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Similarities and differences in cerebellar alterations between youth born preterm and youth born with congenital heart disease

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Individuals born preterm (PT) or with complex congenital heart disease (CHD) present with comparable prevalence of developmental challenges and patterns of neonatal brain injury. Converging evidence also supports that cerebellar development is altered in PT and in CHD survivors. However, no study compared cerebellar integrity between these two groups. This study aims to assess total and regional cerebellar development between youth born PT or with CHD as compared to controls. Participants aged 16–27 years born before 33 weeks of gestational age or who underwent open-heart surgery for CHD during infancy and a group of healthy term-born controls, underwent a brain MRI. Cerebellums were segmented at the lobular level. Youth born PT or with CHD exhibited a comparable pattern of volume reduction affecting total, regional and lobular cerebellar volumes. After adjusting for total brain volume, no significant differences remained between CHD and controls. Only regions and lobules in the anterior cerebellum remained significantly smaller than controls in the youth born PT. Atypical cerebellar development is present in youth born PT and in youth with CHD. However, our results suggested that premature exposure to the extra-uterine environment alters cerebellar development selectively while the cumulative effect of CHD globally hinders brain development.

Keywords Preterm birth, Congenital heart disease, Cerebellum, Magnetic resonance imaging

With advances in medicine, the mortality rate of infants born with critical health conditions, such as prematurity or complex congenital heart disease (CHD), has dropped sharply these past 40 years[1,2]. This decrease in mortality has been accompanied by an increased recognition of the frequent neurodevelopmental difficulties observed in these populations. Indeed, survivors of prematurity or CHD often face developmental challenges affecting the motor, cognition, language, and/or socio-emotional abilities[3,4]. Recent evidence suggests that in modern cohorts, these two distinct clinical populations present with comparable developmental profiles when assessed during childhood[5,6] or adolescence[7]. Neonates born preterm (PT) or with CHD also frequently present with white matter cerebral injury, altered microstructure and delayed growth when compared to term-born infants[8]. Due to their comparable pattern of brain alterations, it was proposed that these two populations present with a similar neonatal dysmaturation syndrome[9]. Recently, we reported similar widespread cerebral white matter alterations in youth born PT or with CHD suggesting that this dysmaturation syndrome persists into adulthood[10].

The past twenty years of research have highlighted that the cerebellum is an important key player in not only in motor functions, but also in high-level cognitive processes such as language and socio-emotional regulation[11–13]. Furthermore, it is understood that alterations of typical cerebellar development mediate many neurodevelopmental disorders by interfering with the development of underlying neural circuits[14]. However, our knowledge of the cerebellum and its role in developmental disorders remains limited due to the lack of studies in at-risk populations. Considering that the cerebellum undergoes its most intensive growth period during the

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third trimester of pregnancy[15], any adverse event occurring during this critical period of development, such as premature birth or the cumulative burden of CHD, should be considered as an important risk factor. Indeed, cerebellar injury and subsequent volume loss is a common complication of PT birth[16]. Even in the absence of a known injury, third-trimester global and regional cerebellar growth is noted to be altered in PT neonates when compared to healthy fetuses and term born neonates[17,18]. Smaller than controls cerebellum has also been observed in older children and adolescents born PT[19,20].

Although less extensively studied than in the PT population, cerebellar altered development has also been described in survivors with complex CHD. When compared to healthy controls, smaller total cerebellar volume has been observed in fetuses and in neonates with CHD during the peri-operative period[21–23]. Newborns with complex CHD are also at higher risk of preoperative cerebellar hemorrhage[24]. Smaller anterior and posterior cerebellar volumes as well as reduced overall cerebellar white matter volume has also been observed in adolescents and young adults with CHD when compared to controls of the same age[25]. However, a lack of statistical difference in cerebellar volume between survivors of CHD and controls has also been reported[21,22,26,27].

Despite compelling evidence supporting that both PT birth and complex CHD are important risk factors of altered cerebellar development, no study to date has directly compared cerebellar integrity between these two clinical groups. Such a comparison may inform on the underlying mechanisms of their cerebellar alterations as well as on their frequent higher cognitive and socio-emotional challenges. Using quantitative MRI, this study will fill this gap in knowledge by comparing lobular cerebellar volumes between youth born PT or with CHD without severe neonatal brain injury and healthy term-born peers.

Results

Participants characteristics

A total of 164 participants were enrolled (56 PT, 52 CHD, and 56 controls, CTL). Participants' characteristics are reported in Table 1. No significant group differences were found for the participants' age at MRI, sex, and body mass index (BMI) or intellectual quotient (IQ). Only socio-economic status (SES) was significantly lower in CHD and PT compared CTL. SES was therefore included in subsequent group comparison analyses as covariate. Only one PT and two CHD participants were identified as having an impaired IQ. Participants with CHD had between 1 and 4 open-heart surgeries (mean = 1.7, SD = 0.9). PT participants were born on average at 28.0 weeks of gestation. Among the 52 participants with CHD, 44 (85%) were born with a two-ventricle cardiac physiology: dextro-transposition of the great arteries ($n = 18$), tetralogy of Fallot ($n = 13$), ventricular septal defects ($n = 5$), double outlet right ventricle ($n = 3$), total anomalous pulmonary venous connection ($n = 2$), Ebstein's pulmonary atresia ($n = 1$), and truncus arteriosus type I ($n = 2$). Eight participants (15%) were born with a single-ventricle cardiac physiology: pulmonary atresia with intact ventricular septum ($n = 4$), double inlet left ventricle ($n = 2$), tricuspid atresia (n = 1), and hypoplastic left heart syndrome ($n = 1$).

MRI findings

No participants were excluded due to motion artifacts after the quality assessment of the T1w images. Mild brain abnormalities located in the cerebrum were found on conventional MRI in 14 PT (25%), 10 CHD (19%),

	PT (N = 56)	CHD (N = 52)	CTL (N = 56)	*p* value
Age at MRI (years)	20.5 (± 3.2)	19.9 (± 2.3)	20.5 (± 2.4)	0.44
Sex				0.78
Male	26 (46%)	21 (40%)	23 (41%)	
Female	30 (54%)	31 (60%)	33 (59%)	
Gestational age at birth (weeks)	28.0 (± 2.18)	39.3 (± 1.2; N = 19)	–	
BMI	22.8 (± 4.3)	23.4 (± 4.7)	23.6 (± 3.88)	0.29
Socioeconomic status	43.3 (± 11.3)	40.2 (± 12.3)	50.1 (± 10.10)	< 0.01
Type of CHD				
Single ventricle		8 (15%)		
Tetralogy of Fallot	–	13 (25%)	–	
Transposition of the great arteries		18 (35%)		
Other two ventricle CHD		13 (25%)		
Number of open-heart surgeries	–	1.7 (± 0.9)	–	
Number of participants presenting brain abnormality	14 (25%)	10 (19%)	5 (9%)	0.08
Abnormalities located in the cerebrum	13 (23%)	14 (27%)	7 (13%)	0.46
Abnormalities located in the cerebellum	2 (4%)	2 (4%)	0 (0%)	0.35
Cerebellar hypoplasia	2 (4%)	–	–	
Cerebellar dysplasia	–	1 (2%)	–	
Chiari I malformation	–	1 (2%)	–	

Table 1. Participants' characteristics. BMI, Body Mass Index; CHD, Congenital Heart Disease; PT, Preterm; CTL, Control.

and 5 CTL (9%) and included asymmetrical ventricles (PT = 2, CHD = 2, CTL = 1), enlargement of perivascular space (CHD = 4), evidence of white matter volume loss (PT = 9), evidence of grey matter volume loss (PT = 1, CHD = 1), noticeable sequelae of periventricular white matter injury (CHD = 2), susceptibility signal abnormality (diffuse in 4 CTL and focal in 2 with CHD) and grey matter heterotopia (PT = 1, CHD = 3, CTL = 1). Cerebellar abnormalities were reported in only 4 participants and included cerebellar hypoplasia (PT = 2), cerebellar dysplasia (CHD = 1) and Chiari 1 malformation (CHD = 1).

Cerebellar volumes

Group similarities and differences

When adjusting for SES, total cerebellar volume was significantly reduced in both youth born PT or with CHD ($p < 0.01$) when compared to CTL participants. At the regional level, the two clinical groups similarly showed smaller cerebellar volume than CTL participants in the bilateral white matter and anterior region and in the left superior posterior and inferior posterior regions (Figure S1, Table 2). At the lobular level, both groups presented with smaller volume in the bilateral lobules III, IV and X, in the left lobules VIIIA and Crus I, and in the right lobules V and Crus II (Figure S1, Table 3). Youth born PT did not present with other regional or lobular cerebellar differences when compared to CTL participants. In contrast, only the youth born with CHD showed smaller than CTL participants right superior posterior, right inferior posterior cerebellar regions, left lobules V, VIIB, and the right lobule VIIIB volumes. No significant total, regional and lobular cerebellar volume differences were found between youth born PT or with CHD.

Group comparisons after adjusting for total brain volume

Because TBV was significantly smaller than CTL participants in the two clinical groups and significantly associated with cerebellar volumes, all our linear models were re-run with TBV as an additional covariate. When adjusting for both SES and TBV, total cerebellar volume was no longer different between the clinical groups

	Mean volume (standard deviation)			**Effect of group & Post hoc analysis**	
	PT	**CHD**	**CTL**	**SES adjusted p value**	**SES + TBV adjusted p value**
Total brain volume	1,357,819.64 (135,227.66)	1,307,881.35 (148,089.92)	1,445,259.46 (121,952.84)	PT vs CHD 0.17 **CHD vs CTL < 0.01** **PT vs CTL < 0.01**	–
Total Cerebellum	125,079.80 (16,297.53)	123,198.64 (16,121.13)	134,872.91 (13,052.20)	PT vs CHD 0.68 **CHD vs CTL < 0.01** **PT vs CTL < 0.01**	Group 0.48 PT vs CHD 0.31 CHD vs CTL 0.97 PT vs CTL 0.31
Cerebellar Regions				**Post hoc analysis of the effect of group FDR correction (q value)**	
				SES adjusted	**SES + TBV adjusted**
Left anterior region	8311.53 (1248.02)	8381.23 (1354.39)	9215.22 (996.69)	PT vs CHD 0.81 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	PT vs CHD 0.22 CHD vs CTL 1.00 PT vs CTL 0.13
Right anterior region	8858.53 (1282.51)	9063.47 (1248.77)	10,002.56 (987.72)	PT vs CHD 0.81 **CHD vs CTL < 0.01** **PT vs CTL < 0.01**	PT vs CHD 0.09 CHD vs CTL 1.00 **PT vs CTL < 0.01**
Left superior posterior region	33,584.88 (4400.60)	32,809.11 (4492.75)	35,785.18 (3913.67)	PT vs CHD 0.81 **CHD vs CTL < 0.05** **PT vs CTL < 0.05**	PT vs CHD 0.71 CHD vs CTL 1.00 PT vs CTL 0.89
Right superior posterior region	27,430.79 (3577.16)	26,781.48 (3831.27)	29,095.57 (3170.44)	PT vs CHD 0.81 **CHD vs CTL < 0.05** PT vs CTL 0.06	PT vs CHD 0.71 CHD vs CTL 1.00 PT vs CTL 0.89
Left inferior posterior region	12,682.72 (1978.77)	12,550.80 (1829.94)	13,520.52 (1522.69)	PT vs CHD 0.81 **CHD vs CTL < 0.05** **PT vs CTL < 0.05**	PT vs CHD 0.37 CHD vs CTL 1.00 PT vs CTL 0.89
Right inferior posterior region	14,141.13 (2092.97)	13,762.94 (1900.85)	14,878.25 (1662.06)	PT vs CHD 0.81 **CHD vs CTL < 0.05** PT vs CTL 0.08	PT vs CHD 0.71 CHD vs CTL 1.00 PT vs CTL 0.89
Left white matter	8574.88 (1242.67)	8483.70 (1122.92)	9665.46 (984.35)	PT vs CHD 0.81 **CHD vs CTL < 0.0001** **PT vs CTL < 0.01**	PT vs CHD 0.37 CHD vs CTL 1 **PT vs CTL < 0.01**
Right white matter	11,495.40 (1584.03)	11,365.91 (1415.35)	12,710.14 (1230.47)	PT vs CHD 0.81 **CHD vs CTL < 0.001** **PT vs CTL < 0.01**	PT vs CHD 0.37 CHD vs CTL 1 **PT vs CTL < 0.05**

Table 2. Total and regional volumes comparisons adjusted for SES only and both SES and TBV. The first three columns report brut mean cerebellar volumes and standard deviation. The fourth column reports group differences after adjusting for SES only while the fifth column presents the differences adjusted for both SES and TBV. CHD, Congenital Heart Disease; CTL, Control; PT, Preterm; SES, Socioeconomic. Significant values are in bold.

	Mean volume (standard deviation)			Post hoc analysis of the effect of group FDR correction (q value)	
	PT	CHD	CTL	SES adjusted	SES + TBV adjusted
Cerebellar Lobules					
Left lobule III	851.40 (167.67)	884 (188.31)	990.54 (156.36)	PT vs CHD 0.83 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	PT vs CHD 0.11 CHD vs CTL 1.00 **PT vs CTL < 0.05**
Left lobule IV	1993.84 (364.82)	2097.59 (396.13)	2358.50 (360.55)	PT vs CHD 0.62 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	**PT vs CHD < 0.05** CHD vs CTL 1.00 **PT vs CTL < 0.01**
Left lobule V	5370.20 (811.08)	5305.35 (850.23)	5759.14 (645.29)	PT vs CHD 0.95 **CHD vs CTL < 0.05** PT vs CTL 0.07	PT vs CHD 0.68 CHD vs CTL 1.00 PT vs CTL 0.82
Left Crus I	9409.69 (1488.03)	9259.31 (1323.18)	10,244.75 (1418.98)	PT vs CHD 0.95 **CHD vs CTL < 0.05** **PT vs CTL < 0.05**	PT vs CHD 0.82 CHD vs CTL 1.00 PT vs CTL 0.61
Left lobule VIIB	4696.70 (715.46)	4468.67 (696.65)	4930.75 (652.34)	PT vs CHD 0.62 **CHD vs CTL < 0.05** PT vs CTL 0.25	PT vs CHD 0.81 CHD vs CTL 0.99 PT vs CTL 0.82
Left lobule VIIIA	4678.41 (723.30)	4617.30 (742.42)	5127.76 (657.71)	PT vs CHD 0.95 **CHD vs CTL < 0.05** **PT vs CTL < 0.05**	PT vs CHD 0.66 CHD vs CTL 1.00 PT vs CTL 0.65
Left lobule X	628.88 (112.14)	648.55 (115.25)	716.64 (103.87)	PT vs CHD 0.84 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	PT vs CHD 0.05 CHD vs CTL 1.00 PT vs CTL 0.06
Right lobule III	812.25 (153.40)	850.34 (164.25)	952.11 (155.16)	PT vs CHD 0.62 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	PT vs CHD 0.05 CHD vs CTL 1.00 **PT vs CTL < 0.05**
Right lobule IV	2437.80 (390.42)	2601.37 (429.30)	2950.26 (444.39)	PT vs CHD 0.61 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	**PT vs CHD < 0.01** CHD vs CTL 0.99 **PT vs CTL < 0.01**
Right lobule V	5537.47 (825.95)	5541.48 (761.66)	6025.50 (584.71)	PT vs CHD 0.95 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	PT vs CHD 0.66 CHD vs CTL 1.00 PT vs CTL 0.33
Right Crus II	5645.50 (915.26)	5609.20 (1045.36)	6172 (898.77)	PT vs CHD 0.96 **CHD vs CTL < 0.05** **PT vs CTL < 0.05**	PT vs CHD 0.66 CHD vs CTL 1.00 PT vs CTL 0.64
Right lobule VIIIB	3526.08 (610.54)	3434.07 (522.82)	3800.76 (544.98)	PT vs CHD 0.88 **CHD vs CTL < 0.05** PT vs CTL 0.050	PT vs CHD 0.86 CHD vs CTL 1.00 PT vs CTL 0.82
Right lobule X	597.35 (106.57)	623.41 (107.07)	690.81 (97)	PT vs CHD 0.62 **CHD vs CTL < 0.05** **PT vs CTL < 0.01**	**PT vs CHD < 0.05** CHD vs CTL 1.00 **PT vs CTL < 0.05**

Table 3. Lobular cerebellar volumetric comparisons adjusted for SES only and both SES and TBV. The first three columns reports the brut mean cerebellar volumes and standard deviation. The fourth column presents significant group differences after adjusting for SES only. The fifth column present significant group differences when adjusting for both SES and TBV. The threshold of significance was set at $q < 0.05$. CHD, Congenital Heart Disease; CTL, Control; PT, Preterm; SES, Socioeconomic Status; TBV, Total Brain Volume. Significant values are in bold.

and the CTL participants. Only some of the differences detected between youth born PT and CTL participants remained significant after this adjustment and included smaller bilateral cerebellar white matter, the right anterior cerebellar region, the bilateral lobules III and IV, and the right lobule X (Fig. 1, Tables 2, 3). None of the previously identified differences between youth born with CHD and CTL participants remained significant. New group differences emerged between the two clinical groups. Specifically, youth born PT presented with significantly smaller bilateral lobule IV, and right lobule X volumes than youth with CHD (Fig. 1, Table 3).

Magnitude of the volumetric differences

Overall, percentage of volumetric differences (VD) calculated after TBV adjustment were generally of small magnitude with the exception of a few lobules in youth born PT that demonstrated a volume reduction of at least 10% when compared to CTL participants (Table S1). These included the right lobule III (VD = 10.2%) and the bilateral lobules IV (Left VD = 11.2%, Right VD = 13.7%). VD between youth born PT and with CHD were less than 10% except in the right lobule IV (VD = 10.8%) (Table S1).

Associations between risk factors and cerebellar volume

To explore if the observed cerebellar differences were driven by the presence of cerebellar abnormalities, we reran our analyses excluding the five concerned participants. The results remained overall the same with the exception of the differences observed between youth born PT and youth born with CHD. Indeed, of the three

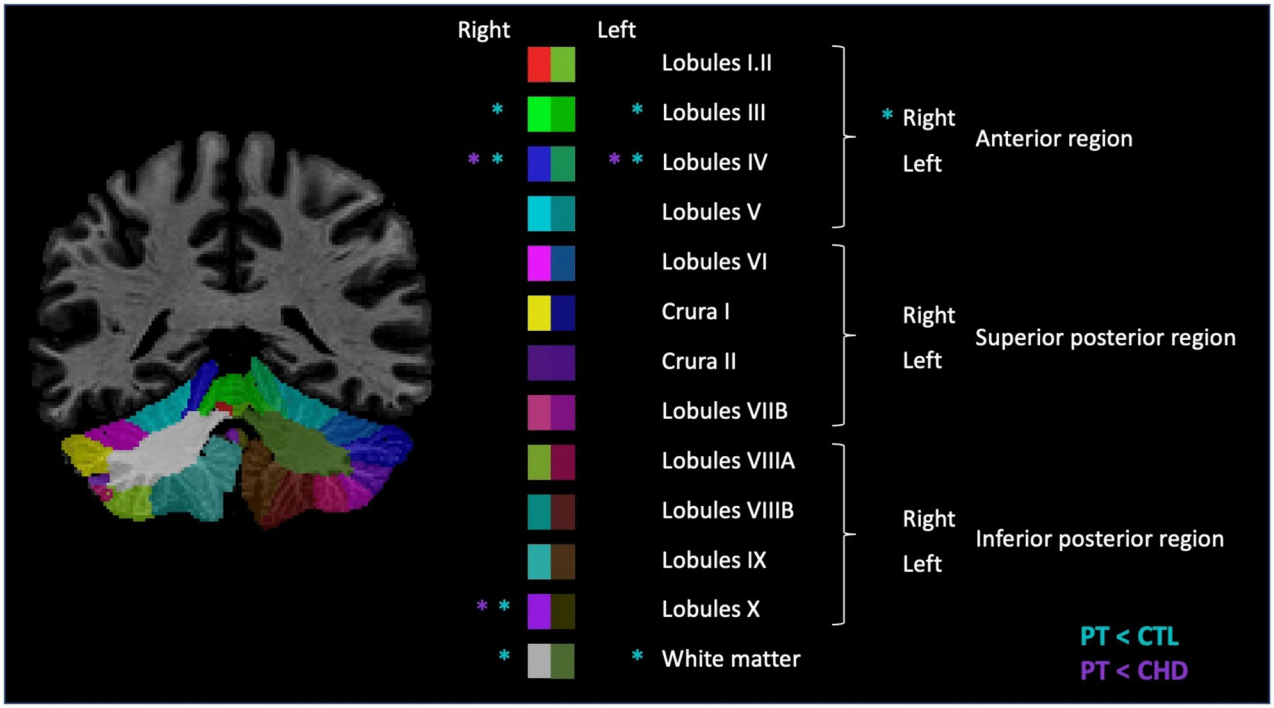

Fig. 1. Between group cerebellar volumetric differences. Linear models were adjusted for SES and TBV and FDR correction were applied. Turquoise asterisks (*) represent regions and lobules significantly smaller in PT than in CTL. Purple asterisks (*) represent lobules significantly smaller in PT than in CHD. The threshold of significance was set at $q < 0.05$. Legend: CHD: Congenital Heart Disease; CTL: Controls; PT: Preterm.

lobules previously found to be significantly smaller in the PT group compared to the CHD group, only the right lobule IV remained significantly smaller in this updated sample.

Lastly to investigate the presence of potential aggravating or protective factors for cerebellar development, associations between clinical variables and total and regional cerebellar volumes were assessed separately in the two clinical groups. In participants born PT, lower gestational age at birth and birth weight were both significantly associated with smaller total cerebellum, bilateral anterior, superior, posterior and inferior posterior region and right white matter. Reduced cerebellar left white matter was only associated with lower gestational age at birth (Table S2). In contrast, no significant associations between cerebellar volumes and severity of the cardiac physiology or any of the other clinical risk factors were detected in youth born with CHD.

Discussion

This study directly compared cerebellar volumes between youth born PT, youth born with CHD, and healthy term-born peers. Using a validated segmentation pipeline, we precisely segmented the cerebellum at the lobular level. Our findings first suggested that youth born PT or with CHD presented smaller cerebellum volumes as compared to CTL participants, with an overall similar pattern of total, regional, and lobular volume reduction. However, when controlling for TBV, these alterations became more diffuse and group-specific differences emerged. Indeed, when compared to CTL participants, significantly smaller cerebellar volumes were initially found in 6/8 regions and in 10/24 lobules in the youth born PT, and across all eight regions and in 13/24 lobules in the youth with CHD. However, after adjusting for TBV, only three regions and five lobules remained significantly smaller than CTL participants in youth born PT, and none of the differences remained significant for the CHD group. Our results stress the importance of adequately controlling for TBV when evaluating regional volume differences and suggests that distinct early life experiences differently affect cerebellar development.

The body of the literature in survivors of PT birth mainly reported the presence of smaller than term-born peers total and regional cerebellar volume when assessed in neonates, children, adolescents and adults[16–19,28–32]. Many studies that have adjusted for TBV also reported significant volumetric differences in total cerebellum and large regions[33–37]. However, others have reported an absence of statistical difference in total or hemispheric cerebellar volume[37–40]. In the current study, only a limited number of regions and lobules remained significantly smaller than controls after adjusting for TBV. The discrepancy between studies may lie in the heterogeneity of segmentation techniques used (i.e., toolbox, number of regions/lobules segmented) and in the difference in the clinical sample (i.e., spectrum of neonatal brain injury, gestational age at birth).

Our findings of limited cerebellar volume reductions in youth with CHD when compared to controls concurred with the few previous studies in adolescents and adults with CHD and who adequately controlled for TBV[26,27]. Other studies have reported smaller cerebellar volumes in fetuses, neonates and young children with CHD when compared to healthy controls, however, many of these studies did not correct for TBV[21–23,41–45]. It

is possible that altered cerebellar development may be a transient phenomenon in the CHD population that is of particular importance during antenatal and early postnatal life. However, this would need to be confirmed in future longitudinal studies using serial MRI.

The similar pattern of acquired brain injury and brain dysmaturation in neonates born PT or with CHD has been well recognized[8]. Our initial observation supported a possible similar increased vulnerability of the cerebellum. However, after controlling for TBV, we observed a different pattern of altered cerebellar development between the two clinical groups. Indeed, the results of the present study suggested that in survivors with CHD, cerebellar alterations take place into the context of a more diffuse phenomenon of altered brain development affecting both the cerebrum and the cerebellum. In comparison, in individuals born PT, cerebral growth was likewise affected when compared to term-born peers, but the cerebellum presents with areas of heightened regional vulnerability. Although cerebellar volumetric differences were overall of small magnitude, the PT participants presented clinically meaningful (i.e., more than 10% volume difference as compared to CTL group) volume loss in the left lobule III and the bilateral lobule IV. The lobules III and IV are anterior regions known for their involvement in motor control[46]. This is interesting considering the high prevalence of developmental coordination disorders in the preterm populations[7,47] and this may be a promising hypothesis to explore in future structure–function studies.

With respect to risk factors, we found only smaller gestational age at birth and lower birth weight to be significantly associated with smaller cerebellar volumes in participants born PT. This is not surprising considering the well-known associations between these two factors and altered cerebral development[19]. Although these factors primarily reflects the infant's level of immaturity at birth, smaller babies are often the sickest and are more likely to experience multiple complications such as chronic lung disease, necrotizing enterocolitis, sepsis, painful procedure, and longer drug exposures which are also known to be associated with cerebellar underdevelopment[8,17]. Despite our efforts, only a limited number of clinical variables could be extracted from retrospective chart review in our cohort as these were born and treated more than 20 years ago, before the digitization of the medical chart, limiting our ability to examine the complex interaction between these factors and brain development.

Limitations

The main limitation of this study pertains to our sample size. Although our overall sample was substantial, the reduced number of participants in each of the groups and the high number of cerebellar regions explored may have reduced the statistical power of our analyses. In addition to hindering the detection of subtle differences, this also prevented the conduction of sub-group analyses to explore, for example, the effect of the different types of CHD diagnoses. Also, while the MAGeT Brain Algorithm provides a precise and reliable segmentation of the cerebellar lobules, it does not delineate the vermal region, which prevented us from studying the vulnerability of this region in individuals born PT or with CHD.

Conclusion

This study is the first to directly compare cerebellar development between youth born PT or born with CHD as compared to healthy controls. We found that premature exposure to the extra-uterine environment alters cerebellar development selectively while the cumulative effect of CHD may hinder brain development more globally. Our results also stress the importance of controlling for total brain volumes when comparing brain regions between different populations, a methodology that is surprisingly still often overlooked.

Material and methods

Participants

Our sample consisted of three groups of youth participants aged 16–27: youth born with CHD, youth born PT and healthy term-born controls. Individuals with CHD were born at term (> 36 weeks gestational age) and underwent open heart surgery utilizing cardiopulmonary bypass for CHD during their first two years of life. PT individuals were born < 33 weeks of gestation, without CHD or a severe developmental impairment (e.g., cerebral palsy, sensory impairment). The recruitment and enrollment procedures of our sample have been previously described[48]. Exclusion criteria for both clinical groups included history of congenital infection, genetic or chromosomal abnormalities, multi-organ dysmorphic conditions, history of moderate to severe neonatal brain injury or malformation, severe developmental impairment (e.g., cerebral palsy, sensory impairment), contraindications for MRI (e.g., pacemaker), and inability to communicate in English or French. Healthy term-born CTL individuals with no history of neurological or developmental conditions who did not receive rehabilitation or special education services during childhood or adolescence were recruited from the community and local educational institutions through advertisements and word of mouth. Written informed consent was obtained from each participant or their legal guardians if younger than 18 years at the time of the study. The study received McGill University Health Centre Pediatric Research Ethics Committee approval. All aspects of the study were performed in accordance with the relevant institutional guidelines and regulations.

Image acquisition

Participants underwent a brain MRI at the Montreal Children's Hospital on a 3 T MRI System (Achieva X, Philips Healthcare, Best, The Netherlands) using a 32-channel head coil. A high-resolution T1 weighted (T1w) anatomical image was acquired for each participant with a three-dimensional 1 mm isotropic voxel, TE = 3.7 ms, TR = 8.1 ms, TI = 1010 ms, shot interval = 3000 ms, bandwidth = 191.4 Hz/pixel, FOV = 240 × 240 × 180 mm, slice thickness 1 mm, flip angle = 8°, acquisition time = 6 min 20 s). Images were reviewed by an experienced neuroradiologist blinded to participants' medical history.

Image processing

T1w images were preprocessed using iterativeN3, a multi-stage MRI preprocessing pipeline (https://github.com/CoBrALab/iterativeN3). Anatomical images underwent the following stages: the "nu_correct" algorithm was applied to correct for bias field contrast inhomogeneity, and a brain extraction was performed using "BEaST" to remove all non-brain tissue[49]. Cerebellar segmentation on the individual T1-w images was performed using the well-validated and standardized Multiple Automatically Generated Templates brain segmentation algorithm (MAGeT-brain, https://github.com/CobraLab/MAGeTbrain,[50,51]. This pipeline uses a multi-atlas voting procedure using a template built on a subset of the subject's images and allows a fine segmentation of the cerebellum into 26 bilateral parts: the bilateral central white matter and the following bilateral lobules: lobule I /II, III, IV, V, VI, Crus I, Crus II, VIIB, VIIIA, VIIIB, IX and X. Lobules were thereafter combined into three bilateral anatomical regions: anterior, superior and inferior posterior cerebellum (Fig. 1). Total brain volume (TBV) estimates were assessed on all T1-w images using the BEaST mask (Eskildsen et al., 2012).

Quality assessment

A visual inspection was done on all individual raw T1w images before pre-processing to include only participants with no or very few motion or scanner artifacts. Quality assessment was also performed at each step of the segmentation pipeline to ensure the quality and accuracy of the preprocessing. Lastly, manual corrections were performed where needed using the Display software implemented in MINC Toolkit (https://bic-mni.github.io, V2 version 1.9.18).

Individual and clinical variables

On the day of the study visit, height and weight of each participant were measured to calculate the BMI. An abbreviated IQ was obtained in both clinical groups using either the Leiter International Performance Scales-Revised[52] as part of a previous study at our center[7], or the Wechsler Abbreviated Scaled of Intelligence[53] on the day of the MRI. IQ scores were dichotomized as normal or impaired using the cut-off of 70 on both scales. SES was assessed using the Hollingshead Four Factor Index questionnaire[54]. Furthermore, the medical records of each participant in the two clinical groups were reviewed to extract the most important perinatal clinical risk factors.

Statistical analysis

Descriptive analyses were first performed to characterize the sample. After evaluating the normality of the data distribution with Shapiro–Wilk test, group differences in participants' characteristics for continuous variables were accessed using ANOVA or Kruskal–Wallis test as appropriate and Chi-square test for categorical variables. Total, regional and lobular cerebellar volumes between the three groups were compared using general linear models with SES as covariates. Cook's distance was calculated to evaluate the influence of each total cerebellar volume on the linear model exploring group differences at the total cerebellar level, and none were deemed as outliers (i.e., all inferior to three times the mean Cook's distance for the model). To investigate whether the volume differences observed were specific to the cerebellum, the models were subsequently re-run with TBV as an additional covariate. Linear models were fit twice, first with the CTL group as a reference to access group differences between PT and CTL, and between CHD and CTL. Models were re-fit with the CHD group as a reference to access group differences between CHD and PT. Multiple comparison corrections were performed using the False Discovery Rate (FDR) correction. The significance threshold was set at $q < 0.05$. Analyses were carried out twice with and without adjustment for TBV. Percentage of volumetric differences between the three groups were calculated on the ratio cerebellar/TBV volume, for regions showing a significant group effect in the linear model adjusting for both SES and TBV, using the following formula: (mean volume Group1—mean volume Group2)/(mean volume Group2) × 100. Lastly, to explore the potential effect of clinical risk factors on cerebellar volumes, Spearman correlations were conducted in both clinical groups separately. For PT participants, the clinical risk factors pertained to gestational age at birth, birth weight, and length of hospital stay following birth. For CHD participants the clinical risk factors assessed included cardiac physiology (i.e., single- vs. two-ventricle), age at first surgery, number of open-heart surgeries, bypass and aortic cross-clamp times, number of catheterizations and number of balloon atrial septostomy procedures before first surgery. Statistical analyses were performed using R software (version 4.1.1 released in 2021; https://www.R-project.org/).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Sarah Palmis: Conceptualization; formal analysis; visualization; writing original draft; review and editing. Kaitlyn Easson: Data collection and curation; review and editing of the final draft. Gabriel Devenyi: software; review and editing of the final draft. Guillaume Gilbert: Resources; review and editing of the final draft. Christine Saint-Martin: Resources; review and editing of the final draft. Mallar M. Chakravarty: Resources; review and editing of the final draft. Marie Brossard-Racine: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing original draft; review and editing.

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Declarations

Competing interests

G. Gilbert is an employee of Philips Healthcare Canada. The authors have no other interests to disclose.

Additional information

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