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Cerebellar Development in Preterm Infants at Term-Equivalent Age Is Impaired after Low-Grade Intraventricular Hemorrhage

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Objectives To investigate cerebellar development in preterm infants at term-equivalent age compared with healthy full-term infants and to examine the effect of a low-grade intraventricular hemorrhage (IVH) on cerebellar development.

Study design This study used 3T magnetic resonance and diffusion tensor imaging (DTI) at 36-41 weeks' postmenstrual age (PMA) in 72 preterm infants without severe brain injury and 16 full-term infants. Cerebellar volumes and DTI parameters of the cerebellar peduncles including fractional anisotropy (FA), apparent diffusion coefficient (ADC), axial diffusivity, and radial diffusivities were measured. Clinical variables that may affect brain development were collected.

Results Compared with full-term infants, preterm infants showed smaller cerebellar volumes and a lower FA, greater ADC, and increased radial diffusivities in the cerebellar peduncles (all $P < .05$). This cerebellar impairment was associated significantly with PMA and IVH grade 2 but was independent of gestational age at birth. When we adjusted for clinical variables, an IVH grade 2 was related with 1.73 cm^3 reduction in cerebellar volumes and altered DTI parameters in the cerebellar peduncles, including decreased FA and increased radial diffusivities in the superior cerebellar peduncle and increases in ADC, axial diffusivity, and radial diffusivities of the middle cerebellar peduncle (all $P < .05$). Cerebellar hemispheric volumes were associated with both ipsilateral and contralateral IVH grade 2.

Conclusion Preterm infants without severe brain abnormalities showed impaired cerebellar development at term-equivalent age after we controlled for PMA at the time of the scan, and this is associated with IVH grade 2. These findings suggest that even a low-grade IVH has potential harmful effects on cerebellar development. *(J Pediatr 2016;175:86-92)*.

The cerebellum has been suggested as a pivotal region that is linked with motor, cognitive, and behavioral impairment in preterm infants.[1,2] The third trimester of a pregnancy is characterized by dynamic cerebellar development. Preterm infants are susceptible to cerebellar injury because they are exposed to an extrauterine environment during this vulnerable period.[3] Therefore, preterm birth often is associated with an underdeveloped cerebellum, specifically cerebellar volume reduction.[3,4] It remains unclear, however, whether this impaired cerebellar development is affected by prematurity itself or by postnatal complications encountered during a hospital stay. Because the cerebellum is influenced by extensive connections with the cerebrum, impaired cerebellar development frequently is observed in preterm infants with a severe intraventricular hemorrhage (IVH).[4-6]

To our knowledge, only one study has mentioned the effect of a low-grade IVH on cerebellar microstructure.[7] Recently, several studies have demonstrated that even a low-grade IVH has a significant effect on neurodevelopmental outcomes.[8,9] Therefore, it is meaningful to demonstrate the effects of a low-grade IVH on cerebellar development. Advanced magnetic resonance imaging (MRI), including diffusion tensor imaging (DTI) or 3-dimensional (3D) volumetric MRI, allows for the assessment of gross or microstructural brain development in preterm infants.[10,11] The apparent diffusion coefficient (ADC) and fractional anisotropy (FA) are common DTI parameters that reflect myelin maturation or axonal integrity.[12] Recently, it has been suggested that the axial diffusivity, which is parallel to an axonal fiber, λ_1, and the radial diffusivity, which is the averaged diffusivity perpendicular to axonal fiber, $(\lambda_2 + \lambda_3)/2$, can elucidate specific pathology leading to changes in FA or ADC.[13-15]

ADC	Apparent diffusion coefficient
BPD	Bronchopulmonary dysplasia
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
IVH	Intraventricular hemorrhage
MCP	Middle cerebellar peduncle
MP RAGE	Magnetization-prepared rapid gradient-echo
MRI	Magnetic resonance imaging
PMA	Postmenstrual age
SCP	Superior cerebellar peduncle
3D	3-dimensional

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The aim of our study was to investigate cerebellar development in preterm infants compared with healthy full-term infants, with adjustment for clinical risk factors that may affect cerebellar development. We also aimed to examine the effect of a low-grade IVH on cerebellar development. We hypothesized that preterm infants would demonstrate impaired cerebellar development and that a low-grade IVH would negatively affect cerebellar development. To assess cerebellar development, this study used 3D volumetric MRI and DTI parameters at term-equivalent age in preterm and full-term infants without severe brain abnormalities.

Methods

The Institutional Review Board from the Gachon University Gil Hospital approved this study (GIRBA 2404), and the parents of all infants gave written informed consent. Infants were recruited between October 2010 and December 2013. MRI and DTI scans were obtained from enrolled preterm infants at 36-41 weeks' postmenstrual age (PMA). As a control group, healthy full-term infants (≥37 weeks' gestational age at birth) also underwent MRI and DTI scans within 1 month of birth (PMA 37-41 weeks). For preterm infants, a cranial ultrasound scan was performed within 7 days of birth, 2 weeks later, and then 4 weeks later. All MRI and cranial ultrasound scans were evaluated for brain injury, including IVH, by a single pediatric radiologist who was blinded to the infants' medical history and the present study.

IVH was determined with the grading system of Papile et al.[16] Infants with any congenital malformations and/or apparent brain abnormalities identified by early ultrasound or subsequent MRI scans, including periventricular leukomalacia, severe IVH (grade 3 and 4), white matter volume loss, corpus callosum thinning, and cerebellar lesions, were excluded. Clinical variables, including gestational age, birth weight, sex, PMA at imaging, low-grade IVH (defined as having an IVH grade of 1 or 2), bronchopulmonary dysplasia (BPD, defined as the need for supplemental oxygen at 36 weeks PMA), culture-proven sepsis, and postnatal glucocorticoid exposure, were recorded.

Imaging Data Acquisition

T1-magnetization-prepared rapid gradient-echo (MP RAGE), T1-and T2-weighted imaging, and DTI scans were obtained with a 3.0-T MRI scanner (Magentom Verio [Siemens, Munich, Germany], with a Siemens matrix coil) under the supervision of an attending pediatrician. The infants were scanned during natural sleep after feeding. If feeding failed to induce natural sleep, a low dose of chloral hydrate (30 mg/kg) was given orally. The 3D T1-MP RAGE imaging parameters used were as follows: repetition time = 1900 ms, echo time = 2.97 ms, flip angle = 9°, pixel bandwidth = 170 Hz/pixel, total acquisition time = 3 minutes, 2 seconds, iso-voxel resolution 1.0 mm. The DTI sequence parameters were as follows: b = 0 and 700 s/mm^2, number of diffusion gradient directions = 30, repetition time = 6600 ms, echo time = 74 ms, slice thickness = 1.8 mm, field of view = 230 mm, matrix = 128 × 128, total acquisition time = 7 minutes, 36 seconds.

Quantitative DTI Analysis

The DTI scanning protocol was the same as described previously.[17] DTI images were processed offline with the FMRIB Software Library (FSL, Oxford, United Kingdom).[18] To investigate the relationship between clinical variables and cerebellar volume or DTI parameters, such as FA, ADC, and axial and radial diffusivities, we selected regions of interest in the superior cerebellar peduncle (SCP, at the level of the decussation) and middle cerebellar peduncle (MCP; at the level of the pons) from the FA color map (**Figure 1**; available at www.jpeds.com).

Volume Measurement

Cerebellar volume was measured by manually outlining the cerebellar tissue on each cerebellar section with the 3D Slicer, version 4.3.1 (http://www.slicer.org/; Surgical Planning Laboratory, Harvard Medical School, Boston, Massachusetts) (**Figure 1**). The cerebellar boundaries were illustrated, from medial to lateral, on each subject's T1-MP RAGE image. Cerebellar volume was calculated by counting the number of voxels in the segment and multiplying by the volume of each voxel.[19] Intracranial volume was measured with Statistical Parametric Mapping 8.0 (http://www.fil.ion.ucl.ac.uk/spm; Wellcome Department of Imaging Neuroscience, London, UK).

Statistical Analyses

For continuous variables, the means and SDs are reported. Individual *t* tests and Fisher exact tests were used for comparisons between the 2 groups. Two researchers measured volumes and DTI parameters. For interobserver and intraobserver reliability, intraclass correlation coefficients were calculated. To investigate the association between a low-grade IVH and the cerebellar imaging measurements, multiple regression analyses were performed, with adjustment for clinical variables, including gestational age, PMA at imaging, sex, BPD, culture-proven sepsis, and postnatal glucocorticoid exposure. In those models, IVH grade 1 and 2 were estimated separately, in comparison with no IVH, by the use of dummy variables. To investigate whether the association between low-grade IVH and cerebellar hemispheric volumes was ipsilateral or contralateral, a mixed random effects model was used. The outcome variable was hemispheric cerebellar volume, and the predictor variables were contralateral and ipsilateral IVH grade 1 or 2. PMA at imaging was adjusted. For all analyses, a P value < .05 was considered statistically significant.

Results

Ninety-four preterm infants and 18 healthy full-term infants were enrolled; 15 preterm infants were excluded because of brain abnormalities (4 periventricular leukomalacia, 5 severe