Diffusion Tensor Imaging (DTI) of the Developing Human Cerebellar Cortex with Immunohistological Correlation

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Introduction: The histogenesis of cortical layers during the development of human fetal cerebellum has been studied using various histological and immunohistochemical methods[1,2]. The normal cerebellar development during the fetal period has been visualized on conventional magnetic resonance imaging (MRI)[3]. Diffusion tensor imaging (DTI) provides information about the water diffusivity and microstructural organization within the neural tissue. Increased fractional anisotropy (FA) values have been observed in human fetal cerebral cortex from 15-26 weeks gestational age (GA), followed by a decrease through 36 weeks using DTI[4]. The early increase in the cortical FA values is attributed to neuronal migration from the germinal matrix to the pial surface along the radial glial fibers[4]. However, diffusion anisotropy changes of human fetal cerebellar cortex have not been reported so far. The aim of this study was to demonstrate the temporal changes in the cortical anisotropy in developing human fetal cerebellum and to further correlate these findings with immunohistochemical expression of neuroglial markers.

Materials and Methods: ***Subjects:*** Conventional MRI and DTI were performed on 28 human fetuses with 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[5]. Elliptical region-of-interest(s) of 2 × 2 to 4 × 4 pixels were placed on the cortical regions of fetal cerebellum for FA quantification.

Immunohistochemical Analysis: Twenty-two fetal brains ranging from 20-36 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 overnight with primary antibody, Glial Fibrillary Acid Protein (GFAP, DAKO) 1:100 diluted in a humid chamber. After rinsing, they were incubated in the corresponding biotinylated secondary antibodies (DAKO), washed, and incubated in the conjugated streptavidin horseradish peroxidase complex (DAKO). Bound peroxidase was revealed using 0.05% 3, 3'diaminobenzidine tetrahydrochloride (DAB). The sections were counterstained with hematoxylin and were dehydrated, cleared, and mounted.

Results: Figure 1 shows pattern of increasing FA values from 20 weeks GA to late second trimester, reaches a peak value at early third trimester, and then gradually decreases in the cortical region until 36 weeks. Figure 2 shows GFAP expressed radial glial fibers within the molecular layer in a 28 and 36 weeks fetal cerebellar cortex. At 28 weeks GA, GFAP expression in the radially oriented Bergmann glial fibers was more intense, while the intensity of GFAP expression decreased at 36 weeks GA.

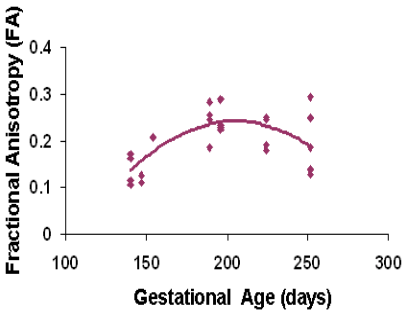

Figure 1

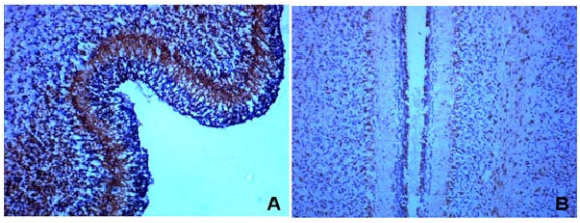

Figure 2

Figure 1: Scatter plot of FA values from the cerebellar cortex (red) as a function of GA from 140 to 252 days in the human fetal brain.

Figure 2: GFAP immunostained sections from the cortical region of 28 weeks (A) and 36 weeks (B) GA fetal brain shows the expression in the glial fibers within the molecular layer. The intensity of GFAP expression is maximum at 28 weeks which decreases at 36 weeks.

Discussion: According to Rakic et al[1], the differentiation of the glial cells starts relatively late i.e. by the end of the 25 weeks GA in the developing human fetal cerebellum. It is known that postmitotic young granule neurons migrate across the molecular layer during late developmental stages along the radially oriented Bergmann glial fibers to form the internal granular layer of the developing fetal cerebellar cortex. These radial glial fibers facilitate the granule cell migrations at later stages of development when many cell processes are well developed and molecular layer has increased in thickness[1,6]. In the current study, the observed increase in cortical FA values during the early third trimester (>27 weeks) appears to be associated with the radial organization of granule cell neurons seen on GFAP staining migrating form the external granular layer across the molecular layer to the internal granular layer. The formation of mossy fiber synapses, climbing fibers, transition of radial glia into astrocytes and proliferation of glial cells might be responsible for the observed decline in cortical FA values until 36 weeks. The present study demonstrates the migrational and maturation changes in the developing cerebellar cortex using DTI and its confirming on GFAP immunostaining should greatly improve our understanding of normal development of cerebellar cortex.

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