DTI Reveals Neuroanatomical Abnormalities in Gbx2-CKO Mouse Model of Cerebellar Hypoplasia

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Background: Although the cerebellum (Cb) is best known for its involvement in motor coordination and vestibular function [1] there is a rapidly growing body of evidence indicating Cb involvement in higher level cognitive processes [2]. Interestingly, abnormalities in Cb morphology have been shown to accompany a number of neurodevelopemental disorders, including autism spectrum disorders (ASDs), in which hypoplasia of the vermis, the central region of the cerebellum, has been reported [3]. Previously we have shown that *Gbx2*-conditional knockout (CKO) mice have hypoplasia of the vermian lobules, anatomical defects in vestibulo-cerebellum and vestibulo-cochlear organ, and abnormalities in the deep cerebellar nuclei (DCN) [4, 5, 6]. DCN are a major relay center from the Cb to other parts of the brain and contain large projection neurons that form the cerebellar peduncles, which comprise the major input and output circuitry of the Cb. We hypothesized that abnormal morphology of the DCN, previously revealed by Mn-Enhanced MRI (MEMRI) and confirmed by tissue staining, is likely to be accompanied by microstructural abnormalities in the cerebellar peduncles, which was investigated using diffusion tensor imaging (DTI). Our work is of particular importance as several clinical studies of autistic individuals found chromosomal abnormalities in 2q37 region in which human homologue of Gbx2 gene is known to be located [7].

Methods: Mice were anesthetized with ketamine/xylazine (0.3ml/30g) and perfused intracardially. After initial flush with 50ml of PBS containing heparin (50U/ml) 50ml of ice cold 4% PFA was used to fix the samples. Brains were extracted from the skull and post-fixed in 4% PFA containing 1mM gadopentetate dimeglumine (Magnevist, Bayer HealthCare Pharmaceuticals) for 24hrs at 4°C. Samples were stored in PBS mixed with Magnevist (1 mM) and left at 4°C for rehydration from 3 to 7days. Prior to scanning, brains were placed in a custom holder and submerged in proton-free perfluoropolyether (Fomblin, Solvay Solexis) for the duration of the scan. Three to four week old (P26±2) wild type (WT) and *Gbx2*-CKO mice were used in this study (WT: n=11; *Gbx2*-CKO: n=11) with 6 males and 5 females in each group. High-resolution DTI scans were performed using a 7T micro-MRI system, consisting of a Biospec Avance II console (Bruker Biospin MRI, Ettlingen, Germany) interfaced to a 200-mm horizontal bore superconducting magnet (Magnex Scientific, Yarnton, UK) with an actively shielded gradient coil (Bruker BGA-9S; 20-mm inner diameter, 750 mT/m gradient strength, 100 us rise time) and a quadrature Litz coil (Doty Scientific, Columbia, SC). A 3D DW-GRASE sequence with twin-navigator echo phase correction [8] was used with 10 diffusion weighting directions, TR/TE=1.2s/37 ms, matrix size = 128x80x144 with isotropic acquisition resolution of 110 um (zero-padded to have 55 um after image reconstruction), and b-values=100 and 1100 s^2/mm. Total scan time was 14h. DTIStudio (John Hopkins University, Baltimore, MD) was used to generate rotationally invariant DTI parameter maps of fractional anisotropy (FA) and mean diffusivity (MD). A whole brain, voxel by voxel, statistical parametric mapping approach was used to asses for statistically significant differences in FA and MD between WT and *Gbx2*-CKO mice. Results of these analyses were compared with the morphological differences, assessed by means of deformation based morphometry (DBM). Jacobian determinant maps, derived from deformation fields generated in the process of nonlinear registration of b0 anatomical images with no diffusion weighting, to an unbiased population average of WT and mutant mice were used [9, 10]. Additionally, an automated region of interest (ROI) analysis approach employing a pre-existing MRI brain atlas was used to calculate volume, FA, and MD of 62 pre-defined brain regions [11]. Statistical analyses were performed on absolute volumes as well as after correcting for differences in a global brain volume. To control for multiple comparisons the False Discovery Rate (FDR) was used [12].

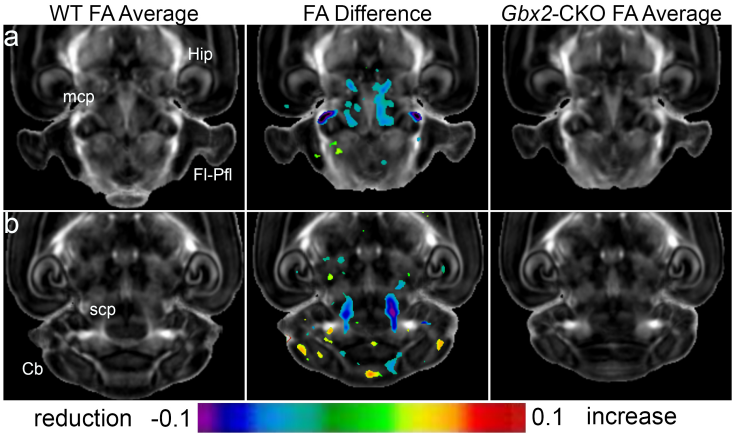

Fig. 1 Results of VBM analysis of fractional anisotropy (FA) maps (FA Difference = *Gbx2*-CKO - WT). Notice statistically significantly (FDR<5%) lower FA (blue) in regions corresponding to mcp and scp which may indicate disrupted white matter integrity in these white matter tracts. Lower FA may be an indicator of fewer or less organized axonal projections.

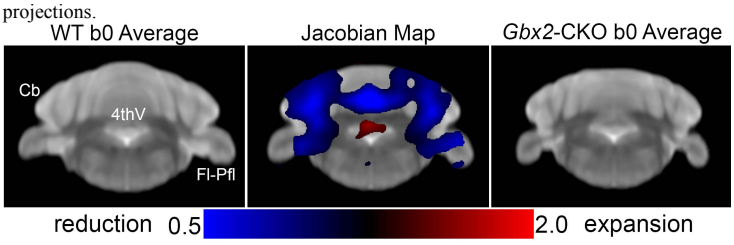

Fig. 2 Results of DBM analysis based on b0 anatomical DTI images. Statistically significant after correcting for differences in overall brain volume (FDR 5%) reduction in size (blue) of cerebellar subregions could be seen on Jacobian maps. Enlargement (red) of the 4thV was apparent. Abbreviations: Cb - cerebellum, Fl-Pfl - flocculus-paraflocculus complex, Hip - hippocampus, mcp - middle cerebellar peduncle, scp - superior cerebellar peduncle, 4thV – 4th Ventricle

Results and Conclusion: Three pairs of nerve tracts, the inferior, middle and superior cerebellar peduncles (icp, mcp, scp), allow the Cb to communicate with other parts of the central nervous system. Voxel based analyses, in concordance with ROI analysis, revealed a statistically significant (FDR<5%) decreased FA in the scp and mcp (Fig. 1) as well as along several other white matter tracts traversing the midbrain that relay further information carried by the scp. Statically significant (FDR<5%) increases in MD were seen only in the vicinity of 4th ventricle (data not shown). These increases were accompanied by 8% enlargement of this region and can be seen in results of the analysis of Jacobian maps (Fig. 2). It is well known that normal communication between brain regions is critical for normal information processing. Decreased FA in cerebellar peduncles is a likely indicator of abnormal relay of information between the Cb and other parts of the brain. Large number of cerebellar morphological abnormalities reported in studies of human neurodevelopmental disorders, including ASDs, argues for comprehensive screening of mouse models with specific cerebellar defects for additional volumetric and microstructural abnormalities in brain regions related to Cb circuitry. This in turn will shed more light on biological underpinnings and roles of genes important for normal Cb development in giving rise to specific behavioral phenotypes. Additional work is ongoing to verify and better understand these MRI findings with histology and immunohistochemistry (IHC).

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