Cerebellar Microstructural Organization is Altered by Complications of Premature Birth: A Case-Control Study

Marie Brossard-Racine, PhD[1,2], Andrea Poretti, MD[3], Jonathan Murnick, MD, PhD[4], Marine Bouyssi-Kobar, MSc[4,5], Robert McCarter, ScD[6], Adre J. du Plessis, MBChB[7], and Catherine Limperopoulos, PhD[4,5]

Objectives To compare regional cerebellar microstructure, as measured by diffusion tensor imaging (DTI), between preterm infants at term-equivalent age and healthy term-born control neonates, and to explore associations between DTI findings and clinical risk factors.

Study design In this case-control study, DTI studies were performed in 73 premature infants born ≤32 weeks and ≤1500 g birth weight and 73 full-term-born controls from healthy pregnancies. Using a region of interest approach, fractional anisotropy (FA) and mean diffusivity (MD) were extracted in 7 cerebellar regions including the anterior vermis, the right/left superior cerebellar peduncles, the middle cerebellar peduncle, and the dentate nuclei. To validate further our DTI measurements, we measured FA and MD in the genu of the corpus callosum and splenium. FA and MD were compared between groups using analyses of multiple linear regression models.

Results Preterm infants at term-equivalent age presented with higher FA in the dentate nuclei (<.001) and middle cerebellar peduncle (.028), and lower MD in the vermis (.023) compared with controls. Conversely, preterm infants showed reduced FA and increased MD in both the genu of the corpus callosum and splenium ($P < .001$). Independent risk factors associated with altered FA and MD in the cerebellum included low Apgar score, supratentorial injury, compromised cardiorespiratory function, and surgery for necrotizing enterocolitis and patent ductus arteriosus.

Conclusions This DTI study provides evidence that complications of premature birth are associated with altered cerebellar microstructural organization when compared with term-born control infants. *(J Pediatr 2017;182:28-33).*

See editorial, p 11

Prematurity-related brain injury is associated with a high prevalence of neurodevelopmental disabilities.[1-4] However, neurodevelopmental disorders are also commonly reported in children despite normal conventional neonatal magnetic resonance imaging (MRI) studies.[5,6] Diffusion tensor imaging (DTI) is highly sensitive in detecting subtle cerebral white matter injury, often missed by conventional anatomic MRI and associated with less favorable outcomes.[7] DTI provides in vivo quantification of brain tissue microstructure including axonal density and white matter integrity.[8] Fractional anisotropy (FA) measures the predominant directionality of water molecule diffusion, and mean diffusivity (MD) is a measure of average water molecule diffusion.[8]

Cerebral white matter microstructural development has been studied extensively in preterm infants.[9,10] Available evidence indicates that by term-equivalent age (TEA), preterm infants demonstrate decreased FA and higher MD in a number of cerebral regions (eg, corpus callosum and posterior limb of the internal capsule), even in the absence of cerebral parenchymal injury.[11-17] These observations presumably represent cerebral white matter dysmaturation currently thought to play a central role in the high prevalence of neurodevelopmental disabilities in preterm infants.[18]

Cerebellar microstructural development following premature birth remains largely unexplored. The third trimester of pregnancy is a dynamic period for cerebellar development and coincides with the timing of most premature births.[19] Cerebellar

DTI	Diffusion tensor imaging
FA	Fractional anisotropy
MCP	Middle cerebellar peduncle
MD	Mean diffusivity
MRI	Magnetic resonance imaging
NEC	Necrotizing enterocolitis
PDA	Patent ductus arteriosus
ROIs	Regions of interest
SCP	Superior cerebellar peduncle
TEA	Term-equivalent age
3D	3-dimensional

From the [1]Montreal Children's Hospital, Division of Pediatric Neurology, McGill University Health Centre, Montreal, Quebec, Canada; [2]School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada; [3]Section of Pediatric Neuroradiology, Division of Pediatric Radiology, Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD; [4]Division of Diagnostic Imaging and Radiology; [5]Developing Brain Research Laboratory; [6]Center for Translational Science; and [7]Fetal and Transitional Medicine, Children's National Health System, Washington, DC

M.B.-R. received postdoctoral fellowship support from the Canadian Institute of Health Research at the time of the data collection and analyses. This research was also funded by an Intellectual and Developmental Disabilities Research Center grant (1U54HD090257). The authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2016.10.034

hemorrhage as detected by conventional MRI has an estimated prevalence of up to 19% in premature infants born before 32 weeks of gestation,[20] with higher rates among extremely low birth weight (<750 g) infants.[21,22] Moreover, even in the absence of destructive cerebellar injury, cerebellar volumetric growth impairment measured by 3-dimensional (3D) volumetric MRI is prevalent in preterm infants[23,24] and is associated with an elevated risk of adverse developmental outcomes.[25] Potential mechanisms for impaired cerebellar development include the presence of severe supratentorial injury (ie, through the cerebro-cerebellar diaschisis),[26-28] and genetic or chromosomal anomalies.[29]

The objective of our study was to compare regional cerebellar microstructural development between preterm-born infants evaluated at TEA with a sample of healthy full-term-born newborns using DTI and, to examine the association between regional cerebellar DTI scalars and neonatal risk factors.

Methods

As part of a prospective study initiated in June 2012, we studied premature infants born ≤32 weeks and of ≤1500 g birth weight, admitted to the neonatal intensive care unit of the Children's National Health System. Infants with congenital malformations or dysmorphic features suggestive of a genetic syndrome, confirmed metabolic disorder, central nervous system infection, or chromosomal abnormality were excluded. We recruited controls from a parallel study evaluating serial brain development in utero and postnatally in healthy full-term-born infants (≥37 weeks) from healthy pregnant volunteers.[30] Controls with multiple pregnancies, fetal ultrasound/neonatal brain MRI abnormalities, congenital infection, documented chromosomal abnormalities, and/or multiorgan dysmorphic conditions were excluded. Ante-, peri-, and postnatal information for all enrolled subjects was collected through review of medical records. Written parental consent was obtained from every participant, along with Children's National Health System institutional review board approval.

MRI Acquisitions

Unless clinically indicated for preterm infants, no sedation or intravenous injection of gadolinium-based contrast agents were used during the neonatal MRIs. The nonsedated neonatal MRIs were performed during natural sleep after feeding and swaddling the infants. All infants were immobilized using an Infant Vacuum Immobilizer (Newmatic Medical, Caledonia, Michigan) and provided with double ear protection (ear plugs and neonatal noise guards). A nurse monitored heart rate and oxygen saturation levels during the entire scan. All MRIs were performed on a 3 Tesla MRI scanner (Discovery MR750; General Electric Medical, Systems, Waukesha, Wisconsin) with an 8-channel receiver head coil. Preterm infants were scanned when medically stable at TEA. Healthy controls completed their brain MRIs as outpatients, shortly after birth.

The MRI acquisition protocol included structural imaging (T2 3D-cube and T1 3D-spoiled gradient recalled), susceptibility-weighted imaging, and a DTI acquisition consisting of a single shot echo-planar sequence with 27 noncollinear direction diffusion gradients with an effective high b-value of 1000 s/mm^2 (3 b = 0 s/mm^2) 80 ms echo time, 8000 ms repetition time, field of view 200 × 200 mm, 3 mm slice thickness, and no gap with a 128 × 128 acquisition matrix. Each brain MRI study was reviewed by an experienced pediatric neuroradiologist (J.M.). An overall score (from 0 to 40) for MRI abnormalities was given using the scoring system of Kidokoro et al[31] for preterm infants at TEA. Abnormalities were subsequently classified as normal, mild, moderate, or severe according to the scoring system's guidelines. Germinal matrix hemorrhage and intraventricular hemorrhage were graded according to the Papile grading system.[32] We excluded preterm infants with cerebellar parenchymal injury detected on conventional MRI.

DTI Processing

Each DTI acquisition was preprocessed using our previously validated DTI pipeline.[33] DTI acquisitions with more than one-half of the volumes corrupted were discarded from subsequent analyses. Using the B0 image and its registered FA map (**Figure 1**; available at www.jpeds.com), 9 cubic regions of interest (ROIs, 16.5-49.4 mm^3) were manually placed by a single observer on 2-3 consecutive slices to maximize the coverage of the evaluated structure, using predefined anatomical landmarks. ROIs were placed in the anterior vermis, in the left/right: superior cerebellar peduncle (SCP), middle cerebellar peduncle (MCP), dentate nuclei, and 2 symmetrically placed ROIs in the pons at the level of the corticospinal tract (**Figure 2**; available at www.jpeds.com). To further validate our DTI measurements in the cerebellum, we added ROIs in 2 well-documented cerebral regions: genu and splenium of the corpus callosum. The 3 eigen values were then extracted for each ROI.

Ten subjects were analyzed twice following a 24-hour interval by the principal evaluator to calculate intrarater reliability and intraclass correlation coefficient ranged between 0.94 and 0.99 for all regions. The principal evaluator and a pediatric neurologist with experience in DTI postprocessing of cerebellar structures (A.P.) independently processed 18 subjects (9 preterm infants/9 controls) to establish interrater reliability. Intraclass correlation coefficient for inter-rater reliability ranged between 0.84 and 0.98.

Statistical Analysis

Descriptive statistics were used to characterize the sample and the outcomes of interest. Assumptions of normality were evaluated and normalizing transformations were applied when necessary to permit parametric analyses. Laterality in bilateral ROIs was assessed using paired *t* tests between the left and right sides. When there was a statistical difference, each side was evaluated separately.

Subsequently, multiple linear regression models were developed to evaluate the relationship between regional microstructural integrity and prematurity. Controlling for postconceptual age at MRI, each model compared the mean FA and MD measurements in ROI between preterm infants vs controls.

Finally, to identify risk factors related to variations in microstructural integrity in the preterm group, we began by