PART FOUR

THE ROLE OF NEONATAL BRAIN INJURY

CHAPTER 2

Associations between neonatal magnetic resonance imaging and short- and long-term neurodevelopmental outcomes in a longitudinal cohort of very preterm children

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Abstract

Objective: To assess associations between neonatal brain injury assessed by magnetic resonance imaging and cognitive, motor, and behavioral outcomes at 2 and 10 years of age, in a longitudinal cohort of children born very preterm.

Study design: There were 112 children born at $<$32 weeks of gestation who participated in a longitudinal prospective study on brain injury and neurodevelopmental outcome. Using the Kidokoro score, neonatal brain injury and altered brain growth in white matter, cortical and deep gray matter, and the cerebellum were assessed. Cognitive, motor, and behavioral outcomes were assessed during follow-up visits at both 2 (corrected) and 10 years of age.

Results: After adjusting for perinatal factors and level of maternal education, the global brain abnormality score was associated with cognition ($B = -1.306$; $P = .005$), motor skills ($B = -3.176$; $P < .001$), and behavior ($B = 0.666$; $P = .005$) at 2 years of age, but was not associated with cognition at 10 years of age. In the subgroup of children with a moderate-severe global brain abnormality score, magnetic resonance imaging was independently associated with cognitive impairment at 10 years of age. For children with milder forms of brain injury, only birth weight and level of maternal education were associated with cognitive outcomes.

Conclusions: Neonatal brain injury, assessed by a standardized scoring system, was associated with short-term neurodevelopmental outcomes, but only with motor skills and behavior in childhood. Environmental factors, such as level of maternal education, become more important for cognitive development as children grow older, especially for children with relatively mild neonatal brain injury.

Introduction

Being born prematurely comprises a number of developmental challenges, especially as infants reach childhood and adolescence [1]. Children born preterm are at risk of a broad spectrum of neurodevelopmental impairments, including cognitive impairments, motor deficits, and behavioral difficulties [2-5]. For clinicians, it remains challenging to predict the short- and long-term outcomes for children born preterm and to identify those at risk for an adverse outcome.

One factor related to the developmental prognosis of children born preterm is neonatal brain injury. Owing to the disruption in development of brain structures and brain maturation caused by preterm birth, the brain is often organized differently compared with children born at full term [6]. The brain of children born preterm frequently shows white matter injury and subsequent dysmaturation of white and gray matter structures [7]. Neonatal brain injury can be assessed using magnetic resonance imaging (MRI). MRI has the ability to identify subtle forms of brain injury, especially diffuse noncystic white matter injury and small cerebellar lesions, and to precisely detect altered brain growth [8-10]. Although brain injury as seen on neonatal MRI has been related to neurodevelopmental outcomes, there is no agreement whether subtle MRI abnormalities have prognostic implications [11-13].

Although neonatal brain abnormalities have been associated with neurodevelopmental outcomes in numerous studies of children born preterm, no studies have investigated the prognostic implications on both short- and long-term cognitive, motor, and behavioral outcomes within the same cohort of children [13-17]. Children may grow into their deficits as they become older, leading to a better prediction of long-term outcomes [18]. Or environmental factors play an increasing role in development as a child grows older and may become of greater influence compared with neonatal brain injury or perinatal factors [19].

To determine if the prognostic implications of neonatal brain injury differ at different timepoints in a child's life, the aim of this study was to assess the associations between brain injury on the neonatal MRI for cognitive, motor, and behavioral outcomes at both 2 and 10 years of age, in a longitudinal cohort of children born very preterm. The Kidokoro score, a commonly used scoring system for conventional MRI at term-equivalent age, was used for this study because it incorporates the assessment of both altered brain growth and abnormalities in different brain regions [20].

Methods

This study was performed as part of a larger single-center longitudinal prospective study on neuroimaging and outcome after preterm birth (PReterm brain injury, long-term OUtcome and brain Development study; PROUD study). An unselected cohort of 112 infants (<32 weeks of gestation), who were admitted to the tertiary neonatal unit of Leiden University Medical Center between May 2006 and November 2007 and underwent an MRI at term-equivalent age, was included. Children were excluded if they had congenital anomalies of the central nervous system, severe other congenital anomalies, chromosomal disorders, metabolic disorders, or neonatal meningitis [21, 22]. All children were invited for follow-up assessments at 2 years of age corrected for prematurity and at 10 years of age (uncorrected). For this particular study, children were included if follow-up assessment was available for at least 1 timepoint.

Brain Injury Assessment on MRI

An MRI of the neonatal brain was performed around term-equivalent age using a 3.0 Tesla MR system (Achieva 3T; Philips Medical Systems), according to the procedure described previously [23]. All MRI examinations included a T1-weighted 3-dimensional turbo field-echo sequence, a T2-weighted turbo spin-echo sequence, and a T2* fast field-echo sequence. Neonatal MRI scans were reviewed by at least 2 experienced investigators. The MRI investigators reviewed the scans together, and any discrepancies in interpretation were solved by consensus or by asking the opinion of a third reviewer. They were blinded to any clinical characteristics or outcome data of the children except for their postmenstrual age (PMA) at the time of scanning. The MRI examinations were performed preferably around term-equivalent age (40-44 weeks of PMA). For infants who were in a unstable condition around that age or still on respiratory support, the MRI was performed as soon as the child was in a stable condition [21]. The median PMA during scanning of the initial cohort was 43.3 weeks (IQR, 42.3-46.0 weeks). The neonatal MRI scans were assessed using a standardized scoring system to assess abnormal brain metrics and the presence and severity of abnormalities in the cerebral white matter, cortical and deep gray matter, and cerebellum [20]. The sum of these subscores leads to a Global Brain Abnormality Score (GBAS), which can be further classified as normal (0-3), mildly abnormal (4-7), moderately abnormal (8-11), and severely abnormal (≥12).

Outcome Assessment

Children were invited for follow-up visits at 2 timepoints: at 2 years of age corrected for prematurity and at 10 years of age (uncorrected) according the national guideline of the Dutch working group on follow-up for preterm infants. During both visits, standardized cognitive and motor functioning examinations were administered, and parents reported on the presence of behavioral problems. Children were examined by a paediatrician at 2 and 10 years of age, and additionally by a child neurologist at 10 years of age. Parents were questioned on the physical and medical history of their child. Children who experienced severe illnesses or additional brain injury were excluded from the study.

At 2 years of age, cognitive and motor development was assessed using the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) [24, 25]. Composite scores have a mean of 100 and a SD of 15. The motor composite score is based on fine and gross motor scaled scores with a mean of 10 and SD of 3. At the time of assessment, US norms were used owing to the lack of a Dutch norm group. Using the US norms leads to an underestimation of developmental delays at 2 years of age; therefore, Bayley-III cognition and motor scores were subsequently corrected for the current Dutch norms [26-28].

At 10 years of age, cognitive development was assessed by the Wechsler Intelligence Scale for Children (WISC-III) [29]. Full scale, verbal, and performance IQs were obtained, with a mean of 100 and SD of 15. Motor development was examined using the Movement Assessment Battery for Children, second edition (M-ABC-II), with a total scaled score based on 3 scaled subscores (manual dexterity, balance, and catch and throw), all with a mean of 10, and SD of 3 [30].

During both follow-up visits, parents reported on behavior using the Child Behavioral Checklist (CBCL) [31]. Age-standardized t-scores were obtained for internalizing, externalizing, and total problem behavior, where higher scores indicate higher levels of problem behavior.

All assessments were performed according to the national guideline of the Dutch working group on follow-up for preterm infants (<32 weeks of gestation). The institutional review board approved this prospective study and parental consent was obtained from both parents. Outcomes of this cohort at 2 years of age, in relation to brain imaging findings, have been published previously [21, 32-34].

Perinatal Risk Factors and Maternal Education

Perinatal data were retrieved for all children, as published earlier, and included the child's sex, gestational age, birth weight, small for gestational age, postnatal sepsis, necrotizing enterocolitis (NEC), and bronchopulmonary dysplasia (BPD) [22]. Small for gestational age was based on a birth weight of <10th percentile [35]. The presence of infection/inflammation was defined as either the presence of a positive blood culture, and/or NEC stage ≥2 [36]. BPD was categorized as none/mild or moderate-severe BPD, defined as oxygen dependence at 36 weeks PMA [37]. Because of the known negative impact of a low level of maternal education on both cognitive and motor outcomes, the level of maternal education was obtained during the first follow-up visit at 2 years of age corrected for prematurity [38, 39]. It was classified as low (primary school and lower general secondary school), intermediate, or high (higher vocational school and university) [40].

Statistical Analyses

Statistical analyses were conducted using SPSS (version 23.0, IBM). To assess if selective loss to follow-up occurred, perinatal risk factors and the MRI GBAS at term-equivalent age for children with and without follow-up were compared using a $\chi 2$ or Fisher exact test for categorical variables and t test for continuous variables.

Neonatal MRI measurements of the biparietal diameter, deep gray matter area, and transcerebellar diameter were corrected for PMA at scanning using linear regression analysis (ie, corrected measurement = original measurement + slope [40 – PMA]).21 Corrected measures were used in subsequent analyses.

To assess differences within the subscores of cognitive, motor, and behavioral outcomes, paired t tests were conducted for fine and gross motor skills on the Bayley-III, verbal and performance IQ on the WISC-III, manual dexterity, aiming and catching and balance on the M-ABC-II, and internalizing and externalizing behavioral problems at both timepoints on the CBCL. If a significant difference ($P < .05$) was present, subscores, instead of total scores, were used as outcome measures in subsequent analyses.

To investigate the effect of MRI scores on cognitive, motor, and behavioral outcomes at 2 and 10 years of age, univariable linear regressions were first conducted unadjusted for any other possible contributing factors. Second, using multivariable linear regressions, the effect of MRI scores on cognitive, motor, and behavioral outcomes was adjusted for both perinatal risk factors and level of maternal education. Finally, multivariable regression analyses were conducted to determine the independent contributions of the GBAS, perinatal risk factors, and the effect of maternal education. MRI scores were used in both univariable and multivariable analyses as continuous variables. To adjust for the effect that observations in twins are not independent, the univariable and multivariable analyses were conducted in a generalized estimated equations model [41].

Results

Of the 112 children who underwent MRI at term-equivalent age, follow-up at 2 and/ or 10 years of age was available for 99 children (88%). Of these, 69 (70%) underwent follow-up assessment at both timepoints and 30 (30%) at 1 timepoint (15 children [15%] at 2 years of age and 15 [15%] at 10 years of age). The baseline characteristics of the participating children are shown in Table I. The PMA at the time of the MRI was older for children with follow-up assessments (median, 43.4 weeks; IQR, 42.4-47.9 weeks) compared with those lost to follow-up (median, 42.3 weeks; IQR, 41.9-42.8 weeks); otherwise, there were no differences in clinical measures or the GBAS on MRI at term-equivalent age. Level of maternal education was registered during the first follow-up at 2 years of age. Therefore, no information on level of maternal education is available for children without follow-up.

Table I. Perinatal characteristics and level of maternal education of the study population.

Perinatal characteristics	**Participants (n=99)**	**No follow-up available (n = 13)**	**p**
Male sex (%)	60 (60%)	7 (54%)	.476
Part of twins or triplets (%)	33 (33%)	2 (15%)	.088
GA (weeks), mean ± SD	28.9 ± 2.0	29.4 ± 1.9	.660
Birth weight (g), mean ± SD	1205 ± 357	1278 ± 413	.646
SGA (%)	12 (12%)	-	.585
BPD (%)			
Moderate-severe	25 (25%)	2 (15%)	.207
Mechanical ventilation >7 days	35 (35%)	3 (23%)	.289
Sepsis (%)	37 (37%)	6 (46%)	.691
NEC (%)	3 (3%)	-	.083
High grade IVH and/or PVHI	8 (8%)	1 (8%)	.814

Table I. Continued

Perinatal characteristics	**Participants (n=99)**	**No follow-up available (n = 13)**	**p**
Maternal education[+]	(n = 97)		
Low (%)	23 (24%)	-	
Intermediate (%)	32 (33%)	-	
High (%)	42 (43%)	-	
PMA in weeks, median (IQR)	43.4 (42.4 – 47.9)	42.3 (41.9 – 42.8)	.014*
GBAS, median (IQR)	4 (2 - 6)	3 (2 – 5)	.772
Normal <4, n (%)	49 (50%)	7 (54%)	
Mildly abnormal 4-7, n (%)	34 (34%)	5 (38%)	
Moderate-severe abnormal >7, n (%)	16 (16%)	1 (8%)	

***** p <.05
[+] Level of maternal education was registered during the first follow-up at two years of age. Therefore, no information on level of maternal education is available for children without follow-up.

Figure I shows the distribution of the GBAS and the subscores as seen on MRI around term-equivalent age. An abnormal GBAS was present in one-half of the children (n = 50 [50%]), with 34 children (34%) having a mild GBAS and 16 children (16%) a moderate-severe GBAS. White matter abnormalities were the most common (mild, n = 25 [25%]; moderate-severe, n = 25 [25%]), followed by cortical gray matter abnormalities (mild, n = 26 [26%]; moderate-severe, n = 19 children [19%]) and cerebellar injury (mild, n = 12 [12%]; moderate-severe, n = 11 [11%]). Three children (3%) had deep gray matter abnormalities (mild, n = 1 [1%]; moderate-severe, n = 2 [2%]), accompanied by severe abnormalities in at least one of the other subscores.

Outcomes at 2 Years of Age

Table II shows the outcome of the 2-year follow-up assessment. In 84 children (85%; mean age, 31.2 ± 4.8 months), at least one of the cognitive, motor, and/or behavioral assessments was available. Children who participated in only the 2-year follow-up assessment performed more poorly on both cognitive (t = −3.698; P = .001) and motor tasks (t = −3.730; P = .002) tasks, compared with children who participated in follow-up at both timepoints. There was no difference in behavioral outcome.

When evaluating the motor subscores, a significant difference was found between fine and gross motor outcomes on the Bayley-III (t = −2.463; P = .017), with lower scores for gross motor outcomes. There was also a difference between internalizing and externalizing behavior on the CBCL (t = −2.194; P = .031), with more reported externalizing behavior.

Figure I. Distribution of the GBAS and the subscores as seen on MRI around term-equivalent age.

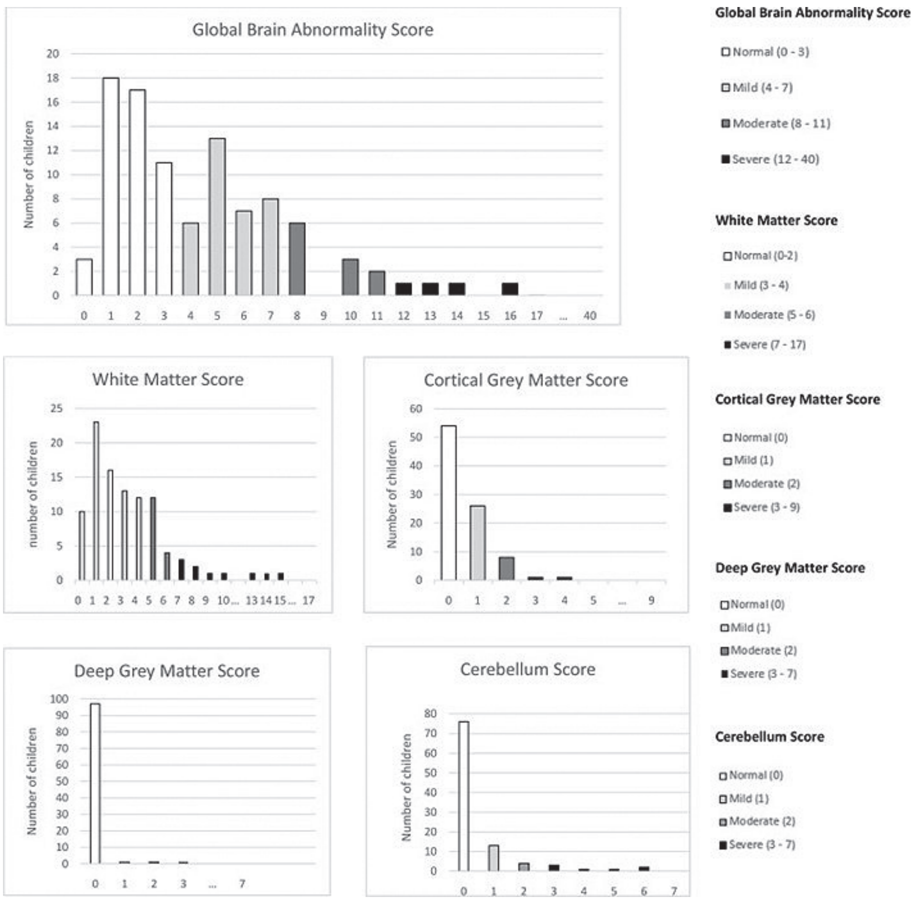

Table II. Outcome of the study population at two and ten years of age.

	Two years of age		Ten years of age
Age at follow-up in months	31.2 ± 4.8		117.2 ± 7.7
Cognition	*Bayley-III (n = 83)*		*WISC-III (n = 83)*
Total (m ± SD)	88.1 ± 12.4	Full Scale IQ (m ± SD)	94.7 ± 16.6
		Verbal IQ (m ± SD)	99.0 ± 16.6
		Performance IQ (m ± SD)	91.2 ± 16.8
Motor outcome	*Bayley-III (n = 74)*		*M-ABC-II (n = 79)*
Motor Composite ± SD	93.8 ± 15.0	Total (m ± SD)	9.1 ± 3.1
Fine motor (m ± SD)	9.9 ± 1.9	Manual Dexterity (m ± SD)	9.7 ± 2.2
Gross motor (m ± SD)	9.2 ± 2.0	Balance (m ± SD)	9.4 ± 2.9
		Aiming and Catching (m ± SD)	9.4 ± 3.0

Table II. Continued

	Two years of age		Ten years of age
Behavior	*CBCL (n = 77)*		*CBCL (n = 75)*
Total (m ± SD)	49.1 ± 9.1	Total (m ± SD)	51.9 ± 11.9
Internalizing (m ± SD)	48.1 ± 9.6	Internalizing (m ± SD)	54.3 ± 11.4
Externalizing (m ± SD)	50.5 ± 9.5	Externalizing (m ± SD)	48.5 ± 10.6

Table III shows the associations between brain injury (MRI) at term-equivalent age on outcome at 2 years of age. Unadjusted, both the GBAS and white matter abnormality scores were associated with lower cognitive outcomes, impaired motor composite scores, and total behavioral problems. Lower motor composite scores were further related to deep gray matter abnormality scores. When considering the motor and behavioral subscores, gross motor skills were related to white matter and cerebellar scores, and fine motor skills to deep gray matter abnormality scores. Additionally, internalizing behavior was associated with white matter and deep gray matter abnormality scores, externalizing behavior could not be predicted at 2 years of age. After adjusting for perinatal risk factors and the level of maternal education these results persisted, except for the association between gross motor skills and cerebellar scores.

Outcomes at 10 Years of Age

Outcomes at 10 years of age are reported in Table II. In 84 children (85%; mean age, 117.2 ± 7.7 months), at least one of the cognitive, motor, and/or behavioral assessments was available. Children who participated in only the 10-year follow-up assessment performed more poorly on both cognitive ($t = -2.225$; $P = .038$) and motor tasks ($t = -3.390$, $P = .002$), compared with children who participated in both follow-up timepoints. There was no difference in behavioral outcomes.

When evaluating the cognitive and behavioral subscores, there was a significant difference between verbal and performance IQ on the WISC-III ($t = 4.587$; $P \leq .001$), with lower performance IQ scores, and between internalizing and externalizing behavior on the CBCL ($t = -4.413$; $P < .001$), with higher levels of internalizing behavior. No differences were found within the different subscores of the M-ABC-II.

Table III. Unadjusted and adjusted associations between neonatal brain injury as seen on MRI at TEA and cognitive, motor and behavioral outcomes at two years of age.

	Cognition (n = 83)		Motor Composite (n = 74)		Fine motor (n = 74)		Gross motor (n = 74)		Total behavior (n = 74)		Internalizing behavior (n = 74)		Externalizing behavior (n = 74)	
	B	**p**	**B**	**p**	**B**	**p**	**B**	**P**	**B**	**p**	**B**	**P**	**B**	**p**
GBAS														
Unadjusted	- 1.458	<.001**	-3.294	<.001**	-.108	.155	-.288	.006**.016*	.721	.003**	.756	.011*	.349	.129
Adjusted	-1.306	.005**	-3.176	<.001**	-.062	.431	-.253		.666	.005**	.680	.021*	.297	.198
WM														
Unadjusted	-1.835	<.001**	-4.124	<.001**	-.123	.254	-.345	.015*	1.000	.001**	1.048	.003**	.541	.062
Adjusted	-1.707	<.001**	-4.034	<.001**	-.094	.381	-.317	.018*	.932	.001**	1.019	.002**	.446	.126
Cerebellum														
Unadjusted	-1.328	.054	-.865	.490	-.139	.347	-.637	.037*	.726	.191	.924	.374	.120	.848
Adjusted	-1.107	.120	.019	.991	.000	.998	-.525	.074	.545	.435	.195	.841	.142	.831
Cortical GM														
Unadjusted	-2.192	.238	-3.571	.202	.082	.756	-.016	.966	.790	.475	.256	.824	.540	.569
Adjusted	-1.551	.350	-2.576	.326	.244	.333	.165	.595	.553	.620	-.041	.973	.493	.617
Deep GM														
Unadjusted	-3.335	.350	-14.375	<.001**	-.851	<.001**	.245	.067	.884	.611	2.579	.008**	-.525	.813
Adjusted	-4.336	.214	-16.829	.002**	-.919	<.001**	.138	.620	1.218	.386	3.121	.050	-.886	.622

Adjusted for sex, birth weight, bronchopulmonary dysplasia, sepsis/necrotizing enterocolitis and the effect of maternal education.
***** p <.05
****** p <.01

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Table IV. Unadjusted and adjusted associations between neonatal brain injury as seen on MRI at TEA and cognitive, motor and behavioral outcomes at ten years of age.

	Full Scale IQ (n = 83)		**Verbal IQ (n = 83)**		**Performance IQ (n = 83)**		**Motor (n = 79)**		**Total behavior (n = 75)**		**Internalizing behavior (n = 75)**		**Externalizing behavior (n = 75)**	
	B	**p**	**B**	**p**	**B**	**p**	**B**	**P**	**B**	**p**	**B**	**P**	**B**	**p**
GBAS														
Unadjusted	-1.344	.008**	-1.094	.082	-1.639	.002**	-.409	.001**	.578	.165	.413	.285	.181	.650
Adjusted	-.532	.228	-.358	.500	-.834	.108	-.304	.021*	.694	.044*	.568	.166	.275	.485
WM														
Unadjusted	-1.344	.069	-.540	.501	-2.116	.011*	-.512	.003**	1.108	.061	.895	.127	.419	.478
Adjusted	-.450	.520	.290	.668	-1.254	.139	-.431	.019*	1.304	.011*	1.143	.050	.553	.332
Cerebellum														
Unadjusted	-3.564	.008**	-4.725	.005**	-2.145	.104	-.592	.014*	-.233	.716	-.318	.584	.554	.537
Adjusted	-2.242	.036*	-3.793	.012*	-.570	.550	-.301	.200	-.097	.876	-.586	.316	.545	.552
Cortical GM														
Unadjusted	.326	.872	.320	.884	-.648	.738	-.091	.815	-.531	.716	-.491	.742	-1.256	.341
Adjusted	1.451	.361	1.343	.422	.340	.843	.225	.525	-.432	.769	-.333	.837	-1.066	.440
Deep GM														
Unadjusted	-3.873	.278	.542	.886	-8.140	.006**	-1.981	<.001**	2.759	.360	3.989	.004**	1.113	.755
Adjusted	-3.096	.053	1.124	.553	-7.021	<.001**	-2.302	.019*	3.437	.124	4.609	.001**	1.254	.693

Adjusted for sex, birth weight, bronchopulmonary dysplasia, sepsis/necrotizing enterocolitis and the effect of maternal education.
***** p <.05
****** p <.01

Table IV shows associations between brain injury (MRI) at term-equivalent age and outcome at 10 years of age. Unadjusted, the GBAS was associated with lower full scale IQ scores and poorer motor outcome. Lower full scale IQ scores were further associated with cerebellar scores. Poorer motor outcome was also related to cerebellar and deep gray matter abnormality scores. Considering the cognitive and behavioral subscores, lower verbal IQ scores were related to cerebellar scores, whereas lower performance IQ was related to the GBAS, white matter and deep gray matter abnormality scores. Deep gray matter abnormality scores were also related to internalizing behavior; externalizing behavior could not be predicted at 10 years of age.

Table V shows the independent contributions of the GBAS, perinatal risk factors, and level of maternal education on cognitive, motor, and behavioral outcomes. In a multivariable analysis, the GBAS and low level of maternal education predicted lower cognition scores at 2 years of age, but at 10 years of age, the GBAS predicted neither full scale, verbal, nor performance IQ. However, full scale, verbal, and performance IQ were associated with lower birth weight and low/intermediate levels of maternal education. Only the GBAS was independently associated with the motor composite score at 2 years of age (Table III). Gross motor skills were associated with the GBAS and lower birth weight at both 2 and 10 years of age. Sepsis and NEC were associated with motor skills at 10 years of age. At 2 years of age, the total CBCL scores were only associated with the GBAS (Table III). At 10 years of age, both the GBAS and male sex were related to higher CBCL scores. Additionally, internalizing behavior at 2 years of age was related to the GBAS and BPD, but at 10 years of age only to a low level of maternal education, whereas externalizing behavior, related to sepsis and NEC at 2 years of age, no longer had any associations at 10 years of age.

Table V. Independent contributions of perinatal risk factors and maternal education in a multivariable analysis on cognitive, motor, and behavioral outcomes at two and ten years of age.

Two years of age	B	p	Ten years of age	B	p
Cognition (n = 83)			**Full Scale IQ (n = 83)**		
GBAS	-1.306	.005**	Birth weight	.013	.013*
Low vs high Mat Edu	-7.112	.018*	Low vs high Mat Edu	-15.474	<.001**
			Int vs high Mat Edu	-11.818	.001**
			Verbal IQ (n = 83)		
			Birth weight	.014	.013*
			Low vs high Mat Edu	-17.697	<.001**
			Int vs high Mat Edu	-11.109	.002**

Table V. Continued

Two years of age	B	p	Ten years of age	B	p
			Performance IQ (n = 83)		
			Birth weight	.013	.015*
			Low vs high Mat Edu	-10.636	.020*
			Int vs high Mat Edu	-11.771	.001**
Gross motor (n = 74)			**Motor (n = 79)**		
GBAS	-.253	.016*	GBAS	-.304	.021*
Birth weight	.002	.042*	Birth weight	.003	.010*
			Sepsis/NEC	1.724	.010*
			Total behavior score (n = 75)		
			GBAS	.694	.044
			Male sex	6.530	.013
Internalizing behavior (n = 74)			**Internalizing behavior (n = 75)**		
GBAS	.680	.021*	Low vs high Mat Edu	-6.881	.035*
BPD	8.111	.003**			
Externalizing behavior (n = 74)					
Sepsis/NEC	4.955	.046*			

***** p <.05
****** p <.01

Discussion

We investigated the associations between neonatal brain injury on cognitive, motor, and behavioral outcomes at 2 different ages in a longitudinal cohort of children born very preterm using a comprehensive, objective scoring system to assess neonatal brain injury, and its associations with short- and long-term developmental outcomes. We showed that neonatal MRI was independently associated with cognition, motor skills, and behavior in early childhood, but at 10 years of age, neonatal MRI scores and cognition were not correlated. In the long-term, environmental risk factors, such as maternal education, were shown to exert a stronger influence on the cognitive abilities of the child.

We found, in line with other studies, that cognitive development was associated with neonatal MRI at term-equivalent age at 2 years of age, persisting after adjusting for perinatal risk factors and level of maternal education [42-44]. However, after adjusting at 10 years of age, neonatal MRI was no longer associated with cognitive abilities, except that cerebellar scores were still independently associated with lower full scale IQ scores. In a stepwise regression analysis, first adding only perinatal risk factors and then adding the level of maternal education, it was the latter with the most important influence on cognitive outcome. We showed that children of mothers with a low level of education performed on average 18 points lower on their verbal IQ and 11 points lower on their performance IQ, indicating that maternal factors are more important for cognitive development at 10 years of age compared with neonatal brain injury or other perinatal risk factors. This finding highlights the importance of maternal education. Different pathways might explain why children of mothers with higher levels of education have a higher cognitive performance. For example, genetic inheritance of maternal IQ could contribute, but mothers with a higher level of education may also be more capable of creating learning opportunities for their children than mothers with lower levels of education [38].

Within our cohort, a relatively high number of children experienced mild neonatal brain injury. It is possible that environmental factors play a more important role in determining the outcome for this group of children, whereas the more severe forms of brain injury may have a long-lasting and independent effect on outcomes. Therefore, in our cohort we evaluated cognitive development for the subgroup of children with a moderate-severe GBAS. In this subgroup, the association between neonatal MRI abnormalities and cognitive development persisted at 10 years of age, independent from perinatal factors and level of maternal education. Combining a moderate-severe GBAS with a low level of maternal education did not lead to an increased risk of an adverse development of cognitive capacities at 10 years of age. Of the 34 children in our cohort who performed $\geq$1 SD below the mean, there were only 3 children with both a moderate-severe GBAS and a mother with a low level of education. These findings should be confirmed in larger samples of children born preterm or with higher grades of brain injury, because this may help when counseling parents of preterm infants, and might provide opportunities for targeted interventions for mothers with lower educational levels and on the other hand for children with a moderate-severe neonatal brain injury.

Motor skills were independently associated at both 2 and 10 years of age with similar abnormality scores at term-equivalent age MRI, namely, the GBAS, white matter, and deep gray matter abnormality scores. This finding is consistent with other studies assessing the capability of the neonatal MRI to predict short- and long-term motor functioning, and is also supported by the associations between Bayley-III motor scores and later motor functioning in very preterm children [14, 43-45].

Children born preterm are at risk for developing behavioral difficulties (especially attentional deficits and internalizing problems) that persist into late adolescence [4]. However, neonatal MRI seems to play only a limited role in the prediction of these behavioral problems later on. At 10 years of age, we found an association between total reported behavioral problems with the GBAS and white matter abnormality score, but only after adjustment for perinatal factors and level of maternal education. Considering the independent contributions of the perinatal factors (Table V), behavioral problems were reported considerably more often for boys compared with girls in our cohort. Additionally, a higher level of maternal education and deep gray matter abnormalities independently contributed to more reported internalizing behavior at 10 years of age. Future research should explore the risk factors for behavioral problems in children born preterm.

The strength of this study is the use of a standardized neonatal MRI assessment tool to indicate brain injury at term-equivalent age combined with prospective long-term follow-up data at different time-points, within the same longitudinal cohort of children born very preterm. This provides valuable information for counseling of parents and the use of neonatal MRI in predicting future outcomes.

Owing to the original design of the study in 2006-2007 (investigating brain imaging findings in an unselected cohort of children born very preterm), no sample size or power analysis was performed for loss to follow-up at 2 and 10 years of age. The group of children seen at 2 years of age and the group at 10 years of age were not completely identical. However, repeating the analyses for the 69 children who were assessed at both timepoints did not change the main results or conclusions of the study. Although the outcome assessments used were age appropriate and based on the general population, the fact that this was a single-center study might affect the generalizability of our results. The use of different neurodevelopmental assessment tools for children at 2 and 10 years of age makes comparison at the 2 points in time difficult, although the tests used in this study reflect current clinical practice. When assessing the associations between outcomes at 2 and 10 years of age, we found significant, but mediocre associations. Within this study, general measures of intellectual abilities have been used. It is possible that the use of more specific measures of cognition and learning strategies, for example, executive functioning, will reveal other, possible stronger, associations with neonatal brain injury [18]. Finally, the Kidokoro scoring system was designed for infants scanned between 36 and 42 weeks of gestation and the neonatal MRI scans in our cohort were performed with a median of 43.4 weeks of gestation. Given brain growth is rapid during the first year of life, the older age at scanning in this cohort may have decreased the sensitivity of the scale, in particular with respect to the growth measures, based on the slope from linear regression. In agreement with the data of Brouwer et al, our cohort of preterm infants (gestational age of <32 weeks of gestation) consisted of a relatively large number of infants with milder forms of brain injury [44]. Therefore, it would be of interest to investigate whether the predictive ability of MRI at term-equivalent age differs for both short- and long-term outcome in other longitudinal cohorts with larger numbers of extremely preterm infants and/or higher brain abnormality scores.

With children born preterm likely to grow into their deficits, and with the increasing influence of social and environmental factors, this study showed that in this longitudinal cohort of children born very preterm, brain injury on neonatal MRI was associated with short-term cognitive, motor, and behavioral outcomes, whereas in the long-term, associations were limited and mainly restricted to the motor domain. Predicting long-term outcomes and identifying those at risk for adverse outcomes remains challenging, especially for cognitive and behavioral development. In these domains, environmental factors, such as maternal education, play an increasingly important role, particularly in children with milder forms of brain injury.

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