

High resolution diffusion tensor imaging to assess brain microstructural abnormalities in a Neuroligin-3 knockin mouse model associated with Autism spectrum disorders

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Introduction: Autism spectrum disorders (ASD) are a set of neurodevelopmental disorders characterized by impairments in communication and social interactions, along with repetitive behaviors and restricted interests and behaviors¹. Mutations in genes encoding regulators of synapse function in neurons, including neuroligin-3 (NL-3) have been implicated in certain forms of monogenic heritable ASD in humans². NL-3-deficient mice display a behavioral phenotype reminiscent of the lead symptoms of ASD; however, behavioral studies on this mouse model have reported conflicting findings^{4,5}. High resolution morphometric and diffusion tensor imaging (DTI) studies in adult (108 day-old) NL-3 knockin mice brains reported significantly reduced volume in different regions of the brain; however no significant changes in DTI were reported⁶. As ASD symptoms are more severe at the young age, the present study was performed in the NL-3 knockin model at a young age to evaluate whether DTI and volumetric MRI can be used to characterize this mouse model and whether the imaging findings correlate with mouse behavior. We have recently reported differences in DTI and social-behavior at pre-pubescent^{7,8} and post pubescent ages in the inbred BALB/cJ mouse strain, which exhibits certain behavioral phenotypes of ASD, and thus the motivation of the study was to establish DTI as a surrogate in a transgenic mouse model relevant to ASD.

Materials and Methods: Behavioral Testing: Age/sex matched NL-3 knockin mice [n=25 (10 at 30 day, 10 at 50 day and 5 at 70 day)] and wild-type [n=24 (7 at 30 day, 9 at 50 day and 8 at 70 day)] were included. Sociability test was performed at 28 (prepubescence), 48 (post pubescence) and 68 (early adulthood) days of age and test for anxiety-related behavior was performed on the following day. A Social Approach Test (a.k.a. Social Choice Test) using a 3-chambered apparatus^{9,10} was used to measure sociability, while anxiety-related behavior was measured using the elevated zero-maze test¹¹. One day following the zero maze test, the animals were perfused and brains were extracted and fixed in 4% para-formaldehyde.

Ex-vivo DTI: After brain tissue fixation, high resolution DTI was performed on a 9.4T, 8.9cm vertical bore magnet using a 20mm inner diameter loop-gap transmit receive coil. DTI was acquired using a 3D multi-echo pulsed-gradient spin echo sequence¹² by using: TR=800ms; TE=29.50ms; FOV=17mmx8.5mmx10mm; acquisition matrix size=136x68x80; number of acquisitions=6 and b-value=902mm²/s. The diffusion-weighted images were acquired with diffusion weighting in 6 non-collinear directions in a total acquisition time of 13.19 hrs per brain sample.

Data processing and quantification: For each sample, the Camino Toolkit¹³ was used to reconstruct the diffusion tensor from the original diffusion weighted images. To obtain anatomical labels for each subject, a set of five manually labeled mouse brains were used along a multi-atlas approach¹³. The Advanced Normalization Toolkit¹⁴ was used to match each of the manually labeled data sets to each subject's B0 image. Next, the STAPLE¹⁵ method was used to obtain a final set of probabilistic anatomical labels for each image (Fig. 2). These probabilistic labels were then used to obtain volumes and fractional anisotropy, mean diffusivity ($\times 10^3$ mm²/s), axial and radial diffusivity values for each labeled structure.

Results: No significant differences in sociability were noted (Fig. 1A), however significant differences in anxiety were observed between the two groups at 50 day (Fig. 1B). Significantly reduced volume was observed in 16 of the 40 gray and white matter regions in NL-3 mice brain when data from all 3 time points was combined (Fig. 3 A, B). Independent t-test was performed to compare different DTI indices and tissue volume from NL-3 and wild type mice at each time point and also by combining the data from at all 3 time points. Similar to the earlier report in the NL-3 model⁶ we did not observe any significance changes in DTI indices between NL-3 knock in and wild type mice (p>0.05) as shown in figure 3 C, D. No significant correlation was noted between DTI, brain volume and behavioral data at any time points.

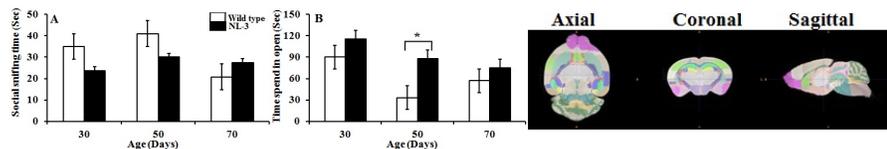


Fig 1: Bar graph showing sociability (A) and anxiety (B) score between wild type and NL-3 mice at 3 different time points. Asterisk (*) represents significant differences. Fig 2: Demonstrating segmented mouse brain on axial, coronal and sagittal orientation.

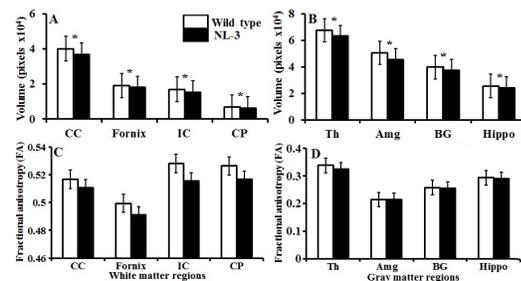


Fig. 3: Representative bar graphs showing changes in volume (A and B) and fractional anisotropy (C and D) in NL-3 and wild type from white (A and C) and gray (B and D) matter regions of the brain. CC=corpus callosum, IC= internal capsule, CP=cerebral peduncle, Th=thalamus, Amg= amygdala, BG=basal ganglia, Hippo=hippocampus.

Discussion: We observed significantly reduced volume of several gray and white matter regions in NL-3 mice. The volumetric differences are unlikely due to abnormal myelination or break down of white matter tissue as we did not observe any differences in DTI indices, similar to what has been reported before⁶. Since no significant differences were observed in FA values in any of the white matter structures, significantly smaller volumes in these regions may be caused by reduced number of axons, or due to less oriented/less mature axons in the white matter regions with similar axonal density and microstructure. We have earlier reported longitudinal changes in DTI and its correlation with sociability in the inbred BALB/cJ mouse model and observed maximum changes in DTI indices and sociability at early age^{7,8}. The sociability test showed no difference in behavior between NL-3 and wild type mice⁷ no correlation was observed between DTI and social behavior. While the volumetric differences in the brain and differences in anxiety at 50 day old NL-3 knockin mice were noted, these differences were not correlated questioning the validity of this model for ASD as reported earlier⁴. However, it is also possible that DTI is not a sensitive technique in picking these subtle differences. Further studies involving MRI and histological correlation along with other behavioral assays may be necessary to establish the validity of this model as being relevant to ASD.

References: [1] Reiss AL. *J Child Psychol Psychiatry*. 2009;50:87-98; [2] Jamain S, et al. *Proc Natl Acad Sci U S A*. 2008;105:1710-5; [4] Chadman KK, et al., *Autism Research*. 2008;1:147-58; [5] Tabuchi K, et al., *Science*. 2007;318:71-76; [6] Ellegood J, et al., *Autism Res*. 2011;4:368-76; [7] Kim S, et al., *NMR Biomed*. 2012;25:104-12. [8] Kumar M, et al., *Brain Res*. 2012;1455:56-67; [9] Sankoorikal GMV, et al. *Biol Psychiatry* 2006;59:415-23; [10] Fairless AH, et al. *Brain Res* 2008;1230:211-17; [11] Stein JM, et al. *J Neurosci* 2006;26:2184-96; [12] Mori S, et al., *Magn. Reson. Med*. 1998;40:511-16; [13] Cook PA, et al, *ISMRM*, 2006, p. 2759. [14] Bai J, et al. *Magn Reson Imaging*. 2012; 30:789-98; [15] Warfield SK et al., *IEEE Trans Med Imaging*. 2004;23:903-21.

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