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The research described in this thesis was performed at the Willem-Alexander Children's Hospital, the Department of Pediatrics; the Department of Obstetrics; and the Department of Biomedical Data Sciences of the Leiden University Medical Center, the Netherlands.

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Voor mijn ouders

Voor opa Hans en oma Gonny

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General introduction and outline of thesis

General introduction

Twinning on the rise

Worldwide, more twins are being born now than ever before. Approximately 12 out of every 1000 deliveries is a twin pair, with even higher rates in North America, Europe and Africa ranging from 15 to 30 twins per 1000 deliveries, resulting in roughly 1.6 million twin pairs globally each year[1]. This is a threefold increase compared to the 1980s and numbers keep rising steadily[1]. The surge in twinning rates is highly relevant for present day health care as twin pregnancies are at greater risk of adverse perinatal outcome, including obstetric complications, prematurity and mortality, and therefore require additional antenatal care[2-4]. This is particularly so for monochorionic (MC) twin pregnancies.

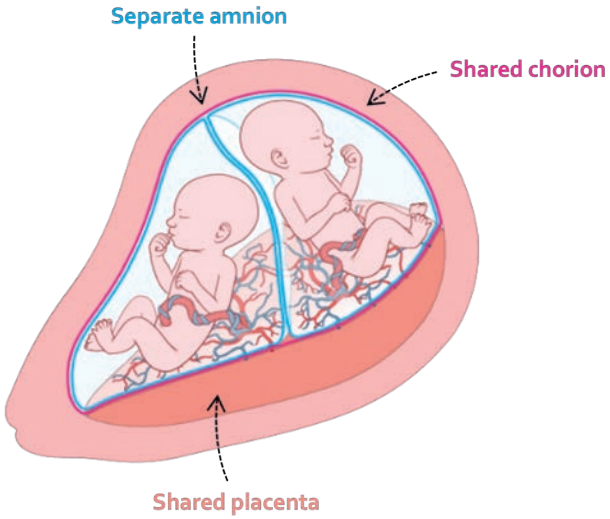

Figure 1. A schematic representation of a MC diamniotic twin pregnancy. Both the placenta, an organ that develops in utero during pregnancy and supplies oxygen and nutrients to the fetus through the umbilical cord, and the chorion, the outermost membrane surrounding the fetus, are shared between the twins. The amnion, the innermost membrane surrounding the fetus filled with amniotic fluid, can either be separate (diamniotic) or shared (monoamniotic) (illustration © Amanda Gautier).

	Uncomplicated monochorionic diamniotic twins	**Twin-twin transfusion syndrome**	**Twin anemia polycythemia sequence**	**Selective fetal growth restriction**
Clinical characteristics	Twins share the placenta equally and intertwin transfusion is balanced.	*Pathophysiology:* Unbalanced transfusion through large vascular anastomoses *Presentation:* Oligohydramnios in donor and polyhydramnios in recipient	*Pathophysiology:* Chronic unbalanced transfusion through miniscule vascular anastomoses *Presentation:* Anemia in donor and polycythemia in recipient	*Pathophysiology:* Unequal placental sharing *Presentation:* Large intertwin growth discrepancy with one twin being growth-restricted
Placenta				

Figure 2. An overview of the pathophysiology and clinical presentation of MC twin complications (top-left illustrations © Amanda Gautier).

Monochorionic twins

Of all twins, approximately two-thirds are dizygotic, that is originating from the fertilization of two separate egg cells. One-third is monozygotic, originating from the fertilization of a single egg cell. All fraternal twins are dichorionic, meaning that they each have their own placenta. Identical twins can also be dichorionic, but the majority of identical twins is MC and thereby shares a single placenta. On the surface of this shared placenta there are vascular connections allowing for intertwin blood flow[5] (Figure 1). This shared placenta can give rise to a spectrum of complications, either due to unbalanced intertwin transfusion through the vascular connections or due to unequal sharing of the placenta (Figure 2). These complications are not mutually exclusive and can overlap during the course of a pregnancy. They also elevate the risk of perinatal morbidity and mortality even higher for MC twins when compared to dichorionic twins[6]. Over the past decades, great progress has been made in understanding, management and outcome of conditions pertaining to unbalanced feto-fetal transfusion. Now, another distinct entity with substantial rates of adverse outcomes is increasingly recognized within the MC twin complications: selective fetal growth restriction (sFGR).

Figure 3. sFGR is characterized by a large intertwin growth discrepancy during pregnancy. The children in the picture were born at a gestational age of 32 weeks with birth weights of approximately 750 grams and 1750 grams (birth weight discordance of nearly 60%). (illustration ©Amanda Gautier).

Selective fetal growth restriction

Isolated sFGR occurs in up to 10-15% of MC twin pregnancies and is characterized by a large intertwin growth discrepancy in which one twin is growth-restricted[7,8] (Figure 3-

5). A large birth weight discordance (BWD) ensues, of which cut-offs vary between 20-25% in literature. sFGR is generally thought to be caused by unequal placental sharing, resulting in discordant access to oxygen and nutrients *in utero* leading (Figure 4)[9]. In 2007 Gratacós et al. proposed an antenatal classification system for sFGR based on the umbilical artery Doppler flow in the smaller twin[9]. Three types can be distinguished: Type I is characterized by positive end-diastolic flow (pEDF), Type II by persistent absent or reversed end-diastolic flow (A/REDF) and Type III by intermittent absent or reversed end-diastolic flow (iA/REDF) (Figure 6).

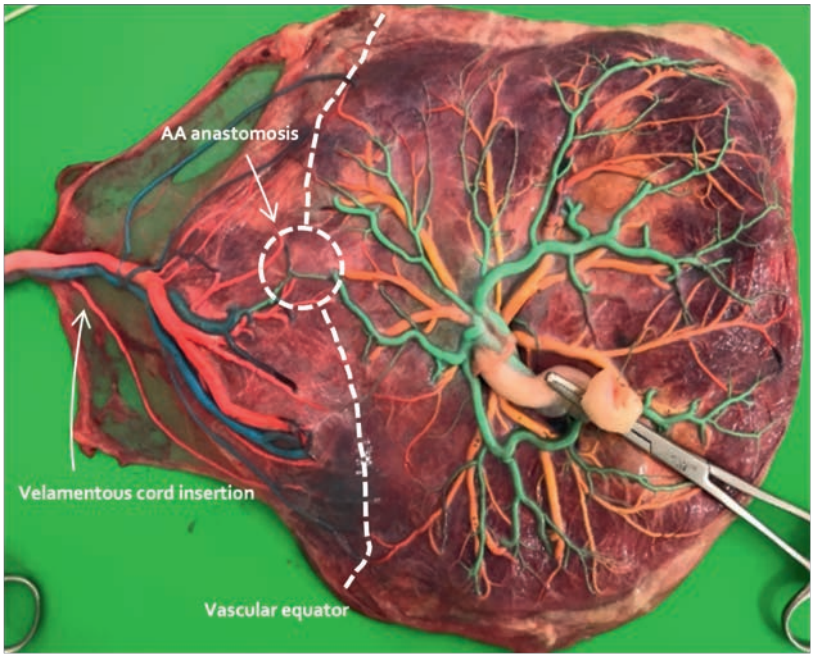

Figure 4. A MC twin placenta after sFGR, with unequal sharing in which the firstborn twin (blue/pink) had 20% of the placenta and the second born twin (green/yellow) 80%, amounting to a placental share discordance of 75%. These twins were born at a gestational age of 28 weeks, with 800 grams for the firstborn twin and 1500 grams for the second-born twin (birth weight discordance of nearly 50%). The firstborn twin has a velamentous cord insertion and the diameter of the arterio-arterial (AA) anastomosis (blue-green dye connection) was 1.3 mm.

From unequal sharing to long-term outcome

Despite the marked surge in published studies on sFGR over the past decade, many questions remain unanswered to date. This impedes proper parent counseling and short- and long-term risk assessment for MC twins with sFGR. The studies in this thesis aimed to fill this gap in knowledge to improve care for this vulnerable patient population.

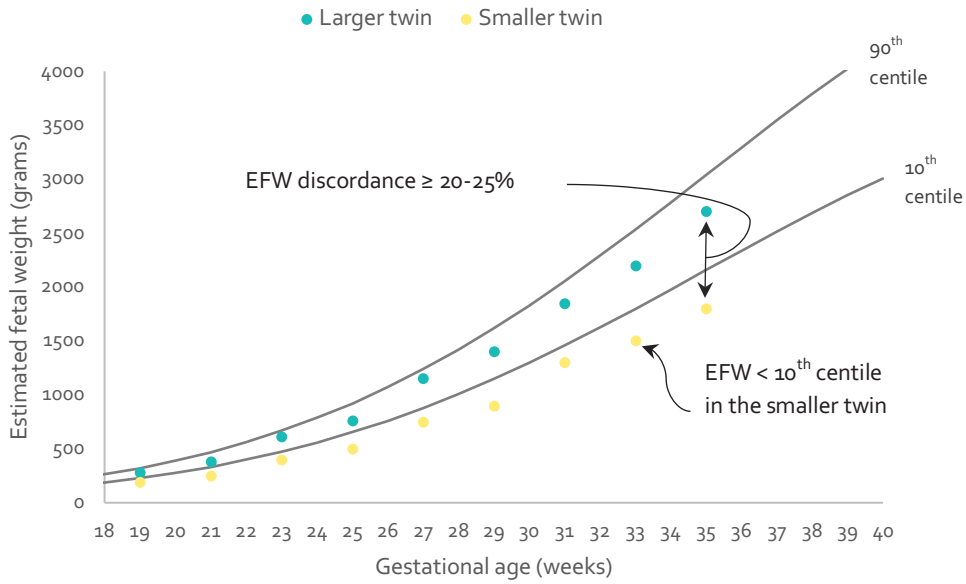

Figure 5. Selective fetal growth restriction is generally diagnosed based on an estimated fetal weight (EFW) discordance ≥ 20-25% in combination with one twin being growth-restricted, i.e., with an EFW < 10th centile. Fetal growth in MC twin pregnancies is measured every other week and EFW is calculated based on head circumference, abdominal circumference, and femur length.

Firstly, placental angioarchitecture in relation to the Gratacós classification is not yet fully understood, even though this classification has been widely used since its introduction. This in turn essentially hampers the formation of adequate antenatal management strategies. We have therefore performed two placental studies using color dye injection. The first study evaluated the placental characteristics of each Gratacós type, including the presence and diameter of bidirectional anastomoses (Chapter 1). The second study assessed if blood flow through the large bidirectional anastomoses can compensate for unequal placental sharing (Chapter 2).

Secondly, MC twins with sFGR are still complicated by high rates of (iatrogenic) prematurity and its sequelae, especially in type II and type III. We performed a systematic literature review to illustrate this international heterogeneity in timing of delivery of MC twins with sFGR (Chapter 3). In addition, available literature on perinatal outcomes after sFGR reports on an inconsistent variety of outcome parameters, chiefly concentrating on mortality and cerebral injury. Within-twin pair comparisons are scarce, resulting in an incomplete assessment of the size (smaller or larger twin) specific risks that clinicians should be wary of after birth. Hence, we

performed three studies on the short-term outcomes of MC twins with sFGR, focusing on respiratory outcomes (Chapter 4), cardiac structure (Chapter 5) and brain growth (Chapter 6) respectively.

Lastly, current research is primarily focused on perinatal outcome and should increasingly be shifted towards long-term outcome as well, in view of the importance for a child's daily functioning. We bundled available studies on long-term neurodevelopmental outcome after sFGR in a systematic literature review (Chapter 7). Additionally, we set up a large cohort study to investigate the long-term neurodevelopmental outcome (Chapter 8), social-emotional development (Chapter 9) and growth patterns (Chapter 10) after sFGR, called the LEMON study (Long-term Effects of selective fetal growth restriction in MONochorionic twins).

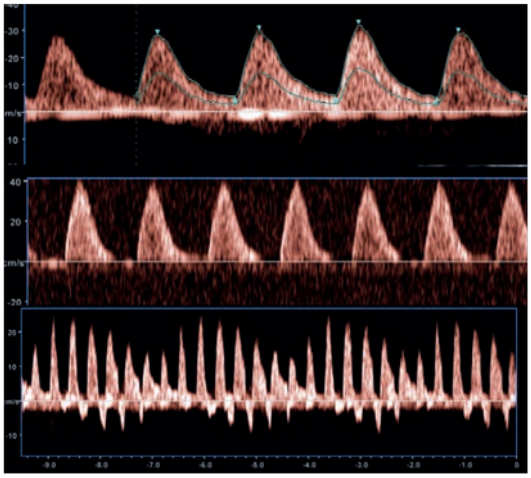

Figure 6. The Gratacós classification based on the Doppler flow pattern in the UA of the smaller twin.

A natural experiment

Lifelong cardiovascular and neurodevelopmental risk may be partially set before birth. FGR, low birth weight and preterm birth are associated with dyslipidemia, adiposity, type 2 diabetes, cardiovascular parameters and neurodevelopmental disorders[10-12]. The common denominator of these factors is that they represent an adverse intrauterine environment, resulting in greater long-term health risk. Epigenetic mechanisms are widely perceived to be a strong candidate to explain the mediation of prenatal adversity in later health[13]. Despite promising findings, it can be argued that the field still has to deliver robust mechanistic insight of the relationship between

unfavorable intrauterine circumstances and long-term health outcomes. This may be achieved by using a study population of MC twins with sFGR, in which a growth-restricted twin can be compared to an appropriately-grown, genetically identical co-twin with the same obstetrical and familial factors. Therefore, we have set up the Twinlife study (Twin Longitudinal Investigation of FEtal discordance), to longitudinally follow these twins from womb to adolescence. The results presented in this thesis will therefore not only be of immediate relevance to MC twins, but the impact may extend to the general population of singletons experiencing severe pregnancy complications such as fetal growth restriction.

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Aim and outline of this thesis

The aim of this thesis is to investigate sFGR in MC twins from womb to adolescence, to provide essential information on pathophysiology and clinical outcomes in this vulnerable patient population while simultaneously exploring the early origins of disease in this unique natural experiment in identical twins with discordant fetal growth.

Part I focuses on the placental mechanisms behind sFGR, including unequal sharing and patterns of vascular anastomoses that are associated with the antenatal classification system. *Part II* focuses on the short-term outcomes by highlighting the international discussion on optimal timing of delivery and evaluating early respiratory, cardiovascular and neurological outcomes. *Part III* focuses on the long-term health outcomes throughout childhood. Lastly, *Part IV* consist of a summary and general discussion of the results presented in this thesis.

General introduction and outline of this thesis.

Part I: From unequal placental sharing to a discordant intrauterine environment

Chapter 1: Placental characteristics in monochorionic twins with selective fetal growth restriction in relation to umbilical artery Doppler classification.
Placenta. 2018 Nov;71:1-5.

Chapter 2: Impact of placental sharing and large bidirectional anastomoses on birth weight discordance in monochorionic twins: a retrospective cohort study in 449 cases.
American Journal of Obstetrics and Gynecology. 2022 Nov 1;227(5):755.E1-755.E10.

Part II: From fetus to newborn

Chapter 3: Gestational age at birth and outcome in monochorionic twins with different types of selective fetal growth restriction: a systematic literature review.
Prenatal Diagnosis. 2022 Aug;42(9):1094-1110.

Chapter 4: Respiratory distress syndrome and bronchopulmonary dysplasia after fetal growth restriction: lessons from a natural experiment in identical twins.
EClinicalMedicine. 2021 Jan 29;32:100725.

Chapter 5: Early structural cardiovascular changes after adverse intrauterine circumstances in identical twins.
Submitted to Archives of Disease in Childhood – Fetal & Neonatal Edition. 2022 Sep.

Chapter 6: Changes in structural brain development after selective fetal growth restriction in monochorionic twins.
Ultrasound in Obstetrics and Gynecology. 2022 Jun;59(6):747-755.

Part III: From infant to adolescent

Chapter 7: The impact of selective fetal growth restriction or birth weight discordance on long-term neurodevelopment in monochorionic twins: a systematic literature review.
Journal of Clinical Medicine. 2019 Jun 28;8(7):944.

Chapter 8: Long-term effects of selective fetal growth restriction (LEMON): a cohort study of neurodevelopmental outcome in growth discordant identical twins in the Netherlands.
The Lancet Child and Adolescent Health. 2022 Sep;6(9):624-632.

Chapter 9: Insecure attachment and internalizing behavior problems in birth weight discordant identical twins.
Early Human Development. 2022 Nov;174:105679.

Chapter 10: Fetal growth restriction inhibits childhood growth despite catch-up: in discordant identical twins.
Under revision in Pediatrics. 2022 Sep.

Part IV: Summary and discussion

Summary and general discussion

Nederlandse samenvatting

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Curriculum Vitae

Dankwoord

Part I

From unequal placental sharing to a discordant intrauterine environment

Chapter 1

Placental characteristics in monochorionic twins with selective fetal growth restriction in relation to the umbilical artery Doppler classification.

Placenta. 2018 Nov;71:1-5.

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Abstract

Introduction: The objective of this study was to evaluate the placental characteristics of monochorionic twin pregnancies with selective fetal growth restriction (sFGR) classified according to the Gratacós classification based on umbilical artery Doppler measurements.

Methods: All consecutive placentas from monochorionic twin pregnancies with sFGR, (defined as a birth weight discordance > 25% and/or an estimated fetal weight in one twin <10th centile) examined at our center between May 2002 and February 2018 were included in the study. Each placenta was injected with colored dye to study the angioarchitecture. Primary outcomes were placental share discordance and diameter of the arterio-arterial anastomoses in relation to the umbilical artery Doppler types of sFGR (Gratacós classification).

Results: Of the 83 sFGR twins included, 27 were classified as Gratacós type I, 24 as type II and 32 as type III. The median gestational age at delivery was 34.3 weeks for type I, compared to 31.2 weeks and 31.6 weeks for type II and type III respectively. A trend towards a higher placental share discordance in type III sFGR was observed. The median arterio-arterial diameter was 1.7 mm (0.8-2.6) in type I, 1.7 mm (0.8-2.6) in type II and 2.8 (2.0-3.5) mm in type III ($p < 0.01$).

Conclusions: Type III sFGR placentas appear to be characterized by a larger diameter of the arterio-arterial anastomoses in type III and a larger placental share discordance compared to type I and II sFGR. The insights in the placental architecture of sFGR placentas may offer new views on the pathophysiology of the disease.

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Introduction

Monochorionic (MC) twin pregnancies are at increased risk of adverse perinatal outcome when compared to dichorionic twin pregnancies[1]. This increased risk is mainly caused by the vascular anastomoses on the surface of the shared placenta, allowing intertwin blood transfusion between the two fetuses, which can lead to complications such as twin-twin transfusion syndrome (TTTS), twin anemia polycythemia sequence (TAPS) or selective fetal growth restriction (sFGR)[2,3]. sFGR occurs in 10-15% of MC twin pregnancies and results from both intertwin blood flow and unequal placental sharing leading to severe growth restriction in the twin with the small placenta share.

In 2007, Gratacós et al proposed a classification system for sFGR based on the umbilical artery (UA) Doppler flow in the smaller twin[4]. Type I is characterized by positive UA Doppler flow and is considered to have a benign prognosis. Type II is defined as a persistently absent/reversed UA end-diastolic flow (A/REDF) and is associated with the highest perinatal mortality and morbidity. Lastly, type III is characterized by intermittent absent/reversed end-diastolic flow (iA/REDF) and has an atypical clinical evolution with an increased risk of unexpected fetal demise of the smaller twin and an increased risk of cerebral injury in the larger twin[4-6].

Several studies previously described the placental angioarchitecture in MC twins with sFGR[2,3,7,8]. However, no other studies, aside from Gratacós et al. in 2007, evaluated the association between placental characteristics and UA Doppler classification. The aim of the study is to evaluate the placental characteristics in MC twins with sFGR according to the Gratacós classification.

Methods

All placentas from MC pregnancies with a birth weight discordance > 25% and/or an estimated fetal weight in one twin < 10th centile consecutively examined at our specialized center between May 2002 and February 2018 were eligible for the study. We excluded MC pregnancies with co-existing TTTS or TAPS, cases where the UA Doppler classification was not recorded, cases with incomplete placental data (either due to placental damage or loss of the placenta) and cases in which placental measurements on the digital picture could not be performed. Cases with single or double intrauterine fetal demise (IUFD), defined as fetal death before 24 weeks of gestational age, were excluded when severe placental maceration made measurements impossible.

The following baseline characteristics were collected from our database: maternal age, gravidity, parity, sex, gestational age at diagnosis, gestational age at birth, mode of delivery, birth weight, birth weight discordance, intertwin hemoglobin (Hb) difference, perinatal survival and severe cerebral injury, defined as periventricular leukomalacia (PVL) ≥ grade 2, intraventricular hemorrhage (IVH) ≥ grade 3, ventricular dilatation, arterial or venous infarct or other severe cerebral injury. Birth weight discordance was calculated as follows: (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100%. The Gratacós classification was established based on routine UA Doppler evaluations, with type I defined as a positive end-diastolic flow (pEDF), type II as A/REDF and type III as iA/REDF. iA/REDF was identified within the same acquisition of UA Doppler and checked within a short interval in the same exam. The cord was assessed at the insertion site of the placenta. In case this was not possible, a free loop close to the insertion of the placenta was assessed. When the classification changed over time, the most prevalent type was chosen with help of an ultrasound operator.

Each of the MC placentas was routinely injected with colored dye to examine the pattern of placental anastomoses. Specific colors correlated with specific vessels, allowing for careful observation of different types of anastomoses. The cords of the twins were marked differently at birth: one clamp for the firstborn and two clamps for the second-born twin. The fetal territories were demarcated by the margins of the twin-specific colored dyes and expressed by a percentage of the total placental surface. After the colored dye injection, the placentas were photographed and the images digitally saved for computer analysis. The placental measurements were

conducted retrospectively and unblinded by the primary investigator using Image J version 1.57.

We measured the diameter of each arterio-arterial (AA) anastomosis and veno-venous (VV) anastomosis and we recorded the proportion of cases with an AA anastomosis > 2 mm in diameter. This specific cut-off was solely chosen in analogy with Gratacós et al. to compare our results[4]. The total AA or VV diameter was calculated in case the placenta possessed multiple AA or VV anastomoses by adding the subsequent diameters together. The umbilical cord insertion ratio was determined by dividing the total distance of the placenta by the distance between the two cord insertions. The umbilical cord insertions were divided into velamentous, marginal and (para)central[9]. The fetal weight ratio was calculated using the following formula: fetal weight larger twin/fetal weight smaller twin. Similarly, placental territory ratio was calculated by dividing the larger placental territory by the smaller placental territory.

Primary outcomes were the placental share discordance and the diameter of the AA anastomoses. The primary outcomes were compared according to the Gratacós classification system.

Data are presented as median (range). Data were analyzed using a Chi-square test for categorical variables, a Kruskal Wallis test for numerical variables and a GEE-analysis for survival data. A p-value < 0.05 was considered statistically significant. Statistical data was analyzed using IBM statistics v23.0 (SPSS, Inc., an IBM company, Chicago, IL, USA).

Results

A total of 109 placentas were eligible for the study based on the aforementioned inclusion criteria. Fifteen cases were excluded because a Gratacós classification was not recorded and eleven cases were excluded because measurements could not be performed due to damage of the placenta, leaving 83 placentas to be included in the study (Figure 1).

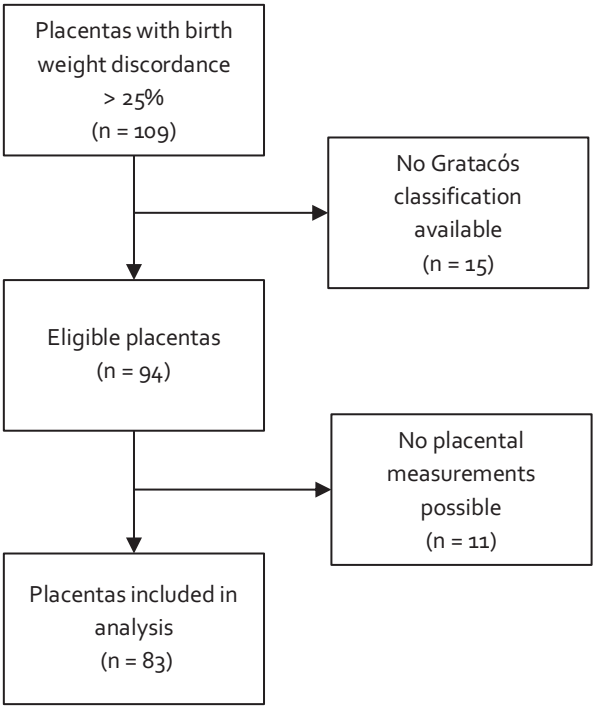

Figure 1. Flowchart of placenta inclusion.

Of the 83 pregnancies and placentas included, 28 were classified as type I, 24 as type II and 31 as type III. Table 1 summarizes the baseline characteristics of the pregnancies according to the Gratacós classification. Type II and type III had a significantly lower gestational age at birth than type I. Gestational age at birth for type I was 34.3 (32.7-35.9) weeks compared to 31.2 (28.4-34.0) and 31.4 (28.8-34.1) weeks for type II and type III respectively ($p < 0.01$). Type II sFGR cases demonstrated the highest birth weight discordance, namely 38.2% (31.7-44.7) as opposed to 32.8 % (27.8-37.8) in type I and 31.9% (26.4-37.4) in type III ($p = 0.035$).

Table 1. Baseline characteristics according to umbilical artery Doppler classification.

Characteristics	Gratacós type I (n=28)	Gratacós type II (n=24)	Gratacós type III (n=31)	*p*-value
Age mother – *years*	32.5 (29.0-36.0)	32.0 (27.0-37.0)	31.0 (29.0-33.0)	0.610
Gravidity – *n*	2 (1-2)	2 (1-3)	2 (1-3)	0.512
Parity – *n*	0 (0-1)	0 (0-1)	0 (0-1)	0.800
GA at diagnosis – *weeks*	17.0 (13.7-20.4)	17.9 (13.1-22.8)	18.7 (16.9-20.6)	0.805
GA at birth – *weeks*	34.3 (32.7-35.9)	31.2 (28.4-34.0)	31.4 (28.8-34.1)	**0.001**
Caesarian section	16 (57)	18 (75)	24 (77)	0.273
Female	12 (42)	8 (33)	18 (58)	0.226
Birth weight – *grams*				
Larger twin	2356 (1966-2746)	1635 (1270-2001)	1540 (1015-2065)	**<0.0001**
Smaller twin	1474 (1117-1830)	941 (571-1311)	968 (671-1266)	**<0.0001**
BWD – %	32.8 (27.8-37.8)	38.2 (31.7-44.7)	31.9 (26.4-37.4)	**0.035**
Hb difference at birth – *mmol/L*	0.9 (0.0-1.8)	1.5 (0.5-2.5)	0.5 (-0.1-1.1)	0.194
Perinatal survival	49/54 (91)	40/48 (83)	53/62 (86)	0.577
Cerebral injury	0/39 (0)	1/41 (2)	2/47 (4)	0.433

GA: gestational age, BWD: birth weight discordance.

Data are median (IQR), n/N(%) or n (%); Cerebral injury defined as periventricular leukomalacia (PVL) ≥ grade 2, intraventricular hemorrhage (IVH) ≥ grade 3, ventricular dilatation, arterial or venous infarct or other severe cerebral injury.

Table 2 summarizes the placental characteristics according to the Gratacós classification. At least one AA anastomosis was detected in all sFGR placentas. In a few placentas (6.0%, 5/83), more than one AA anastomosis were present.

The diameter of the AA anastomoses was significantly higher in type III compared to type I and II, namely 2.8 mm (2.0-3.5) in type III versus 1.7 (0.8-2.6) in type I and 1.7 (0.8-2.6) in type II ($p < 0.01$). Moreover, type III demonstrated the highest proportion of AA anastomoses with a diameter larger than 2 mm, namely 77.4% as opposed to 42.9% in type I and 29.2% in type II ($p < 0.01$). The median fetal weight ratio was 1.5 (1.4-1.6) in type I, 1.6 (1.4-1.8) in type II and 1.5 (1.3-1.6) in type III ($p = 0.027$). The median placental territory ratio differed between the groups with 2.4 (1.7-2.9) in type I, 2.2 (1.5-2.9) in type II and 2.8 (2.2-3.5) in type III ($p = 0.044$). When dividing these (fetal weight ratio/placental territory ratio), type III sFGR cases had a significantly lower ratio ($p = 0.025$), meaning that type III sFGR cases have a lower fetal weight discordance than expected for the amount of placental territory discordance.

Mortality rate and the incidence of cerebral injury was low and similar in the three groups (Table 1). One twin pair (type I) had missing data concerning the perinatal mortality. In all cases, the cerebral injury affected the larger twin. The first case (type II) experienced an arterial infarction on the first day after birth. In the second case (type III), cerebral injury was caused by post-hemorrhagic ventricle dilatation one week after birth due to IVH grade 3. The last case (type III) suffered from a PVL grade III which was not present antenatally but developed several weeks after birth.

Table 2. Placental features and anastomoses according to umbilical artery Doppler classification.

Characteristics	**Gratacós type I (n=28)**	**Gratacós type II (n=24)**	**Gratacós type III (n=31)**	***p*-value**
Placental share – %				
Larger twin	70.9 (64.4-77.3)	69.4 (62.7-76.2)	73.9 (69.6-78.3)	0.091
Smaller twin	29.1 (22.7-35.6)	30.6 (23.8-37.3)	26.1 (22.0-30.6)	0.091
Placental territory ratio	2.4 (1.7-2.9)	2.2 (1.5-2.9)	2.8 (2.2-3.5)	**0.044**
Fetal weight ratio	1.5 (1.4-1.6)	1.6 (1.4-1.8)	1.5 (1.3-1.6)	**0.027**
Fetal weight ratio/placental territory ratio	0.6 (0.4-0.8)	0.7 (0.4-0.9)	0.5 (0.4-1.6)	**0.025**
Arterio-arterial anastomoses				
1 AA	27 (96.4)	23 (95.8)	28 (90.3)	0.730
>1 AAs	1 (3.6)	1 (4.2)	3 (9.7)	
Total AA diameter – *mm*	1.7 (0.8-2.6)	1.7 (1.2-2.2)	2.8 (2.0-3.5)	**0.002**
AA diameter > 2 mm	12 (42.9)	7 (29.2)	24 (77.4)	**0.001**
Veno-venous anastomoses				
0 VV	22 (75.9)	18 (75.0)	24 (77.4)	0.764
1 VVs	6 (20.7)	5 (20.8)	5 (16.1)	
>1 VVs	1 (3.4)	1 (4.2)	2 (6.4)	
Total VV diameter – *mm*	2.2 (1.3-3.2)	2.7 (1.9-3.6)	3.0 (1.5-4.4)	0.884
Velamentous/marginal cord insertion				
Larger twin	4 (7.1)	2 (4.2)	1 (1.6)	0.293
Smaller twin	20 (35.7)	22 (45.8)	26 (41.9)	0.117
Umbilical cord insertion ratio	61.5 (46.5-86.5)	64.5 (56.0-73.0)	59.6 (43.6-75.6)	0.741

Data are median (IQR), n/N(%) or n (%).

Discussion

This study shows that the placental characteristics vary according to the type of sFGR, in particular the diameter of the AA anastomoses. We found that the AA diameter in type III pregnancies was significantly larger (almost double the size) compared to type I and II. In addition, we found a trend towards a higher placental share discordance in type III sFGR. Our data thus confirms that placentas in type III sFGR cases have larger AA anastomoses and a higher degree of sharing discordance.

The placental characteristics in type I and II placentas appear to be largely similar, with almost identical placental territory ratios (2.4 and 2.2, respectively) and equal size of the AA anastomoses (diameter of 1.7 mm in both types). Moreover, seven (24.1%) of the type I placentas and six (25.0%) of the type II placentas demonstrated VV anastomoses. Lastly, the umbilical cord insertions were farther apart in both groups compared to type III, as observed in the umbilical cord ratios of 61.5 in type I, 64.5 in type II and 59.6 in type III.

Due to the similarities of the placental characteristics between type I and type II sFGR, it is difficult to distinguish a type I sFGR from a type II based solely on observation of the placentas. So far, it is unclear why the UA Doppler of type II is abnormal.

In contrast, type III placentas reveal a different architecture as opposed to type I and II placentas, with a significantly larger median diameter of the AA anastomoses (2.8 mm) and a trend towards a higher placental share discrepancy, with a placental territory ratio of 2.8. Additionally, the umbilical cord ratio was smaller compared to type I and II (Figure 2).

When interpreting the size of the AA anastomoses, one should take into account the significantly lower gestational age at birth in type II and III sFGR. Placental vessels grow with advancing gestational age[10], which could bias the comparison of the AA diameter, as type I twins have a higher gestational age allowing for a longer period of growth. This might lead to an underestimation of the discrepancy in diameter of type I AA anastomoses versus type II and III. However, more extensive research is required to confirm the correlation between gestational age and chorionic vessel diameter with more certainty.

Our results are largely similar to the results of Gratacós et al.[4]. The diameter of AA anastomoses in their study was larger than 2 mm, predominantly in type III sFGR compared to type I and II, namely 98% of the AA anastomoses in type III and 70% and

18% in type I and II respectively ($p < 0.01$). This correlates with our findings that type III sFGR has the largest AA diameter and in our study 77.4% of AA anastomoses also had a diameter > 2 mm. However, exact measurements of the AA anastomoses were not reported in the study from Gratacós et al. limiting the comparisons between our studies.

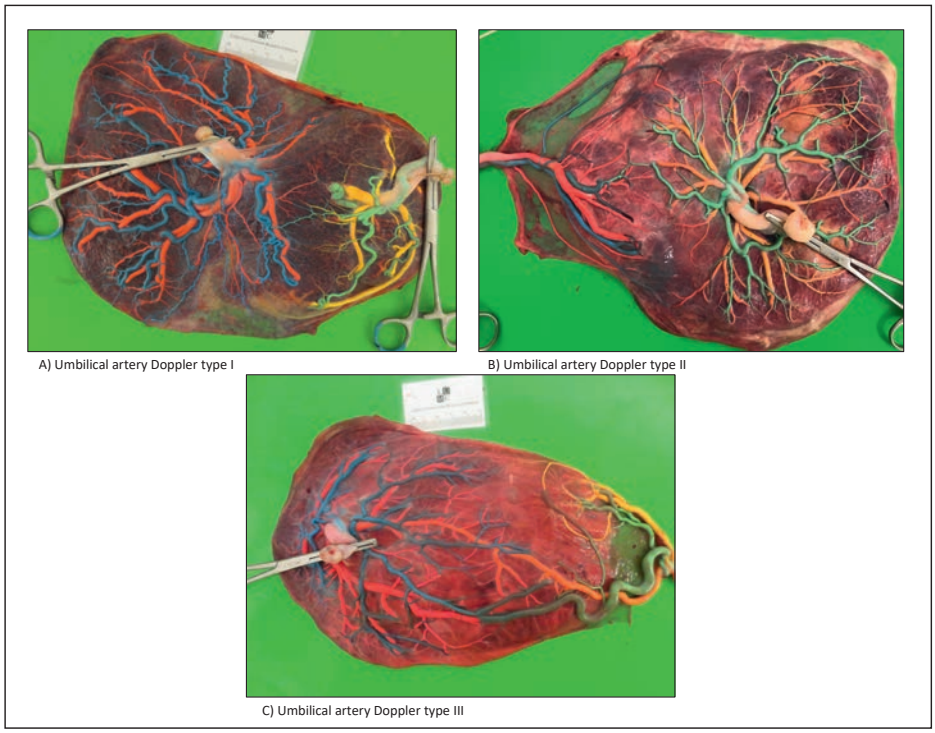

Figure 2. Pictures of injected placentas of pregnancies with sFGR with UA Doppler type I (A), type II (B) and type III (C). Type I demonstrates a small AA anastomosis. In type II the diameter of the AA anastomosis is slightly larger. Type III shows the largest diameter.

Gratacós et al. also found that the placental territory ratio increased significantly from type I to type III, namely from 1.8 in type I, 2.6 in type II and 4.4 in type III, which is not in agreement with our study results. They also concluded that the ratio between fetal and placental discordance followed a similar pattern. This is complementary to our results, even though the differences are not as broad. The reasons for the differences between the studies are not entirely clear. Heterogeneity in study populations may have contributed to the discrepancies between our results, however the true reasons remain elusive.

The larger diameter of the AA anastomoses and higher degree of sharing discordance in type III sFGR found in these two studies may explain the high rate of adverse outcome. This in turn leads to more detrimental clinical consequences, such as cerebral injury[5,6] or neurodevelopmental impairment[11,12]. Our study results elicit a possible pathophysiology of sFGR. Due to the AA anastomoses, there is a compensatory flow of the large twin to the smaller twin. This flow can serve as a rescue transfusion for the smaller twin, since its placental share is temporarily perfused by the flow of the larger twin through the AA anastomosis after the systole[4]. These flow patterns can be recognized in UA Doppler measurements. As the diameter of the AA anastomoses increases, there is more feto-fetal transfusion, resulting in an increased compensatory flow. However, larger AA anastomoses might also result in acute feto-fetal transfusion which leads to IUFD or neurological damage in either of the twins[6,13]. Therefore, the larger diameter of AA anastomoses in type III is the most likely cause of the unpredictable clinical outcomes.

The lower gestational age at birth of type III sFGR twins is probably the direct consequence of the atypical clinical course, which leads to earlier iatrogenic delivery as compared to type I and type II as was observed in our results.

Furthermore, our results show that there is a larger discrepancy between fetal weight and placental share discordance in type III, suggesting that the placental anastomoses have less effect on the fetal weight than expected. A possible explanation for this is the rescue transfusion of the larger twin to the smaller twin. Since the AA anastomoses in type III have a significantly larger diameter, there is a larger net transfusion which compensates for the amount of placental share discordance and allows for growth of the smaller twin.

According to a study performed by Rustico et al.[14], the UA Doppler classification is not static, but rather dynamic and may change over time into another type. This presents difficulty in determining the Gratacós type. The dynamic character of the flows could also have consequences for the outcomes and management and should be taken into account when assessing the individual risk per pregnancy. It stresses the importance of frequent and consistent monitoring of the UA flow, to document changes in flow patterns.

When interpreting our data, there are certain limitations that should be taken into account. Firstly, the retrospective character of the data collection and the relatively small sample size could introduce bias into our results. Since our institution is a

specialized center, referral bias could interfere with our results as well. Generally, only the complicated cases of sFGR are referred to our center for further diagnosis and therapy. Nevertheless, the number of cases with type I, II and III was evenly distributed in our study without an overrepresentation of the more severe cases. Additional studies with a prospective character and a larger study population of MC twin pregnancies with sFGR might present more evidence on the placental characteristics in relation to the UA Doppler classification with superior quality.

In conclusion, this study shows that the types of the Gratacós classification are associated with specific placental features, with type I and II having a relatively similar architecture as opposed to type III. These placental features can in turn determine the level of severity of the sFGR and perinatal outcomes. Type III sFGR has a larger AA anastomosis diameter and a larger placental share discordance and therefore has the most unpredictable clinical outcome due to the risk of acute feto-fetal transfusion, leading to IUFD or neurological damage. More research may lead to a better understanding of how the placental architecture contributes to the pathophysiology and clinical outcomes in MC twins with sFGR according to the UA Doppler measurements. In the future, early antenatal visualization of the AA anastomoses identifying the Gratacós classification with its associated risks might lead to timely and appropriate management.

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Chapter 2

Impact of placental sharing and large bidirectional anastomoses on birth weight discordance in monochorionic twins: a retrospective cohort study in 449 cases.

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Abstract

Background. In monochorionic twin pregnancies the fetuses share a single placenta. When this placenta is unequally shared, a discordant antenatal growth pattern ensues resulting in high rates of perinatal morbidity and mortality. Understanding of placental pathophysiology is paramount in devising feasible antenatal management strategies. Unequal placental sharing is not the sole determinant of a birth weight discordance as there is no one-to-one relationship with placental share discordance. Placental angioarchitecture, especially the presence of large bidirectional anastomoses, is thought to affect this relationship by allowing for a compensatory intertwin blood flow.

Objective(s). To assess whether placental angioarchitecture can affect birth weight discordance in live-born monochorionic twins, the aim of our study is twofold: 1) to assess the relationship between birth weight discordance and placental share discordance and 2) to examine to what extent large bidirectional anastomoses can compensate for the effect of unequal placental sharing on birth weight discordance, with a subgroup analysis for umbilical artery Doppler flow patterns in cases with a birth weight discordance ≥ 20%.

Study design. Retrospective cohort study including monochorionic twin pregnancies followed in our center between March 2002-June 2021, in which twins with a birth weight discordance ≥ 20% were classified according to umbilical artery Doppler flow patterns of the smaller twin. We excluded cases with twin-twin transfusion syndrome and twin anemia polycythemia sequence. Monochorionic placentas of live-born twins were injected with dye and images were saved for computer measurements of placental sharing and diameter of anastomoses. Univariate linear regression of the relationship between placental share discordance and birth weight discordance (both calculated as larger weight or share – smaller weight or share / larger weight or share x 100%), and the relationship between arterio-arterial and veno-venous diameter and birth weight ratio/placental territory ratio were performed.

Results. A total of 449 placentas were included in the analysis. Placental share discordance was positively correlated with birth weight discordance (β-coefficient 0.325; 95% CI; 0.254-0.397, $p < 0.0001$). Arterio-arterial diameter was negatively correlated with birth weight ratio/placental territory ratio (β-coefficient -0.041; 95% CI -0.059--0.023, $p < 0.0001$), meaning that an increase in arterio-arterial diameter leads to less birth weight discordance than expected for the amount of placental share

discordance. There was no relationship between veno-venous diameter and birth weight ratio/placental territory ratio (β-coefficient -0.007; 95% CI -0.027-0.012, p = 0.473).

Conclusions. Birth weight discordance in monochorionic twins is strongly associated with placental share discordance. Large arterio-arterial anastomoses can mitigate the effect of unequal placental sharing.

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Introduction

In monochorionic (MC) twin pregnancies the fetuses share a single placenta[1]. This shared placenta can give rise to different complications due to vascular anastomoses on its surface[2]. The most prevalent complication is a discordant antenatal growth pattern resulting in a birth weight discordance (BWD)[3,4]. A large BWD is associated with increased rates of neonatal morbidity as well as impaired long-term neurodevelopment[5-7]. The antenatal classification of the severity of discordant antenatal growth (generally termed selective fetal growth restriction (sFGR)) proposed by Gratacós et al. in 2007 is based on umbilical artery (UA) end-diastolic flow patterns of the smaller twin and allows clinicians to estimate the prognosis[8]. Type I is characterized by positive end-diastolic flow (pEDF), type II by persistent absent or reversed end-diastolic flow (A/REDF) and type III by intermittent A/REDF (iA/REDF). Type II and type III have the most unpredictable clinical course and thereby still exhibit the highest rates of perinatal morbidity and mortality.

Understanding of placental pathophysiology is paramount in devising feasible antenatal management strategies for pregnancies with discordant growth. The primary cause of a BWD in MC twins is generally considered to be unequal placental sharing[9]. However, it is not the sole determinant as there is no one-to-one relationship between placental share discordance and BWD[10]. Placental angioarchitecture, especially the presence of large bidirectional anastomoses, is thought to affect this relationship by allowing for a compensatory intertwin blood flow. This hypothesis was put forward by the previous finding that type III sFGR placentas have a relatively lower degree of BWD than expected for the amount of placental share discordance whilst also having a large arterio-arterial (AA) anastomosis[11]. Yet, this large AA anastomosis also increases the risk of acute feto-fetal transfusion after demise of either twin. Hence, intensive fetal surveillance in type III is advised. This illustrates that each UA Doppler flow pattern as described by Gratacós is an expression of a distinct placental mechanism that affects clinical decision making, particularly in type II and type III. By further studying placental sharing and angioarchitecture we can gain more etiological knowledge on the origin of discordant antenatal growth that allows us to enhance our risk assessment and subsequent management approach in the future.

Therefore, the aim of this study is twofold: 1) to assess the relationship between BWD and placental share discordance (a measure for unequal placental sharing) and 2) to examine to what extent large bidirectional anastomoses (AA and VV anastomoses) can compensate for the effect of unequal placental sharing on BWD, with a subgroup analysis for each UA Doppler flow pattern as diagnosed prenatally in twin pairs with a BWD ≥ 20%.

Materials and methods

All MC twin placentas of live-born twins injected with colored dye in our center between March 2002 and June 2021 were eligible for inclusion. Placentas of monoamniotic (MA) twins, twins with twin-twin transfusion syndrome (TTTS) or twin anemia polycythemia sequence (TAPS) were excluded due to their distinct pathophysiology and corresponding placental characteristics[2,12]. We also excluded MC triplet pregnancies, cases with twin reversed arterial perfusion (TRAP) and/or other congenital abnormalities. Cases in which placental measurements were impossible due to maceration after fetal death (either single/double intrauterine fetal demise (IUFD), selective reduction or termination of pregnancy (TOP)) or damage to the placenta due to manual removal were further excluded, as well as cases with unknown birth weights.

The following maternal and neonatal baseline characteristics were collected: maternal age, gravidity, parity, UA Doppler flow pattern (for cases with a BWD ≥ 20% as this can be considered a postnatal expression of discordant antenatal growth), gestational age at birth, sex, delivery mode, birth weight, BWD (calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100[13]), birth weight ratio (calculated as birth weight larger twin / birth weight smaller twin), proportion of neonates born small for gestational age (SGA) (defined as birth weight < 10th centile on a singleton chart)[14,15] and the incidence of neonatal mortality (defined as death within 28 days after birth). The UA Doppler flow pattern was established in line with the Gratacós classification based on antenatal ultrasound with routine UA Doppler evaluations for MC twin pregnancies, distinguishing between pEDF, persistent A/REDF and iA/REDF. As the UA Doppler flow pattern can change during the course of a pregnancy, the most prevalent type was chosen[16]. In our center, pregnancies with discordant antenatal growth are managed expectantly. In severe cases, a selective reduction is considered. Fetoscopic laser coagulation is not performed.

MC placentas are routinely injected with dye in our center as previously described[17]. After dye injection, placentas are photographed and the images are digitally saved for computer analysis using Image J version 1.57. The total number of anastomoses and the number of arterio-venous (AV) and veno-arterial (VA) anastomoses were recorded from the firstborn twin to the second-born twin, as well as the presence, number and total diameter of arterio-arterial (AA) and veno-venous (VV) anastomoses. The proportion of fetuses with a velamentous or marginal (< 1 centimeter from the margin of the placenta) cord insertion was documented for the smaller and larger twin. Fetal

territories were demarcated by the margins of the twin-specific colored dyes and expressed by a percentage of the total placental surface. Placental share discordance was calculated as (larger placental share – smaller placental share)/larger placental share x 100. Placental territory ratio was calculated in a similar manner as birth weight ratio: larger placental share / smaller placental share. Part of this data was previously published in 2018[11]. Birth weight ratio/placental territory ratio was calculated. A value below 1 suggests a lower BWD for the given placental share discordance (i.e., equal birth weights with an unequally shared placenta). A value above 1 suggest a higher BWD for the given placental share discordance (i.e., discordant birth weights with an equally shared placenta)[18]. We have chosen to report on both BWD and birth weight ratio as well as placental share discordance and placental territory ratio for comparability to other available studies reporting on similar parameters.

Statistical data was analyzed using IBM Statistics Version 25.0 (SPSS, Inc., an IBM company, Chicago, IL, USA). Data are presented as median (interquartile range), n/N (%) or n (%). To assess the first aim of our study, multivariate linear regression was performed to examine the relationship between placental share discordance, total AA diameter, total VV diameter and BWD. To assess the second aim of our study, univariate linear regression was performed to examine the relationship between both total AA and total VV diameter and birth weight ratio/placental territory ratio. We chose a different outcome measure than in the first aim, as the strong effect of placental share discordance on BWD can cloud the compensatory effect of AA and VV diameter we want to research. Birth weight ratio/placental territory ratio eliminates this strong effect from the analysis by looking at BWD relative to placental share discordance and is an outcome parameter that is consistent with previous literature[8,10,18]. When a significant association was found for both AA and VV diameter in univariate analysis, they were included in a multivariate linear regression model. A subgroup analysis per UA Doppler flow pattern in twin pairs with a BWD ≥ 20% was performed for both aims. As VV anastomoses are rare, we did not include total VV diameter in this subgroup analysis due to probable insufficient power. A *p*-value of < 0.05 was considered statistically significant. The relationship between placental share discordance, BWD, total AA and VV diameter and birth weight ratio/placental territory ratio for the total population and per UA Doppler flow pattern were plotted using RStudio Version 2021.9.2.382 (RStudio, PBC, Boston, MA, USA).

This retrospective study was approved and waived of the requirement for written informed consent by the ethics committee of the Leiden University Medical Center (LUMC) (protocol number G21.184) and funded by The Dutch Heart Foundation (grant number 2017T075). The funding source had no role in conducting the research or writing the research paper.

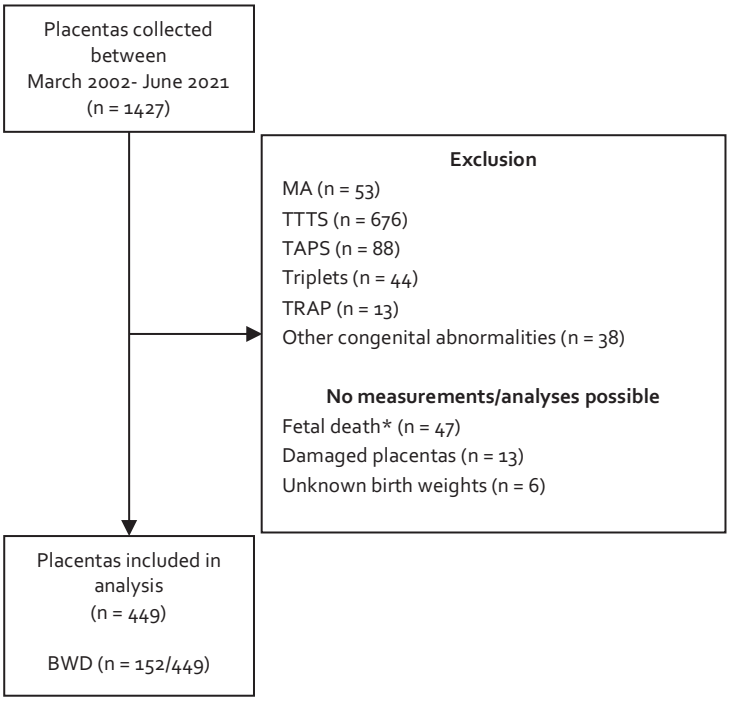

Figure 1. Flowchart of study inclusion. Of the fetal death cases, 26 were diagnosed with sFGR antenatally (estimated fetal weight (EFW) < 10th centile and EFW discordance ≥ 25%) of which 4 were type I, 10 type II and 9 type III (unknown in two cases). MA: monoamniotic, TTTS: twin-twin transfusion syndrome, TAPS: twin anemia polycythemia sequence, TRAP: twin reversed arterial perfusion, BWD: birth weight discordance. *Either single/double IUFD or selective reduction/TOP.

Results

Between March 2002 and June 2021, 1427 placentas were injected with colored dye. After applying the aforementioned exclusion criteria, 449 placentas were included for analysis (Figure 1). Of these 449 placentas, 152 (34%) had a BWD ≥ 20%.

Baseline characteristics

Baseline characteristics for the total population and the subgroup of MC twins with a BWD ≥ 20% are summarized in Table 1. Median gestational age at birth was 35.3 (IQR 32.1-36.3) weeks for the total population. Median birth weight in the smaller twin was 1956 (IQR 1415-2350) grams as opposed to 2350 (IQR 1792-2670) grams in the larger twin. BWD was 13.3% (IQR 6.3-25.2), with a birth weight ratio of 1.2 (IQR 1.1-1.3) meaning the larger twin had a 1.2 times higher birth weight than the smaller twin. Neonatal mortality occurred in 3% (11/423) of smaller twins and 1% (4/282) of larger twins.

In the subgroup of MC twins with a BWD ≥ 20% (n = 152), 71 pairs (50%) presented with pEDF, 28 pairs (20%) with A/REDF and 44 pairs (33%) with iA/REDF. The UA Doppler flow patterns was unknown in nine twin pairs as no antenatal ultrasound was available. The median gestational age at birth for the subgroup was 33.5 (IQR 31.0-35.8) weeks and 70% (212/304) were delivered by way of caesarean section. The smaller twin had a median birth weight of 1381 (IQR 996-1796) grams and the larger twin of 2566 (IQR 1540-2010) grams. Of the smaller twins, 95% (144/152) were born SGA compared to 16% (25/152) of the larger twins. Median BWD was 30.2% (IQR 25.1-36.9) and the birth weight ratio was 1.4 (IQR 1.3-1.6) implicating that the larger twin had a 1.4 times higher birth weight compared to the smaller twin. Neonatal mortality occurred in 5% (7/141) of smaller twins (of which 1/7 from a pregnancy with pEDF, 2/7 from a pregnancy with A/REDF and 4/7 from a pregnancy with iA/REDF) and 1% (2/140) of larger twins (of which 1/2 from a pregnancy with pEDF and 1/2 from a pregnancy with iA/REDF).

Placental characteristics

Placental characteristics of the 449 MC twin pregnancies and the subgroup of 152 MC twin pregnancies with a BWD ≥ 20% are presented in Table 2. AA anastomoses were present in the majority of placentas (92% (411/449)) and VV anastomoses in 24% (109/449) of the total population. Median AA diameter was 2.2 (IQR 1.3-3.0) mm and median VV diameter was 3.1 (IQR 1.8-4.3) mm. Of the smaller twins, 63% (282/449) had a

velamentous or marginal cord insertion, of which 62% (175/282) velamentous and 38% (107/282) marginal. This was the case for 23% (104/449) of the larger twins, with 33% (34/104) velamentous and 67% (70/104) marginal. Median placental share discordance was 35.1% (IQR 18.2-52.8) with 41% (IQR 33-50) of the placenta for the smaller twin and 59% (IQR 50-67) for the larger twin. Placental territory ratio was 1.5 (IQR 1.2-2.10), meaning that the larger twin had a 1.5 times larger placental share as opposed to the smaller twin. The birth weight ratio/placental territory ratio of 0.8 (IQR 0.6-0.9), implicating 0.2 times lower BWD than expected for the given placental share discordance.

Table 1. Baseline maternal and neonatal characteristics for the analyzed placentas, with the subgroup of MC twins with a BWD ≥ 20%.

Characteristics	**MC twins (n=898; 449 pregnancies)**	**BWD ≥ 20% (n=304; 152 pregnancies)**
Maternal age – *years*	32 (28-34)	31 (28-34)
Gravidity	2 (1-3)	1 (1-3)
Parity	1 (0-1)	0 (0-1)
UA Doppler flow pattern*		
pEDF		71 (50)
A/REDF		28 (20)
iA/REDF		44 (31)
Gestational age at birth – *weeks*	35.3 (32.1-36.3)	33.5 (31.0-35.8)
Female	448/894 (50)	150/304 (49)
Caesarean	415/890 (46)	212/304 (70)
Birth weight – *grams*		
Smaller twin	1956 (1415-2350)	1381 (996-1796)
Larger twin	2350 (1792-2670)	2566 (1540-2010)
Small for gestational age		
Smaller twin	273/447 (61)	144/152 (95)
Larger twin	67/447 (15)	25/152 (16)
Birth weight discordance – *%*	13.3 (6.3-25.2)	30.2 (25.1-36.9)
Birth weight ratio	1.2 (1.1-1.3)	1.4 (1.3-1.6)
Neonatal mortality		
Smaller twin	11/423 (3)	7/141 (5)
Larger twin	4/282 (1)	2/140 (1)

MC: monochorionic, UA: umbilical artery, pEDF: positive end-diastolic flow, A/REDF: persistent absent/reversed end-diastolic flow, iA/REDF: intermittent absent/reversed end-diastolic flow, BWD: birth weight discordance. Outcomes are presented as median (interquartile range (IQR)) or n (%).
*Unknown in nine twin pairs.

Similarly, nearly all placentas of MC twins with a BWD ≥ 20% had AA anastomoses (97% (147/152). VV anastomoses were present in 22% (33/152) of these placentas. Median AA diameter was 2.2 (IQR 1.3-3.1) and median VV diameter was 3.3 (IQR 1.9-3.9). The majority of the smaller twins had a velamentous or marginal cord insertion (82% (124/152), of which 69% (85/124) velamentous and 31% (39/124) marginal. Of the larger twins, 11% (17/152) had a velamentous or marginal cord insertion, of which 29% (5/17) velamentous and 75% (12/16) marginal. Median placental share discordance was 55.4% (IQR 36.8-66.4), with 31% (IQR 25-40) of the placenta for the smaller twin and 69 (IQR 60-75) for the larger twin. Placental territory ratio was 2.2 (IQR 1.6-3.0), meaning that the larger twin had a 2.2 times larger placental share compared to the smaller twin. Birth weight ratio/placental territory ratio was 0.7 (IQR 0.5-0.9), implicating a 0.3 times lower BWD than expected for the given placental share discordance.

Table 2. Placental characteristics of the analyzed placentas, including the subgroup of MC twins with a BWD ≥ 20%.

Characteristics	**MC twins (n=898; 449 pregnancies)**	**BWD ≥ 20% (n=304; 152 pregnancies)**
Total anastomoses – *n*	10 (6-16)	10 (6-16)
AV anastomoses – *n*	4 (2-7)	4 (2-8)
VA anastomoses – *n*	4 (1-7)	4 (2-7)
Presence of AA anastomoses	411 (92)	147 (97)
> 1 AA anastomoses	22 (5)	8 (6)
Total AA diameter – *mm*	2.2 (1.3-3.0)	2.2 (1.3-3.1)
Presence of VV anastomoses	109 (24)	33 (22)
> 1 VV anastomoses	17 (4)	4 (3)
Total VV diameter – *mm*	3.1 (1.8-4.3)	3.3 (1.9-3.9)
Velamentous or marginal cord insertion		
Smaller twin	282 (63)	124 (82)
Larger twin	104 (23)	17 (11)
Placental share – *%*		
Smaller twin	41 (33-50)	31 (25-40)
Larger twin	59 (50-67)	69 (60-75)
Placental share discordance – *%*	35.1 (18.2-52.8)	55.4 (36.8-66.4)
Placental territory ratio	1.5 (1.2-2.1)	2.2 (1.6-3.0)
Birth weight ratio/Placental territory ratio	0.8 (0.6-0.9)	0.7 (0.5-0.9)

MC: monochorionic, BWD: birth weight discordance, AV: arterio-venous, VA: veno-arterial, AA: arterio-arterial, VV: veno-venous.

Outcomes are presented as median (interquartile range (IQR)) or n (%).

Relationship placental sharing, AA and VV diameter and BWD

Results from the multivariate linear regression of placental share discordance, total AA diameter and total VV diameter and BWD (aim 1) for the total population and the subgroup with a BWD ≥ 20% and available UA Doppler flow patterns (n = 143) are shown in Table 3 and depicted in Figure 2. An increase in placental share discordance was associated with an increase in BWD (β coefficient 0.325; 95% CI; 0.254-0.397, p < 0.0001) in the total population. Cases with pEDF demonstrated a similar positive correlation for placental share discordance and BWD (β coefficient 0.214; 95% CI 0.102-0.326, p = 0.001). In cases with A/REDF and iA/REDF, there was no significant association between placental share discordance and BWD, but for cases with A/REDF there was a significant negative correlation between total AA diameter and BWD (β coefficient -4.143; 95% CI -7.103--1.182, p = 0.006).

Relationship birth weight ratio/placental territory ratio, AA and VV diameter

Results from the univariate linear regression of total AA and VV diameter and birth weight ratio/placental territory ratio (aim 2) are shown in Table 4 and depicted in Figure 3. AA diameter, but not VV diameter, was correlated with birth weight ratio/placental territory ratio (β coefficient -0.041; 95% CI -0.059—0.023, p < 0.0001) for the total population, meaning that an increase in total AA diameter leads to less BWD than expected for the amount of placental share discordance. This was similar for cases with pEDF (β coefficient -0.055; 95% CI -0.098--0.011, p = 0.013) and cases with A/REDF (β coefficient -0.180; 95% CI -0.297--0.063, p = 0.002). The association between total AA diameter and birth weight ratio/placental territory ratio in cases with iA/REDF approached statistical significance (β coefficient -0.053, 95% CI -0.111-0.004, p = 0.070).

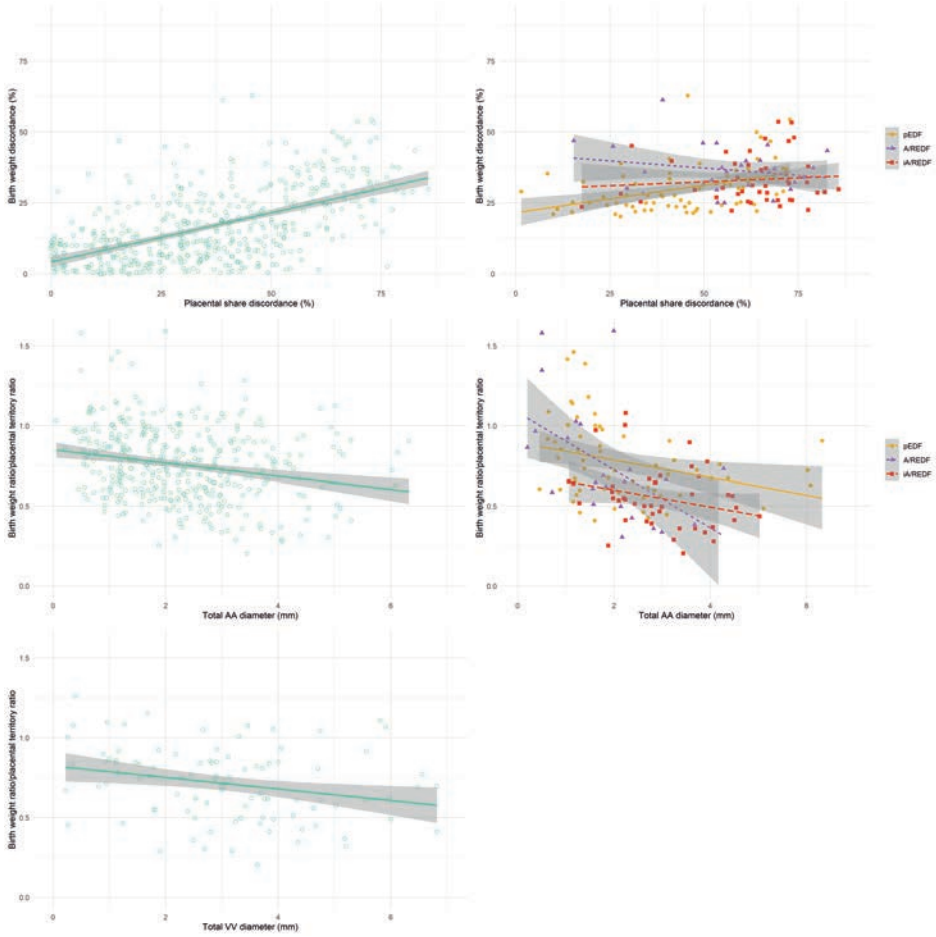

Figure 2. Scatterplots depicting the relationship between placental share discordance and BWD with 95% confidence interval bands; total AA diameter and birth weight ratio/placental territory ratio; and total VV diameter and birth weight ratio/placental territory ratio for the total population and per UA Doppler flow pattern.

Table 3. Multivariate linear regression to evaluate the association between birth weight discordance and placental share discordance for the total population and per antenatal UA Doppler flow pattern for the twin pairs with a BWD ≥ 20%.

	Total population			pEDF			A/REDF			iA/REDF		
Characteristics		β coefficient (95% CI)	*p*-value		β coefficient (95% CI)	*p*-value		β coefficient (95% CI)	*p*-value		β coefficient (95% CI)	*p*-value
Placental share discordance – *%*	35 (18-53)	0.325 (0.254-0.397)	**<0.0001**	45 (30-60)	0.214 (0.102-0.326)	**0.001**	56 (50-68))	0.018 (-0.157-0.193)	0.840	66 (58-72)	0.136 (-0.037-0.310)	0.124
Total AA diameter – *mm*	2.2 (1.3-3.0)	0.470 (-1.106-2.047)	0.559	1.7 (1.2-2.9)	-1.039 (-2.515-0.326)	0.167	1.8 (1.1-2.4)	-4.143 (-7.103--1.182)	**0.006**	2.8 (2.2-3.6)	-1.726 (-4.191-0.740)	0.170
Total VV diameter – *mm*	3.1 (1.8-4.3)	-0.180 (-1.071-0.712)	0.693									

pEDF: positive end-diastolic flow, A/REDF: persistent absent/reversed end-diastolic flow, iA/REDF: intermittent absent/reversed end-diastolic flow, AA: arterio-arterial, VV: veno-venous. Outcomes are presented as median (interquartile range (IQR)).

Table 4. Univariate linear regression to evaluate the association between birth weight ratio/placental territory ratio and total AA and VV diameter per antenatal UA Doppler flow pattern in twin pairs with a BWD ≥ 20%.

	Total population			pEDF			A/REDF			iA/REDF		
Characteristics		β coefficient (95% CI)	*p*-value		β coefficient (95% CI)	*p*-value		β coefficient (95% CI)	*p*-value		β coefficient (95% CI)	*p*-value
Total AA diameter – *mm*	2.2 (1.3-3.0)	-0.041 (-0.059--0.023)	**<0.0001**	1.7 (1.2-2.9)	-0.055 (-0.098--0.011)	**0.013**	1.8 (1.1-2.4)	-0.180 (-0.297--0.063)	**0.002**	2.8 (2.2-3.6)	-0.053 (-0.111-0.004)	0.070
Total VV diameter – *mm*	3.1 (1.8-4.3)	-0.007 (-0.027-0.012)	0.473									

pEDF: positive end-diastolic flow, A/REDF: persistent absent/reversed end-diastolic flow, iA/REDF: intermittent absent/reversed end-diastolic flow, AA: arterio-arterial, VV: veno-venous. Outcomes are presented as median (interquartile range (IQR)).

Comment

Principal findings

This study shows that there was a strong association between placental share discordance and BWD in live-born MC twins. Yet, the amount of BWD was smaller than the amount of placental share discordance. A larger AA diameter was shown to mitigate the effect of unequal placental sharing on BWD as reflected by a lower birth weight ratio/placental territory ratio with increasing diameter. With regard to the different UA Doppler flow patterns in twin pairs with a BWD ≥ 20%, cases with pEDF demonstrated similar associations as the total population in line with type I sFGR pregnancies also having a relatively uncomplicated course. As expected, cases with A/REDF and iA/REDF showed a distinct placental pathophysiology in which both did not show a significant association between placental share discordance and BWD, while compensation through a larger AA diameter (approaching statistical significance for iA/REDF) was present. This suggests an increased importance of placental angioarchitecture.

Results in the context of what is known

Our results are in line with previous studies performed by Lewi et al. and Couck et al. including 100 and 247 MC placentas respectively[10,18]. We confirmed the strong linear relationship between placental share discordance and BWD as well as the effect of a larger AA diameter in reducing the birth weight ratio/placental territory ratio in a substantially larger study population with a subgroup analysis per antenatal UA Doppler flow pattern. Couck et al. found that a larger VV diameter also decreases the amount of BWD for any given placental share discordance, independent of the AA diameter[18]. We did not find this effect in our population, potentially due to the nearly double amount of placentas with VV anastomoses we were able to include. In a study performed by Wang et al., the presence of VV anastomoses was found to be negatively correlated with BWD in type III sFGR when corrected for gestational age at diagnosis and delivery[19]. We were unable to draw conclusions about the effect of VV diameter in the subgroup analysis of the UA Doppler flow patterns, as only nine pEDF, six A/REDF and twelve iA/REDF placentas had a VV anastomosis. More research is necessary, preferably in a multicenter setting, to study the role of VV anastomoses.

In previous literature on the placental characteristics of the Gratacós types in sFGR, the large AA diameter was considered to be the compensation mechanism for unequal placental sharing primarily in type III placentas, as type III had both the largest AA diameter and the lowest birth weight ratio/placental territory ratio in comparison

with type I and type II[8,11]. Our study now demonstrates that in type I (pEDF) and II (A/REDF), there is also compensation through the AA anastomoses. However, as the AA diameter is smaller in these types, they still demonstrate a higher birth weight ratio/placental territory ratio than reported in type III (iA/REDF).

The hazard in comparing studies using the Gratacós classification is the different scoring methods that are widely used. Some studies classify a pregnancy as type II or type III when abnormal UA Doppler flow patterns were observed on a single occasion[20], others use the final classification prior to delivery[19], and others use the most prevalent type of flows as we have done now and in the past[11]. UA Doppler flow patterns are dynamic in nature and can change over time, presenting difficulty in determining the 'definitive' Gratacós type[16]. International consensus is urgently needed to minimize this variation in diagnosis, as this currently clouds the exploration of pathophysiological mechanisms and hampers comparisons between studies.

Clinical implications

These findings support the hypothesis that large bidirectional anastomoses, AA anastomoses in particular, allow for an increased feto-fetal blood flow and can thereby compensate for unequal placental sharing by way of a rescue transfusion from the larger to the smaller twin. Whilst being beneficial for the growth of the smaller twin during pregnancy, large anastomoses can also pose a threat to either twin due to the risk of acute feto-fetal transfusion potentially leading to fetal demise or neurological damage[8,21,22]. This is especially thought to be the cause of the unpredictable clinical course of type III sFGR, which is reported to have the largest AA diameter[8,11]. At present, this also determines the current management protocol in which fetal surveillance is advised. The knowledge from our current study can now lead to a more accurate risk assessment, especially if antenatal visualization of large, bidirectional anastomoses is further improved in the future.

Fetoscopic laser coagulation has been suggested for sFGR pregnancies with abnormal UA Doppler flow patterns to eliminate the risk of acute feto-fetal transfusion by coagulating the large anastomoses[23]. However, our study further substantiates that the smaller twin also relies on these anastomoses for an additional blood supply from its co-twin. This rescue transfusion is lost when anastomoses are coagulated, resulting in high rates of fetal demise in the smaller twin (60-77%)[24-27]. This phenomenon was also observed in TTTS pregnancies where sFGR prior to laser was identified as a risk factor for fetal demise of the smaller twin[28]. In addition, fetoscopic laser coagulation

in sFGR pregnancies comes with more technical challenges than in TTTS due to the absence of an amniotic fluid discordance.

Research implications

This study provides us a glimpse in the black box that is the MC placenta. However, its exact internal mechanisms are not yet fully understood. Future research should focus on volumetric measurements to quantify placental sharing more accurately. By early antenatal visualization of placental sharing and angioarchitecture (e.g., with placental mapping by three-dimensional color Doppler ultrasound or MRI[29-32]), the knowledge from this study can be applied to formulate an individualized risk assessment and adapt the management strategy accordingly in the future. Moreover, pathological examination of placental tissue including placental weight can provide more information on other potential causes of a BWD, such as antenatal placental insufficiency or maternal disease as reported in singletons with fetal growth restriction[33].

Strengths and limitations

Our study has limitations that should be considered when interpreting the data. Firstly, its retrospective nature can introduce bias in the results. Moreover, as we are a specialized center there might be an overrepresentation of severe cases with a large BWD. Importantly, reliable dye injection is only possible in cases with double survivors, automatically resulting in a selected population with relatively favorable outcome as cases in which fetal demise or selective reduction occurred are generally the most severe cases. Lastly, it should be noted that we solely look at the placental surface in determining the sharing and not placental volume. Nevertheless, our study is strengthened by its large study population, inclusion of placentas from twins with a broad range of BWD to explore, and the subgroup analyses per UA Doppler flow pattern in twins with a BWD ≥ 20% allowing for in depth investigation of the distinct placental mechanisms for each type. As dye injection of placentas has been part of standard care in our center for nearly twenty years, we have a large dataset of placentas available including digitally saved pictures that can be reviewed.

Conclusions

This study shows that BWD in MC twins is strongly associated to placental share discordance but that large bidirectional anastomoses, particularly AA anastomoses, can mitigate the effect of unequal placental sharing. Placentas from pregnancies with

UA Doppler abnormalities show a distinct mechanism with a greater importance of placental angioarchitecture.

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Part II

From fetus to newborn

Chapter 3

Gestational age at birth and outcome in monochorionic twins with different types of selective fetal growth restriction: a systematic literature review.

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Abstract

This systematic review aims to assess the gestational age at birth and perinatal outcome (intrauterine demise (IUD), neonatal mortality and severe cerebral injury) in monochorionic (MC) twins with selective fetal growth restriction (sFGR), according to Gratacós classification based on umbilical artery Doppler flow patterns in the smaller twin. Seventeen articles were included. Gestational age at birth varied from 33.0-36.0 weeks in type I, 27.6-32.4 weeks in type II, and 28.3-33.8 weeks in type III. IUD rate differed from 0-4% in type I to 0-40% in type II and 0-23% in type III. Neonatal mortality rate was between 0-10% in type I, 0-38% in type II, and 0-17% in type III. Cerebral injury was present in 0-2% of type I, 2-30% of type II and 0-33% of type III cases. The timing of delivery in sFGR varied substantially among studies, particularly in type II and III. The quality of evidence was moderate due to heterogenous study populations with varying definitions of sFGR and perinatal outcome parameters, as well as a lack of consensus on the use of the Gratacós classification, leading to substantial incomparability. Our review identifies the urgent need for uniform antenatal diagnostic criteria and definitions of outcome parameters.

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Introduction

Selective fetal growth restriction (sFGR), defined as estimated fetal weight (EFW) of one twin < 10th centile and an EFW discordance > 25%, is a complication affecting 10-15% of monochorionic (MC) twin pregnancies resulting in an intertwin growth discordance[1]. The pathophysiology is primarily due to unequal placental sharing, in which the growth-restricted twin has a smaller share of the placenta leading to suboptimal growth[2]. sFGR is associated with high perinatal morbidity and mortality rates[3]. Even if both twins are born alive, there is still a risk of neurological impairment due to increased rates of prematurity.

The extent of the perinatal morbidity and mortality risk depends on the type of sFGR. sFGR can be classified into three types according to Gratacós[4]. Type I is characterised by a continuous positive end-diastolic flow (pEDF) in the umbilical artery (UA) of the smaller twin and is generally associated with a relatively good outcome[1-4]. Type II is distinguished by a persistently absent or reversed EDF (A/REDF) in the UA and is associated with increased perinatal mortality and morbidity[1-4]. Lastly, type III is characterised by an intermittent absent/reversed EDF (iA/REDF) in the UA and has an unpredictable clinical course due to a large arterio-arterial (AA) anastomosis on the placenta, resulting in an unstable and fluctuating blood flow between the fetuses[1-4].

The current management of sFGR consists mainly of expectant management including fetal monitoring and medically induced preterm birth in case of fetal distress. In some cases, fetal interventions may be considered, including selective feticide using cord occlusion or fetoscopic laser coagulation. However, management in sFGR is not based on robust evidence, but mainly on expert opinion. Hence, uncertainty regarding the optimal management strategy persists. sFGR twins are often delivered electively at an early gestational age (GA) due to the fear of intrauterine demise (IUD). Preterm birth is in turn associated with an increased risk of adverse neonatal outcomes. The balance between the risk of IUD and the risk of adverse neonatal outcomes following prematurity remains a clinical dilemma. Due to a lack of robust evidence to guide a consensus regarding the optimal GA at birth for these infants, practice varies across fetal medicine centers. To evaluate the international variation in the GA at birth in sFGR twins and to gain more understanding of worldwide differences in perinatal outcome in sFGR pregnancies, we performed a systematic review and studied the differences in GA at birth in twin pregnancies complicated by sFGR according to the Gratacós classification.

Methods

Search strategy

This systematic review was conducted according to PRISMA guidelines[5]. An information specialist was involved in the development of the search terms. The online electronic PubMed database, EMBASE, Web of Science and Cochrane Library was searched in June 2022 by using the Boolean combination of: "Fetal Growth Retardation" AND "Twins, Monozygotic" AND "Gestational Age". Additionally, a variety of synonyms were added as free text words and MESH terms (Supplement I). A publication date restriction was applied to select studies published between 2007, the year the Gratacós classification was introduced, and 2022. Lastly, reference lists of reviewed articles were manually searched to identify relevant missed articles.

Study selection

All articles were assessed for eligibility through screening of the title and abstract. Subsequently, the full text was evaluated. Articles (clinical trials, cohort studies and case-control studies, both prospective and retrospective in nature) were eligible for inclusion when the cohort consisted of MC twin pregnancies complicated by sFGR, classified into the three Gratacós types and expectantly managed. Articles were excluded when they did not distinguish between isolated sFGR and sFGR with twin-twin transfusion syndrome (TTTS) and/or twin anemia polycythemia sequence (TAPS)[6,7]. Additionally, articles were excluded when fetoscopic laser coagulation or selective reduction were the only management options. Further exclusion criteria were case reports, case series (N<3), reviews, editorials, conference abstracts and unavailable full text. To identify eligibility of inclusion, two reviewers (S.E., S.G.) independently assessed the search results and discrepancies were resolved through discussion.

The primary outcome was GA at birth in the three types of sFGR, as reported in the various cohorts. The secondary outcomes were IUD, neonatal mortality and severe cerebral injury. Definitions of sFGR and delivery indications were reported when present. To compare the various cerebral injuries described in the articles, one definition was formulated. Severe cerebral injury was defined as the presence of intraventricular hemorrhage (IVH) ≥ grade II, periventricular leukomalacia (PVL) ≥ grade II, porencephalic cysts and/or intraparenchymal bleeding.

Quality assessment

The "Users Guides to the Medical Literature" and the "GRADE working group" method were used to assess the validity of the included articles with regards to the research question and the overall quality of evidence[8,9]. The validity assessment is based on two primary and two secondary guides. The primary guides were whether there was a representative and well-defined sample at a similar point in the course of disease and whether the follow-up was sufficient and complete. The secondary guides were whether objective and unbiased outcome criteria were used and whether there was adjustment for important prognostic factors. The overall quality of evidence was determined based on the four key elements reported by the "GRADE working group": study design, study quality (in this case the validity assessment), consistency and directness.

Results

The search strategy yielded 723 results. After excluding duplicates, 434 abstracts were screened. The primary assessment led to the exclusion of 399 articles based on above-mentioned in- and exclusion criteria. Manual search of the reference lists provided one additional article. Of the remaining 35 articles, 18 were excluded after full text assessment, resulting in a total of 17 articles to be included in this systematic review (Figure 1). The methodology of the studies is presented in Table 1. The study characteristics and neonatal outcomes of sFGR twins with type I, II and III are presented in Table 2, 3 and 4, respectively. The mean or median GA at birth in the three subgroups varied greatly per cohort and is shown in Figure 2. The results for all sFGR types are described separately here below.

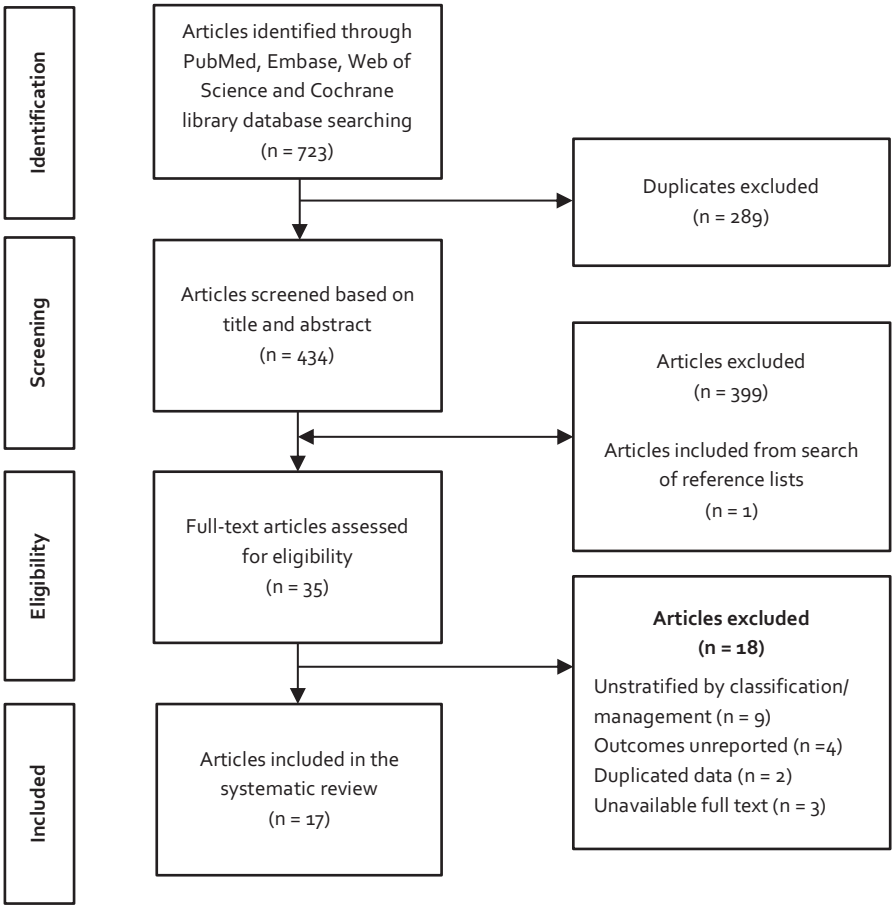

Figure 1. Flowchart of study inclusion.

In summary, the included studies were all published between 2007 and 2021 (mainly after 2016). The majority of studies (10/17) were conducted in Europe, and the others in North/South America and Asia. Thirteen studies were retrospective and four prospective. All studies focused on MC twin pregnancies diagnosed with sFGR in the absence of TTTS or TAPS, with 6/17 focusing on all management options and 11/17 on expectant management. 7/17 studies reported on all sFGR types and at least two secondary outcomes.

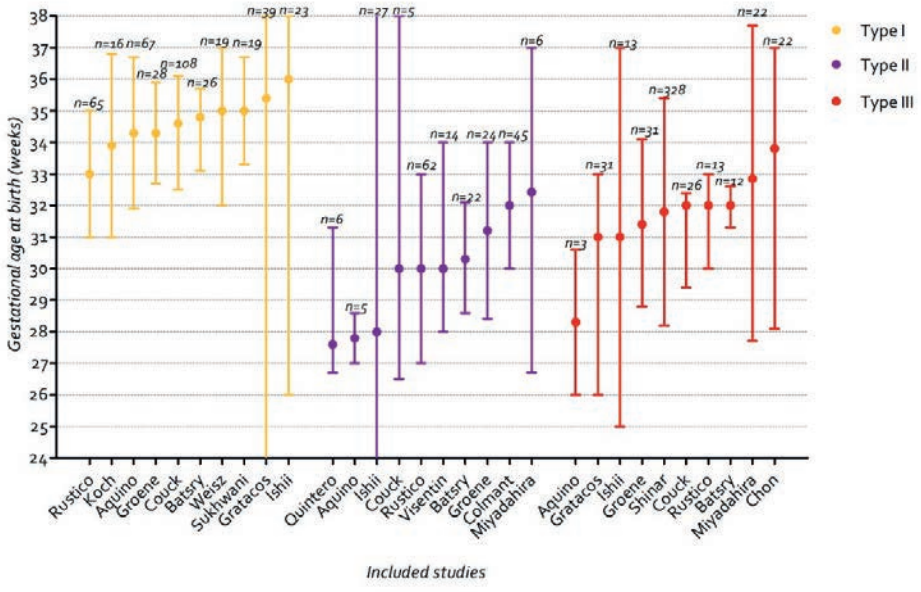

Figure 2. GA at birth per included study in twin pregnancies complicated by sFGR type I, II and III twins. This figure should be interpreted with care due to the heterogeneity of available studies, reporting GA at birth in either mean or median, using different definitions of outcomes measures and having small sample sizes.

Quality assessment and level of evidence

The validity of the included studies with regards to our primary research question is presented in Table 1. Three studies were deemed to have a low validity: the study by Visentin et al., the study by Koch et al. and the study by Quintero et al.[10-12]. This was primarily due to their different research questions focusing on, respectively, cord insertion and fetoscopic laser coagulation in sFGR as a treatment option, resulting in only a small population that could be included in this review. Moreover, Visentin et al. solely included sFGR diagnosed in the first trimester and did not fully define their outcome measures[10]. Reported outcomes by Koch et al. were combined for type II and

III and cases with IUD at time of diagnosis were excluded, leading to a potential underestimation of mortality[11]. Twelve out of the fourteen other studies were considered to have adequate validity, primarily due to either small study populations, sole inclusion of early onset sFGR or limited availability of the outcomes of interest in this review. The two studies with high validities, Couck et al. and Shinar et al., presented the largest cohorts diagnosed with sFGR irrespective of GA with the most complete perinatal outcome data[13,14].

Overall, the definitions of sFGR and the application of the Gratacós classification differed substantially among studies. While six studies defined sFGR as an EFW < 10th centile in the smaller twin and/or EFW discordance ≥ 25%, eight studies only focused on an EFW of one twin < 10th centile, one study focused on an AC < 5th centile and EFW < 10th centile (Colmant et al.) and two studies used the new Delphi consensus definition (Couck et al. and Aquino et al., Table 2-4[13,15]). Moreover, there was no uniformity in the application of the Gratacós classification and reported outcome measures. This resulted in heterogenous methodologies, and thereby incomparability between studies. Hence, the overall quality of evidence of the included articles for our research question was of moderate quality, suggesting that further research is necessary to provide evidence of superior quality.

<u>sFGR type I</u>

Ten cohort studies assessing the GA at birth in sFGR type I were included, with the number of pregnancies per cohort ranging from 16 to 108 (Table 2).

GA at birth

Based on the included literature, sFGR type I cases were born at a GA between 33.0-36.0 weeks' gestation. The lowest GA at birth presented in the type I cohort of Rustico et al. (n=65), which had a median GA at birth of 33 (31-35) weeks[16]. Ishii et al. (n=23) reported the highest median GA at birth of 36 (26–38) weeks[17]. Only 1/10 studies described indication of delivery. The cohort of Ishii et al. was delivered due to fetal deterioration (4/23), growth arrest smaller twin (3/23) or spontaneous labor/maternal indication (20/23)[17].

Perinatal mortality

sFGR type I twins had an IUD rate between 0-4% and neonatal mortality rate between 0-10%. No perinatal mortality occurred in the cohorts of Weisz et al. (n=19), Koch et

al. (n=16), Batsry et al. (n=26) and Sukhwani et al. (n=19)[11,18-20]. The lowest neonatal mortality rate was reported in the study with the highest GA at birth (Ishii et al.[17]). In addition, the study with the highest perinatal mortality rate (Rustico et al. (n=65) with 4% (5/130) IUD and 10% (12/118) neonatal mortality[16]) had the lowest GA at birth. However, this cohort included three bipolar cord coagulations following a change in the Doppler pattern to type II, one termination of pregnancy and one miscarriage. Nearly all studies reported that the smaller twin was the one affected by perinatal death, except for Gratacós et al. (n=39), Ishii et al. (n=23) and Couck et al. (n=108) in which the IUD rate was similar for the larger and smaller twin in type I cases (double IUDs except for Ishii et al.)[4,13,17].

Cerebral injury

Only 7/10 studies reported on cerebral injury, which was only observed in 2% of the cohort of Ishii et al. (1/44) and affected the smaller twin[17].

sFGR type II

Ten cohort studies assessing the GA at birth in sFGR type II were included with the number of pregnancies per cohort ranging from 5 to 62 (Table 3).

GA at birth

sFGR type II cases were born at a GA at birth between 27.6-32.4 weeks. The lowest GA at birth was reported by Quintero et al. (n=6), with a median GA at birth of 27.6 (26.7–31.3) weeks[12]. Miyadahira et al. (n=6) reported the highest median GA at birth of 32.4 (26.7–37.0) weeks[21]. Only 4/10 studies described indication of delivery. The majority of the sFGR type II/III cohort (individual indications not reported) of Miyadahira et al. was delivered due to fetal distress (19/27), and others due to threatened preterm labor (2/27), IUD (4/27) or spontaneous labor ≥34 weeks (2/27)[21]. The cohort of Quintero et al. were all delivered due to fetal indications: A/REDF (2/6), non-reassuring fetal testing (3/6) and preterm premature rupture of membranes (1/6)[12]. The main reasons for delivery in the cohort of Ishii et al. were fetal deterioration (9/27), spontaneous labor/maternal indication (8/27), double IUD (4/27), growth arrest smaller twin (3/27) and miscarriage (2/27). The cohort of Visentin et al. (n=14) was delivered at a median GA at birth of 30 (28–34) weeks either following signs of fetal demise, an abnormal biophysical fetal profile or fetal indications including abnormal cardiotocography or absent or reversed a-wave in ductus venosus[10].

Perinatal mortality

sFGR type II twins demonstrated a relatively high IUD rate between 0-40% and neonatal mortality rate between 0-38%. The cohorts of Visentin et al. (n=14) and Aquino et al. (n=5) were the only two cohorts in which perinatal mortality did not occur, despite the relatively low GA at birth reported by the latter[10,15]. The absence of IUD in the cohort of Aquino et al. could be explained by the late inclusion of pregnancies (median GA at diagnosis = 24.8 weeks). Interestingly, the highest perinatal mortality occurred in the cohort born at a median GA at birth of 30.0 (26.5–38.0) weeks, namely Couck et al. (n=5), who reported an IUD rate of 40% (4/10) and no neonatal mortality[13]. Additionally, the lowest IUD rate was reported in the cohort of Quintero et al. (n=6) delivered at the lowest GA at birth[12]. These results, as well as the results described by Aquino et al. (n=5), can be substantially impacted by their small sample size. Furthermore, almost all studies reported higher perinatal mortality in the smaller twin, except for the cohort of Batsry et al. (n=22) and Couck et al. (n=5) in which the IUD rate was similar for the larger and smaller twin (double IUDs)[13,19].

Cerebral injury

sFGR type II cases had the highest rates of cerebral injury (between 2-30%) of all three types which was documented in 7/10 studies. The lowest severe cerebral injury rate (2% (1/43)) occurred in the type II cohort of Groene et al. (n=24)[22]. The highest severe cerebral injury rate of 30% (3/10) was reported in the cohort of Quintero et al. delivered at the lowest GA at birth[12]. Furthermore, in Ishii et al. (n=27), Batsry et al. (n=22) and Aquino et al. (n=5) the smaller twin presented with more severe cerebral injury than the larger twin, while Miyadahira et al. (n=6), Groene et al. (n=24) and Quintero et al. (n=6) reported the opposite.

<u>sFGR type III</u>

Ten cohort studies assessing the GA at birth in sFGR type III were included, with the number of pregnancies ranging from 3 to 328 (Table 4).

GA at birth

sFGR type III cases were born at a GA at birth between 28.3-33.8 weeks. The lowest GA at birth was presented in the type III cohort of Aquino et al. (n=3), with a mean GA at birth of 28.3 (±2.3) weeks[15]. The highest median GA at birth of 33.8 (28.1–37.0) weeks was described by Chon et al.[20]. Only four studies reported on the indication of delivery. The majority of the cohort of Ishii et al. was delivered due to fetal

deterioration (8/13), while others either due to growth arrest of smaller twin (1/13) or spontaneous labor/maternal indication (4/13)[17]. The cohort of Chon et al. (n=22) was delivered either due to non-reassuring fetal status (10/22), spontaneous delivery (5/22), elective delivery (6/22) or preeclampsia (1/22)[20]. Miyadahira et al. (n=22) reported on the indication of delivery for both type II/III combined as previously described[21]. The main reasons for delivery in the cohort of Shinar et al. (n=328) with a mean GA at birth of 31.8 (±3.6) weeks, were fetal distress including abnormal cardiotocography or absent or reversed a-wave in ductus venosus (106/308), maternal diabetes (20/308), IUD/abnormal biophysical profile (36/308), spontaneous labor (46/308) and elective birth (100/308)[14].

Perinatal mortality

sFGR type III twins had an IUD rate between 0-23% and neonatal mortality rate between 0-17%. The cohorts of Couck et al. (n=26) and Aquino et al. (n=3) were the only two cohorts in which IUD did not occur[13,15]. Neonatal mortality was absent in the cohorts described by Chon et al. (n=22), who described the most advanced GA at birth, and Batsry et al. (n=12) who reported the highest IUD rate of 23% (5/24) in a cohort born at a median GA of 32.0 (31.3–32.6) weeks[19,20]. The highest neonatal mortality rate of 17% (1/6) were reported by Aquino et al. (n=3), who also reported the lowest GA at birth[15]. The majority of studies conclude that the smaller twin more often presented with perinatal mortality than the larger twin, except Groene et al. (n=31) in which the IUD rate was similar for the smaller and larger twin but the larger twin presented with higher risk of neonatal mortality, and Ishii et al. (n=13) and Aquino et al. (n=3) in which the larger twin also presented with a higher neonatal mortality rate[15,17,22].

Cerebral injury

Cerebral injury in sFGR type III cases was documented in 8/10 studies and varied between 0-33%. Batsry et al. (n=12) and Aquino et al. (n=3) were the only cohorts in which severe cerebral injury did not occur[15,19]. The highest severe cerebral injury rate of 33% (8/24) occurred in the cohort of Ishii et al. (n=13), which was born at a median GA of 31 (25–37) weeks[17]. Interestingly, Ishii et al. (n=13), Gratacós et al. (n=31) and Groene et al. (n=31) reported a higher severe cerebral injury rate in the larger twin, while Miyadahira et al. (n=22) and Chon et al. (n=22) identified the smaller twin to be at higher risk[17,20-23].

Table 1. Methodology and validity of included studies.

First author (year)	Study design	Population	Total sFGR cases	Inclusion criteria	Exclusion criteria	Validity
Gratacós (2007)	P	Spain, Belgium, 2003-2006	n = 134 Type I: 39, Type II: 30 (21 EM/9 CO), Type III: 65 (61 EM/4 CO)	MC twins diagnosed with sFGR at 18-26 weeks in three centers	Signs of TTTS	Adequate
Gratacós (2008)	R	Spain, Belgium, 2003-2006	n = 49 Type III: 49 (31 EM/18 FLC)	MC twins diagnosed with sFGR in three centers	-	Adequate
Ishii (2009)	R	Japan, 2001-2008	n = 63 Type I: 23, Type II: 27, Type III: 13	MC pregnancies diagnosed with sFGR <23 weeks of gestation in three centers	Cases with TTTS or the diagnosis of fetal malformation at the time of initial diagnosis	Adequate
Weisz (2011)	P	Israel, 2004-2008	n = 37 Type I: 19, Type II/III: 18	MC pregnancies >24 weeks of gestation followed at a single tertiary center	Cases with TTTS or major fetal anomalies	Adequate
Visentin (2013)	P	Italy, 2008-2011	n = 14 Type II: 14	MCDA pregnancies with sFGR diagnosed during the 1st trimester or during the 2nd trimester when referred from other centers	Unknown last menstrual period and chorionicity, triplet pregnancy, TTTS or related conditions, MCMA pregnancies, and structural/chromosomal abnormalities in either twin	Low
Rustico (2017)	R	Italy, 2004-2012	n = 140 Type I: 65, Type II: 62, Type III: 13	MCDA pregnancies complicated by sFGR examined <26 weeks of gestation at a single tertiary referral center	-	Adequate
Koch (2017)	R	France, 2008-2015	n = 25 Type I: 16, Type II/III: 9	MCDA pregnancies presenting with sFGR >16 weeks of gestation at multiple referral centers	TTTS, TAPS, chromosomal or structural anomalies and IUD at time of diagnosis	Low
Miyadahira (2018)	R	Brazil, 2007-2016	n = 67 Type II: 36 (6 EM/30 FLC) Type III: 31 (22 EM/9 FLC)	MCDA pregnancies with sFGR type II or III diagnosed <26 weeks of gestation and managed at one of the participating centers, with a cervical length of ≥15 mm	TTTS or TAPS	Adequate
Groene (2019)	R	The Netherlands, 2002-2018	n = 83 Type I: 28, Type II: 24, Type III: 31	MC placentas with a BW discordance >25% and/or an EFW in one twin <10th centile, consecutively examined at a single tertiary referral center	Co-existing TTTS or TAPS, no record of UA Doppler classification, incomplete placental data, inadequate placental measurements on digital pictures and single or double IUD when severe placental maceration made measurements impossible	Adequate

Quintero (2019)	P	United States of America	n = 20 Type II: 20 (6 EM/14 FLC)	Surviving MCDA children diagnosed with sFGR between 16-26 weeks of gestation, balanced karyotype, no major congenital anomalies, ≥24 months corrected age (±6 weeks) and ≤7 years and 11 months	Refusion of neurodevelopmental assessment and examination, unable to complete the measures in English or Spanish, and families lost to follow up	Low
Sukhwani (2019)	R	Spain, 2012-2018	n = 55 Type I: 25, Type II/III: 30 *Excluded due to peripheral delivery: 11*	MC pregnancies diagnosed with sFGR at a single tertiary center	-	Adequate
Chon (2019)	R	United States of America, 2006-2017	n = 48 Type III: 22, Type II or TTTS: 26	MCDA pregnancies referred to a single tertiary center for the evaluation of TTTS/sFGR	Cases with both twins having an EFW <10th percentile (dual sFGR)	Adequate
Colmant (2020)	R	France, 2011-2016	n = 108 Type II: 108 (45 EM/50 CC/13 FLC)	MC pregnancies referred to a single center for sFGR with A/REDF (type II) <27 weeks of gestation	Co-existing TTTS and morphological or chromosomal abnormalities detected prenatally	Adequate
Batsry (2020)	R	Israel, 2012-2018	n = 88 (60 EM/ 28 CO) Type I: 26, Type II: 22, Type III: 12	MCDA pregnancies complicated by sFGR <24 weeks of gestation, managed at a single tertiary referral center	TTTS, TAPS and fetal anomalies, including chromosomal abnormalities or genetic abnormalities	Adequate
Couck (2020)	R	Belgium, 2002-2018	n = 177 Type I: 110, Type II: 11, Type III: 33, Subsequent TTTS: 17 *Excluded due to developing TAPS: 6*	MCDA twin pregnancies followed from the first trimester onward and diagnosed with sFGR at 16, 20 or 30 weeks of gestation	MCDA twin pregnancies referred in the 1st trimester for an anomaly or invasive testing, unavailable ultrasound data, single/double demise, TTTS, TAPS, TOP, miscarriage or birth and lethal anomalies diagnosed between the 1st trimester and 16 weeks, 16-20 weeks and 20-30 weeks.	High
Shinar (2021)	R	Canada, China, The Nether-lands, Belgium, Israel, Switzer-land, 2008-2019	n = 328 Type III: 328 *18 pregnancies progressed to TTTS with 7 cases requiring FLC*	MCDA pregnancies complicated by sFGR type III, irrespective of GA at referral or diagnosis, at nine tertiary fetal medicine centers	Higher-order multiple gestations, major fetal structural or genetic anomalies, missing neonatal data, TTTS, TAPS or TRAP sequence at first presentation	High
Aquino (2021)	R	Brazil, 2010-2018	n = 75 Type I: 67, Type II: 5, Type III: 3	MCDA pregnancies affected by sFGR managed expectantly at two referral centers	TTTS > Quintero I, TAPS, congenital anomalies, aneuploidies, genetic syndromes, dual FGR and peripheral institution delivery	Adequate

sFGR: selective fetal growth restriction; P: prospective, R: retrospective, EM: expectant management; CO: cord occlusion; MCDA: monochorionic diamniotic; TTTS: twin-twin transfusion syndrome; FLC: fetoscopic laser coagulation; MCMA: monochorionic monoamniotic; TAPS: twin anemia polycythemia sequence; IUD: intrauterine demise; BW: birth weight; EFW: estimated fetal weight; UA: umbilical artery; CC: cord coagulation; A/REDF: absent or reversed end-diastolic flow; TOP: termination of pregnancy; TRAP: twin reversed arterial perfusion.

Table 2. Study characteristics, gestational age at birth and secondary outcomes in twin pregnancies complicated by sFGR type I.

First author (year)	Patients	Definition sFGR	GA at birth in weeks	IUD	Neonatal mortality	Severe cerebral injury	Delivery indication	Comments
Gratacós (2007)	n = 39	EFW <10th centile in one twin	35.4 (16–38)§	2/78 (3) -Smaller: 1/39 (3) -Larger: 1/39 (3) -Double: 1/39 (3)	-	IVH/PVL on neonatal cUS: 0/39 (0)	-	-
Ishii (2009)	n = 23	EFW <10th percentile in the smaller twin	36 (26–38)†	2/46 (4) -Smaller: 1/23 (4) -Larger: 1/23 (4) -Double: 0/23 (0)	0/44 (0)	Neurological morbidity: 1/44 (2) -Smaller: 1/22 (5) -Larger: 0/22 (0)	Fetal deterioration: 4/23 Growth arrest: 3/23 Spontaneous/maternal indication: 20/23	*Neurological morbidity was defined as IVH Grade III/IV, cystic PVL, blindness and deafness. Variety of postnatal cerebral imaging: both cUS and MRI.*
Weisz (2011)	n = 19	EFW <10th percentile in one twin	35 (32–37)†	0/38 (0)	0/38 (0)	IVH/PVL on neonatal cUS: 0/38 (0)	-	-
Rustico (2017)	n = 65	EFW <10th centile in the smaller twin or EFW discordance ≥25% in the absence of TTTS and TAPS	33 (31–35)†	5/130 (4) -Smaller: 3/65 (5) -Larger: 2/65 (3) -Double: 2/65 (3)	12/118 (10) -Smaller: 8/57 (14) -Larger: 4/61 (7)	-	-	*Three cases of BCC (no larger twins lost), one TOP (both twins lost) and one miscarriage were included in the analysis.*
Koch (2017)	n = 16	EFW <10th centile for one of the twins	33.9 (±2.9)‡	0/32 (0)	0/32 (0)	-	-	-
Groene (2019)	n = 28	BW discordance >25% and/or an EFW in one twin <10th centile	34.3 (32.7–35.9)†	2/56 (4)* -Smaller: 1/28 (4) -Larger: 1/28 (3) -Double: 1/28 (3)	1/52 (2)* -Smaller: 1/26 (4) -Larger: 0/26 (0)	Severe cerebral injury on neonatal cUS: 0/39 (0)*	-	*Severe cerebral injury was defined as PVL ≥grade II, IVH ≥grade III, ventricular dilatation, arterial or venous infarct or other injuries.*
Sukhwani (2019)	n = 19	EFW of one twin <10th centile and EFW discordance >25% in the absence of TTTS	35.0 (±1.7)‡	0/38 (0)	0/38 (0)	PVL: 0/38 (0)	-	*Grade of PVL and cerebral imagining modality not reported.*

Batsry (2020)	n = 26	EFW of one twin <10th centile or EFW discordance ≥25% in the absence of TTTS or TAPS	34.8 (33.1–35.7)†	0/52 (0)	0/52 (0)	Severe brain lesions on fetal MRI: 0/52 (0)	-	*Severe brain lesions were defined as IVH Grade III/IV, PVL or intraparenchymal hemorrhage.*
Couck (2020)	n = 108	EFW <3rd centile in one twin or at least two of the following: -EFW of one twin <10th centile, -AC of one twin <10th centile, -EFW discordance ≥25%, -UA PI of the smaller twin >95th centile	34.6 (32.5–36.1)†	4/216 (2)* -Smaller: 2/108 (2) -Larger: 2/108 (2) -Double: 2/108 (2)	0/210 (0)*	-	-	*Two pregnancies underwent intervention (1 CO, 1 FLC) and these were included in the analysis of GA at birth. One miscarriage was included in this cohort.*
Aquino (2021)	n = 67	EFW <3rd centile in one twin or at least two of the following: -EFW of one twin <10th centile, -AC of one twin <10th centile, -EFW discordance ≥25%, -UA PI of the smaller twin >95th centile	34.3 (±2.4)‡	0/134 (0)	1/134 (1) -Smaller: 1/67 (1) -Larger: 0/67 (0)	Severe cerebral injury on neonatal cUS: 0/134 (0)	-	*Severe cerebral injury was defined as IVH grade III/IV, PVL grade II/III or porencephalic cysts.*

sFGR: selective fetal growth restriction; GA: gestational age; IUD: intrauterine demise; EFW: estimated fetal weight; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia; cUS: cerebral ultrasound; MRI: magnetic resonance imaging; TTTS: twin-twin transfusion syndrome; TAPS: twin anemia polycythemia sequence; BCC: bipolar cord coagulation; TOP: termination of pregnancy; BW: birthweight; AC: abdominal circumference; UA:umbilical artery; PI: pulsatility index; CO: cord occlusion; FLC: fetoscopic laser coagulation.
Data are presented as median (interquartile range) † , mean (± standard deviation)‡ , mean (min-max) or *n*/N (%).
*Authors have been approached for additional data.

Table 3. Study characteristics, gestational age at birth and secondary outcomes in twin pregnancies complicated by sFGR type II

First author (year)	Patients	Definition sFGR	GA at birth in weeks	IUD	Neonatal mortality	Severe cerebral injury	Delivery indication	Comments
Ishii (2009)	n = 27	EFW <10th percentile in the smaller twin	28 (18–40)†	14/54 (26) -Smaller: 8/27 (30) -Larger: 6/27 (22) -Double: 4/27 (15)	8/40 (20) -Smaller: 5/19 (26) -Larger: 3/21 (14)	Neurological morbidity: 7/40 (18) -Smaller: 4/19 (21) -Larger: 3/21 (14)	Fetal deterioration: 9/27 Growth arrest: 3/27 Double IUD: 4/27 Miscarriage: 2/27 Spontaneous/maternal indication: 8/27	*Neurological morbidity was defined as IVH Grade III/IV, cystic PVL, blindness and deafness. Variety of postnatal cerebral imaging: both cUS and MRI.*
Visentin (2013)	n = 14	EFW <10th percentile in one twin	30 (28–34)†	0/28 (0)	0/28 (0)	Neonatal cUS: IVH ≥ grade III: 0/28 (0) PVL: 45%	IUD/abnormal biophysical profile/fetal indications: 14/14	*Grade of PVL and proportion of neonates with PVL not reported.*
Rustico (2017)	n = 62	EFW <10th centile in the smaller twin or EFW discordance ≥25% in the absence of TTTS and TAPS	30 (27–33)†	22/124 (18) -Smaller: 14/62 (23) -Larger: 8/62 (13) -Double: 8/62 (13)	13/78 (17) -Smaller: 8/31 (26) -Larger: 5/47 (11)	-	-	*Fifteen cases of BCC (four larger twins lost) and three TOP (two both twins and one larger twin lost) were included in the analysis.*
Miyadahira (2018)	n = 6	EFW of one twin <10th centile and intertwin EFW discordance ≥25%	32.43 (26.71–37)†	4/12 (33) -Smaller: 3/6 (50) -Larger: 1/6 (17) -Double: 1/6 (17)	3/8 (38) -Smaller: 2/3 (67) -Larger: 1/5 (20)	IVH ≥ grade III: 1/6 (17) -Smaller: 0/1 (0) -Larger: 1/5 (20)	Fetal distress: 19/27 Preterm labor: 2/27 IUD: 4/27 Spontaneous: 2/27	*Indication of birth is combined for sFGR type II and III. Cerebral imaging modality not reported.*
Groene (2019)	n = 24	BW discordance >25% and/or an EFW in one twin <10th centile	31.2 (28.4–34.0)†	6/48 (13)* -Smaller: 4/24 (17) -Larger: 2/24 (8) -Double: 2/24 (8)	2/42 (5)* -Smaller: 2/20 (10) -Larger: 0/22 (0)	Severe cerebral injury on neonatal cUS: 1/43 (2)* -Smaller: 0/19 (0) -Larger: 1/22 (5)	-	*Severe cerebral injury was defined as PVL ≥grade II, IVH ≥grade III, ventricular dilatation, arterial or venous infarct or other injuries.*
Quintero (2019)	n = 6	EFW <10th percentile in one twin	27.6 (26.7–31.3)†	0/12 (0)	2/12 (17) -Smaller: 2/6 (33) -Larger: 0/6 (0)	IVH Grade III/IV:3/10 (30) -Smaller: 1/4 (25) -Larger: 2/6 (33)	A/REDF: 2/6 Non-reassuring fetal testing: 3/6 PPROM: 1/6	*Cerebral imaging modality not reported.*

Colmant (2020)	n = 45	AC <5th centile and EFW <10th centile	32 (30–34)†	11/90 (12) -Smaller: 8/45 (18) -Larger: 3/45 (7)	4/74 (5)	-	-	*One TOP (larger twin) and two cases of miscarriage were included in the analysis.*
Batsry (2020)	n = 22	EFW of one twin <10th centile or intertwin EFW discordance ≥25% in the absence of TTTS or TAPS	30.3 (28.6–32.1)†	2/44 (5) -Smaller: 1/22 (5) -Larger: 1/22 (5) -Double: 1/22 (5)	1/42 (2) -Smaller: 1/21 (5) -Larger: 0/21 (0)	Severe brain lesions on fetal MRI: 2/37 (5) -Smaller: 2/18 (11) -Larger: 0/19 (0)	-	*Severe brain lesions were defined as IVH Grade III/IV, PVL or intraparenchymal hemorrhage.*
Couck (2020)	n = 5	EFW <3rd centile in one twin or at least two of the following: -EFW of one twin <10th centile, -AC of one twin <10th centile, -EFW discordance ≥25%, -UA PI of the smaller twin >95th centile	30.0 (26.5–38.0)†	4/10 (40)* -Smaller: 2/5 (40) -Larger: 2/5 (40) -Double: 2/5 (40)	0/6 (0)*	-	-	*Six pregnancies underwent intervention (3 CO, 2 FLC, 1 RFA) and these were included in the analysis of GA at birth.*
Aquino (2021)	n = 5	EFW <3rd centile in one twin or at least two of the following: -EFW of one twin <10th centile, -AC of one twin <10th centile, -EFW discordance ≥25%, -UA PI of the smaller twin >95th centile	27.8 (±0.8)‡	0/10 (0)	0/10 (0)	I IVH Grade III on neonatal cUS: 1/10 (10) -Smaller: 1/5 (20) -Larger: 0/5 (0)	-	*Severe cerebral injury was defined as IVH grade III/IV, PVL grade II/III or porencephalic cysts.*

Data are presented as median (interquartile range) † , mean (± standard deviation)‡ , mean (min-max)§ or *n*/N (%). *Authors have been approached for additional data.
sFGR: selective fetal growth restriction; GA: gestational age; IUD: intrauterine demise; EFW: estimated fetal weight; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia; cUS: cerebral ultrasound; MRI: magnetic resonance imaging; TTTS: twin to twin transfusion syndrome; TAPS: twin anemia polycythemia sequence; BCC: bipolar cord coagulation; TOP: termination of pregnancy; BW: birthweight; A/REDF: absent or reversed end-diastolic flow; PPROM: preterm premature rupture of membranes; AC: abdominal circumference; UA, umbilical artery; PI: pulsatility index; CO: cord occlusion; FLC: fetoscopic laser coagulation; RFA: radiofrequency ablation.

Table 4. Study characteristics, gestational age at birth and secondary outcomes in twin pregnancies complicated by sFGR type III.

First author (year)	Patients	Definition sFGR	GA at birth in weeks	IUD	Neonatal mortality	Severe cerebral injury	Delivery indication	Comments
Gratacós (2008)	n = 31	EFW <10th centile in one twin in the absence of severe TTTS	31.0 (26.0–33.0)†	9/62 (15) -Smaller: 6/31 (19) -Larger: 3/31 (10) -Double: 3/31 (10)	-	IVH: 3/53 (6) -Smaller: 1/25 (4) -Larger: 2/28 (7) PVL: 4/53 (8) -Smaller: 0/25 (0) -Larger: 4/28 (14)	-	*Elective delivery at 32 weeks after maternal administration of corticosteroid therapy for fetal maturation.* *Cerebral brain imaging performed on neonatal cUS.*
Ishii (2009)	n = 13	EFW <10th percentile in the smaller twin	31 (25–37)†	2/26 (8) -Smaller: 2/13 (15) -Larger: 0/13 (0) -Double: 0/13 (0)	3/24 (13) -Smaller: 0/11 (0) -Larger: 3/13 (23)	Neurological morbidity: 8/24 (33) -Smaller: 3/11 (27) -Larger: 5/13 (38)	Fetal deterioration: 8/13 Growth arrest: 1/13 Spontaneous/ maternal indication: 4/13	*Neurological morbidity was defined as IVH Grade III/IV, cystic PVL, blindness and deafness.* *Variety of postnatal cerebral imaging: both cUS and MRI.*
Rustico (2017)	n = 13	EFW <10th centile in the smaller twin or EFW difference ≥25% in the absence of	32 (30–33)†	1/26 (4) -Smaller: 1/13 (8) -Larger: 0/13 (0)	1/21 (5) -Smaller: 1/9 (11) -Larger: 0/12 (0)	-	-	*Two cases of BCC (smaller twin lost) and one TOP (both twins lost) were included in the analysis.*
Miyadahira (2018)	n = 22	EFW of one twin <10th centile and intertwin EFW discordance ≥25%	32.85 (27.71–37.71)†	6/44 (14) -Smaller: 4/22 (18) -Larger: 2/22 (9) -Double: 2/22 (9)	1/38 (3) -Smaller: 1/18 (6) -Larger: 0/20 (0)	IVH ≥ grade III: 1/32 (3) -Smaller: 1/15 (7) -Larger: 0/17 (0)	Fetal distress: 19/27 Preterm labor: 2/27 IUD: 4/27 Spontaneous: 2/27	*Indication of birth is combined for sFGR type II and III.* *Cerebral imaging modality not reported.*
Groene (2019)	n = 31	BW discordance >25% and/or an EFW in one twin <10th centile	31.4 (28.8–34.1)†	4/62 (6)* -Smaller: 2/31 (6) -Larger: 2/31 (6) -Double: 2/31 (6)	3/58 (5)* -Smaller: 1/29 (3) -Larger: 2/29 (7)	Severe cerebral injury on neonatal cUS*: 2/47 (4) -Smaller: 0/23 (0) -Larger: 2/24 (8)	-	*Severe cerebral injury was defined as PVL ≥grade II, IVH ≥grade III, ventricular dilatation, arterial or venous infarct or other injuries.*
Chon (2019)	n = 22	EFW of one twin <10th centile	33.8 (28.1–37.0)†	1/44 (2) -Smaller: 1/22 (5) -Larger: 0/22 (0)	0/43 (0)	IVH: 1/43 (2) -Smaller: 1/21 (5) -Larger: 0/22 (0) PVL: 0/43 (0)	Fetal distress: 10/22 Preeclampsia: 1/22 Spontaneous: 5/22 Elective: 6/22	*Variety of postnatal cerebral imaging: both cUS and MRI.*
Batsry (2020)	n = 12	EFW of one twin <10th centile or intertwin EFW discordance ≥25% in the absence of TTTS or TAPS	32.0 (31.3–32.6)†	5/24 (23) -Smaller: 3/12 (25) -Larger: 2/12 (17) -Double: 2/12 (17)	0/19 (0)	Severe brain lesions on fetal MRI: 0/18 (0)	-	*Severe brain lesions were defined as IVH Grade III/IV, PVL or intraparenchymal hemorrhage.*

Couck (2020)	n = 26	EFW <3rd centile in one twin or at least two of the following: -EFW of one twin <10th centile, -AC of one twin <10th centile, -EFW discordance ≥25%, -UA PI of the smaller twin >95th centile	32.0 (29.4–32.4)†	0/52 (0)*	3/52 (6)* -Smaller: 2/26 (8) -Larger: 1/26 (4)	-	-	*Seven pregnancies underwent intervention (5 CO, 1 RFA, 1 TOP) and these were included in the analysis of GA at birth, but not in the analysis of the secondary outcomes.*
Shinar (2021)	n = 328	EFW of one twin <10th centile and intertwin EFW discordance ≥25%	31.8 (±3.6)‡	51/638 (8) -Smaller: 35/310 (11) -Larger: 16/328 (5) -Double: 16/328 (5)	18/587 (3) -Smaller: 14/275 (5) -Larger: 4/312 (1)	Neonatal cUS: IVH ≥ grade II: 12/532 (2) -Smaller: 3/246 (1) -Larger: 9/286 (3) PVL: 7/532 (1) -Smaller: 4/246 (2) -Larger: 3/286 (1)	Fetal distress: 106/308 Maternal distress: 20/308 IUD/abnormal biophysical profile: 36/308 Spontaneous: 46/308 Elective: 100/308	*Grade of PVL not reported.*
Aquino (2021)	n = 3	EFW <3rd centile in one twin or at least two of the following: -EFW of one twin <10th centile, -AC of one twin <10th centile, -EFW discordance ≥25%, -UA PI of the smaller twin >95th centile	28.3 (±2.3)‡	0/6 (0)	1/6 (17) -Smaller: 0/3 (0) -Larger: 1/3 (33)	Severe cerebral injury on neonatal cUS: 0/6 (0)	-	*Severe cerebral injury was defined as IVH grade III/IV, PVL grade II/III or porencephalic cysts.*

sFGR: selective fetal growth restriction; GA: gestational age; IUD: intrauterine demise; EFW: estimated fetal weight; TTTS: twin-twin transfusion syndrome; IVH: intraventricular hemorrhage; PVL: periventricular leukomalacia; cUS: cerebral ultrasound; MRI: magnetic resonance imaging; TAPS: twin anemia polycythemia sequence; BCC: bipolar cord coagulation; TOP: termination of pregnancy; BW: birthweight; AC: abdominal circumference; UA: umbilical artery; PI: pulsatility index; CO: cord occlusion; RFA: radiofrequency ablation.
Data are presented as median (interquartile range)† , mean (± standard deviation)‡ , mean (min-max)§ or *n*/N (%).
*Authors have been approached for additional data.

Summary

The summarized findings per sFGR type are presented in Table 5. Overall, sFGR type I showed the most favorable outcomes, with GA at birth ranging from 33.0-36.0 weeks, a perinatal mortality rate (IUD and neonatal mortality combined) between 0-10% and 0-2% cerebral injury. sFGR type II presented with the poorest outcomes, with a GA at birth between 27.6-32.4 weeks, a perinatal mortality rate ranging between 0-40% and a cerebral injury rate of 2-30%. sFGR type III is reported to have relatively similar outcomes as type II, albeit slightly better, with a GA ranging from 28.3-33.8 weeks, a perinatal mortality rate of 0-23% and cerebral injury in 0-33%.

Table 5. Summarized perinatal outcome ranges of MC twin pregnancies complicated by sFGR according to Gratacós type.

	sFGR type I	**sFGR type II**	**sFGR type III**
GA at birth	33.0-36.0 weeks	27.6-32.4 weeks	28.3-33.8 weeks
Intrauterine demise	0-4%	0-40%	0-23%
Neonatal mortality	0-10%	0-38%	0-17%
Cerebral injury	0-2%	2-30%	0-33%

sFGR: selective fetal growth restriction, GA: gestational age.
These numbers should be interpreted with care due to the heterogeneity of available studies, reporting GA at birth in either mean or median, using different definitions of outcomes measures and having small sample sizes.

Discussion

Summary of the key findings

This systematic review shows that sFGR type I twins are generally born at a later GA than type II and type III twins and have a lower rate of IUD, neonatal mortality and cerebral injury. Nearly all studies reported that the smaller twin was especially at a disadvantage for adverse perinatal outcomes. However, the reported GA at birth of MC twins complicated by sFGR varies substantially between studies as well as the incidence of IUD, neonatal mortality and cerebral injury, especially in sFGR type II and III cohorts. Importantly, the 17 included studies had heterogenous study populations with different definitions of sFGR and timing of inclusion (between the first and third trimester) and reported on different perinatal outcome measures. Hence, this systematic review primarily demonstrates the knowledge gap regarding the optimal GA at birth and the lack of uniform outcome measures (assessment and management of expectantly managed MC twins complicated by sFGR and the lack of uniformity in various definitions). The application of the Gratacós classification substantially differs between studies, hampering proper comparison of outcomes between the types of sFGR.

Strengths and limitations

Five main recurring limitations can be identified in current literature: 1) information bias due to retrospective study designs, 2) small sample sizes, 3) the use of different antenatal management protocols (including frequency and methods of fetal surveillance) and definitions of sFGR, 4) lack of detailed information on perinatal outcomes categorised per Gratacós type, 5) lack of standardized neonatal and long-term follow-up including uniform definitions of perinatal outcome measures. Additionally, we did not synthesize our data in the form of a meta-analysis. Therefore, evidence of the association between GA at birth and adverse neonatal outcomes in MC twins with sFGR is considered to be of low quality. However, our review provides an elaborate and most recent overview of GA at birth in sFGR twins, demonstrating great variation between centers and emphasizing the uncertainty regarding the optimal timing of delivery after expectant management.

Interpretation of the findings

Our review demonstrates that type II and type III sFGR twins are generally born at a lower GA and have an increased rate of perinatal and neonatal mortality and severe cerebral injury as opposed to type I. However, we also demonstrate the current lack of

knowledge on the average GA at birth for the different types of sFGR due to limitations in the available literature leading to incomparability between studies.

A crucial limitation that is persistently present in current literature is the different scoring methods used for the Gratacós classification. Its dynamic nature hampers the determination of a 'definitive' Gratacós type. At present, available studies base the classification of a pregnancy complicated by sFGR on either a single observation of abnormal UA Doppler flow patterns, the final UA Doppler flow pattern prior to delivery or the most prevalent Doppler flow pattern[14,22,24]. Therefore, the classification of sFGR according to Gratacós is still not uniformly applied in literature, leading to substantial incomparability between studies with regards to outcome per sFGR type. It was recently suggested that a modification of the Gratacós classification is necessary that includes GA at diagnosis, variation in UA Doppler flow patterns, ductus venosus Doppler (has been shown to be a powerful prognostic marker for sFGR and might identify infants with increased risk for neonatal mortality and morbidity[25]) and the co-existence of TTTS[26]. By reaching an international consensus on an update of the current classification system, outcome parameters can be properly compared between studies and antenatal prognostication can be further improved.

A previous systematic review and meta-analysis by Townsend et al. also explored the perinatal outcomes of sFGR categorised according to the Gratacós classification[27]. A noteworthy difference between our two studies is the significantly higher cerebral injury rates after expectant management in type II and type III reported by Townsend et al. This can be the consequence of improved care over the years, as Townsend et al. primarily included older studies (2001-2017), while our review included more recent studies (2007-2021). Yet, accurate comparison of our studies is hampered by different aims and methods. While we focused on the international variation in GA at birth and perinatal outcome in this systematic literature review, Townsend et al. investigated the impact of different management strategies on perinatal outcomes in a meta-analysis. Interestingly, a similar outcome will be investigated by the FERN study with the aim to determine whether it is feasible to conduct a randomized control trial of active intervention versus expectant management[28].

Buca et al. showed similar results in their systematic review and meta-analysis exploring the outcomes of sFGR according to UA Doppler pattern of the smaller twin[29]. sFGR type I twins were also born at a significantly higher GA compared to type II (Median difference: 2.8 (95% CI, 1.83–3.86) weeks) and type III (Median difference:

2.1 (95% CI, 0.97–3.19) weeks). This meta-analysis showed a significantly higher risk of perinatal mortality (OR, 4.1 (95% CI, 1.6–10.3)) and abnormal postnatal brain imaging in sFGR type II and III compared to Type I (Type II: OR, 4.9 (95% CI, 1.9–12.9), Type III: OR, 8.2 (95% CI, 2.0–33.1)). Noteworthy, Buca et al. excluded studies reporting only one type of sFGR and included 13 studies (2007-2017), while our systematic review included 17 more recently published studies (2007-2021) with minimal overlap.

A third study following from the retrospective multicenter cohort study by Shinar et al. (of which data is also included in this review), focusing on outcomes of type III pregnancies, showed a GA dependent decrease in neonatal morbidity in sFGR type III with low rates of neurological morbidity[14]. Remarkably, a large decline in risk was seen from 29 weeks' gestation (74%) to 30 weeks (45%). It should be noted that postnatal brain ultrasound examinations were only routinely performed for neonates delivered before 32 weeks, resulting in a potential underestimation of brain injury. In addition, the study did not take into account the possibility of cases changing Gratacós types during pregnancy, resulting in a potential misclassification (especially in type II/III).

The findings from the study by Shinar et al. and our review are in agreement with the systematic review by Inklaar et al., which showed a significantly increased risk of cerebral injury in cohorts with a lower GA at birth[30]. Inklaar et al. illustrated that the odds of cerebral injury decreased with a factor of 0.65 for each additional increase in week of GA at birth. The increased risk of cerebral injury was thought to be primarily associated with a lower GA at birth but could also be due to an indicated urgent caesarean section in more severe cases. The review by Inklaar et al., however, lacks a distinction between Gratacós types and also reports high heterogeneity between the studies and small sample sizes, which are similar limitations as were found in this systematic review.

Based on our systematic literature review and the previously mentioned review by Inklaar et al., it can be concluded that sFGR type II and type III are especially at increased risk of cerebral injury. The cause of this injury is unknown and could be related to *in utero* adverse environment with abnormal flows and/or it could be a consequence of (iatrogenic) prematurity. In order to determine the timing of cerebral injury, routine and repeated neuroimaging examinations should be performed during fetal and neonatal life. The presence of cerebral injury already *in utero* or directly after birth would point towards a causal relation with adverse in utero environment, whereas cerebral injury which becomes apparent only one/two weeks after birth

would point towards a causal relation with (iatrogenic) prematurity. Importantly, both prematurity and neonatal cerebral injury are associated with an increased risk of long-term neurodevelopmental impairment. The risk for developmental delay is known to increase exponentially with decreasing GA (OR per week' gestation: 1.13, 95% CI 1.08–1.18)[31-33]. Furthermore, the IQ of children delivered <34 weeks' gestation decreases by 2.34 (95% CI: -2.99, -1.70) points with each lower GA week[34].

Clinical and research implications

In conclusion, due to the high heterogeneity of published studies, uncertainty regarding the optimal GA at birth in MC twins complicated by sFGR persists. Our review emphasises the uncertainty regarding the optimal timing of delivery after expectant management. Additionally, it demonstrates the varying GA at birth, rates of IUD and adverse neonatal outcome between international centers in sFGR twins, stratified according to sFGR classification. In order to estimate the optimal timing of delivery, future prospective studies should implement uniform diagnostic criteria for sFGR itself and the Gratacós classification, and objective and uniform management protocols with standardised perinatal outcome measures reported according to Gratacós type prior to delivery. Indication for delivery should be included as well as a description of neonatal morbidity. International collaboration is warranted to increase sample size. In addition, standardized long-term follow-up should be included to assess the effect of perinatal management and timing of delivery on long-term outcome[35]. Subsequently, a meta-analysis can be performed categorising perinatal outcome measures according to GA at birth. In the absence of a randomized controlled trial, larger and standardised data from retrospective and prospective studies can help us elucidate the optimal timing of delivery for MC twins with sFGR and ensure a more favourable perinatal outcome for these vulnerable neonates.

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Chapter 4

Respiratory distress syndrome and bronchopulmonary dysplasia after fetal growth restriction: lessons from a natural experiment in identical twins.

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Abstract

Background: Fetal growth restriction (FGR) is thought to negatively affect lung development resulting in increased respiratory morbidity. However, research performed in singletons is often limited by a certain level of bias caused by individual differences in genetic constitution, obstetrical and maternal factors.

Methods: Respiratory morbidity was compared between the smaller and the larger twin in monochorionic twins with selective fetal growth restriction (sFGR), defined as a birth weight discordance ≥ 20%, born in our center between 2010-2019 in this retrospective study. Respiratory distress syndrome (RDS) was diagnosed based on the clinical picture of a neonate with respiratory failure requiring mechanical ventilation and/or surfactant, confirmed by a chest X-ray. Bronchopulmonary dysplasia (BPD) was diagnosed when the neonate required treatment with >21% oxygen for at least 28 days.

Findings: Median gestational age at birth for the 94 included pregnancies was 32.4 (IQR 30.4-34.3) weeks. Within-pair analyses showed that the prevalence of RDS was lower in the smaller twin compared to the larger twin, 19.1% (18/94) *vs.* 34.0% (32/94), respectively (p = 0.004). The odds of RDS for the larger twin was doubled (OR 2.1 (95% CI 1.3-3.5)). In contrast, the rate of BPD in the smaller twin was higher as opposed to the larger twin, 16.7% (15/90) vs 6.7% (6/89), respectively (p = 0.008), with a more than doubled odds (OR 2.5 (95% CI 1.3-4.9)).

Interpretation: Despite being genetically identical, sFGR twins have different respiratory outcomes. Adverse growth condition *in utero* in the smaller twin is associated with a reduced odds of RDS at birth but a more than doubled odds of BPD, reflecting the pathophysiologic adverse effect of growth restriction on lung development.

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Introduction

Chronic intrauterine stress, as caused by fetal growth restriction (FGR), is thought to influence lung development and thereby lead to lifelong changes in respiratory functioning. Animal models have shown that FGR can induce persistent changes to both the lung and chest wall, already impairing respiratory function in early postnatal life[1]. Moreover, previous research in singletons has identified FGR as a risk factor for both respiratory distress syndrome (RDS), characterized by progressive respiratory insufficiency directly after birth, and bronchopulmonary dysplasia (BPD) which is in turn associated with chronic lung disease in adulthood[2-4]. In contrast, it is also hypothesized that FGR is protective for RDS by way of an increased endogenous surfactant production resulting in enhanced lung maturation[5].

Although many studies have tried to uncover the effect of FGR on respiratory morbidity, results are often contradictory as a multitude of other factors can influence respiratory functioning beyond FGR. Research performed in singletons is therefore often limited by a certain level of bias caused by individual differences in genetic constitution, obstetrical and maternal factors (maternal diseases and medication). Hence, conclusive evidence is still lacking. A unique population in which these potential confounders are naturally eliminated are monochorionic (MC) twins.

MC twins are vulnerable to perinatal complications because of the shared placenta with vascular anastomoses[6]. In 10-15% of MC twin pregnancies, the placenta is unequally shared leading to a large intertwin growth discrepancy described as selective fetal growth restriction (sFGR) (Figure 1)[7,8]. As MC twins are genetically identical and also have identical maternal factors, they present the ideal natural experiment to study the effect of adverse "environmental" intrauterine circumstances. So far, only few small studies have thoroughly explored the respiratory outcomes in MC twins with sFGR[9]. In this study we aim to compare respiratory outcomes, primarily RDS and BPD in which we expect to find consistent associations, between the larger and smaller twin in a large cohort of MC twins with sFGR.

Methods

This report is written according to the STROBE guidelines[10]. The study was approved by the ethics committee of the Leiden University Medical Center (LUMC) and waived of the requirement for written informed consent due to its retrospective design. All consecutive live-born MC twin pairs born in the LUMC between 2010-2019 were eligible for inclusion. The LUMC is the national referral center for complicated MC twin pregnancies and fetal therapy in The Netherlands. The cases were reviewed for the presence of sFGR, defined as a birth weight discordance (BWD) ≥ 20%. BWD was calculated as: (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100%[11]. All MC twins with sFGR were included. We excluded MC triplet pregnancies, cases with twin reversed arterial perfusion (TRAP)[12] and cases with other congenital abnormalities including, but not limited to heart defects, neural tube defects and chromosome abnormalities. Cases with twin anemia-polycythemia sequence (TAPS)[13], twin-twin transfusion (TTTS) cases who underwent treatment other than laser coagulation (no treatment, amniocentesis, and/or selective reduction) and cases with incomplete laser (either recurrent TTTS or post-laser TAPS) were excluded as well due to their added adverse intrauterine circumstances.

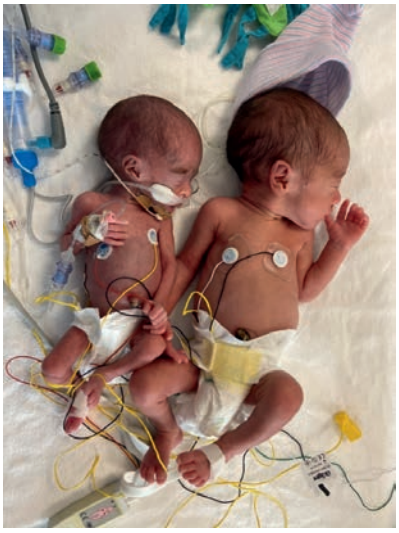

Figure 1. A MC twin pair with sFGR born at a gestational age of 30 weeks with respective birth weights of 500 grams (left) and 1350 grams (right). The picture was taken ten days after birth.

The following maternal and neonatal baseline characteristics were collected from medical records: maternal age, gravidity, parity, presence of underlying maternal health problems, occurrence of preeclampsia (defined as new-onset hypertension in

the setting of proteinuria during pregnancy)[14], occurrence of gestational diabetes (defined as any degree of glucose intolerance with onset during pregnancy)[15], suspected chorioamnionitis (based on the following clinical criteria: maternal intrapartum fever, maternal leukocytosis, purulent cervical drainage, or fetal tachycardia)[16], presence of umbilical artery (UA) Doppler abnormalities (defined as persistent or intermittent absent or reversed end-diastolic flow (A/REDF)[17], occurrence of TTTS (diagnosed according to the Eurofoetus criteria[18]), gestational age at diagnosis of TTTS, gestational age at laser coagulation, amnionicity, gestational at birth, sex, delivery mode, presence of fetal distress at birth (defined as an abnormal CTG), whether a full course of corticosteroids was administered prior to delivery and BWD. Birth weight and the proportion of neonates that were born small for gestational age (SGA) (defined as birth weight < 10th centile) were compared between the larger and the smaller twin. Differences in baseline characteristics for cases with and without TTTS were evaluated as well. All MC twin placentas were routinely injected with colored dye as part of standard care to evaluate vascular patterns and placental share[19]. Fetal territories were distinguished by either the laser demarcation line, or the margins of the twin-specific dyes and expressed as a percentage of the total placental area measured using Image J version 1.57.

Primary outcomes were the prevalence of respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD). RDS was diagnosed based on the clinical picture of a neonate with respiratory failure receiving mechanical ventilation and/or surfactant, confirmed by a chest X-ray[20]. BPD was diagnosed when the neonate required treatment with > 21% oxygen for at least 28 days[21]. Secondary outcomes included persistent pulmonary hypertension of the newborn (PPHN) (defined as the failure of circulatory transition after birth requiring treatment with nitric-oxide (NO)[22]), patent ductus arteriosus (PDA) requiring medical treatment or surgical closure, necrotizing enterocolitis (NEC) ≥ stage 2[23], neonatal sepsis (defined as a clinically ill neonate with positive blood cultures), asphyxia[24] (defined as at least three of the following criteria: signs of fetal distress before delivery; Apgar score < 5 at 5 minutes; arterial pH < 7.1 and base excess ≥ 16 mmol/L or lactate > 10 mmol/L in either arterial umbilical cord blood or capillary blood gas sample within 1 hour after birth; respiratory failure requiring resuscitation measures during at least 5 minutes after birth; multiple organ failure), neonatal mortality (defined as mortality within the first 28 days after birth), Apgar scores at 1, 5 and 10 minutes, proportion of twins requiring major resuscitation at birth (defined as chest compression and/or epinephrine administration), proportion of twins intubated at birth, proportion of twins with

continuous positive airway pressure (CPAP) during transport to the neonatal intensive care unit (NICU), proportion of twins who received surfactant, whether surfactant was administrated via minimally invasive surfactant therapy (MIST), number of doses of surfactant, proportion of twins who received mechanical ventilation, duration of mechanical ventilation, day of start after birth of mechanical ventilation, proportion of twin who received high frequency oscillation (HFO), duration of HFO, proportion of twins who received CPAP, duration of CPAP, proportion of twins who received non-invasive respiratory support (defined as CPAP, high flow and low flow), duration of non-invasive respiratory support, the total duration of respiratory support until discharge to home (including both non-invasive respiratory support and mechanical ventilation), duration of NICU admission and respiratory support at discharge from the NICU (either no respiratory support or non-invasive respiratory support). The outcomes were compared between the larger and the smaller twin within each twin pair.

Statistical analyses were performed using IBM SPSS Statistics Version 25.0 (SPSS, Inc., an IBM company, Chicago, IL, USA). Data are presented as median (interquartile range), n/N (%) or n (%). To test for association between FGR and neonatal morbidity and mortality (categorical data), we used a Generalized Estimated Equation (GEE) to analyze within-pair differences. The association between FGR and type of respiratory support was analyzed similarly. When an outcome was not observed 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, as the GEE cannot be used when an outcome event does not occur in one of the groups. This adjustment generates more conservative *p*-values. The analysis of RDS was corrected for birth order, as it has previously been described that the firstborn in twin pregnancies is more susceptible to RDS[25,26]. To test for association between FGR and start/duration of respiratory support (numerical data), a Wilcoxon Signed Rank Test was used to assess within-pair differences. Both the GEE and the Wilcoxon Signed Rank Test account for the fact that observations between co-twins are not independent. We did not correct for multiple testing, because our primary outcomes (BPD and RDS) share etiological factors and are thereby not independent but related. So, we expect to observe consistent associations between our outcomes of interest. Differences in baseline characteristics between cases with and without TTTS were analyzed using a Mann-Whitney-U (numerical data) or a Chi-square test (categorical data). A *p*-value of < 0.05 was considered statistically significant.

Results

A total of 587 live-born MC twin pairs were delivered at the LUMC between 2010-2019 (Figure 2). After exclusion according to the aforementioned criteria (n = 216) and the cases without sFGR (n = 277), 94 twin pairs with sFGR were included for analysis.

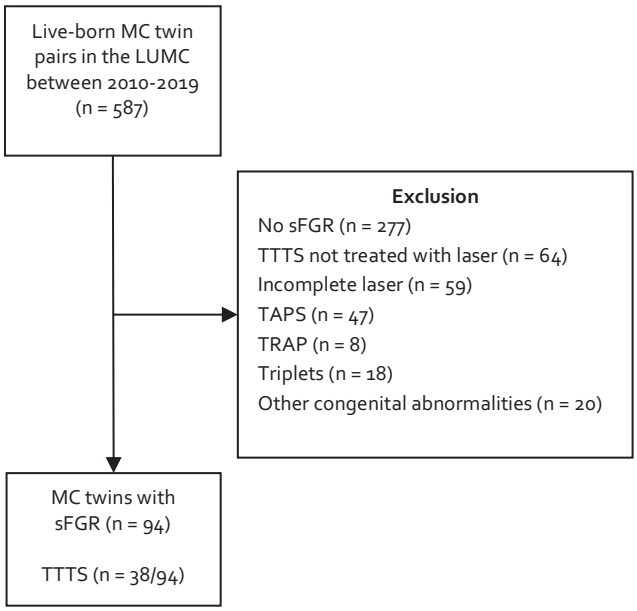

Figure 2. Flowchart of study inclusion.

Maternal and neonatal baseline characteristics are summarized in Table 1. Preeclampsia was diagnosed in 7.4% (7/94) and gestational diabetes in 5.3% (5/94) of pregnancies. Chorioamnionitis was suspected in 8.5% (8/94) of pregnancies. UA Doppler abnormalities were documented in 50 pregnancies, of which 20.2% (19/94) were persistent A/REDF and 33.0% (31/94) were intermittent A/REDF. Nearly half (40.4% (38/94)) of the included twin pairs were diagnosed with TTTS and successfully treated with laser therapy. Median gestational age at birth was 32.3 (30.4–34.3) weeks. The twins were delivered by way of caesarean section in 66.0% (124/188) of cases. Fetal distress was present in 50% (47/94) of cases and 72% (67/93) received a full course of corticosteroids prior to delivery. The median BWD was 31.8% (26.7-40.8). The median birth weight of the larger twin was 1819 (1464-2336) and 1183 (912-1490) grams for the smaller twin. In the smaller twins, the proportion of neonates born SGA was 97.9% (92/94) as opposed to 13.8% (13/94) in the larger twins. The larger twin had a significantly larger placental share (68.9% (63.0-73.9)) compared to the smaller twin (31.1% (26.1-37.0)).

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

Characteristics	sFGR twins (n=188; 94 pregnancies)	Larger twin (n=94)	Smaller twin (n=94)
Maternal age – *years*	31 (28-35)		
Gravidity	2 (1-3)		
Parity	0 (0-1)		
Maternal health problems	7/94 (7.4)		
Pregnancy complications			
Preeclampsia	7/94 (7.4)		
Gestational diabetes	5/94 (5.3)		
Suspected chorioamnionitis	8/94 (8.5)		
UA Doppler abnormalities			
A/REDF	19/94 (20.2)		
iA/REDF	31/94 (33.0)		
TTTS	38/94 (40.4)		
Gestational age at diagnosis TTTS – *weeks*	18.5 (16.4-20.2)		
Gestational age at laser – *weeks*	18.7 (16.7-20.8)		
Monoamniotic twins	3/94 (3.2)		
Gestational age at birth – *weeks*	32.3 (30.4-34.3)		
Gestational age at birth < 32 weeks	43/94 (45.7)		
Female	88/188 (46.8)		
Caesarean	124/188 (66.0)		
Fetal distress	47/94 (50.0)		
Full course of corticosteroids prior to delivery	67/93 (72.0)		
Birth weight discordance – *%*	31.8 (26.7-40.8)		
Birth weight – *grams*		1819 (1464-2336)	1183 (912-1490)
Small for gestational age		13/94 (13.8)	92/94 (97.9)
Placental share – *%*		68.9 (63.0-73.9)	31.1 (26.1-37.0)

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, TTTS: Twin-twin transfusion syndrome. Outcomes are presented as median (interquartile range (IQR)) or n/N (%).

Neonatal morbidity and respiratory outcomes for the larger and smaller twin are presented in Table 2 and 3, respectively. The Apgar score at one minute was 7 (5-8) for the smaller twin and 8 (6-9) for the larger twin ($p < 0.0001$). The prevalence of RDS was lower in the smaller twin compared to the larger twin (19.1% (18/94) vs. 34.0% (32/94), $p = 0.004$). The odds of RDS for the larger twin was doubled (OR 2.1 (CI95% 1.3-3.5). The large twin required surfactant more often, in 27.7% (26/94) of cases, compared to 10.8% (10/93) of cases in the smaller twin ($p < 0.0001$). The median duration of mechanical ventilation for the smaller twin was 8 (1-18) days as opposed to

2 (1-4) days for the larger twin (p = 0.016). The median duration of NICU admission was 6 (2-14) days for the smaller twin and 7 (3-20) days for the larger twin (p = 0.001).

The prevalence of BPD was significantly higher in the smaller twin as opposed to the larger twin (16.7% (15/90) vs 6.7% (6/89), (p = 0.008). The odds of developing BPD for the smaller twin were more than doubled (OR 2.5 (CI95% 1.3-4.9)). The median gestational age at birth of the group with BPD was 28.6 (IQR 28.0-29.8). Of the large twins that developed BPD, 66.7% (4/6) received mechanical ventilation, as opposed to 46.7% (7/15) of the small twins. The median duration of the mechanical ventilation for the large twins with BPD was 3 (2-7) days vs. 14 (2-27) days for the small twins with BPD.

Table 2. Neonatal morbidity in the larger versus the smaller twin.

Outcomes	Larger twin (n=94)	Smaller twin (n=94)	p-value
RDS	32/94 (34.0)	18/94 (19.1)	**0.004***
BPD	6/89 (6.7)	15/90 (16.7)	**0.008**
PPHN	6/92 (6.5)	5/93 (5.4)	0.749
PDA	3/94 (3.2)	7/94 (7.4)	0.167
NEC	0/94 (0.0)	2/94 (2.1)	0.175
Sepsis	11/92 (12.0)	15/91 (16.5)	0.333
Asphyxia	1/94 (1.1)	2/94 (2.1)	0.571
Neonatal mortality	2/94 (2.1)	2/94 (2.1)	1.000

RDS: respiratory distress syndrome, BPD: bronchopulmonary dysplasia, PPHN: persistent pulmonary hypertension of the newborn, PDA: patent ductus arteriosus, NEC: necrotizing enterocolitis, CPAP: continuous positive airway pressure.
Outcomes are presented as n/N (%).
*corrected for birth order

Differences in baseline characteristics were examined for cases with and without TTTS. Significant differences were found for the presence of fetal distress (34.2% (13/38) in cases with TTTS vs. 60.7% (34/56) in cases without TTTS, p = 0.012) and delivery mode (caesarean section in 50.0% (38/76) of cases with TTTS as opposed to 76.8% (86/112) in cases without TTTS, p < 0.0001). Furthermore, the presence of TTTS was associated with three outcomes, namely CPAP during transfer to the NICU (40.3% in TTTS cases as opposed to 64.5% (p = 0.004), still no difference between larger and smaller twin), the duration of mechanical ventilation (no differences between larger (2 (1-6) days) and smaller (3 (1-12) days) twin in TTTS cases (p = 0.285)) and the duration of NICU admission (no differences between larger (6 (2-12) days) and smaller (7 (3-17) days) twin in TTTS cases (p = 0.326)).

Table 3. Respiratory outcomes in the larger versus the smaller twin.

Outcomes	**Larger twin (n=94)**	**Smaller twin (n=94)**	***p*-value**
Apgar			
1 minute	8 (6-9)	7 (5-8)	**<0.0001**
5 minutes	9 (8-9)	9 (8-9)	0.282
10 minutes	9 (8-10)	9 (8-10)	0.816
Major delivery room resuscitation	0/94 (0.0)	1/93 (1.1)	0.324
Intubation at birth	7/92 (7.6)	7/91 (7.7)	0.985
CPAP to NICU	49/92 (53.3)	51/91 (56.0)	0.667
Surfactant	26/94 (27.7)	10/93 (10.8)	**<0.0001**
MIST	12/26 (46.2)	4/10 (40.0)	0.341
Number of doses	1 (1-2)	1 (1-2)	1.000
Mechanical ventilation	23/94 (24.5)	19/93 (20.4)	0.388
Day of start	1 (1-2)	1 (1-3)	0.750
Duration – *days*	2 (1-4)	8 (1-18)	**0.024**
HFO	4/94 (4.3)	7/93 (7.5)	0.314
Duration – *days*	2 (1-4)	7 (4-12)	*
CPAP	57/93 (57.3)	57/91 (51.6)	0.059
Duration – *days*	2 (1-5)	3 (1-11)	0.392
Non-invasive respiratory support	60/94 (63.8)	54/93 (58.1)	0.235
Duration – *days*	4 (1-10)	6 (1-31)	0.076
Total duration of respiratory support – *days*	5 (1-11)	4 (1-32)	0.110
Duration NICU admission	7 (3-20)	6 (2-14)	**0.001**
Respiratory support at discharge NICU			
No support	79/90 (87.8)	73/91 (80.2)	0.077
Non-invasive respiratory support	11/90 (12.2)	18/91 (19.8)	0.139

NICU: neonatal intensive care unit, MIST: minimally invasive surfactant therapy, HFO: high frequency oscillation.

Outcomes are presented as median (IQR) or n/N (%).

*No within-pair comparison possible, due to low number of cases.

Discussion

This study shows that while fetuses suffering from FGR may be 'protected' from acute respiratory morbidity at birth their odds of developing chronic respiratory morbidity is more than doubled. Our results originate from a simple but unique natural experiment in identical twins, comparing the respiratory outcomes in the smaller growth restricted twin to the larger co-twin. Despite their identical genetic constitution and identical maternal factors, their odds of specific respiratory morbidity differ significantly. We are the first to describe detailed respiratory outcomes, including BPD, in a cohort of monochorionic twins. Our data emphasize that an adverse intrauterine environment may have a pathophysiologic effect on neonatal lung development.

Multiple pathways have been implicated to explain the mechanism behind the respiratory pathology associated with FGR. A widely proposed hypothesis is that the smaller twin has an increased corticosteroid production as a result of prolonged exposure to prenatal stress, by which the smaller twin is better prepared for the neonatal transition following elective premature delivery as opposed to the large twin[27]. This is reflected in a lower prevalence of RDS for the smaller twin in our study population. One can speculate that the corticosteroids produced by the smaller twin can be transferred to the large twin through the vascular anastomoses[28]. When separately examining the lasered TTTS cases in our population, the significant difference in RDS rate for the larger and smaller twin is still present (13.5% in the larger twin vs. 28.8% in the smaller twin, p = 0.011), indicating a negligible effect of this transfer.

Simultaneously, FGR is thought to already negatively affect lung development *in utero* by inducing persistent structural and functional changes to the respiratory system associated with the pathophysiology of BPD[5,29]. Firstly, chronic hypoxia as caused by FGR, can impair pulmonary angiogenesis by way of reactive oxygen species and a down- or upregulation of key proteins (such as elastin and collagen, respectively) and growth factors (such as vascular endothelial growth factor)[30]. Pulmonary vascular resistance increases, in turn causing further cardiovascular adaptations amongst which increased vascular stiffness and cardiac hypertrophy. Secondly, the impaired vascularization disrupts the alveolarization as well, resulting in poor alveolar morphology[31]. Combined, gas exchange becomes inefficient[32,33]. So, the abnormal lung development associated with FGR increases the vulnerability for postnatal insults and thereby the risk of BPD.

A factor possibly complicating the interpretation of our data is the prolonged mechanical ventilation in the smaller twin, which has been identified as an independent risk factor for BPD[34]. One may speculate that the increased BPD rate for the smaller twin is not solely attributable to the FGR but also to the extensive mechanical ventilation following the presence of, as is largely the case in our population, severe neonatal morbidity. Nonetheless, animal models have shown that FGR does not further exacerbate ventilation induced lung injury[35]. Moreover, it is important to realize that both the development of complications and thereby the need for prolonged mechanical ventilation are closely interwoven with FGR as well. In addition, caretakers might be more prone to prolong ventilation based on the perceived vulnerability of SGA neonates, regardless of the true necessity for respiratory support at that point. As the etiology of BPD is multifactorial, similarly to the etiology of FGR, it is difficult to discern through which mechanisms the association between FGR and BPD truly runs. Still, there is an apparent association that should be taken into account in clinical practice.

Furthermore, the distinctive etiologies of FGR in singletons and sFGR in MC twins should be considered. While sFGR in MC twins is characterized by unequal placental sharing and thereby a small placental volume for the smaller twin, FGR in singletons comes from placental insufficiency caused by abnormal placentation. Impaired trophoblast invasion results in inadequate remodeling of the uterine spiral arteries, reducing uteroplacental blood flow[36]. So, the mechanisms of chronic hypoxia in FGR and sFGR differ significantly, possibly affecting outcomes.

Regarding twin research, our results are in agreement with previous studies identifying the presence of sFGR and being the larger twin as important risk factors for RDS[25-27,37]. However, these studies all lack a distinction in chorionicity (and zygosity) and are thereby still limited by genetic constitution as a potential confounder. Moreover, information on the prevalence of BPD is often missing from these studies as well as a within-pair comparison. The only study on respiratory morbidity in growth discordant MC twins was performed at our center and found that the larger twin is at increased risk of RDS (32% vs. 6%), compared to the smaller twin[9]. Our results are complementary, albeit in a larger study population with a more thorough examination of additional respiratory outcomes including BPD. Another small study that conducted a within-pair comparison of twins with discordant fetal growth patterns (once more lacking a distinction in chorionicity and zygosity) has shown that the small twin had a significantly decreased lung function and an

increased bronchial reactivity at the age of 16 years[38]. These results affirm the long-lasting effects of FGR that need to be researched more thoroughly.

The retrospective nature of our study should be considered when interpreting the results, as the documentation of BPD was largely based on hospital discharge letters. A timed oxygen reduction test which is the current golden standard in the diagnosis of BPD was only performed in 22% of BPD cases in our population[39]. Additionally, as the LUMC is the national referral center for complicated MC twin pregnancies referral bias may enlarge the proportion of twins with an anticipated severe neonatal course. On the other hand, our population is inherently at low risk of BPD due to the low number of extremely preterm neonates (gestational age at birth < 28 weeks and/or birth weight < 1000 grams), leading to relatively few neonates with BPD. This possibly limits the applicability of our results to higher risk preterm neonates and thereby also the use of newer BPD definitions[40]. Prospective research evaluating both neonatal respiratory morbidity and childhood lung function, not solely focusing on BPD cases as those without BPD are not necessarily free of respiratory morbidity[41], is necessary to provide more conclusive evidence. Nevertheless, the results of our study are strengthened by the extensive documentation of respiratory data and the size and unique nature of our study population, consisting of monozygotic twins discordant for intrauterine exposures that can thereby act as each other's control for genetic, maternal and obstetrical factors. Moreover, all twins were born in a single specialized center, limiting treatment variation and resulting in availability of information on resuscitation, surfactant supplementation and initial respiratory support.

In conclusion, in MC twins FGR is associated with a reduced risk of respiratory distress at birth but an increased the risk of BPD in the smaller twin, emphasizing the early consequences of FGR for lung structure and functioning. Our study helps improve the perhaps counterintuitive anticipation of respiratory problems after birth for both pediatricians and parents. It also provides a basis for risk assessment of chronic lung disease in childhood and throughout adulthood, pressing the need for larger, prospective studies on respiratory morbidity in MC twins with sFGR to further research the early origin of lung disease.

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Chapter 5

Early structural cardiovascular changes after adverse intrauterine circumstances in identical twins: a cohort study using neonatal cardiac ultrasound.

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Abstract

Objective. To investigate early structural cardiovascular remodeling after fetal growth restriction (FGR) in a cohort of identical twins, controlling for confounding of genetic and maternal factors.

Design. Prospective observational cohort study.

Setting. Single-center, population-based.

Patients. Live-born monochorionic twins born between January 2019 – June 2021.

Interventions. Transthoracic cardiac ultrasound within one week after birth.

Main outcome measure. Z-scores for cardiac valve annuli diameters and left ventricle dimensions were calculated based on gestational age at birth and compared between smaller and larger twins. The z-score difference between birth weight and each cardiac structure per twin was tested against the intercept, to assess heart sparing. A value >0 indicates that the cardiac structure is less affected by FGR than birth weight (heart sparing). A value <0 indicates that the cardiac structure is more affected by FGR than birth weight.

Results. Median gestational age at birth of the 100 included twin pairs was 33.8 (interquartile range (IQR) 30.8-36.1) weeks, with birth weights of 1729 (IQR 1200-2115) grams for the smaller twin and 2058 (IQR 1643-2500) grams for the larger twin. All structures showed the same association in which the smaller twin had a lower z-score than the larger twin. The z-score difference in birth weight and cardiac structure was significantly higher than the intercept in all but one structure (tricuspid valve).

Conclusions. While cardiac structures are generally smaller for the twin with the lower birth weight, the deviation in birth weight tends to be more pronounced than the deviation in cardiac structure, indicative of heart sparing.

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Introduction

Adverse intrauterine circumstances are thought to negatively impact lifelong health. Previous research has demonstrated a strong association between fetal growth restriction (FGR) and an increased risk of cardiovascular disease (CVD) in adulthood[1,2]. The mechanism behind this association is thought to be fetal programming: structural and functional adaptations in fetal development in response to a suboptimal environment that are persistent throughout life[3].

Cardiovascular remodeling can already be observed in fetuses with FGR. The morphometry of the heart becomes more spherical with thickened myocardial walls[3]. Cardiomegaly has been implicated as an expression of 'heart sparing', i.e., blood flow redistribution favoring major organs when in a prolonged state of hypoxia[4]. These subtle but disadvantageous changes may persist after birth. Echocardiographic studies in children born after FGR have reported smaller cardiovascular dimensions and mass as well as greater arterial wall stiffness from early childhood until preadolescence[5-7]. Yet, neonatal cardiovascular changes after FGR are largely unreported, while these form the basis for tracking the lifelong cardiovascular consequences of FGR. Moreover, the scarcely available studies are limited by their study design in which small for gestational age (SGA) neonates are compared to appropriately-grown neonates, inherently subject to confounding[8,9]. A population of identical twins offers a solution.

Monochorionic (MC) twins are genetically identical and share a single placenta during pregnancy. This shared placenta can give rise to a range of complications, including a discordant antenatal growth pattern following from an unequally shared placenta[10]. A within-pair comparison in this study population thereby controls for any potential confounding of genetic and maternal factors. Therefore, this study aims to compare structural cardiac measurements and aortic pulse-wave velocity (aPWV) between the smaller and larger twin at birth and to assess whether heart sparing is present using neonatal cardiac ultrasound.

Methods

This study is part of the Twinlife study (Twin Longitudinal Investigation of FEtal discordance; Netherlands Trial Register ID NL7538). This is a prospective, longitudinal cohort study including all MC twins born in the Leiden University Medical Center (LUMC) from January 2019 onwards[11]. The Twinlife study was reviewed and approved by the ethics committee of the LUMC (P18.184) and inclusion is still ongoing. All parents are asked for informed consent. Exclusion criteria are triplet pregnancies, perinatal mortality (intrauterine fetal demise, selective reduction, termination of pregnancy or neonatal death before cardiac ultrasound), cases with twin reversed arterial perfusion or congenital abnormalities.

The following baseline characteristics were collected: maternal age, gravidity, parity, type of MC twin pathology (twin-twin transfusion syndrome (TTTS) diagnosed according to the Eurofetus criteria[12], twin anemia polycythemia sequence (TAPS)[13] and selective fetal growth restriction (sFGR), defined as a birth weight discordance (BWD) ≥ 20% in the absence of TTTS/TAPS with BWD calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100[14]), fetoscopic laser coagulation or other treatment modalities for TTTS/TAPS (amniodrainage, intrauterine transfusion (IUT) with/without partial exchange transfusion (PET)), gestational age at birth, sex, delivery mode, BWD, birth weight with z-score (singleton curves based on gestational age and sex), proportion of neonates born SGA (defined as birth weight < 10th centile[15,16]) and the presence of a patent ductus arteriosus requiring either medical or surgical treatment.

Transthoracic cardiac ultrasound including two-dimensional M-mode was performed by a senior pediatric cardiologist (AAWR) within one week after birth according to the guidelines of the American Society for Echocardiography[17,18]. Mitral valve (MV) annulus diameter and tricuspid valve (TV) annulus diameter were measured in an apical four-chamber view. Aortic valve (AV) annulus diameter was measured in the parasternal long-axis view and pulmonary valve (PV) annulus diameter in the parasternal short-axis view. The following parameters were measured in M-mode: left atrium diameter (LA), aortic root diameter (Ao), LA/Ao ratio, left ventricular internal diameter in diastole (LIVDD) and systole (LVIDS), left ventricular posterior wall thickness in diastole (LVPWD) and systole (LVPWS), LV mass[19], ejection fraction, fractional shortening calculated as (LVIDD – LVIDS)/LVIDD x 100, and relative wall thickness (RWT) calculated as (2 x LVPWD)/LVIDD)[18-20]. All measurements were performed by a single investigator (SGG) under supervision of a senior pediatric

cardiologist (AAWR). Z-scores for the cardiac vale annuli diameters and LV parameters were calculated based on gestational age at birth[21,22].

To determine aPWV, a measure of aortic stiffness, two pulsed Doppler recordings of the aorta were made at the level of the AV and the descending aorta (Figure 1)[23-25]. A similar time point in the electrocardiogram was chosen for both recordings and the time until onset of flow was measured three times in both recordings to calculate an average. The transit time was calculated as: average time of onset of flow at descending aorta – average time of onset of flow at AV. The length of the aorta from the location of the first Doppler recording to the other was determined using Osirix DICOM viewer[26]. aPWV was calculated as: (aortic length)/(100 x transit time)[25].

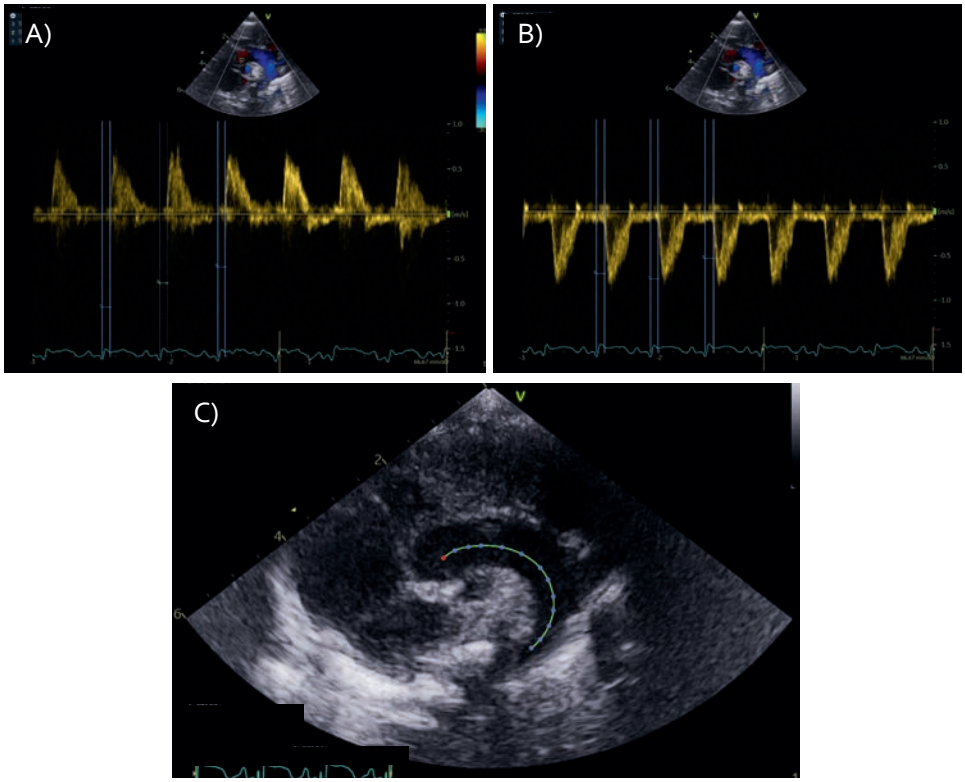

Figure 1. Measurement of the aPWV; A) pulsed Doppler recording at the level of the AV with three measurements of time until flow, B) pulsed Doppler recording at the descending aorta with three measurements of time until flow, C) length of the aorta from the location of pulsed Doppler recording A to pulsed Doppler recording B.

The difference between the z-score of birth weight and the z-score of each cardiac structure was calculated per individual twin to assess the presence of heart sparing. A value >0 indicates that the deviation of the cardiac structure is less than the deviation of birth weight in relation to gestational age at birth, i.e., the cardiac structure is less affected than birth weight (heart sparing). A value <0 indicates that the deviation of the cardiac structure is more than the deviation of birth weight in relation to gestational age at birth, i.e., the cardiac structure is more affected than birth weight. The relationship between the z-scores of birth weight and the cardiac structures was plotted with a line depicting x = y (representing a one-to-one relationship). Values above this line indicate that the cardiac structure was less affected than birth weight relative to gestational age at birth and values below the line indicate that the cardiac structure was more affected than birth weight relative to gestational age at birth.

To test for association between 1) twin size (smaller/larger) and the cardiovascular outcomes and 2) the difference in z-scores of birth weight and cardiac structures and the intercept (representing the absence of a difference in these z-scores), a Generalized Estimating Equation (GEE) was used. This method accounts for the fact that we study paired data (twin pairs) while also allowing us to control for covariates. It is well-known that in MC twins TTTS can influence fetal cardiac development, differentially for the donor and recipient, due to intertwin hemodynamic imbalances[27]. The same is presumed for TAPS, albeit not thoroughly researched. As prenatal growth is our primary focus, we have chosen to examine the effect of twin size (smaller/larger) in this study and regard the group of included MC twins as a whole. To take into account any potential influences of the different types of MC twin complications (TTTS, TAPS, sFGR, uncomplicated) on the cardiac parameters, we first tested this association using a GEE. When there was a statistically significant association, the analysis of twin size for that outcome parameter was corrected for the type of complication. Statistical analyses were performed using IBM Statistics Version 25.0 (SPSS, Inc. an IBM company, Chicago, IL, USA) and R Version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented as median (interquartile range (IQR)), n/N (%) or n (%). A *p*-value of < 0.05 was considered statistically significant.

Results

Between January 2019 and June 2021, 105 parents agreed to participate in the study (inclusion rate 89% (105/118); Figure 2). In four cases, we were unable to perform a cardiac ultrasound for logistic reasons (fast transfer to a peripheral hospital after birth, unavailability of the pediatric cardiologist and parental COVID-19 infection). In one case, a congenital abnormality was detected within the first weeks after birth with subsequent exclusion. aPWV was missing in 24 cases due to unavailability of staff trained to perform this measurement at the time of these ultrasounds.

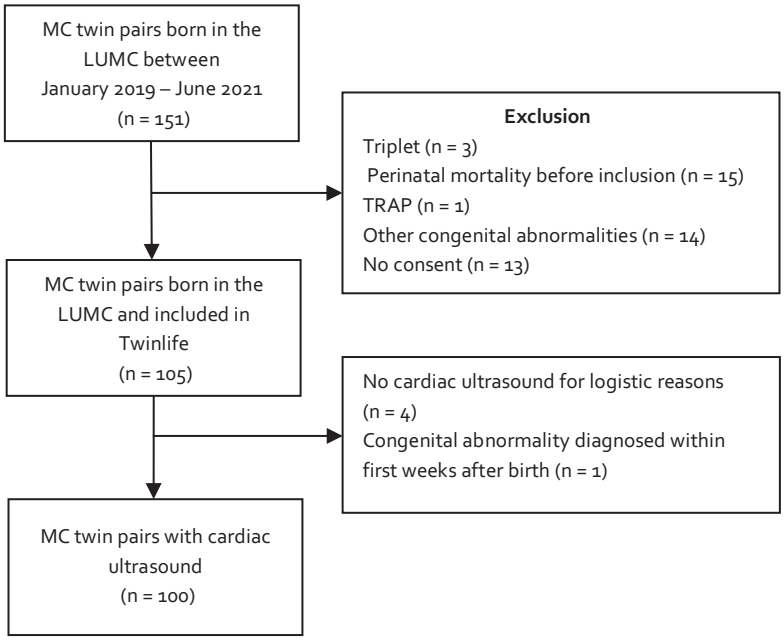

Figure 2. Flowchart of study inclusion.

Median gestational age at birth was 33.8 (IQR 30.8-36.1) weeks (Table 1). The smaller twin had a median birth weight of 1729 (IQR 1200-2115) grams and the larger twin of 2058 (IQR 1643-2500) grams. Patent ductus arteriosus requiring treatment was present in four cases (three smaller twins, one larger twin born between 27-34 weeks (p = 0.340)). Of all pregnancies, 41% (41/100) was complicated by TTTS, of which 90% (37/41) were treated with fetoscopic laser coagulation which was successful in 36/37 cases; 17% (17/100) was complicated by TAPS of which 13% (2/17) were successfully treated with fetoscopic laser coagulation in the TAPS trial (ClinicalTrails.gov ID NCT04432168); 19% (19/100) was complicated by sFGR in the absence of TTTS or TAPS; and 23% (23/100) was uncomplicated.

Table 1. Baseline maternal, obstetrical and neonatal characteristics for included twins.

Characteristics	**MC twins (n=200; 100 pregnancies)**
Maternal age – *years*	32 (29-34)
Gravidity	2 (1-3)
Parity	1 (0-1)
MC twin pathology	
Uncomplicated	23 (23)
TTTS	41 (41)
Fetoscopic laser coagulation	37 (90)
Other treatment	4 (10)
TAPS	17 (17)
Fetoscopic laser coagulation	2 (13)
Other treatment	15 (87)
sFGR	19 (19)
Gestational age at birth – *weeks*	33.8 (30.8-36.1)
Female	96 (48)
Caesarean	123 (62)
Birth weight discordance – *%*	13 (7-27)
Birth weight – *grams*	
Smaller twin	1729 (1200-2115)
z-score	-1.1 (-1.7--0.7)
Larger twin	2058 (1643-2500)
z-score	-0.3 (-0.7-0.1)
Small for gestational age	
Smaller twin	63 (63)
Larger twin	17 (17)

MC: monochorionic, TTTS: twin-twin transfusion syndrome, TAPS: twin anemia-polycythemia sequence, sFGR: selective fetal growth restriction.
Outcomes are presented as median (interquartile range (IQR)).

All cardiac valve annuli diameters showed the same association in which the smaller twin had a lower z-score (0.6 difference for MV, 0.5 difference for TV and AV, 0.3 difference for PV, with $p < 0.0001$ for MV, TV and AV and $p = 0.019$ for PV; Table 2). Similarly, both the LA diameter and Ao diameter were smaller in the smaller twin, with a 0.8 mm ($p = 0.002$) and a 0.4 mm difference ($p = 0.002$). A similar association was observed for the z-scores of the LV dimensions with lower z-scores for the smaller twin (0.3 difference for LVIDD ($p = 0.001$) and LVIDS ($p = 0.002$), 0.1 difference for LVPWD ($p = 0.020$) and LVPWS ($p = 0.010$)). The analysis of LVPWD was corrected for type of complication, following the significant association of $p = 0.033$ (Table S1). LV mass was lower for the smaller twin as opposed to the larger twin (5.1 (IQR 4.0-6.6)

grams vs. 6.0 (IQR 4.5-7.7) grams, $p < 0.0001$), as was RWT (0.34 (IQR 0.29-0.40) vs. 0.36 (IQR 0.29-0.47), $p = 0.020$). Functional parameters did not differ within twin pairs. No significant difference was found for aPWV, with 3.1 (IQR 2.4-5.3) m/s in the smaller twin and 3.3 (IQR 2.5-4.4) m/s in the larger twin ($p = 0.259$).

Table 2. Structural and functional cardiac parameters for the smaller and larger twin.

Outcomes	Smaller twin (n=100)	Larger twin (n=100)	*p*-value
Cardiac valve annuli diameter			
MV – mm	9.9 (9.1-11.1)	10.8 (9.8-11.9)	
z-score	-0.8 (-1.6--0.2)	-0.2 (-0.9-0.5)	**<0.0001**
TV – mm	10.4 (8.9-11.7)	11.0 (9.8-11.9)	
z-score	-1.3 (-2.4--0.3)	-0.8 (-1.6--0.1)	**<0.0001**
AV – mm	5.9 (5.3-6.5)	6.1 (5.8-6.7)	
z-score	-0.5 (-1.2-0.2)	0.0 (-0.7-0.7)	**<0.0001**
PV – mm	6.6 (5.9-7.4)	6.9 (6.2-7.5)	
z-score	0.8 (-0.2-1.7)	1.1 (0.2-2.0)	**0.019**
LA diameter – mm	10.7 (9.4-12.1)	11.5 (10.2-12.7)	**0.002**
Ao diameter – mm	8.2 (7.2-9.0)	8.6 (7.7-9.7)	**0.002**
LA/Ao ratio	1.3 (1.2-1.5)	1.3 (1.1-1.5)	0.347
Left ventricular dimensions			
LVIDD – mm	14.8 (13.3-16.2)	15.5 (13.7-16.8)	
z-score	0.5 (-0.1-1.3)	0.8 (0.2-1.7)	**0.001**
LVPWD – mm	2.5 (2.2-2.9)	2.7 (2.3-3.3)	
z-score	-0.2 (-0.9--0.1)	-0.1 (-0.8-0.2)	**0.020***
LVIDS – mm	9.6 (8.8-11.4)	10.7 (9.4-11.9)	
z-score	0.4 (-0.4-1.1)	0.7 (0.1-1.4)	**0.002**
LVPWS – mm	3.6 (3.1-4.0)	3.7 (3.3-4.6)	
z-score	-0.5 (-1.1-0.0)	-0.4 (-1.2-0.9)	**0.010**
LV mass – grams	5.1 (4.0-6.6)	6.0 (4.5-7.7)	**<0.0001**
LV ejection fraction – %	64.0 (57.7-70.5)	61.2 (56.0-69.2)	0.108
Fractional shortening – %	31.6 (27.9-36.6)	30.0 (26.6-35.7)	0.220
RWT	0.34 (0.29-0.40)	0.36 (0.29-0.47)	**0.020**
aPWV – m/s	3.1 (2.4-5.3)	3.3 (2.5-4.4)	0.259

MV: mitral valve, TV: tricuspid valve, AV: aortic valve, PV: pulmonary valve, LA: left atrium, Ao: aortic root, LV: left ventricular, LVIDD: end-diastolic left ventricular internal diameter, LVPWD: end-diastolic left ventricular posterior wall thickness, LVIDS: end-systolic left ventricular internal diameter, LVPWS: end-systolic left ventricular posterior wall thickness, RWT: relative wall thickness, aPWV: aortic pulse-wave velocity.

RWT calculated as (2 x LVPWD)/LVIDD

Outcomes are presented as median (IQR).

*corrected for type of complication.

Table S1. *p*-values per tested measurement for the association with type of complication (divided into TTTS, TAPS, sFGR or uncomplicated twins).

Outcomes	*p*-value
Cardiac valve annuli diameter	
MV z-score	0.772
TV z-score	0.127
AV z-score	0.377
PV z-score	0.292
LA diameter – mm	0.627
Aortic root diameter – mm	0.196
LA/Ao ratio	0.104
Left ventricular dimensions	
LVIDD z-score	0.141
LVPWD z-score	**0.033**
LVIDS z-score	0.734
LVPWS z-score	0.527
LV mass – grams	0.186
LV ejection fraction – %	0.100
Fractional shortening – %	0.108
RWT	0.059
aPWV – m/s	0.184

MV: mitral valve, TV: tricuspid valve, AV: aortic valve, PV: pulmonary valve, LA: left atrium, Ao: aortic, LV: left ventricular, LVIDD: end-diastolic left ventricular internal diameter, LVPWD: end-diastolic left ventricular posterior wall thickness, LVIDS: end-systolic left ventricular internal diameter, LVPWS: end-systolic left ventricular posterior wall thickness, RWT: relative wall thickness, aPWV: aortic pulse-wave velocity.

The cardiac structures were less affected than birth weight in relation to gestational age at birth, indicated by a significantly higher difference in z-scores compared to the intercept in all but one parameter (Table 3): 0.2 (IQR -0.6-1.2) for MV (p =0.011), 0.5 (IQR -0.4-1.3) for AV (p = 0.002), 1.6 (IQR 0.6-2.7) for PV, 0.4 (IQR -0.2-1.1) for LVIDD, 0.8 (IQR 0.1-1.4) for LVPWD, 1.3 (IQR 0.4-2.1) for LVIDS and 0.3 (IQR -0.4-1.4) for LVPWS, all with $p < 0.0001$. The sole structure that was affected to a greater extent than birth weight in relation to gestational age at birth, i.e., significantly lower than the intercept, was the TV (-0.5 (IQR -1.2-0.5), $p < 0.0001$). These associations are also depicted in Figure 3, illustrating that the majority of twins is above the x = y line.

Table 3. The difference in z-score of birth weight and z-score of the cardiac structure tested against the intercept.

Differences in z-scores	MC twins (n=200)	*p*-value
Cardiac valve annuli diameter		
Birth weight - MV	0.2 (-0.6-1.2)	**0.011**
Birth weight - TV	-0.5 (-1.2-0.5)	**0.002**
Birth weight - AV	0.5 (-0.4-1.3)	**<0.0001**
Birth weight - PV	1.6 (0.6-2.7)	**<0.0001**
Left ventricular dimensions		
Birth weight - LVIDD	0.4 (-0.2-1.1)	**<0.0001**
Birth weight - LVPWD	0.8 (0.1-1.4)	**<0.0001**
Birth weight - LVIDS	1.3 (0.4-2.1)	**<0.0001**
Birth weight - LVPWS	0.3 (-0.4-1.4)	**<0.0001**

MV: mitral valve, TV: tricuspid valve, AV: aortic valve, PV: pulmonary valve, LVIDD: end-diastolic left ventricular internal diameter, LVPWD: end-diastolic left ventricular posterior wall thickness, LVIDS: end-systolic left ventricular internal diameter, LVPWS: end-systolic left ventricular posterior wall thickness, Outcomes are presented as median (IQR).

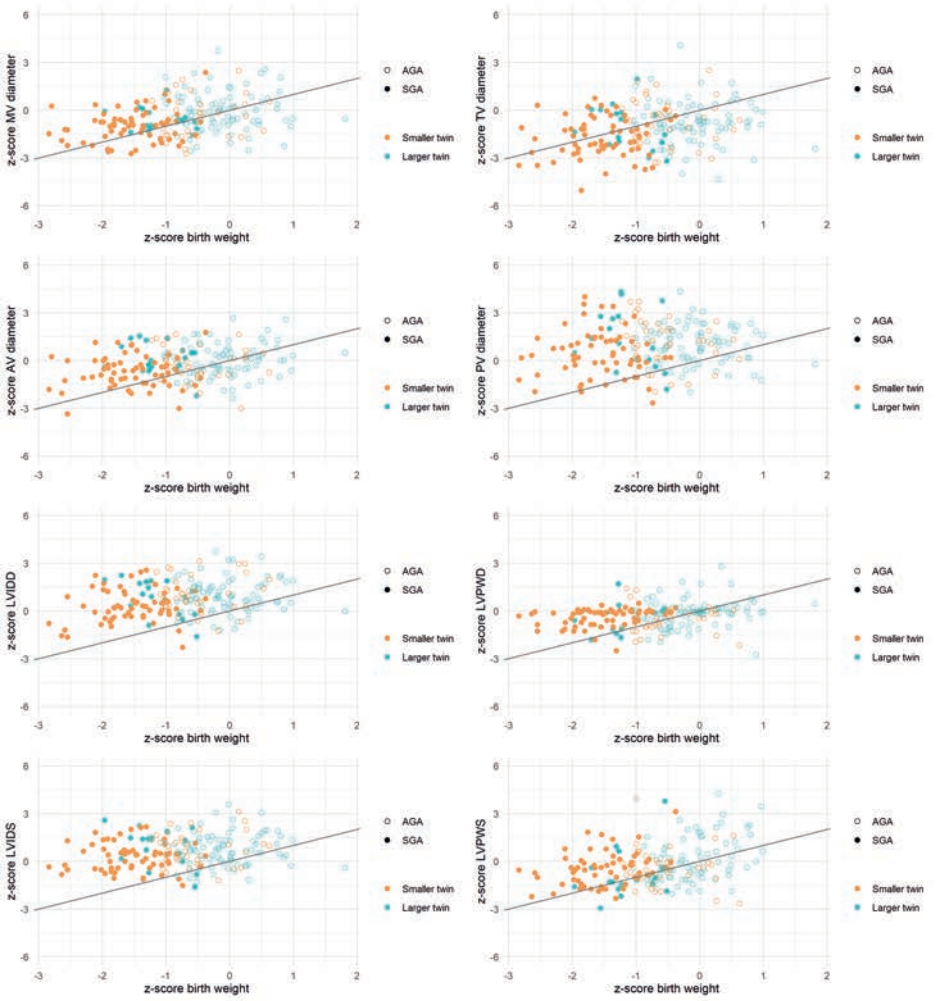

Figure 3. Scatterplots of the relationship between the z-score of the birth weight (x-axis) and the z-score of the cardiac structures (y-axis), with the line depicting x = y. Values above this line indicate that the cardiac structure was less affected than birth weight relative to gestational age at birth and values below the line indicate that the cardiac structure was more affected than birth weight relative to gestational age at birth.

Discussion

In MC twins, the smaller twin presents with a structurally smaller heart (including cardiac valve annuli, LA, Ao and LV dimensions) at birth when compared to the larger twin. Despite having smaller cardiac structures, the heart (except for the TV) appears to be less affected by FGR than body size for a given gestational age. This finding is indicative of heart sparing.

A fetus experiencing FGR adapts by redistributing blood flow towards major organs. This 'sparing' has been elaborately described in the context of the brain and is reflected by a larger head circumference relative to body size[28-30]. We have now observed a similar phenomenon for the heart in which the cardiac structures are less affected by FGR than body size. Redistribution of cardiac output is facilitated by vasodilation and increased blood flow towards essential vascular beds and vasoconstriction and decreased blood flow towards non-essential vascular beds[29]. This may be the cause of the smaller TV annulus diameter relative to birth weight as observed in our study, following preferential blood flow towards the brain through the left side of the heart, thereby favoring the MV over the TV. Yet, one would expect the PV to also be affected and this was not the case. Nonetheless, when these hemodynamic changes continue for an extended period of time, structural cardiovascular remodeling occurs that can in turn result in functional deficits as well[3,29,31].

Our results are in line with available literature in singletons. Preterm SGA neonates are reported to already have an altered cardiac morphology within days after birth when compared to appropriately-grown controls[32]. A similar study at two years of age demonstrated that SGA infants presented with significantly smaller LV dimensions and that any alterations were strongly related to birth weight[5]. The unique aspect of our population in which we compare identical twins that were exposed to different intrauterine environments sets us apart from previous studies, eliminating any other known and unknown influences.

Tracking cardiovascular structure and functioning over a longer period of time is crucial to understand the pathophysiological processes that underlie CVD risk[33,34]. We have now focused on neonatal cardiovascular remodeling. It is plausible that changes in some parameters, such as the aPWV, are yet to occur if this adaptive process is more gradual throughout childhood. This is supported by a study reporting a higher aPWV for preschool children born SGA compared to AGA children, as well as by a

study that found that measures at two years were stronger predictors for outcome at ten years than measures at 1.5 months[33,35]. In addition, we do not yet know whether the observed changes at birth will also persist into childhood or if these are (partially) reversible. This information is essential in devising targeted interventions to potentially induce this reversal in the future.

Our study has limitations. We have included a range of MC twin complications that can each have a different impact on cardiovascular structure. TTTS and TAPS twins experience hemodynamic imbalances in utero, which can affect postnatal cardiac dimensions despite reversibility after fetal therapy[27,36,37]. Similarly, in TAPS the donor experiences anemia and the recipient polycythemia, resulting in distinct circulatory adaptations[13]. In sFGR, hemodynamic changes are different in origin and expressed by abnormal umbilical artery Doppler flow patterns[38]. Additionally, prenatal growth is affected differently by each MC twin complication. Where isolated sFGR is primarily caused by unequal placental sharing, growth discrepancies in TTTS and TAPS can also occur due to oliguria or anemia in the donor. This influences direct extrapolation of our results to FGR in singletons, as this is primarily caused by placental insufficiency. To control for heterogeneity in our population, we assessed whether there was an association between the type of complication and each cardiac measurement. This was only the case LVPWD that stayed significant even after correction for the complications, illustrative of a limited influence.

To conclude, we report a structurally smaller heart for the smaller twins in our population. While cardiac structures are generally smaller, the deviation in birth weight tends to be more pronounced than the deviation in cardiac structure, indicative of heart sparing. These findings are suggestive of early cardiovascular changes after FGR. We used an identical twin cohort controlling for genetic and maternal factors, in which epigenetic profiling and cardiovascular follow-up will also be performed in the near future. Tracking cardiovascular structure and functioning over a longer period of time is crucial to understand the pathophysiological processes that underlie CVD risk[33,34]. Individualized risk assessments can be formed, allowing for consecutive timely and targeted interventions to minimalize the burden of disease in later life.

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Chapter 6

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

Ultrasound in Obstetrics and Gynecology.

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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].

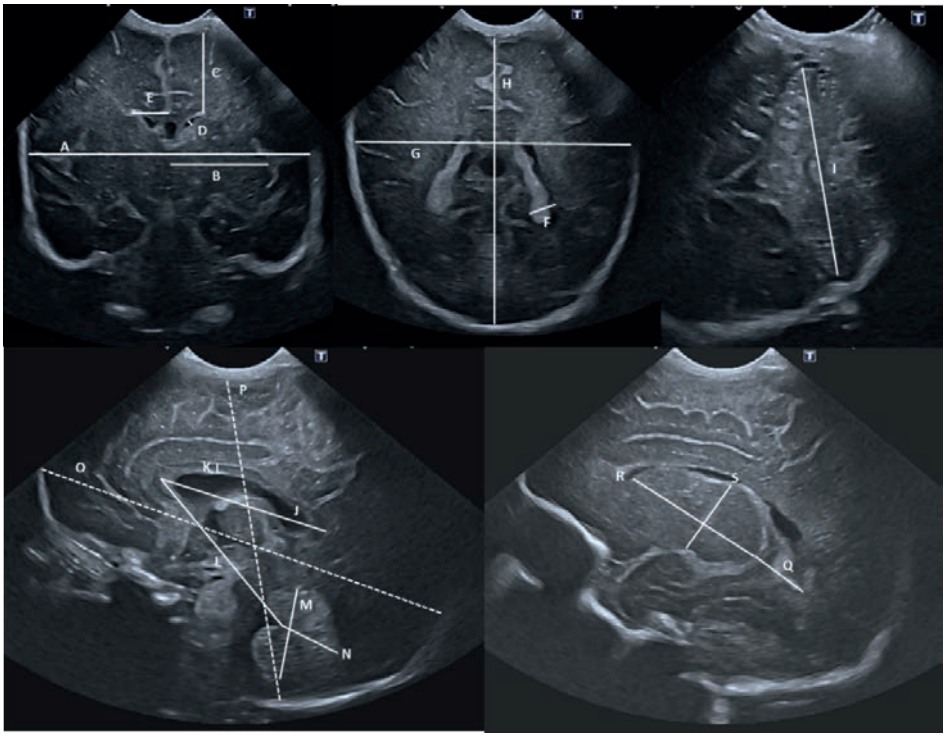

Figure 1. Overview of cerebral measurements: A/G = biparietal diameter; B = deep gray matter width; C = frontal white matter height; D = AHW; E = VI; F = VAW; G-H = used in calculation of intracranial surface; I = TCD; J = corpus callosum length; K = corpus callosum height; L = callosum-fastigium length;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

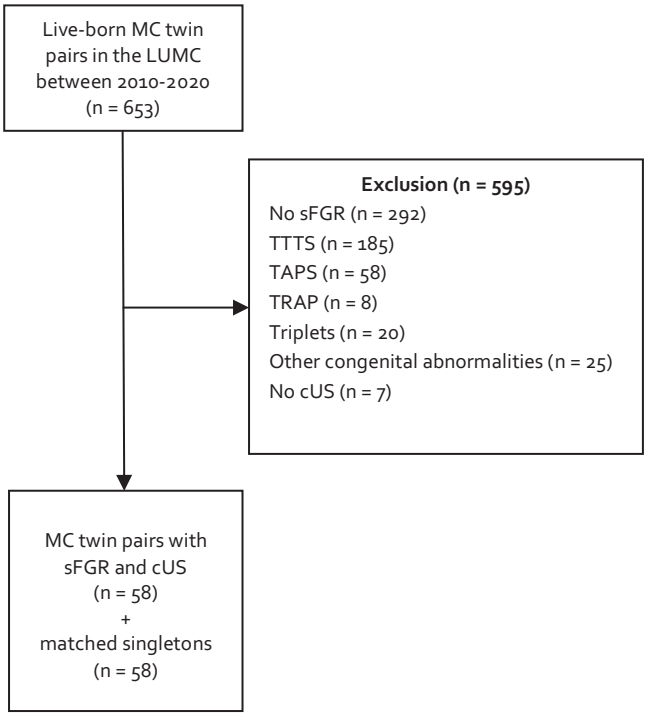

Figure 2. Flowchart of study inclusion.

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 (%).

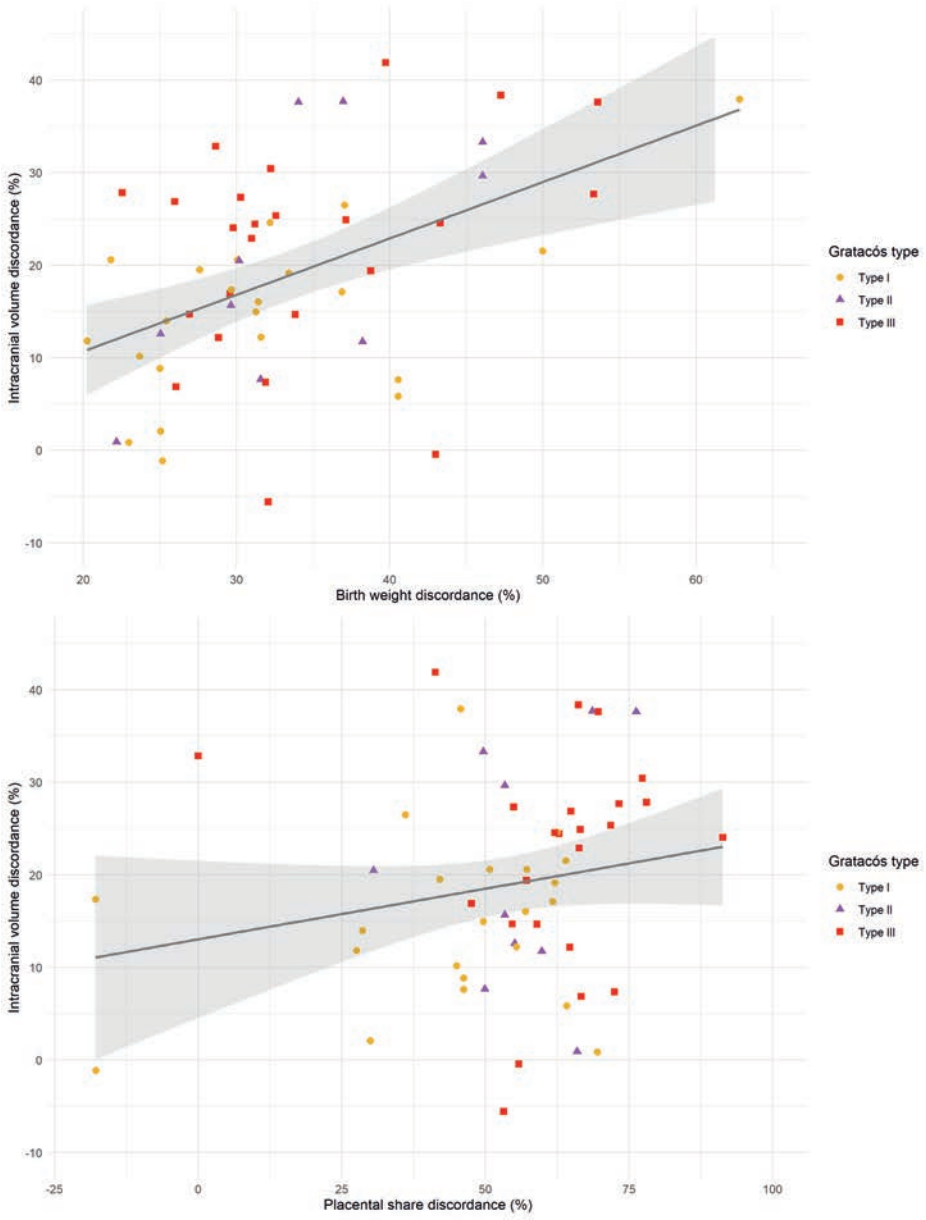

Figure 3. Scatterplots with regression line and 95% confidence interval depicting the association between intracranial volume discordance and BWD and placental share discordance according to Gratacós type. Regression lines are based on the group as a whole. Intracranial volume discordance was missing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

From infant to adolescent

Chapter 7

The impact of selective fetal growth restriction or birth weight discordance on long-term neurodevelopment in monochorionic twins: a systematic literature review.

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Abstract

The aim of this review was to assess the impact of selective fetal growth restriction (sFGR) and/or birth weight discordance (BWD) on long-term neurodevelopment in monochorionic (MC) twins. Five out of 28 articles assessed for eligibility were included. One article concluded that the incidence of long-term neurodevelopmental impairment (NDI) was higher in BWD MC twins (11/26, 42%) than in BWD dichorionic (DC) (5/38, 13%) and concordant MC twins (6/71, 8%). BWD MC twins had a 6-fold higher risk of cerebral palsy compared to DC twins (5/26, 19% vs. 1/40, 3%, $p < 0.05$). Another article described a linear relationship between birth weight and verbal IQ scores, demonstrating a 13-point difference for a 1000 gram BWD between the twins, with a disadvantage for the smaller twin ($p < 0.0001$). Three articles analyzing within-pair differences showed that the smaller twin more frequently demonstrated mild NDI (6/80, 8% vs. 1/111, 1%) and lower developmental test scores (up to 5.3 points) as opposed to its larger co-twin. Although these results suggest that MC twins with sFGR/BWD are at increased risk of long-term NDI as compared to BWD DC or concordant MC twins, with a within-pair disadvantage for the smaller twin, the overall level of evidence is of moderate quality. As only five articles with a high degree of heterogeneity were available, our review mainly demonstrates the current lack of knowledge of the long-term outcomes of MC twins with sFGR/BWD. Insight into long-term outcomes will lead to improved prognostics, which are essential in parent counseling and crucial in the process of forming a management protocol specifically for twins with sFGR to optimally monitor and support their development.

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Introduction

Selective fetal growth restriction (sFGR) is a severe complication of monochorionic (MC) twin pregnancies, characterized by a large inter-twin growth discrepancy. sFGR occurs in 10%–15% of MC twin pregnancies and is defined as an estimated fetal weight (EFW) < 10th percentile in the smaller fetus and/or a birth weight discordance (BWD) of > 20%[1-3]. The pathogenesis is associated with specific patterns of vascular anastomoses allowing for inter-fetal blood exchange and unequal placental sharing, leading to unbalanced access to nutrients.

sFGR can be classified according to umbilical artery (UA) Doppler flow in the smaller twin, as proposed by Gratacós et al. in 2007[4]. Type I is characterized by a positive UA flow, linked to a relatively benign prognosis. The anastomoses in this group are similar to uncomplicated MC twin pregnancies. Type II presents with a persistently absent or reversed UA Doppler flow (A/REDF) and is considered to have the highest perinatal mortality and morbidity. Finally, type III is defined as intermittent absent or reversed end-diastolic flow (iA/REDF). The clinical course is unpredictable and type III is associated with an elevated risk of fetal demise of the smaller twin and severe neurological damage in the larger twin[5,6].

Several studies examined the perinatal outcomes of MC twin pregnancies complicated by sFGR, and high rates of fetal demise (16%–29%) and neonatal morbidities such as cerebral injury (0%–33%) were observed, depending on the UA Doppler classification[6-8]. Studies documenting the long-term neurodevelopmental outcomes of these twins are scarce. Hence, proper information on the long-term prognosis is lacking. We performed a systematic review of the literature on long-term follow-up data to assess the impact of sFGR and/or BWD on long-term neurodevelopment in MC twins.

Methods

This systematic review was conducted according to PRISMA guidelines[9]. The PubMed database was searched electronically in January 2019. A search strategy using a variety of combinations of relevant medical subject heading terms, keywords, and word variations was applied to search for relevant articles on long-term neurodevelopmental outcomes for sFGR pregnancies. The main keywords consisted of "selective fetal growth restriction", "birth weight discordance", "twins", and "neurodevelopmental outcomes". The reference lists of reviewed articles were searched to include potentially missing articles. The search was restricted to articles published in English.

Study Selection

The primary assessment of the articles for potential relevance was based on title and abstract, with additional full-text screening. The articles were reviewed by two independent authors. Inconsistencies were discussed and consensus was reached. An article was eligible for inclusion when the population consisted of MC twins diagnosed with sFGR, the neurodevelopmental tests performed were age appropriate and a description of BWD was given. sFGR was defined as a BWD of ≥ 20% or an EFW < 10th percentile in the smaller twin.

We excluded articles from the review when the study design was either a case report or a case series with fewer than three cases, as these populations were too limited to be of prognostic value. We also excluded articles where intrauterine interventions were performed in the study population. As there is no consensus on appropriate treatment thus far, it is important to evaluate the long-term outcome following the natural course of the disease. Intrauterine interventions may alter this natural course.

Quality Assessment

Quality assessment of the included studies was performed using the "*Users Guides to the Medical Literature*"[10]. The final level of evidence was determined with the use of the GRADE working group method for grading quality of evidence, incorporating the risk of bias and any imprecision or inconsistency between the articles[11].

Results

The search strategy yielded 309 results. The primary assessment led to the exclusion of 281 articles based on the aforementioned inclusion and exclusion criteria. Of the 28 remaining articles, 23 were excluded after thorough full-text assessment by two independent authors, leaving five articles to be included for systematic review (Figure 1).

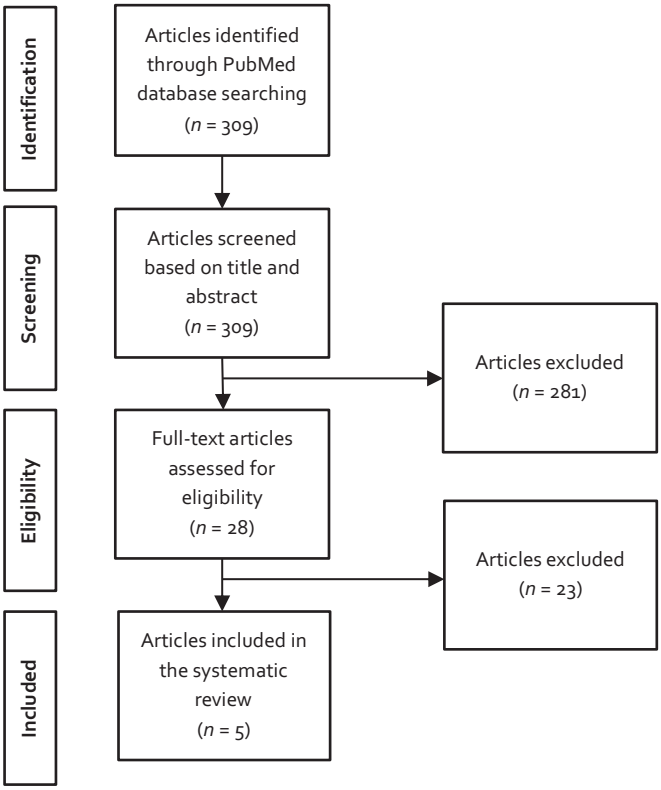

Figure 1. Flowchart of study inclusion.

The characteristics of the studies are presented in Table 1. The number of included MC twins ranged from 13 to 140. The long-term neurodevelopmental results of the included studies are summarized in Table 2. Pooled analysis of the results could not be performed due to the heterogeneity of the inclusion criteria, methods and outcome measures.

The first article by Adegbite et al.[12] in 2004 followed a prospective cohort to determine the incidence of neurologic morbidity in MC and dichorionic (DC) twins

born between 24 and 34 weeks' gestation. Twins were included when the pregnancy was not complicated by fetal aneuploidy, fetal demise of both twins, congenital malformations, embryo reduction, or selective feticide. Incomplete patient data sets were excluded. A total of 76 MC pregnancies were included, of which 13 were classified as birth weight discordant (≥ 20% BWD or abdominal circumference ≤ 5th centile with an abnormal UA Doppler). Of these 13, 10 were delivered by caesarian section (73%). The neonatal course was not described and children were assessed at two years of age. Neurodevelopmental impairment (NDI) was defined as impaired neurologic development including minor disabilities or developmental delay (> 2SD below the mean of the Griffith's mental developmental scale score). Cerebral palsy was diagnosed using standard criteria and was defined as a persistent abnormality of movement and posture resulting from a non-progressive lesion of the immature brain. The incidence of NDI in BWD MC twins was 23% (6/26). The cerebral palsy rate was significantly higher in BWD MC twins than in DC twins, 19% (5/26) vs. 3% (1/40), respectively (p < 0.05). The overall NDI rate, combining minor disabilities or developmental delay and cerebral palsy, in BWD MC twins was 42% (11/26) as opposed to 13% (5/38) in DC twins (p < 0.01). In addition, the overall NDI rate was significantly higher in the BWD MC twins compared to the concordant MC twins, namely, 42% (11/26) vs. 8% (6/71) (p < 0.01). The authors concluded that BWD MC twins have a 6-fold higher risk of cerebral palsy compared to DC twins. No significant differences in developmental test scores were found using the Griffith's Mental Development Scales.

In 2010, Edmonds et al.[13] retrospectively included 71 monozygotic twin pairs born at a gestational age > 32 weeks to study whether poor fetal growth was related to impaired cognitive functions. Chorionicity was, however, not reported. Those with severe chronic disease such as cerebral palsy (contrary to Adegbite et al. who included these cases), those who received treatment at birth for acute twin–twin transfusion syndrome (TTTS) and those who were unwell (not further specified) on the study day were excluded from the analyses. Nevertheless, six twins with reported evidence of TTTS without fetal therapy were still included in the study, possibly affecting the results. However, after removing these six cases from the data analyses, the outcomes did not substantially change. Moreover, three additional twin pairs were excluded because one or both children had autism spectrum disorder. Delivery mode and neonatal course were not described. The dataset exhibited a spectrum of birth weights varying from 1070 to 3500 grams, with birth weight differences ranging from 30 to 1480 grams. The authors reported a relationship between within-twin BWD and

verbal IQ scores with a slope (β) of 13.0 (CI: 7.1–18.9), implying that for 1000 grams of within-twin BWD the within-twin verbal IQ differed by 13 points ($p < 0.01$). In BWD twin pairs the smaller twin demonstrated lower verbal IQ scores as opposed to its larger co-twin ($p = 0.006$).

Table 1. Summary of study characteristics of the included studies.

Author (year)	Study design	Number of MC twins with sFGR (GA at birth)	Definition sFGR/BWD	Outcome measures and neurodevelopmental evaluation	Age at assessment
Adegbite (2004)	Prospective	13 (GA at birth 24–32 weeks)	≥20% BWD or smaller twin with AC ≤5th centile with an abnormal UA Doppler	Overall incidence of CP and minor neurological disabilities Developmental delay (Griffith's mental developmental scale score)	2 years
Edmonds (2010)	Retrospective	Not reported (GA at birth > 32 weeks)	BWD continuous variable	VIQ, PIQ (WISC-III)	7 years, 11 months–17 years, 3 months
Halling (2015)	Prospective	24 (mean GA at birth 35.2 (32.5–37.9) weeks)	≥20% BWD	Bayley-III scores	24–42 months
Rustico (2017)	Retrospective	140 (median GA at birth 32 (29–33) weeks)	EFW <10th percentile in smaller twin or EFW difference ≥25%	Level of neurological impairment (severe, moderate, mild)	12 months–7 years
Swamy (2018)	Retrospective	51 (mean GA at birth 34 (26–40) weeks)	≥20% BWD	BASII scores QNST scores SDQ scores	4–8.7 years

MC: monochorionic, sFGR: selective fetal growth restriction, GA: gestational age, BWD: birth weight discordance, AC: abdominal circumference, UA: umbilical artery, EFW: estimated fetal weight, WISC-III: Wechsler Intelligence Scale for Children third edition, Bayley-III: Bayley Scales of Infant and Toddler Development third edition, BASII: British Ability Scales: second edition, QNST: Quick Neurological Screening Test-III, SDQ: Strengths and Difficulties Questionnaire.
Defined as: Severe: CP level 3–5, developmental quotient <70, severe behavioral disorder (autism), bilateral sensorineural deficit (deafness or blindness); Moderate: CP level 2, developmental quotient 70–84, behavioral disorders (attention deficit and/or hyperactivity), unilateral sensorineural deficit; Mild: minor motor deficits (clumsiness), transient motor delay (with prospect of normalization), isolated language impairment).

In 2016, Halling et al.[14] studied the effect of a BWD ≥ 20% on neurodevelopmental outcomes in MC and DC twins, based on prospective data from "The Neuro-Developmental Outcome for Twins of the ESPRiT Study" (NOTES study). Neurodevelopment was assessed using the Bayley Scales of Infant and Toddler Development third edition (Bayley-III). The study included 119 BWD twin pairs of which 24 were MC twins. All were double survivors. Twins with chromosomal abnormalities were excluded. Of the 119 twin pairs, 70 (59%) were delivered via an elective caesarian section. An emergency pre-labor caesarian section was performed in 21 (18%) cases. The presence of neonatal morbidity (defined as intraventricular hemorrhage (IVH), hypoxic ischemic encephalopathy, radiological evidence of periventricular leukomalacia (PVL) or necrotizing enterocolitis (NEC)) was not different for BWD twin pairs and control twin pairs (17/119, 17% vs. 10/111, 9%, respectively, $p = 0.21$). No separate baseline characteristics were presented for the 24 MC twin pairs. BWD MC twins had lower scores in composite language (3.8-point difference, $p = 0.03$), scaled expressive language (0.8-point difference, $p = 0.02$), composite motor (5.3-point difference, $p = 0.002$) and scaled gross motor scores (1.1-point difference, $p = 0.001$) as compared to DC twins. The smaller twin exhibited lower neurodevelopmental scores across all three domains (cognition, language, and motor) in comparison with their larger co-twin for MC and DC twins combined. An analysis of differences between the smaller and larger twins specifically for MC twins could not be conducted due to the small sample size.

In a recent retrospective cohort study published by Rustico et al.[15] in 2017, the authors examined the correlation between UA Doppler findings and pregnancy course, perinatal outcome, and postnatal follow-up in 140 MC pregnancies complicated by sFGR referred before 26 weeks gestation. No neurodevelopmental test was performed, but all surviving twins were seen by a pediatric neurologist–psychiatrist for follow-up according to routine care in Italy. The delivery mode was not described. The prevalence of severe neonatal morbidity (defined as chronic lung disease, NEC or stage III retinopathy of prematurity) was 5% (7/140) for the small twin and 2% (3/140) for the large twin. The smaller twin more often demonstrated a mild NDI, defined as minor motor deficits (clumsiness), transient motor delay (with the prospect of normalization) or isolated language impairments, as opposed to the larger twin, namely 8% (6/80) versus 1% (1/111) of children, respectively ($p = 0.02$). The intact survival rate, calculated by dividing the number of children without impairments by the total number of children, was 48% (67/140) for the smaller twin and 74% (103/140) for the larger twin ($p < 0.001$).

Lastly, in 2018, Swamy et al.[16] reported the long-term cognitive outcomes of 51 MC twins with a BWD ≥ 20% using a prospectively ascertained database. Six pregnancies were complicated by TTTS, for which one received laser treatment, perhaps affecting the results. Twin pairs with cerebral palsy either in one or both twins, or twins with behavioral issues were excluded (n = 3). Delivery modes and neonatal morbidities were not mentioned. The general conceptual ability (GCA) score assessed with the British Ability Scales (BASII) was 108.4 in the larger twin and 105.4 in the smaller twin (p = 0.005). Moreover, there were significant differences for a mathematics subtest (quantitative reasoning, p = 0.004) and a memory subtest (recall of objects – immediate verbal, p = 0.014) with lower scores for the smaller twin. When an abnormal Doppler flow (AREDF in at least one scan) was present, the GCA score of the smaller twin was 7 points lower than that of the larger twin (p = 0.04).

Quality Assessment and Level of Evidence

The methodological evaluation of the included articles can be found in Table 2. Two of the included studies, Halling et al. and Swamy et al., demonstrated high validity based on the risk of bias assessment. The study by Adegbite et al. obtained an adequate validity rating due to the lack of corrections for possible confounders and a small study population. Rustico et al. did not perform any neurodevelopmental or psychometric testing, resulting in an adequate validity rating. Only the study of Edmonds et al. had low validity. Chorionicity was not reported and there was a broad follow-up range resulting from the retrospective nature of the study.

The included studies have certain limitations, among which were the presence of small study populations (13–140) and the use of retrospective designs (3/5). When comparing the studies, the content of the included articles was relatively similar with regard to study type, study population and the definition of BWD/sFGR. However, the inclusion criteria differed considerably. While Adegbite et al., Halling et al., and Rustico et al. included cases with cerebral palsy, Edmonds et al. and Swamy et al. did not, disregarding an important group of twins with NDI. Adegbite et al. included twins with a gestational age between 24 and 34 weeks, whereas Edmonds et al. focused on twins with a gestational age > 32 weeks. In addition, a wide variety of outcomes and tests were used, namely, IQ scores, neurodevelopmental assessment scores, and the incidence of impairments. Lastly, the follow-up periods differed extensively between the studies, complicating the overall conclusion. These differences in methodology, heterogeneity of the neurodevelopmental evaluations, and the lack of uniform outcome criteria led to incomparability which should be taken into consideration

when comparing and assessing the results presented in this systematic review. Hence, the overall evidence level of the included articles was of moderate quality, suggesting that further research will have a significant impact on confidence in the estimates of the outcomes.

Table 2. Long-term neurodevelopmental outcomes in birth weight discordant twins.

Author (Year)	Results	Methodological Comments	Validity
Adegbite (2004)	Incidence of CP ($p < 0.05$): - BWD MC twins = 5/26 (19%) CP - BWD DC twins = 1/40 (3%) CP Overall neuromorbidity ($p < 0.01$): - BWD MC twins = 11/26 (42%) - BWD DC twins = 5/38 (13%) Overall neuromorbidity ($p < 0.01$): - BWD MC twins = 11/26 (42%) - Concordant MC twins = 6/71 (8%)	Loss-to-follow up of 13%; No corrections for possible confounders; Small study population	Adequate
Edmonds (2010)	1 kg of within-twin birth weight difference = VIQ difference of 13 points (CI: 7.1–18.9) between twins ($p < 0.01$) Birth weight difference < 340 g: larger twin VIQ disadvantage Birth weight difference > 340 g: smaller twin VIQ disadvantage	No chorionicity distinction; Inclusion of six twin pairs with evidence for TTTS; CP cases excluded; Broad follow-up range; Corrections for socioeconomic status, preterm birth, birth weight difference > 0.5 kg	Low
Halling (2015)	BWD MC twins: - 3.8 lower in language composite score ($p = 0.03$), - 0.8 lower in expressive language ($p = 0.02$), - 5.3 lower in composite motor ($p = 0.002$), - 1.1 lower in scaled gross motor categories ($p = 0.001$)	Follow-up rate of 79%; Concordant twin pairs matched for gestation as controls; Corrections for chorionicity, gender, prematurity, and birth weight	High

Rustico (2017)	Mild neurodevelopmental impairment (p = 0.02) - Smaller twin = 6/80 (8%) - Larger twin = 1/111 (1%)	No psychometric test; Complete follow-up; Only within-twin pair comparison	Adequate
Swamy (2018)	BASII GCA scores (p = 0.005) - Larger twin = 108.5 - Smaller twin = 105.4 Difference in GCA in 10 twin pairs with abnormal Doppler flows = 7 points (p = 0.04)	CP cases excluded; Blinded investigator; Corrections for factors shared within-twin pairs; Only within-twin pair comparison; Inclusion of six TTTS twin pairs	High

MC: monochorionic, sFGR: selective fetal growth restriction, CP: cerebral palsy, BWD: birth weight discordant, DC: dichorionic, VIQ: verbal intelligence-quotient, CI: confidence interval, TTTS: twin-twin transfusion syndrome, BASII: British Ability Scales: second edition, GCA: general conceptual ability.

Discussion

According to the current literature, MC twins with sFGR or BWD are at a substantial risk of NDI in the long-term. The incidence of long-term NDI is higher in MC twins with sFGR as opposed to concordant MC twins, or DC twins with sFGR. Three studies showed that the smaller twin has a disadvantage as they frequently demonstrated mild neurodevelopmental impairment and lower developmental test scores. However, as only five articles on this subject were available, all with heterogenous populations and outcome measures, our review mainly demonstrates the current lack of knowledge on the long-term outcomes of MC twins with sFGR or BWD.

The heterogeneity between the included studies was extensive due to differences in definitions for sFGR/BWD, different inclusion and exclusion criteria, and the use of a variety of different outcomes and tests to describe neurodevelopment at diverse follow-up time points. Furthermore, there was a wide range of gestational ages at birth, within and between articles. Along with the relatively small study populations, the high degree of heterogeneity leads to incomparability of results. Thus, strong evidence of long-term outcome of MC twins with sFGR or BWD is currently lacking. This gap in knowledge results in the inability to provide an accurate long-term prognosis and form appropriate management protocols, both topics of MC sFGR twin pregnancies which are widely debated.

There are several explanations for the current findings. MC pregnancies have a higher preterm birth rate, subsequently leading to a higher incidence of adverse neonatal outcomes, such as severe cerebral injury which is a risk factor for long-term NDI[17]. As prematurity is associated with an increased risk of cerebral palsy and cognitive and motor disabilities, also due to the increased prevalence of neonatal severe cerebral injury[18,19], the observed NDI in MC twins with sFGR might not solely be the result of the growth discrepancy between the twins. The outcomes might be influenced by the gestational ages at which the children were born, as these ranged from 24 weeks to 40 weeks in the included studies, and the presence of severe cerebral injury after birth. Additionally, the smaller twins are growth restricted and consequently small for gestational age (SGA). Children born SGA often suffer from impaired brain development likely resulting in long-term cognitive or motor disabilities[20-22]. Hence, being born SGA also negatively affects the neurodevelopmental status of the smaller twin.

The different UA Doppler types are correlated with specific perinatal outcomes[4,5]. This association might also be present for neurodevelopmental outcomes. A systematic review and meta-analysis by Buca et al.[23] evaluated the outcomes of sFGR pregnancies according to the UA Doppler pattern. They concluded that children with type II and type III sFGR are at higher risk of abnormal brain imaging compared with those with type I sFGR based on thirteen included studies. Furthermore, a systematic review by Inklaar et al.[6] identified abnormal UA Doppler measurements as a risk factor for a higher incidence of cerebral injury. One should consider that both reviews also found a lower gestational age in the abnormal UA Doppler group , likely influencing the results as described by the authors. Nevertheless, the outcomes of both reviews demonstrated that specific UA Doppler classifications can be linked to certain cerebral and neurological outcomes. Research on whether this is the case for long-term NDI is still lacking.

In addition, the above-mentioned systematic review by Inklaar et al. documented an incidence of 8% (0%–33%) of cerebral injury in sFGR twins, mainly affecting the larger twin. Interestingly, this implies that the larger twin is at higher risk of cerebral injury, while our study shows that the smaller twin has an elevated risk of long-term NDI. One explanation for this finding is that an abnormal ultrasound visualizing cerebral injury after birth does not necessarily lead to long-term neurodevelopmental impairment. Although the predictive value of neuroimaging is increasing, its predictive accuracy remains a subject of debate[24]. Another theory to explain the discrepancy is that the two studies in this review (Halling et al. and Swamy et al.) that performed a within-pair comparison of neurodevelopmental outcomes solely included double survivors, while the increased risk of cerebral injury in the larger twin likely results from the fetal demise of the smaller twin. Hence, there is a difference in the population analyzed, namely, single fetal demise cases versus double survivors.

In conclusion, the incidence of long-term neurological or cognitive impairment in MC sFGR/BWD twins appears to be higher compared to uncomplicated MC or DC twins, with a disadvantage for the smaller twin. As there were only five articles available, and the overall evidence level of the included articles was of moderate quality due to a high degree of heterogeneity between studies, conclusive evidence on the long-term outcomes of MC sFGR twins is lacking. Our review is the first to demonstrate this shortage of knowledge. More extensive research should be performed, preferably in a prospective follow-up setting with a large cohort of MC twins and a long follow-up period at standardized time points until at least school age. The incidence of both

cerebral palsy and of NDI should be documented and clearly defined. Additionally, stratification according to UA Doppler classification might offer insight into the risk of long-term neuromorbidity per type of sFGR and subsequently lead to proper antenatal management options. This stratification can only be achieved when UA Dopplers are structurally measured and reported. Insight into long-term outcomes will lead to improved prognostics, which are essential in parent counseling. In addition, the outcomes will be crucial for the process of forming a management protocol specifically for twins with sFGR to optimally monitor and support their development.

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Chapter 8

Long-term effects of selective fetal growth restriction (LEMON): a cohort study of neurodevelopmental outcome in growth discordant identical twins in the Netherlands.

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Abstract

Background. Singletons born after fetal growth restriction (FGR) are at increased risk of poor neurodevelopmental outcomes. Studies of singletons with FGR usually compare outcomes with those without FGR, a comparison that is inherently biased by obstetrical, parental, and genetic factors. We aim to compare neurodevelopmental outcomes between the smaller and larger twin in a population of discordant identical twins who shared a single placenta, naturally eliminating these confounders.

Methods. This study is part of the LEMON cohort study of monochorionic diamniotic twins with selective FGR. All monochorionic diamniotic twins with selective FGR who were born in Leiden University Medical Center (Leiden, Netherlands) between March 1, 2002, and Dec 31, 2017, were eligible for inclusion. Twin pregnancies that were complicated by twin–twin transfusion syndrome, twin anemia polycythemia sequence, or monoamnionicity were excluded. Cognitive performance was evaluated with two standardised psychometric age-appropriate tests, producing a full-scale intelligence quotient (FSIQ). Motor functioning was assessed with a standardized neurological examination. A composite outcome of neurodevelopmental impairment (NDI) was used, subdivided into mild NDI (defined as FSIQ <85, minor neurological dysfunction or cerebral palsy grade 1, or mild visual or hearing impairment) and severe NDI (defined as FSIQ < 70, severe neurological dysfunction, or severe visual or hearing impairment).

Findings. Between Jan 25, 2021, and March 15, 2022, 47 twin pairs were enrolled in the study and underwent neurodevelopmental assessment. The median gestational age at birth was 33.9 weeks (IQR 31.3–36.0) for the 47 included twin pairs, with median birthweights of 1400 g (1111–1875) in the smaller twin and 2003 g (1600–2680) in the larger twin. The median age at neurodevelopmental assessment was 11 years (8–13). Median FSIQ was 94 (86–101) for the smaller twin and 100 (92–108) for the larger twin ($p < 0.0001$). More smaller twins had mild NDI (36% (17/47)) than did the larger twins (11% (5/47); odds ratio 4.8 (95% CI 1.6–14.1); $p = 0.0049$). There was no difference in the proportion of children with severe NDI (4%(2/47) in both groups, $p = 1.0$).

Interpretation. As mild NDI can impede children in their daily functioning, we recommend standardized long-term follow-up, including neurodevelopmental testing, for monochorionic diamniotic twins with selective FGR to facilitate early identification of children at risk.

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Introduction

The intrauterine environment sets the foundation for lifelong health. Unfavorable intrauterine circumstances, such as fetal growth restriction (FGR), in which the fetus does not reach its growth potential, are associated with health disadvantages[1]. High rates of perinatal morbidity and substantial long-term neurodevelopmental impairment (NDI), with poor cognitive performance and neurological dysfunction, have been reported for singletons with FGR[2,3]. In these studies, however, singletons with FGR are primarily compared with singletons without FGR. This comparison is inherently biased by obstetrical, parental, and genetic factors, impeding a proper risk assessment. A study population of identical twins who are discordant for fetal growth naturally eliminates these confounders.

Monochorionic diamniotic (MCDA) twins are genetically identical and share a single placenta. In 15% of MCDA twins, this placenta is unequally shared: one twin has a much smaller placental share than their co-twin, causing FGR for the twin with the smaller share, which is termed selective FGR (sFGR)[4,5]. Similar to FGR in singletons, the severity of sFGR in twins is classified according to the umbilical artery Doppler flow pattern in the smaller twin, as proposed by Gratacós and colleagues, with poorer outcomes in children from pregnancies with persistent (type II) or intermittent (type III) absent or reversed end-diastolic flow (A/REDF) than in children from pregnancies with positive end-diastolic flow (type I)[6]. Assessment of MCDA twins with sFGR can be considered a unique natural experiment in which a twin with restricted growth can be compared with its genetically identical co-twin without growth restriction, allowing evaluation of the true effect of FGR on neurodevelopmental outcomes. Little is known about the long-term outcomes of these twins at present.

Neonatal neurological outcomes of MCDA twins with sFGR have been widely reported, with a high incidence of cerebral injury (i.e., up to 33%) and an overall restriction in brain growth for the smaller twin on cerebral ultrasound[7,8]. Yet, well designed studies of long-term neurodevelopmental outcomes are scarce. The existing studies are underpowered; differ extensively in methodology, timing, and type of neurodevelopmental evaluation; and do not give detailed perinatal information[9]. The aim of this study is to compare neurodevelopmental outcomes between the smaller and larger twin in MCDA twin pairs with sFGR.

Methods

Study design and participants

This study is part of the LEMON study (Long-Term Effects of selective fetal growth restriction in MONochorionic twins), which is a cohort study, including all MCDA twin pairs with sFGR born in the Leiden University Medical Center (LUMC), Leiden, Netherlands, the national referral center for complications specific to monochorionic twins, such as twin–twin transfusion syndrome, twin anemia polycythemia sequence, and sFGR. The LEMON study was reviewed and approved by the ethics committee of the LUMC (P20.089). For children younger than 12 years, only parents were asked for written informed consent. For children 12 years and older, both children and parents were asked for written informed consent. Patient recruitment began in January 2021, and inclusion was finalized in January 2022.

All MCDA twins with sFGR who were born in the LUMC between March 1, 2002, and Dec 31, 2017, were eligible for this study, with sFGR defined as a birth weight discordance (BWD) ≥ 20% (calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100)[10]. Twin pregnancies complicated by twin-twin transfusion syndrome, twin anemia polycythemia sequence or monoamnionicity were excluded[11,12]. Cases in which one or both twins died were excluded as within-pair analyses impossible. Lastly, twins with twin reversed arterial perfusion or other congenital abnormalities were excluded.

Procedures

The following baseline characteristics were collected: Gratacós type, with type I defined as positive end-diastolic flow, type II defined as persistent A/REDF, and type III defined as intermittent A/REDF in the umbilical artery of the smaller twin[6]; gestational age at diagnosis of sFGR (i.e., the first moment that the combination of an estimated fetal weight in <10th centile and an estimated fetal weight discordance of ≥20% was observed, categorized into early onset (< 24 weeks) and late onset (≥ 24 weeks)[13]); gestational age at birth; sex; delivery mode; birth weight and birthweight discordance; whether the child was small for gestational age (i.e., birth weight in <10th centile[14]); severe neonatal morbidity; current weight and BMI; and maternal education (primary and secondary school; intermediate vocational education; or higher vocational education and university). The perinatal baseline characteristics were retrospectively collected from patient files by SGG and KJJS. Weight and BMI as well as maternal education were documented at the time of the neurodevelopmental assessment (which is described in the next paragraph). Over the span of this study,

the sole change in management of MCDA twin pregnancies in the Netherlands was the advice to induce delivery between 36 and 37 weeks, which was gradually introduced between 2007 and 2008 (before 2007, there was no advice on delivery of MDCA twins). Severe neonatal morbidity was defined as at least one of the following: respiratory distress syndrome (i.e., respiratory failure needing mechanical ventilation or surfactant); persistent pulmonary hypertension of the neonate (i.e., the failure of circulatory transition after birth requiring treatment with nitric oxide); patent ductus arteriosus requiring medical treatment or surgical closure; necrotizing enterocolitis of at least stage 2; neonatal sepsis (i.e., a clinically ill neonate with positive blood cultures); bronchopulmonary dysplasia (i.e., supplemental oxygen for ≥ 28 days) [15]; and severe cerebral injury (i.e., intraventricular hemorrhage ≥ grade 3, cystic periventricular leukomalacia ≥ grade 2, ventricular dilatation > 97th percentile, arterial or venous infarction, or porencephalic or parenchymal cysts).

When informed consent was obtained, a follow-up appointment was scheduled. At this follow-up appointment, cognitive performance was evaluated with two standardised psychometric tests: the Wechsler Preschool and Primary Scale of Intelligence, Fourth Edition, for children aged 4–6 years[16] and the Wechsler Intelligence Scale for Children, Fifth Edition, for children older than 6 years[17]. These tests generate a full-scale intelligence quotient (FSIQ) score representing a child's general intellectual ability and five primary index scores measuring intellectual functioning in five cognitive areas: the Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index. The index scores and the FSIQ are on a standard score metric with a mean of 100 and an SD of 15. Mild cognitive delay was defined as a test score of less than 1 SD and severe cognitive delay as a test score of less than 2 SD. Motor functioning was assessed using a standardised neurological examination developed by Touwen and colleagues[18], modified by Hadders-Algra [19], to establish the presence of dysfunction in the following domains: posture, reflexes, involuntary movements, coordination, fine manipulation, associated movements, sensory function, and cranial nerve function. Simple minor neurological dysfunction was defined as the presence of one or two dysfunctional domains before the onset of puberty or an isolated presence of dysfunctional posture and tone regulation, choreiform dyskinesia, excessive associated movements, mild sensory dysfunction, or mild cranial nerve dysfunction after onset of puberty. Complex minor neurological dysfunction was defined as the presence of three or more dysfunctional domains before onset of puberty or the presence of mild coordination problems or fine manipulative disability after onset of

puberty[19]. Cerebral palsy was classified according to the Gross Motor Function Classification System[20]. Severe neurological dysfunction was defined as any severe motor impairment, including cerebral palsy of at least grade 2. The presence of any visual or hearing impairment was recorded, graded as mild visual impairment (i.e., requiring treatment by an ophthalmologist, strabismus, a correction of a maximum of plus or minus 3.0 with glasses or contact lenses, or a correction of more than plus or minus 3.0 adequately corrected with glasses or lenses), severe visual impairment (i.e., blindness or partially sighted), mild hearing impairment (i.e., hearing loss up to 30 decibels with or without amplification), or severe hearing impairment (i.e., bilateral deafness). Data on neurodevelopmental outcomes and visual or hearing impairments were collected by SGG and KJJS at follow-up examination.

NDI was used as primary composite outcome and subdivided into two categories of severity: mild NDI, defined as FSIQ less than 85, the presence of simple or complex minor neurological dysfunction (or a cerebral palsy grade 1), or mild visual or hearing impairment; and severe NDI, defined as FSIQ less than 70, the presence of severe neurological dysfunction, or severe visual or hearing.

Statistical analysis

Statistical analyses were performed with IBM Statistics version 25.0. Data are presented as median (IQR), n (%) of N, or n (%). To test for an association between sFGR and the intelligence quotient scores (numerical values), motor and sensory functioning (categorical values), and NDI (categorical values), a generalized estimating equation was used. This analysis considers that observations between co-twins are not independent. An unstructured covariance matrix was used. As the generalized estimating equation cannot be used when an outcome event does not occur in one of the groups (i.e., smaller or larger twin), an adjustment to the data was applied, in which an unaffected twin (i.e., outcome not present) was changed into an affected twin (i.e., outcome present) for both the smaller and larger twin. This adjustment generates more conservative p values than other available analyses for paired data. To test for association between Gratacós type and gestational age at birth and within-pair difference in FSIQ, a generalized estimating equation was also used. A univariate linear regression model was applied for identification of pair-related risk factors for a lower FSIQ. The Gratacós type and amount of birth weight discordance were included as well as gestational age at birth and maternal education level. When a significant association was found in the univariate analysis, the variable was included in a multivariate linear regression model. A *p*-value of less than 0.05 was

considered significant. The differences in within-pair intelligence quotient scores between the larger and smaller twin for the primary indexes and FSIQ scores and within-pair difference in FSIQ were depicted in a sinaplot using RStudio version 2021.9.2.382.

This study is registered with the Netherlands Trial Register, ID NL9833.

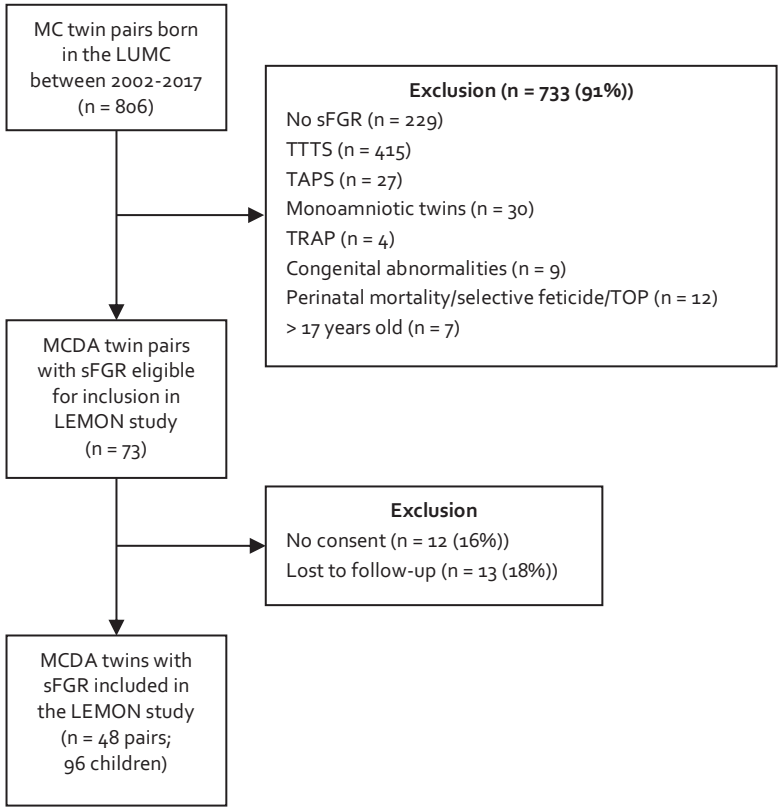

Figure 1. Flowchart of LEMON study inclusion**.** LUMC: Leiden University Medical Center. MCDA: monochorionic diamniotic. sFGR: selective fetal growth restriction. TOP: termination of pregnancy.
*Twin pairs aged 18 years or older at the start of this study (January 2021) were excluded.
†One of 48 twin pairs completed only questionnaires and did not complete the follow-up (and is therefore not included in the analysis).

Results

Between March 1, 2002, and Dec 31, 2017, 806 MCDA twin pairs were born in the LUMC, of which 73 were eligible for inclusion in the LEMON study. Of these twin pairs, 12 (16%) did not want to participate in the study, 13 (18%) were lost to follow-up (five twin pairs moved abroad and eight pairs could not be reached for inclusion), and one (1%) twin pair participated only in the questionnaire assessment of the LEMON study, leaving 47 twin pairs to be included in the neurodevelopmental follow-up (an inclusion rate of 64% for the present study; the inclusion rate for the LEMON study overall, including the twin pair who participated only in the questionnaire assessment, was 66% (48/73); Figure 1). Recruitment, data collection, and neurodevelopmental assessment took place between Jan 25, 2021, and March 15, 2022. Baseline characteristics were compared between the group of children who were included and the group of children who were lost to follow-up, and no significant differences were identified (Table A1).

Table A1. Comparison of perinatal baseline characteristics for the included twin pairs and the twin pairs that were lost to follow-up or who did not give consent for follow-up.

Characteristic	Included twins (n=47 pairs, 94 children)	Lost to follow-up/no consent (n=25 pairs, 50 children)	*p*-value
Gratacós type*			0.294
Type I	24/47 (51)	9/23 (39)	
Type II	10/47 (21)	5/23 (22)	
Type III	13/47 (28)	9/23 (39)	
Gestational age at birth – *weeks*	33.9 (31.3-36.0)	34.0 (31.4-36.0)	0.867
Sex			0.224
Female	48 (51)	18 (36)	
Male	46 (49)	32 (64)	
Caesarean	54 (94)	34 (68)	0.383
Birth weight discordance – *%*	30.1 (26.1-33.4)	36.0 (26.4-40.2)	0.085
Birth weight – *grams*			
Smaller twin	1400 (1111-1875)	1345 (925-1660)	0.475
Larger twin	2003 (1600-2680)	2140 (1455-2620)	0.973
Small for gestational age			
Smaller twin	46 (98)	24 (96)	0.651
Larger twin	11 (23)	6 (24)	0.955

Outcomes are presented as median (interquartile range (IQR)) or n (%).

*Gratacós type was unknown in two pregnancies of the lost to follow-up/no consent group.

Baseline characteristics

Baseline maternal, obstetrical, and neonatal characteristics are presented in table 1. Maternal education level was comparable to the general Dutch population[21]. In one twin pair, cognitive testing could not be performed due to a language barrier; a neurological examination was performed and, combined with their above-average school performance, no NDI was found.

Table 1. Maternal, obstetrical and neonatal characteristics for the included sFGR twins.

Characteristics	MCDA twins (n=94; 47 pregnancies)	Smaller twin (n=47)	Larger twin (n=47)
Gratacós type*			
Type I	24/47 (51)		
Type II	10/47 (21)		
Type III	13/47 (28)		
Gestational age at diagnosis of sFGR[†] – *weeks*	20.9 (16.9-24.6)		
Early onset (< 24 weeks)	29/39 (74)		
Late onset (≥ 24 weeks)	10/39 (26)		
Gestational age at birth – *weeks*	33.9 (31.3-36.0)		
Female	48/94 (51)		
Caesarean	54/94 (57)		
Birth weight discordance – *%*	30.1 (26.1-33.4)		
Birth weight – *grams*		1400 (1111-1875)	2003 (1600-2680)
Small for gestational age		46/47 (98)	11/47 (23)
Severe neonatal morbidity		10/47 (21)	10/47 (21)
RDS		3 (6)	10 (21)
PPHN		1 (2)	0 (0)
PDA		2 (4)	3 (6)
NEC		0 (0)	1 (2)
Sepsis		6 (13)	4 (9)
BPD		7 (15)	3 (6)
Severe cerebral injury		0 (0)	0 (0)
Maternal education			
Primary and secondary school	5/47 (11)		
Intermediate vocational education	20/47 (43)		
High vocational education or university	22/47 (47)		
Weight at assessment[‡] – *kg*		34.2 (22.9-49.5)	37.5 (27.8-52.3)
BMI at assessment[‡] – *kg/m²*		16.0 (14.9-19.2)	17.1 (16.0-20.2)

MCDA: monochorionic diamniotic. sFGR: selective fetal growth restriction, BMI: body mass index.
Outcomes are presented as median (interquartile range (IQR)), n/N (%) or n (%).
[†]Gestational age at diagnosis was unknown in eight twin pairs.
[‡]Weight not measured in two twin pairs for logistic reasons.

Table 2. Neurodevelopmental outcomes compared between the smaller and larger twin in sFGR twins.

Outcomes	Smaller twin (n=47)	Larger twin (n=47)	*p*-value
Age at participation	11 (8-13)	11 (8-13)	
Cognitive test score*			
FSIQ	94 (86-101)	100 (92-108)	**<0.0001**
Verbal Comprehension	96 (86-103)	103 (92-113)	**<0.0001**
Visual Spatial	92 (85-104)	97 (89-110)	**0.0012**
Fluid Reasoning	97 (90-105)	100 (93-109)	**0.016**
Working Memory	91 (85-100)	99 (88-110)	**<0.0001**
Processing Speed	95 (86-106)	100 (94-108)	**<0.0001**
Higher FSIQ‡	9/46 (20)	34/46 (74)	**<0.0001**
Cognitive delay			
Mild (score < 1 SD)	8/46 (17)	2/46 (4)	0.073
Severe (score < 2 SD)	1/46 (2)	0/46 (0)	0.322
Neurological examination			
Simple MND	7/47 (14)	2/47 (4)	0.069
Complex MND	1/47 (2)	2/47 (4)	0.571
Cerebral palsy	1/47 (2)	0/47 (0)	0.322
Severe neurological dysfunction	1/47 (2)	0/47 (0)	0.322
Visual impairment			
Mild	1/47 (2)	1/47 (2)	1.000
Severe	2/47 (4)	0/47 (0)	0.178
Hearing impairment			
Mild	4/47 (9)	1/47 (2)	0.101
Severe	0/47	0/47	1.000
Neurodevelopmental impairment			
Mild	17/47 (36)	5/47 (11)	**0.0049**
Severe	2/47 (4)	2/47 (4)	1.000

FSIQ: full scale intelligence quotient, SD: standard deviation, MND: minor neurological dysfunction. Outcomes are presented as median (interquartile range (IQR)), n/N (%) or n (%).

*Cognitive test scores were not available in one twin pair. ‡The FSIQ was the same in three twin pairs.

The median age at neurodevelopmental assessment was 11 (IQR 8–13 years). Median FSIQ was significantly lower for the smaller twin (Table 2). All index scores were affected similarly with a disadvantage for the smaller twin (Figure 2): verbal comprehension was 7 points lower, visual spatial was 5 points lower, fluid reasoning was 3 points lower, working memory was 8 points lower, and processing speed was 5 points lower (Table 2). Median within-pair differences for FSIQ and the indexes are presented in Table A2. Age (p = 0.85), weight (p = 0.50), and BMI (p = 0.165) at follow-up did not affect the size of the within-pair FSIQ difference. The smaller twin had a higher FSIQ than the larger twin in nine (20%) of 46 twin pairs for whom an FSIQ could be generated, whereas the larger twin had a higher FSIQ than the smaller twin in 34

(74%) twin pairs (p < 0.0001). The FSIQ was the same in three twin pairs. Mild cognitive delay was present in eight (17%) of 46 smaller twins as opposed to two (4%) of 46 larger twins (p = 0.073).

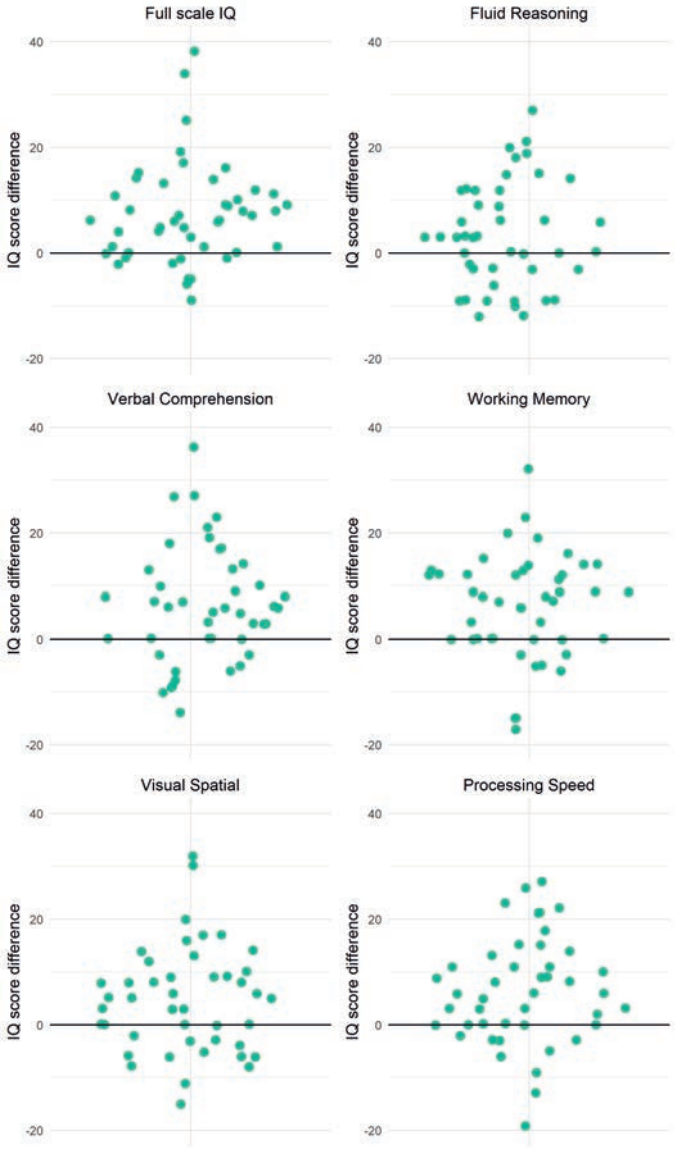

Figure 2. Sinaplot of the within-pair IQ score differences between the larger and the smaller twin per index. The calculation for the within-pair difference was: IQ score of larger twin minus IQ score of smaller twin. A positive score difference indicates that the smaller twin had a lower IQ score than the larger twin. A negative score difference indicates that the larger twin had a lower IQ score than the smaller twin.

Two factors were univariately associated with FSIQ: Gratacós type (β coefficient –12.2 (95% CI –20.8 to –3.5) for type II and β coefficient –9.5 (–17.3 to –1.8) for type III)—i.e., an FSIQ that was 12.2 points lower for type II and 9.5 points lower for type III than with type I (p = 0.0062)—and gestational age at birth (β coefficient 2.1 (0.8–3.5); i.e., for each additional week in gestational age at birth, FSIQ increases by 2.1 points (p = 0.0019); Table 3). Multivariate analysis did not identify these two factors as independent.

Table 3. Uni- and multivariate risk factor analysis for lower FSIQ in MC twins with sFGR.

	Univariate analysis			**Multivariate analysis**		
Characteristic	**β coefficient (95% CI)**	**SE**	***p*-value**	**β coefficient (95% CI)**	**SE**	***p*-value**
Gratacós type			**0.0062**			0.289
Type I	-	-		-	-	
Type II	-12.2 (-20.8--3.5)	4.416		-6.7 (-15.1-1.8)	3.985	
Type III	-9.5 (-17.3--1.8)	3.933		-3.8 (-15.3-6.7)	5.528	
Gestational age at birth – *weeks*	2.1 (0.8-3.5)	0.690	**0.0019**	1.5 (-0.3-3.3)	0.919	0.109
Birth weight discordance – %	-0.3 (-0.8-0.2)	0.233	0.191			
Maternal education			0.234			
Primary and secondary school	-5.3 (-14.7-3.5)	4.623				
Intermediate vocational education	3.0 (-4.7-10.7)	3.925				
High vocational education or university	-	-				

CI: confidence interval, SE: standard error.
Outcomes are presented as median (interquartile range (IQR)).

Regarding the different Gratacós types, gestational age at birth and FSIQ were significantly lower in children from pregnancies classified as type II and type III than children from pregnancies classified as type I. Children from pregnancies classified as type I were born at a median gestational age of 35.7 weeks (IQR 34.0–36.7) with a median FSIQ of 102 (94–109), those from pregnancies classified as type II were born at a median gestational age of 31.3 weeks (30.4–32.6) with a median FSIQ of 94 (85–99), and those from pregnancies classified as type III were born at a median gestational age of 31.7 weeks (29.7–34.1; p < 0.0001) with a median FSIQ of 93 (86–100; p = 0.0062; Table A3; Figure 3). The within-pair difference in FSIQ was numerically larger,

although not significantly so (p = 0.086), for type II pregnancies than for type I and III pregnancies (6 points (IQR 4–9) for type I, 14 points (4–27) for type II, and 6 points (1–10) for type III).

Simple minor neurological dysfunction was more often present in the smaller twin than in the larger twin (Table 2). One smaller twin presented with cerebral palsy grade I and another smaller twin presented with severe neurological dysfunction (epilepsy and severe developmental delay substantially impeding the neurological examination). The two observed severe visual impairments in the smaller twins in our population consisted of a correction of –10.0 following extensive retinopathy of prematurity and a unilateral coloboma (i.e., a congenital defect in the iris of the eye). Of the five children with a mild hearing impairment (four were smaller twins, one was a larger twin), four presented with a unilateral hearing aid (three were smaller twins, one was the larger twin). The hearing loss was congenital in origin (in two of five children), caused by chronic inner ear infections (in two children), or a cholesteatoma (in one child).

Table A2. Overview of within-pair differences in IQ scores and rate of NDI per twin pair.

Characteristic	MCDA twins (n=47 pregnancies)
Within-pair difference in cognitive test scores	
FSIQ	6 (0-11)
Verbal Comprehension	6 (0-14)
Visual Spatial	5 (-3-10)
Fluid Reasoning	3 (-3-12)
Working Memory	8 (0-13)
Processing Speed	6 (0-13)
Mild NDI per twin pair	
No mild NDI	27/47 (57)
Only smaller twin	15/47 (32)
Only larger twin	3/47 (6)
Both twins	2/47 (4)
Severe NDI per twin pair	
No severe NDI	44/47 (94)
Only smaller twin	1/47 (2)
Only larger twin	1/47 (2)
Both twins	1/47 (2)

MCDA: monochorionic diamniotic, FSIQ: full scale intelligence quotient. NDI: neurodevelopmental impairment.
Outcomes are presented as median (interquartile range (IQR)) or n/N (%).

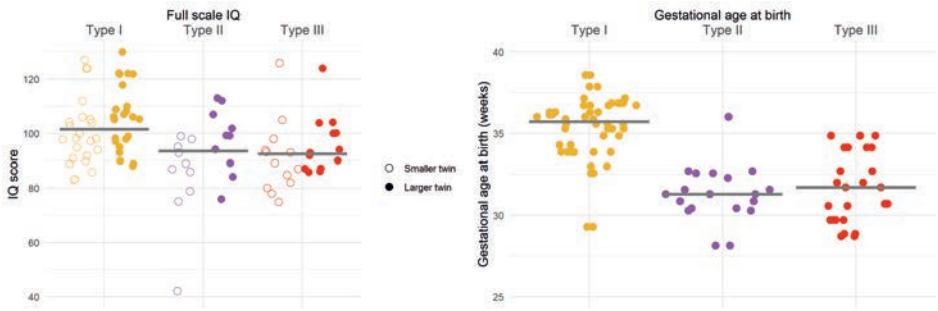

Figure 3. Sinaplot of the FSIQ and gestational age at birth per Gratacós type, with the grey line representing the median.

Smaller twins presented with significantly more frequent mild NDI than the larger twins (Table 2), and a higher odds of developing mild NDI than larger twins (odds ratio 4.8, 95% CI 1.6–14.1) based on the generalized estimating equation model. Of the children with mild NDI, 14% (3/22) children presented with multiple impairments on different domains (all smaller twins). Age (p = 0.28), weight (p = 0.45), and BMI (p = 0.22) at follow-up did not affect the presence of mild NDI. There was no difference in the presence of severe NDI (4% (2/47) children in both groups; p = 1.0). Of the children with severe NDI, 75% (3/4) children presented with multiple impairments (two were smaller twins). The proportions of mild and severe NDI per twin pair are presented in Table A2.

Table A3. Gestational age at birth and FSIQ scores in sFGR twins per Gratacós type.

Characteristic	Type I (n = 24)	Type II (n = 10)	Type III (n =13)	*p*-value
Gestational age at birth – *weeks*	35.7 (34.0-36.7)	31.3 (30.4-32.6)	31.7 (29.7-34.1)	**<0.0001**
FSIQ	102 (94-109)	94 (85-99)	93 (86-100)	**0.006**
Difference in FSIQ	6 (4-9)	14 (4-27)	6 (1-10)	0.086

FSIQ: full scale intelligence quotient.
Outcomes are presented as median (interquartile range (IQR).

Discussion

In MCDA twins with sFGR, the smaller twin presents with a lower intelligence quotient across all indexes and an increased rate of mild NDI compared with the larger co-twin. To our knowledge, we are the first to show that FGR poses a substantial risk for long-term neurodevelopment in this unique identical twin model controlling for maternal, obstetrical, and genetic factors.

We report that the prevalence of mild NDI in smaller twins with sFGR (36%) was more than double that of the general population (14%; intelligence follows a normal distribution), stressing the clinical importance of our results. This increased prevalence of mild NDI could be considered a consequence of prematurity, as research has shown an exponential increase in prevalence of developmental delay as gestational age at birth decreases[22]. However, in a large population (n = 1461) with a similar gestational age at birth (i.e., 30–34 weeks) to the twins in our study, the prevalence of mild NDI was estimated at 16%[23]. The prevalence of mild NDI for the smaller twins in our study was more than double this estimate, supporting our hypothesis that FGR also affects neurodevelopmental outcomes for a given gestational age. The larger twins in our study had a lower rate of mild NDI than did the participants in this same population (11% vs 16%), which suggests that the larger twin might be spared from adverse neurodevelopmental outcomes to a greater extent than are singletons without FGR. Being a twin is often thought to be a risk factor for NDI, but studies report no differences for twins and singletons when matched for gestational age and birthweight[24]. As we have not included a group of singletons in our study, no well-founded statements can be made.

Our findings agree with the scientific literature on neurodevelopmental outcome after FGR in singletons. A systematic review by Murray and colleagues described a 0.5 SD difference in cognitive test score for children with FGR when compared with children without FGR, exacerbated to 0.7 SD in children born at less than 35 weeks gestational age[25]. This finding is consistent with the difference of 6 points in FSIQ between the smaller and larger twin in our study. Another systematic review by Sacchi and colleagues concluded that preterm children with FGR were 1.6 times more likely to have mild cognitive delay and 2.8 times more likely to have severe cognitive delay than were children without FGR[26]. This association did not reach statistical significance in our study population due to the small sample size, but eight (17%) smaller twins had mild cognitive delay compared with two (4%) larger twins. On the

basis of the available scientific literature, we present the most complete overview of long-term neurodevelopmental outcome in a cohort of MCDA twins with sFGR.

The observed deficits for the smaller twin are hypothesised to be the consequence of prenatal adversity. The development of the brain during pregnancy is an intricate process requiring a stable and favourable environment. When this environment is suboptimal, as is the case in FGR, it can induce major changes in brain development[27]. White matter injury, a persistent reduction in grey matter volume, and altered brain connectivity on MRI have been reported in singletons with FGR[27]. In a previous study about structural changes on cerebral ultrasound, we showed that the smaller twin presents with an overall reduction in brain growth.8 All of these structural adaptations have been linked to increased rates of NDI in children with FGR.

Regarding the different Gratacós types specific for MCDA twins, our analysis shows that twins born after a pregnancy classified as type II (persistent A/REDF) or type III (intermittent A/REDF) have significantly lower FSIQ scores than those born after a pregnancy classified as type I, supporting previous research[28]. The changing umbilical artery doppler flow pattern as observed in type III pregnancy is thought to be the consequence of large arterio-arterial anastomoses on the shared placenta[5,6]. These large anastomoses can cause episodes of acute feto-fetal transfusion, which can affect brain development through either vascular overload or hypovolemic events[6]. These results should be interpreted cautiously. Children from type II and type III pregnancies were also born significantly earlier than children from type I pregnancies. It is well known that prematurity is one of the most important determinants of long-term neurodevelopmental outcomes[29]. The lower FSIQ score for children with abnormal umbilical artery Doppler flow patterns could therefore be a direct consequence of the increased rate of iatrogenic prematurity, as also reflected by our univariate and multivariate linear regression analyses for FSIQ. Both Gratacós type and gestational age at birth were univariately associated with FSIQ in our population. On multivariate analysis, these associations ceased to exist, suggesting a relationship between Gratacós type and gestational age at birth.

Our study has limitations that should be considered when interpreting the results. First, because we included live twin pairs (i.e., both twins had to be alive to be eligible) and have a low number of children from type II and type III pregnancies, we might have an under-representation of severe cases (i.e., children with more adverse perinatal outcomes). Additionally, our inclusion rate of 66% (64% in the present study)

might introduce bias into the results. Baseline characteristics were compared between the group of children who were included and the group of children who were lost to follow-up, and no significant differences were identified. Lastly, our twin design might not serve as an infallible proxy for FGR in singletons due to different pathophysiological mechanisms. FGR in singletons is primarily caused by placental insufficiency (i.e., multifactorial in origin), whereas the mechanism in MCDA twins is associated with unequal placental sharing[5]. Similarly, the neurodevelopmental outcomes of MCDA twins can differ from those of both dichorionic twins and singletons because MCDA twins share a placenta with vascular anastomoses connecting the circulatory systems of the twins[30]. Nevertheless, we present an extensive long-term follow-up in a cohort of MCDA twins with sFGR, including a broad spectrum of neurodevelopmental outcomes throughout childhood. Whereas previous studies primarily reported on neurodevelopment at the age of 2 years assessed with a surrogate questionnaire, we performed actual neurodevelopmental testing at an older age (median age 11 years), thereby increasing the reliability of our results. Moreover, in using this identical twin model, we were able to uncover the true effect of FGR on neurodevelopmental outcomes by eliminating fundamental confounders, such as gestational age at birth and genetic predisposition.

The information provided by our study allows clinicians to more accurately counsel parents about the future development of their child than before. Even though the impairments in our study population are mainly classified as mild, children are still impeded in their daily functioning. Children at risk can now be identified at an early stage after birth and in childhood, and targeted interventions can be administered to optimise development. The next step in research on neurodevelopmental outcomes in MCDA twins with sFGR involves linking the functional consequences of FGR to probable alterations in brain growth, maturation, and connectivity on MRI. Finally, the insights presented in this study are also crucial in forming a specific management protocol for MCDA twins with sFGR and emphasise that survival should not be the sole indicator of successful perinatal management.

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Chapter 9

Insecure attachment and internalizing behavior problems in growth discordant identical twins.

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Abstract

Background. Psychosocial development in monochorionic twins born after selective fetal growth restriction (sFGR) has been unreported to date, despite its importance for daily functioning and future relationships.

Aims. We aim to investigate psychosocial development, attachment and school functioning in sFGR twins and compare outcomes with the general population and between the smaller and larger twin.

Study design. Observational cohort study.

Setting. Single tertiary center.

Subjects. Monochorionic twin pairs with sFGR born between 2002-2017 (3-17 years).

Outcome measures. Multiple parent-report questionnaires: the Child Behavior Checklist (social-emotional development and behavior), the (Early) Childhood Behavior Questionnaire Very Short Form (temperament), the Attachment Insecurity Screening Inventory (attachment) and a school functioning questionnaire.

Results. Median age for the 48 twin pairs was 11 (interquartile range (IQR) 8-13) years. Attachment insecurity for both twins was significantly higher than in the general population for ambivalence/resistance (34% (21/62) vs. 16%, p = 0.024) and total attachment insecurity (35% (22/62) vs. 16%, p = 0.016). The smaller twin had more internalizing behavioral problems, i.e., negative emotions and behaviors turned inwards (22% (10/46) vs. 11% (5/46), p = 0.021) and a higher negative affect, i.e., more likely to experience negative emotions (3.2 (2.9-3.7) vs. 2.9 (2.2-3.2), p = 0.009) than the larger twin, as well as a lower secondary school level (p = 0.031).

Conclusions. Monochorionic twins with sFGR have more ambivalent/resistant attachment insecurity following the complicated pregnancy course. The smaller twin has a tendency towards negative emotions and internalizing behaviors compared to the larger twin, indicating an increased sensitivity for depression and anxiety.

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Introduction

Monochorionic (MC) twins are identical twins who share a single placenta during pregnancy, which can give rise to multiple complications due to the vascular anastomoses[1]. The placenta can also be unequally shared, causing a discordant distribution of nutrients and oxygen leading to a large intertwin growth discrepancy[2]. This condition is called selective fetal growth restriction (sFGR) and is reported to have high rates of perinatal morbidity and mortality as well as long-term neurodevelopmental impairment (NDI)[3,4]. While cognitive and motor outcomes have recently been elaborately described[4], psychosocial development in these twins is unreported so far, despite its importance in a child's day-to-day ability to cope with environmental and social tasks and to reach important milestones.

Psychosocial development encompasses the development of social skills and learning how to behave and respond in different social environments[5]. The main domains include behavior, emotional well-being and social competence. At the foundation of early psychosocial development are temperament, i.e., individual differences in behavioral tendencies, and attachment to caregivers[6]. The majority of children has secure attachment with at least one caregiver. Insecure attachment can be subdivided into three styles: avoidant (avoiding seeking comfort from caregivers), ambivalent/resistant (constantly seeking attention while also resisting contact) and disorganized (inconsistent mixture of avoidance and ambivalence/resistance)[7]. Impaired psychosocial functioning can significantly affect both school functioning and academic performance[8].

FGR in singletons has already been associated with more psychosocial difficulties[9]. This suggests that the smaller twin potentially experiences more challenges than its larger co-twin. This unique identical twin model allows us to eliminate any confounding of genetic, obstetrical or maternal factors that can affect psychosocial development, such as gestational age at birth or maternal stress[10,11]. We hypothesize that as these twins and their parents are faced with a complicated pregnancy course and high rates of prematurity, this can negatively impact their early psychosocial development and attachment relations. Therefore, the aim of our study is to evaluate psychosocial development including behavior, temperament and attachment and subsequent school functioning and academic performance throughout childhood in a cohort of MC twin pairs with sFGR and to compare these outcomes 1) for the group as a whole with the general population and 2) between the smaller and larger twin within each twin pair.

Methods

This study is part of the 'Long-term Effects of selective fetal growth restriction in MONochorionic twins' (LEMON) study (Netherlands Trial Register ID NL9833), which was reviewed and approved by the ethics committee of the LUMC (P20.089). The LEMON study is a cohort study focusing on all MC twin pairs with sFGR born in the Leiden University Medical Center (LUMC), the national referral center for complicated MC twins in the Netherlands. Parents and/or children ≥ 12 years of age were asked for informed consent and inclusion was finalized in January 2022.

MC twin pairs with sFGR born in the LUMC between 2002-2017 aged 3-17 years were eligible for this study, with sFGR defined as a birth weight discordance (BWD) ≥ 20% (calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100)[12]. Cases with twin-twin transfusion syndrome (TTTS), twin anemia polycythemia sequence and monoamnionicity were excluded. Cases with mortality of the co-twin did not allow for within-pair comparison and were excluded, as well as twins with twin reversed arterial perfusion or other congenital abnormalities.

The following maternal, obstetrical and neonatal baseline characteristics were collected: maternal age, gravidity, parity, Gratacós type (based on umbilical artery Doppler flow patterns, with type I positive end-diastolic flow, type II persistent absent/reversed end-diastolic flow and type III intermittent absent/reversed end-diastolic flow[13]), gestational age at birth, sex, delivery mode, BWD, birth weight, small for gestational age (SGA) (birth weight < 10th centile[14]), severe neonatal morbidity[15] and maternal education level, divided into primary and secondary school, intermediate vocational education and higher vocational education and university.

When informed consent was obtained, parents were asked to fill in multiple questionnaires about their twins applicable to different age groups. To assess psychosocial development, three questionnaires reporting on social-emotional and behavioral functioning, temperament and attachment were used. Social-emotional and behavioral functioning was recorded using the Child Behavior Checklist (CBCL) for ages 2-5 years and 6-18 years, reporting standard T-scores using a Dutch normative sample (mean T-score of 50 with a standard deviation (SD) of 10). T-scores were considered borderline to clinical if the T-score ≥ 60 on one of the broadband scales: internalizing problems (negative emotions and behaviors turned inwards), externalizing problems (negative emotions or behaviors turned outwards) or total problems[16]. To assess temperament, the early childhood behavior questionnaire very

short form (ECBQ-VSF) for children aged 2-3 years and the children's behavior questionnaire very short form (CBQ-VSF) for children aged 4-5 years were used, reporting on three broadband scales: negative affect, i.e., the experience and expression of negative emotions, surgency, i.e., tending towards increased expression of positive emotions, and effortful control, i.e., self-regulation of attention, activity and behavior[17,18]. Lastly, the Attachment Insecurity Screening Inventory (AISI) for ages 2-5 years and 6-12 years were used to screen for any attachment insecurity based on three subscales and a total scale: avoidance ((sub)clinical with a score ≥ 20), ambivalence/resistance ((sub)clinical with a score ≥ 17), disorganization ((sub)clinical with a score ≥ 16) and total attachment insecurity ((sub)clinical with a score ≥ 46)[19,20]. The (sub)clinical scores are based on standardized T-scores with a mean 50 and SD 10.

Parents were asked to report on school functioning. The type of education (regular or special needs) was recorded, as well as any parent-reported learning problems (communication/language problems, reading problems amongst which dyslexia, writing problems, arrhythmic problems amongst which dyscalculia). The primary school system in the Netherlands consists of eight grades ranging from grade 1 (four years old) to grade 8 (twelve years old), in which group 1 and 2 are comparable to kindergarten. From group 3 onwards, children learn reading, writing and arithmetic. Grade repetition in either group 1-2 or group 3-8 of primary school was documented[21-23]. From twelve years onwards, children go to secondary school that is divided into three levels: pre-vocational education, senior general education and pre-university education. Academic performance was assessed using the latest standardized test scores from the Dutch Pupil Monitoring System developed by the National Institute for Educational Measurement as requested from teachers by parents themselves[24-26]. These academic tests encompass three domains: arithmetic, spelling and reading comprehension. The test results are translated in ability scores, which are in turn divided into five levels (I-IV) with I being the top 20% highest scoring children and V being the 20% lowest performing children.

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 (%). To test for association between sFGR and behavior, attachment, temperament, school functioning, academic performance and quality of life a Generalized Estimating Equation (GEE) was used. This analysis considers that observations between co-twins are not independent. A *p*-value of < 0.05 was considered statistically significant.

Results

Between 2002-2017, 73 MC twin pairs with sFGR were eligible for inclusion. Of these twin pairs, 12 (16%) did not want to participate and 13 (18%) were lost to follow-up (5 twin pairs moved abroad and 8 could not be reached for inclusion), leaving 48 twin pairs to be included in the LEMON study.

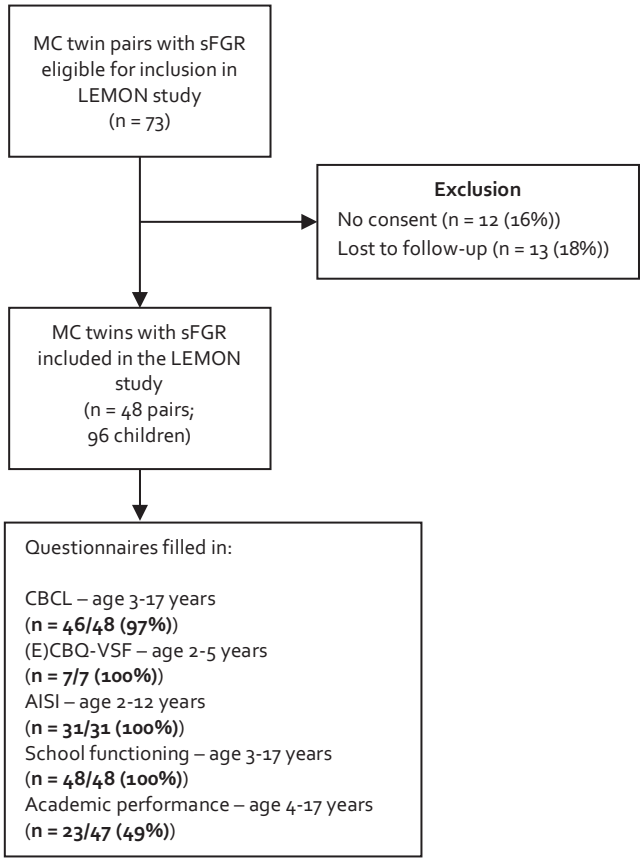

Figure 1. Flowchart of LEMON study inclusion. MC: monochorionic, sFGR: selective fetal growth restriction, CBCL: Child Behavior Checklist, (E)CBQ-VSF: (Early) Childhood Behavior Questionnaire – Very Short Form, AISI: Attachment Insecurity Screening Inventory.

Baseline characteristics are presented in Table 1. As the questionnaires are applicable to different ages and the age in our study population ranged from 3-17 years (median age at participation was 11 (IQR 8-13) years), not every questionnaire was applicable to each twin pair. One CBCL was not filled in and one CBCL could not be filled in due to a language barrier. Academic test scores (applicable to children from grade 3 onwards, 47/48) were available for 23/47 (49%) of twin pairs (Figure 1).

Table 1. Maternal, obstetrical and characteristics for the 48 included sFGR twin pairs.

Characteristics	MC twins (n=96; 48 pregnancies)	Smaller twin (n=48)	Larger twin (n=48)
Maternal age at delivery – *years*	32 (29-35)		
Gravidity	2 (1-2)		
Parity	0 (0-1)		
Gratacós type			
Type I	25 (52)		
Type II	10 (21)		
Type III	13 (27)		
Gestational age at birth – *weeks*	34.0 (31.3-36.0)		
Female	48 (50)		
Caesarean	54 (56)		
Birth weight discordance – *%*	30.2 (26.3-33.3)		
Birth weight – *grams*		1433 (1112-1879)	2025 (1608-2695)
Small for gestational age		46 (96)	11 (23)
Severe neonatal morbidity		10 (21)	10 (21)
Maternal education			
Primary and secondary school	5 (10)		
Intermediate vocational education	20 (42)		
High vocational education or university	23 (48)		

MC: monochorionic.
Outcomes are presented as median (interquartile range (IQR)), n/N (%) or n (%).

MC twin pairs with sFGR versus the Dutch general population

Social-emotional and behavioral functioning in MC twin pairs with sFGR did not differ from the Dutch norm population (Table 2). Temperament could not be compared to the Dutch norm population as this was only available for 7 twin pairs and the data was not normally distributed. (Sub)clinical attachment insecurity was significantly higher for MC twin pairs with sFGR as opposed to the Dutch norm population with 35% (22/62) vs. 16%, p = 0.016. This was primarily attributable to a higher rate of ambivalent/resistant attachment (34% (21/62) vs. 16%, p = 0.024).

School functioning and academic performance are presented in Table 3. As there is no reliable estimation of learning problems in Dutch children at present, this comparison could not be made. MC twin pairs with sFGR more often repeated group 1-2 of primary school than the Dutch norm population with 10% (10/96) as opposed to 3% (p = 0.014). The median gestational age at birth of the twins who repeated group 1-2 was 30 (29-35) weeks.

Table 2. Behavioral functioning, attachment and temperament as measures of psychosocial development in MC twin pairs with sFGR.

Outcomes	MC twins (n=96)	Dutch norm population	*p*-value	Smaller twin (n=48)	Larger twin (n=48)	*p*-value
Age at participation – *years*	11 (8-13)					
Borderline to clinical behavioral problems (n = 46 pairs)						
Internalizing	15/92 (16)	16%	0.960	10/46 (22)	5/46 (11)	**0.021**
Externalizing	7/92 (8)	16%	0.103	5/46 (11)	2/46 (4)	0.189
Total	9/92 (10)	16%	0.283	5/46 (11)	4/46 (9)	0.563
Temperament (n = 7 pairs)						
Negative affect	3.0 (2.4-3.3)	-	-	3.2 (2.9-3.7)	2.9 (2.2-3.2)	**0.009**
Surgency	4.5 (3.8-4.8)	-	-	4.3 (3.8-4.7)	4.6 (3.8-5.4)	0.232
Effortful control	5.0 (4.5-5.2)	-	-	4.8 (4.5-5.0)	5.0 (4.7-5.3)	0.110
(Sub)clinical attachment insecurity (n = 30 pairs)						
Avoidance	9/62 (15)	16%	0.805	4/31 (13)	5/31 (16)	0.706
Ambivalence/ resistance	21/62 (34)	16%	**0.024**	11/31 (36)	10/31 (32)	0.654
Disorganization	11/62 (18)	16%	0.797	5/31 (16)	6/31 (19)	0.654
Total	22/62 (35)	16%	**0.016**	11/31 (36)	11/31 (36)	1.000

Outcomes are presented as median (interquartile range (IQR)) or n/N (%)

The smaller versus the larger twin: a within-pair comparison

The smaller twin demonstrated significantly more internalizing problems in the borderline to clinical range as opposed to the larger twin, namely 22% (10/46) vs. 11% (5/46) with p = 0.021 (Table 2). The analysis of temperament showed a significantly higher negative affect for the smaller twin (3.2 (2.9-3.7) vs. 2.9 (2.2-3.2), p = 0.009). Attachment did not differ between the larger and smaller twin within twin pairs (i.e., the same insecure attachment styles were observed within one family). Both presented with a high rate of ambivalence/resistance (36% (11/31)) and total attachment insecurity (36% (11/31)).

The level of secondary education differed significantly between the larger and smaller twin: the larger twin more often followed pre-university education (41% (7/17)) compared to the smaller twin (18% (3/17)), and the smaller twin more often followed

senior general education (29% (5/17)) compared to the larger twin (18% (3/17)), with *p* = 0.031 (Table 3). Arrhythmic and spelling level were similar for the smaller and larger twin but reading comprehension levels showed that most smaller twins were in either level II (27% (6/22)) or level III (36% (8/22) while most larger twins were in either level I or II (both 32% (7/22), *p* = 0.025).

Table 3. School functioning and academic performance in MC twin pairs with sFGR.

Outcomes	MC twins (n=96)	Dutch norm population	*p*-value	Smaller twin (n=48)	Larger twin (n=48)	*p*-value
Special needs education	4/96 (4)	3%	0.424	3/48 (6)	1/48 (2)	0.171
Learning problems	16/96 (17)	-	-	10/48 (21)	6/48 (13)	0.155
Grade repetition						
Group 1-2	10/96 (10)	3%	**0.014**	6/48 (13)	4/48 (8)	0.316
Group 3-8	10/96 (10)	8%	0.471	5/48 (10)	5/48 (10)	1.000
Secondary education level (n = 17 pairs)						**0.031**
Pre-vocational	16/34 (47)	-	-	9/17 (53)	7/17 (41)	
Senior general	8/34 (24)	-	-	5/17 (29)	3/17 (18)	
Pre-university	10/34 (29)	-	-	3/17 (18)	7/17 (41)	
Arrhythmic level[±] (n = 23 pairs)			0.349			0.113
I	13/46 (27)	20%		3/22 (14)	10/22 (46)	
II	10/46 (22)	20%		8/22 (36)	2/22 (9)	
III	6/46 (13)	20%		3/22 (14)	2/22 (9)	
IV	5/46 (10)	20%		2/22 (5)	4/22 (18)	
V	12/46 (25)	20%		7/22 (32)	4/22 (18)	
Spelling level[±] (n = 23 pairs)			0.295			0.483
I	13/46 (27)	20%		4/22 (18)	9/22 (41)	
II	12/46 (25)	20%		8/22 (36)	4/22 (18)	
III	10/46 (21)	20%		6/22 (27)	4/22 (18)	
IV	5/46 (10)	20%		1/22 (5)	4/22 (18)	
V	6/46 (13)	20%		3/22 (14)	1/22 (5)	
Reading comprehension level[±] (n = 23 pairs)			0.106			**0.025**
I	10/46 (22)	20%		3/22 (14)	7/22 (32)	
II	13/46 (28)	20%		6/22 (27)	7/22 (32)	
III	13/46 (28)	20%		8/22 (36)	4/22 (18)	
IV	4/46 (9)	20%		2/22 (9)	2/22 (9)	
V	6/46 (13)	20%		3/22 (14)	2/22 (9)	

Outcomes are presented as n/N (%).

[±]Two smaller twins went to special education and therefore had no regular education levels available. These pairs were not included in the within-pair comparison.

Discussion

Our study shows that MC twin pairs with sFGR present with substantially higher (sub)clinical attachment insecurity when compared to the general population, particularly for ambivalent/resistant attachment. In addition, the smaller twin had more internalizing behavioral problems (negative emotions and behaviors turned inwards) and a higher negative affect (more likely to experience negative emotions) when compared to the larger twin, indicating an increased sensitivity for depression and anxiety.

The process of attachment already starts during pregnancy. As previously described for TTTS, increased uncertainty about the health of the twins towards their birth results in more depressive symptoms (72%), anxiety (50%) and post-traumatic stress disorder (30%) with a subsequent lower prenatal attachment for prospective parents[27-29]. Similarly, parents of MC twins complicated by sFGR often experience a difficult pregnancy full of uncertainty and are confronted with an increased risk of perinatal loss and the options to perform a selective reduction of the smaller twin. This can unconsciously impair early attachment between parents and children. Prematurity is known to further impact the parent-child relationship and is associated with an increased rate of ambivalent/resistant attachment (23%) as also observed in our study population (34%)[30]. The median gestational age at birth of twins that were found to have (sub)clinical attachment insecurity was 31 weeks and larger and smaller twins were equally affected, indicative of an influence of prematurity/complicated pregnancy course rather than a twin-specific effect. Nonetheless, parents and children can benefit from further guidance during pregnancy and in the first year after birth to identify problems in an early stage and minimize attachment insecurity.

With regard to within-pair differences, we found that the smaller twin presents with a tendency towards negative emotions and internalizing behaviors, as also described in previous research in singletons with FGR or born SGA[9]. By using this unique, discordant identical twin model we have now established that these neurobehavioral deficits after FGR are irrespective of genetic predisposition, obstetrical complications or gestational age at birth. The two identified characteristics in our study are closely intertwined and have been linked to the development of psychopathology in adolescence and adulthood, especially depression and anxiety[31]. The detected deficits may be the result of an abnormal brain development following FGR. The chronic state of hypoxia that the fetus experiences inhibits brain growth and maturation in utero, as evidenced by previous studies reporting on decreased brain volumes, altered

gyrification, delayed myelination and reduced connectivity[32,33]. These structural changes are thought to have functional neurobehavioral consequences: poor attention, altered mood, irritability and anxiety [32]. In the future, MRI studies are necessary to look more closely at the changes in structural brain development that underlie the findings in this study.

In a prior analysis of the LEMON study, we have shown that the smaller twin had a significantly lower IQ across all indexes[4]. Working Memory was most affected with an 8 point within-pair difference and is at the basis of learning and essential for remembering and processing new information. We have now demonstrated that smaller twins did not have more learning problems, but that they did attend a lower secondary school level than their larger co-twin despite their identical genetic predisposition. In addition, even though arrhythmic and spelling levels were similar, the smaller twin did score lower for reading comprehension. Yet, it should be noted that only 17/48 twin pairs attended secondary school in our population and information on academic performance was only available in 49% of the participating twin pairs, possibly resulting in an overestimation of overall performance by response bias. In general, it can be concluded that as long as children attend a level of education that fits their needs and capacities, both the smaller and larger twin can function adequately at school.

Our study has limitations that should be taken into account when interpreting our data. Firstly, we only included double survivors in this study which potentially leads to an underestimation of problems. Parents of twin in which single fetal demise has occurred or parents who opted for selective reduction of the smaller twin experience more anxiety, depression and posttraumatic stress, presumably affecting the early psychosocial development of the surviving twin to a greater extent[34]. Secondly, as the questionnaires were not applicable to every twin pair due to the wide age range, groups per outcome measure were relatively small. Thirdly, only parent-reported questionnaires were used, potentially introducing response bias in the results as parents may be prone to give more positive evaluations about their children[35]. Lastly, a comparison of outcomes with a group of uncomplicated twins may be better suited than the Dutch norm population to take into account twin interaction in childhood that can influence psychosocial development and attachment to mothers and fathers[36]. This group is unavailable at present. Similarly, a comparison with a population of preterm, SGA singletons with the same gestational age range would allow us to explore whether our findings are twin-specific. Yet, current literature does

not allow for such a comparison due to heterogeneity in methodology of assessments of psychosocial development. So, future prospective research should include both parent- and teacher-reported questionnaires at standard time points in childhood, an additional qualitative assessment and a control group of uncomplicated twins as well as preterm, SGA singletons to provide more conclusive evidence. Nevertheless, our study is strengthened by the extensive follow-up evaluating different domains of psychosocial development and the consequences for school functioning and academic performance and by the unique identical twin model controlling for genetic, obstetrical and maternal factors. At present, we are the first to describe these outcomes in MC twin pairs with sFGR, including a within-pair comparison.

Conclusion

The insights presented in this study allow for improved parent counseling about the more fine-grained aspects of development throughout childhood. Early detection of problems and subsequent targeted interventions can further optimize the circumstances surrounding early psychosocial development. We recommend parent-child guidance throughout pregnancy and the first year after birth to promote the formation of secure attachment with both twins. In addition, we provide favorable information on school functioning and academic performance, which are outcomes that have not previously been reported for this cohort but that are of importance to parents. Our results stress the fact that there is more to the development of a child than cognition and motor functioning alone.

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Chapter 10

Fetal growth restriction inhibits childhood growth despite catch-up in discordant identical twins.

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Abstract

Background and objectives. Research suggests that postnatal catch-up growth after fetal growth restriction (FGR) occurs frequently and is completed within two years. Yet, postnatal growth in singletons may be influenced by multiple factors. Identical twins with discordant prenatal growth, termed *selective* FGR (sFGR), can be regarded as a natural experiment eliminating these sources of bias.

Methods. Monochorionic twins with sFGR born in our center between 2002-2017 were eligible for inclusion. Growth measurements (height, weight, head circumference) were performed at follow-up. Detailed growth curves as documented by a systematic primary care system in the Netherlands were retrospectively collected. A mixed-effects model was used to assess within-pair standard deviation score (SDS) difference and individual height SDS relative to target height SDS.

Results. Forty-seven twin pairs (94 children) were included at a median age of 11 (8-13) years. At the time of the last measurement, the smaller twin at birth had a lower height SDS (-0.6 vs. -0.3, $p < 0.001$, median difference 0.5 (95% CI 0.4-0.7)), lower weight SDS (-0.5 vs. -0.1, $p < 0.001$, median difference 0.8 (95% CI 0.5-1.0)) and lower head circumference SDS (-0.5 vs 0.2, $p < 0.001$, median difference 0.6 (95% CI 0.6-0.9)) compared to larger twins. These differences persisted at least until the age of seventeen. Smaller twins catch-up to a height within their target range between 8-11 years.

Conclusions. Identical twins with discordant prenatal growth maintain a modest but significant difference in height, weight and head circumference until, indicating a persistent, inhibitory effect of an adverse intrauterine environment on childhood growth.

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Introduction

Fetal growth restriction (FGR) is a condition in which the fetus is unable to reach its intrinsic growth potential due to unfavorable intrauterine circumstances[1]. A period of accelerated growth usually follows after birth as compensation, termed catch-up growth. This is regarded as completed when height is within normal range. Multiple definitions of completed catch-up growth can be identified in literature, including height above -2 standard deviation score (SDS) on population growth curves, or height within target height (TH) range, based on parental height[2]. The former is useful for tracking childhood growth in a clinical setting, to evaluate whether height deviates substantially from the norm and an intervention may be necessary. The latter also explores growth in relation to an individuals' actual intrinsic growth potential. Children born small for gestational age (SGA) generally complete catch-up growth within two years after birth and approximately 90% has reached a normal height, i.e., above -2 SDS at eight years[3,4]. At twelve years, the mean height of children born after FGR falls within 0.5 SDS of the population mean and only 5% had a height below TH range[5]. Yet, comparisons of childhood growth measurements of appropriately-grown singletons cannot control for known and unknown factors that influence postnatal growth, including maternal, obstetrical, genetic factors, and postnatal family environment. The study of monochorionic (MC) twin pairs affected by selective fetal growth restriction (sFGR) provides a direct opportunity to circumvent these limitations.

MC twins are monozygotic twins, who share a single placenta in utero. This placenta is unequally shared in 10-15% of pregnancies which is thought to cause a disproportionate oxygen and nutrient supply resulting in a growth discrepancy. When the difference in birth weight of the twins is more than 20%, this is defined as sFGR[6-8]. Within such twin pairs a growth-restricted twin can be compared with a larger co-twin who is genetically identical and who shared similar maternal and obstetric factors as well as postnatal family environment. Therefore, the study of sFGR twins results in a robust estimate of the long-term effect on growth of FGR due to an adverse intrauterine environment.

At present, research on catch-up growth in birth weight discordant monozygotic twins is scarce (Table 1)[9-14]. In the available studies sample sizes are often limited, chorionicity is largely unknown and neither BMI nor pubertal status were recorded. Additionally, the timing and number of growth measurement varied substantially and importantly, multiple definitions of catch-up growth have been used. Therefore,

detailed analysis of catch-up growth patterns in MC twins with sFGR is unavailable at present. Hence, the aim of this study is to assess the childhood growth patterns of MC twins with sFGR to evaluate to what extent catch-up growth (i.e., height within TH range) occurs in the smaller twin, using comprehensive growth measurements from birth up to seventeen years of age. Our definitions of catch-up growth thereby differs from previous studies focusing on population growth curves and allows for the analysis of more subtle differences in growth.

Table 1. An overview of available literature on catch-up growth in monozygotic twins.

Authors (year)	Study population	Follow-up	Findings
Babson et al. (1973)	9 discordant MZ twin pairs of which 3 MC	Three measurements between: 7.5-11.5 yrs, 12-16 yrs, 18-22 yrs	Smaller twin 5.6-6.8 cm shorter than larger twin at each follow-up moment.
Buckler et al. (2009)	38 discordant MZ twin pairs	One measurement between 2-9 yrs	Smaller twin 0.5 SDS shorter and 0.8 SDS lighter than larger twin.
Henrichsen et al. (1986)	14 discordant MZ twin pairs	One measurement between 9-17 yrs	Smaller twin 0-8 cm shorter and 0-1.5 kg lighter than larger twin.
Keet et al. (1986)	14 discordant MZ twin pairs	Nine measurements from birth until 6 yrs	Within-pair percentage difference at 6 years of age was 0.2% for height, 8.0% for weight and 1.0% for head circumference.
Schulte et al. (2016)	16 discordant MC twin pairs after TTTS	Three measurements at a mean age of 2, 4 and 10 yrs	Smaller twin 0.53 SDS shorter than larger twin at age 14.6 yrs.
Wilson (1978)	10 discordant MZ twin pairs	One measurement at 6 yrs	Smaller twin was 1.85 cm shorter and 2.19 kg lighter than larger twin at 6 years of age.

MZ: monozygotic, MC: monochorionic, TTTS: twin-twin transfusion syndrome.
Outcomes are presented as median (interquartile range (IQR)) or n (%).

Methods

This study is part of the LEMON study (Long-term Effects of selective fetal growth restriction in MONochorionic twins, International Clinical Trial Registry Platform ID NL9833), a longitudinal cohort study including all MC twins with sFGR born in the Leiden University Medical Center (LUMC) in the age range of 3-17 years with available growth measurements from birth onwards[15]. The LUMC is the national referral center for complicated MC twins in the Netherlands, so data of a large cohort of MC twins is available. The LEMON study was reviewed and approved by the ethics committee of the LUMC (P20.089). All parents and/or children ≥ 12 years of age have provided written informed consent. The neurodevelopmental outcomes, including cognitive test scores, of the twins included in the LEMON study have previously been described[15].

All MC twins with sFGR born in the LUMC between 2002-2017 were eligible for this study, with sFGR defined as a birth weight discordance ≥ 20% (calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin x 100)[8]. Cases with twin-twin transfusion syndrome (TTTS), twin anemia polycythemia sequence or monoamnionicity were excluded, as well as cases complicated by perinatal mortality in one or both twins before inclusion, since this would preclude within-pair analyses[16,17]. Cases with twin reversed arterial perfusion (TRAP) or other congenital abnormalities were excluded as well.

The following maternal, obstetrical and neonatal baseline characteristics were collected from digital patient files: maternal age, gravidity, parity, Gratacós type based on umbilical artery (UA) Doppler flow patterns in the smaller twin (type I positive end-diastolic flow, type II persistent absent/reversed end-diastolic flow, type III intermittent absent/reversed end-diastolic flow[18], gestational age at birth, sex, delivery mode and birth weight from which birth weight discordance and small for gestational age (SGA) (birth weight < 10th centile) were derived.

After informed consent was obtained, a follow-up examination was scheduled in which standardized growth measurements (height, weight, body mass index (BMI), head circumference, arm span and sitting height) were obtained of each twin. Parents were asked to bring the childhood growth curves as documented by the primary care system, to the examination. The primary care system in the Netherlands consists of regular follow-up appointments for every child, including height, weight and head circumference measurements at standard time points (3 months, 5-6 months, 10-12

months, 12-15 months, 22-26 months, 22-29 months and 42-48 months). If twins were simultaneously followed up in a local hospital in case of prematurity or dysmaturity, these growth measurements were retrieved as well. Only measurements of both twins on the same day were used for further analysis. Prior to the follow-up examination, both parents were asked to report their own height and weight in a questionnaire. Children ≥ 8 years of age were asked to fill out the Pubertal Development Scale, a standardized and validated self-assessment on pubertal status in children, classifying them on an ordinal scale from 1 = prepubertal, 2 = early pubertal, 3 = mid pubertal, 4 = late pubertal to 5 = post pubertal[19].

All growth measurements throughout childhood were plotted in Dutch growth curves, generating appropriate standard deviation scores (SDS)[20]. No correction for gestational age was applied, as this is not generally performed in clinical practice. BMI was regarded as an absolute value in line with clinical practice and as appropriate Dutch SDS are currently unavailable. Within-pair differences in height SDS, weight SDS and BMI were calculated as SDS or BMI larger twin – SDS or BMI smaller twin. TH was calculated according to the Dutch guidelines taking ethnicity into account and plotted in the growth curves as well[21]. TH range was defined as -0.8 to +0.8 SDS. Subsequently, catch-up growth was defined as growth into TH range[2].

Statistical analyses were performed using IBM Statistics Version 25.0 (SPSS, Inc. an IBM company, Chicago, IL, USA) and RStudio Version 2021.9.2.382 (RStudio, PBC, Boston, MA, USA). Data are presented as median (interquartile range (IQR)), n/N (%) or n (%). To test for association between FGR and the growth measurements/pubertal status at follow-up examination, a Wilcoxon signed-rank test was used (non-parametric data). This analysis takes into account that observations between co-twins are not independent. A *p*-value of < 0.05 was considered statistically significant. Multiple mixed-effects models were compared and tested (Supplement). Ultimately, mixed-effects models using a third-degree natural cubic spline to fit the curves were used to assess 1) within-pair difference in height SDS, BMI and head circumference SDS in relation to age to evaluate catch-up growth relative to the larger twin and 2) individual height SDS minus TH SDS in relation to age (a negative value indicates height below TH), to evaluate catch-up growth of both twins to their TH range. These models included a twin-specific random effect (second degree spline).

Results

Between 2002-2017, 73 twin pairs were eligible for inclusion. Of these twin pairs, 12 (16%) did not want to participate in the study and 13 (18%) were lost to follow-up (5 twin pairs moved abroad and 8 could not be reached for inclusion). Ultimately, 47 twin pairs were included.

Table 2. Maternal, obstetrical and characteristics for the 47 included sFGR twin pairs.

Characteristics	**MC twins (n=94; 47 pregnancies)**
Maternal age at delivery – *years*	32 (29-35)
Gravidity	2 (1-2)
Parity	0 (0-1)
Gratacós type	
Type I	24 (51)
Type II	10 (21)
Type III	13 (28)
Gestational age at birth – *weeks*	33.9 (31.3-36.0)
Female	48 (51)
Caesarean	54 (57)
Birth weight discordance – *%*	30.1 (26.1-33.4)
Birth weight – *grams*	1744 (1219-2184)
Smaller twin	1400 (1111-1875)
Larger twin	2003 (1600-2680)
Small for gestational age	57 (61)
Smaller twin	46 (98)
Larger twin	11 (23)

MC: monochorionic.

Outcomes are presented as median (interquartile range (IQR)) or n (%).

Baseline characteristics are presented in Table 2. Two smaller twins had an indication to start with recombinant growth hormone therapy. One of them (age 5 years) was scheduled to start recombinant growth hormone therapy after the follow-up examination, so all growth measurements could still be included in this study. The other one (age 11 years) had started recombinant growth hormone therapy at age four, so only growth measurements up to this point of both the smaller and larger twin were included in the analysis. Moreover, in one twin pair growth measurements at follow-up examination could not be performed due to severe cognitive impairment and subsequent resistance to anthropometric measurements in the smaller twin.

The SDS scores of the growth measurements at the follow-up examination are shown in Table 3. All SDS scores differed significantly between the smaller and the larger

twin, with persistently lower SDS for the smaller twin for all three main outcome measurements (height, weight and head circumference). The smaller twin had a 0.3 lower SDS in height as opposed to the larger twin (-0.6 vs. -0.3, $p < 0.0001$; median difference 0.5 (95% CI 0.4-0.7)); weight was 0.4 SDS lower (-0.5 vs. -0.1, $p < 0.0001$; median difference 0.8 (95% CI 0.5-1.0)) and head circumference was 0.7 SDS lower (-0.5 vs. 0.2, $p < 0.0001$; median difference 0.8 (95% CI 0.6-0.9)). Median BMI was 16.0 (IQR 14.9-19.4) kg/m^2 for the smaller twin and 17.2 (IQR 16.0-20.3) kg/m^2 for the larger twin ($p < 0.0001$). Pubertal status did not differ between the smaller and larger twin ($p = 0.915$). In the majority of twin pairs, the smaller twin was smaller (91% (41/45)), lighter (93% (41/44) and had a smaller head circumference (88% (38/43)) at the follow-up examination, with $p < 0.0001$).

Table 3. Childhood growth measurements in the smaller vs. the larger twin in sFGR twin pairs.

Outcomes	Smaller twin (n=45)	Larger twin (n=45)	*p*-value
Age at participation	11 (8-13)	11 (8-13)	
Height – *SDS*	-0.6 (-1.7--0.1)	-0.3 (-1.3-0.3)	**<0.0001**
Weight – *SDS*	-0.5 (-1.4-0.3)	-0.1 (-0.6-1.0)	**<0.0001**
Head circumference – *SDS*	-0.5 (-1.4-0.3)	0.2 (-0.4-0.8)	**<0.0001**
BMI – *kg/m²*	16.0 (14.9-19.4)	17.2 (16.0-20.3)	**<0.0001**
Pubertal status‡			0.915
Pre-pubertal	10 (22)	10 (22)	
Early pubertal	19 (42)	17 (38)	
Mid-pubertal	6 (13)	9 (20)	
Late pubertal	8 (18)	7 (16)	
Post-pubertal	2 (4)	2 (4)	
Within-pair size differences at follow-up			
Smaller height	41 (91)	4 (9)	**<0.0001**
Lower weight	41 (93)	3 (7)	**<0.0001**
Smaller head circumference*	38 (88)	5 (12)	**<0.0001**

SDS: standard deviation score, BMI: body mass index, kg: kilograms, m: meters, TH: target height.
Outcomes are presented as median (interquartile range (IQR)) or n (%).
‡Pubertal status was unknown in one twin pair.
*Two twin pairs had the same head circumference at follow-up.

Next, we investigated all 1072 growth measurements available for both twins on the same date, starting at birth followed by all standardized measurements by the primary care system and any other follow-up appointments by physicians, up until the final follow-up study visit. Within-twin pair difference in height SDS decreased steadily from 0- 17 years, with the most rapid decrease in the first two years after birth (Figure 1). At the age of 17, a within-pair difference in height of 0.3 SDS remained. Similarly, the within-twin pair difference in BMI decreased predominantly in the first

year to subsequently stabilize around 1 kg/m^2. The within-pair difference in head circumference SDS also decreased most in the first year and stabilized at approximately 0.7 SDS.

Finally, we compared the individual height SDS minus TH SDS between the smaller and larger twin according to age (Figure 2). The larger twin was found to rapidly catch-up to its TH range at six months. This rapid catch-up growth continued until the age of two. The smaller twin showed a similar rapid catch-up growth in the first two years of life, albeit still incomplete in the majority of cases at this age. Further catch-up growth slowed down from two years onwards and was completed between ages 8-11 years. Both the smaller and larger twin displayed an additional gradual increase in height SDS between ages 10-18 years.

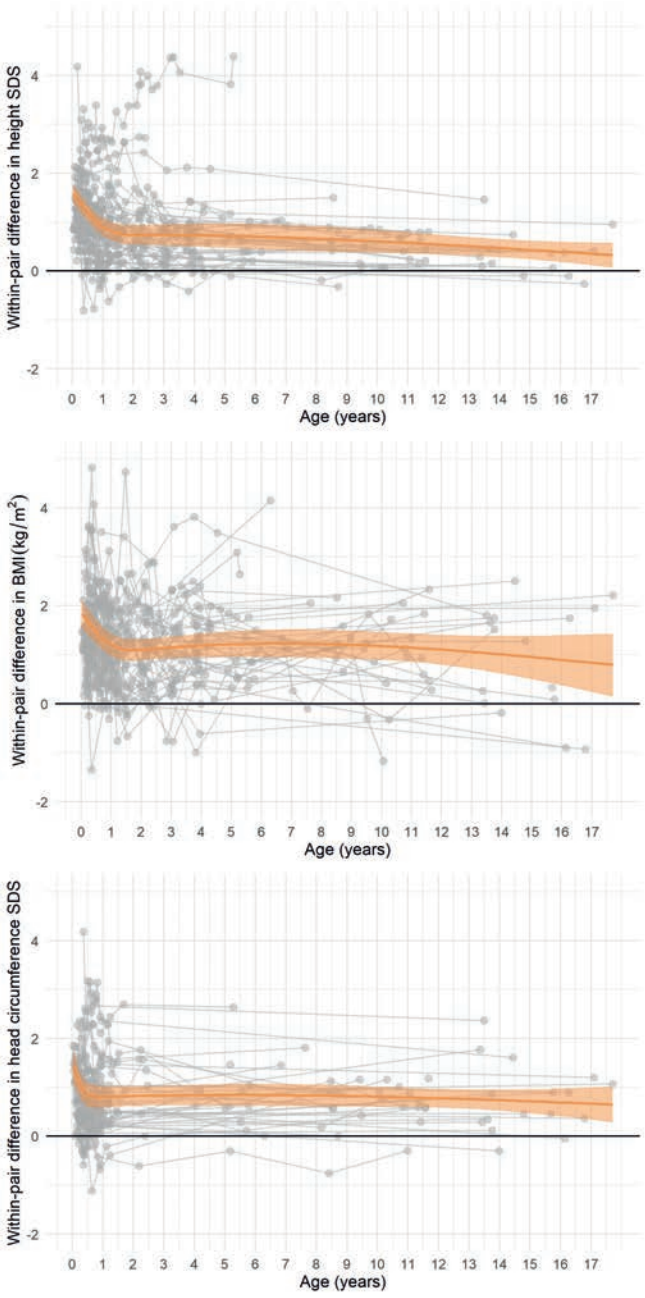

Figure 1. Mixed effects-model depicting the within-pair difference in height SDS, BMI and head circumference SDS according to age.

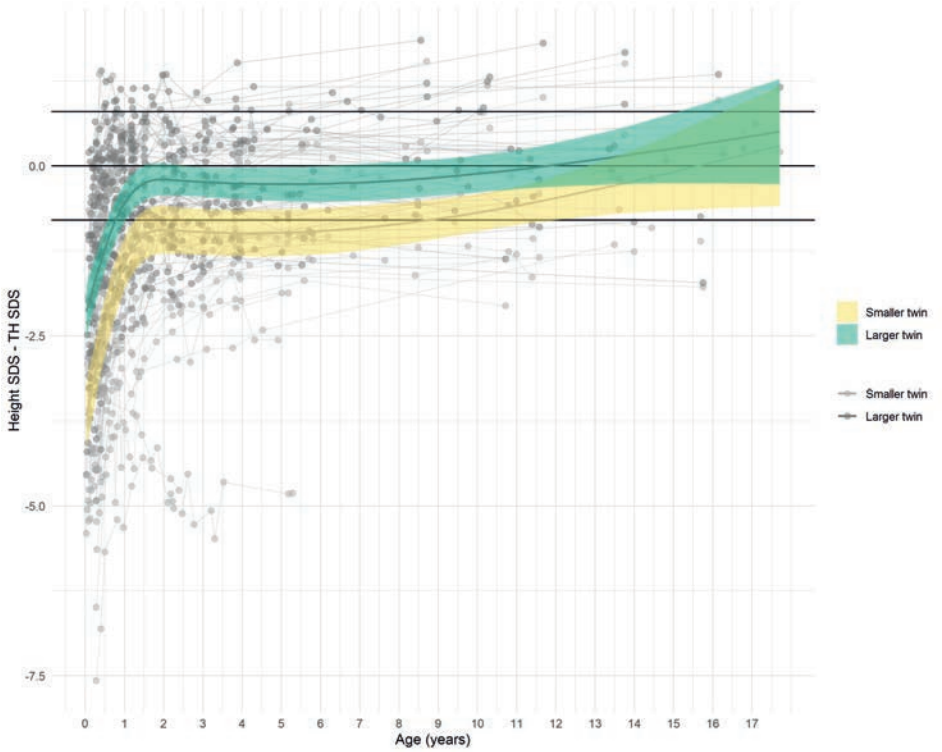

Figure 2. Mixed-effects model depiciting the difference in height SDS and TH SDS according to age for the smaller and larger twin. The horizontal lines represent the TH range of -/+ 0.8 SDS.

Discussion

Our analysis of genetically identical twins with sFGR shows that FGR results in modest but persistent differences in height, weight and head circumference throughout childhood, despite rapid catch-up growth in the first two years after birth. This is indicative of lasting growth-inhibitory effects of an adverse intrauterine environment. The median persistent height difference in our study between the smaller and the larger twin is 0.3 SDS at seventeen years, which corresponds to approximately 2-3 cm at adult height.

Our results are in line with previous studies on singleton SGA children: rapid catch-up growth generally occurs in the first two years after birth but near-adult height tends to still be below TH[3]. Similarly, we found that both twins rapidly catch-up within two years after birth following premature birth. While the larger twin already reaches its TH range during this period, the smaller twin continues to catch-up, albeit much slower, until completion between 8-11 years. The within-pair difference in height, weight and head circumference persists well into adolescence. Importantly, two previous dizygotic twin studies report an increasingly discordant growth with advancing age[11,13]. This further substantiates the use of our monozygotic twin model.

At present, research on growth patterns of discordant monozygotic twins is limited (Table 1). Available studies are largely in line with our results and describe a normal growth pattern for monozygotic twins with a birth weight discordance in which the smaller twin remains only marginally (between 0-8 cm) shorter, albeit using different definitions of catch-up growth[9-14]. However, we did not replicate being born SGA or low birth weight (< 1.95 kg) as risk factors for absence of catch-up growth as previously described[11]. We now provide strong evidence on catch-up growth and childhood growth patterns in a cohort of identical twins with known chorionicity and extensive longitudinal growth measurements from birth until late adolescence, including individual height relative to genetically determined TH range.

It is reassuring for physicians and parents alike to know that the vast majority of the smaller twins end up with a near-adult height in their genetic target range without the need for additional growth-promoting therapies such as recombinant growth hormone. Our data suggests that catch-up growth may take longer than previously expected (at least in MC twins with sFGR) and may not be completed until 8-11 years. Interestingly, both the smaller and larger twin seem to further grow into their TH range between ages 10-18 years. It should be noted, however, that relatively few

growth measurements in our study were available during adolescence, resulting in a wider confidence interval for this particular period. Growth hormone therapy is often considered when catch-up growth in SGA children is still insufficient between ages two and four. The 'late' catch-up growth in our cohort may support a more expectant approach, because part of these children will eventually catch-up with time. This is especially relevant for borderline cases in which parents or other caregivers are hesitant to start growth hormone therapy and burden their 2–4-year-old child with daily subcutaneous injections[22]. Our data suggests that in some cases a prolonged watchful waiting approach beyond four year may be perfectly feasible, thereby substantially reducing the time pressure that some parents may face while having to make this complicated decision together with their child's health care provider.

Several limitations of our study design should be taken into account when interpreting our results. Firstly, growth measurements were retrospectively retrieved from our national, standardized primary care system, potentially introducing information bias. Secondly, height measurements before the age of two (which are the predominant data in our study) tend to be less accurate due to interobserver measurement variation[23]. Lastly, it is important to consider that the etiological mechanisms of FGR in singletons and sFGR in MC twins may differ, thereby possibly affecting the direct extrapolation of our results to singletons. Where sFGR is presumed to primarily be caused by unequal sharing of a healthy placenta, with a smaller placental share and volume for the smaller twin, FGR in singletons is the result of impaired trophoblast invasion with subsequent placental insufficiency[7,24]. In addition, MC twin placentas have vascular connections allowing for intertwin blood flow during pregnancy. Even though we have excluded cases with evident imbalanced transfusion (TTTS and twin anemia polycythemia sequence), there is always a certain level of blood exchange that may affect the outcomes. Furthermore, it is unknown whether the growth trajectory of the larger twin accurately reflects the growth of an appropriately-grown singleton. Future research is necessary to determine whether these factors actually influence comparability between singletons and twins, as this is currently unknown. We now report similar outcomes in our twin population as were found for singletons with FGR, corroborating the use of our monozygotic twin model as well as the impact of FGR in itself. We were able to identify the more subtle but persistent differences in postnatal growth by conducting a within-pair comparison instead of solely focusing on growth within normal range on population growth curves.

It is currently unknown which mechanisms underlie the long-term effects of an adverse prenatal environment on growth, although epigenetic programming is considered a plausible candidate[25,26]. Likewise, questions remain about the impact of FGR on overall health in adulthood. Several studies have reported increased rates of obesity and metabolic disease due to a permanently altered insulin sensitivity[27]. This can in turn render individuals more susceptible to cardiovascular disease at later in life[28]. In addition, a smaller head circumference has been shown to be an important, independent predictor of adverse neurodevelopmental outcome[29,30]. This is substantiated by our study as well, as we have shown that the smaller twin (with the smaller head circumference) presents with significantly lower cognitive test scores as opposed to the larger twin in a previous analysis of the neurodevelopmental outcomes of the LEMON study[15]. The size of the within-pair difference in head circumference SDS and the within-pair difference in full scale IQ did not correlate significantly (p = 0.374).

Conclusion

This study provides a detailed description of childhood catch-up growth from birth until late puberty in a large cohort of genetically identical twins with discordant prenatal growth. We show that the majority of smaller twins born after sFGR will remain shorter and lighter than their larger co-twin throughout childhood, suggestive of a persistent inhibitory effect of FGR on growth which may affect neurodevelopmental outcome and adult health. The smaller twin will reach a height within their target range between ages 8-11 years. This information may reassure parents of newborn MC twins who are concerned about the future growth potential of their children. Moreover, these results provide guidance to treating physicians, favoring a more expectant approach in the early years after birth.

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Part IV

Summary and general discussion

Summary

This thesis consists of studies in monochorionic (MC) twins with selective fetal growth restriction (sFGR) investigating placental mechanisms (part I), short-term outcomes (part II) and long-term outcomes (part III). sFGR is a complication characterized by a large intertwin growth discrepancy during pregnancy, resulting in a large birth weight discordance (BWD) that is associated with an increased risk of perinatal morbidity and mortality as well as adverse long-term outcomes. Yet, a large gap in knowledge persists, impeding proper parent counseling and risk assessment. Simultaneously, this unique identical twin model can be used to investigate the early origins of disease after adverse intrauterine circumstances, by comparing a growth-restricted twin with a genetically identical, appropriately-grown co-twin with similar parental and obstetrical factors.

From unequal placental sharing to a discordant intrauterine environment (Part I)

In **Chapter 1** we investigated the placental characteristics after color-dye injection according to the classification for sFGR proposed by Gratacós based on the Doppler flow pattern in the umbilical artery (UA) of the smaller twin, delineating three types: type I with positive end-diastolic flow, type II with persistent absent/reversed end-diastolic flow and type III with intermittent absent/reversed end-diastolic flow. We found that type III placentas presented with the largest diameter of arterio-arterial (AA) anastomoses and the largest placental share discordance compared to type I and type II placentas. The larger AA anastomosis was thereby thought to primarily be responsible for the unpredictable clinical course of type III pregnancies, by allowing for acute feto-fetal transfusion in case of fetal demise in the smaller twin.

Chapter 2 further explored the relationship between BWD and placental share discordance, as well as the compensatory mechanism of large bidirectional anastomoses (AA and veno-venous anastomoses) in 449 MC placentas. The twins with a BWD ≥ 20% were classified according to the Gratacós classification. BWD appeared to be strongly associated with placental share discordance. Yet, the amount of BWD was relatively smaller than expected for the given amount of placental share discordance. A larger AA diameter was found to mitigate the effect of unequal placental sharing on BWD. In type II and type III, a distinct pathophysiology was identified with an increased importance of AA diameter as opposed to placental sharing. So, larger AA anastomoses are also beneficial for prenatal growth of the smaller twin, by allowing for a rescue transfusion from the larger to the smaller twin.

From fetus to newborn (Part II)

Chapter 3 comprises a systematic literature review of twelve articles on the optimal timing of delivery in MC twins with sFGR according to the Gratacós classification. We described that type I pregnancies are generally delivered at a later gestational age (33.0-35.0 weeks) and have lower rates of fetal demise, neonatal mortality and cerebral injury when compared to type II (27.8-32.4 weeks) and type III (28.3-33.8 weeks). Timing of delivery varied greatly in type II and type III, which was the result of heterogenous studies using different antenatal diagnostic criteria and definitions of outcome measures. This illustrated that uncertainty regarding optimal timing of delivery in MC twins with sFGR persists.

In **Chapter 4**, we showed that the larger twin in MC twins with sFGR has a doubled risk of developing respiratory failure at birth requiring mechanical ventilation and/or surfactant, while the smaller twin has a more than doubled risk of developing bronchopulmonary dysplasia characterized by respiratory insufficiency requiring treatment with >21% oxygen for at least 28 days, highlighting a pathophysiological effect of fetal growth restriction (FGR) on fetal lung development.

Chapter 5 presents the first results of the Twinlife study (Twin Longitudinal Investigation of FEtal discordance), in which MC twins are longitudinally followed up starting antenatally until eight years of age to uncover the early origins of disease using this unique discordant identical twin model. We analyzed the neonatal cardiac ultrasounds of 100 twin pairs, measuring the cardiac valve annuli diameters, left ventricular dimensions and aortic pulse-wave velocity as a surrogate marker for aortic stiffness. Z-scores were calculated based on gestational age at birth, describing the relationship between the measurement and the mean of a reference population. We found that the z-scores of the cardiac structures were all lower for the smaller twin when compared to the larger twin. Yet, the birth weight difference tended to be more pronounced than the difference in cardiac structure, indicative of heart sparing. These findings are suggestive of early structural cardiovascular remodeling after FGR.

In **Chapter 6** we retrospectively analyzed the first neonatal cerebral ultrasound after birth in 58 MC twins with sFGR, performing structural cerebral measurements to assess brain growth. These measurements were compared between the smaller and larger twin and with a sex- and gestational age-matched appropriately-grown singleton. The smaller twin presented with an overall restriction in brain growth, including smaller cerebral structures (corpus callosum, vermis, cerebellum),

white/deep gray matter and overall brain size, when compared to both the larger twin and the matched singleton. These differences remained after correction for intracranial volume, indicating a proportional decrease in brain growth.

From infant to adolescent (Part III)

Chapter 7 consists of a systematic literature review on the impact of sFGR on long-term neurodevelopmental outcomes. Five articles were included, all pointing in the same direction: substantial rates of neurodevelopmental impairment (NDI) for MC twins with sFGR with a trend towards a within-pair disadvantage for the smaller twin. These studies, however, had a high degree of heterogeneity resulting from substantially different methodologies, study populations and outcome measures. Therefore, this review primarily stresses the lack of knowledge of the long-term neurodevelopment after sFGR.

Chapter 8 presents the first results of the LEMON study (Long-term Effects of selective fetal growth restriction in MONochorionic twins), in which MC twins with sFGR born between 2002-2017 in our center and aged between 3-17 years were invited for follow-up. An age-appropriate neurodevelopmental test and a standardized neurological examination were performed as part of this follow-up in 47 included twin pairs. A within-pair comparison revealed that the smaller twin has a significantly lower intelligence quotient (IQ) across all domains of intelligence (6-point lower full scale IQ) as well as a substantially higher rate of mild NDI, with 36% vs. 11% (defined as a full scale IQ < 85, simple or complex minor neurological dysfunction or any mild visual or hearing impairments) when compared to the larger twin. The odds of developing mild NDI for the smaller twin was nearly five-fold higher than for the larger twin (OR 4.8). These findings indicated that FGR poses a substantial risk for long-term neurodevelopment, irrespective of genetic predisposition or obstetrical factors.

In **Chapter 9** we investigated the psychosocial development and school functioning of the LEMON study population using multiple parent-report questionnaires. We established that MC twins with sFGR (equally for the smaller and larger twin) presented with significantly more attachment insecurity (34%) than the general population (16%). Ambivalent/resistant attachment was most prevalent, in which the child constantly seeks attention from caregivers while also being resistant to contact. In addition, the smaller twin experienced more negative behaviors and emotions turned inwards (internalizing problems) and had a temperament that predisposes to

experiencing more negative emotions when compared to the larger twin. This information facilitates early detection, prevention and intervention strategies.

In **Chapter 10,** we analyzed the childhood growth patterns of the LEMON study population using a mixed-effects model to assess whether catch-up growth (defined as growth within target height range (target height +/- 0.8 standard deviation score)) occurs. Growth measurements as documented by a systematic primary care system in the Netherlands were collected and height, weight and head circumference were measured at follow-up. On average, smaller twins catch-up to a height within their target height range within 8-11 years after birth. A within-pair analysis showed that differences in height, body mass index and head circumference generally persisted, with smaller twins remaining smaller, lighter and lower in head circumference than their larger co-twins. These findings were suggestive of a persistent inhibitory effect of FGR on childhood growth.

General discussion

Selective fetal growth restriction (sFGR) in monochorionic (MC) twins is a prevalent complication in which the placenta is unequally shared, leading to a discordant antenatal growth pattern with a subsequently large birth weight discordance (BWD). These twins are at risk for adverse short- and long-term outcomes. Research on sFGR has been on the rise over the past decade (Figure 1). sFGR in the absence of twin-twin transfusion syndrome (TTTS) and twin anemia polycythemia sequence (TAPS) is increasingly recognized as a distinct entity in MC twin complications with its own course of disease. The uniqueness of these twins is also increasingly acknowledged: them being monozygotic (i.e., genetically identical) and in the same womb while experiencing a vastly discordant environment. The prenatal malnutrition that the smaller twin experiences is thought to predispose to disease in adulthood according to the early origins of disease hypothesis.

The aim of this thesis was to investigate the placental pathophysiology, short- and long-term outcomes of MC twins with sFGR as well as the early origins of disease after adverse intrauterine circumstances in this unique identical twin model.

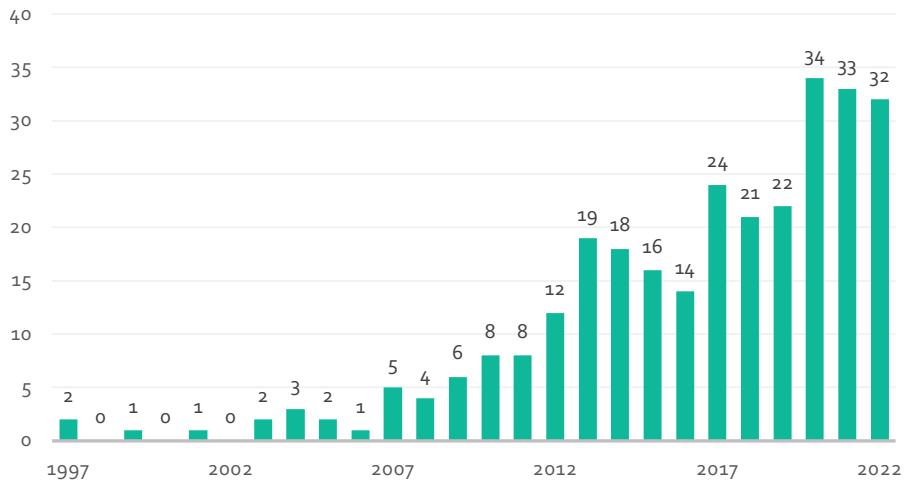

Figure 1. An overview of PubMed search results for the combination of the keywords 'selective fetal growth restriction', 'selective intrauterine growth restriction and 'monochorionic twins' (date 17-10-2022).

Placental pathophysiology

The importance of colored dye injection

Colored dye injection of MC twin placentas visualizing placental sharing and vascular anastomoses is paramount in understanding the pathophysiological processes behind the complications that can occur. Placenta injection with colored dye is part of standard care for all MC twins born in the Leiden University Medical Center (LUMC), including twins born after uncomplicated pregnancies to discern between what is normal and abnormal. This ensures that both clinicians involved in the day-to-day care for these twins and twin researchers gain familiarity with the complications that can occur in this vulnerable patient group. This thesis began with nearly twenty years of data gathered from the injection of placentas (n = 1168). The ensuing four years have led to an additional 445 placentas that have been added to this track record (Figure 2).

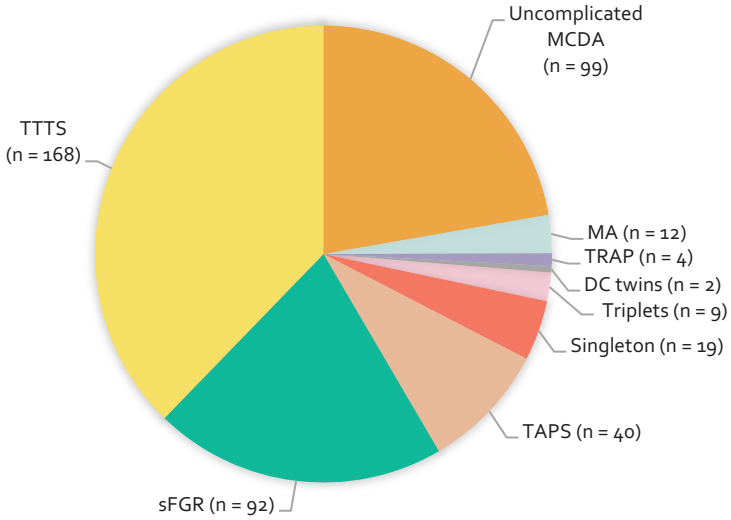

Figure 2. The injected placentas (n = 445) per category in the four years of this thesis (date 17-10-2022). Singleton placentas were from friends and colleagues or used as a demonstration in educational courses (n = 19).

But what lies beneath?

The placenta in MC twin pregnancies with sFGR is still a black box despite the colored dye injection: its exact internal mechanisms remain relatively unknown. We have now studied sharing by measuring the placental surface and have found that it strongly correlates to birth weight. Yet, much lies beneath the surface that has scarcely been

researched. Future studies should investigate the role of placental weight in determining birth weight in MC twins, to assess whether placental surface is indeed an adequate proxy for sharing. It is well-known that placental weight is correlated to birth weight[1-3]. Low placental weight is considered an expression of reduced functional mass in which exchange of nutrients and oxygen can occur, thereby leading to FGR. Yet, when separating the MC placentas along the vascular equator the fetal weight/placental weight ratio appeared to be higher for the smaller twin indicative of a more efficient perfusion despite the smaller share[4]. Studying the correlation between individual placental surface and placental weight in MC twins with sFGR can potentially lead to a more precise method of quantifying the actual placental sharing.

A crucial limitation in the generalizability of the results from our twin studies to singletons that was repeatedly considered in this thesis was the substantially different pathophysiological mechanisms of (s)FGR. Where sFGR in MC twins is thought to primarily be caused by unequal placental sharing, FGR in singletons generally finds its origin in placental insufficiency characterized by malperfusion of the placenta multifactorial in origin[5]. These differences may lead to incomparability of outcomes due to a distinct course of disease that has its own impact on fetal development. It should be investigated whether placental insufficiency is also at play in the smaller share of sFGR placentas. Current literature on the pathology of discordant MC placentas is conflicting: some report low placental weight (an expression of unequal placental sharing) combined with a velamentous cord insertion as the main culprit, while others also find more vascular thrombotic lesions (common in FGR placentas) in the smaller share[2,3,6]. Additionally, if pathophysiological mechanism do differ, it should be researched whether this does in fact lead to different health outcomes or if the consequences for health and development are the same. As of September 2020, we have included a pathological examination of the placenta in our standard care for complicated MC twins and/or participants in our prospective study (Twinlife).

Placental imaging: personalized medicine for monochorionic twins

Our new knowledge of placental mechanisms in sFGR can be used in clinical practice by improving antenatal visualization of the placenta and anastomoses. Current available imaging modalities include ultrasound and MRI. Three-dimensional color Doppler ultrasound can be used to assess the vascularization of the placenta as well as placental volumes[7-10]. Functional MRI can provide information on placental perfusion[11]. Ideally, imaging of the placenta would allow for quantification of exact placental sharing and the amount of transfusion that passes over anastomoses. With

this knowledge, the risk of fetal death in case of severe placental share discordance as well as the risk of acute feto-fetal transfusion can be estimated more accurately per pregnancy. When imaging modalities are further improved in the future, prognostication and management strategy can in turn become increasingly individualized.

Diagnosis

Fifteen years of the Gratacós classification – time for an update?

In 2007, Gratacós et al. introduced the classification system for sFGR in MC pregnancies based on the Doppler flow patterns in the umbilical artery (UA) of the smaller twin[12]. Fifteen years later, this classification system is still widely used, marking persistent (type II) or intermittent (type III) absent/reversed end-diastolic flow (A/REDF) as especially at risk for perinatal morbidity and mortality[13]. Management of these pregnancies is adapted accordingly, with daily fetal surveillance (in our center from 28 weeks onwards). If fetal distress of either twin is observed, the twins are delivered.

Even though this classification system has been widely used for fifteen years, the cause of the abnormal Doppler flow patterns is still speculated about. We described the distinct placental characteristics of each type in Chapter 2, primarily identifying type III placentas as 'the odd one out'. The intermittent A/REDF as observed in type III pregnancies is thought to be the consequence of the large AA anastomoses in which the two systolic waveforms collide. The cyclical nature of this flow pattern can be explained by the fluctuation in synchronicity of fetal heart rates[14]. Interestingly, we did not identify any specific placental characteristics for type II placentas. These cannot be discerned from a type I placenta with the naked eye. It remains unclear how the type II flow pattern arises, but it may be indicative of underlying placental insufficiency.

Importantly, chapter 2 and 3 of this thesis have touched upon a great obstacle in using the current Gratacós classification: the insurmountable international differences in its application in relation to the dynamic nature of the UA Doppler flow patterns. The changing patterns throughout pregnancy, sometimes fluctuating between type I, type II and type III, impede a proper determination of the 'definitive' Gratacós type[15]. As each center has its own methods of dealing with these changing flows in the classification (which are often not recorded in published studies), this ultimately leads to fundamental incomparability of reported outcomes for each type. Additionally,

intra- and interobserver variability in the interpretation of flow patterns (especially of type II and type III) have never been assessed. Hence, an update of the Gratacós classification is drastically necessary to further improve antenatal prognostication and management of sFGR pregnancies. A key element of a new system should be a guideline on how to interpret and classify changes in Doppler flow patterns throughout pregnancy. The first step in devising such a guideline is to research how placental characteristics relate to these changing patterns, to gain more understanding of underlying pathophysiology and consecutive risks. Moreover, it is desirable to incorporate brain sparing, measured by the cerebroplacental ratio, into a new classification as well, as it is considered a marker for a greater severity of FGR in singletons. Uniformity in the formation of a new classification for sFGR can only be achieved by international collaboration between fetal therapy centers. Multicenter research is necessary to 1) properly establish the variability in the application of the former classification, 2) identify relevant parameters that should be included in a new classification, 3) build a large retrospective cohort with available ultrasound imaging to re-classify cases and assess the differences in clinical outcomes with the former classification and 4) to start a large prospective cohort to apply the new classification.

Antenatal management

Overlapping complications

An important consideration in the antenatal management of MC twin complications, is that these are not mutually exclusive. Figure 3 shows the possibilities of overlap between the various complications. A previous study by our research group has confirmed that 60% of TTTS cases also present with an intertwin growth discordance before fetoscopic laser coagulation (Table 1)[16,17]. After birth, this drops to 25% that presents with a BWD ≥ 20% as intertwin transfusion stops when treatment is successful and the donor twin can largely recover. It is unknown how many TAPS cases present with a growth discordance at diagnosis, but this was estimated at 15-25%. In contrast with TTTS, the percentage of TAPS cases born with a BWD ≥ 20% increases to 35-45% due to the chronic nature of the intertwin transfusion. The donor becomes increasingly growth-restricted following an extended period of progressive anemia and hypoalbuminemia[18]. This is also illustrative of the different pathophysiological mechanisms behind growth discrepancies in TTTS or TAPS. Yet, the exact numbers for these combinations are still lacking. Future research must make out the true overlap between TTTS, TAPS and sFGR at diagnosis and at birth also specified according to treatment modality. Importantly, the overlapping complications can result in confusing terminology, e.g., the Gratacós classification

being applied to TTTS pregnancies. This may lead to substantial international differences in diagnosis and management. We need to speak the same language to stay on the same page. The distinct complications and classifications should be considered in the antenatal management of MC twin pregnancies: TTTS using the Quintero staging system, TAPS using the classification based on delta middle cerebral artery peak systolic velocity and isolated sFGR using the Gratacós classification[12,19,20]. A TTTS case that presents with simultaneous anemia-polycythemia or sFGR should always be primarily treated as TTTS.

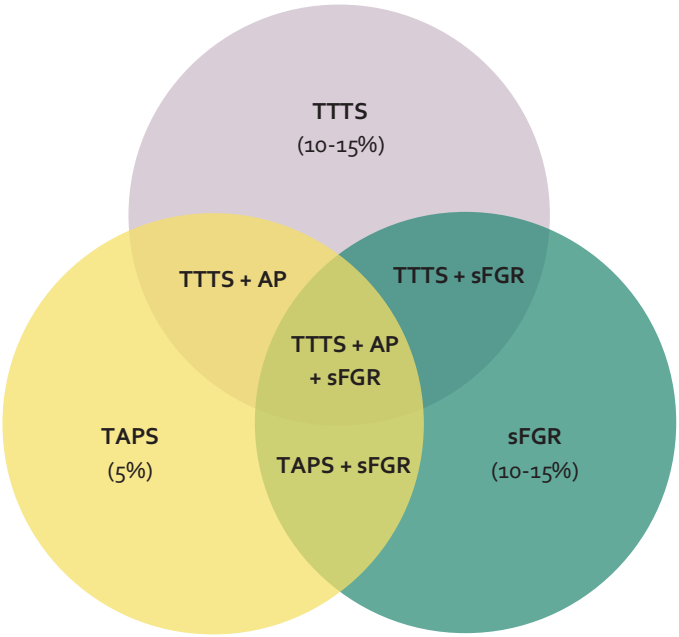

Figure 3. A Venn-diagram of the concurrent complications in MC twin pregnancies.

Management for sFGR

Two primary management strategies for sFGR can currently be identified: expectant management with fetal monitoring in case of abnormal UA Doppler flows or selective feticide of the smaller twin. Expectant management is the most widely used strategy to date, but frequency of ultrasounds and the threshold for admittance varies greatly internationally. A uniform management protocol is therefore still necessary, especially in light of the recent discussion on fetal viability in the Netherlands that may be adjusted to 23 weeks of gestation. In our center, fetal growth is monitored fortnightly including Doppler flow measurements. The frequency of these Doppler flow measurements can be intensified to weekly or even biweekly in case of type II or type

III sFGR. Ultrasounds are primarily performed at the outpatient clinic. In type II and type III, or in case of severe growth restriction (EFW < 3rd centile) and/or stagnating growth, it can be decided (in consultation with parents) to admit the mother for daily fetal surveillance using cardiotocography once or twice a day. The threshold for admittance in our center is 28 weeks of gestation, yet this may be earlier in other centers.

Table 1. An approximation of TTTS and TAPS with a concurrent intertwin growth discrepancy at diagnosis and at birth.

Intertwin growth discordance	**TTTS**	**TAPS**
At diagnosis *EFW < 10th centile + EFW discordance ≥ 20%*	60%	15-25% (?)
Pathophysiology	Combination of unequal sharing and amniotic fluid imbalance, disadvantageous for growth of donor.	Chronic intertwin transfusion resulting in anemia and hypoalbuminemia in donor.
At birth *BWD ≥ 20%*	20-30% after laser (?) 40-50% if no/unsuccessful laser (?)	35-45% (?)
Pathophysiology	Unequal placental sharing, intertwin transfusion no longer present if laser is successful. If no/unsuccessful laser, intertwin transfusion and amniotic fluid imbalance persists.	Progressive anemia and hypoalbuminemia in donor; donor generally has a larger placental share in TAPS.

TTTS: twin-twin transfusion syndrome, TAPS: twin anemia polycythemia sequence, sFGR: selective fetal growth restriction, EFW: estimated fetal weight, BWD: birth weight discordance.

Selective feticide is discussed with parents in cases in which the smaller twin is already severely growth-restricted in an early stage in pregnancies with abnormal UA Doppler flow patterns and/or presenting with additional signs of compromise (e.g., further stagnation in growth, cerebral abnormalities, cardiac compromise or echogenic bowels). As previously mentioned, there is a risk of acute feto-fetal transfusion through large bidirectional anastomoses after sudden fetal demise of the smaller twin

that may lead to neurological damage or death of the larger twin. Selective feticide aims to protect the larger twin, who is given the best chances of survival.

Another available treatment modality is fetoscopic laser coagulation. Yet, this is still a disputable option for sFGR to date. The idea behind performing fetoscopic laser coagulation in pregnancies with abnormal UA Doppler flow patterns (type II/type III) is that it eliminates the risk of acute feto-fetal transfusion in case of demise of the smaller twin, similar to selective feticide. As stressed by chapter 2, however, the compensatory function of the large AA anastomoses is also lost after coagulation, leading to high rates of fetal demise in the smaller twin (67-77%)[21-24]. In addition, the cause of sFGR is not an imbalance of blood flow as in TTTS or TAPS, so coagulation of anastomoses does not solve the underlying problem. Lastly, fetoscopic laser coagulation in sFGR comes with more technical challenges due to the absence of an amniotic fluid discordance. Overall, we do not deem it a feasible treatment option for sFGR in our center.

Optimal timing of delivery

Chapter 3 has illustrated that there is still no guideline for optimal timing of delivery in MC twins with sFGR. The risk of fetal demise of the smaller twin is thought to increase over the course of the pregnancy, as the placental share can become more insufficient in providing nutrients and oxygen. Simultaneously, the risk of neonatal morbidity and mortality increases with decreasing gestational age, thereby substantially impairing long-term neurodevelopment as well. Preferably, we must identify the moment at which the risk of fetal demise outweighs the risk of severe neonatal morbidity and mortality for each Gratacós type as has previously been done for monoamniotic twin pregnancies (Figure 4)[25]. Finding this balance proves difficult as many other factors play a role during sFGR pregnancies, such as a deterioration of fetal condition or stagnation of growth in the third trimester and, importantly, fetal distress. The first step in devising a guideline is to equalize reported outcome measures to uniformly assess clinical outcomes at birth and in childhood. As mentioned in Chapter 3, a wide variety of outcome measures is used in available literature leading to incomparability between studies. Reason for delivery is generally not recorded while this is crucial information. Moreover, as mentioned earlier, a new uniformly applicable classification for sFGR is a prerequisite for proper prognostication.

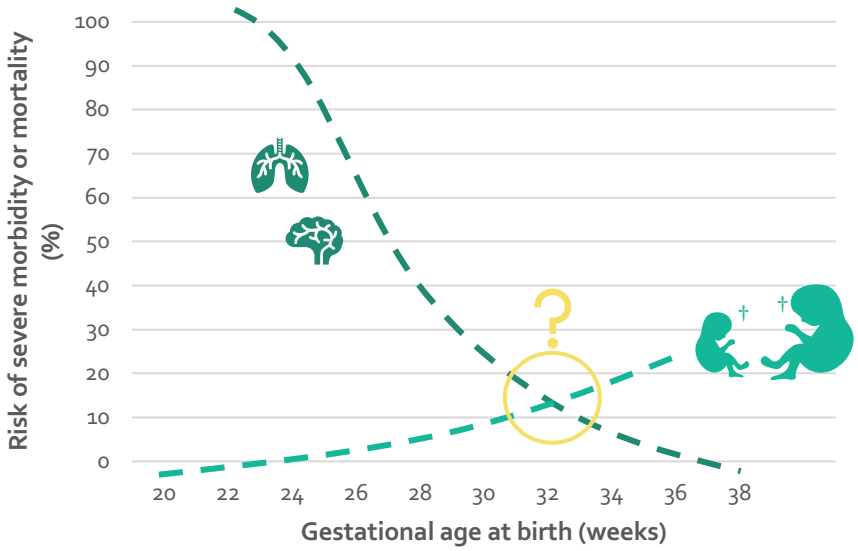

Figure 4. How to find the optimal timing of delivery in MC twins with sFGR.

Respiratory health

FGR has repeatedly been linked to adverse respiratory outcomes through abnormal fetal lung development, in which persistent structural (impaired vascularization and alveolarization) and functional (inefficient oxygenation) changes are induced following the chronic state of hypoxia fetus experiences[26,27]. Bronchopulmonary dysplasia (BPD), a chronic form of respiratory insufficiency, is thought to be an expression of this impaired lung development. As shown in Chapter 4, the smaller twin had a more than doubled odds of developing BPD. BPD as well as FGR in itself can in turn predispose to increased respiratory morbidity in childhood. Studies describing lung function after FGR at school age show conflicting results and primarily focus on very preterm infants[28-31]. Many other confounding factors can be at play, such as genetic susceptibility and parental smoking habits[32,33]. Identical twin models are ideally suited to control for these confounding factors. A large twin study including 752 twins (both mono- and dizygotic) found that lung function was reduced with decreasing birth weight. The smaller twin had five-fold higher odds of having a clinically relevant deficit. Yet, chorionicity and discordance were unreported[34]. It remains to be seen whether our observed within-pair differences in short-term respiratory morbidity also persist into childhood. We have included spirometry with add on of lung diffusion capacity and volumetric measurements for MC twins with sFGR aged between 4-17 years in the LEMON study (Netherlands Trial Register ID

NL9833), which are currently still being scheduled. These data will allow us to assess the prevalence of respiratory diseases, such as asthma, but also provides information on subtler changes in lung structure and functioning that are primarily associated with FGR[29].

Cardiovascular health

The early origins of disease hypothesis all began with cardiovascular disease (CVD). David Barker laid the foundation for this hypothesis in 1986 by proposing a link between prenatal nutrition and ischemic heart disease after he observed that the rate of ischemic heart disease was higher in areas with poor living conditions[35]. Research on the Dutch Hunger Winter, a severe famine at the end of World War II, has further substantiated this hypothesis by demonstrating that individuals who were exposed to prenatal famine in this period presented with an adverse risk profile of metabolic disease in adulthood[36-40]. MC twins now present the ideal model to further study the effect of early life adversity on lifelong health outcomes in the Twinlife study, limiting confounding by genetic, obstetrical or parental factors to an even greater extent than siblings [41]. By longitudinally following both concordant and discordant MC twins from fetus to adolescent, we can follow early cardiovascular differences after adverse intrauterine circumstances and consecutive development of risk factors for CVD[42,43].

The first step in cardiovascular tracking of these twins is presented in Chapter 5, reporting that early structural cardiovascular remodeling after FGR is already visible on neonatal cardiac ultrasound within one week after birth. These findings form the baseline for the Twinlife cohort. Previous research indicates that the adaptive process following prenatal adversity is more gradual throughout childhood[44,45]. Follow-up in the Twinlife study can explore whether the cardiovascular adaptations at birth are still present throughout childhood or whether new adaptations can be observed over time. Ideally, twins will be tracked well into adulthood to investigate whether CVD indeed occurs at a later age. Similarly, the older MC twins with sFGR included in the LEMON study are still being seen for a cardiac ultrasound. This will provide us with a look into the future to identify outcomes of interest for Twinlife. A measurement of the carotid intima-media thickness is performed in this population as well, a widely used surrogate marker for atherosclerosis and predictor of cerebral and cardiovascular events[46]. Ultimately, MRI studies including cardiovascular functioning and extensive metabolic imaging (e.g., body fat distribution) could provide novel insights into the effects of prenatal adversity on CVD risk.

Neurodevelopmental health

Structural brain development – Imaging

As mentioned, 'brain sparing' in FGR constitutes the redistribution of cardiac output towards major organs like the brain. It is considered as misnomer as the brain is not in fact spared. Brain sparing is indicative of a more severe form of FGR in which the fetus experiences chronic hypoxia that impairs the development of major organs. We have shown this in Chapter 7 in which the smaller twin of sFGR twins presented with an overall restriction in brain growth on neonatal cerebral ultrasound (cUS) when compared to the larger co-twin and a matched singleton. We were unable to find any differences in cerebral maturation on cUS, possibly because we used a relatively old scoring system that looks at a few rough markers of maturation in different planes. cUS is widely accepted as an important bedside tool to screen for brain injury and follow brain growth during the neonatal period. However, compared to cUS, MRI is able to detect injurious and maturational changes in the developing brain in far more detail as it allows the quantification of brain volume and cortical thickness, and can be used to assess functional brain development and connectivity[47-50]. All these parameters are important markers of brain development and maturation and all can be altered by early-life brain insults such as FGR.

Previous studies on structural cerebral adaptations after FGR in singletons have shown atypical brain development, including reduced total and cortical gray matter volumes, reduced cortical complexity, reduced and delayed myelination, altered hippocampal and cerebellar development, and reduced connectivity of specific brain networks[51-53]. However, the available literature is scarce. There are also many other early life adversities that can have an impact on brain development, such as socio-economic status, maternal stress, pregnancy complications and genetic factors[54]. Both fetal and neonatal term age equivalent MRI in MC twins with sFGR would be the next step to provide more robust mechanistic insight into brain development after FGR.

Functional brain development – Neurodevelopment

Chapter 7 has identified the poignant shortage of long-term neurodevelopmental outcomes of MC twins with sFGR in literature, with only five available studies reporting on a variety of outcome measures using different assessments and study populations. This review was the foundation for the LEMON study that ultimately provided the answer to a pressing question: are MC twins with sFGR at risk of long-term NDI? In Chapter 8 we have established that the smaller twin had a considerably

higher rate of mild neurodevelopmental impairment (NDI) as well as a lower intelligence quotient (IQ) across all domains of intelligence when compared to the larger twin. Additionally, the smaller twin was found to have a tendency towards negative emotions and internalizing behaviors, as well as a lower secondary school level than the larger twin, described in Chapter 9. These findings have validated that (s)FGR indeed has a long-lasting effect on functional brain development. It remains to be researched which structural cerebral changes lead to these functional consequences and whether these changes can already be identified in an early stage. Aside from the earlier proposed fetal and neonatal term age equivalent MRIs, an MRI at school age in the LEMON study population would allow us to directly link the observed functional changes we found to changes in brain growth, maturation and connectivity on MRI. This would in turn identify points of interest for longitudinal MRIs in a prospective setting.

The valuable viewpoint of parents

sFGR not only affects gross neurodevelopment, but also the more fine-grained aspects of childhood development as shown in Chapter 9. Importantly, (sub)clinical attachment insecurity rate was high (34%). We hypothesized that this resulted from the complicated pregnancy course and subsequent prematurity, impairing the formation of the parent-child relationship. Conversations with parents in the LEMON study have brought forward the impact of the option to perform selective reduction that was presented to them during pregnancy. This has, in their own words, resonated with them even years later. Therefore, we see added value of qualitative studies about the impact of a MC twin pregnancy and its sequelae on parents. This can establish major themes and problems that can then be addressed during pregnancy and in the first years after birth. Parents can be offered appropriate psychological support to ensure safe attachment and to identify any problems, such as post-traumatic stress or depression, in an early stage to facilitate early intervention.

Single survivors

An essential limitation of the LEMON study is the sole inclusion of double survivors. This may result in the selection of cases with relatively favorable outcome, as both twins have survived. Single fetal demise is thought to potentially lead to severe neurological damage of the surviving twin due to acute feto-fetal transfusion. Similarly, neonatal mortality is primarily caused by the complications of extreme prematurity. The neurodevelopment and psychosocial development of the surviving twin may therefore be more severely affected. This would mean that our reported

outcomes are only the tip of the iceberg. Research on long-term outcomes in this subgroup of single survivors after sFGR is necessary.

Childhood growth patterns

Once smaller, always smaller

Postnatal catch-up growth has been a prominent topic in research on FGR over the past years. It is well-known that the majority of infants born after FGR catches up to a normal height range within a few years after birth. Yet, comparisons with population growth curves do not tell the whole story. In Chapter 10, we have shown that the smaller twin at birth will remain smaller throughout childhood in the majority of cases, suggesting a persistent growth-perturbing effect of FGR on lifelong growth. Nonetheless, significant catch-up growth was observed in the smaller twin that continued up to ten years after birth. While catch-up growth after FGR is generally considered to be a positive phenomenon reflecting recovery, it is also thought to come at a cost. A previous study in twins (mono- and dizygotic) found that catch-up growth in weight SDS relative to birth weight SDS in the first two years of life was negatively correlated with IQ at age 12 and 18 years[55]. Chapter 8 supports this hypothesis, as the smaller twin had a lower IQ across all indexes[56]. Moreover, in Chapter 10 we have demonstrated that head circumference of the smaller twin remains significantly smaller throughout childhood, which is considered a predictor of adverse neurodevelopmental outcome in itself[57,58]. Similarly, Chapter 10 shows that within-pair difference in BMI decreases in the first year and then stabilizes with the smaller twin continuing to have a lower BMI throughout childhood. Multiple studies report high rates of obesity in adulthood after FGR[59]. It is thought that both fetal malnutrition and subsequent catch-up growth in early life alter insulin sensitivity and result in an adverse body composition with a more central body fat distribution, increasing susceptibility to metabolic syndrome and cardiovascular disease[60]. The relationship between neurodevelopmental, cardiovascular and metabolic outcomes and catch-up growth will be explored in the LEMON study in the near future.

The importance of long-term outcomes

Long-term outcomes are fundamental to reflect on the decisions clinicians make in daily practice. In available literature on MC twins with sFGR, follow-up generally extended up to two years of age (often only for research purposes) and largely encompassed questionnaires as opposed to an actual examination of the children. This thesis has illustrated that there is more to clinical outcome than survival and cerebral injury alone. Our research on neurodevelopmental outcome, psychosocial

and school functioning and growth patterns has substantiated that MC twins with sFGR experience more adversity later in life, with a disadvantage for the smaller twin. With the knowledge from this thesis we can now adequately counsel parents and remain watchful throughout the pregnancy and childhood to timely intervene and optimize the development of these children and address any psychological difficulties in parents in an early stage. Standardized follow-up in both the smaller and larger twin is essential to facilitate this, as the larger twin is not exempt from impairments in this vulnerable patient group.

Epigenetics: the link between prenatal adversity and lifelong health

What remains is to now explore the link between prenatal adversity and lifelong health in the epigenome. Epigenetics encompasses modifications to the DNA that can change the regulation of gene expression without altering the genetic code itself. The two primary molecular mechanisms underlying epigenetic programming are DNA methylation and modifications of histones[61]. Epigenetic changes after malnutrition in early fetal development are persistent into adulthood, predisposing individuals to health deficits at a later age as previously described in the Dutch Hunger Winter research[36,40]. Chapter 4-6 and Chapter 8-10 of this thesis have identified these short- and long-term health deficits in the smaller twins after sFGR. The epigenetic profiles of the umbilical cord derived MSCs, that can in turn be cultured into a spectrum of cell types that can be found in the human body, collected in Twinlife can now shine a light on how these adverse outcomes are indeed programmed in utero.

An identical twin model

The 'ideal' twin model?

There is a major pitfall in using MC twins as a model for the early origins of disease: the intertwin blood flow through the vascular anastomoses. When unbalanced, in case of TTTS, TAPS or other hematological imbalances at birth, intertwin blood transfusion can dilute the true effect of FGR we primarily aim to uncover[62]. In addition, it hampers extrapolation of our results to singletons, that do not experience these hematological shifts throughout pregnancy. Hence, another twin model may be even better suited: monozygotic dichorionic (DC) twins with sFGR. These twins are also genetically identical, but do not share a single placenta and therefore do not have vascular anastomoses. In this study design, the interference of intertwin transfusion would be eliminated. In addition, the pathophysiology of sFGR in DC twins is supposedly more similar to the pathophysiology in singletons, namely placental insufficiency instead of unequal sharing. Yet, using this twin model poses many challenges. Monozygotic DC

twins with sFGR are extremely rare. Approximately 175.000 children are born in the Netherlands each year, of which 3 to 4 in 1000 is monozygotic. This amounts to 700 monozygotic twins on a yearly basis, of which only a quarter (175) is DC[63]. sFGR occurs in 10-15% of all DC twins, regardless of zygosity[64]. Ultimately, this would lead to the birth of 18-27 monozygotic DC twin pairs with sFGR yearly, in the 'best case' scenario. Moreover, zygosity need to be determined after birth as same-sex DC twins can also be fraternal[65]. In the context of research this would mean additional costs (zygosity tests cost approximately €100 per twin pair) and either antenatal inclusion of cases that appear to be fraternal after birth or postnatal inclusion with limited antenatal measurement. So, despite the potential superiority of a monozygotic DC twin model, it is not as 'practical' as the MC twin model we have used.

The bare necessities for a longitudinal twin study

To facilitate a prospective study in a MC twin model that aims to investigate the early origins of disease, a few criteria must be met. Firstly, collaboration between research disciplines is essential. Studies should be translational to warrant the link between fundamental research (in this case epigenetics) and clinically relevant outcomes in all fields of health research. Secondly, extensive antenatal documentation of fetal condition (including Doppler flow measurements, fetal growth and imaging of the heart and brain) should be performed as this forms the basis of the assessment of what an 'adverse' intrauterine environment actually entails. Lastly, follow-up throughout childhood and into adulthood is crucial. Many surrogate markers can already be investigated in childhood, but whether these actually unearth into health problems at later age should ideally be examined as well.

Final conclusions

To conclude, this thesis has provided novel insights into the short- and long-term outcomes of MC twins with sFGR, including placental pathophysiology. We have thoroughly examined the clinical course of sFGR from womb to adolescence, thereby improving our knowledge regarding this vulnerable patient group. Additionally, we have explored the early origins of disease in this unique identical twin model discordant for intrauterine environment, eliciting the effects of prenatal adversity on lifelong health.

As mentioned, this thesis has also raised new questions that form the basis for future research in MC twins with sFGR. The following themes can be identified (Figure 5):

- **Placental mechanisms**, including:
    - The combination of placental surface and weight as an enhanced proxy for placental sharing.
    - Pathological examination of placental tissue to unearth any abnormalities that can play a role in the development of sFGR in MC twins, including (dis)similarities to FGR in singletons.
    - Antenatal imaging (ultrasound and MRI) of the shared placenta and its angioarchitecture to quantify the amount of placental share discordance and intertwin transfusion.
- **Antenatal management strategies**, including:
    - A much-needed update of the Gratacós classification that incorporates the changing UA Doppler flow patterns throughout pregnancy and brain sparing.
    - A guideline on optimal timing of delivery at which the risk of fetal demise outweighs the risk of neonatal morbidity and mortality.
- **Childhood outcomes**, including:
    - Childhood spirometry with added lung diffusion capacity and measurement of lung volumes, to uncover the persistent consequences of (s)FGR for lung development.
    - Cardiovascular follow-up, including more extensive metabolic imaging at school age, from fetus to adult to track surrogate markers for CVD throughout childhood and subsequent incidence of CVD in adulthood after (s)FGR.
    - MRI studies to provide the missing link between structural and functional brain development after (s)FGR.
    - Qualitative research on the impact of a complicated MC twin pregnancy and its sequelae on parents.
    - The possible deleterious effects of catch-up growth after (s)FGR for neurodevelopmental, metabolic and cardiovascular outcomes.
    - The role of the epigenome in the fetal programming of lifelong respiratory, cardiovascular and neurodevelopmental health, and growth patterns after (s)FGR.

In short, we are far from done. There is still so much to learn about sFGR, from womb to adolescence, to improve current management strategies and thereby perinatal and childhood outcomes for MC twins with sFGR. Simultaneously, we can use this unique identical twin model to take new steps in uncovering the early origins of disease. By

combining the expertise from different research disciplines within our center as well as from internationally renowned fetal therapy centers, we can join forces to do what is necessary to offer MC twins with sFGR the best possible care.

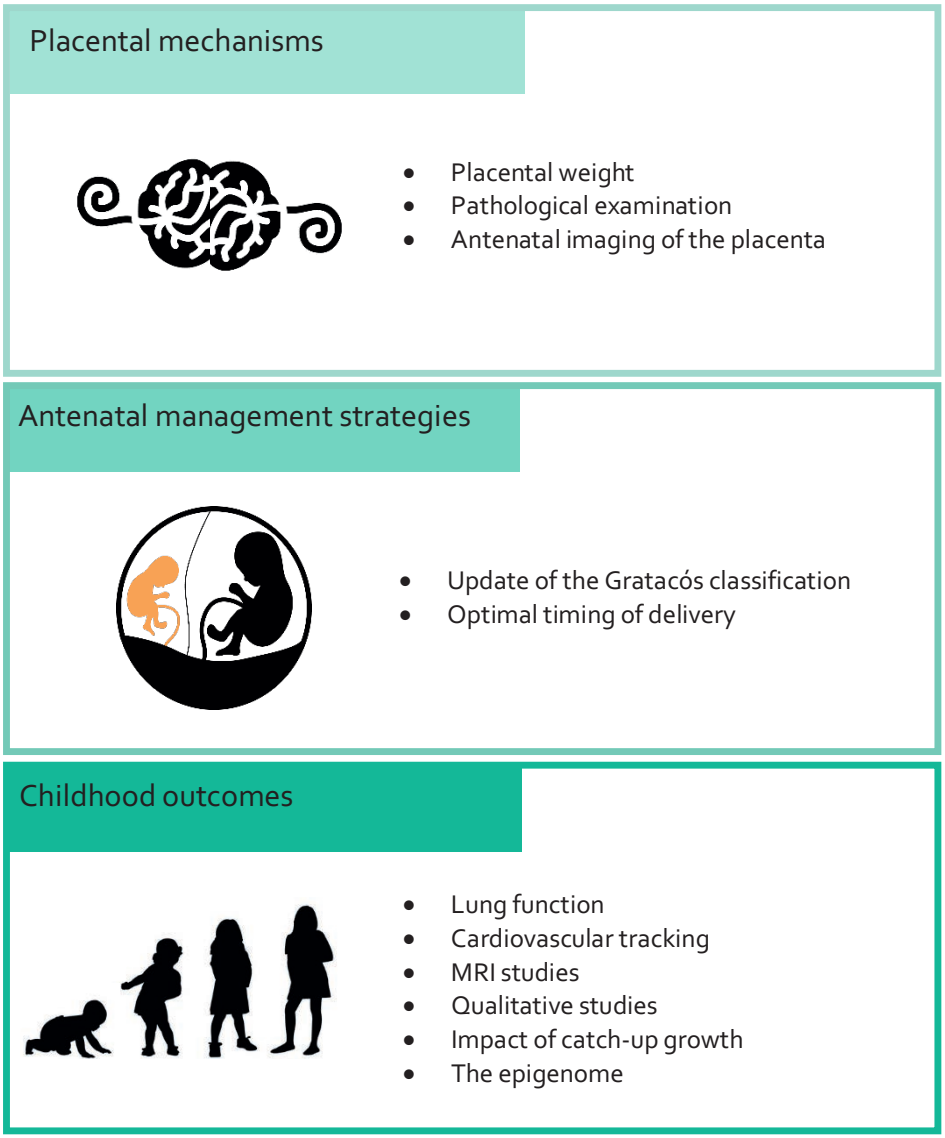

Figure 5. Future perspectives in research on MC twins with sFGR.

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Nederlandse samenvatting

Dit proefschrift bestaat uit studies naar monochoriale (MC) tweelingen met selectieve foetale groeirestrictie (sFGR) naar de placenta mechanismes (Deel I), korte termijn uitkomsten (Deel II) en lange termijn uitkomsten (Deel III). sFGR is een complicatie die wordt gekenmerkt door een groot verschil in groei binnen de tweeling tijdens de zwangerschap, wat resulteert in een groot verschil in geboortegewicht. Dit gaat gepaard met een verhoogd risico op perinatale morbiditeit en mortaliteit en andere nadelige gevolgen voor de lange termijn gezondheid, waar weinig bekend over is. Toch blijft er een groot gat in kennis bestaan, wat goede informatievoorziening voor ouders en risicoschatting op morbiditeit bij deze tweelingen door zorgverleners in de weg staat. Tegelijkertijd kan dit unieke, identieke tweelingmodel worden gebruikt om de vroege oorsprong van ziekte na ongunstige intra-uteriene omstandigheden te onderzoeken. Het groei-vertraagde kind kan worden vergeleken met een genetisch identieke, normaal gegroeide tweelingbroer/zus die in dezelfde baarmoeder van dezelfde moeder heeft gezeten.

<u>Van een oneerlijke verdeling van de placenta naar een verschil in intra-uteriene omgeving (deel I)</u>

De oorzaak van sFGR ligt in de placenta. In **Hoofdstuk 1** onderzochten we de placenta kenmerken met behulp van kleurverf injectie volgens de classificatie van sFGR voorgesteld door Gratacós. Deze classificatie is gebaseerd op het Doppler flow patroon in de navelstrengslagader van het kleine kind, waarbij er onderscheid wordt gemaakt tussen drie types: type I met positief eind-diastolische flow, type II met continue afwezige/omgekeerde eind-diastolische flow en type III met intermitterende afwezige/omgekeerde eind-diastolische flow. We vonden dat type III placenta's de grootste diameter van arterio-arteriële (AA) anastomosen en de grootste discordantie in de placenta verdeling hadden in vergelijking met type I en type II placenta's. De grotere AA anastomose wordt verantwoordelijk geacht voor het onvoorspelbare klinische beloop van type III zwangerschappen, gezien de mogelijkheid tot acute foeto-foetale transfusie in geval van overlijden van de kleine foetus.

Hoofdstuk 2 evalueerde de relatie tussen geboortegewichtsdiscordantie en discordantie in de placenta verdeling, evenals het compensatiemechanisme van grote bidirectionele anastomosen (AA en veno-veneuze anastomosen) in 449 MC placenta's. Tweelingparen met een geboortegewichtsdiscordantie ≥ 20% werden retrospectief geclassificeerd volgens Gratacós. Geboortegewichtsdiscordantie bleek

sterk geassocieerd te zijn met discordantie in de placenta verdeling. Toch was de mate van geboortegewichtsdiscordantie relatief kleiner dan verwacht voor de mate van discordantie in placenta verdeling. Een grotere AA diameter bleek het effect van ongelijke placenta verdeling op geboortegewichtsdiscordantie te verminderen. Bij type II en type III speelde de angioarchitectuur van de placenta een belangrijkere rol in de pathofysiologie dan placenta verdeling. Grotere AA anastomosen blijken ook gunstig te zijn voor de prenatale groei van het kleine kind, door 'reddings'transfusies van het grote naar het kleine kind tijdens de zwangerschap.

Van foetus tot pasgeborene (Deel II)

Hoofdstuk 3 betreft een systematisch literatuuronderzoek van twaalf artikelen over de optimale timing van bevalling bij MC tweelingen met sFGR geclassificeerd volgens Gratacós. We beschreven dat type I zwangerschappen over het algemeen bij een latere zwangerschapsduur worden geboren (33.0-35.0 weken) en minder perinatale mortaliteit en hersenschade hebben in vergelijking met type II (27.8-32.4 weken) en type III (28.3- 33.8 weken). De timing van de bevalling varieerde sterk bij type II en type III, wat het resultaat was van heterogene studies met verschillende prenatale diagnostische criteria en definities van uitkomstmaten. Dit laat zien dat er nog steeds onzekerheid is over de optimale zwangerschapsduur bij geboorte bij MC tweelingen met sFGR.

In **Hoofdstuk 4** lieten we zien dat het grote kind in MC tweelingen met sFGR een twee keer zo hoog risico heeft op het ontwikkelen van respiratoire insufficiëntie bij de geboorte dan het kleine kind waarbij het mechanische ventilatie en/of surfactant nodig heeft. Daarentegen heeft het kleine kind een tweeëneenhalf keer zo hoog risico op het ontwikkelen van bronchopulmonale dysplasie, gekenmerkt door respiratoire insufficiëntie waarvoor behandeling met >21% zuurstof gedurende ten minste 28 dagen. Ondanks een lager risico op korte termijn respiratoire problematiek, heeft het kleine kind op de lange termijn dus een hoger risico op chronische respiratoire problematiek. Dit wijst op een pathofysiologisch effect van foetale groeirestrictie (FGR) op de ontwikkeling van de foetale long.

Hoofdstuk 5 bevat de eerste resultaten van de Twinlife studie (Twin Longitudinal Investigation of FEtal discordance), waarin MC tweelingen longitudinaal worden gevolgd van foetus tot achtjarige leeftijd om de vroege oorsprong van ziekte te onderzoeken in dit unieke discordante identieke tweelingmodel. We analyseerden neonatale echocardiografie van 100 tweelingparen, waarbij de diameters van de

hartklep annuli, de linkerventrikel afmetingen en de aorta-polsgolfsnelheid (surrogaatmarker voor aortastijfheid) werden gemeten. Z-scores werden berekend op basis van de zwangerschapsduur bij de geboorte en beschrijven daarmee de relatie tussen de meting en het gemiddelde van een referentiepopulatie. We ontdekten dat de z-scores van de hartstructuren allen lager waren voor het kleine kind in vergelijking met het grote kind binnen het tweelingpaar. Toch was het verschil in geboortegewicht meer uitgesproken dan het verschil in hartstructuur, wat kan wijzen op 'heart-sparing'. Deze bevindingen wijzen op vroege structurele cardiovasculaire remodellering na FGR.

In **Hoofdstuk 6** analyseerden we retrospectief de eerste neonatale echo cerebrum na de geboorte in 58 MC tweelingparen met sFGR, waarbij we structurele cerebrale metingen uitvoerden om de hersengroei te beoordelen. Deze metingen werden vergeleken tussen het kleine en grote kind binnen de tweeling en met een op geslacht en zwangerschapsduur bij geboorte gematchte eenling. Het kleine kind vertoonde een algehele restrictie in hersengroei, waaronder kleinere cerebrale structuren (corpus callosum, vermis, cerebellum), witte/diep grijze stof en totale hersengrootte, vergeleken met zowel het grote kind als de gematchte eenling. Deze verschillen bleven bestaan na correctie voor intracraniaal volume, wat erop wijst dat alle hersenstructuren in dezelfde mate zijn aangedaan.

Van zuigeling tot adolescent (Deel III)

Hoofdstuk 7 bestaat uit systematisch literatuuronderzoek naar de impact van sFGR op de lange termijn psychomotore ontwikkeling. Er werden vijf artikelen geïdentificeerd, die allen in dezelfde richting wezen: aanzienlijke percentages van psychomotore ontwikkelingsachterstand voor MC tweelingen met sFGR met een nadeel voor het kleine kind. Deze studies hadden echter een hoge mate van heterogeniteit als gevolg van substantieel verschillende methodologieën, onderzoekspopulaties en uitkomstmaten. Daarom benadrukt deze review het gebrek aan kennis van de lange termijn psychomotore ontwikkeling na sFGR, maar ook het gebrek aan eenduidige definities, meetmoment en meetinstrumenten.

Hoofdstuk 8 bevat de eerste resultaten van de LEMON-studie (Long-term Effects of selective fetal growth restriction in MONochorionic twins), waarin MC tweelingen met sFGR geboren in ons centrum tussen 2002-2017 en in de leeftijdscategorie 3-17 jaar werden uitgenodigd voor vervolgonderzoek. Als onderdeel van dit vervolgonderzoek werd er bij 47 tweelingparen een ontwikkelingstest en een gestandaardiseerd

neurologisch onderzoek uitgevoerd. Een vergelijking binnen de tweeling liet zien dat het kleine kind een significant lager intelligentiequotiënt (IQ) had op alle domeinen van intelligentie (6 punten lager totaal IQ), evenals een aanzienlijk hoger percentage milde psychomotore ontwikkelingsachterstand, met 36% vs. 11% (gedefinieerd als een totaal IQ < 85, simpele of complexe 'minor neurological dysfunction' of milde visuele of gehoorsproblemen) in vergelijking met het grote kind. Het risico op het ontwikkelen van een milde psychomotore ontwikkelingsachterstand was bijna vijf keer hoger voor het kleine kind dan voor het grote kind (OR 4.8). Deze bevindingen geven aan dat FGR een aanzienlijk risico vormt voor de lange termijn psychomotore ontwikkeling, ongeacht genetische aanleg of obstetrische/maternale factoren.

In **Hoofdstuk 9** hebben we de psychosociale ontwikkeling en het school functioneren van de LEMON studiepopulatie onderzocht met behulp van vragenlijsten ingevuld door ouders. We hebben gevonden dat de hechting van MC tweelingen met sFGR (zowel het kleine als het grote kind) met de ouders anders lijkt te verlopen dan bij kinderen uit de zogenaamde algemene bevolking. Zo werd er significant meer onveilig hechtingsgedrag (34%) gerapporteerd dan in de algemene bevolking (16%). Ambivalent/resistent hechtingsgedrag kwam het meest voor, waarbij het kind voortdurend aandacht vraagt van ouders/verzorgers en tegelijkertijd zich afzet tegen contact. Daarnaast werd er meer negatief en naar binnen gericht gedrag gerapporteerd bij het kleine kind en heeft hij/zij vaker een temperament dat predisponeert voor het ervaren van meer negatieve emoties in vergelijking met het grote kind. Een goede hechting met ouders of andere primaire opvoeders in de eerste levensjaren draagt bij aan een voorspoedige sociaal-emotionele, taal en cognitieve ontwikkeling van een kind. De informatie uit deze studie faciliteert dan ook vroege opsporing, preventie en interventie om veilige hechting te bevorderen in een vroeg stadium.

In **Hoofdstuk 10** analyseerden we de groeipatronen van de LEMON studiepopulatie met behulp van een mixed-effects model om te beoordelen of het kleine kind inhaalgroei (gedefinieerd als groei binnen de target height range (target height +/- 0.8 standaarddeviatiescore)) laat zien. We verzamelden de groeimetingen die zijn gedaan op het consultatiebureau in Nederland. Daarbij werden er bij het vervolgonderzoek ook lengte, gewicht en hoofdomtrek gemeten. Gemiddeld halen kleinere tweelingen binnen 8-11 jaar na de geboorte een lengte in binnen hun target height range. Een vergelijking binnen het tweelingpaar liet zien dat verschillen in lengte, gewicht en hoofdomtrek aanhielden gedurende de kindertijd, waarbij het kleine kind een kleinere

lengte, lichter gewicht en kleinere hoofdomtrek bleef houden dan hun grotere tweelingbroer of -zus. Deze bevindingen wijzen op een persisterend remmend effect van FGR op groei in de kindertijd.

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Appendices

Abbreviations

AA: arterio-arterial
AC: abdominal circumference
AGA: appropriately-grown for gestational age
AISI: Attachment Insecurity Screening Inventory
AHW: anterior horn width
Ao: aortic root
aPWV: aortic pulse-wave velocity
AV: arterio-venous
AV: aortic valve
A/REDF: absent or reversed end-diastolic flow
BASII: British Ability Scales
Bayley: Bayley Scales of Infant and Toddler Development
BMI: body mass index
BPD: bronchopulmonary dysplasia
BWD: birth weight discordance
CBCL: Child Behavior Checklist
CI: confidence interval
CP: cerebral palsy
CPAP: continuous positive airway pressure
CPR: cerebro-placental ratio
cUS: cerebral ultrasound
CVD: cardiovascular disease
DC: dichorionic
(E)CBQ-VSF: (Early) Childhood Behavior Questionnaire – Very Short Form
EFW: estimated fetal weight
FGR: fetal growth restriction
FOD: fronto-occipital diameter
FSIQ: full scale intelligence quotient
GA: gestational age
GCA: general conceptual ability
GEE: generalized estimated equation
GMFCS: Gross Motor Function Classification System
HFO: high frequency oscillation
Hb: hemoglobin
iA/REDF: intermittent absent or reversed end-diastolic flow
IFW: interhemispheric fissure width
IQ: intelligence quotient
IQR: interquartile range
IUD: intrauterine demise
IUFD: intrauterine fetal demise
IUT: intrauterine transfusion
IVH: intraventricular hemorrhage
LA: left atrium
LEMON: Long-term Effects of selective fetal growth restriction in MONochorionic twins
LSV: lenticulostriate vasculopathy
LUMC: Leiden University Medical Center
LVIDD: left ventricular internal diameter in diastole
LVIDS: left ventricular internal diameter in systole

LVPWD: left ventricular posterior wall thickness in diastole
LVPWS: left ventricular posterior wall thickness in systole
MC: monochorionic
MCDA: monochorionic diamniotic
MIST: minimally invasive surfactant therapy
MND: minor neurological dysfunction
MRI: magnetic resonance imaging
MV: mitral valve
NDI: neurodevelopmental impairment
NEC: necrotizing enterocolitis
NICU: neonatal intensive care unit
OR: odds ratio
PDA: patent ductus arteriosus
pEDF: positive end-diastolic flow
PET: partial exchange transfusion
PIQ: performal intelligence quotient
PPHN: persistent pulmonary hypertension of the newborn
PV: pulmonary valve
PVL: periventricular leukomalacia
QNST: Quick Neurological Screening Test
RDS: respiratory distress syndrome
RWT: relative wall thickness
SDQ: Strengths and Difficulaties Questionnaire
SD(S): standard deviation (score)
sFGR: selective fetal growth restriction
SGA: small for gestational age
sIUGR: selective intrauterine growth restriction
TAPS: twin anemia polycythemia sequence
TCD: transverse cerebellar diameter
TH: target height
TOD: thalamo-occipital distance
TRAP: twin reversed arterial perfusion
TTTS: twin-twin transfusion syndrome
TV: tricuspid valve
Twinlife: Twin Longitudinal Investigation of FEtal discordance
UA: umbilical artery
VA: veno-arterial
VAW: ventricular atrium width
VI: ventricular index
VIQ: verbal intelligence quotient
VV: veno-venous
WISC: Wechsler Intelligence Scale for Children
WPPSI: Wechsler Preschool and Primary Scale of Intelligence

List of publications

First author

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Ultrasound Obstet Gynecol. 2022 Mar;59(3):371-376.

Curriculum vitae

Sophie Groene werd geboren op 12 juli 1997. In 2015 haalde zij cum laude haar diploma aan het Haarlemmermeerlyceum in Hoofddorp, inclusief International Baccalaureate Higher Level in de Engelse taal. Datzelfde jaar begon zij aan haar Bachelor Geneeskunde aan de Universiteit Leiden. Op eigen initiatief zocht zij in 2017 contact met Prof.dr. Enrico Lopriore om naast haar studie onderzoek te doen in het kader van het Honours traject voor excellente studenten. Na het afronden van haar Bachelor met Honours in juli 2018, startte zij in september 2018 met een promotietraject op de afdelingen Neonatologie en Biomedical Data Sciences van het Leids Universitair Medisch Centrum (LUMC), onder begeleiding van Prof.dr. Enrico Lopriore, Prof.dr. Bas Heijmans en Dr. Jeanine van Klink. Dit promotietraject heeft zij in 2022 afgerond. In februari 2023 zal Sophie haar master Geneeskunde aan het LUMC voortzetten en gaan starten met haar coschappen. Ze zal gelijktijdig doorgaan met wetenschappelijk onderzoek in een parttimefunctie als postdoctoraal onderzoeker.

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Arno, we hebben veel uren samen naast het bed van tweelingen doorgebracht: het bewonderingswaardig hoe jij altijd tijd maakt voor deze wetenschapsecho's. Je bent een essentiële schakel binnen Twinlife, en niet alleen vanwege je opbrengst bij wanguitstrijkjes.

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