Voxel Based Morphometric Analysis of the Gbx2 Mutant Mouse Phenotype *via* MEMRI

K. U. Szulc[1,2], B. J. Nieman[1,2], R. V. Sillitoe[3], A. L. Joyner[3], and D. H. Turnbull[1,2]

[1]Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY, United States, [2]Radiology, NYU School of Medicine, New York, NY, United States, [3]Developmental Biology, Sloan-Kettering Institute, NY, New York, NY, United States

***Introduction*:** The cerebellum (Cb) is a highly patterned brain structure with a stereotypical foliation pattern, connected to multiple regions throughout the brain and the spinal cord, and playing an essential role in normal motor and cognitive function [1]. Mouse models have been produced through gene targeting to analyze the roles of a number of genetic and molecular factors in cerebellum development, including *Gbx2* conditional knockout (*Gbx2*-CKO) mice that have variable deletions of the vermis, the central cerebellum [2]. Previously we demonstrated the feasibility of *in vivo* longitudinal Manganese-Enhanced MRI (MEMRI) of Cb development in normal and *Gbx2*-CKO mice during critical neonatal stages of foliation, including volumetric analysis of the vermis deletion and abnormalities in the flocculus-paraflocculus (FL-PFL) complex and deep cerebellar nuclei (DCN) that were not previously reported [3]. Despite these successes, the semi-automated methods of segmentation and volumetric analysis used previously are extremely time-consuming and can be subject to user-dependent bias. In the current study, we extended our analysis to whole brain voxel-based morphometry (VBM) in order to provide a comprehensive, unbiased characterization of the *Gbx2*-CKO phenotype.

***Methods*:** Mn was delivered to neonates through the milk after maternal IP injection of $MnCl_2$ in isotonic saline (40mg/kg). The neonatal mice were imaged 24h after the maternal injection of the $MnCl_2$ solution at 5 developmental stages every other day from postnatal day P3 to P11. A 7T Bruker Biospec system with 750mT/m actively shielded gradients and a 25-mm (ID) quadrature Litz coil (Doty) was used to acquire 3D T1-weighted gradient echo images (TE/TR=3.6/50 ms, FA=40°, FOV=(2.56 cm)^3, matrix=256^3, NEX=2, time=~2h). The neonates were positioned in a custom-built holder, including isoflurane anesthesia delivery and warm air to maintain homothermic body temperatures. VBM analyses were performed at P7 and P11, including the following steps [4]: generation of unbiased averages of mutant and wildtype (WT) images, nonlinear registration of individual images to the average, generation of a deformation field for each individual image. Statistical tests were performed on a voxel-by-voxel basis to indentify significant differences in volume (false discovery rate, FDR < 5%) between *Gbx2*-CKO mice and WT littermates. Additional volumetric analyses and 3D renderings were produced using AMIRA for 6 *Gbx2*-CKOs and 6 WT littermates at each developmental stage.

***Results and Conclusions*:** The VBM analysis revealed abnormalities in the *Gbx2*-CKO cerebellar vermis, hemispheres, FL-PFL complex and DCN at both at P7 and P11. The DCN abnormalities were more pronounced at the later stage. Expansion of adjacent midbrain structures, including the inferior (IC) and superior colliculus (SC), was also evident on the VBM parametric maps. These results were in excellent agreement with the previous semi-automated volumetric analysis [3]. Additionally, clear enlargement of the fourth ventricle at both developmental stages was observed that was not included in the initial volumetric analysis. Interestingly, abnormalities in several non-cerebellar nuclei were also detected at both developmental stages. Specifically, VBM showed a reduced interpeduncular nucleus, and bilateral defects in the vicinity of the red nucleus, oculomotor nucleus, retro rubral field and dopaminergic group 8. In addition to these abnormalities, which may reflect changes in cerebellar circuitry, VBM also showed a reduction in the volume in entorhinal cortex at P7. Traditional manual or semi-automated volumetric analyses of MRI data typically require an *a priori* hypothesis as to which regions are affected and therefore should be analyzed. They are not only labor intensive but are also sensitive to intra- and inter-observer variability, which can bias the results and affect data interpretation. VBM provides an alternative approach, which has found numerous applications within the field of human imaging and has recently been applied for phenotype analysis in a number of genetically engineered mouse models [4-7]. In our study, VBM analysis identified several brain regions already known to be altered in *Gbx2*-CKO mice, as well as a number of novel phenotypes that will be further investigated with MEMRI and validated *via* histological methods.

Acknowledgements: This work was supported by NIH grant RO1 N5038461. We thank Drs. Mark Henkelman, John Sled and Jason Lerch at the Mouse Imaging Center (MICe) in Toronto Canada for providing us with the software used for the VBM analyses.

References: [1] Sillitoe RV, Joyner AL (2007). *Ann Rev Cell Dev Bio* 23:549-77; [2] Li JYH et al. (2002). *Neuron* 36: 31-43; [3] Szulc KU et al. (2008). *Proc ISMRM* 16: 529**;** [4] Kovacevic N et al. (2005). *Cereb Cortex* 15: 639-45; [5] Nieman BJ et al. (2007). *Human Brain Mapp* 28(6):567-75; [6] Lerch JP et al. (2008). Neuroimage 39(1):32-9; [7] Lau C et al. (2008). Neuroimage 42(1):19-27

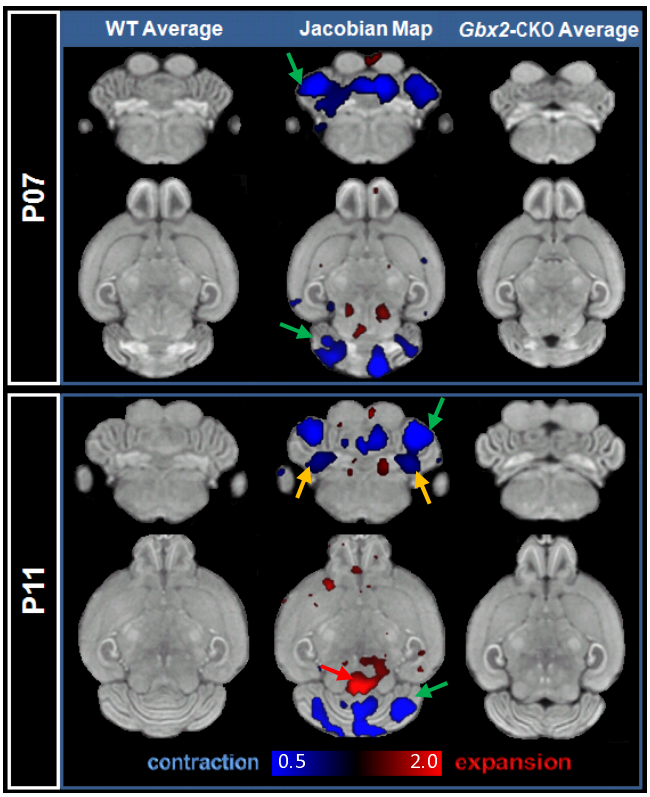

Fig 1. VBM analysis revealed differences in brain volume and shape in a number of regions. These included reduction in size of the cerebellum (green arrow), FL-PFL (not shown) complex and DCN (yellow arrows). Expansion of dorsal midbrain structures (not shown) end the 4th ventricle (red arrow) was also evident.