Incidence and patterns of abnormal corpus callosum in fetuses with isolated spina bifida aperta

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Bulleted statements:

What's already known about this topic?

- In children with spina bifida aperta, the corpus callosum is often abnormal
- CC abnormalities have a functional impact on children's cognitive function, language and social skills.
- The CC is fully developed and can be visualized by prenatal ultrasound at approximately 16 weeks' gestation.

What does this study add?

- In three out of four fetuses with isolated spina bifida aperta who are referred for fetal surgery assessment, the CC is structurally abnormal.

Abstract

Objective: To determine the incidence and characterize corpus callosum (CC) abnormalities in fetuses with spina bifida aperta (SBA) between 18 and 26 weeks of gestation.

Methods: This was a retrospective study on fetuses with isolated SBA and who were assessed for fetal surgery. Digitally stored ultrasound images of the brain were reviewed for the presence/absence of the CC, and the length and diameter of its constituent parts (rostrum, genu, body and splenium). We used regression analysis to determine the relationship between CC abnormalities and gestational age, head circumference, ventricle size, lesion level and lesion type.

Results: Nearly three-quarters of fetuses with isolated SBA had an abnormal CC (71.7%, 76/106). Partial agenesis was most common in the splenium (18.9%, 20/106) and the rostrum (13.2%, 14/106). The most common abnormal pattern was of a short CC with normal diameter throughout. Of note, 20.8% (22/106) had a hypoplastic genu and 28.3% (30/106) had a thick body part. Larger lateral ventricle size was associated with partial agenesis of the CC (OR 0.14, $p<0.001$) and inversely associated with a shorter CC (OR 2.60, $p<0.01$).

Conclusion: An abnormal corpus callosum is common in fetuses with isolated SBA who are referred for fetal surgery.

Introduction

Spina bifida aperta (SBA) is a congenital anomaly caused by failure of posterior neural tube closure early in gestation. This leads to exposure of the neural placode and the nerves to the intrauterine environment. The *in utero* neurodegeneration of the exposed spinal cord has lifelong consequences including sensory problems, muscle paralysis, skeletal deformities, bowel and bladder dysfunction (1, 2). Besides a malformation of the spine and spinal cord, several abnormal findings in the brain have been described in association with SBA. This includes ventriculomegaly, a small and dysplastic cerebellum, beaking of the tectal plate, neuronal migration, cortical organization disorders and malformations of the corpus callosum (CC) (3, 4).

Although the embryonic development of the corpus callosum is not yet completely understood, evidence from pathology studies revealed that the structure of the CC adopts an adult morphology and position at approximately 18 weeks of gestation (5). This is in accordance with what was observed in several prenatal ultrasound studies, showing that CC structure can be visualised and its length and diameter can be measured as early as 16 weeks' gestation (5-8). The CC is the largest white matter structure connecting and coordinating neurons in the left and the right hemispheres. It facilitates the integration of motor and sensory information and also plays a role in higher cognitive functions and social skills. In case of *isolated* agenesis of the CC, the spectrum of neurological deficits is wide (9). This includes poor performance in tasks that require integration of complex cognitive operations and/or rapid processing time; for example, abstract reasoning, problem solving, comprehensive and expressive language (10-13). Furthermore, it can lead to impaired social skills and poor

personal insight, which by parents are reported as prominent features that disrupt their children's lives (9).

Several postnatal studies have documented that the majority of children with SBA (57.1%-95.9%) have an abnormal CC; the most prevalent patterns being generalised hypoplasia and partial agenesis (14-17). These abnormalities have been shown to adversely affect the intelligence quotient (IQ) and interhemispheric transferring functions, leading to delayed reaction time to stimuli, slower bimanual coordination and poor idiom comprehension. This is similar pattern as neurocognitive findings observed in children with isolated agenesis of the CC (18-20). The exact incidence of CC abnormalities in SBA fetuses remains unknown, including in fetuses who are candidates for fetal surgery. Identifying abnormalities prenatally can help with parental counselling, planning for pre- and postnatal imaging follow-up, and neurological management after birth.

The primary objective of this study was to establish the incidence of, and, if present, to characterise CC abnormalities in fetuses with isolated SBA and who are assessed for fetal surgery. The secondary objective was to investigate the correlation between CC biometry and the level or type of lesion, head size as well as ventricular size.

Material and methods

Study settings

This observational cohort study was conducted at the fetal medicine units of the University Hospitals Leuven (UZL), Leuven, Belgium and University College London Hospital (UCLH), London, UK. A clinical service offering SBA fetal surgery has been available since January 2012 at UZ Leuven, and since January 2018 at UCLH. Cases were included from March 2014, from what time point systematic clinical ultrasound documentation of the CC was acquired and

archived, until 31st December 2019. All US assessments were performed and/or supervised by highly experienced neurosonographers (LDC, FU), who assessed patients either with a Voluson E6, E8 or E10 machine (GE Medical Systems, Zipf, Austria) equipped with a convex 2-5 MHz transabdominal and a 5-13 MHz transvaginal transducer. The midsagittal plane of the fetal brain was assessed according to the International Society in Obstetrics and Gynecology (ISUOG) guideline for fetal neurosonography (21). Digital US images and clinical information of pregnant women and their fetuses were obtained from the Picture Archiving and Communication System (PACS V3.2.1002, DOBCO Medical Systems, Belgium) and electronic patient record for UZ Leuven or ViewPoint digital software (V5.6.16.917~~,~~ GE Healthcare GmBH, Zipf, Austria) for UCLH. This study was approved by the Ethics Committee for clinical studies (S60814) at UZ Leuven. The UK Health Research Authority identified that this study was not considered to be research and ethical review by the National Health Service (NHS) Ethics Committee was therefore not required. Audit approval was received.

Study population

Inclusion criteria were singleton pregnant women carrying a fetus with SBA who were referred for assessment for fetal surgery, between 18-25+6 weeks' GA and in whom there was at least one adequate image of the mid-sagittal plane of the brain. This was defined as a sagittal fetal brain image demonstrating the total length of the CC, the cavum septum pellucidum, thalamus and part of the midbrain, according to the ISUOG guideline (Figure 1) (21). If multiple images were available, the latest one during the second trimester was used for the analysis.

Recorded maternal characteristics included obstetric history and GA at assessment. Information on the fetus included US findings such as fetal biometry, biparietal diameter

(BPD), head circumference (HC), abdominal circumference (AC), femur length (FL), estimated fetal weight (EFW), additional structural/genetic anomalies, lesion type (myelomeningocele [MMC; i.e. with presence of a meningeal sac] or myeloschisis [MS; i.e. when such a meningeal sac is absent]), the upper level of the defect, and ventricular size. The upper level of the lesion was determined on the sagittal plane of the spine; either counting down from T12 (most caudal vertebrae with corresponding rib) or counting up from the S4 or S5 (the lowest ossified vertebrae according to GA [S4 if GA<24 weeks and S5 if GA≥24 weeks]). The defect was also confirmed on the axial plane, as a divergence of the posterior arch of the vertebrae (22). Ventricle size was measured at the atrium level perpendicular to the ventricle cavity (21). A value of 10 mm or greater was considered ventriculomegaly (23).

Ultrasound measurements of the corpus callosum

Digitally stored images were reassessed offline by or under the supervision of two senior sonographers (FU, LDC) who were blinded to the patients' clinical information. They checked the following (Figure 1):

1. Presence of an adequate image of the mid-sagittal plane of the fetal brain characterised according to the ISUOG guideline for fetal neurosonography (21).
2. Presence of the CC and its parts; the rostrum, the genu, the body and the splenium, were determined. The CC was defined as a hypoechoic structure lying above the cavum septum and the thalamus. The rostrum was the anterior part with a beak shape. The genu was the most anterior part curving downward in front of the cavum septum. The body was the horizontal slightly curved part while the splenium was the most rear part with round shaped configuration (5, 8).

3. Measurement of the straight antero-posterior (AP) length of the CC from the most anterior part of the genu, to the most posterior part of the splenium (5, 8).
4. Measurement of the diameter of the genu, body and splenium portions. No attempt was made to measure the rostrum diameter because of its thin dimensions; however, its presence was noted (5, 8).

The reproducibility of these measurements has been previously documented (8). Measurements of the length and diameter were categorised as either <5th percentile (thin), 5th-95th percentile or >95th percentile (thick) according to the normal reference ranges provided by Pashaj, et al (8).

Statistical analysis

Continuous variables were summarized as mean (standard deviation, SD) or median (interquartile ranges, IQR). Normal distribution was assessed by the Shapiro Wilk test and normality plots. Categorical variables or binary variables were reported as percentages. Fetal biometric variables were adjusted for gestational age according to published formula (24). Corpus callosum biometry was also corrected for GA (8). All the biometric variables were converted to Z-scores for GA. One sample student's t-test was used to compare Z-scores of several variables against a standard normal distribution. Correlation matrices were obtained by Pearson's rho.

We performed multiple logistic regression analysis to define variables associated with partial agenesis of CC. The area under the curve was computed to assess our model. A multiple linear regression analysis was also performed to determine the effect of GA, fetal biometry, lesion level and lesion type on the CC length and the diameter of the genu, body and splenium. Initially we obtained a matrix of coefficients by variables' forced entry; subsequently we

carried out backward elimination on the basis of the higher p value and R square differences for addition or removal of the examined variables. Residual diagnostics were used to check the model's adequacy and regression assumptions. The statistical software R (version 3.6.3) was used for data analysis (25).

Results

We assessed 172 fetuses with SBA (UZ Leuven: 74%, 128/172; UCLH: 26%, 44/172) (Figure 2). An adequate image of the mid-sagittal plane was available in 70% (121/172). Of those that were inadequate, 74.5% (38/51) did not have any sagittal image stored and 25.5% (13/51) had an image which did not completely represent the mid-sagittal plane. Fetuses with an abnormal array comparative genomic hybridization (CGH) study or additional major structural anomalies were excluded (12.4%, 15/121) (supplementary information 1). This left a total of 106 fetuses including eight with minor structural anomalies (7.4%, supplementary information 1). Almost half of cases were evaluated by both transabdominal and transvaginal ultrasound (47%, 50/106). There were no differences in patient characteristics between the two centres; thus, we pooled the data. Demographic data are summarised in table 1. The majority of fetuses had an MMC (72.2%) as opposed to 27.8% with an MS lesion. Almost half had a lesion above or equal to lumbar level 3 (44.3%). Ventriculomegaly (65.1%) and small BPD ($<5^{th}$ centile) (64.4%) were common findings.

Table 2 summarises the characteristics of the CC in our cohort. The CC biometry was overall smaller than the reference distribution (total CC length Z-score -1.4, SD 2.0, $p<0.01$; genu diameter Z-score -0.9, SD 1.6, $p<0.01$; splenium diameter Z-score -1.14, SD 1.66, $p<0.01$). The only exception was the diameter of the body, which was increased after correction for GA (Z-score 0.9, SD 1.7) (supplementary information 2). Only 28.3% (30/106) of the cohort had a

normal CC, defined as a complete CC with its length and diameter of all parts between the 5^{th}-95^{th} centile for GA. We did not identify a single case with total absence of the CC; however, the splenium was the most common part of the CC to be absent (18.9%, 20/106) followed by the rostrum (13.2%, 14/106). Of note, 7.5% (8/106) of fetuses had both splenium and rostrum parts absent. In approximately 20% (22/106) of cases, the genu was thin, while in almost 30% (30/106) of cases, the body of the CC was thickened. Common examples of CC abnormalities are shown in Figure 3.

We also investigated the patterns of abnormalities in 76 fetuses that were identified to have an abnormal CC (Table 3). The most common patterns were a short CC with normal diameter of all parts (21.1%, 16/76) and a normal length CC with a thick body part (9.2%, 7/76). We did not find any other specific patterns in the rest of the cohort.

Multiple logistic regression analysis revealed that partial agenesis of the CC was associated with a larger ventricle size (supplementary information 3). The lateral ventricle of fetuses with partial agenesis of CC was significantly wider than those with complete CC (mean Z-score, 8.05 vs 3.56, $p<0.001$). We found an interaction between the ventricle size and the GA at assessment in fetuses with complete CC (Figure 4); the ventricle size was strongly correlated with GA (rho=0.30, p=0.006). However, there was no correlation between the two factors in the partial agenesis group (rho=0.22, p=0.28) (Figure 4). It is worth mentioning that, in the latter group, the z-score of the lateral ventricle was 8.05, which is equivalent to approximately 15 mm (severe ventriculomegaly) across gestation.

Multiple linear regression analysis on the dimensions of the CC revealed that GA, other CC biometric indices, lesion type and ventricle size had an impact on the CC length (supplementary information 4). Fetuses with myeloschisis had an almost 1SD (-0.88 SD)

shorter CC length than fetuses with a cystic lesion (MMC) (supplementary information 4). In addition, GA and ventricle size also had an impact on the diameter of the splenium and the genu (supplementary information 5).

Discussion

In this study, we found that only one-quarter of SBA fetuses who were referred for fetal surgery had a normal CC. The dimensions of the CC were smaller with the exception of a thicker body part. Partial agenesis was most commonly observed in the splenium and the rostrum. Common patterns of CC abnormalities were a short CC and a thick body part. Fetuses with severe ventriculomegaly were more likely to have partial agenesis of the CC regardless of GA at examination. Conversely, a shorter length of the CC and thin splenium and/or genu was associated with advanced GA at examination and wider lateral ventricles.

In this cohort, nearly three-quarters of fetuses with isolated SBA were found to have an abnormal CC, without a single case of complete agenesis. This is consistent with findings in postnatal studies reporting a 57-96% rate of CC malformation, without any cases of complete CC agenesis (14-17). One common abnormality we observed was partial agenesis, in particular of the splenium (18.9%) and the rostrum (13.2%). Partial agenesis of those parts is also commonly found in postnatal cases with shunted hydrocephalus (15, 17). However, the incidence was higher in these postnatal series; with 25-42% and 32-56% of children with SBA having agenesis of the rostrum and the splenium, respectively (15, 17).

Considering patterns of CC abnormalities, in postnatal series, a generalised thinning was the most prevalent pattern documented in 21-50% of cases (14-17). In contrast, we only observed thin genu in 20.8% rather than a generalised thinning, and often a more ~~hyperplastic~~ thickened body (28.3%). We did not find any postnatal studies reporting a thickened

appearance of the CC in children with spina bifida, and very rarely it is reported in fetuses with other pathology of the central nervous system. A generalised thickening of the CC is typically found in conjunction with other brain abnormalities such as macrocephaly, neurofibromatosis type 1 and abnormal cortical development (26-28). When a thickened CC co-exists with an additional brain anomaly, the finding usually persists into the postnatal period (27). On the contrary, a small prenatal study, though not in fetuses with SBA, reported a thickened CC as a single abnormal finding in the second trimester, though later becoming normal during the third trimester (29). We documented a thickened body part of the CC in our cohort, which is in line with the recent publication of Maurice et al. In that study, 18.6% (13/70) of the prenatal cohort of spina bifida fetuses had a thickened CC (>97th centile); although, the authors did not state in which part the CC becomes thickened (30). In the same study, the authors described a stretched CC in almost 1/10 of their cases (5/70); however, we did not study this feature because of the absence of a clear definition for "stretched" (30). The thickened appearance of the CC in spina bifida fetuses might change with advancing GA, as the feature was not reported in postnatal studies (14-17). In this respect, it would be interesting to look at later images or even better to longitudinally follow up cases, both in the third trimester and in postnatal life. The latter is more difficult as patients undergoing fetal surgery are referred back to their local centres.

Although the pathophysiology of partial agenesis of the CC in SBA remains unknown, it is postulated that leakage of cerebrospinal fluid (CSF) through the vertebral defect early in gestation may disturb brain development (4). CSF circulation is vital for the process of neuronal migration, and once neurons are present in certain cortical layers, they extend their axons forming deep white matter structures including the CC (31, 32). Disruption of the CSF circulation may cause cortical thinning, disproportionally dilated ventricles and an abnormal

formation of the CC (4, 5, 33). In our cohort, we did not identify a single case with complete agenesis of the CC. This observation would underpin that the key processes in CC development (the formation of midline substrates and the crossing over of the pioneering axons) remain intact in SBA (26). However, partial agenesis is commonly identified in the rostrum and the splenium and it was associated with severe ventriculomegaly. As the two parts are the last to develop, they may be exposed longer to disrupted neuro-embryogenesis and perhaps be more sensitive to disturbances of CSF circulation (15, 17, 33).

Regarding hypoplasia of the CC, it is hypothesized that with increasing ventriculomegaly, as is often the case in SBA later in gestation, the CC inherently becomes thinner. Distended lateral ventricles might stretch the CC causing a more generalised hypoplasia of the structure (15, 17). Our data also support this view, because as gestation advances and the ventricles become larger, the total length of the CC, genu and splenium became shorter and thinner. As fetal surgery is able to reverse hindbrain herniation and slow down ventricular enlargement (34), the surgery may arrest the stretching process in the CC. However, to confirm this, and an eventual cause-and-effect relationship, longitudinal imaging studies will be needed and are under way.

In terms of clinical application, we have shown the relevance of acquiring a good mid-sagittal plane of the fetal brain in fetuses with SBA, as CC abnormalities are common. Also, it is recommended to assess the CC thoroughly when there is severe ventriculomegaly, because it was associated with both partial agenesis of CC and as well as abnormal biometry.

A strength of the study is that it includes a large number of fetuses, considering the incidence of spina bifida, and also reports on a typical fetal surgery population. The images were reviewed by experts blinded to the clinical details, and we used strict inclusion criteria and

reference ranges to adjust the measurements so as to reduce operator perception bias. One of the limitations however was that an adequate mid-sagittal view of the brain was not available for all cases in our cohort; thus, we acknowledge that we may have overestimated the true incidence of the abnormalities as fetuses with a suspicion of an abnormal CC may be more likely to have complete images stored. However, our findings are comparable to those of postnatal studies, suggesting that our findings were representative (14-17). It is also possible that additional magnetic resonance imaging (MRI) might have detected additional CNS and brain abnormalities that are associated with abnormal CC such as disorders of neuronal migration, grey matter heterotopia and polymicrogyria, which are better detected by MRI rather than US (35). Another limitation was the inherent selection bias, as we report findings in two fetal surgery centres. Therefore, fetuses with very high/low lesions, additional major anomalies or with severe secondary changes such as kyphosis, were not included, as these may not have been referred for consideration of fetal surgery. The most important limitation of our study is obviously that it remains descriptive, and lacks any follow-up details, either in the short of long-term.

Conclusion

Three-quarters of fetuses with spina bifida aperta who were referred for consideration of fetal surgery, have an abnormal CC on second trimester US. We propose further study to document changes longitudinally and to assess the functional impacts of CC abnormalities.

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Disclosure of interest

The authors declare that there is no conflict of interest

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Ethical approval

UZ Leuven: The ethics Committee Research UZ Leuven, study number S60814, approval date 12/12/2017

UCLH: The UK Health Research Authority identified that this study was not considered to be research and ethical review by the National Health Service (NHS) Ethics Committee was therefore not required.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table 1. Patient characteristics

Characteristics	n=106
Gestational age at diagnosis (weeks)‡	23^{+2} (22-24^{+4})
Gravida‡	2 (1-3)
Parity‡	1 (0-2)
Open spina bifida lesion type, % (n)	
▪ Myelomeningocele (MMC)	71.7% (76/106)
▪ Myeloschisis (MS)	28.3% (30/106)
Biparietal diameter (BPD)†, % (n)	
▪ <5th percentile according to GA	64.4% (67/104)
▪ 5-95th percentile according to GA	35.6% (37/104)
▪ >95th percentile according to GA	0% (0/104)
Head circumference (HC)†, % (n)	
▪ <5th percentile according to GA	36.5% (38/104)
▪ 5-95th percentile according to GA	63.5% (66/104)
▪ >95th percentile according to GA	0% (0/104)
Abdominal circumference (AC)†, % (n)	
▪ <5th percentile according to GA	0% (0/104)
▪ 5-95th percentile according to GA	98.1% (102/104)
▪ >95th percentile according to GA	1.9% (2/104)
Femur length (FL)†, % (n)	
▪ <5th percentile according to GA	0% (0/104)
▪ 5-95th percentile according to GA	95.2% (99/104)
▪ >95th percentile according to GA	4.8% (5/104)
Ventriculomegaly, % (n)	
▪ No ventriculomegaly	34.9% (37/106)
▪ Mild-to-moderate ventriculomegaly	38.7% (41/106)
▪ Severe ventriculomegaly	26.4% (28/106)
Lesion levels	
▪ S2 and below	4.7% (5/106)
▪ S1-L4	51.0% (54/106)
▪ L3 and above	44.3% (47/106)

Abbreviation: S, body of sacrum; L, body of lumbar

Data presented as median (IQR) or % (n)

‡ Median (IQR); † Data available in 104 fetuses

Table 2. Characteristics of corpus callosum in fetuses with open spina bifida

	Absence	<5th percentile†	5th-95th percentile†	>95th percentile†
Total length of corpus callosum	0% (0/106)	**44.3% (47/106)**	50.0% (53/106)	5.7% (6/106)
Rostrum	**13.2% (14/106)**	Presence=86.8% (92/106)		
Genu	6.6% (7/106)	**20.8% (22/106)**	69.8% (74/106)	2.8% (3/106)
Body	0% (0/106)	3.8% (4/106)	67.9% (72/106)	**28.3% (30/106)**
Splenium	**18.9% (20/106)**	7.5% (8/106)	72.6% (77/106)	1.0% (1/106)

†According to gestational age

Table 3. Patterns of corpus callosum abnormalities

Total length	Rostrum	Genu	Body	Splenium	% (n)
Short	Absence	Absence	Normal	Absence	3.9% (3/76)
Short	Absence	Absence	Thick	Absence	1.3% (1/76)
Short	Absence	Absence	Thick	Normal	2.6% (2/76)
Short	Absence	Thin	Normal	Absence	1.3% (1/76)
Short	Absence	Thin	Thick	Absence	1.3% (1/76)
Short	Absence	Thin	Thick	Normal	2.6% (2/76)
Short	Absence	Normal	Normal	Normal	1.3% (1/76)
Short	Presence	Thin	Normal	Absence	1.3% (1/76)
Short	Presence	Thin	Normal	Thin	2.6% (2/76)
Short	Presence	Thin	Normal	Normal	6.5% (5/76)
Short	Presence	Thin	Thick	Absence	1.3% (1/76)
Short	Presence	Thin	Thick	Normal	1.3% (1/76)
Short	Presence	Normal	Thin	Absence	1.3% (1/76)
Short	Presence	Normal	Thin	Normal	2.6% (2/76)
Short	Presence	Normal	Normal	Absence	1.3% (1/76)
Short	Presence	Normal	Normal	Thin	1.3% (1/76)
Short	Presence	Normal	Normal	Normal	**21.1% (16/76)**
Short	Presence	Normal	Thick	Absence	2.6% (2/76)
Short	Presence	Normal	Thick	Thin	1.3% (1/76)
Short	Presence	Normal	Thick	Normal	2.6% (2/76)

Short	Presence	Thick	Normal	Normal	1.3% (1/76)
Normal	Absence	Absence	Normal	Absence	1.3% (1/76)
Normal	Absence	Thin	Thin	Normal	1.3% (1/76)
Normal	Absence	Thin	Thick	Absence	1.3% (1/76)
Normal	Presence	Thin	Normal	Absence	1.3% (1/76)
Normal	Presence	Thin	Normal	Thin	1.3% (1/76)
Normal	Presence	Thin	Normal	Normal	2.6% (2/76)
Normal	Presence	Thin	Thick	Thin	1.3% (1/76)
Normal	Presence	Thin	Thick	Normal	1.3% (1/76)
Normal	Presence	Normal	Normal	Absence	1.3% (1/76)
Normal	Presence	Normal	Normal	Thin	1.3% (1/76)
Normal	Presence	Normal	Thick	Absence	3.9% (3/76)
Normal	Presence	Normal	Thick	Normal	**9.2% (7/76)**
Normal	Presence	Thick	Normal	Normal	1.3% (1/76)
Long	Presence	Thin	Normal	Normal	1.3% (1/76)
Long	Presence	Normal	Normal	Normal	1.3% (1/76)
Long	Presence	Normal	Thick	Absence	1.3% (1/76)
Long	Presence	Normal	Thick	Thin	1.3% (1/76)
Long	Presence	Normal	Thick	Normal	1.3% (1/76)
Long	Presence	Thick	Thick	Thick	1.3% (1/76)

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