of frontal tracts at the youngest ages tended to predict greater symptom severity at final evaluation, whereas a trend toward the opposite relationship (lower FA and volume related to greater severity) was seen for frontal tracts measured at older ages, suggesting that greater early growth abnormality may be associated with greater later symptom severity.

Early developmental changes in tract volume and FA are affected by many factors, including number of axons, growth in axon caliber, increases in myelination, changes in the packing density of axons, and coherence of axon orientation. Multiple lines of evidence support the view that several pathologies—excess neurons and axons, reduced cell and axon growth, and reduced myelination—likely underlie the abnormal volume and FA trajectories we describe here. First, postmortem, genetic, genomic, and animal and cell model studies of ASD (18,31,33–35,38–40,42,43) collectively point to increased prenatal excess of prefrontal neurons, and therefore axons, in enlarged ASD brains. Many high-confidence ASD gene defects are associated with excess neuron proliferation and brain overgrowth (e.g., *CHD8, PTEN, EIF4A, WDFY3, KCTD13-CUL3-RHOA*) (29–36). *CHD8*, a gene of high importance in ASD, targets cell cycle networks (37) and is associated with excess neurons and brain overgrowth (34,35). Computational data (41) indicate increases in neuron numbers can quadruple axon numbers, and an excess of short and medium distance prefrontal axons has been reported postmortem in ASD (71). We therefore hypothesize that early but transient increased frontal tract volume and FA are a result of prenatal prefrontal axon excess due to prenatal neuron excess. Aberrant connectivity could also ensue from abnormal early developmental spine and synapse formation and function, which can be a direct result of a prenatal excess of layer 2 and 3 neurons according to a novel mouse model of ASD (71). In that model of ASD, prenatal neuron excess produces a developmentally transient excess of spines, reduction in excitatory synapses and altered excitatory/inhibitory ratio, all of which reverse with age (i.e., decreased spines, reduction in excitatory synapses and reversed excitatory/inhibitory ratio). Much evidence also points to genetic factors in synapse functioning in ASD (72,73). Thus, we view early overconnectivity in ASD as having a prenatal origin likely due to neuron excess. Second, postmortem studies of ASD point to 1) dysregulation of gene expression and gene networks that affect cell differentiation and growth (71); 2) reduction in cell and minicolumn size that would be expected to affect axon caliber (40,74–76); 3) reduction in very large caliber long-distance axons in ASD adults (71); and 4) presence of thin short and medium distance prefrontal axons (71). Third, postmortem studies of ASD adults find decreased myelination of axons in some prefrontal regions (71). In sum, postmortem and genomic evidence support the conclusions of Zikopoulos and Barbas (71), Casanova and Trippe (76), and Courchesne and Pierce (77) that there is an early excess of axons and overconnectivity in autism but later underdevelopment of axons in multiple prefrontal regions. Here, we propose that different neural developmental pathologies—excess axons and misconnectivity, axon underdevelopment, and developmental dysmyelination—acting at different ages underlie the changing FA and volume patterns seen during early development in ASD. Ultimately, these lead to reduced volume and FA as reported in most DTI studies of ASD adolescents and adults.

The frontal tracts that develop abnormally in ASD—fronto-amygdala, fronto-temporal, fronto-frontal, and fronto-striatal—are important in social, emotion, language, and behavior control functions (61–70). For example, the uncinate fasciculus is a key pathway connecting frontal and amygdala regions. Frontal and amygdala structures are strongly and interactively involved in complex social behavior (4,78) and the evolution of social behavior (68). In a literature review on how and where social knowledge is represented, Olson *et al.* (61) concluded that orbital frontal and amygdala regions are key sites and hypothesized the uncinate fasciculus is a principal interconnecting pathway. Recently, Oishi *et al.* (62) examined fiber

tracts in adults with lesions and found that of eight different tracts studied, lesions of only the uncinate significantly altered emotional empathy. In the present study, uncinate fasciculus FA and volume in ASD toddlers, in whom social and emotion functions are significantly impaired, were highly significantly abnormal at early ages and showed a much shallower slope of age-related change. In magnetic resonance imaging studies at young ages in ASD, overgrowth of amygdala volume is correlated with variation in deficits in joint attention and social behavior (79–81). Early abnormalities in fronto-amygdala growth and uncinate connectivity undoubtedly play key roles in disturbances in early autism social behavior. As a second example, the arcuate is critically involved with human language and its size and broad temporal and frontal projection regions are unique in humans, being much smaller and narrowly restricted in apes and monkeys (82). The expanded arcuate pathway in humans underlies transmission of word-meaning information stored in temporal cortices to several frontal regions for sentence comprehension and sentence construction during spontaneous speech (82). Frontal callosal axons within the forceps minor enable integration of higher order language and prosodic information. Microstructural development of arcuate and forceps minor pathways is abnormal in ASD toddlers in the present study as well as in Wolff *et al.* (24). These defects in language pathways are accompanied at young ages in ASD by failure of language to normally activate temporal cortices, especially in ASD toddlers who later have poor language and communication abilities (51,83,84), and by abnormal brain-language relationships (83). Furthermore, in our study, young ASD children with higher FA in these pathways (i.e., who were more abnormal) had poorer social and communication symptoms at their final evaluation. Thus, critical language cortical regions and pathways are already aberrant in ASD at the very earliest ages, and this may be an important characteristic of ASD, as it occurs in the general pediatric population (the present study) and in multiplex families (24).

We conclude that at the age of first clinical signs of ASD, the frontal lobes in a majority of individuals already display axonal overconnectivity and growth pathology that are likely due to neuron excess and lead to underfunctional connectivity and impaired social and communication behavior. Further imaging and postmortem studies of early structural hyperconnectivity in ASD (such as frontal u-fibers) will be important in formulating computational models of connectivity development in ASD, interpreting functional overconnectivity and underconnectivity data at young ages and guiding the development of accurate cellular and animal models of the disorder. Potentially, a combination of measures of axon development, cortical growth trajectories, and clinical abnormalities (83) may lead to biosignatures of early risk for autism.

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ARTICLE INFORMATION

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REFERENCES

1. Zwaigenbaum L, Bryson S, Rogers T, Roberts W, Brian J, Szatmari P (2005): Behavioral manifestations of autism in the first year of life. Int J Dev Neurosci 23:143–152.
2. Wetherby AM, Woods J, Allen L, Cleary J, Dickinson H, Lord C (2004): Early indicators of autism spectrum disorders in the second year of life. J Autism Dev Disord 34:473–493.
3. Jones EJ, Gliga T, Bedford R, Charman T, Johnson MH (2014): Developmental pathways to autism: A review of prospective studies of infants at risk. Neurosci Biobehav Rev 39C:1–33.
4. Adolphs R (2009): The social brain: Neural basis of social knowledge. Annu Rev Psychol 60:693–716.
5. Dubois J, Dehaene-Lambertz G, Kulikova S, Poupon C, Huppi PS, Hertz-Pannier L (2014): The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. Neuroscience 276:48–71.
6. Damasio AR, Maurer RG (1978): A neurological model for childhood autism. Arch Neurol 35:777–786.
7. Geschwind DH, Levitt P (2007): Autism spectrum disorders: Developmental disconnection syndromes. Curr Opin Neurobiol 17:103–111.
8. Just MA, Keller TA, Malave VL, Kana RK, Varma S (2012): Autism as a neural systems disorder: A theory of frontal-posterior underconnectivity. Neurosci Biobehav Rev 36:1292–1313.
9. Carper RA, Moses P, Tigue ZD, Courchesne E (2002): Cerebral lobes in autism: Early hyperplasia and abnormal age effects. Neuroimage 16:1038–1051.
10. Herbert MR, Ziegler DA, Makris N, Filipek PA, Kemper TL, Normandin JJ, *et al*. (2004): Localization of white matter volume increase in autism and developmental language disorder. Ann Neurol 55:530–540.
11. Schumann CM, Bloss CS, Barnes CC, Wideman GM, Carper RA, Akshoomoff N, *et al*. (2010): Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. J Neurosci 30:4419–4427.
12. Muller RA, Shih P, Keehn B, Deyoe JR, Leyden KM, Shukla DK (2011): Underconnected, but how? A survey of functional connectivity MRI studies in autism spectrum disorders. Cereb Cortex 21:2233–2243.
13. Kumar A, Sundaram SK, Sivaswamy L, Behen ME, Makki MI, Ager J, *et al*. (2010): Alterations in frontal lobe tracts and corpus callosum in young children with autism spectrum disorder. Cereb Cortex 20:2103–2113.
14. Jeong JW, Kumar AK, Sundaram SK, Chugani HT, Chugani DC (2011): Sharp curvature of frontal lobe white matter pathways in children with autism spectrum disorders: Tract-based morphometry analysis. AJNR Am J Neuroradiol 32:1600–1606.
15. Walker L, Gozzi M, Lenroot R, Thurm A, Behseta B, Swedo S, Pierpaoli C (2012): Diffusion tensor imaging in young children with

autism: Biological effects and potential confounds. Biol Psychiatry 72: 1043–1051.
16. Aoki Y, Abe O, Nippashi Y, Yamasue H (2013): Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: A meta-analysis of diffusion tensor imaging tractography studies. Mol Autism 4:25.
17. Travers BG, Adluru N, Ennis C, Tromp do PM, Destiche D, Doran S, *et al*. (2012): Diffusion tensor imaging in autism spectrum disorder: A review. Autism Res 5:289–313.
18. Stoner R, Chow ML, Boyle MP, Sunkin SM, Mouton PR, Roy S, *et al*. (2014): Patches of disorganization in the neocortex of children with autism. N Engl J Med 370:1209–1219.
19. Bailey AJ (2008): Postmortem studies of autism. Autism Res 1:265.
20. Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, *et al*. (2010): The neuropathology of autism: Defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol 119: 755–770.
21. Dinstein I, Pierce K, Eyler L, Solso S, Malach R, Behrmann M, Courchesne E (2011): Disrupted neural synchronization in toddlers with autism. Neuron 70:1218–1225.
22. Sundaram SK, Kumar A, Makki MI, Behen ME, Chugani HT, Chugani DC (2008): Diffusion tensor imaging of frontal lobe in autism spectrum disorder. Cereb Cortex 18:2659–2665.
23. Ben Bashat D, Kronfeld-Duenias V, Zachor DA, Ekstein PM, Hendler T, Tarrasch R, *et al*. (2007): Accelerated maturation of white matter in young children with autism: A high b value DWI study. Neuroimage 37: 40–47.
24. Wolff JJ, Gu H, Gerig G, Elison JT, Styner M, Gouttard S, *et al*. (2012): Differences in white matter fiber tract development present from 6 to 24 months in infants with autism. Am J Psychiatry 169:589–600.
25. Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Ben Itzhak E, Artzi M, *et al*. (2011): Abnormal white matter integrity in young children with autism. Hum Brain Mapp 32:534–543.
26. Barnea-Goraly N, Lotspeich LJ, Reiss AL (2010): Similar white matter aberrations in children with autism and their unaffected siblings: A diffusion tensor imaging study using tract-based spatial statistics. Arch Gen Psychiatry 67:1052–1060.
27. Holmboe K, Elsabbagh M, Volein A, Tucker LA, Baron-Cohen S, Bolton P, *et al*. (2010): Frontal cortex functioning in the infant broader autism phenotype. Infant Behav Dev 33:482–491.
28. Belmonte MK, Gomot M, Baron-Cohen S (2010): Visual attention in autism families: 'Unaffected'; sibs share atypical frontal activation. J Child Psychol Psychiatry 51:259–276.
29. Yang G, Smibert CA, Kaplan DR, Miller FD (2014): An eIF4E1/4E-T complex determines the genesis of neurons from precursors by translationally repressing a proneurogenic transcription program. Neuron 84:723–739.
30. O'Roak BJ, Vives L, Fu W, Egertson JD, Stanaway IB, Phelps IG, *et al*. (2012): Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 338:1619–1622.
31. Orosco LA, Ross AP, Cates SL, Scott SE, Wu D, Sohn J, *et al*. (2014): Loss of Wdfy3 in mice alters cerebral cortical neurogenesis reflecting aspects of the autism pathology. Nat Commun 5:4692.
32. Dong Z, Liu Z, Cui P, Pincheira R, Yang Y, Liu J, Zhang JT (2009): Role of eIF3a in regulating cell cycle progression. Exp Cell Res 315:1889–1894.
33. Sugathan A, Biagioli M, Golzio C, Erdin S, Blumenthal I, Manavalan P, *et al*. (2014): CHD8 regulates neurodevelopmental pathways associated with autism spectrum disorder in neural progenitors. Proc Natl Acad Sci U S A 111:E4468–E4477.
34. Prontera P, Ottaviani V, Toccaceli D, Rogaia D, Ardisia C, Romani R, *et al*. (2014): Recurrent approximately 100 Kb microdeletion in the chromosomal region 14q11.2, involving CHD8 gene, is associated with autism and macrocephaly. Am J Med Genet A 164A:3137–3141.
35. Bernier R, Golzio C, Xiong B, Stessman HA, Coe BP, Penn O, *et al*. (2014): Disruptive CHD8 mutations define a subtype of autism early in development. Cell 158:263–276.
36. Lin GN, Corominas R, Lemmens I, Yang X, Tavernier J, Hill DE, *et al*. (2015): Spatiotemporal 16p11.2 protein network implicates cortical late mid-fetal brain development and KCTD13-Cul3-RhoA pathway in psychiatric diseases. Neuron 85:742–754.
37. Cotney J, Muhle RA, Sanders SJ, Liu L, Willsey AJ, Niu W, *et al*. (2015): The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment. Nat Commun 6:6404.
38. Le Belle JE, Sperry J, Ngo A, Ghochani Y, Laks DR, Lopez-Aranda M, *et al*. (2014): Maternal inflammation contributes to brain overgrowth and autism-associated behaviors through altered redox signaling in stem and progenitor cells. Stem Cell Reports 3:725–734.
39. Fang WQ, Chen WW, Jiang L, Liu K, Yung WH, Fu AK, Ip NY (2014): Overproduction of upper-layer neurons in the neocortex leads to autism-like features in mice. Cell Rep 9:1635–1643.
40. Courchesne E, Mouton PR, Calhoun ME, Semendeferi K, Ahrens-Barbeau C, Hallet MJ, *et al*. (2011): Neuron number and size in prefrontal cortex of children with autism. JAMA 306:2001–2010.
41. Ringo JL (1991): Neuronal interconnection as a function of brain size. Brain Behav Evol 38:1–6.
42. Chow ML, Pramparo T, Winn ME, Barnes CC, Li HR, Weiss L, *et al*. (2012): Age-dependent brain gene expression and copy number anomalies in autism suggest distinct pathological processes at young versus mature ages. PLoS Genet 8:e1002592.
43. Santos M, Uppal N, Butti C, Wicinski B, Schmeidler J, Giannakopoulos P, *et al*. (2011): von Economo neurons in autism: A stereologic study of the frontoinsular cortex in children. Brain Res 1380:206–217.
44. van Kooten IA, Palmen SJ, von Cappeln P, Steinbusch HW, Korr H, Heinsen H, *et al*. (2008): Neurons in the fusiform gyrus are fewer and smaller in autism. Brain 131:987–999.
45. Dini LI, Vedolin LM, Bertholdo D, Grando RD, Mazzola A, Dini SA, *et al*. (2013): Reproducibility of quantitative fiber tracking measurements in diffusion tensor imaging of frontal lobe tracts: A protocol based on the fiber dissection technique. Surg Neurol Int 4:51.
46. Pierce K, Carter C, Weinfeld M, Desmond J, Hazin R, Bjork R, Gallagher N (2011): Detecting, studying, and treating autism early: The one-year well-baby check-up approach. J Pediatr 159: 458–465. e1–e6.
47. Lord C, Rutter M, DiLavore PC, Risi S (2001): Autism Diagnostic Observation Schedule (ADOS). Los Angeles: Western Psychological Services.
48. Sparrow S, Cicchetti D, Balla D (2005): Vineland-II Scales of Adaptive Behavior: Survey Form Manual. Circle Pines, MN: American Guidance Service.
49. Mullen EM (1995): Mullen Scales of Early Learning, AGS ed. Circle Pines, MN: American Guidance Service Inc.
50. Redcay E, Kennedy DP, Courchesne E (2007): fMRI during natural sleep as a method to study brain function during early childhood. Neuroimage 38:696–707.
51. Redcay E, Courchesne E (2008): Deviant functional magnetic resonance imaging patterns of brain activity to speech in 2-3-year-old children with autism spectrum disorder. Biol Psychiatry 64:589–598.
52. Pierce K (2011): Early functional brain development in autism and the promise of sleep fMRI. Brain Res 1380:162–174.
53. Hagler DJ Jr, Ahmadi ME, Kuperman J, Holland D, McDonald CR, Halgren E, Dale AM (2009): Automated white-matter tractography using a probabilistic diffusion tensor atlas: Application to temporal lobe epilepsy. Hum Brain Mapp 30:1535–1547.
54. Basser PJ, Mattiello J, LeBihan D (1994): MR diffusion tensor spectroscopy and imaging. Biophys J 66:259–267.
55. Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di Chiro G (1996): Diffusion tensor MR imaging of the human brain. Radiology 201: 637–648.
56. Eicher JD, Powers NR, Miller LL, Akshoomoff N, Amaral DG, Bloss CS, *et al*. (2013): Genome-wide association study of shared components of reading disability and language impairment. Genes Brain Behav 12: 792–801.
57. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, *et al*. (2002): Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355.

58. Xu R (2003): Measuring explained variation in linear mixed effects models. Stat Med 22:3527–3541.
59. Benjamini Y, Hochberg Y (1995): Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300.
60. Gotham K, Pickles A, Lord C (2012): Trajectories of autism severity in children using standardized ADOS scores. Pediatrics 130:e1278–e1284.
61. Olson IR, McCoy D, Klobusicky E, Ross LA (2013): Social cognition and the anterior temporal lobes: A review and theoretical framework. Soc Cogn Affect Neurosci 8:123–133.
62. Oishi K, Faria AV, Hsu J, Tippett D, Mori S, Hillis AE (2015): Critical role of the right uncinate fasciculus in emotional empathy. Ann Neurol 77:68–74.
63. Schmahmann JD, Smith EE, Eichler FS, Filley CM (2008): Cerebral white matter: Neuroanatomy, clinical neurology, and neurobehavioral correlates. Ann N Y Acad Sci 1142:266–309.
64. Catani M, Mesulam M (2008): The arcuate fasciculus and the disconnection theme in language and aphasia: History and current state. Cortex 44:953–961.
65. Wahl M, Lauterbach-Soon B, Hattingen E, Jung P, Singer O, Volz S, *et al*. (2007): Human motor corpus callosum: Topography, somatotopy, and link between microstructure and function. J Neurosci 27: 12132–12138.
66. Lehericy S, Ducros M, Van de Moortele PF, Francois C, Thivard L, Poupon C, *et al*. (2004): Diffusion tensor fiber tracking shows distinct corticostriatal circuits in humans. Ann Neurol 55:522–529.
67. Langen M, Leemans A, Johnston P, Ecker C, Daly E, Murphy CM, *et al*. (2012): Fronto-striatal circuitry and inhibitory control in autism: Findings from diffusion tensor imaging tractography. Cortex 48: 183–193.
68. Dunbar RI, Shultz S (2007): Evolution in the social brain. Science 317: 1344–1347.
69. Friederici AD (2009): Pathways to language: Fiber tracts in the human brain. Trends Cogn Sci 13:175–181.
70. Petrides M (2005): Lateral prefrontal cortex: Architectonic and functional organization. Philos Trans R Soc Lond B Biol Sci 360:781–795.
71. Zikopoulos B, Barbas H (2010): Changes in prefrontal axons may disrupt the network in autism. J Neurosci 30:14595–14609.
72. Voineagu I, Eapen V (2013): Converging pathways in autism spectrum disorders: Interplay between synaptic dysfunction and immune responses. Front Hum Neurosci 7:738.
73. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, *et al*. (2014): Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 515:209–215.
74. Casanova MF (2004): White matter volume increase and minicolumns in autism. Ann Neurol 56:453; author reply 454.
75. Buxhoeveden DP, Semendeferi K, Buckwalter J, Schenker N, Switzer R, Courchesne E (2006): Reduced minicolumns in the frontal cortex of patients with autism. Neuropathol Appl Neurobiol 32:483–491.
76. Casanova MF, Trippe J (2009): Radial cytoarchitecture and patterns of cortical connectivity in autism. Philos Trans R Soc Lond B Biol Sci 364:1433–1436.
77. Courchesne E, Pierce K (2005): Why the frontal cortex in autism might be talking only to itself: Local over-connectivity but long-distance disconnection. Curr Opin Neurobiol 15:225–230.
78. Forbes CE, Grafman J (2010): The role of the human prefrontal cortex in social cognition and moral judgment. Annu Rev Neurosci 33:299–324.
79. Munson J, Dawson G, Abbott R, Faja S, Webb SJ, Friedman SD, *et al*. (2006): Amygdalar volume and behavioral development in autism. Arch Gen Psychiatry 63:686–693.
80. Mosconi MW, Cody-Hazlett H, Poe MD, Gerig G, Gimpel-Smith R, Piven J (2009): Longitudinal study of amygdala volume and joint attention in 2- to 4-year-old children with autism. Arch Gen Psychiatry 66:509–516.
81. Schumann CM, Barnes CC, Lord C, Courchesne E (2009): Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. Biol Psychiatry 66:942–949.
82. Rilling JK, Glasser MF, Preuss TM, Ma X, Zhao T, Hu X, Behrens TE (2008): The evolution of the arcuate fasciculus revealed with comparative DTI. Nat Neurosci 11:426–428.
83. Lombardo MV, Pierce K, Eyler LT, Carter Barnes C, Ahrens-Barbeau C, Solso S, *et al*. (2015): Different functional neural substrates for good and poor language outcome in autism. Neuron 86:567–577.
84. Eyler LT, Pierce K, Courchesne E (2012): A failure of left temporal cortex to specialize for language is an early emerging and fundamental property of autism. Brain 135:949–960.