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

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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<sup>1</sup>. The placenta can also be unequally shared, causing a discordant distribution of nutrients and oxygen leading to a large intertwin growth discrepancy<sup>2</sup>. 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)<sup>3,4</sup>. While cognitive and motor outcomes have recently been elaborately described<sup>4</sup>, 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<sup>5</sup>. 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<sup>6</sup>. 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)<sup>7</sup>. Impaired psychosocial functioning can significantly affect both school functioning and academic performance<sup>8</sup>.

FGR in singletons has already been associated with more psychosocial difficulties<sup>9</sup>. 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<sup>10,11</sup>. 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  $\geq 12$  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)  $\geq 20\%$  (calculated as (birth weight larger twin – birth weight smaller twin)/birth weight larger twin  $\times 100$ )<sup>12</sup>. Cases with twin-twin transfusion syndrome (TTTS), twin anemia polycythemia sequence and monoamnicity 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<sup>13</sup>), gestational age at birth, sex, delivery mode, BWD, birth weight, small for gestational age (SGA) (birth weight  $< 10^{\text{th}}$  centile<sup>14</sup>), severe neonatal morbidity<sup>15</sup> 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  $\geq 60$  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<sup>16</sup>. To assess temperament, the early childhood behavior questionnaire very

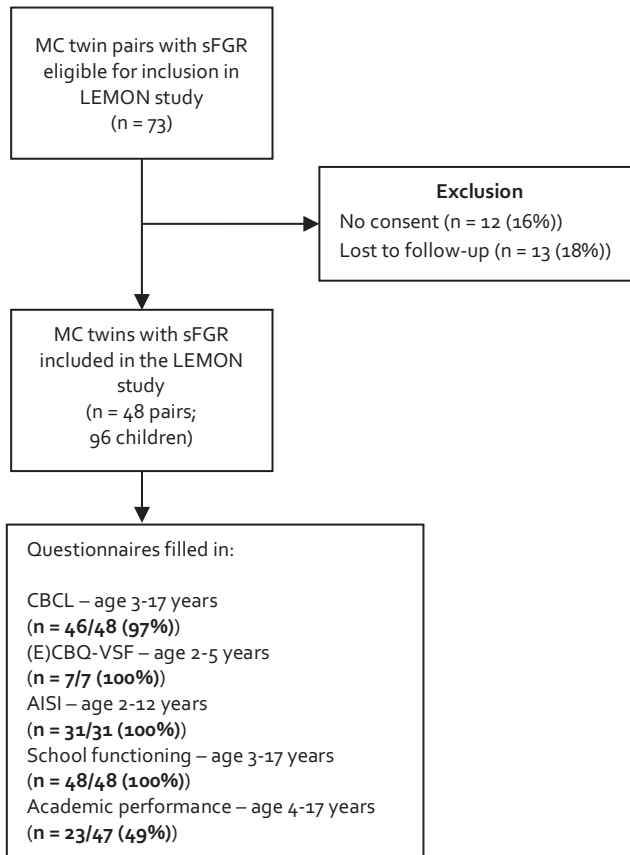
short form (ECBQ-VSF) for children aged 2-3 years and the children's behavior questionnaire very short form (CBQ-VSF) for children aged 4-5 years were used, reporting on three broadband scales: negative affect, i.e., the experience and expression of negative emotions, surgency, i.e., tending towards increased expression of positive emotions, and effortful control, i.e., self-regulation of attention, activity and behavior<sup>17,18</sup>. 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  $\geq 20$ ), ambivalence/resistance ((sub)clinical with a score  $\geq 17$ ), disorganization ((sub)clinical with a score  $\geq 16$ ) and total attachment insecurity ((sub)clinical with a score  $\geq 46$ )<sup>19,20</sup>. 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<sup>21-23</sup>. 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<sup>24-26</sup>. 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)	<b>0.021</b>
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)	<b>0.009</b>
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%	<b>0.024</b>	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%	<b>0.016</b>	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	<i>p</i> - value	Smaller twin (n=48)	Larger twin (n=48)	<i>p</i> - 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%	<b>0.014</b>	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)						<b>0.031</b>
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 <sup>‡</sup> (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 <sup>‡</sup> (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 <sup>‡</sup> (n = 23 pairs)			0.106			<b>0.025</b>
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 (%).

<sup>‡</sup>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<sup>27-29</sup>. 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%)<sup>30</sup>. 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<sup>9</sup>. 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<sup>31</sup>. 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<sup>32,33</sup>. These structural changes are thought to have functional neurobehavioral consequences: poor attention, altered mood, irritability and anxiety<sup>32</sup>. 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<sup>4</sup>. 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<sup>34</sup>. 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<sup>35</sup>. 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<sup>36</sup>. 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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