Selective fetal growth restriction in identical twins: from womb to adolescence

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Chapter 6

Changes in structural brain development after selective fetal growth restriction in monochorionic twins.

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Abstract

Objectives. Fetal growth restriction (FGR) may permanently alter brain development resulting in lifelong structural and functional changes. However, in studies addressing this research question, FGR singletons are primarily compared to matched appropriately-grown singletons, a design which is inherently biased by differences in genetic and maternal factors. To overcome these limitations, we conducted a within-pair comparison of structural cerebral measurements in identical twin pairs discordant for fetal growth.

Methods. Structural cerebral measurements on neonatal cerebral ultrasound were compared between the smaller twin and larger twin of monochorionic twins with selective fetal growth restriction (sFGR), defined as a birth weight discordance ≥ 20%, born in our center between 2010-2020. Each twin pair was also matched to an appropriately-grown singleton based on sex and gestational age at birth.

Results. We included 58 twin pairs with sFGR, with a median gestational age at birth of 31.7 (IQR 29.9-33.8) weeks and a median birth weight for the smaller twin and the larger twin of respectively 1155 grams versus 1725 grams (median birth weight discordance of 32%). The smaller twin had significantly smaller cerebral structures (corpus callosum, vermis, cerebellum), white/deep gray matter and intracranial surface and volume. Intracranial volume discordance and birth weight discordance correlated significantly ($r = 0.443$, $p = 0.004$). Intracranial volume discordance was smaller as opposed to birth weight discordance (19% vs. 32% respectively, $p < 0.0001$). After correction for intracranial volume, all observed differences (except for biparietal diameter) ceased to exist.

Conclusions. sFGR in monochorionic twins is associated with an overall, proportional restriction in brain growth on neonatal cerebral ultrasound for the smaller twin, in line with previous singleton studies. The amount of birth weight discordance translates into a discordance in the size of brain structures as well, albeit smaller as opposed to the amount of birth weight discordance.

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Introduction

Approximately 10% of all pregnancies are affected by fetal growth restriction (FGR), characterized by the inability of the fetus to reach its growth potential[1]. FGR in singletons is multifactorial in origin by way of maternal, fetal or placental determinants and is responsible for a large portion of both perinatal morbidity and mortality[2]. It is hypothesized that FGR can permanently alter fetal development, including brain development, resulting in lifelong structural and functional changes.

The hemodynamic adaptation of the brain to suboptimal growth conditions can already be detected antenatally as "brain sparing", a redistribution of blood flow to the brain indicated by a lowered cerebral-placental ratio (CPR)[3]. Despite this supposedly protective mechanism, deficits in brain structures are prevalent in FGR singletons, amongst which a reduced intracranial volume, corpus callosum size and cerebellar diameter[4,5]. These structural deficits are known to have significant consequences for brain functioning in childhood for FGR singletons, such as lower cognitive test scores and impaired motor skills[6].

So far, in the available studies regarding the impact of FGR on brain structure and functioning, FGR singletons are primarily compared to matched appropriate for gestational age singletons[4,7]. However, this study design is inherently biased by differences in genetic and maternal factors possibly influencing outcomes and thereby limiting comparability. These limitations are not present when research is performed in an identical twin model with discordance in fetal growth[8].

Monochorionic (MC) twins share a single placenta that can be unequally shared, resulting in an unbalanced nutrient and oxygen supply and a subsequent discordant growth pattern called selective fetal growth restriction (sFGR)[9]. These twins allow us to compare a growth-restricted twin with its genetically identical appropriately-grown co-twin with identical maternal characteristics. At present, no studies have evaluated cerebral ultrasound (cUS) parameters in this specific twin population. The aim of this study is to conduct a within-pair comparison of structural cerebral measurements on neonatal cUS in MC twin pairs with sFGR.

Methods

This study was approved and waived of the requirement for written informed consent by the ethics committee of the Leiden University Medical Center (LUMC) as it concerns a retrospective analysis of clinically indicated ultrasound examinations (protocol G21.011). All consecutive MC twin pairs with sFGR, defined as a birth weight discordance (BWD) ≥ 20%, born in our center (the national referral center for complicated MC twin pregnancies) between 2010-2020 were eligible for inclusion. BWD was calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100[10]. Cases with twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) were excluded due to the likely additional effect of these complications on brain development[11,12]. We also excluded MC triplet pregnancies, cases with twin reversed arterial perfusion (TRAP) and/or other congenital abnormalities[12]. Structural measurements could not be performed when no cUS was available for either one or both neonates. Each twin pair was matched to one appropriate for gestational age singleton without cerebral injury to account for differences between twins and singletons. The singletons were selected from our Neonatology patient database and were born in the same period as the included twins. Per twin pair, a singleton was selected with the same sex and gestational age at birth. In order to minimize factors that can influence cerebral outcomes for this group, singletons with asphyxia, congenital abnormalities or infections, or singletons born after alloimmunization (with/without fetal therapy) during pregnancy were not included.

Clinical characteristics

The following maternal and obstetrical baseline characteristics were recorded: maternal age, gravidity, parity¸ Gratacós classification (Type I defined as positive end-diastolic flow (pEDF), Type II defined as persistent absent or reversed end-diastolic flow (A/REDF) and Type III defined as intermittent absent or reversed end-diastolic flow (iA/REDF))[13], the presence of brain sparing, (defined as a CPR < 1 for at least two weeks, with CPR calculated as the pulsatility index of the medial cerebral artery divided by the pulsatility index of the umbilical artery) and if the case, the gestational age at start and duration of brain sparing[14], the proportion of monoamniotic twins and delivery mode. The neonatal baseline characteristics that were recorded were: gestational age at birth in weeks, sex, BWD, birth weight in grams and proportion of neonates born small for gestational age (defined as birth weight < 10th centile)[15]. Placental share was calculated and expressed as a percentage of the total placental

area, based on the margins of the twin-specific dyes after standard color dye injection of MC twin placentas[16]. The percentages were calculated using Image J version 1.57.

cUS measurements

Before 2015, cUS was performed using an Aloka α ultrasound system (Hitachi Medical Systems Holding AG, Switzerland). From 2015 onwards, a Canon Aplio 400 or Aplio i700 system (Canon Medical Systems B.V., the Netherlands) was used. A cUS was performed between day 1-3 after birth by the attending neonatologist, all of which have extensive experience with this imaging modality as it is part of standard care in the LUMC. Head circumference at birth and corresponding z-score were documented[17]. Cerebral measurements were performed offline on the first available cUS after birth (Clinical Assistant, RVC B.V., the Netherlands). The resistance index of the anterior cerebral artery (RI-ACA) was recorded and calculated as (peak systolic velocity – end-diastolic velocity)/peak systolic velocity. The following structural measurements were performed by one researcher (SG) to limit interobserver variability[4]: anterior horn width (AHW), ventricular index (VI), ventricular atrium width (VAW), thalamo-occipital distance (TOD), interhemispheric fissure width (IFW), corpus callosum length, corpus callosum height, callosum-fastigium length, vermis height, vermis width, transverse cerebellar diameter (TCD), frontal white matter height, deep gray matter width, deep gray matter surface, biparietal diameter, intracranial fronto-occipital diameter (FOD), the axial intracranial area and the intracranial volume[18] (Table S1 and Figure 1). Intracranial volume discordance was calculated as (intracranial volume larger twin – intracranial volume smaller twin)/intracranial volume larger twin x 100). The researcher was not blinded for group (smaller twin, larger twin or singleton). The measurements were compared between the smaller and larger twin, the smaller twin and singleton, and the larger twin and singleton. To examine whether certain structures were affected to a greater extent than others, the analyses were also corrected for intracranial volume[18]. Both uncorrected and corrected measurements are presented, as having a smaller brain in itself might have consequences for future neurodevelopment as well. To evaluate reliability, measurements were repeated by the same researcher in a random sample of 18 neonates (10% of the population) after which an intraclass correlation coefficient (ICC) was calculated for every measurement. Values < 0.50 were indicative of poor reliability and values between 0.50-0.75 of moderate reliability[19].

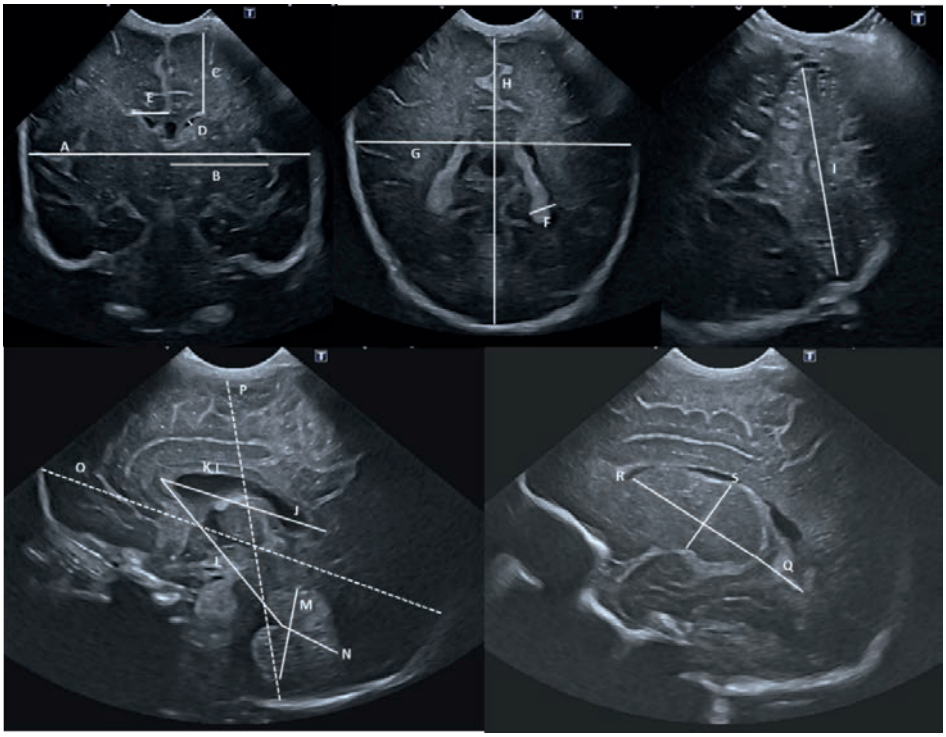

Figure 1. Overview of cerebral measurements: A/G = biparietal diameter; B = deep gray matter width; C = frontal white matter height; D = AHW; E = VI; F = VAW; G-H = used in calculation of intracranial surface; I = TCD; J = corpus callosum length; K = corpus callosum height; L = callosum-fastigium length; M = vermis height; N = vermis width; O = FOD; P = intracranial height; Q = TOD; R-S = used in calculation of deep gray matter surface.

Brain lesions seen on cUS

The presence of brain lesions was recorded, including pseudocysts, germinolytic cysts, subependymal cysts or choroid plexus cysts, lenticulostriate vasculopathy (LSV), intraventricular hemorrhage (IVH) grade 1-4[20], periventricular leukomalacia (PVL) grade 1-4[21], ventricular dilatation > 97th percentile[22] and parenchymal hemorrhage. Severe cerebral injury was defined as IVH ≥ grade 3; cystic PVL (c-PVL) ≥ grade 2; ventricular dilatation > 97th percentile, arterial or venous infarction, or porencephalic or parenchymal cysts.

Brain maturation

Brain maturation in the twin pairs was assessed by two other researchers (LV and SS) with expertise in neonatal neuroimaging. These researchers did not perform any structural measurements and were blinded for group (smaller or larger twin) and gestational age at birth. Maturation was scored in three planes according to the

appearance and increasing complexity of the principal sulci, as described by Murphy, Rennie and Cooke[23]. Overall maturity was determined on the first cUS after birth and based on the comparison of actual gestational age at birth with the maturation score of at least two out of three planes and was categorized either according to the norm, 2-4 weeks behind or > 4 weeks behind.

Statistical analyses

Statistical analyses were performed using IBM Statistics Version 25.0 (SPSS, Inc., an IBM company, Chicago, IL, USA). Data are presented as median (interquartile range (IQR)), n/N (%) or n (%). Given the nature of the study population (twin pairs), the analyses take into account that observations between co-twins are not independent, by using the Wilcoxon signed-rank test (non-parametric test for related samples) and Generalized Estimating Equations (GEE). To test for association between sFGR and the structural cerebral measurements, the Wilcoxon signed-rank test was used. A GEE was used to test for association between sFGR and the structural cerebral measurements, corrected for intracranial volume. Lastly, a GEE was also used to test for association between sFGR and the presence of brain lesions. As the GEE cannot be used when an outcome event does not occur in one of the groups, an adjustment to the data was applied in which an unaffected twin was changed into an affected twin for both groups; this approach result is a conservative estimate of *p*-values.

Intracranial volume discordance was tested for correlation with BWD and placental share discordance and plotted against BWD and placental share discordance in a graph per type of sFGR. The ICC of each structural measurement was calculated in a two-way mixed effects model based on a single measurement.

A *p*-value of < 0.05 was considered statistically significant. For every structural measurement, three comparisons were performed, namely the smaller twin vs. the larger twin, the smaller twin vs. the singleton and the larger twin vs. the singleton. Therefore, a Bonferroni adjustment was applied to correct for multiple testing, resulting in a significance level set at $p < 0.017$ (0.05/3) for the structural measurements. The association between intracranial volume discordance, BWD and placental share discordance were plotted using RStudio Version 2021.9.2.382 (RStudio, PBC, Boston, MA, USA).

Table S1. Definitions of the structural measurements on neonatal cUS.

Measurement	Abbreviation	Definition
Anterior horn width	AHW	The diagonal width of the anterior horn measured at its widest point in the coronal plane at the level of Monro
Ventricular index	VI	The distance between the falx and the lateral wall of the anterior horn in the coronal plane at the level of Monro
Ventricular atrium width	VAW	The distance between the lateral walls of the ventricles in a coronal plane at the level of the atria
Thalamo-occipital distance	TOD	The distance between the outermost point of the thalamus at its junction with the choroid plexus and the outermost part of the occipital horn in the parasagittal plane
Interhemispheric fissure width	IFW	The maximum horizontal distance between the hemispheres, measured from the depth of the sulci in the coronal plane at the level of Monro
Frontal white matter height	-	The length/distance from the highest point of the ventricular roof to the surface of the cortex, taken parallel to the midline in the coronal plane at the level of Monro
Corpus callosum length	-	The distance from the outer border of the genu to the outer border of the splenum on a midsagittal plane
Corpus callosum height	-	The thickness of the body of the corpus callosum on a midsagittal plane
Callosum-fastigium length	-	The distance from the outer border of the genu of the CC to the fastigium on a midsagittal plane
Vermis height	-	The distance from the anterosuperior portion to the infero-posterior portion of the vermis in a midsagittal plane
Vermis width	-	The longest diameter of the anterosuperior vermis from the fastigial point to the posterior border in a midsagittal plane
Transverse cerebellar diameter	TCD	The widest diameter of the cerebellum in the coronal plane obtained through the mastoid fontanel
Deep gray matter width	-	Measured in the coronal plane from midline to the border of the insula
Deep gray matter surface	-	Measured in the parasagittal plane and calculated using the formula for an ellipse surface
Biparietal diameter	-	The diameter of the head between the inner part of the parietal bones of the skull in the coronal plane at the level of Monro
Intracranial fronto-occipital diameter	FOD	The antero-posterior diameter in the midsagittal plane
Intracranial height	-	The height from the posterior aspect of the foramen magnum to the inner aspect of the fontanel below the transducer
Axial intracranial area	-	Calculated according to the method of Graca [18]
Intracranial volume	-	Calculated according to the method of Graca [18]

Results

Of the 653 live-born MC twin pairs delivered at the LUMC between 2010-2020, pairs who did not have sFGR (n = 292) or met the aforementioned exclusion criteria (n = 296) were excluded. Of the remaining pairs, seven did not have a cUS available for either one or both twins. So, 58 twin pairs with sFGR and an available cUS were included in the analyses (Figure 2). Hence, 58 appropriate for gestational age singletons without cerebral injury and matched for sex and gestational age at birth were included as well.

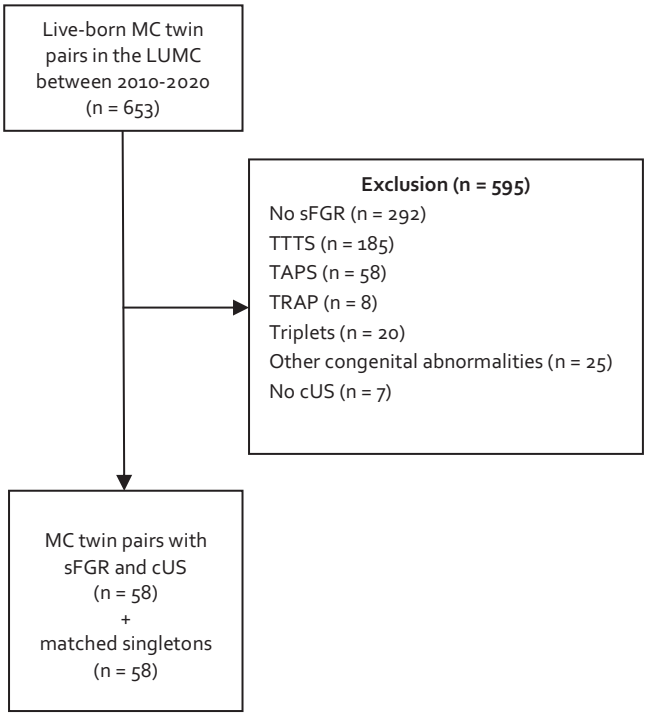

Figure 2. Flowchart of study inclusion.

Clinical characteristics

Baseline maternal, obstetric and neonatal characteristics are presented in Table 1. As expected, antenatal brain sparing was primarily observed in the smaller twins (76.8% (43/56)), with a median duration of 7 (4-9) weeks, as a sign of hemodynamic adaptation of the brain to suboptimal growth conditions. Brain sparing was only observed in 2% (1/56) of larger twins with a duration of 4 weeks. Of the 58 included pregnancies, 37% (23/58) were classified as Gratacós Type I, 17% (10/58) as Type II and

43% (25/58) as Type III. The median gestational age at birth was 31.7 (29.9-33.8) weeks and nearly 80% of twin pairs were delivered by caesarean section. The median BWD was 31.5% (26.7-38.1), with the smaller twin weighing 1155 (886-1433) grams and the larger twin weighing 1725 (1386-2145) grams. In line with the difference in birth weight, the proportion of neonates born small for gestational age was 94.8% (55/58) for the smaller twin and 13.8% (8/58) for the larger twin. Conforming to the pathophysiology of sFGR, the smaller twin had a smaller placental share as opposed to the larger twin, namely 30.0% (25.3-34.7) vs. 70.0% (65.3-74.7). The median gestational age at birth and birth weight for the matched singleton were 31.7 (29.9-33.8) weeks and 1758 (1528-2164) grams respectively.

Table 1. Baseline maternal, obstetric and neonatal characteristics for sFGR twins.

Characteristics	**sFGR twins (n=116; 58 pregnancies)**	**Smaller twin (n=58)**	**Larger twin (n=58)**	**Matched singleton (n=58)**
Maternal age – *years*	31 (28-34)			
Gravidity	1 (1-2)			
Parity	0 (0-1)			
Gratacós type				
Type I	23 (39.7)			
Type II	10 (17.2)			
Type III	25 (43.1)			
Brain sparing		43/56 (76.8)	1/56 (1.8)	
Start brain sparing – *weeks*		19.6 (17.4-21.4)	15.9 (15.9-15.9)	
Duration brain sparing – *weeks*		7 (4-9)	4 (4-4)	
Monoamniotic twins	6 (10.3)			
Gestational age at birth – *weeks*	31.7 (29.9-33.8)			31.7 (29.9-33.8)
Female	52 (44.8)			26 (44.8)
Caesarean	92 (79.3)			
Birth weight discordance – *%*	31.5 (26.7-38.1)			
Birth weight – *grams*		1155 (886-1433)	1725 (1386-2145)	1758 (1528-2164)
Small for gestational age		55 (94.8)	8 (13.8)	0 (0.0)
Placental share – *%*		30.0 (25.3-34.7)	70.0 (65.3-74.7)	

sFGR: selective fetal growth restriction, UA: umbilical artery, A/REDF: absent or reversed end-diastolic flow, iA/REDF: intermittent absent or reversed end-diastolic flow. Outcomes are presented as median (interquartile range (IQR)), n (%) or n/N (%).

cUS measurements

Structural cUS measurements are summarized in Table 2. The median values are presented for the groups as a whole. As expected, based on the difference in birth weight, head circumference at birth and corresponding z-score were lowest for the smaller twin as opposed to the larger twin and singleton, namely 27.1 (25.0-29.3) cm with z-score -1.3 (-1.9- -0.1) for the smaller twin, 29.0 (27.5-30.0) cm with z-score 0.5 (-0.5-1.2) for the larger twin and 29.0 (27.5-30.0) cm with z-score 0.1 (-0.5-0.9) for the singleton (p < 0.0001). The structural measurements can be divided into four categories: ventricular parameters, brain structures, white/deep gray matter and overall brain size parameters.

Ventricular parameters did not differ between groups, except for the right TOD which was smaller for the singleton (12.8 (10.7-15.9) mm) in comparison with both the smaller (15.6 (13.5-18.4 mm, p < 0.0001) and the larger twin (16.0 (12.7-18.0) mm, p = 0.007). This difference was independent of intracranial volume (Table S3). All other structural measurements (brain structures, white/deep gray matter and overall brain size parameters) were significantly smaller for the smaller twin as opposed to the larger twin, in line with the difference in head circumference. So, there was an overall restriction in brain growth for the smaller twin. When corrected for intracranial volume, only the right frontal white matter height (p = 0.003) and biparietal diameter (p < 0.0001) remained significantly different. Similarly, the measurements of brain structures, white/deep gray matter and overall brain size parameters that differed between the smaller and larger twin also differed between the smaller twin and singleton (indicative of comparability between the larger twin and singleton), except for vermis height (p = 0.364) and width (p = 0.215) and left deep gray matter surface (p = 0.106). The differences that persisted after correction for intracranial volume were corpus callosum height (p < 0.0001), biparietal diameter (p < 0.0001) and FOD (p = 0.014). Vermis width became significantly different after correction (p = 0.007).

Measurements that were significantly different between the larger twin and the singleton (thereby indicative of possible incomparability between these groups) were in two brain structures and in deep gray matter, namely corpus callosum height (p < 0.0001), vermis height (p = 0.003) and both right and left deep gray matter surface (p = 0.001 and p < 0.0001, respectively). Three of these differences between the larger twin and singleton persisted after correction for intracranial volume, namely corpus callosum height (p < 0.0001), vermis height (p = 0.005) and left deep gray matter surface (p < 0.0001).

Table 2. Neonatal cUS parameters in sFGR twins and matched singletons.

Outcomes		Smaller twin (n=58)	Larger twin (n=58)	*p*-value (smaller vs. larger)	Matched singleton (n=58)	*p*-value (smaller vs. singleton)	*p*-value (larger vs. singleton)
GA at cUS – *weeks*		31.9 (29.9-34.0)	31.9 (29.9-34.0)		31.7 (30.0-34.0)	0.615	0.608
Postnatal age at cUS – *days*		2 (1-2)	2 (1-2)		2 (1-3)	0.063	0.060
HC – *cm*		27.1 (25.0-29.3)	29.0 (27.5-30.0)	**<0.0001**	29.0 (27.5-30.0)	**<0.0001**	0.435
HC z-score		-1.3 (-1.9--0.1)	0.5 (-0.5-1.2)	**<0.0001**	0.1 (-0.5-0.9)	**<0.0001**	0.481
RI-ACA		0.7 (0.6-0.8)	0.8 (0.7-0.8)	0.062	0.7 (0.6-0.8)	0.441	0.177
Ventricular parameters							
AHW – *mm*							
	Right	0.7 (0.3-1.4)	0.6 (0.3-1.0)	0.136	0.6 (0.0-1.1)	0.048	0.820
	Left	0.8 (0.3-1.4)	0.6 (0.3-1.4)	0.797	0.6 (0.0-1.3)	0.382	0.593
VI – *mm*							
	Right	9.6 (8.6-10.9)	9.5 (8.8-10.7)	0.991	9.8 (8.8-10.8)	0.462	0.341
	Left	9.5 (8.8-10.5)	9.6 (8.8-10.4)	0.486	9.9 (9.2-10.5)	0.241	0.188
VAW – *mm*							
	Right	6.1 (5.2-7.4)	6.4 (5.5-7.6)	0.809	6.4 (5.6-7.5)	0.554	0.874
	Left	6.2 (5.2-7.4)	6.8 (6.1-7.8)	0.036	7.0 (5.9-8.0)	0.105	0.863
TOD – *mm*							
	Right	15.6 (13.5-18.4)	16.0 (12.7-18.0)	0.385	12.8 (10.7-15.9)	**<0.0001***	**0.007***
	Left	16.0 (14.1-18.1)	16.2 (13.7-18.9)	0.750	14.6 (11.8-18.7)	0.078	0.109
IFW – *mm*		0 (0-0)	0 (0-0)	0.347	0 (0-0)	0.386	0.875
Brain structures							
Corpus callosum – *mm*							
	Length	37.6 (35.6-41.1)	39.8 (37.7-43.1)	**<0.0001**	40.8 (38.4-42.0)	**0.001**	0.461
	Height	2.1 (1.8-2.4)	2.3 (2.0-2.6)	**0.003**	1.8 (1.5-2.0)	**<0.0001***	**<0.0001***
Callosum-fastigium length – *mm*		42.0 (39.8-45.0)	43.2 (41.6-46.0)	**<0.0001**	43.4 (42.1-45.1)	**0.014**	0.585
Vermis – *mm*							
	Height	18.3 (16.6-20.3)	19.2 (18.1-21.1)	**<0.0001**	18.7 (17.2-19.8)	0.364	**0.003***
	Width	11.7 (10.3-13.1)	12.0 (10.2-14.2)	**<0.0001**	11.1 (10.2-12.4)	0.215*	0.132
TCD – *cm*		3.5 (3.1-4.0)	3.8 (3.5-4.3)	**<0.0001**	3.8 (3.5-4.1)	**<0.0001**	0.851
White/deep gray matter							
Frontal white matter height – *mm*							
	Right	18.5 (16.9-20.2)	19.4 (18.1-20.6)	**0.002***	19.9 (18.3-21.0)	**0.006**	0.429
	Left	18.8 (16.8-20.1)	19.4 (17.7-21.0)	**<0.0001**	19.8 (18.4-20.7)	**0.001**	0.530
Deep gray matter width – *mm*							
	Right	22.4 (20.8-24.9)	24.0 (22.4-27.2)	**<0.0001**	24.0 (23.0-26.2)	**<0.0001**	0.993
	Left	22.8 (21.1-24.7)	24.3 (22.3-26.7)	**<0.0001**	24.4 (22.5-25.8)	**<0.0001**	0.969
Deep gray matter surface – mm^2							
	Right	379 (330-460)	436 (393-499)	**<0.0001**	417 (372-466)	**0.003**	**0.001**
	Left	378 (331-452)	447 (403-486)	**<0.0001**	418 (385-448)	0.106	**<0.0001***
Overall brain size parameters							
Biparietal diameter – *cm*		6.6 (6.1-7.0)	7.0 (6.5-7.7)	**<0.0001***	7.2 (6.8-7.5)	**<0.0001***	0.706
Intracranial							
	Surface – cm^2	34.9 (30.9-43.3)	41.1 (37.1-47.5)	**<0.0001**	40.5 (36.3-44.1)	**<0.0001**	0.088
	FOD – *cm*	8.3 (7.5-9.0)	8.7 (8.3-9.2)	**<0.0001**	8.7 (8.3-9.1)	**<0.0001**	0.718
	Height – *cm*	6.7 (6.3-7.3)	7.1 (6.8-7.7)	**<0.0001**	7.3 (6.9-7.6)	**<0.0001**	0.619
	Axial surface – cm^2	42.6 (37.2-49.1)	49.3 (43.4-55.3)	**<0.0001**	49.3 (45.5-52.6)	**<0.0001**	0.794
	Volume – cm^3	191 (155-240)	231 (199-283)	**<0.0001**	245 (210-266)	**<0.0001**	0.730

GA: gestational age,cUS: cerebral ultrasound, HC: head circumference, RI-ACA: resistance index anterior cerebral artery, AHW: anterior horn width, VI: ventricular index, VAW: ventricular atrium width, TOD: thalamo-occipital distance, IFW: interhemispheric fissure width, FWMH: frontal white matter height, TCD: transverse cerebellar diameter, DGMW: deep gray matter width, DGMS: deep gray matter surface, FOD: fronto-occipital diameter.
Outcomes are presented as median (IQR).
*significant after correction for intracranial volume discordance (Table S3).

Table S3. Neonatal cUS parameters in sFGR twins and matched singletons, corrected for intracranial volume.

Outcomes		Smaller twin (n=58)	Larger twin (n=58)	*p*-value (smaller vs. larger)	Matched singleton (n=58)	*p*-value (smaller vs. singleton)	*p*-value (larger vs. singleton)
Ventricular parameters							
AHW – *mm*							
	Right	0.7 (0.3-1.4)	0.6 (0.3-1.0)	0.062	0.6 (0.0-1.1)	0.126	0.859
	Left	0.8 (0.3-1.4)	0.6 (0.3-1.4)	0.965	0.6 (0.0-1.3)	0.477	0.442
VI – *mm*							
	Right	9.6 (8.6-10.9)	9.5 (8.8-10.7)	0.111	9.8 (8.8-10.8)	0.818	0.131
	Left	9.5 (8.8-10.5)	9.6 (8.8-10.4)	0.095	9.9 (9.2-10.5)	0.901	0.135
VAW – *mm*							
	Right	6.1 (5.2-7.4)	6.4 (5.5-7.6)	0.318	6.4 (5.6-7.5)	0.457	0.918
	Left	6.2 (5.2-7.4)	6.8 (6.1-7.8)	0.884	7.0 (5.9-8.0)	0.638	0.663
TOD – *mm*							
	Right	15.6 (13.5-18.4)	16.0 (12.7-18.0)	0.754	12.8 (10.7-15.9)	**0.003**	**0.005**
	Left	16.0 (14.1-18.1)	16.2 (13.7-18.9)	0.832	14.6 (11.8-18.7)	0.164	0.075
IFW – *mm*		0 (0-0)	0 (0-0)	0.213	0 (0-0)	0.236	0.693
Brain structures							
Corpus callosum – *mm*							
	Length	37.6 (35.6-41.1)	39.8 (37.7-43.1)	0.439	40.8 (38.4-42.0)	0.327	0.137
	Height	2.1 (1.8-2.4)	2.3 (2.0-2.6)	0.060	1.8 (1.5-2.0)	**<0.0001**	**<0.0001**
Callosum-fastigium length – *mm*		42.0 (39.8-45.0)	43.2 (41.6-46.0)	0.245	43.4 (42.1-45.1)	0.329	0.862
Vermis – *mm*							
	Height	18.3 (16.6-20.3)	19.2 (18.1-21.1)	0.391	18.7 (17.2-19.8)	0.102	**0.005**
	Width	11.7 (10.3-13.1)	12.0 (10.2-14.2)	0.313	11.1 (10.2-12.4)	**0.007**	0.077
TCD – *cm*		3.5 (3.1-4.0)	3.8 (3.5-4.3)	0.573	3.8 (3.5-4.1)	0.583	0.872
White/deep gray matter							
Frontal white matter height – *mm*							
	Right	18.5 (16.9-20.2)	19.4 (18.1-20.6)	**0.003**	19.9 (18.3-21.0)	0.260	0.202
	Left	18.8 (16.8-20.1)	19.4 (17.7-21.0)	0.036	19.8 (18.4-20.7)	0.813	0.123
Deep gray matter width – *mm*							
	Right	22.4 (20.8-24.9)	24.0 (22.4-27.2)	0.351	24.0 (23.0-26.2)	0.191	0.677
	Left	22.8 (21.1-24.7)	24.3 (22.3-26.7)	0.577	24.4 (22.5-25.8)	0.157	0.309
Deep gray matter surface – mm^2							
	Right	379 (330-460)	436 (393-499)	0.839	417 (372-466)	0.057	0.028
	Left	378 (331-452)	447 (403-486)	0.210	418 (385-448)	0.017	**<0.0001**
Overall brain size parameters							
Biparietal diameter – *cm*		6.6 (6.1-7.0)	7.0 (6.5-7.7)	**<0.0001**	7.2 (6.8-7.5)	**<0.0001**	0.107
Intracranial							
	Surface – cm^2	34.9 (30.9-43.3)	41.1 (37.1-47.5)	0.528	40.5 (36.3-44.1)	0.627	0.111
	FOD – *cm*	8.3 (7.5-9.0)	8.7 (8.3-9.2)	0.038	8.7 (8.3-9.1)	**0.014**	0.482
	Height – *cm*	6.7 (6.3-7.3)	7.1 (6.8-7.7)	0.342	7.3 (6.9-7.6)	0.200	0.541
	Axial surface – cm^2	42.6 (37.2-49.1)	49.3 (43.4-55.3)	0.058	49.3 (45.5-52.6)	0.085	0.522

AHW: anterior horn width, VI: ventricular index, VAW: ventricular atrium width, TOD: thalamo-occipital distance, IFW: interhemispheric fissure width, TCD: transverse cerebellar diameter, FOD: fronto-occipital diameter.
Outcomes are presented as median (IQR).

Intracranial volume discordance and BWD correlated significantly with a Pearson correlation coefficient of 0.477 (R^2 = 0.228, p < 0.0001). Figure 3A depicts the relationship between intracranial volume discordance and BWD. Intracranial volume discordance was smaller as opposed to BWD (19.3% vs. 31.5% respectively, p < 0.0001). Intracranial volume discordance and placental share discordance did not correlate significantly (Pearson correlation coefficient of 0.198 (R^2 = 0.039, p = 0.144)). This relationship is depicted in Figure 3B. There was no significant difference between the three sFGR types for intracranial volume discordance (p = 0.080).

The majority of the structural measurements had an ICC indicative of good to excellent reliability, except for the left VI, right VAW and vermis width which had a moderate reliability (Table S2).

Brain lesions seen on cUS

The observed brain lesions on neonatal cUS are presented in Table 3. If PVL was present, the smaller twin more often presented with a PVL grade 1 (transient periventricular densities > 7 days after birth) as opposed to the larger twin (100% (10/10) and 86% (12/14) respectively, p < 0.0001). Severe cerebral injury was present in 7% (4/58) of larger twins and 0.0% (0/58) of smaller twins, with p = 0.065. These four twins presented with 1) c-PVL grade 3 fifteen days after birth (gestational age at birth 28.9 weeks, birth weight 1262 grams, severe respiratory morbidity and patent ductus arteriosus, passed away fifteen days after birth following redirection of care because of severity of cerebral injury); 2) a periventricular hemorrhagic infarction with ventricular dilatation three days after birth (gestational age at birth 28.4 weeks, birth weight 1210 grams, severe respiratory and cardiovascular morbidity, passed away six days after birth following redirection of care because of severity of cerebral injury); 3) a periventricular hemorrhagic infarction three days after birth (gestational age at birth 30.4 weeks, birth weight 1740 grams, severe respiratory morbidity) and 4) c-PVL grade 3 and IVH grade 2 seven days after birth (gestational age at birth 29.6 weeks, birth weight 1450 grams, severe respiratory morbidity). The first case was from a pregnancy antenatally classified as sFGR Type II and the other three cases were from pregnancies classified as Type III.

Table S2. ICC for the neonatal cUS parameters.

Outcomes	ICC	Reliability
Ventricular parameters		
AHW		
Right	0.98 (0.94-0.99)	Excellent
Left	0.91 (0.78-0.97)	Excellent
VI		
Right	0.89 (0.74-0.96)	Good
Left	**0.73 (0.41-0.89)**	**Moderate**
VAW		
Right	**0.61 (0.21-0.83)**	**Moderate**
Left	0.78 (0.51-0.91)	Good
TOD		
Right	0.96 (0.89-0.99)	Excellent
Left	0.96 (0.90-0.99)	Excellent
IFW	0.93 (0.81-0.97)	Excellent
Brain structures		
Corpus callosum		
Length	0.93 (0.81-0.97)	Excellent
Height	0.75 (0.45-0.90)	Good
Callosum-fastigium length	0.94 (0.85-0.98)	
Vermis		
Height	0.80 (0.54-0.92)	Good
Width	**0.51 (0.07-0.78)**	**Moderate**
TCD	0.97 (0.93-0.99)	Excellent
White/deep gray matter		
Frontal white matter height		
Right	0.94 (0.85-0.98)	Excellent
Left	0.87 (0.69-0.95)	Good
Deep gray matter width		
Right	0.95 (0.86-0.98)	Excellent
Left	0.78 (0.51-0.91)	Good
Deep gray matter surface		
Right	0.80 (0.54-0.92)	Good
Left	0.88 (0.71-0.96)	Good
Overall brain size parameters		
Biparietal diameter	0.97 (0.93-0.99)	Excellent
Intracranial		
Surface	0.99 (0.98-1.00)	Excellent
FOD	0.97 (0.91-0.99)	Excellent
Height	0.93 (0.83-0.97)	Excellent
Axial surface	0.98 (0.95-0.99)	Excellent
Volume	0.98 (0.94-0.99)	Excellent

ICC: intraclass correlation coefficient, AHW: anterior horn width, VI: ventricular index, VAW: ventricular atrium width, TOD: thalamo-occipital distance, IFW: interhemispheric fissure width, TCD: transverse cerebellar diameter, FOD: fronto-occipital diameter.

Brain maturation

Overall brain maturation was 2-4 weeks behind in 9% (5/55) of smaller twins as opposed to 16% (9/57) of larger twins (p = 0.281), and > 4 weeks behind in 2% (1/55) of smaller twins as opposed to 2% (1/58) of larger twins (p = 0.979). Maturation could not be scored in three cases due to insufficient quality of the cUS. In two twin pairs (both born at a gestational age of 31 weeks), the maturation of both the smaller and larger twin was behind.

Table 3. Brain lesions as seen on neonatal cUS for sFGR twins.

Outcomes	Smaller twin (n=58)	Larger twin (n=58)	*p*-value
Pseudocysts	9/58 (16)	3/58 (5)	0.065
LSV	1/58 (2)	2/58 (3)	0.571
IVH	6/58 (10)	6/58 (10)	1.000
Grade 1	5/6 (83)	2/6 (33)	0.519
Grade 2	1/6 (17)	2/6 (33)	0.683
Grade 3	0/6 (0)	0/6 (0)	1.000
Grade 4 (venous infarction)	0/6 (0)	2/6 (33)	0.190
PVL	10/53 (19)	14/53 (26)	0.333
Grade 1	10/10 (100)	12/14 (86)	**<0.0001**
Grade 2	0/10 (0)	0/14 (0)	1.000
Grade 3	0/10 (0)	2/14 (14)	0.482
Grade 4	0/10 (0)	0/14 (0)	1.000
Ventricular dilatation > 97th centile	0/58 (0)	1/58 (2)	0.323
Parenchymal hemorrhage	0/58 (0)	1/58 (2)	0.323
Severe cerebral injury	0/58 (0)	4/58 (7)	0.065

LSV: lenticulostriate vasculopathy, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia. Outcomes are presented as n/N (%).

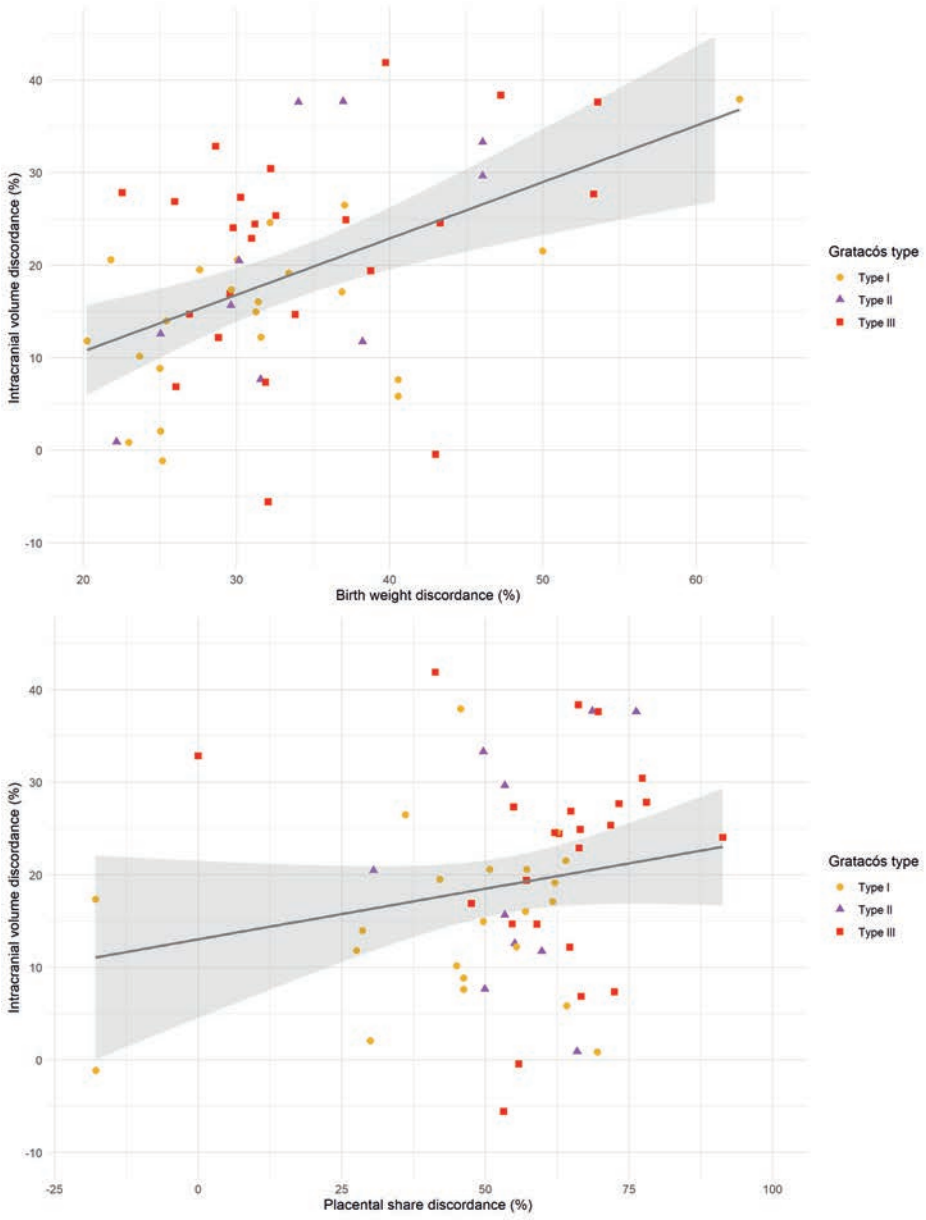

Figure 3. Scatterplots with regression line and 95% confidence interval depicting the association between intracranial volume discordance and BWD and placental share discordance according to Gratacós type. Regression lines are based on the group as a whole. Intracranial volume discordance was missing in two cases due to insufficient cUS quality.

Discussion

This is the first study evaluating cerebral measurements in a unique identical twin population, controlling for genetic and maternal factors. Our study shows that the smaller twin presents with an overall restriction in brain growth, with smaller cerebral structures (corpus callosum, vermis, cerebellum), white/deep gray matter and overall brain size parameters. The measurements were primarily different for the smaller twin in comparison with both the larger twin and the matched singleton, demonstrating that the larger twin has similar outcomes to the matched singleton. After correction for intracranial volume, all observed differences (except for biparietal diameter) ceased to exist, indicating a global, proportional decrease in brain growth. Lastly, there was a positive linear relationship between the amount of BWD and intracranial volume discordance. The intracranial volume discordance appeared to be smaller than BWD, indicating a certain degree of brain preservation.

The observed structural deficits were previously linked to functional consequences in singletons. Small head circumference can be considered an important predictor of adverse neurodevelopmental outcome, with increased rates of cerebral palsy and impaired cognitive and motor development[6,24]. Smaller corpus callosum size in preterm infants has been associated with speech and language difficulties, motor delay, cerebral palsy, and a lower full scale intelligence quotient (IQ) in adolescence[25-27]. Moreover, a study in monozygotic twins has shown that even subtle differences in birth weight within normal range in full term twins can result in alterations in brain structure which persist into adolescence and can be correlated with neurodevelopmental outcome[28]. It is important to realize that any neurodevelopmental consequences of (s)FGR are influenced by their onset and severity, as well as the gestational age at birth. Preterm birth is predominantly considered to exacerbate any deficits.

Our results are similar to a pilot study on cUS measurements in singletons, reporting that FGR neonates with antenatal brain sparing have a smaller corpus callosum and cerebellum[4]. In our study, no additional effect of brain sparing was found. However, only 77% of the smaller twins presented with antenatal brain sparing. Brain sparing can be considered a marker of the severity of FGR, as there is redistribution of blood to vital organs in response to unfavorable intrauterine circumstances and has been linked to adverse neurobehavioral outcome[14]. The term 'brain sparing' is therefore a misnomer, as it appears to be an indication of a type of FGR with an even greater impairment of brain growth[4]. This is also illustrated by the fact that the sole

parameter that remained significantly different between the smaller and larger twin and singleton after correction for intracranial volume was the biparietal diameter, indicative of smaller head growth and associated with adverse neurodevelopmental outcomes[29].

With regard to cerebral injury, a systematic literature review has previously reported an incidence of approximately 8% in MC twins with sFGR, with a particularly increased risk for the larger twin[30]. We found a similar incidence of cerebral injury in larger twins (7% (4/58)). All these larger twins were born between 28-30 weeks of gestation, were from pregnancies with sFGR Type II and III, presented with severe respiratory morbidity, did not have cerebral abnormalities *in utero* and developed cerebral injury 3-15 days after birth. It has been suggested that the larger twin is already at increased risk of cerebral injury *in utero* due to feto-fetal shifts in blood volume through large anastomoses resulting in hypoxic injury[13,31,32]. Hence, one would expect the injury to already be visible antenatally or within one or two days after birth. As this was not the case, our data is more in line with the hypothesis that (iatrogenic) prematurity plays an additional role in the development of cerebral injury in the larger twin[30]. As of yet, there is no consensus on timing of delivery for sFGR twins. The benefits of prolonging pregnancy, with the risk of fetal demise of the smaller twin and subsequent demise or neurological damage of the larger twin, are weighed against the risks of prematurity[33].

Our study is limited by its retrospective design. As cUS was performed for the detection of cerebral injury, the quality was not always optimal to conduct all measurements or to score maturation. Moreover, a control group including uncomplicated MC twin pregnancies would have been desirable to include, as these can also present with brain injury and may show cerebral growth alterations without any known antenatal complications. Lastly, we were unable to find differences in cerebral maturation on cUS, possibly because we used a scoring system that looks at a number of rough markers of maturation. A more detailed, validated scoring system for cUS scans is currently unavailable. Nevertheless, our results are strengthened by the unique population of identical twins discordant in birth weight, controlling for sex, gestational age at birth and genetic and maternal factors. By including matched singletons, we were able to investigate changes that are specific for MC twins and may also be present in the larger twin.

More research is necessary to investigate the effects of the observed structural differences on brain functioning. A systematic review published by our group

concluded that the smaller twin of MC twins with sFGR is at increased risk of neurodevelopmental impairment[34]. However, this was based on merely five articles with varying degrees of validity. Long term follow-up with neurodevelopmental testing is needed to provide more conclusive evidence. Ideally, MRI at term age should be performed to get a better understanding of alterations in brain growth, maturation and connectivity. Longitudinal neuroimaging beyond the neonatal period should be used to assess whether these alterations are permanent or whether there is catch-up growth over time.

To conclude, sFGR in MC twins is associated with an overall restriction in brain growth on neonatal cUS. The severity of BWD and intracranial volume discordance are positively correlated, suggesting that the BWD translates into a discordance in brain size as well (smaller as opposed to the amount of BWD). Our results reinforce the hypothesis that FGR has significant implications for brain development.

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