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Diffusion Tensor Tractography of the Cerebellar Peduncles in Prematurely Born 7-Year-old Children

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Abstract

The objective of this study was to correlate neurodevelopmental outcome of preterm-born children and their perinatal clinical and imaging characteristics with diffusion magnetic resonance imaging (MRI) measures of the three cerebellar peduncles at age 7. Included in this prospective longitudinal study were 140 preterm born children (< 30 weeks' gestation) who underwent neurodevelopmental assessment (IQ, motor, language, working memory) and diffusion weighted imaging (DWI) at age 7 years. White matter tracts in the superior, middle, and inferior cerebellar peduncles were delineated using regions of interest drawn on T2-weighted images and fractional anisotropy (FA) maps. Diffusion measures (mean diffusivity (MD) and FA) and tract volumes were calculated. Linear regression was used to assess relationships with outcome.

The severity of white matter injury in the neonatal period was associated with lower FA in the right superior cerebellar peduncle (SCP) and lower tract volumes of both SCPs and middle cerebellar peduncles (MCPs). In the MCP, higher IQ was associated with lower MD in the whole group and higher FA in right-handed children. In the SCP lower motor scores were associated with higher MD and higher language scores were associated with higher FA. These associations remained significant in multivariable models. This study adds to the body of literature detailing the

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importance of cerebellar involvement in cognitive function related to reciprocal connections with supratentorial structures.

Keywords

Cerebellum; neurodevelopmental outcome; premature infants; magnetic resonance imaging

1. Introduction

The cerebellum has traditionally been known to play a critical role in learning, planning and coordination of movement [1]. More recent studies have demonstrated its additional involvement in higher cognitive functions [2, 3]. Its role in these processes is established during the neonatal period, a critical window of cerebellar development, when a vast increase in structural and functional connections occurs [4, 5, 6]. During this interval, the cytoarchitecture of the cerebellum develops uniformly [4, 7], and the number of cells in the cerebellum reaches an adult level before the age of 18 months [8].

Afferent (inferior and middle cerebellar peduncles [ICP and MCP]) and efferent (superior cerebellar peduncle [SCP]) tracts connect with the cerebrum and the spinal cord through multisynaptic pathways relayed in the deep cerebellar nuclei. Increasingly, advanced neuroanatomical and neuroimaging techniques have helped to characterize the role of the cerebellum in cognitive function. Neuronal tracing methods have revealed both efferent and afferent cerebellar projections involving the prefrontal cortex [6]. In addition, functional magnetic resonance imaging (MRI) investigations have demonstrated coupling of the cerebellum with cerebral association areas [9]. Reciprocal effects between the cerebellum and cerebrum have also been established, as cerebellar volume [10] and microstructure [11] are altered by supratentorial cerebral injury, and, neonatal cerebellar lesions have been shown to correlate with supratentorial brain development, neurobehavioral and cognitive outcomes [12, 13, 14].

Diffusion tensor imaging (DTI), an MRI technique that models the random thermal movement of water molecules as a diffusion tensor, can be used to visualize white matter tracts (diffusion tensor tractography [DTT]) [15] and to measure their diffusion parameters (including mean diffusivity [MD] and fractional anisotropy [FA]). Diffusion measures in the brain have been shown to change consistently with maturation (most notably as decreasing MD and increasing FA in the white matter) [16, 17, 18]. Further, abnormalities in diffusion measures have been found to be associated with brain injury [19, 20] and neurodevelopmental disorders [21, 22, 23], rendering it a well-suited technique for studying the developing brain.

Preterm children have high rates of neurodevelopmental impairment, including lower IQ, motor problems, delayed language and working memory deficits [24, 25, 26]. It has also been reported that this population is at increased risk for cerebellar lesions [27]. Given the important role of the cerebellum in motor and cognitive functioning, we hypothesized that preterm-born children with abnormal neurodevelopmental outcome may have altered white matter tract diffusion measures and volumes within the cerebellar peduncles.

The objective of this study was to correlate the diffusion characteristics of the three cerebellar peduncles (SCP, MCP and ICP) in preterm-born children with neurodevelopmental outcome, specifically IQ, motor functioning, language and working memory.

2. Methods

2.1. Participants

Two hundred and twenty seven children born at less than 30 weeks' gestation and/or with a birth weight of less than 1250 g with no genetic or congenital abnormalities were recruited into the Victorian Infant Brain Study (VIBeS) cohort between 2001 and 2003 at the Royal Women's and the Royal Children's Hospitals, Melbourne, Australia [28]. Two children subsequently died and one child was excluded due to a late diagnosis of a congenital disorder, leaving a cohort of 224 subjects. Parental informed consent was obtained for all subjects and the study was approved by the Human Research Ethics Committees at the Royal Women's and the Royal Children's Hospitals.

2.2. Perinatal/Neonatal Data

Clinical information was recorded for all subjects. This included antenatal and postnatal corticosteroid treatment, culture proven sepsis, necrotizing enterocolitis, ultrasound diagnoses of intraventricular hemorrhage and leukomalacia, exposure to morphine and oxygen requirement at 36 weeks postmenstrual age (PMA).

All participants underwent brain MRI at term equivalent PMA (median 40, range 38–42 weeks) to evaluate the presence of injury in the neonatal period. MRI scans were completed using a 1.5T General Electric Signa LX Echospeed System MR scanner (Milwaukee, WI, USA) without the use of sedating medications [29]. Acquired sequences included 3D T1 spoiled gradient recalled (SPGR) images [0.8–1.6 mm coronal slices, flip angle 45°, TR 35 ms, TE 9 ms, FOV 210 × 150 mm², matrix 256 × 192] and T2 dual echo (interleaved acquisition) fast recovery fast spin echo images [1.7–3.0 mm coronal slices, TR 4000 ms, TE 60/160 ms, FOV 220 × 165 mm², matrix 256 × 192 interpolated to 512 × 512]. Anatomical scans were assessed for white matter injury by a rater who was blinded to the infants' clinical course. Each infant was given a summary injury score that was categorized as none, mild, moderate or severe white matter injury. Moderate and severe white matter injury were collapsed into one group as only two infants were categorized with severe injury. The reader is referred to Kidokoro et al., [30] for the description of the standardized scoring system used.

2.3. Neurodevelopmental Assessment

At 7 years of age, children underwent a detailed neurodevelopmental assessment by trained psychologists who were blinded to the medical history and imaging results. For the purposes of this study, we focused on domains that are considered to be core deficits for preterm children and that have been linked to the cerebellum [12] including: general intelligence (IQ) (Wechsler Abbreviated Scale of Intelligence (WASI) [31]), motor functioning (Movement Assessment Battery for Children, 2nd Edition (M-ABC2) [32]), language development

(Clinical Evaluation of Language Fundamentals, 4th Edition (CELF-4) Core Language Index (CLI) [33]), and working memory (Backward Digit Recall subtest from the Working Memory Test Battery for Children (WMTB-C) [34]).

The neurological examination included an assessment of muscle tone, power and reflexes. Cerebral palsy (CP) and its severity were diagnosed with the use of standard criteria: location or body part impaired (e.g. diplegia or hemiplegia), degree of impairment of muscle tone and reflexes, and the effects on ambulation [35]. A structured psychiatric interview [36, 37] with parents was used to determine the diagnosis of autism and attention deficit/hyperactivity disorder (ADHD). Handedness was classified as writing hand preference and the hemisphere contralateral to child's preferred hand was designated as dominant.

Social risk was assessed on a 12 point scale as previously described for this cohort [38], with a higher score indicating higher social risk.

2.4. 7-Year-Old Magnetic Resonance Imaging

At 7 years of age, consenting participants who passed a mock MRI session underwent neuroimaging using a 3T Siemens scanner at the Royal Children's Hospital, Melbourne. Data acquisition included sagittal 3D turbo spin-echo T2-weighted images [0.9 mm³ isotropic voxels, TR 3200 ms, TE 447 ms, FOV 240 × 215 mm², matrix 256 × 230] and a DTI spin-echo echo-planar sequence with 25 non-collinear gradient directions with b value amplitudes ranging from 50 to 1200s/mm², and additionally a baseline image with b value 0 s/mm² [1.7 mm³ isotropic voxels, TR 12000 ms, TE 96 ms, FOV 250 × 250 mm², matrix 144 × 144].

2.5. Diffusion Tensor Tractography Analysis

2.5.1. Tract Processing—White matter tracts were identified from 26-directions DTI data linearly spaced between b=0 and b=1200s/mm². The directions of the 26 vectors were uniformly spaced across the unit sphere. Details of the data processing were previously published [39] and were achieved with locally developed software [40]. The process included brain mask extraction, motion and eddy current correction, and registration of the DTI data to age appropriate atlas. Deterministic tracking algorithms and tract reconstructions were based on the streamline method [15, 41], tailored for short tracts without crossing fibers. Tract termination criteria included fractional anisotropy (FA) < 0.13 and radius of curvature < 2.5 mm (about a 25 degree angle). These stopping criteria were used in order to optimize tracking, as white matter in children is not fully developed. Full brain tractography was filtered using regions of interest (ROIs) manually placed perpendicular to the length of the tract based on anatomical landmarks in native space using Analyze 10 (Mayo Clinic, Rochester MN) and visualized on Slicer 2.5 (<http://www.slicer.org/>). Diffusion eigenvalues were calculated, and diffusion parameters and tract volume (number of voxels) were extracted. Measured diffusion parameters included MD and FA.

2.5.2. Tracts identification (Figure 1)—Tracts were identified using the following ROIs: For the inferior cerebellar peduncle (ICP) (Figure 1A), superior ROIs were placed at

the level of the pons and roof of the 4th ventricle and inferior ROIs in the medulla at the level of the inferior temporal lobes in axial planes. For the superior cerebellar peduncle (SCP) (Figure 1B), superior ROIs were placed at the level of the midbrain just lateral to the SCP decussation in an axial plane and inferior ROIs lateral to the posterior portion of the 4th ventricle in a coronal plane. For the middle cerebellar peduncle (MCP) (Figure 1C), posterior ROIs were placed lateral and inferior to the posterior SCP ROIs and anterior ROIs prior to SCP decussation in the pons in coronal planes.

All three tracts were visualized in order to ascertain their correct relative positions (Figure 1D). Of note, both the SCP and MCP decussation were easily located on the FA map as condensed horizontal fibers. Processing and measurements of tracts were performed by investigators blinded to perinatal factors, outcome and gestational age (GA) at birth.

2.5.3. Reliability of tracts extraction (see supplemental table)—Intra-rater reliability for tracking was assessed using the intraclass correlation coefficient. Approximately 15% of cases (ICP: n=26, SCP: n=29, MCP: n=34) were randomly chosen and tracking was repeated according to the same protocol for each of the cerebellar peduncles. For the ICP, the coefficient for the different diffusion values ranged from 0.66 to 0.95, for the MCP from 0.83 to 0.96 and for the SCP from 0.88 to 0.95 for the left and right sides, respectively.

2.6. Data analysis and Statistics

Continuous data are presented as mean $\pm$ standard deviation (SD) and assessed by t test or Mann–Whitney. Categorical data are presented as proportions and compared by Chi Square or Fisher exact tests.

The normality of the four main outcomes (full scale IQ, movement assessment, language and working memory) was investigated by normal distribution attributes, skewness, kurtosis and inverse normal plot.

For each outcome, we have examined the associations of the cerebellar peduncle tracts as observed at the age of 7 by a linear regression. We have also assessed neonatal clinical characteristics and neonatal imaging characteristics, which their relevance for neurodevelopment outcome is well established or suspected to confound the results. Subgroup analysis according to handedness was performed.

For each significant association between MRI tract and outcome, we have performed multivariable linear regression. The final models contain covariates based on the finding of the univariate analysis. “Goodness of fit” for a model was assessed using likelihood-ratio R squared. We have reported final parsimonious models with the best “goodness of fit”.

As the study is of an exploratory nature, we did not correct for multiple comparisons and an adjusted alpha level of 0.05 was set as significant [42].

SPSS version 21 (IBM Corporation, 1989, 2010) was used for statistical analyses.

3. Results

3.1. Cohort characteristics

3.1.1. General—Of the 224 children in the cohort, 198 (87%) underwent neurodevelopmental testing at 7 years of age, with the remaining 26 either declining to participate or uncontactable. One hundred and sixty of these children also underwent an MRI scan at 7 years of age. The reasons for not having an MRI scan were poor test tolerance by the children (n=20), severe neurodevelopmental impairment (n=6), lack of consent (n=6) and other (n=6). DTI data of sufficient quality were available to enable tracking from 140 (63%) preterm-born children (median age at follow up 7.5y, range: 6.6-8.4y).

3.1.2. Basic characteristics and comparison with children not included in the cohort (Tables 1 and 2)—The cohort had equal number of males and females, typically received antenatal corticosteroids, had low incidence of severe white matter injury and cerebral hemorrhage and a high incidence of oxygen requirement at 36 weeks PMA (Table 2). At 7 years of age, there was a low incidence of cerebral palsy and autism, with 20% left handedness (Table 2). Children of the original cohort that did not participate in this study had more complicated neonatal course (more days on parenteral nutrition, higher incidence of necrotizing enterocolitis and longer hospital stay although they had fewer proven septic episodes) and lower cerebellar volumes without a difference in cerebellar injury. Also these children had lower IQ scores, scored lower on the M-ABC2 test, had a higher rate of cerebral palsy and a higher social risk score.

3.1.3 Correlation with Outcome (Table 3)—Outcome parameters were significantly correlated to several characteristics of this cohort. Higher full scale IQ was significantly correlated with shorter hospital stay ($p < 0.001$), higher birth weight z-score ($p=0.009$), less white matter ($p=0.041$) and cerebellar injury ($p=0.029$), larger cerebellar volume ($p=0.044$) and lower social risk (0.001). Higher M-ABC2 score was associated with higher birth weight z-score ($p=0.008$), less white matter ($p < 0.001$) and cerebellar injury ($p=0.039$). Higher CELF-4 CLI score was associated with shorter length of hospital stay ($p=0.001$), higher birth weight z-score ($p < 0.001$), less cerebellar injury ($p=0.004$) and lower social risk ($p < 0.001$). Better working memory was associated with higher birth weight z-score ($p=0.032$).

3.2. Diffusion Tensor Tractography

3.2.1. Correlation with Perinatal Factors—Mean diffusivity and fractional anisotropy results are presented in table 4. Higher grade of white matter injury was correlated with lower FA in the right SCP ($p=0.003$), as well as with smaller SCP (left $p < 0.001$, right $p=0.003$) and MCP volumes (left $p=0.003$, right $p=0.01$). The male preterm group had higher MD in the right MCP (0.7038 ± 0.36 vs 0.6882 ± 0.36 , $p=0.013$) and lower FA in left SCP (1.5649 ± 0.22 vs 1.656 ± 0.21 , $p=0.016$) when compared with the female group. No other perinatal factors were associated with diffusion measures (data not shown).

3.2.2. Left versus right tracts (Table 4)—Differences between left and right tracts were detected, with higher MD in the left SCP and MCP and higher FA values and larger volumes in left compared with right peduncles.

3.2.3. Correlation with Neurodevelopmental outcome (table 5)—Of the six cerebellar tracts only the right MCP and right SCP diffusion parameters were associated with neurodevelopment at 7 years of age.

3.2.3.1. Right Middle Cerebellar Peduncle (tables 5, 6, 7): Lower MD of the right MCP was significantly associated with higher full scale IQ ($p=0.001$) and higher CELF-4 CLI score ($p=0.005$) (table 5). These associations remained significant in multivariable models (table 6).

Higher fractional anisotropy was associated with higher full scale IQ ($p=0.022$) and higher working memory scores (backward digit recall) ($p=0.029$) (table 5). See handedness paragraph for the report on the multivariable models.

Larger tracts volume was significantly associated with higher M-ABC2 scores ($p=0.015$) and higher working memory scores (backward digit recall) ($p=0.010$) (table 5). In the multivariable models (table 7) the association with M-ABC2 scores trended to significance ($p=0.055$) and remained significant for working memory scores (backward digit recall) ($p=0.013$).

3.2.3.1. Right Superior Cerebellar Peduncle (table 5 and 8): Higher mean diffusivity in the right SCP was significantly associated with lower M-ABC2 scores ($p=0.009$). Higher FA in the right SCP was significantly associated with higher full scale IQ ($p=0.0180$), higher M-ABC2 scores ($p=0.009$) and higher CELF-4 CLI score ($p=0.003$). Only the correlations of MD with M-ABC2 ($p=0.030$) and of FA with CELF-4 CLI score ($p=0.033$) remained significant in the multivariable models (Table 8).

3.2.4. Handedness (table 9 and 10, figure 2)—Subgroup analysis according to handedness was of interest only in the regression analysis for the right MCP fractional anisotropy where the regression coefficient of right and left handed children were of opposite directions in three outcome measures (full scale IQ, M-ABC2 score and CELF-4 CLI) (table 9 and figure 2) but only for full scale IQ these results were significant in both groups with higher right MCP FA values in right handed children significantly associated with higher IQ ($p < 0.001$) and in left handed children associated with lower IQ scores ($p=0.010$). This finding suggested an effect modification of handedness as demonstrated in the multivariable regression analysis (table 10) were the variables: “handedness” and “handedness by right MCP FA” were added to other predictors and yielded three significant models.

No effect of handedness was demonstrated on working memory scores (backward digit recall) but only the association in the right handed group was significant. Controlling for weight z-score and cerebellar injury in a multivariable model for this domain did not change the association (results not shown).

4. Discussion

In view of recent findings on the importance of the cerebellum in cognitive development [12, 43, 44], we explored diffusion measures of the cerebellar peduncles and related them to neurodevelopment in prematurely born, 7 year old children. The main finding of this study was that both the MCP and SCP were associated with neurodevelopmental outcome. In the MCP we demonstrated a robust relationship between lower MD and higher IQ and a relationship between FA and IQ, with an effect of handedness on this relationship. In the SCP we demonstrated a robust relationship between higher MD and lower M-ABC2 and between higher FA and higher CELF-4 CLI. We also found higher MD, FA and track volume in the left peduncles when compared with those on the right.

4.1. Cerebellar DTI Measures and Perinatal Factors

Correlation of cerebral injury with cerebellar structural changes has been previously described. Tam et al., reported an association between intraventricular hemorrhage in prematurely-born infants and decreased cerebellar volume at term [10]. They also described an association between higher MD in the MCP and lower MD in the cerebellar parenchyma as well as an association of mild white matter injury and lower MD in the MCP [11]. Our finding, that perinatal white matter injury was associated with smaller cerebellar tract volumes (SCP and MCP) and lower FA in the right SCP, may suggest that white matter injury in the perinatal period, however mild, may have a persisting effect on the development of the cerebellum via changes in the main connecting pathways.

4.2. Cerebellar DTI Measures and Outcome

Prematurity increases the risk for cognitive and behavioral impairment, even in the absence of noticeable cerebral injury [24, 26, 45]. Concordant with these data, changes of diffusion measures in the corpus callosum, posterior limb of internal capsule, superior longitudinal fasciculus and corona radiata [46, 47], uncinate fasciculus [45] and white matter [48] have been related to cognitive and behavioral development of preterm infants through childhood and early adulthood.

This implication of the role of the cerebellum in cognitive functions has fostered studies that explored associations of neonatal cerebellar injury with developing cognitive functions. Limperopoulos et al., found a higher risk of motor disabilities, expressive and receptive language and cognitive deficits in a cohort of 35 preterm infants with isolated cerebellar injury [12]. Allin et al., [43] found smaller cerebellar volumes in a cohort of 67 adolescents born preterm compared to 50 matched controls born at term, with a significant positive association between cerebellar volume and cognitive function. Similarly van Kooij et al., (2012) reported an association between cognitive outcome and cerebellar volume and cerebellar NAA/Cho ratio at term equivalent in a cohort of 112 preterm infants, most of them without major cerebellar lesions.

Although no prior diffusion study has related cerebellar tract measures in prematurely born subjects with neurocognitive outcome, some research has been reported in other high-risk populations. For example, decreased FA values in the cerebellar parenchyma and ROIs in the

MCP have been related to ADHD in children with epilepsy [23], and lower FA values were found to be correlated to lower M-ABC2 scores in children with autism spectrum disorders [49]. As the cerebellum is intimately connected to associative areas in the cerebrum, our findings of decreasing MD and increasing FA of the right MCP predicting higher IQ scores are consistent with these studies. It should also be noted, that the right cerebellum is connected to the left cerebral hemisphere which is usually dominant for language functions and right hand control, which could be a plausible reason for the concentration of our significant findings in the right MCP and SCP.

4.3. Handedness

Differences in laterality between right and left handed individuals are mainly described within language related areas both in the cerebrum and in the cerebellum [50]. In our cohort a significant effect modification of handedness was found with higher FA predicting higher IQ in right handed children while lower FA predicting higher IQ in left handed children. Though higher FA is usually considered a sign of improved microstructural organization of tracts, other factors such as crossing fibers, fiber density and neuronal architecture may influence FA values [51]. These results may relate to the greater variability of laterality in left handed children similar to their variability in language areas [52]. The number of left handed children in this cohort is relatively small (n=28), thus, the above discussion serves only as a hypothesis for further investigation on handedness and the MCP. It should also be noted that a similar effect modification of handedness was detected for the relation of MCP FA with M-ABC2 and CELF4 CLI but in the subgroup analysis not all associations were significant. Also, no other differences were found between diffusion measures of right and left handed children for the MCP FA and SCP.

4.4. Hemispheric Asymmetry

Asymmetry of diffusion indices has been previously reported in the cerebral tracts of children. Johnson et al., [53] used a multiple ROI tractography technique in 76 normally developing toddlers (mean age of 35 months) and reported higher left FA and lower left MD in most of the examined cerebral tracts. Similarly, Trivedi et al., [54], in a cross sectional cohort DTT study that included 51 normal developing individuals aged one month to over 18, reported higher left FA values in most of the cerebral tracts explored. In view of these studies and others, it would have been expected that cerebellar tracts would show a reversed laterality. However, in two small studies that compared normal children to those with autism spectrum disorders, left laterality was demonstrated in the normal developing children's ICP (11 children aged 10 years using whole track assessments) [49], and in their SCP and MCP (16 children aged 6 years using single ROI measurements) [55]. These results are similar to those in our cohort, where higher FA was found in the left SCP and MCP. Some of these differences may be related to small group sizes and differences in technique (ROI versus tracking), and thus additional larger studies are needed to explore this issue.

4.5. Strengths and limitations

Strengths of this study relate to the relatively large cohort and its longitudinal design, enabling exploration of the relationship between MRI results from the neonatal period with neuroimaging and neurodevelopmental outcome measures at 7 years of age. However, the

study focused on a specific subset of subjects from a larger cohort, and further studies of children with unilateral injury and those with severe white matter injury are needed. Also, we used a strict and reproducible strategy for tract delineation, and thus were able to use and compare diffusion measures from the whole segment of the tract and not just a single ROI.

Limitations of this study relate to the difficulty of tracking in the cerebellum, although this is felt to be a more reliable approach for reporting diffusion measures than those derived from ROIs in this region. Another limitation relates to the difficulty scanning more severely neurologically impaired children in this cohort as is implied from both the baseline characteristics (table 1) and outcome of the children that were not included in the study (table 2). Were we able to scan severely impaired children, other important or stronger correlations of diffusion parameters with outcome might have been revealed. Lastly, we were unable to compare 7 years old tract parameters with data from the neonatal period, as unfortunately, diffusion scans that were done at term equivalent were not appropriate for tractography.

4.6. Conclusions

This study explored the characteristics of efferent and afferent pathways of the cerebellum in a cohort of prematurely born subjects at 7 years of age, demonstrating their relationship to neurodevelopmental outcome. We identified significant correlations between diffusion parameters and outcome measures at 7 years of age, as well as differences between left and right tracts and a relationship to white matter injury. These results suggest an enduring effect of prematurity related cognitive impairment on the brain at the microstructural level of the cerebellar peduncles. As the cerebellum continues to develop through childhood [56], additional longitudinal evaluations of this cohort may provide greater understanding of the role of the cerebellum in the developmental outcome of this high risk population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The figure is derived from a 7 year old child born prematurely at 29 weeks' gestation with a normal outcome.

Columns A, B, C: ROIs on Fractional anisotropy map (red: left/right, green: anterior/posterior, blue: rostral/caudal).

A: Inferior cerebellar peduncle (ICP). Upper image, coronal slice: Superior region of interests (ROIs) at the level of the pons and roof of the 4th ventricle. Middle image, axial slice: Inferior ROIs in the medulla at the inferior level of the temporal lobes. Lower image:, the relative position of the ROIs on a coronal slice.

B: Superior cerebellar peduncle (SCP). Upper image, axial slice: Superior ROIs drawn at the midbrain laterally to level of the decussation of the SCP at the midbrain (red dot between the two ROIs). Middle image, axial slice: Inferior ROIs drawn laterally to the posterior level of the 4th ventricle. Lower image: The relative position of the ROIs on a sagittal slice.

C: Middle cerebellar peduncle (MCP). Upper image, coronal slice: Posterior ROIs were drawn at the same plane as the posterior SCP's ROIs (1B middle image) laterally and inferiorly to them. Middle image, coronal slice: The anterior ROIs were drawn just prior to their decussation in the pons. Lower image: The relative position of the ROIs on an axial slice.

D: The 3 tracts placed on a 3 dimensional T2W image. Tracts color code: Blue: Right ICP, yellow: Left ICP, Green: Right SCP, Azure: Left SCP, Pink: Right MCP, Violet: Left MCP

Figure 2.

Regression line of the right middle cerebellar peduncle fractional anisotropy (right vs left handed prematurely born children) with the full scale IQ (see text for details). R^2 : right handed: 0.248, Left handed: 0.124

Table 1

Child Characteristics at the Neonatal Period

	MRI Performed	MRI not Performed	p
Clinical Characteristics			
Gestational age (weeks)	27.0 ± 1.7 (n=140)	26.9 ± 1.7 (n=80)	0.747
Birth Weight (grams)	966 ± 229 (n=140)	931 ± 288 (n=80)	0.275
Days on O2	47.5 ± 76.0 (n=140)	75.4 ± 133.4 (n=76)	0.051
Days on parenteral nutrition	12.2 ± 9.5 (n=140)	18.4 ± 24.4 (n=76)	0.008
Male	70 (50%) (n=140)	40 (50%) (n=80)	1.0
Narcotics	32 (23%) (n=140)	27 (35%) (n=78)	0.061
Antenatal Steroids	124 (89%) (n=140)	74 (93%) (n=80)	0.318
Postnatal steroids	8 (6%) (n=139)	13 (16%) (n=80)	0.011
Proven Sepsis	37 (30%) (n=128)	31 (48%) (n=64)	0.008
Necrotizing Enterocolitis	13 (9%) (n=140)	15 (19%) (n=80)	0.043
O2 at 36 weeks post conceptual age	46 (33%) (n=140)	33 (41%) (n=80)	0.174
Length of hospital stay	85 ± 28.0 (n=140)	105 ± 61 (n=80)	0.001
Imaging Characteristics			
Sonographic intracerebral Hemorrhage Grade 3 or Intraparenchymal	5 (4%) (n=135)	4 (5%) (n=76)	0.727
Sonographic periventricular leukomalacia	4 (3%) (n=136)	7 (10%) (n=73)	0.103
MRI white matter injury	98 (70%) (n=140)	49 (61%) (n=80)	0.185
Mild	76 (54%)	36 (45%)	
Moderate	20 (14%)	9 (11%)	
Severe	2 (1%)	4 (5%)	
Cerebellar Volume (cm ³) (MRI)	21.93 ± 4.81 (n=134)	20.63 ± 3.79 (n=75)	0.045
Cerebellar Injury	8 (6%) (n=138)	5 (7%) (n=71)	0.776

Values are presented as means $\pm$ standard deviations or numbers (percentage).
n differs due to missing data on some of the variables

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Table 2

Outcome at 7y of Age

Domain	MRI Performed	MRI not Performed	p
Full scale Intelligence Quotient	99.0 (n=140) ±13.4	92.0±14.7 (n=43)	0.002
Movement Assessment Battery for Children, 2nd Ed (Standard Score)	8.9±3.3 (n=133)	7.1±4.0 (n=31)	0.010
Clinical Evaluation of Language Fundamentals, 4th Ed (Core Language Index)	93.7 ± 16.8 (n=137)	88.5±28.6 (n=38)	0.112
Working Memory Test Battery for Children (Backward Digital Recall)	9.2±3.4 (n=134)	8.0±3.8 (n=51)	0.062
Right Handed	112 (80%) (n=140)	33 (75%) (n=44)	0.427
Cerebral palsy	2 (1%) (n=140)	10 (21%) (n=47)	<0.001
Social risk	1.97±1.8 (n=135)	2.74±1.9 (n=35)	0.008
Autism spectrum Disorders	4 (3%) (n=131)	2 (6%) (n=36)	0.618
Attention Deficit and Hyperactivity disorders	12 (9%) (n=131)	2 (5%) (n=38)	0.568

Values are presented as means ± standard deviations or numbers (percentage).

n differs since not all children were able to undergo the full battery of test and not all children from the original cohort were followed up to the age of 7y

Table 3
Correlations of Neonatal Clinical Characteristics and Neonatal Imaging with 7 years old Outcome

Neonatal Clinical Characteristics												
Outcome Measures	Full Scale IQ			Movement Assessment Battery for Children, 2 nd Ed			Clinical Evaluation of Language Fundamentals, 4 th Ed			Working Memory Test Battery for Children		
	N	B	p	N	B	p	N	B	p	N	B	p
GA at birth (weeks)	140	0.674	0.307	133	0.196	0.243	137	0.724	0.388	134	-0.108	0.532
Length of hospital Stay	140	-0.152	<0.001	133	-0.018	0.079	137	-0.166	0.001	134	0.0	0.973
Birth Weight Z-Score	140	3.8	0.009	133	0.98	0.008	137	8.1	<0.001	134	0.824	0.032
Gender	140	3.0	0.185	133	0.179	0.757	137	2.9	0.312	134	0.168	0.774
Antenatal steroids	140	1.7	0.252	133	0.171	0.651	137	1.1	0.580	134	0.065	0.867
Oxygen at 36 weeks	140	-5.0	0.036	133	0.04	0.948	137	-3.6	0.241	134	0.342	0.587
Sepsis	140	0.005	0.998	133	0.208	0.722	137	-0.191	0.947	134	0.464	0.432
Neonatal Imaging Characteristics												
MRI white matter injury	140	-6.327	0.041	133	-2.628	<0.001	137	-4.0	0.316	134	-0.738	0.351
Total Cerebellar Volume	134	0.880	0.044	127	0.142	0.204	131	0.365	0.515	128	0.032	0.777
Cerebellar Injury	140	-10.6	0.029	133	-2.8	0.039	137	-18.3	0.004	134	-2.164	0.097
7 years												
Social Risk	135	-2.08	0.001	130	-0.29	0.062	132	-3.29	<0.001	129	-0.27	0.106
Handedness	139	-2.283	0.428	132	-0.674	0.362	136	1.724	0.640	133	0.978	0.198

N varies as not all outcome variables were available for all children and not all cerebellar volumes were available.

B: regression coefficient, GA: Gestational age, p: p value

Table 4

Diffusion Parameters and comparisons Between Left and Right Tracts

Parameters		Mean Diffusivity (micM ² /s)			Fractional Anisotropy			Volume (voxels)		
		Lt	Rt	Paired t-tests	Lt	Rt	Paired t-tests	Lt	Rt	Paired t-tests
Tracts										
ICP	Preterm	0.990±0.151	0.945±0.136	0.012	0.541±0.041	0.536±0.046	0.320	43.0±17.61	32.2±14.93	<0.001
Number of tracks traced		Lt: 124, Rt: 113, Lt vs Rt: 106								
SCP	Preterm	1.607±0.222	1.064±0.098	<0.001	0.583±0.033	0.568±0.033	<0.001	97.5 ±5.76	89.5±26.07	<0.001
Number of tracks traced		Lt: 138, Rt: 136, Lt vs Rt: 135								
MCP	Preterm	0.710±0.028	0.696±0.037	<0.001	0.634±0.034	0.620±0.032	<0.001	366.5±69.95	343.5±71.57	<0.001
Number of tracks traced		Lt: 136, Rt: 136, Lt vs Rt: 136								

Values are presented as means ± standard deviations

Table 5
Correlations of Cerebellar Peduncles Tracts Indices at the age of 7y with Long Term Outcome Univariable analysis

Outcome Measures	Full Scale IQ*			Movement Assessment Battery for Children, 2 nd Ed*			Clinical Evaluation of Language Fundamentals, 4 th Ed*			Working Memory Test Battery for Children		
	N	B	p	N	B	p	N	B	p	N	B	p
Right Middle Cerebellar Peduncle												
Mean Diffusivity	136	-103.4	0.001	129	-8.9	0.294	133	-110.9	0.005	130	-7.2	0.409
Fractional Anisotropy*	136	81.4	0.022	129	-1.4	0.877	133	61.9	0.177	130	20.2	0.029
Volume	136	0.015	0.352	129	0.01	0.015	133	0.028	0.170	130	0.011	0.010
Right Superior Cerebellar Peduncle												
Mean Diffusivity	136	-14.1	0.223	130	-7.5	0.009	133	-25.8	0.081	131	-4.4	0.148
Fractional Anisotropy	136	81.1	0.018	130	22.6	0.009**	133	129.0	0.003**	131	3.8	0.684

Only 136 right middle cerebellar peduncles were tractables. N varies as not all outcome variables were available for all children.

B: regression coefficient, p: p value, Volume: Tracts volume in voxel

* Significant effect modification of handedness with Rt middle cerebellar peduncle fractional anisotropy detected in those outcome domains (see text and table 10)

** Regression Constant not significant

Table 6

Correlation of Right Middle Cerebellar Peduncle Mean Diffusivity with 7y Outcome Multivariable Models

	B	p	95% Confidence Interval	
			Lower Bound	Upper Bound
Full Scale IQ R ² =0.271				
Rt MCP MD	−92.1	0.001	−147.8	−36.4
Social Risk	−2.97	0.001	−3.10	−0.85
Length of hospital stay	−0.22	<0.001	−0.31	−0.13
Gestational Age at birth	−1.97	0.011	−3.48	−0.46
Clinical Evaluation of Language Fundamentals, 4 th Ed IQ R ² =0.349				
Rt MCP MD	−83.5	0.015	−150.5	−16.5
Social Risk	−3.1	<0.001	−4.50	−1.72
Weight Z-Score	6.28	<0.001	2.98	9.59
Length of Hospital Stay	−0.13	0.005	−0.21	−0.14
Cerebellar Injury	−11.8	0.081	−25.0	1.46

Rt MCP: Right Middle Cerebellar Peduncle, B: regression coefficient, p: p value

Table 7

Correlation of Right Middle Cerebellar Volume with 7y Outcome Multivariable Models

	B	p	95% Confidence Interval	
			Lower Bound	Upper Bound
M-ABC IQ ($R^2=0.168$)				
Rt MCP Vox	0.008	0.055	-0.0001	0.016
Cerebellar Injury	-2.40	0.088	-5.16	0.36
White Matter Injury	-2.46	0.001	-3.90	-1.02
Social Risk	-0.35	0.020	-0.65	-0.06
Working Memory Test Battery for Children IQ ($R^2=0.097$)				
Rt MCP Vox	0.011	0.013	0.002	0.020
Social Risk	-0.29	0.086	-0.61	0.04
Weight Z-Score	0.388	0.347	-0.43	0.20
Cerebellar Injury	-2.25	0.159	-5.38	0.89

Rt MCP: Right Middle Cerebellar Peduncle, B: regression coefficient, p: p value, Vox: Voxel

Table 8

Correlation of Right Superior Cerebellar Peduncle Diffusion Parameters with 7y Outcome Multivariable Model

	B	p	95% Confidence Interval	
			Lower Bound	Upper Bound
M-ABC IQ, R ² =0.192				
Right SCP MD	-5.99	0.030	-11.392	-0.605
Weight z-score	0.825	0.023	0.115	1.536
White Matter Injury	-2.529	0.001	-3.959	-1.098
Cerebellar Injury	-2.149	0.102	-4.735	0.436
Clinical Evaluation of Language Fundamentals, 4 th Ed. R ² =0.249				
Right SCP FA	85.793	0.033	6.851	164.736
Social Risk	-2.779	<0.001	2.826	9.638
Weight Z-Score	6.232	<0.001	-4.230	1.237

FA: Fractional Anisotropy, MD: Mean Diffusivity, SCP: Superior Cerebellar Peduncle

Table 9

Correlations of Right Middle Cerebellar Peduncle Tract Fractional Anisotropy Indices at the age of 7y with Long Term Outcome Split by Handedness[#]

Outcome Measures	Full Scale IQ			Movement Assessment Battery for Children, 2 nd Ed			Clinical Evaluation of Language Fundamentals, 4 th Ed			Working Memory Test Battery for Children		
	N	B	p	N	B	p	N	B	p	N	B	p
Fractional Anisotropy (Rt)	109	141.4	<0.001	104	6.1	0.516	107	113.4	0.014	106	25.4	0.008
Fractional Anisotropy (Lt)	26	-246.2	0.010	24	-53.8	0.075	25	-211.7	0.143	23	2.8	0.928

[#] Numbers don't add up to those in table 5 as one child is ambidextrous
N varies as not all outcome variables were available for all children and not all cerebellar volumes were available.
B: regression coefficient, Lt: Left handed children, p: p value, Rt: Right handed children

Table 10

Correlation of Right Middle Cerebellar Peduncle Fractional anisotropy with 7y Outcome Multivariable Models

	B	p	95% Confidence Interval	
			Lower Bound	Upper Bound
Full Scale IQ (R ² =0.420)				
Rt MCP FA	−677.2	<0.001	−976.4	−377.9
Weight Z-Score	2.4	0.060	1.072	6.840
Gender	7.4	<0.001	3.4	11.3
Total Neonatal Cerebellar Volume	0.809	0.025	0.105	1.513
Length of Hospital Stay	−0.145	<0.001	−0.213	−0.078
Social Risk	−2.0	<0.001	−3.1	−0.9
Handedness	−252.6	<0.001	−352.9	−152.3
Rt MCP FA × Handedness	407.4	<0.001	247.2	567.7
Movement Assessment Battery for Children, 2 nd Ed (R ² =0.216)				
Rt MCP FA	−115.4	0.011	−204.0	−26.7
Weight Z-Score	0.887	0.013	0.194	1.579
Social risk	−0.262	0.079	−0.555	0.031
White matter injury	−2.726	<0.001	−4.142	−1.311
handedness	−39.1	0.010	−68.445	−9.729
Rt MCP FA × Handedness	61.5	0.011	14.640	108.381
Clinical Evaluation of Language Fundamentals, 4th Ed, (R ² =0.402)				
Rt MCP FA	−583.2	0.003	−970.6	−195.9
Weight Z-Score	5.534	0.001	2.3	8.8
Gender	5.526	0.030	0.54	10.5
Cerebellar injury	−14.8	0.025	−27.7	−1.9
Length of hospital stay	−0.144	0.001	−0.230	−0.058
Social risk	−3.389	<0.001	−4.766	−2.013
handedness	−204.867	0.002	−334.398	−75.336
Rt MCP FA × Handedness	335.913	0.002	129.047	542.778

B: regression coefficient, FA: Fractional anisotropy, MCP: Middle cerebellar peduncle, p: p value