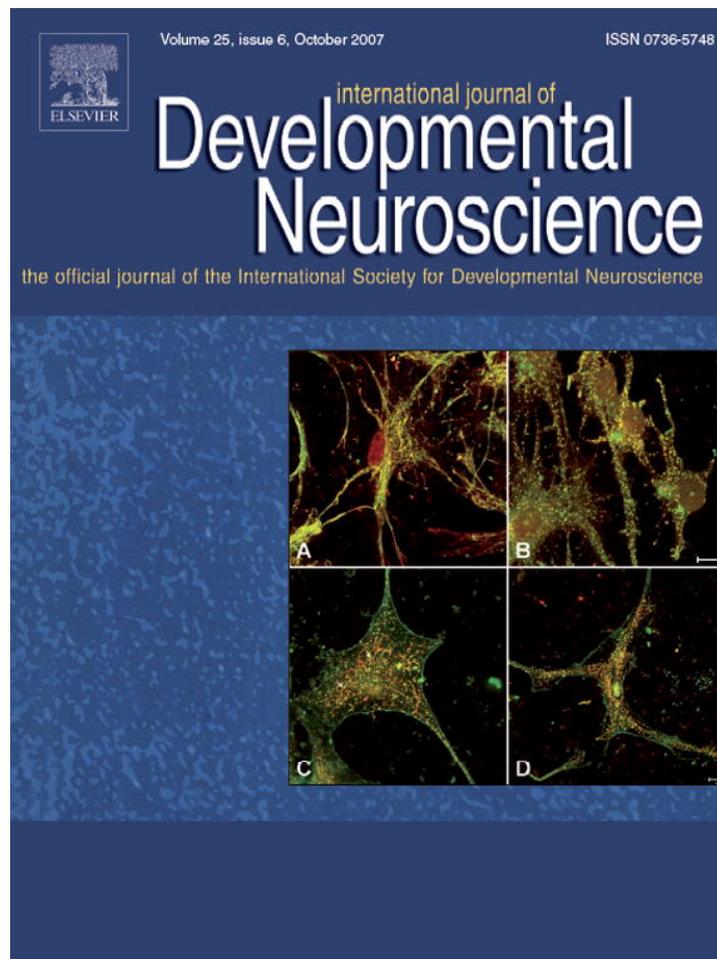


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## Visualization of maturation of the corpus callosum during childhood and adolescence using T2 relaxometry

Eung Y. Kim<sup>a,\*</sup>, Dong-Hyun Kim<sup>b</sup>, Eunhye Yoo<sup>a</sup>, Hae-Jeong Park<sup>a,e</sup>, Xavier Golay<sup>c,d</sup>,  
 Seung-Koo Lee<sup>a</sup>, Dong J. Kim<sup>a</sup>, Jinna Kim<sup>a</sup>, Dong I. Kim<sup>a</sup>

<sup>a</sup> Department of Radiology and Research Institute of Radiological Sciences, Yonsei University College of Medicine,  
 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea

<sup>b</sup> School of Electrical and Electronic Engineering, Yonsei University, Seoul, South Korea

<sup>c</sup> Department of Neuroradiology, National Neuroscience Institute, Singapore

<sup>d</sup> Laboratory of Molecular Imaging, Singapore Biomed Imaging Consortium, Singapore

<sup>e</sup> Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul, South Korea

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### Abstract

Previous studies have shown that maturation of the white matter in terms of its relative signal intensity changes on MRI is almost complete at 2–3 years of age. We hypothesized that quantitative analysis may show maturation of the white matter during childhood and adolescence. In the present study we performed multi-echo T2 relaxometry in 33 healthy subjects (girls, 15; boys, 18) aged 3–15 years. T2 relaxation times of the genu and splenium were measured. In healthy subjects, the T2 relaxation times were significantly correlated with age in both girls ( $r = 0.611$ ,  $p = .016$ ) and boys ( $r = 0.721$ ,  $p = .001$ ) in the splenium, but not in the genu ( $p > .05$ ). To further confirm genu-to-splenium signal intensity ratio changes, a total of 389 brain MRIs were retrospectively selected from the patients who had normal results (189 girls/women, 200 boys/men; age range, 3–20 years). The genu-to-splenium signal intensity ratio was obtained from the T2-weighted images. In patients with normal MRI, the genu-to-splenium signal intensity ratio was significantly decreased with age ( $p < .001$ ) by 16 years. The T2 relaxation times gradually increase in the splenium during childhood and adolescence, suggestive of maturation.

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**Keywords:** Human development; Corpus callosum; Magnetic resonance imaging

### 1. Introduction

It has been suggested that the splenium and genu mature on T2-weighted images of magnetic resonance imaging (MRI) at 6 months and 8 months, respectively (Barkovich et al., 1988). The authors also suggested that the whole white matter except the peripheral arborization reaches an adult appearance at approximately 18 months. A study using relaxometry suggested that both T1 and T2 relaxation times of gray and white matter of the human brain show rapid decrease during infancy, and

decrease slowly during adolescence (Holland et al., 1986). In this study, the corpus callosum matures at 1 year, and that the internal capsule is the last to develop, with maturation occurring at 10 years. Myelination of the white matter was considered responsible for this signal intensity change, which was not observed after 2–3 years of age except at the internal capsule (Barkovich et al., 1988; Holland et al., 1986).

The water molecules in the white matter are located within myelin sheath, axon, and interstitial tissue. A large proportion of water molecules within the axon and interstitial tissue are likely unbound to macromolecules, and thus have longer T2 relaxation times (Whittall et al., 1997). Therefore, thicker axons and/or larger interstitial space may give rise to longer T2 relaxation times. The axonal diameter increase with decrease of density gradually occurs in the splenium of rhesus monkey (LaMantia and Rakic, 1990a,b), which may be true in human. Accordingly, it can be assumed that the gradual increase of

*Abbreviations:* MRI, magnetic resonance imaging; CST, corticospinal tract; SENSE, sensitivity-encoding; ROI, region of interest; G–S ratio, genu-to-splenium signal intensity ratio; DTI, diffusion-tensor imaging; FA, fractional anisotropy.

\* Corresponding author. Tel.: +82 2 2228 2377; fax: +82 2 393 3035.

E-mail address: eungkim@yuhs.ac (E.Y. Kim).

axonal diameter in the splenium, which is part of the maturation, may parallel an increase of interstitial space, i.e. decrease of axonal density, thus giving rise to gradual increase of T2 relaxation times.

During childhood and adolescence, previous studies using T2 relaxometry showed gradual, but insignificant decrease of T2 relaxation times in the splenium with age (Hassink et al., 1992; Holland et al., 1986). However, they used dual-echo T2 relaxometry, which may not measure T2 relaxation times accurately (Graham et al., 1996; Whittall et al., 1997). Therefore, multi-echo T2 relaxometry may demonstrate different results as compared with the previous studies. The purpose of this study was to investigate whether the T2 relaxation times change in the splenium of the corpus callosum on multi-echo T2 relaxometry during childhood and adolescence.

## 2. Materials and methods

We obtained T2 relaxometry in healthy volunteers at 3.0 T first due to its higher signal-to-noise ratio. Thereafter, we retrospectively validated our results by using the data from patients with normal T2-weighted MRI. It is desirable to evaluate the T2-weighted MRI obtained at the same 3.0 T scanner. However, the patients with normal brain MRI obtained at 3.0 T were limited in number. Therefore, we analyzed patients with normal T2-weighted MRI obtained at 1.5 T.

### 2.1. T2 Relaxometry in healthy normal volunteers

Thirty-three healthy subjects (girls, 15; boys, 18) aged 3–15 years (mean, 10.9 years) were enrolled in this study as volunteers. The parents of all healthy subjects gave consent for MRI. No sedation was required. The institutional review board of our hospital approved this study. Subjects having abnormalities discovered while obtaining the MRI were excluded in imaging analysis.

#### 2.1.1. T2 relaxometry

All healthy subjects were examined on a 3.0 T scanner (Intera Achieva, Philips Medical Systems, Best, Netherlands) with an eight-channel sensitivity-encoding (SENSE) head coil. A multi-echo 3-D Carr-Purcell-Meiboom-Gill sequence (Georgiades et al., 2001) was used for transverse T2 relaxometry with the following parameters:  $256 \times 128$  matrix ( $256 \times 256$  after reconstruction); repetition time, 4294 ms; 8 echoes (20–160 ms) with an increment of 20; echo train length, 8; field of view, 220 mm; SENSE factor 2. The thickness was 3 mm without gap.

### 2.2. MR imaging in patients

To further validate our study using T2 relaxometry, we selected normal brain MRIs to validate whether signal intensity of the splenium on T2-weighted imaging gradually increase with age. From the records of our institute between January 2002 and October 2006, a total of 389 normal brain MRIs were selected from patients who underwent MRI for minor neurologic symptoms and signs (headache, 198; fever, 125; dizziness, 34; eye diseases, 18; syncope, 10; facial palsy, 4). The patients were 189 girls/women and 200 boys/men aged 3–20 years (mean, 11.8 years  $\pm$  5.2 [S.D.]). Two neuroradiologists verified the normality of all MRIs in consensus. The number of patients at any given age was greater than 20.

#### 2.2.1. Conventional imaging

All patients were examined with 1.5 T scanners (Signa Horizon, G.E. Medical Systems, Milwaukee, Wis [ $n = 226$ ]; Intera, Philips Medical Systems,

Best, Netherlands [ $n = 172$ ]) equipped with a manufacturer-supplied quadrature head coil (G.E.) and a six-channel SENSE head coil (Philips). The following conventional sequences were performed: sagittal T1-weighted (333 (G.E.) or 400 (Philips)/11 [repetition time ms/echo time ms], one signal acquired), transverse fast spin-echo T2-weighted (4000–4058/80–114, one signal acquired), and transverse T1-weighted (311 (G.E.) or 420 (Philips)/11, one signal acquired) MR imaging. The thickness and slice gap were 5 mm and 2–2.5 mm, respectively. The field of view was 220 or 240 mm. The matrix of T2-weighted imaging was  $256 \times 256$  (G.E.) or  $512 \times 267$  ( $512 \times 352$  after reconstruction, Philips).

### 2.3. Data analyses

#### 2.3.1. T2 relaxometry maps in healthy volunteers

To avoid cerebrospinal fluid contamination, the regions of interest (ROIs) in each subject were carefully drawn on the transverse images with echo time of 100 ms in each center of the genu and splenium by a neuroradiologist who was blind to the subject's age. The areas of the ROIs ranged from 15 to 50.0 mm<sup>2</sup> according to the size of the genu or splenium. These ROIs were transferred onto the T2 relaxometry maps, and T2 relaxation times were recorded in each center of the genu and splenium. The absolute signal intensity on T2-weighted imaging may be different between 3.0 and 1.5 T. Therefore, we additionally measured genu-to-splenium signal intensity ratios (G–S ratios) on the images obtained with echo time of 100 ms. The same ROIs for T2 relaxation time measurement were used. The G–S ratios were recorded in all subjects.

#### 2.3.2. Conventional T2-weighted images in patients with normal MRI

A neuroradiologist who did not involve in measurement of T2 relaxation times drew ROIs in the center of the genu and splenium of all patients on the transverse T2-weighted images without information regarding the age of patients. The areas of the ROIs ranged 15 mm<sup>2</sup> to 60 mm<sup>2</sup> according to the size of the genu or splenium. The G–S ratios were recorded in all patients.

### 2.4. Statistical analyses

The Spearman rank correlation test was performed to assess whether T2 relaxation times of the genu and splenium or G–S ratios may correlate with age in healthy subjects. To test whether the G–S ratios change with age (3 to 20 years of age) in patients, a linear regression analysis was performed. The variable of gender was included in the analysis to test its difference in the G–S ratio change pattern. The Kruskal–Wallis test was performed to test differences in the G–S ratio among older adolescents. The Mann–Whitney test was performed to test differences in the G–S ratio between the two age groups identified with the Kruskal–Wallis test. The age range of the older adolescents was determined by a box plot. A  $p$ -value less than .05 was considered to indicate statistical significance. All the statistical analyses were performed with statistical software (SPSS, version 12; Chicago, IL).

## 3. Results

### 3.1. T2 Relaxometry in healthy subjects

The T2 relaxation times of the genu and splenium of all subjects ranged from 64.09 to 73.06 (mean, 68.74; S.D., 2.59) and from 69.56 to 83.93 (mean, 77.50; S.D., 3.77), respectively. The T2 relaxation times were significantly correlated with age in both girls ( $r = 0.611$ ,  $p = .016$ ) and boys ( $r = 0.721$ ,  $p = .001$ ) in the splenium, but not in the genu ( $p > .05$ ) (Fig. 1). The G–S ratios on the images with echo time of 100 ms were also significantly correlated with age in both girls ( $r = -0.873$ ,  $p < .001$ ) and boys ( $r = -0.806$ ,  $p < .001$ ). The signal intensity on T2 relaxation maps was gradually increased in the splenium (Fig. 2).

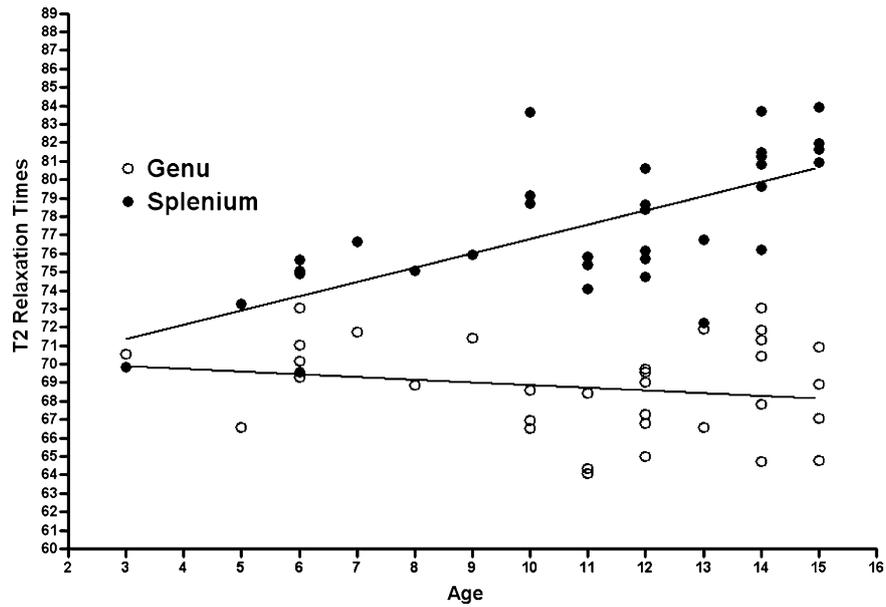


Fig. 1. T2 relaxation times of the splenium obtained from healthy subjects (filled circles) are significantly correlated with age ( $r = 0.703$ ,  $p < .001$ ), but not those in the genu (empty circles).

### 3.2. G–S ratio on T2-weighted imaging in patients

The G–S ratio significantly decreased with age ( $p < .001$ ). The scatter plots and regression lines for correlation between age and G–S ratio are shown in Fig. 3. When the gender variable was included in the linear regression analysis, the G–S ratio was not significantly correlated with gender ( $y = 1.01 - 0.01x_1 - 0.004x_2$ ,  $r^2 = 0.776$ ,  $p < .001$  [ $x_1$ , age],  $p = .191$  [ $x_2$ , gender]).

The box plot revealed a gentle decline among the patients aged 17–20 (Fig. 4). The Kruskal–Wallis test showed no significant difference in the G–S ratio among these patients ( $p > .05$ ), but a significant difference among the patients aged 16–20 ( $p = .006$ ). The Mann–Whitney test showed a significant difference between the patients aged 16 and 17 ( $p = .032$ ). Consequently, no significant decrease of G–S ratio in patients over 16 years of age was determined.

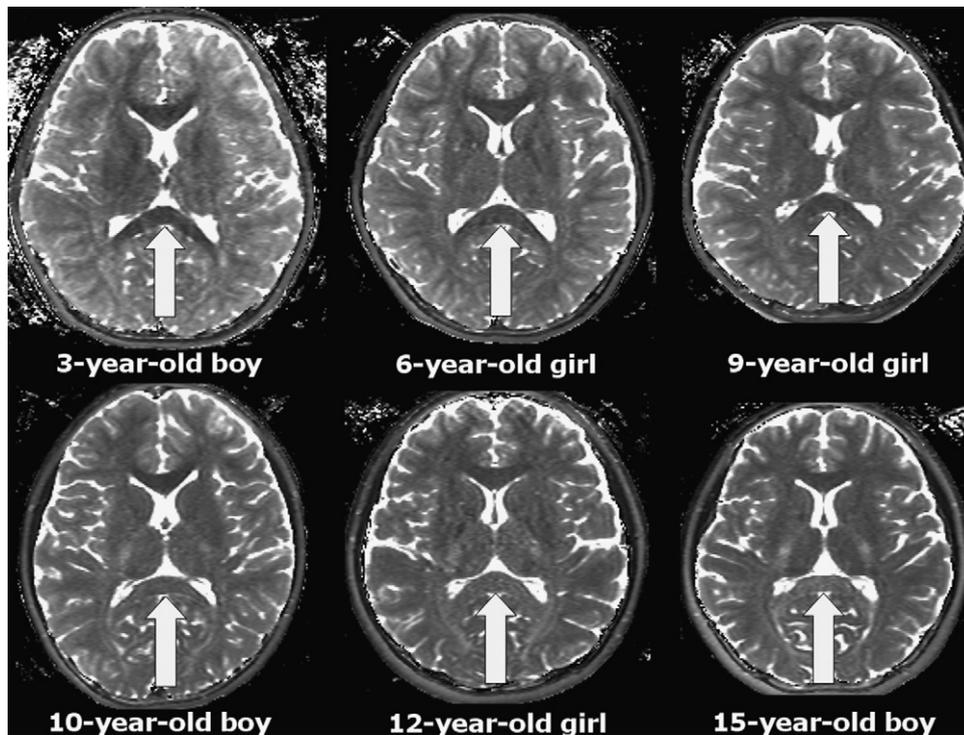


Fig. 2. Axial T2 relaxometry maps obtained from healthy subjects show a gradual increase of signal intensity in the splenium with age (arrows), but are not prominent in the genu.

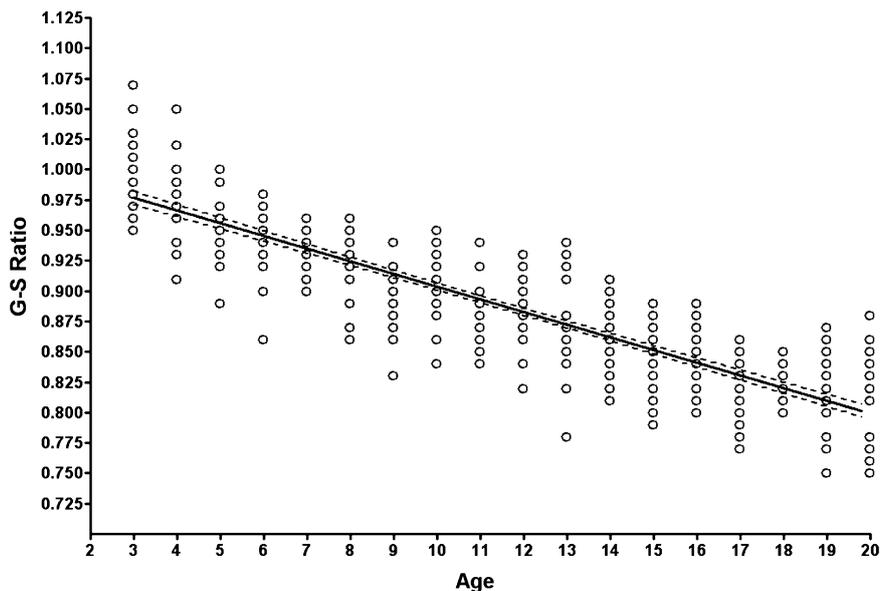


Fig. 3. The genu-to-splenium ratio (G–S ratio) acquired from the patients with normal MRIs. Scatter plot and regression lines show a positive correlation between age and G–S ratio ( $y = 1.008 - 0.01x$ ,  $r^2 = 0.775$ ,  $p < .001$ ). The solid line is the regression line and the dashed line is the 95% confidence interval.

#### 4. Discussion

No complete autopsy studies have been reported with regard to microstructural maturation of the corpus callosum in human children and adolescents. However, we can infer this from the previous autopsy studies of rhesus monkeys and adult humans (Aboitiz et al., 1992; Highley et al., 1999; LaMantia and Rakic, 1990a,b). A study of rhesus monkeys demonstrated that the number of axons in the corpus callosum of the newborn monkey is 3.5 times higher than in the adult monkey, and myelin in the corpus callosum gradually increases with age (LaMantia and Rakic, 1990a). Interestingly, the genu has larger axons than the splenium by 90 days of life in contrast to the relationship seen in the adult monkeys in which the splenium has larger axons than the genu. In addition, axonal density is gradually

decreased with age, which is prominent in the posterior body and splenium (LaMantia and Rakic, 1990b). This relationship of axonal density and diameter in adult monkeys is very similar to the results from human autopsy studies (Aboitiz et al., 1992; Highley et al., 1999). In human, the axons in the splenium are less dense than those in the genu (Highley et al., 1999), but they are larger with more myelin (Aboitiz et al., 1992). Therefore, larger axons with lower density in the splenium may be a consequence of maturation in human as well as in rhesus monkey.

It has not been reported whether the signal intensity change can be observed in the white matter during childhood and adolescence on T1-weighted imaging. That is, the signal intensities of the genu and splenium are indistinguishable on conventional T1-weighted imaging in children or adolescents. Consequently, one may dispute our results because the splenium with longer T2 relaxation times did not parallel longer T1 relaxation times, i.e. low signal intensity on T1-weighted imaging. According to the study measuring T1 relaxation times in the white matter corresponding to age (Steen et al., 1997), such values decrease gradually after 4 years. In this study, age regression normalizes at 4 and 6.4 years in the genu and splenium, respectively. Therefore, signal intensity change on T1-weighted images after 7 years cannot be observed, while signal intensity on T2-weighted images gradually changes in the splenium in our study. This could be explained with the assumption that the splenium has more myelinated axons than the genu (Whittall et al., 1997), which might offset the increase in T1 relaxation times. However, we still do not know the relationship between myelin quantity and T1 relaxation times because myelin comprises only about 50% of the central nervous system white matter (Norton and Cammer, 1984).

One may also question our results because the splenium, with more myelin than the genu, shows higher signal intensity

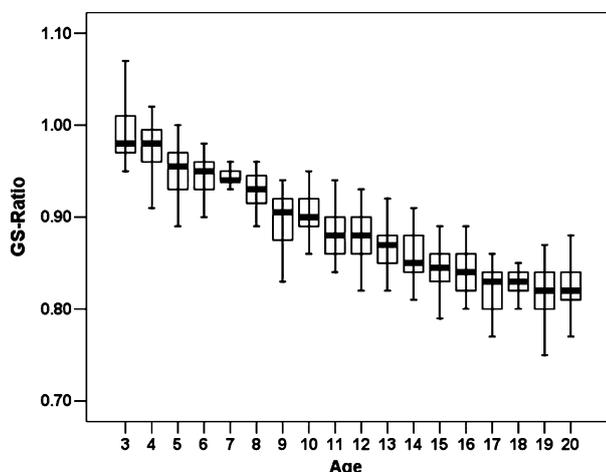


Fig. 4. The genu-to-splenium ratio (G–S ratio) acquired from the patients with normal MRIs. Box plot shows a gradual decrease of G–S ratio with age. A gentle decline is seen at late adolescence. No significant difference of G–S ratio is noted among the subjects aged 17–24 (Kruskal–Wallis test,  $p > .05$ ).

on T2-weighted imaging than the genu. This is the opposite of the findings in childhood less than 18 months of age when more myelin may give rise to darker signal intensity on T2-weighted imaging. The water fraction of myelin is about 10% (Whittall et al., 1997). Therefore, it seems possible that larger interstitial space due to lower density and/or thicker axons having much water may offset the decrease of T2 relaxation times and may result in higher signal intensity in the splenium. However, further study should be followed to validate this assumption.

It has been suggested that the corpus callosum shows caudo-rostral growth (Barkovich et al., 1988); however, a rostro-caudal wave of peak growth demonstrated by using continuum mechanical tensor maps has been suggested in the corpus callosum after 3 years (Thompson et al., 2000). The aforementioned animal and human studies may suggest that the axonal growth patterns are different in the genu and splenium in human as well as rhesus monkey, i.e. the axons with more myelin and larger in diameter are in the posterior body and splenium, supporting the assertion of rostro-caudal growth (Aboitiz et al., 1992; Highley et al., 1999; LaMantia and Rakic, 1990a,b). Our study also showed gradual changes in signal intensity in the splenium as compared with the genu, comparable to the previous studies.

Several diffusion-tensor imaging (DTI) studies dealing with maturation of the brain of childhood have recently been published (Barnea-Goraly et al., 2005; Mukherjee et al., 2001; Schmithorst et al., 2002; Snook et al., 2005, 2007). However, these studies showed conflicting results among each other in terms of maturation of the corpus callosum. The fractional anisotropy of the splenium was significantly correlated with age only in the studies by Snook et al. (Snook et al., 2005, 2007). An increase of axonal diameter may parallel an increase in fractional anisotropy from DTI. However, it is important to note that the determinants of FA include coherence and axonal density as well as axonal diameter (Pierpaoli and Basser, 1996). Therefore, further studies should be followed on this issue.

The limitations of our study are fourfold. First, we did not directly compare the axonal diameter and density of the splenium or genu of the corpus callosum between adults and children with autopsy studies. However, few pediatric autopsy studies have been reported to date because of their scarcity (Witelson and Kigar, 1988). Second, measurement of the signal intensity ratio, not absolute relaxation time, was performed in patients. However, we collected data from a relatively large number of patients, which may make up for this drawback. Third, we used different MR imagers in this study; i.e. T2 relaxometry in healthy subjects at 3.0 T and the G–S ratio measurement in patients at 1.5 T. However, we also measured the G–S ratio at 3.0 T and obtained results comparable to those at 1.5 T, which may support our assertion. Lastly, although it may be desirable to isolate myelin water fraction by using more echo times as shown in the previous study (Whittall et al., 1997), we did not obtain these data because pediatric MRI studies without sedation are rather time constrained.

In summary, by using multi-echo T2 relaxometry, we demonstrated that the T2 relaxation times in the splenium gradually increase during childhood and adolescence, sugges-

tive of maturation. The signal intensity changes on T2-weighted images were validated in large number of patients with normal MRI findings.

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