

High-Resolution 3D MRI to Identify Neurodegeneration in a Huntington Disease Mouse Model Brain

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Introduction

Magnetic resonance imaging (MRI) is emerging as a powerful tool for phenotyping mice in biological studies of gene expression, development and disease progression. In specific cases, MRI can be used to follow a known biomarker of a mouse model of human disease. In Huntington Disease (HD), a yeast artificial chromosome (YAC)128 mouse model has been developed which recapitulates the neurodegenerative phenotype observed in human HD¹. This manifests as a specific loss of neurons in the striatum, eventually progressing to include selective regions of the cortex. Until recently, specific neuropathology has been detected with the use of immunohistochemical and stereological evaluation. However such techniques are not ideal in monitoring disease progression in a single mouse over time, in the case of a clinical trial, for example. For this reason, the ability of MRI to identify striatal degeneration, a potential biomarker in the mouse model of HD, was examined. In the mouse model of HD, striatal atrophy is apparent with the use of stereological methods in mice at 9 months of age with a 15% striatal volume decrease compared to non-diseased counterparts¹. To determine whether MRI could detect neurodegeneration at an earlier stage of disease progression and shorten a potentially expensive clinical trial, neuroanatomical differences between fixed brains of eight month old YAC128 and wild-type mice were analyzed. With the usefulness of MRI in the identification of specific neuropathology established, the use of the technique in a longitudinal study, using live mice, would be warranted.

Materials and Methods

Eight-month-old FVB/N (Charles River, Wilmington, MA) and YAC128 transgenic HD model mice maintained on FVB/N background strain¹ were anaesthetized with a combination of Ketamine (100 mg/kg) and Rompun (20 mg/kg) via intraperitoneal injection. Thoracic cavities were opened and animals were perfused through the left ventricle with 30 mL of phosphate buffered saline (PBS) (pH 7.4) at room temperature (25°C). This was followed by infusion with 30 mL of iced 4% paraformaldehyde (PFA) in PBS. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures were allowed to postfix in 4% PFA at 4°C for 12 hours. Following an incubation period of 5 days in PBS and 0.01% sodium azide at 15°C, the skulls were transferred to a PBS and 2 mM Prohance® (Bracco Diagnostics Inc., Princeton, NJ) solution for at least 7 days at 15°C. MR imaging occurred 12 to 21 days post-mortem. Nine brains for each normal and diseased mouse group were analyzed.

A four-channel 7.0-T MR scanner (Varian Inc, Palo Alto USA) with a 6-cm inner bore diameter gradient set was used to acquire anatomical images of brains within skulls. Prior to imaging, the skulls were removed from the contrast agent solution and placed into plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp., St. Paul, MN). Custom-built, 12-mm over-wound uniform solenoid coils were used to image three brains in parallel. The parameters used in the scans were optimized for grey/white matter contrast: T2-weighted, 3D fast spin-echo sequence, with TR/TE= 325/32 ms, four averages, field-of-view 12 x 12 x 25 mm and matrix size = 780 x 432 x 432 giving an image with 32 µm isotropic voxels. The total imaging time was 14 hours.

All mice underwent a rigid body registration towards a pre-existing model². All possible pairwise 12 parameter registrations were then carried out to create a linear model of the entire dataset. Next, a non-linear and unbiased average of the wild-type mice was created using an iterative deformation procedure. All mice were subsequently non-linearly registered towards this model, the deformation magnitudes computed, and the two groups compared using linear models at every grid point of the deformation field, multiple comparisons controlled for using a False Discovery Rate of 10%.

Results

Significant differences in deformation magnitudes were found in the bilateral striatum, the right cerebral cortex, and isolated regions of the thalamus, as shown in figure 1. The greatest difference occurred at the cortex/caudate boundary in the right hemisphere (180µm, SEM=30µm, t=6.7, p=1.7x10⁻¹¹ uncorrected). There were no significant differences in overall brain size between the two groups.

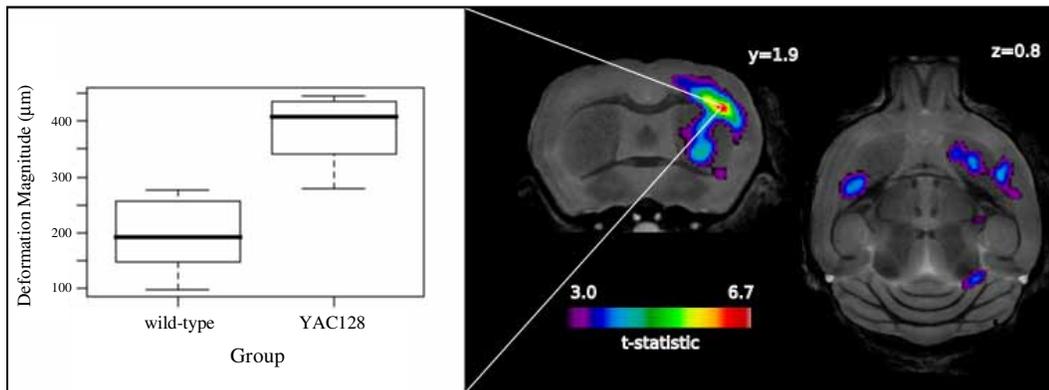


Figure 1. Group differences in deformation magnitudes between wild-type and YAC128 mutant mice.

Conclusion

Automated analysis of high-resolution MR images of a murine model of HD were able to identify significant shape changes in the striatum and cortex of the HD mutant mice at eight months of age. Furthermore, a statistically significant striatal volume decrease of approximately 5% was measured in the mutant model of HD³. These results suggest that HD-related neurodegeneration can be detected earlier than initially believed, and suggest that MR-based measures can provide essential information about neuroanatomical integrity for clinical trials in HD mouse models.

References

- ¹ Slow E. *et al.* Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. *Hum Mol Genet* 2003. 12:1555-1567.
- ² Kovacevic *et al.* A three-dimensional MRI atlas of the mouse brain with estimates of the average and variability. *Cereb Cortex* 2004. 15:639-645.
- ³ Carroll J. *et al.* in preparation.