Mouse model of ADA deficiency shows similar brain abnormalities as human patients.

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Target Audience

When establishing an animal model for a human disease or disorder, it is critical to know whether it accurately recapitulates the human picture. Brain morphology is often altered in pathological conditions. Here we report the combined use of *ex vivo* high-resolution MRI (40µm) and tensor-based morphometry (TBM) as a tool for investigating volumetric changes in the brain and how this method has uncovered the same pattern of local shrinkage of brain structures in a mouse model of adenosine-deaminase (ADA) deficiency as those seen in human patients. Our finding should be of interest to researchers who study diseases that have an origin in the brain or an effect on the brain, as well as those who use MRI as a tool for phenotyping.

Purpose

Our aim was to investigate whether a mouse model of ADA deficiency exhibits abnormalities in brain morphology. ADA deficiency is an autosomal recessive disorder that damages the immune system and accounts for approximately 20% of all cases of severe combined immunodeficiency (SCID). Patients with SCID are prone to life-threatening infections and the main symptoms include pneumonia, chronic diarrhea, and widespread skin rash. However, unlike other forms of SCID, patients lacking ADA also present abnormalities that may not stem directly from a loss of immune protection: notably, they were found to display behavioural impairment compared with case control patients[1]; and very interestingly, a volume loss in the basal ganglia and thalamus was reported in a study of three ADA-deficient patients[2]. These findings prompted us to look into the brain morphology of our ADA-deficient mouse strain, using the emerging technique of high-resolution MRI combined with advanced image processing, which is capable of detecting local and subtle volumetric changes in the rodent brain.

Methods

Animals. ADA-deficient mice were generated using a two-stage genetic engineering strategy[3]. Male mice of two age groups were studied: one at postnatal day 9 (P9, n = 9 for $ADA^{-/-}$ mutants, n = 9 for $ADA^{+/+}$ wild-type controls), and the other at P18 (n = 5 for $ADA^{-/-}$, n = 6 for $ADA^{+/+}$). When they reached either P9 or P18, animals were culled and decapitated. Then the brains with intact skulls were extracted and immersion-fixed in 4% PFA solution doped with 8mM Gd-DTPA (Magnevist, Bayer-Schering Pharma, Newbury, UK) for 9 weeks.

Imaging. A Varian 9.4 T DirectDrive VNMRS system (Varian Inc., Palo Alto CA, USA) and a 26mm quadrature birdcage coil (RAPID Biomedical GmbH, Würzburg, Germany) were used. A gradient-echo 3D sequence, which has been optimised to give high signal-to-noise ratio and histology-like anatomical delineation, was used: TR = 17ms, TE = 4.54ms, flip angle = 51°, averages = 6.

Image analysis. Images are put through a pipeline[4]: after preprocessing, the brain images were registered, first affinely using a block-matching strategy, then non-rigidly using a fast free-form deformation method, to a common target. For TBM, deformation fields from the last round of non-rigid registration were used to calculate Jacobian determinants, on which voxelwise *t* tests were performed within a general linear model, with false discovery rate (FDR) correction to account for multiple comparisons. P9 and P18 cohorts were analysed individually.

Results

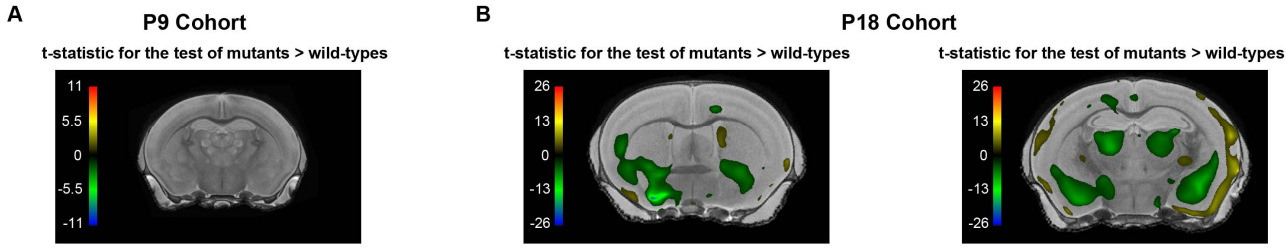

Figure 1 TBM results for the P9 cohort (**A**) and the P18 cohort (**B**). Colour bars indicate the value of t-statistic.

For the post-natal day 9 cohort (P9), TBM analysis revealed no significant difference between the $ADA^{-/-}$ and $ADA^{+/+}$ mice (Fig. 1A). However, extensive local volumetric differences had emerged by P18. Two example slices of the statistical map are shown in Figure 1B. Notably, volume loss in part of the striatum (Fig. 1B, left) and thalamus (Fig. 1B, right) can be seen in P18 mutants compared with controls. Other areas of change include basal forebrain septum (Fig. 1B, left), amygdala (Fig. 1B, right), and cortex (Fig. 1B, right).

Discussion and Conclusion

Our study of a mouse model of ADA deficiency substantiates the finding of volume loss in the basal ganglia and thalamus of the brain reported in human patients. This confirms that mice generated using a two-stage genetic engineering strategy provide a comparative model of ADA deficiency. We also identified other affected brain regions and the progressive nature of brain pathology in this disease. This suggests that abnormalities in the brain are more severe and complicated than previously thought, and calls for further research. Histological studies are underway to provide insight into the causes of the changes, and there are also plans for longitudinal studies that use morphological changes as a biomarker to track disease development and evaluate therapy. This is the first report of brain morphological abnormalities in an animal model of ADA deficiency and our finding highlights the usefulness of brain phenotyping with high resolution MRI and TBM.

References

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