A technique for neuroanatomical screening of mutant mice by MRI

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Introduction

Magnetic resonance imaging (MRI) holds enormous potential as an investigative tool in many biological studies in which soft-tissue structure and morphology can be used for assessment of gene expression, development or disease progression. With the advent of high throughput multiple mouse imaging techniques[1], such studies include large-scale screening of new or incompletely characterized mutant mice. Anatomical abnormalities discovered through screening may then be further studied to improve our understanding of the genetic basis for human diseases. However, significant challenges lie in the initial detection and characterization of mutant mice. This abstract presents results from a screening study of gja1 mice—a mutant mouse recently discovered to model a rare human disease called oculodentodigitalis dysplasia (ODDD)—with emphasis on the imaging and analysis techniques appropriate for anatomical screening for the purpose of mutant characterization.

Imaging Methods

The present study included the analysis of ten mice, five gja1 mutant mice and five littermate controls. MR imaging was performed on three to four mice simultaneously with parallel receive coils in a 7.0T magnet operated by a Varian Inova console. Images were acquired using a fast spin-echo sequence with a reduced excitation angle (40°). Imaging parameters included TEeff = 36 ms, TR = 900 ms, 12 ms echo spacing, 40×24×24 mm field-of-view, and 384×208×208 matrix giving a resolution of 104×115×115 µm. Scan duration was 2 hrs 45 min. Mice were maintained under anesthesia throughout the imaging session using 1.0% isoflurane gas after induction at 4.0%. Image reconstruction was performed using Matlab software.

Data Analysis

Following image reconstruction, data were processed through in-house automated registration software[2] (based on both AIR5.22 and ANIMAL registration packages). The registrations aligned individual brains and produced an unbiased "average" brain representative of all mice. Output from the software included: average control and average gja1 images; deformation fields transforming each individual into its average; and deformation fields transforming the average control image into each of the gja1 mice. The nonlinear components of the deformation fields were subsequently smoothed using b-splines with 2 mm separation between knot points. Further analyses considered only the smoothed deformation fields transforming the average control image to each of the ten mouse images.

Local shape and volume differences between the two mouse groups were examined for potential abnormalities. Shape changes were determined directly from the deformation fields. In this analysis, the average vector displacement to the gja1 mice were considered with respect to the expected biological variation drawn from the control deformation fields. This was quantified through calculation of Hotelling's T^2 fields. Local volume differences can be determined as well by calculation of the Jacobian. Again, any volume differences in the gja1 mice must be evaluated with respect to the variation observed in control data. In this case, a simple t-test was performed on the logarithm of the Jacobian field. To visualize the most important changes between mutant and control data, a threshold was set on Hotelling's T^2 and t-test fields corresponding to a p-value of 0.01.

Results

Figure 1 shows results of the MR imaging and analyses. Panels (a) and (b) show the average control mouse. In (a), an overlay displays the magnitude of the mean deformations required to produce the mutant phenotype. Similarly, panel (b) shows an overlay of the logarithm of the jacobian. The image of the mutant average is presented in panel (c). Regions of notable difference between the control and mutant groups include the cerebellum and forebrain, where displacements on the order of 400 µm are present with local volume changes as large as -20 and +30% respectively. The number of voxels above the threshold level was 8-times and 3-times larger than would be expected by a 1% false positive rate for the Hotelling's T^2 and Jacobian data respectively.

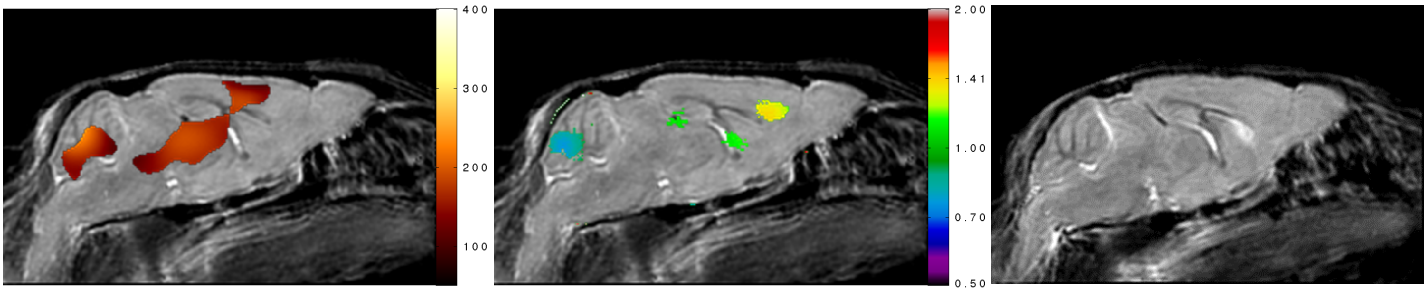

Figure 1: The average control image is shown in (a) and (b). An overlay of the magnitude of the average deformations required to produce the mutant is shown (a) (scale in µm). Likewise, the log jacobian values are overlaid in (b). The average mutant image is also shown for comparison (c).

Conclusions

This study demonstrates a methodology for automated *in vivo* secondary screening of mutant mice. Initial results in the gja1 mouse model of ODDD show significant shape and size differences in the brain. This screening technique is expected to be applicable in other studies comparing groups of mutant mice with control populations and will eventually extend to primary screening of individual mice against a normal population.

References

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