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Abnormal Development of the Corpus Callosum in Autism Spectrum Disorder: An MRI Study

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Abstract

Background: Altered size in the corpus callosum (CC) has been reported in individuals with autism spectrum disorder (ASD), but few studies have investigated younger children. Moreover, knowledge about the age-related changes in CC size in individuals with ASD is limited.

Objectives: Our objective was to investigate the age-related size of the CC and compare them with age-matched healthy controls between the ages of 2 and 18 years.

Methods: Structural-weighted images were acquired in 97 male patients diagnosed with ASD; published data were used for the control group. The CC was segmented into 7 distinct subregions (rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus, and splenium) as per Witelson's technique using ITK-SNAP software. We calculated both the total length and volume of the CC as well as the length and height of its 7 subregions. The length of the CC measures was studied as both continuous and categorical forms. For the continuous form, Pearson's correlation was used, while categorical forms were based on age ranges reflecting brain expansion during early postnatal years. Differences in CC measures between adjacent age groups in individuals with ASD were assessed using a Student *t*-test. Mean and standard deviation scores were compared between ASD and control groups using the Welch *t*-test.

Results: Age showed a moderate positive association with the total length of the CC (r = 0.43; P_{adj} = 0.003) among individuals with ASD. Among the subregions, a positive association was observed only in the anterior midbody of the CC (r = 0.41; P_{adj} = 0.01). No association was found between the age and the height of individual subregions or with the total volume of the CC. In comparison with healthy controls, individuals with ASD exhibited shorter lengths and heights of the genu and splenium of the CC across wide age ranges.

Conclusion: Overall, our results highlight a distinct abnormal developmental trajectory of CC in ASD, particularly in the genu and splenium structures, potentially reflecting underlying pathophysiological mechanisms that warrant further investigation.

Keywords: MRI, corpus callosum, autism spectrum disorder, ASD, Witelson

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INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder that manifests during early childhood, defined by varying degrees of deficits in social and communication skills, accompanied by stereotyped or repetitive behaviors and interests.[1] Over the last 2 decades, there has been a significant global increase in the prevalence of ASD. For example, in 2020, 1 in 36 children aged 8 years in the United States was diagnosed with ASD.[2] Despite this rise in prevalence, the exact causes remain largely unknown; however, it is considered to be a complex interplay between genetic and environmental factors.[3,4] Emerging theories from neuroimaging studies have hypothesized that ASD is associated with disrupted brain connectivity rather than localized impairment, that is, hypoconnectivity between distant brain regions and overconnectivity within local regions.[5–7] As a result, white matter connectivity, particularly, the corpus callosum (CC) has been the focus of interest.[8]

The CC is the largest white matter commissural pathway in the brain consisting of more than 200 million axons, connecting the cerebral hemispheres of the brain.[9] This structure plays an important role in facilitating interhemispheric communication with some callosal fibers projecting to homotopic regions, while others projecting to heterotopic regions.[10–13] It is topographically arranged across the callosum, consisting of approximately 70% myelinated fibers, along with 30% unmyelinated fibers, astrocytes, oligodendrocytes, and neurons.[11,14]

Multiple cross-sectional magnetic resonance imaging (MRI) studies have documented a smaller area, volume, and thickness of the corpus callosum, along with its subdivision in individuals with ASD.[15–20] These studies have showcased diverse results, for example, some studies have reported smaller sizes in anterior and posterior regions of the CC compared with controls,[18,19] while others have shown smaller sizes in midbody structures of the CC.[16,17] Intriguingly, certain studies have even reported either no differences or larger volumes of CC in boys with low-functioning and high-functioning ASD compared with controls.[21,22] Taken together, the findings of CC in ASD across studies are mixed and inconclusive, which might be due to heterogeneity of the disorder or the varied age range studied. Moreover, most studies have grouped all individuals with ASD together without considering the developmental stages of the brain and have given limited focus to young individuals with ASD.

Our study aimed to investigate the development of CC size using a large sample of individuals with ASD across an age range from 2 to 18 years. The objectives of our investigation were twofold:

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The authors declare that they have no conflicts of interest.

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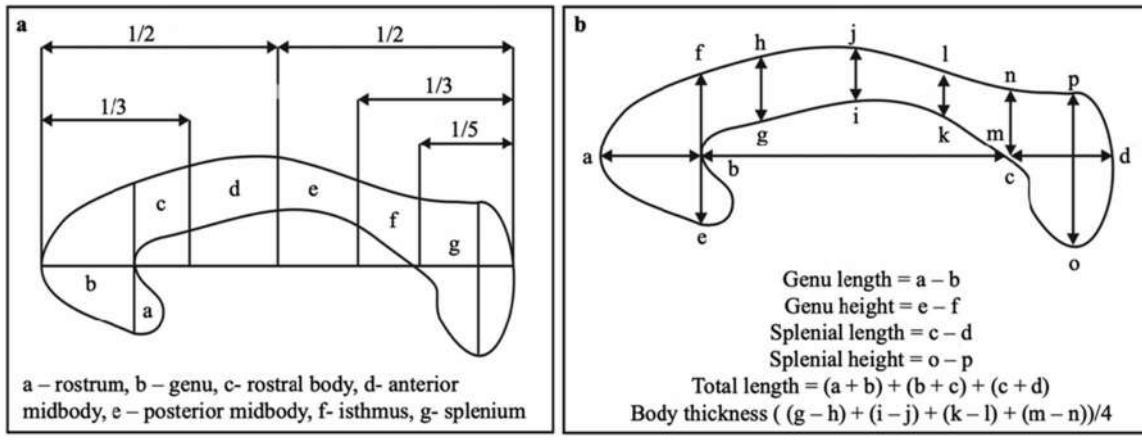

FIGURE 1. Schematic representation of midsagittal corpus callosum classification developed by Witelsons (A),[26] and the new classification technique for measuring the length and height of the corpus callosum as developed by Vannucci and his colleagues (B).[24]

1) to comprehend the age-related changes in CC size among individuals with ASD and 2) to discern the age-related differences in CC size between individuals with ASD and healthy controls.

MATERIALS AND METHODS

Participants

A total of 97 male individuals with ASD, aged between 2 and 18 years, were recruited from Neurogen Brain and Spine Institute, India, as part of a prospective study. The inclusion of only boys aimed to limit structural variability associated with sex. Patients were diagnosed through independent clinical interviews conducted by an experienced neurologist and psychologist, ensuring that all included individuals met the DSM-V (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) criteria for ASD.[23] Those presenting evidence of genetic condition or syndrome, any comorbidities associated with ASD, such as attention-deficit hyperactivity disorder (ADHD), significant vision or hearing impairment, a history of preterm birth, or any contraindication for an MRI scan were excluded from the study. Written informed consents were obtained from all parents of individuals with ASD. The Institutional Ethics Committee of the Hospital approved the study.

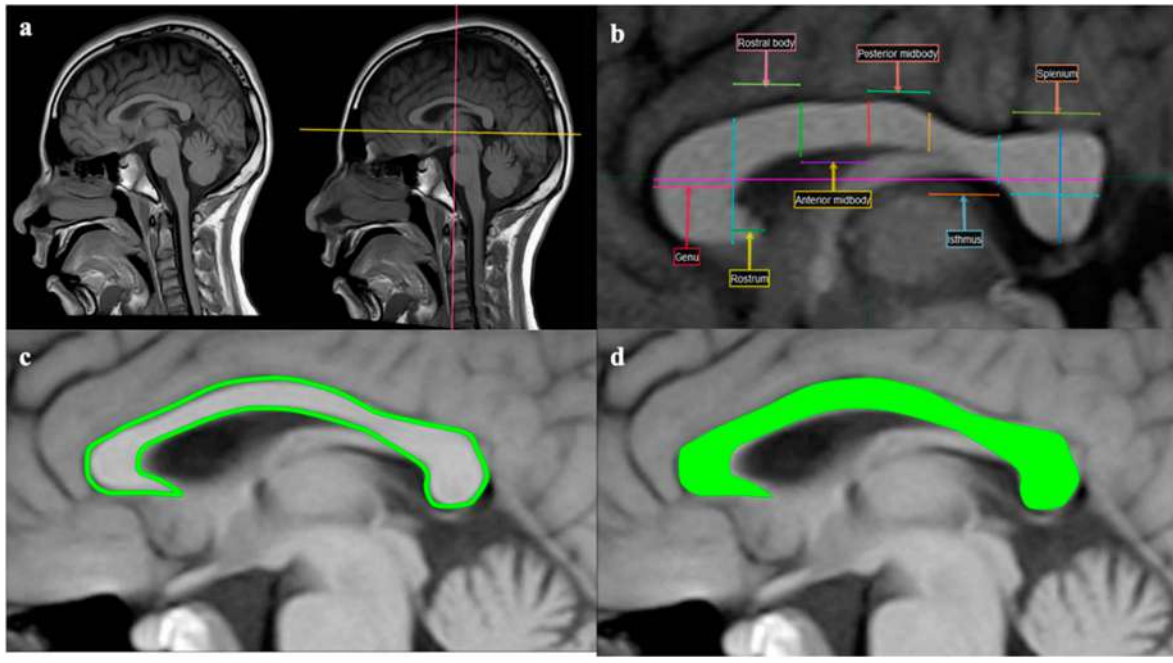

FIGURE 2. Representative anatomical image of T1-weighted image (A); the reoriented image are zoomed three-fold, wherein the corpus callosum (CC) is traced along the midsagittal section, confirmed by the presence of the cerebral aqueduct, septum pellucidum, and the distinctness of the thalamus (B); the CC was segmented into 7 subregions (rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus, and splenium) following Witelson guidelines using the ITK-SNAP tool, an image processing software (B). The line drawn along the borders of the CC (C) and the measurement of volume using the number of pixels (voxels) within the CC (D).

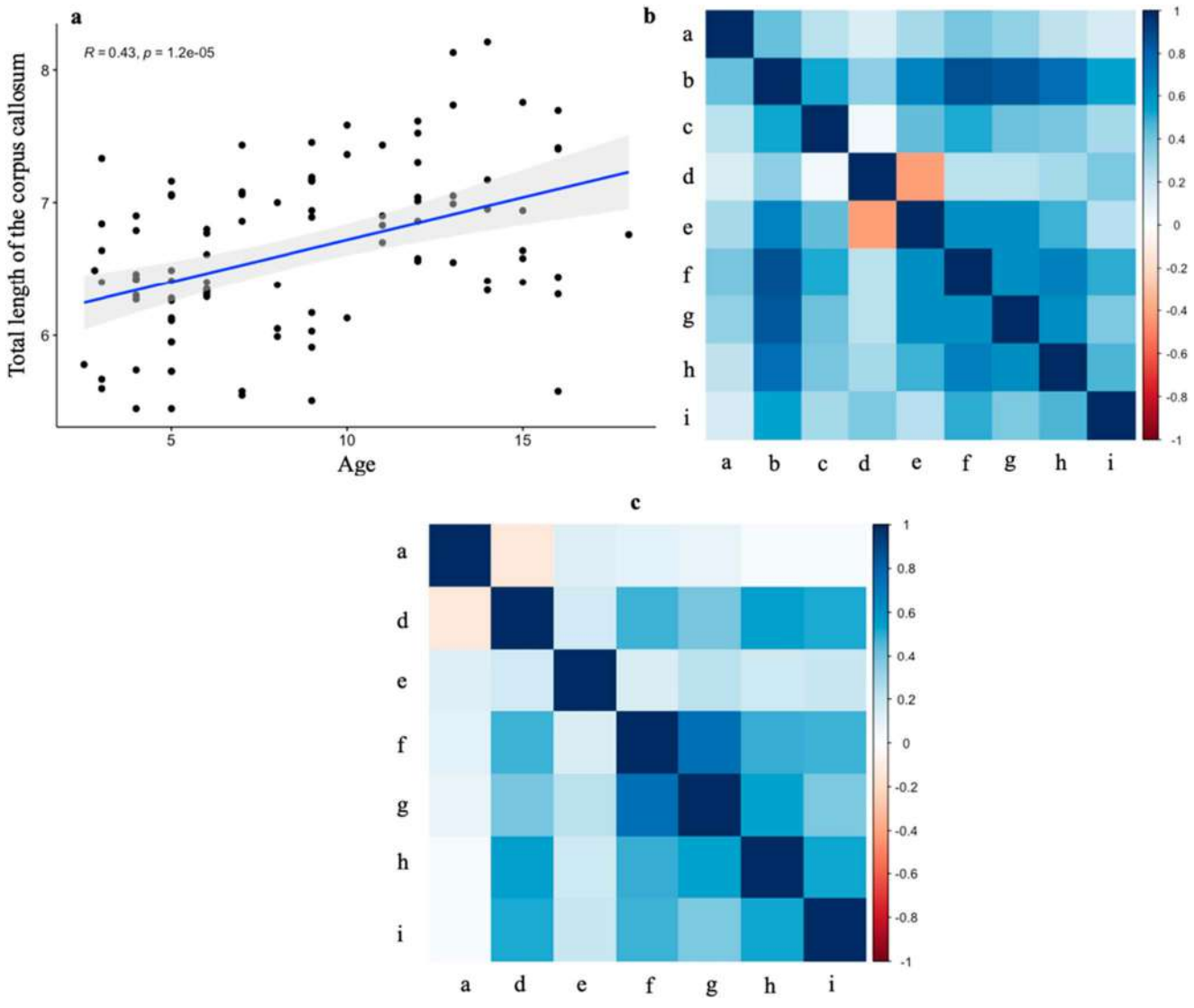

FIGURE 3. The association between age and (A) total length of the corpus callosum (B) length of the individual subregions of the corpus callosum and (C) height of the individual subregions of the corpus callosum. The abbreviations used in the correlation plots are described as follows: a, age; b, the total length of the corpus callosum; c, rostrum; d, genu; e, rostral body; f, anterior midbody; g, posterior midbody; h, isthmus; and i, splenium.

Image Acquisition

The individuals with ASD underwent an MRI brain scan using a 1.5T GE Optima machine (General Electric Healthcare, Milwaukee, Wisconsin) in Swastik Imaging Centre, India, using an 8-channel head coil. The imaging protocol encompassed a sagittal three-dimensional T1-weighted sequence (repetition time = 2000 ms, echo time = 60 ms, slice thickness = 3 mm, the field of view = 256 mm), and for clinical screening, axial T2-weighted and T2 FLAIR sequences were acquired. All acquired images were transmitted from the acquisition center to the image analysis laboratory at Neurogen using OSIRIX. Individuals were sedated with propofol (0.5–1.0 mL/kg) if required before the scans and were regularly monitored for heart rate to maintain their safety and well-being by an interventionist (qualified anesthesiologist).

Details on the acquisition parameter of control data can be found elsewhere.[24]

Corpus Callosum Measurements

The length, height, and volume of the CC structures were computed from all the scans by meticulously tracing the CC along the midsagittal slice obtained from the T1-weighted image. All analyses were conducted by a perinatal neuroscientist with expertise in developmental neuroimaging, under the supervision of an experienced radiologist.

The procedures were as follows: first, delineating the borders of the CC in the midsagittal section at the level of the anterior and posterior commissure. The midsagittal orientation criterion was ensured by observing the presence of the cerebral aqueduct, septum pellucidum, and the clear distinction of the thalamus. Subsequently, the CC was segmented into 7 distinct subregions (rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus, and splenium) as proposed by Witelson (Fig. 1),[25] using ITK-SNAP (Penn Image Computing and Science Laboratory, University of

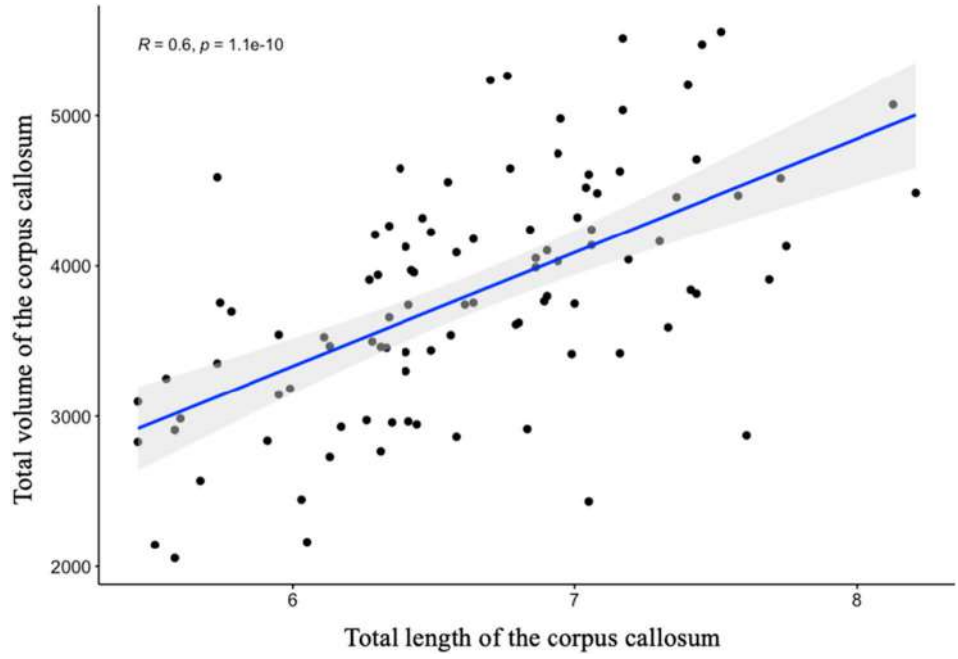

FIGURE 4. Scatterplot demonstrating the association between the total length of the corpus callosum and the total volumes of the corpus callosum. Shorter lengths are associated with smaller volumes.

Pennsylvania, Philadelphia),[26] a semiautomated segmentation software. Furthermore, the length and height of genu, splenium, and body thickness of all individuals with ASD were calculated as outlined in (Fig. 2). This process was repeated to determine the total length and total volume of the CC (Fig. 2).

To ensure accuracy and minimize measurement error, the analysis was repeated 3 times, and the average values were considered for subsequent evaluations. Intraclass correlation coefficients (ICC) were calculated to estimate test-retest reliability, and ICC values were higher than 0.9 (for example, ICC values for CC volume was 0.97).

Statistics

All the normally distributed values are presented as the mean ± standard deviation (SD) or mean (SD). Pearson correlation coefficients were used to examine the relationships between age and both total length/height, as well as individual subregions, of the CC, and to explore the relationships between age and total volumes of the CC. A Student *t* test was applied to compare the differences in both total CC and subregion measurements between the nearest age groups among individuals with ASD.

We further compared the mean and standard deviation of the length and height of the genu, splenium, and body thickness between individuals with ASD and age-matched controls using the Welch *t* test. Reference data from Vannucci et al.[24] served as health controls, categorized based on age ranges reflecting brain expansion during early postnatal years: 2–4 years, 5–6 years, 7–9 years, 10–12 years, 13–15 years, and 16–18 years.

Our initial plan was not to implement multiple comparison adjustments because of the exploratory nature of the study, with the aim of not overlooking potential significant observations. However, the Bonferroni correction was applied separately for correlation and group analyses to account for multiple comparisons; we report both adjusted and unadjusted p-values exclusively. *P*-values less than 0.05 were deemed statistically significant after corrections.

All the statistical analyses were performed using GraphPad Prism version 7 (GraphPad Software, San Diego, CA), and for plots, R studio (version 4.0.3) was used.

RESULTS

Out of the 97 individuals with ASD studied, 23 (23.7%) were aged 2–4 years, 17 (17.5%) were between 5 and 6 years, 20 (20.6%) fell within the 7–9 year range, 15 (15.5%) were aged 10–12 years, 15 (15.5%) were between 13 and 15 years, and 7 (7.2%) were aged between 16 and 18 years.

Association Between Age and Length of the CC

Among individuals with ASD, when we explored age as a continuous variable, we observed a moderate positive association between age and the total length of the CC ($r = 0.43$, $P_{unadj} < 0.001$) (Fig. 3A). When we studied the individual subregions of the CC, a positive association was still observed between age and the anterior and posterior midbody of CC (ranging from 0.31 to 0.4) (Fig. 3B). However, we did not find any association between age and rostrum, genu, isthmus, and splenium of CC.

After Bonferroni correction, only the results for total length ($P_{adj} = 0.003$) and anterior midbody ($P_{adj} = 0.01$) of CC with age were significant.

Association Between Age and Height of the CC

There was no association observed between the age and height of the individual subregions of the CC (Fig. 3C).

Association Between Age and Volume of the CC

There was no association observed between the age and volume of the total CC ($r = 0.17$; $P_{unadj} = 0.09$).

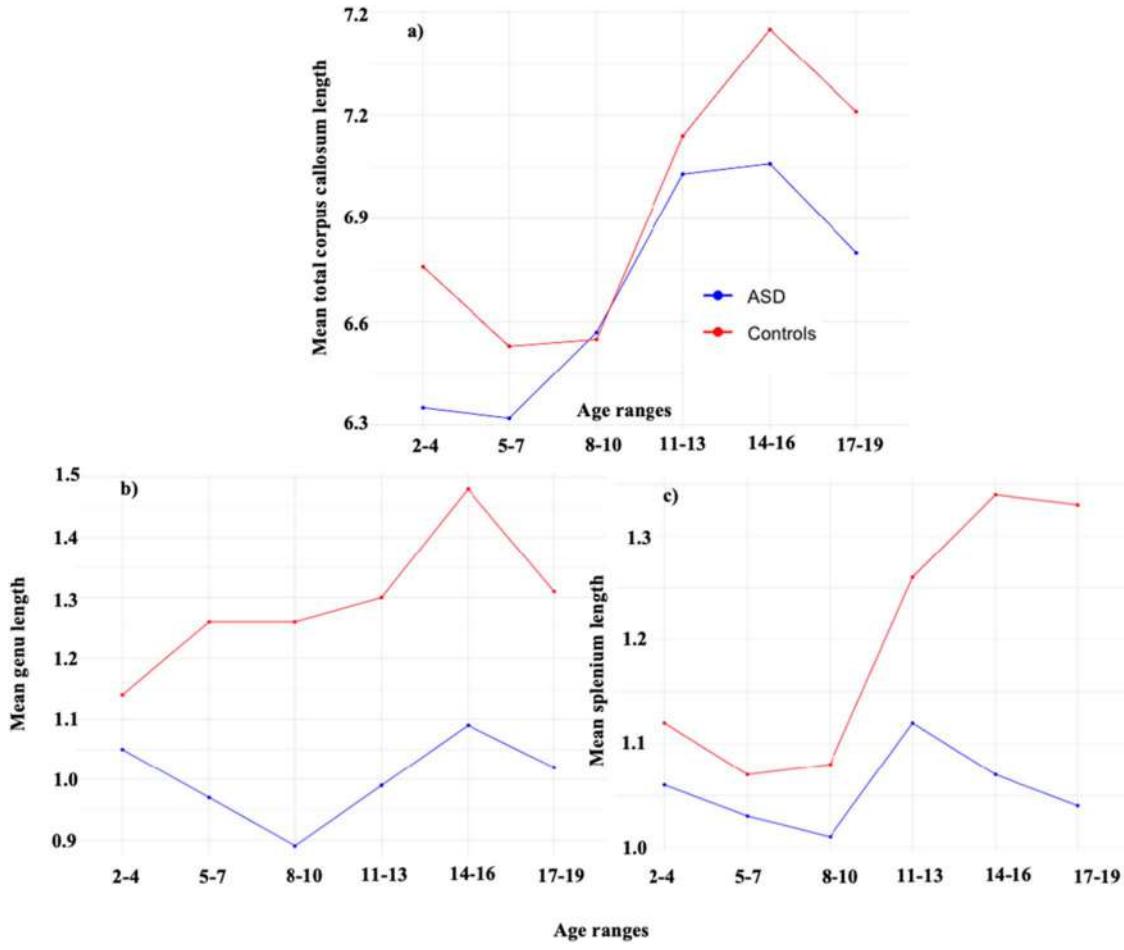

FIGURE 5. A line plot comparison of mean length of the total (A) genu (B) and splenium (C) of corpus callosum across groups in ASD and controls.

Association Between Length and Volume of the CC

There was a strong positive association observed between the length and volume of the total CC ($r = 0.60$; $P_{unadj} < 0.001$) (Fig. 4), and the results survived multiple comparison corrections.

Length and Height of the Corpus Callosum Between Age Groups

When we categorized age based on the developmental pattern and studied the differences in length and height of the CC, along with subregions of the CC, no consistent patterns were observed between the age groups studied (Table 1).

Comparison of Corpus Callosum Structures Between ASD and Healthy Controls

Overall, individuals with ASD exhibited a smaller total length of the CC at 2–4 and 16–18 years in comparison with healthy controls (Fig. 5A). Furthermore, they demonstrated shorter lengths of the genu and splenium of the CC across the studied age range (Table 2; Figs. 5B and C). Importantly, after multiple comparison corrections, most of the significant findings observed in the length of the genu and splenium remained significant.

DISCUSSION

Here we explored the development of CC sizes in individuals with ASD between early childhood and late adolescence. We found that the total length of the CC increases linearly with age, and this trend was also observed in different subregions of CC. However, we did not observe an age-related increase in the size of the rostrum, genu, posterior midbody, isthmus, and splenium of the CC. We also did not observe an age-related increase in the height of the subregion of CC or the total volumes of CC. As expected, compared with typically developing controls, individuals with ASD exhibited smaller CC structures, particularly in genu and splenium, across different age groups reaffirming the involvement of altered CC in the pathophysiology of ASD.

Prior MRI studies have demonstrated an age-related increase in the size of CC between childhood and young adulthood in typically developing controls.[27–29] Consistent with these, our study observed an age-related linear increase in the total length of CC between early childhood and young adulthood in individuals with ASD, aligning with other studies showing a similar trend of increase in total CC area in individuals with ASD.[29] However, we observed atypical age-related changes in some of the subregions studied, specifically the rostrum, genu, posterior midbody, isthmus, and splenium of CC, suggesting a diffuse pattern of CC development in ASD.

TABLE 1. Differences in Length, Height, and Volumes of the Corpus Callosum in Individuals With ASD According to Age Ranges

	Age Ranges					
	2 to 4 Years	5 to 6 Years	7 to 9 Years	10 to 12 Years	13 to 15 Years	16 to 18 Years
Measures	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Length (cm)						
Total length of CC	6.35 ± 0.43	6.32 ± 0.52	**6.57 ± 0.66***†	7.03 ± 0.44	7.06 ± 0.63	6.80 ± 0.75
Rostrum	0.57 ± 0.21	0.62 ± 0.20	0.66 ± 0.21	0.74 ± 0.25	0.73 ± 0.20	0.70 ± 0.19
Genu	1.05 ± 0.16	0.97 ± 0.10	0.89 ± 0.20	0.99 ± 0.17	1.09 ± 0.19	1.02 ± 0.17
Rostral body	1.07 ± 0.15	**1.12 ± 0.12**‡§	1.28 ± 0.24	**1.38 ± 0.18**†‖	1.22 ± 0.23	1.24 ± 0.16
Anterior midbody	1.03 ± 0.11	1.04 ± 0.09	1.10 ± 0.11	1.15 ± 0.11	1.17 ± 0.11	1.11 ± 0.11
Posterior midbody	1.07 ± 0.09	1.07 ± 0.13	**1.12 ± 0.11***†	1.18 ± 0.07	1.17 ± 0.14	1.16 ± 0.18
Isthmus	0.87 ± 0.09	0.86 ± 0.11	0.95 ± 0.16	0.92 ± 0.08	0.95 ± 0.08	0.90 ± 0.12
Splenium	1.01 ± 0.14	**0.98 ± 0.12**‡§	0.96 ± 0.18	1.07 ± 0.19	1.02 ± 0.13	0.99 ± 0.16
Height (cm)						
Genu	1.69 ± 0.23	1.79 ± 0.18	1.64 ± 0.27	1.67 ± 0.22	1.68 ± 0.24	1.58 ± 0.23
Rostral body	0.62 ± 0.06	0.61 ± 0.09	0.56 ± 0.12	0.60 ± 0.11	0.98 ± 1.55	0.61 ± 0.16
Anterior midbody	0.55 ± 0.10	0.56 ± 0.08	0.54 ± 0.09	0.58 ± 0.10	0.57 ± 0.08	0.56 ± 0.18
Posterior midbody	0.52 ± 0.12	0.49 ± 0.10	0.46 ± 0.11	0.53 ± 0.11	0.51 ± 0.12	0.53 ± 0.20
Isthmus	0.76 ± 0.18	**0.73 ± 0.13**†‡	0.62 ± 0.19	0.69 ± 0.15	0.77 ± 0.21	0.66 ± 0.24
Splenium	1.61 ± 0.28	1.71 ± 0.26	1.49 ± 0.23	1.58 ± 0.27	1.71 ± 0.32	1.63 ± 0.42
Volume (mm^3)						
Total CC	3686 ± 585	3733 ± 494	3626 ± 911	4092 ± 824	4092 ± 824	3812 ± 1156

Student *t* test was performed between the nearest age groups.
*The significant difference between the 7–9 years group and the 10–12 years group.
†$P < 0.05$.
‡The significant difference between 5–6 years group and 7–9 years group.
§$P < 0.01$.
‖The significant difference between the 10–12 years group and 13–15 years group.
CC, corpus callosum; SD, standard deviation.

We observed that the total length of CC was smaller in individuals with ASD compared with typically developing controls, with this difference being evident only during early childhood and young adulthood. However, these findings did not survive multiple comparison corrections. Interestingly, we also observed a smaller length of the genu and splenium of CC in individuals with ASD compared with controls. These findings align with earlier studies that have reported smaller sizes in the midsagittal area of CC in both children and adults with ASD relative to their typically developing controls.[18,30–35] Furthermore, some studies have indicated a negative association between the CC size and symptom severity in both school-aged children with ASD and adults with ASD.[19,29]

In addition, we observed a weak and nonsignificant age-related linear increase in the volumes of CC in individuals with ASD. Furthermore, when we categorized individuals with ASD based on different age ranges, we still did not observe any differences between groups. However, prior cross-sectional studies on CC volumes have reported smaller CC volumes from early childhood in individuals with ASD.[34,36–38] However, we cannot comment on this, since we did not had details of CC volumes from the healthy control data.

The anterior regions of CC, namely the rostrum and genu, contain fibers originating from the prefrontal cortices. The posterior region, the splenium comprises fibers from inferior temporal and occipital cortices.[25] Considering the extensive connections between the genu and splenium of CC and the cortex, our results imply a likelihood of impaired brain connectivity across various regions, substantiated by alterations in CC subdivisions. This interpretation is further supported by functional studies implicating multiple cortical regions, including but not limited to the frontal and parietal areas.[6,39]

There are multiple explanations for the observed smaller CC size in individuals with ASD, particularly in genu and splenium. One possibility is that it may stem from smaller fiber size or reduced myelination. Another alternative explanation is that the altered development of brain connectivity could lead to a decrease in the number of large axons responsible for intrahemispheric or interhemispheric regional communication.[34]

While we noted reduced CC sizes in individuals with ASD, these deviations are not exclusive to ASD but are also observed in individuals with obsessive-compulsive disorder, where compulsive behavior correlates with CC size.[40] Similarly, a smaller CC size is noted in individuals with Tourette disorder, with the size of the CC correlating with the severity of tics.[41] In addition, studies on individuals with ADHD have reported a similar pattern of smaller CC size.[42] Future studies investigating CC size should include individuals with ASD, both with and without comorbidities, to determine whether the presence of comorbidities worsens CC size with age.

Despite achieving an adequate sample size across various age subgroups, it is essential to acknowledge certain potential limitations. First, we did not stratify individuals based on high or low-functioning ASD. Second, the generalizability of our findings is confined to the Indian cohort and males with ASD, limiting extrapolation to other populations. Third, the cohort design spanning early childhood to young adulthood, while extensive, may exhibit reduced sensitivity to age-related changes during developmental phases. Fourthly, we were unable to conduct statistical analysis for CC measures between individuals with ASD and healthy controls, due to the unavailability of raw data of healthy controls. Fifthly, the CC consists of white matter pathways between brain regions suggesting that advanced techniques like functional and diffusion imaging could enhance sensitivity in investigating ASD-related brain changes. For example, diffusion studies investigating male individuals with high-functioning ASD have reported lower FA and higher MD values in the genu, rostral body, and splenium of the CC compared with controls.[43,44] Functional studies have demonstrated decreased interhemispheric functional connectivity during resting

TABLE 2. Age-Appropriate Comparison of Corpus Callosum Structures Between ASD and Controls

	Corpus callosum											
	Total Length		Genu Length		Genu Height		Body Thickness		Splenium Length		Splenium Height	
Age Range (y)	ASD	Controls	ASD	Controls	ASD	Controls	ASD	Controls	ASD	Controls	ASD	Controls
2 to 4	6.35* (0.43)	6.76 (0.42)	1.05 (0.16)	1.14 (0.17)	1.69 (0.23)	1.67 (0.26)	0.59 (0.07)	0.59 (0.12)	1.01 (0.14)	1.07 (0.16)	1.61† (0.28)	1.44 (0.17)
5 to 6	6.32 (0.52)	6.53 (0.71)	**0.97‡ (0.10)**	1.26 (0.17)	1.79 (0.18)	1.88 (0.26)	0.61 (0.10)	0.64 (0.13)	0.98 (0.12)	1.02 (0.14)	1.71† (0.26)	1.52 (0.32)
7 to 9	6.57 (0.66)	6.55 (0.46)	**0.89‡ (0.20)**	1.26 (0.18)	1.64 (0.27)	1.73 (0.26)	0.55† (0.10)	0.62 (0.10)	0.96 (0.18)	1.03 (0.13)	1.49 (0.23)	1.47 (0.27)
10 to 12	7.03 (0.44)	7.14 (0.51)	**0.99‡ (0.17)**	1.30 (0.19)	1.67 (0.22)	1.79 (0.30)	0.59* (0.09)	0.67 (0.12)	1.07 (0.19)	1.21 (0.20)	1.58 (0.27)	1.61 (0.18)
13 to 15	7.06 (0.63)	7.45 (0.67)	1.09* (0.19)	1.48 (0.19)	1.68 (0.24)	1.76 (0.31)	0.61 (0.10)	0.67 (0.06)	**1.02‡ (0.13)**	1.29 (0.18)	1.71† (0.32)	1.61 (0.25)
16 to 18	6.80* (0.75)	7.21 (0.50)	**1.02‡ (0.17)**	1.31 (0.16)	1.58† (0.23)	1.75 (0.34)	0.59 (0.17)	0.68 (0.09)	**0.99‡ (0.16)**	1.28 (0.17)	1.63 (0.42)	1.63 (0.25)

Data are presented as mean (SD). The healthy age-matched control data used here are reported elsewhere.[24]
n of individuals across age range groups: 2–4 years, ASD = 17, controls = 11; 5–6 years, ASD = 23, controls = 13; 7–9 years, ASD = 20, controls = 14; 10–12 years, ASD = 15, controls = 11; 13–15 years, ASD = 15, controls = 11; and 16–18 years, ASD = 7, controls = 18.
*$P < 0.01$;
†$P < 0.05$;
‡$P < 0.001$. P-values surviving multiple comparison corrections were indicated in bold.
ASD, autism spectrum disorders.

state compared to controls.[45,46] Sixthly, prenatal factors like birth weight,[47] and postnatal factors such as socioeconomic status, known to influence outcomes, were not accounted for in this study. Finally, we did not correlate CC length or volumes with either clinical symptoms or behavioral scores, necessitating further investigations in this aspect. Understanding the negative effect of atypical changes in CC with symptoms and behavioral performance would enable researchers to tailor interventions to enhance overall well-being.

In conclusion, our study sheds light on the age-related dynamics of CC development in individuals with ASD. While a linear increase in total CC size is observed among individuals with ASD, mirroring the pattern seen in typically developing controls, atypical age-related changes were noted in specific subregions of the CC, including the rostrum, genu, posterior midbody, isthmus, and splenium. Importantly, our results highlight a consistent trend of smaller sizes, particularly in the genu and splenium of CC, across wide age ranges in individuals with ASD compared with typically developing controls, suggesting a distinct abnormal developmental trajectory of CC in ASD, potentially reflecting underlying pathophysiological mechanisms that warrant further investigation.

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