MRI findings and neuropathology in a mouse model of maple syrup urine disease

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Introduction: Maple syrup urine disease (MSUD) is an inborn error of metabolism, resulting from branched-chain keto acid dehydrogenase (BCKADH) enzyme deficiency. This enzyme is involved in branched-chain amino acid (BCAA) catabolism. MSUD presents acutely with cerebral edema, and "dismyelination-like" white matter changes later in life. Classic MSUD presents with severe neonatal encephalopathy leading to a coma within the first few days of life. An intermediate form of MSUD exists where a low level of residual enzyme activity delays presentation of encephalopathy. Children with MSUD are well managed on a low BCAA diet, and liver transplant has been shown to be curative. However, encephalopathic crisis and brain injury may still occur in the context of metabolic decompensation from catabolic stress. Therefore additional protective strategy is needed in addition to low BCAA diets during critical periods of brain growth and development to prevent brain damage [2]. Here we use MRI and a novel mouse model of MSUD, with 5-6% of residual liver BCKADH activity [1], similar to intermediate MSUD, to determine long-term outcomes with normal and low BCAA diets.

Methods: The complete deletion of branched-chain keto acid dehydrogenase activity, E2, resulted in neonatal lethality of homozygous knockout mice [1], and several fold increase in circulating BCAA. Transgenic expression of a human E2 cDNA in the liver of the E2 knockout animals produced a model of intermediate MSUD (iMSUD). Branched-chain keto acid dehydrogenase activity was 5-6% of normal and was sufficient to allow 4-6 weeks survival. A group of N=6 iMSUD and N=6 heterozygote iMSUD mice was maintained on regular diet. A group of N=6 iMSUD mouse was maintained on a low BCAA diet (consisting of BCAA free diet and regular diet, so that animals was able to choose). Amino acids were separated and measured from HPLC of brain extracts by UV detection after phenyl-isothiocyanate derivatization. MRI was performed on a 7.0 T Bruker system using a 2 mm birdcage coil. Mice were imaged at 4 and 6 weeks of age. During the imaging, mice were kept anesthetized with isoflurane (1-1.5%). A T2-weighted multi-echo spin echo sequence was used (twelve 1.0 mm thick slices, TR/TE=3000/8.7-121.8 ms, 14 echoes, 117X117 μm^2 resolution, 2 averages). Transverse relaxation time constants (T2) were calculated on a pixel-by-pixel basis from the corresponding exponential fits. A 10 µm thick slices and antibody against glial fibrillary acidic protein (GFAP), neuronal-specific nuclear protein (NeuN), and neurofilament (N52) were used for immunohistochemistry. All slides were counterstained with DAPI.

Results: A significant accumulation of BCAAs in the serum and brain was measured in iMSUD mice on a normal diet compared to those on a low BCAA diet at 6 weeks of age (serum leucine: 204±21 vs 1528±231 µmole/l, brain leucine 18020±1430 vs. 4030±350 µmole/kg wet wt, $p<0.001$). All mice on the low BCAA diet had normal MRI findings at 4 and 6 weeks of age (Fig. 1), with similar T2-values as heterozygote iMSUD mouse. A T2-weighted MRI revealed two distinct neuropathologies among iMSUD mice on a normal diet (Fig. 1 B, C). Moderately injured iMSUD mouse had elevated T2-values in the striatum compared to iMSUD mouse on the low BCAA diet (94.9±7.1 vs 54.3±4.6 ms, $p<0.01$). Severely injured iMSUD mice had highly elevated T2-values in the occipital lobes compared to iMSUD mouse on a normal diet (198.3±15.1 vs 51.3±4.2 ms, $p<0.001$), and periventricular leukomalacia strikingly similar to human MSUD. Increased signal was observed in the caudate/putamen area, thalamus, occipital lobes and cerebellum (Fig. 1 B, C). There were no significant changes in T2-values from 4- to 6-weeks of age in iMSUD mice maintained on a low BCAA diet. Histology (H&E staining) of iMSUD mice on a normal diet revealed intense vacuole-like changes in the striatum where elevated T2-values (~94.9±7.1 ms) were observed (Fig. 2 F). These changes were mostly confined to the white matter tracts. Immunohistochemistry confirmed severe loss of neuronal process (Fig. 2 D, E) in these white matter tracts from similar sections. Gait disturbances and ataxia were apparent in all iMSUD mice on a normal diet, while not obvious in iMSUD mice on the low BCAA diet.

Conclusions: Residual BCKADH activity (5-6%) in the liver significantly improves survival from neonatal lethality to 4-6 week survival in iMSUD mice, suggesting a key role of liver in regulating circulating levels of BCAA. However, this residual liver activity is not sufficient to prevent MSUD associated neuropathology, and BCAA restriction is still necessary to avoid brain injury. A combination of MRI, histology and immunohistochemistry confirms that elevated T2-values often attributed to "dysmyelination" are due to intense vacuolation and loss of neuronal processes that make up white mater tracts within caudate/putamen, thalamus and cerebellum. Intermediate MSUD mice allowed to choose a low BCAA diet had improved neuroprotection and reduced serum BCAA. Presented results indicate the necessity of BCAA control to provide normal brain growth and development in the context of MSUD.

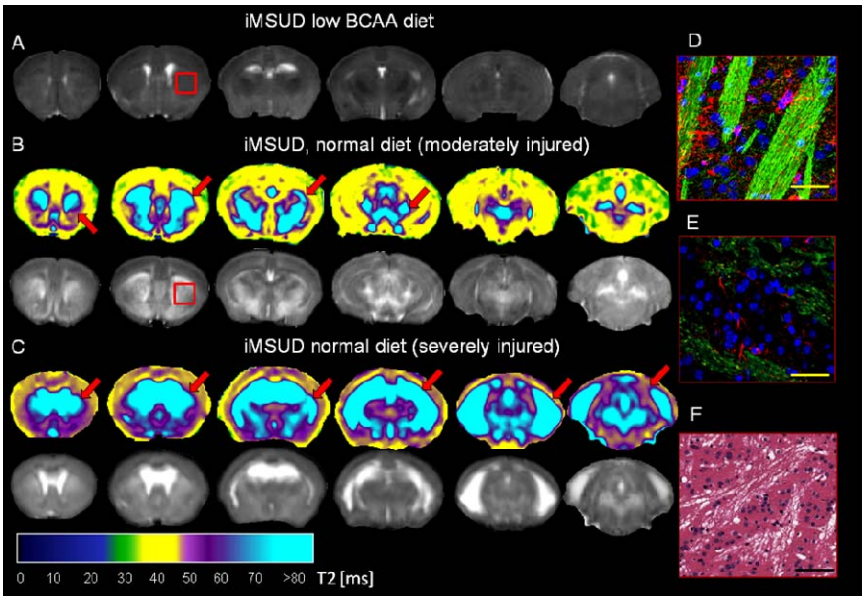

Figure 1. Representative T2-WI for iMSUD mice on low BCAA diet (A). Representative T-WI and with corresponding T2 maps for moderately injured (B) and severely injured (C) iMSUD mouse at 4 weeks of age. The injured areas are indicated with red arrow. Immunohistochemistry revealed loss of neuronal processes (N52 staining in green) in injured iMSUD mouse (E), but not in iMSUD on low BCAA (D). The approximate brain area examined for immunohistochemistry is shown as red square on MRI image. H&E histology revealed intense vacuolation of the white mater tracts within the striatum.
Shown scale bar= 37.5 µm.

References:

1. Homanics et al. BMC Med Genet, 2006. 7:p. 33.

2. Watford. Nutr Rev, 2007. 65(4):p. 167-72.