# Proton MRS shows cerebral accumulation of neurotoxic 3-hydroxyisovaleric acid in 3-methylcrotonyl-CoA-carboxylase deficiency

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## Introduction

3-Methylcrotonyl-CoA-carboxylase (MCC; EC 6.4.1.4) is a biotin-dependent enzyme in the L-leucine degradation pathway (Fig. 1). 3-Methylcrotonyl-CoA-carboxylase deficiency (MCCD) affects leucine metabolism and results in an accumulation of 3-hydroxyisovaleric acid (3HIVA) and 3-hydroxymethylcrotonylglycine. Reported outcomes range widely: from asymptomatic to death in infancy. As the majority of identified MCCD patients (>90%) develop no symptoms and it largely represents as a nondisease, it is under debate if newborn screening for MCCD is justified [1].

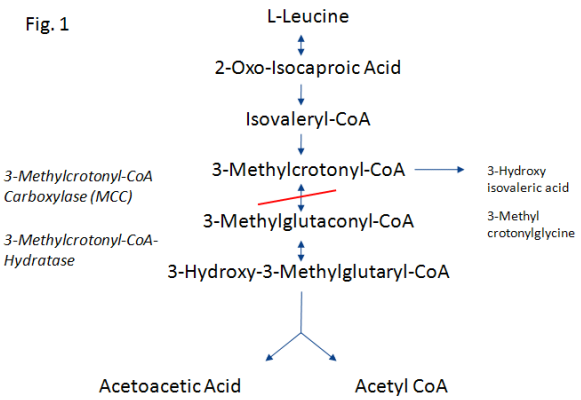
A previous *in vivo* MRS and *in vitro* NMR study showed an MRS-detectable resonance of 3HIVA in cerebral white matter of a patient presenting with a slowly progressing leukoencephalopathy [2]. This accumulation of neurotoxic 3HIVA turned out to originate from a defect in the enzyme 3-methylglutaconyl-CoA-hydratase (EC 4.2.1.18; Fig.1). As the enzymes MCC and 3-methylglutaconyl-CoA-hydratase play a role in two consecutive reactions in the L-leucine catabolic pathway, it was hypothesized that also MCCD may result in 3HIVA accumulation in the brain. The **aim of this study** was to investigate if proton MRS could detect altered brain metabolism - in particular elevated 3HIVA - in a patient with proven MCCD.

## Methods

*Subject*: A female patient positive for MCCD in newborn screening was investigated by MRI and MRS at the age of 5 months. After this investigation she started with a low-protein diet (1 gr/kg/day) supplemented with an amino acid mixture. The patient underwent another MRI/MRS investigation at the age of 21 months.

*MR measurements:* High resolution one-dimensional *in vitro* $^1$H-NMR spectra of urine (pH adjusted to 2.5) from the patient at approximately 1 month after birth were acquired at 500 MHz on a Bruker DRX spectrometer; for experimental details see [1]. MRI and *in vivo* MRS measurements were performed on a Siemens Magnetom Tim-Trio System (Siemens, Erlangen, Germany) operating at 3T. The patient underwent a standard MRI protocol including T1w, T2w, TIR, FLAIR, and at the age of 21 months also an diffusion-weighted MRI measurement. At both ages, MRS was performed using a 2D multivoxel semi-LASER sequence [3] (TE 30 ms, TR 2000 ms) with and without water suppression to obtain multiple spectra in gray and white matter in a slab through the ventricles. In addition, single voxel MRS measurements were performed in a voxel positioned in periventricular occipital white matter using a PRESS sequence with short and long echo times (TE 30 ms, TR 5000 ms and TR 136 ms, TR 2000 ms). The data was analyzed with jMRUI.

Fig. 1



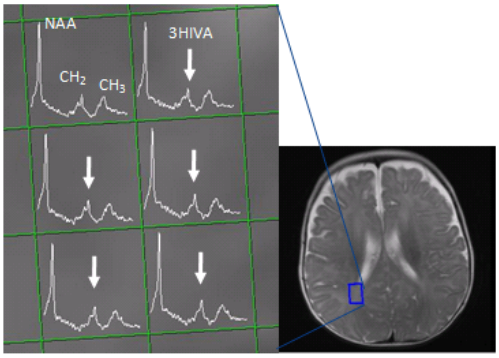




Fig. 2A Part of a 2D MRSI measurement obtained at 5 months.

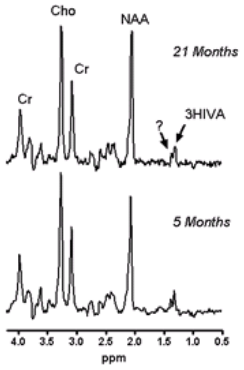



Fig. 2B PRESS spectra (TE 136 ms) obtained from a comparable location as shown in 2A.

## Results and Discussion

High resolution $^1$H NMR spectra of urine of the patient showed highly increased concentrations of 3HIVA (5860 µmol/mmol creatinine vs < 10 in controls) and 3-methyl crotonylglycine (1290 µmol/mmol creatinine vs < 20 in controls)

MRI obtained at the age of 5 months indicated a slightly relayed myelination in frontal and occipital areas, which was normalized at 21 months.

MRS showed predominantly in white matter a clear single resonance at 1.28 ppm superimposed on the broader signal of the methylene protons of macromolecules, which could be assigned to 3HIVA (Fig. 2A). Based upon the relative intensity of the 3HIVA signal originating from two methyl groups and the methyl proton signal of creatine, the brain 3HIVA concentration was roughly estimated to be in the order of magnitude of 0.4 – 0.6 mmol/l. At the age of 21 months, the 3HIVA resonance is still detectable with comparable intensity (Fig. 2B). Note that the singlet signal of HIVA has a positive signal intensity at TE 136 ms. In addition to the 3HIVA resonance, the PRESS spectra acquired with TE 136 ms shows another - still unidentified - signal at 1.35 ppm. No *in vivo* signal of 3-methylcrotonyl glycine could be detected, but this is hardly possible due to the multiplet character of its resonances and overlap with signals of other brain metabolites. Considering normal brain metabolites, Figure 2B shows that the ratio NAA/Cho has increased from 5 to 21 months as can be expected for a developing brain. However, the spectrum obtained at 21 months still shows a relative low NAA/Cho ratio for this age, which may indicate that myelination has not yet completely normalized.

## Conclusion

To the best of our knowledge this is the first time that MRS has shown the presence of 3HIVA in the brain of a patient with MCCD. Since 3-HIVA is considered to be neurotoxic [4], early detection is relevant as diet adjustments can be made to minimize its accumulation, which justifies newborn screening for MCCD.

**References: [1]** Stadler SC et al., *Hum Mutat*, 2006;27:748-759, **[2]** Engelke UFH et al., *NMR Biomed*, 2006;19:271-278, **[3]** Scheenen TW et al. *Magn Reson Med*, 2008;59:1-6. **[4]** Duran M et al., *J Inherit Metab Dis* 1993;16:513-6.