Diffusion Tensor Imaging and Immunohistochemical Studies in the Developing Human Cerebellar Peduncles

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Introduction: The cerebellum communicates with other brain regions via the cerebellar peduncles. Myelination of white matter is an important component of the brain maturation process. The onset and progression of myelination in fiber tracts of the neuronal system occur at different time intervals during development[1,2]. The cerebellar pathways begin the myelination process during the fetal period and continue to myelinate after birth[3]. The process of myelination in the cerebellar peduncles during the fetal period has been visualized using conventional magnetic resonance imaging (MRI)[4,5]. Diffusion tensor imaging (DTI) provides information about the organization and architecture of the white matter fibers in vivo. Diffusion anisotropy has been used to assess the cerebral white matter development before and after the onset of myelination in healthy neonates[6,7]. The development of cerebellar white matter tracts in human fetuses using DTI has not been reported so far. The purpose of this study was to demonstrate the diffusion anisotropy changes in the developing human fetal cerebellar white matter and to further correlate these findings with the immunohistochemical expression of myelin basic protein (MBP).

Materials and Methods: ***Subjects:*** Conventional MRI and DTI were performed on 23 human fetuses with gestational age (GA) of 20-37 weeks. The younger fetuses were obtained after spontaneous abortion, whereas the older ones were obtained after medical abortion for incurable renal malformation, osseous dysplasia, or intrauterine death from unknown causes. None of the fetuses had detectable central nervous system malformations on antenatal ultrasound. All the MRI studies were performed on unfixed brains. The age of each fetus was based on a combination of postovulatory age and early ultrasonographic GA estimation.

Image Acquisition: Whole-brain conventional MRI and DTI data were acquired on a 1.5 Tesla GE MRI scanner using a standard quadrature knee coil for both transmission of radio frequency pulses and signal reception. DTI data were acquired by using a single-shot echo planar dual spin echo sequence with ramp sampling. The diffusion weighting b-factor was set to 700 sec mm^{-2}. The other acquisition parameters were TR=8 sec, TE=100 msec, number of axial sections=30–34, slice thickness of 3 mm with no gap, field-of-view varying from 160 to 240 mm depending on the size of the fetal head, image matrix of 256 × 256 (following zero-filling) and NEX=8. The DTI data was processed and evaluated using JAVA based program[8]. For middle cerebellar peduncles (MCPs), elliptical region-of-interest(s) of 2 × 2 to 4 × 4 pixels were placed at the level of mid pons in the axial plane for fractional anisotropy (FA) quantification.

Immunohistochemical Analysis: Nine fetal brains ranging from 19-37 weeks GA were removed after imaging and fixed in 10% formalin. After fixation, brain was sliced in axial plane (for the purpose of comparison with DTI images); these slices were embedded in paraffin, and cut in series of 10-µm-thick sections. Sections were incubated with anti-Myelin Basic Protein antibody (Santacruz lab, USA) in a humid chamber for overnight. Immunostaining was done by Universal HRP kit (Diagnostics Biosystems, USA) using 3, 3'diaminobenzidine tetrahydrochloride (DAB) as substrate. The sections were counterstained with hematoxylin and were dehydrated, cleared, and mounted.

Results: An increasing pattern of FA values from 20 weeks GA to late third trimester of gestation, reaching a plateau in the MCP upto 37 weeks was observed in DTI studies (Figure 1). MBP expression was not observed in the cerebellar peduncles at 19 weeks GA. Myelination had commenced in the medial longitudinal fasciculus at this age (Figure 2A). Expression of MBP at 32 weeks in the cerebellar peduncles was high which continued to express strongly in the term fetus at 37 weeks GA (Figure 2B and 2C).

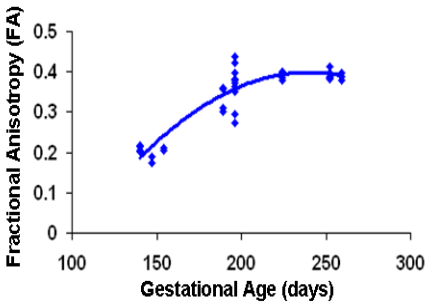
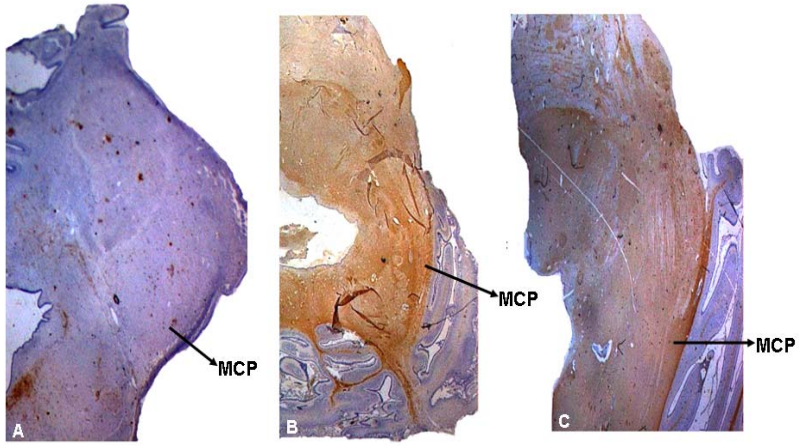


Figure 1



19 weeks GA **32 weeks GA** **37 weeks GA**

Figure 2

Figure 1: Scatter plot of FA values from the middle cerebellar peduncles (blue) as a function of GA from 140 to 259 days in the human fetal brain.

Figure 2(A-C): MBP immunostained sections of 19 weeks (A), 32 weeks (B) and 37 weeks (C) GA. Fetal brain shows expression in middle cerebellar peduncles (MCP). At 19 weeks, no myelinated fibers are seen while at 32 and 37 weeks strong MBP expression is observed.

Discussion: In this study, using DTI and MBP immunostaining we demonstrate the progression of myelination in the cerebellar white matter in the developing human fetal brain. In the current study, the observed initial increase in FA values in the MCP (>20 weeks) appears to be associated with the increase in the concentration of microtubule-associated proteins in axons and proliferation of immature oligodendroctyes during the premyelinating period. These premyelinating changes are supported by the absence of MBP expression in the cerebellar peduncles at 19 weeks GA. Histological studies have shown that myelination in the MCP begins during the late third trimester of gestation[1,2]. In our study, we observed maximum increase in FA values during the late third trimester of gestation, reaching a plateau by 37 weeks in MCP. These findings are consistent with the previous studies[1,2], reflecting myelination of axons during the late third trimester which is again confirmed by the expression of myelinated fibers in the MCP at 32 and 37 weeks GA. This study will provide a normative database of the cerebellar white matter development using DTI and MBP expression in the fetal brain.

References: 1. Yakovlev PI, et al. *Regional Development of the Brain in Early Life*, Minkowski A (ed.). 3-70, 2. Brody BA, et al. J. Neuropathol. Exp. Neurol. 1987;46:283-301, 3. Gilles FH, et al. The *Developing Human Brain Growth and Epidemiologic Neuropathology* Gilles FH, Leviton A, Dooling EC (eds). 117-183, 4. Counsell SJ, et al. Am. J. Neuroradiol. 2002;23:872-881, 5. Triulzi F, et al. Seminars in Fetal and Neonatal Medicine 2005;10:411-420, 6. Neil JJ, et al. Radiology 1998;209:57-66, 7. Huppi PS et al, Pediatr. Res. 1998;44:584-590, 8. Purwar A, et al. Proc. Euro. Mag. Reson. Med. 2006, Abstract #644.