Neuroanatomical Abnormalities in a Neuroligin3 R451C Knockin Mouse Model of Autism

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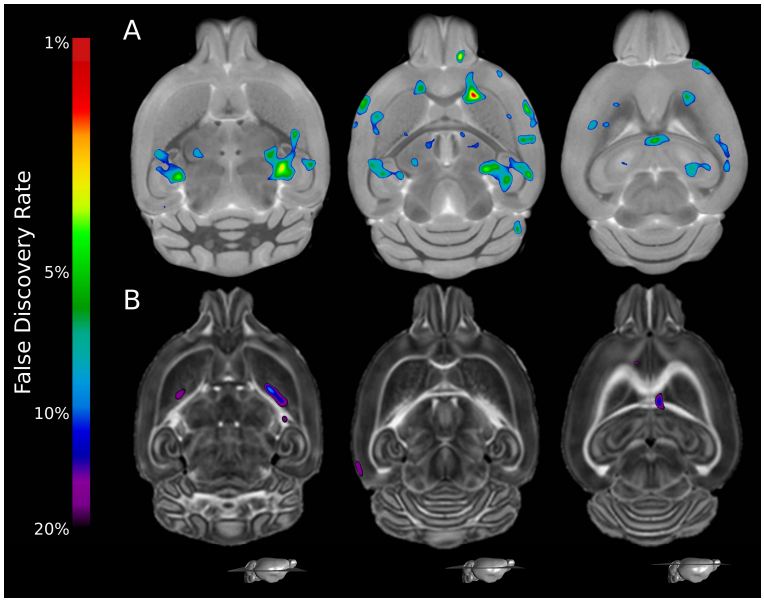
Introduction – Autism is highly heritable; twin studies have shown that there is a 90% concordance rate with identical twins (1,2). The neuroligin and neurexin genes have been recognized in human autism association studies (3,4). One of these studies found a mutation of Neuroligin3 (NL3) in a family with two brothers, one with typical autism and the other with Asperger's syndrome. In this mutation a highly conserved arginine residue was replaced with cysteine at amino acid position 451 (R451C) (3). Tabuchi et al. introduced the R451C substitution into a mouse model, creating the Neuroligin3 R451C knockin (NL3 KI) mouse model of autism. The purpose of this study was to examine the volumetric and white matter structural changes in the brain of the NL3 KI mouse using high resolution MRI and detailed statistical analysis.

Methods - *Specimen Preparation* – 16 fixed mouse brains were examined, 8 wild-type and 8 NL3 KI mice. Mice were anesthetized and intracardially perfused. The brain was removed (left in the skull) and placed in 4% PFA and 2mM Prohance (a Gadolinium contrast agent) overnight and then transferred to PBS, 0.02% sodium azide, and 2mM Prohance for at least 7 days prior to the MRI acquisition. *MRI Acquisition* - A multi-channel 7.0 Tesla MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient set (max gradient strength 100 G/cm) was used to acquire anatomical images of brains within skulls. Three custom-built solenoid coils were used to image three brains in parallel. Parameters used in the anatomical conventional MRI scans were optimized for gray/white matter contrast: a T2- weighted, 3-D fast spin-echo sequence, with a TR of 325 ms, and TEs of 10 ms per echo for 6 echos, four averages, field-of-view of 14 × 14 × 25 mm^3 and matrix size = 432 × 432 × 780 giving an image with 0.032 mm isotropic voxels. Total imaging time was ~11 h. An additional 3-D diffusion weighted fast spin-echo sequence with an echo train length of 6 was used with a TR of 325 ms, first TE of 30 ms, and a TE of 6 ms for the remaining 5 echos, ten averages, field-of-view 14 x 14 x 25 mm^3 and a matrix size of 120, 120, 214 yielding an image with 0.117 mm isotropic voxels. One b=0 s/mm^2 image (with minimal diffusion weighting) and 6 high b-value images (b=1956 s/mm^2) in six different directions [(1,1,0),(1,0,1),(0,1,1),(-1,1,0),(-1,0,1),(0,1,-1)] (G_x,G_y,G_z) were acquired. Total imaging time was ~16 hours. *Data Analysis* - To visualize and compare volumetric changes and white matter structural changes, the brains were registered together. For the volume measurements, the registration resulted in deformation fields for each individual brain, which were used to calculate individual volumes from the segmented population average. From this data the volume of 62 different structures (5) was assessed for all 16 brains. As well, voxel by voxel changes were evaluated. For the DTI measurements, the mean fractional anisotropy (FA) was measured in each of the same 62 structures and also voxelwise differences in FA.



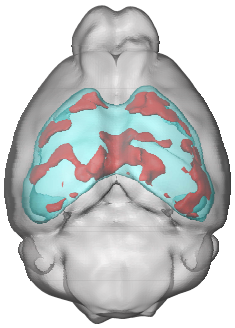


Figure 1 (above) – Three axial slices from the consensus average of the 16 brains used in this study. A) Significant volume decreases thresholded at an FDR of 10%. B) Significant FA decreases thresholded at an FDR of 20%.

Figure 2 (left) – 3D representation of the mouse brain (gray) showing a surface rendering of the corpus callosum (blue). Significant decreases are highlighted in red.

Results and Discussion – Brain volume, while showing an 8% decrease in the mutant, did not reach statistical significance compared with intragroup variation. However, when separated into gray matter, white matter, and ventricular volume, the total white matter significantly decreased by 10%. Moreover, specific gray matter structures significantly decreased in volume, such as the hippocampus, striatum and thalamus (decreases of 12%, 12%, and 15%, respectively). Figure 1A shows 3 images where the significant decreases in volume are highlighted for the NL3 KI mouse when compared with the wild-type (false discovery rate (FDR) <10%). Bilateral differences in the hippocampus can be seen as well as decreases in the striatum and cortex. Large scale volume changes were also found in white matter structures, with the cerebral peduncle, corpus callosum, fornix/fimbria bundle, and the internal capsule all significantly decreasing. The corpus callosum decrease (14%) is consistent with the callosal thinning reported in the human literature (5-7). Fig. 2 shows a 3D representation of the corpus callosum (blue) with the significant decreases highlighted (red). Along the midline of the corpus callosum changes are localized in the posterior, which has also been shown in human autism (8,9). No mean FA changes were found in the 62 regions, which indicates that, while there is a loss of white matter volume, the tract density and structure remains intact. Figure 1B shows FA maps of the same axial slices as 1A. Small significant decreases were found when the FDR was thresholded at 20%, where there is a bilateral decrease in FA in the globus pallidus seen in Figure 1B (left).

Conclusion – The NL3 KI mouse has marked volume differences in many different structures in the brain, a number of which are identified with human autism. The corpus callosum changes are particularly interesting as they are frequently found in human studies.

References - 1) Steffenburg et al., J. Child Psychol Psychiatry, (1989) 30:405-416, 2) Bailey et al., Psychol Med, (1995) 25:63-77, 3) Jamain et al., Nat Genetics, (2003) 34:27-29, 4) Laumonnier et al., Am J Hum Genet, (2004) 74:553-557, 5) Cody et al., Int J Dev Neurosci, (2002) 20:421-438, 6) Stanfield et al. Eur Psychiatry, (2008) 23:289-299, 7) Verhoeven et al., Neuroradiology (2010) 52:3-14, 8) Egaas et al. Arch Neurol, (1995) 52:795-801, 9) Piven et al., Am J Psychiatry, (1997) 24:154-162.