Quantitative phenotype characterization of developing mouse brains by diffusion tensor imaging: Application for the Frizzled-4/- mutant mice

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Introduction: It has been shown that high resolution diffusion tensor imaging (DTI) is a powerful tool for characterizing mouse brain phenotype1. DTI can provide unique neuroanatomical information not readily available by histology, and has been used to characterize developmental abnormalities in several mutant mouse strains2. We are developing quantitative approaches that can efficiently examine phenotypic changes throughout the entire brain. In this study, we report population-based quantitative analyses of DTI datasets of developing mouse brains, applied to the Frizzled-4 knockout (Fz4/-) mouse model. Histological analyses of the Fz4/- mouse brains have shown progressive cerebellar degeneration, with apoptosis of granule cells after the second postnatal week, followed by progressive loss of Purkinje cells through adulthood3. We used high resolution DTI to study the phenotypic changes during development in the Fz4/- mouse brain.

Methods: MRI of ex vivo perfusion-fixed brains from Fz4/- mice at postnatal day 7 (P7), P14, P21 and P30 (n = 3 at each age), and age-matched wild-type (WT) control mice (n ≥ 3 at each age) was performed. DTI images were acquired on an 11.7T NMR spectrometer, using a diffusion weighted multiple spin echo sequence (ETL = 4, TE/TR = 28700 ms, NA = 2, b value = 1200-1500 s/mm2, at least six diffusion directions, total scan time ≥ 24 h per sample). The isotropic native resolution was 75 µm for P7, 120 µm for P14, and 125 µm for P21 and P30 brain images (uniform relative resolution with respect to the total cerebellar volume). The diffusion tensor was diagonalized to obtain three pairs of eigenvalues and corresponding eigenvectors. Direction encoded color (DEC) maps were computed from the primary eigenvector and fractional anisotropy (FA) images. For each voxel, the ratio between the red, green and blue components was defined by the ratio of the absolute values of x, y and z components of the primary eigenvector, and the intensity was proportional to FA. Average diffusion weighted (aDW) images were the sum of all diffusion weighted images. At each developmental age, a WT brain image was chosen as the reference. Images of Fz4/- and WT mouse brains were spatially normalized to the age-matched reference image, using non-linear two-channel large deformation diffeomorphic metric mapping (LDDMM3) based on the aDW and FA contrasts. At each developmental stage, the spatially normalized tensor data (after re-orientation) were averaged for each of the two groups (wt and Fz4/-), and the average FA and DEC maps were computed from the average tensor data. A FA difference map was generated by computing the difference of average FA images for the WT and Fz4/- groups at each age. The difference map quantitatively captures the sample-averaged regional differences in diffusion anisotropy between age matched WT and Fz4/- mouse brains.

Results & Discussion: Fig. 1 shows the population-averaged mid-sagittal DEC maps of the WT and Fz4/- mouse cerebellum, at P7, P14, P21 and P30. In Fig. 1, WT mouse cerebellar cortex has relatively high FA (0.35 ± 0.04 at P30) and a distinct pattern. The red layer corresponds to the molecular layer of the cerebellar cortex, which contains massive granule cell parallel fibers running parallel to the pia surface, the green layer contains the Purkinje cell fibers, and the blue layer contains the cerebellar white matter. At P7, no significant differences could be observed between the WT and Fz4/- cerebellums. From P14 to P30, a progressive decrease in FA as well as a reduction in the thickness of the red layer in the cerebellar cortex was observed in the Fz4/- mice, as compared to age matched WT controls. At P30, the average DEC map of the Fz4/- cerebellum was marked by reduced FA and an almost complete absence of the red layer in the cerebellar cortex (indicated by white arrows in Fig. 1). The FA difference maps revealed regions in the developing Fz4/- brain with anomalies in FA as compared to WT brains. Fig. 2 shows coronal sections from average FA maps at P7, P14 and P30, showing semi-sections from the WT (left) and mirrored Fz4/- (right) brains in the left panel. The difference map [FAwt - FAfz4/-] of the entire sections are shown in the right panel. At P7, no significant differences were observed between the two groups. However, starting at P14, the Fz4/- brains showed increased FA in the dentate gyrus compared to WT brains (FA increase of 0.16 ± 0.04 and 0.17 ± 0.05 for the P14 and P30 age groups respectively). This increase in FA was observed consistently in all individual Fz4/- brains compared to their age-matched controls up to the fourth postnatal week. In addition, the mean FA was consistently lowered in the Fz4/- mice in the olfactory bulb, the optic tract and the fasciculus retroflexus compared to WT controls.

The DTI results suggest a progressive disorganization of the cerebellar structure and a temporal pattern of loss of granule cell parallel fibers in the developing Fz4/- cerebellum, consistent with the histological findings previously reported. Wang et al. have reported apoptosis of granule cells in the Fz4/- cerebellum begins between P14 and P19. In our study, at P14 DTI revealed a marked thinning of the red layer accompanied by a decrease in FA starting at P14 (Fig. 1). While no apparent abnormality was reported in regions other than the cerebellum in the Fz4/- brain previously, our DTI results show potential microstructural changes in several forebrain regions. While the significance of these changes and the precise correlations between structural phenotype and DTI signals remain to be investigated, our results show that high resolution DTI can be valuable for quantitative characterization of developmental changes in mutant mouse brains.