Absolute Quantification of Brain metabolites in a Mouse model of Huntington's Disease

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Synopsis: Analysis of magnetic resonance spectroscopy spectra using peak-area ratios is unsatisfactory because of the uncertain stability of the concentration of the reference metabolite (e.g. creatine (Cr)) and possible relaxation changes. Absolute quantification of metabolite concentrations avoids these problems. Previous studies of a transgenic mouse model (R6/2) of Huntington’s disease have identified the N-acetylaspartate (Naa)/Cr and Choline (Cho)/Cr ratios as surrogate markers for disease progression. These results demonstrate that [Naa] decreases and [Cho] increases with age supporting their use as more rigorous markers of disease progression.

Introduction: The R6/2 mouse is transgenic (TG) for the causative gene (IT15) in Huntington’s Disease. Magnetic resonance spectroscopy (MRS) studies of this model have shown that the peak-area ratio Naa/Cr decreases, whilst that of Cho/Cr increases, with age[1]. The uncertain stability of [Cr] and the possibility of relaxation changes suggest the use of both [Naa] and [Cho] as more readily interpretable surrogate markers for disease progression than peak-areas.

Purpose: To use absolute quantification to establish normal ranges for brain metabolite concentrations in wild-type (WT) control mice, investigate the temporal stability of [Cr], and test the hypothesis of decreasing [Naa] and increasing [Cho] in R6/2 TG mice.

Methods: 13 R6/2 mice and 11 age-matched littermate WT controls were studied between 6 and 15 weeks of age; a further 5 WT control mice were studied between 28 and 37 weeks of age. Halothane anaesthesia was used with continuous physiological monitoring[2]. A 7T Bruker spectrometer was used with a 12mm transmit/receive surface coil with the brain centered above the coil. After obtaining scout images in three orthogonal planes an 8 µl voxel (2x2x2 mm³) was positioned in either the left or right striatum. ¹H PRESS spectra were obtained using CHESS water suppression and with 256 summed transients, repetition time (TR) 5 s and echo time (TE) 10, 25, 135 and 270 ms in order to correct peak areas for transverse (T₂) relaxation. A further series of 11 spectra, acquired without water suppression (TE 10 to 400 ms), were used to calculate the voxel CSF fraction using a bi-exponential fit to the water peak amplitudes. Spectra were analysed using LCModel[3]. The water signal from an external concentration reference (ECR), obtained in a separate experiment, was used to facilitate the absolute quantification of metabolite concentrations using the method of Danielsen and Henriksen[4]. In this technique separate measurements of the first and second optimal pulse powers for a single water suppression pulse allows the calculation of the flip angle of the first pulse power. Knowledge of the pulse power and flip angle allows use of the principle of reciprocity to correct for differences in inter-subject and subject-ECR coil loading.

Results: The results from the TG mice were analysed in 3 groups: the entire age range (6 to 14 weeks); young mice (6 to 10 weeks, when HD symptoms were less severe); and older mice (10 to 14 weeks). Age-matched WT mice were used for comparisons.

Discussion: The use of metabolite ratios is attractive as they can be simply and automatically obtained using packages such as LCModel. However, rigorous interpretation assumes that the reference metabolite, such as Cr, has a concentration invariant with respect to pathology evolution. Furthermore, peak-area ratios can be modulated by changes in T₂ relaxation. Therefore, absolute quantification enables a more accurate characterisation of the model. The quantification technique used in this study reduces or eliminates several systematic and random errors. The ECR avoids assumptions about the concentration of an internal reference, such as brain water, which may alter with pathology. The principle of reciprocity removes assumptions about the equivalence of coil-loading and voxel-location conditions when comparing in-vivo and ECR data. [Cr] remained constant with pathology progression. However, [Cr] was higher in TG mice aged 6 to 14 weeks than in WT controls. For this reason, and potential relaxation effects, the rigorous comparison of peak-area ratios from TG and WT mice is problematic. The hypothesis of decreasing [Naa] and increasing [Cho] is confirmed by our results. [Naa] and [Cho] in young TG mice were comparable to those in age-matched WT mice whilst older TG mice showed significantly decreased [Naa] and significantly increased [Cho]. Reduced [Naa] and increased [Cho] may reflect a reduction in neuronal cell volume and either glial proliferation or increased membrane turnover respectively. (The increased [Cr] in the TG mice is less easy to interpret.) Our results therefore further advance the characterisation of the HD model and support the use of [Naa] and [Cho] as improved surrogate markers for disease progression and the effectiveness of putative therapeutic agents in the R6/2 strain.