Increased dietary lysine and associated age dependent bilateral striatal damage in glutaryl-CoA dehydrogenase deficient mice

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INTRODUCTION: Glutaryl-CoA dehydrogenase (GCDH) activity is required for normal intramitochondrial oxidation of glutaryl-CoA produced by lysine and tryptophan catabolism. Autosomal recessive inheritance of mutant GCDH results in glutaric aciduria Type I (GA-1). The key neuropathologic feature of GA-1 is bilateral striatal degeneration with acute or chronic onset resulting in severe disabling dystonia and choreoathetosis (Goodman et al. 1977). Spongiform vacuolation, subdural hemorrhages and astrogliosis are common additional findings. The vacuolation may be present regardless of striatal injury. Some children, however, suffer striatal degeneration regardless of adherence to therapy (Strauss and Morton 2003). A mouse deficient in GCDH has been created (Koeller et al., 2002), however the mouse does not spontaneously develop neuropathology typical of the human disease. We hypothesized that elevated dietary lysine will lead to increased accumulation of glutaric acid and consequently trigger neuropathology in *Gcdh* -/- mice.

METHODS: *Gcdh* -/- mice and wild type (WT) littermate controls, both of mixed C57BL6/J X 129SvEv genetic background were used. The high lysine diet was prepared by adding free lysine to a standard diet to achieve 4.7% total lysine. MRI was performed on a 3.0 T Medspec S300 (Bruker). MR images were obtained from nine 4-week old *Gcdh* -/- mice, three WT and five 8-week old *Gcdh* -/- mice. Young 4-week old *Gcdh* -/- mice were imaged prior to and 3-5 days following high lysine diet exposure as they became hypoactive and hypothermic. Older (8-week-old) *Gcdh* -/- mice remained asymptomatic and were imaged after 6 weeks of high lysine, and sacrificed afterwards. Prior to imaging mice were anesthetized with 2 mg/kg xylazine and 15 mg/kg ketamine (i.p.). Each animal was imaged with a T_2-weighted multi-echo spin echo sequence (ten 0.5 mm thick slices, TR/TE=3000/10-152 ms, 15 echoes, 156X78 μm^2 resolution, 2 averages) and diffusion-weighted imaging (four 1 mm-thick slices, 0.5 mm slice separation, TR/TE=1500/70 ms, big delta=30 ms, small delta=16 ms, b-value=1030, 208X208 μm^2 resolution, 1 average, 288 s total imaging time). Transverse relaxation time constant (T_2) was calculated on a pixel-by-pixel basis from the exponential fit using CCHIPS software (Schmithorst et al., 2001). Calculated T_2 values are displayed as color-coded maps. Following imaging the mice were kept warm and allowed to recover. A neuron specific nuclear protein (NeuN) was detected using deparrafinized 10 μm thick coronal brains slices. The slides were incubated overnight with mouse monoclonal α-NeuN, and processed further using Vectastain ABC kits (Vector Labs, Burlingame, CA) per manufacturer's instructions and developed with DAB.

RESULTS: MRI findings were normal for all WT mice 3 weeks following the high lysine diet (Figure 1A). High lysine resulted in vasogenic edema within the striatum, hemorrhages, paralysis, seizures and death in 75% of 4-week-old *Gcdh* -/- mice after 3-12 days. All *Gcdh* -/- mice that became hypoactive exhibited loss of cerebral ventricles, deep cortical laminae and striatal signal hyperintensity on diffusion-weighted and T_2-weighted images 5-9 days after high lysine exposure (Figure 1B). Three *Gcdh* -/- mice that were symptomatic after 9 days of high lysine had elevated T_2-values in the cortex and significantly ($p < 0.02$) elevated T_2-values in the striatum compared to WT. A *Gcdh* -/- mouse developed bilateral striatal injury evidenced by highly elevated T_2-values (~105 ms) and diffusion-weighted signal hyperintensity in the striatum, deep cortical laminae and lateral hypothalamus after 12 days of high lysine (Figure 1C). Acute hemorrhage was present in this and three other Gcdh -/- mice prior to death (Figure 1C, red arrow). Older (8-week-old) *Gcdh* -/- mice were imaged prior to and 6 weeks following exposure to the high lysine diet. All 8-week-old *Gcdh-/-* mice developed bilateral signal hyperintensity in the caudate/putamen (Figure 1D) with significantly ($p < 0.001$) increased T_2 values in the striatum and cortex compared to WT. Histological examination of striatum in 8-week old animals on the high lysine diet revealed numerous vacuoles within striatal patches (Figure 2) and selective loss of cortical neurons was evident in 8-week old animals on a high lysine diet for 6 weeks.

CONCLUSIONS: Presented results indicate that the *Gcdh* -/- mouse exposed to increased dietary lysine may be a good model to study the development of neuropathology that characterizes human GA-1. Presented data support life long protein and lysine restrictions. Further study of this model may provide the basis underlying the neuropathology, which might then be used to protect children identified by newborn screening from devastating striatal damage.

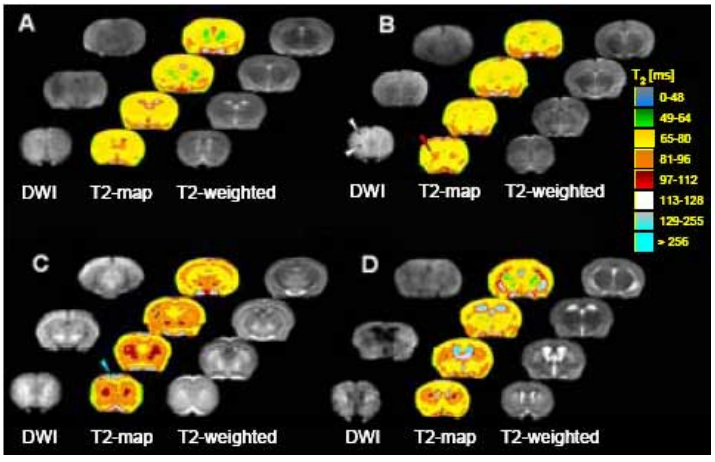

Figure 1. (A) WT mice on the high lysine diet for 3 weeks. (B) Gcdh -/- mice 4-weeks old after 9 days on the high lysine, and after 12 days on the high lysine (C). (D) Gcdh-/- mice, 8-week old after 6 weeks of the high lysine diet. Color scale represents different T2 values.

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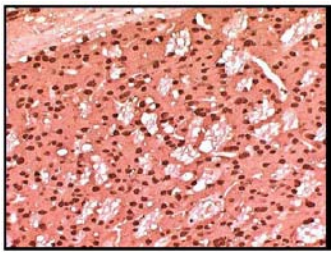

Figure 2. Prominent vacuolation of the striatal white matter patches in 8-week old Gcdh -/- mice after 6 weeks on the high lysine.

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