1H MR Spectroscopy of Creatine Deficiency Syndrome Caused by a Novel Mutation

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Introduction: In the recent past, several patients with inborn errors found in the creatine (Cr) metabolic pathway – AGAT (arginine/glycine amidinotransferase), GAMT (guanidinoacetate methyltransferase) and creatine transporter (CRTR) deficiencies[1,2] – have been described. MR imaging of the brain in these cases is usually without visible pathologic changes, but in MR spectra there is a lack of Cr signal whereas other metabolites remain unchanged.
This study deals with the potential of ^{1}H MR spectroscopy in differential diagnosis and quantitative description of creatine deficiency disease in two siblings with a new, as yet unknown, mutation of the CRTR syndrome.

Subjects and Methods: Two siblings, (male, 6 years old; female, 7 years old), underwent MR examination because of suspected creatine deficiency syndrome. In the female, mild psychomotor delay had been described. Her brother suffered from severe psychomotor retardation, speech delay, muscle hypotonia, epilepsy and signs of autism. Following enzymatic tests, CRTR deficiency was confirmed in both siblings. A second MR examination was done one year later after the male received several months of Cr monohydrate supplementation treatment.
MR examination was performed using a Siemens Vision 1.5T system. After standard MRI examination ^{1}H MR spectroscopy using CSI sequence (TR/TE=1500/40 ms, 2 acquisitions, 18 mm slice thickness) in the transversal plane was applied. Single voxel STEAM sequence in the parieto-occipital white matter was used to confirm the CSI results.
Spectra were evaluated automatically using the Culich[1] program and concentrations of 14 metabolites were calculated. Signals of lipids and macromolecules were eliminated in the LCModel procedure. Corrections on CSF, relaxation times and actual quality control were applied. The patients' data were compared to those of control groups used for these purposes.
Genetic analysis was performed using the PCR method from genomic DNA of the affected probands. The *SLC6A8* gene located on the X chromosome was sequenced.
Results: MR imaging repeatedly did not show any significant pathological changes in either patient. Contrarily, the MR spectroscopic examination of the male revealed a significantly reduced signal of Cr (see Figure 1) whereas spectra in his sister appeared normal. Decreased Cr concentration was independent of voxel position and similar in both hemispheres. To quantify differences between the siblings, the mean values of N-acetylaspartate (NAA), total creatine (Cr) and choline compounds (Cho) concentrations in the whole measured areas are summarized in Table 1. The concentration of Cr in the male was found to be approximately three times lower than in his sister. The concentrations of other metabolites between the siblings differed around 10%. The results of the second examination showed another decrease in Cr concentration in the male despite creatine supplementation; NAA remained stable, and choline compounds slightly increased. In the female, all studied compounds remained in the range of controls.
Sequence analysis of the *SLC6A8* gene revealed a single base deletion del c.219C in our male patient. This nonsense mutation causing frameshift and a premature stop codon at position 96 is clearly pathogenic. The patient's sister, presenting with a mild phenotype of the disease, carried the same mutation in the heterozygous state. Presence of the mutation was confirmed by sequencing a cloned corresponding fragment of genomic DNA of the affected male.

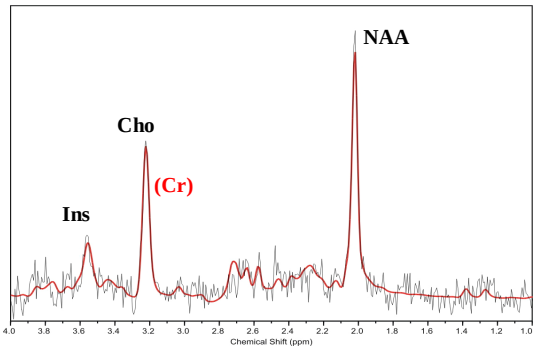

Figure 1. Brain ^{1}H MR spectrum (TE=40 ms) with calculated fit using LCModel of the male patient. The striking decrease in the Cr signal cannot be overlooked. N-acetylaspartate (NAA), total creatine (Cr), choline compounds (Cho), myo-inositol (Ins).

Table 1. Mean concentrations [mM] of selected metabolites over the whole examined area in the brain obtained in the first and second MR spectroscopic examinations. Standard deviations are in parentheses.

		NAA+NAAG	Cr	Cho
Male	**First**	8.22 (1.6)	1.49 (0.4)	0.95 (0.2)
	Second	8.29 (1.3)	1.34 (0.5)	1.11 (0.3)
Female	**First**	9.07 (1.5)	4.92 (1.3)	1.06 (0.2)
	Second	9.76 (1.8)	5.55 (1.0)	1.24 (0.2)
Controls		9.47 (1.2)	7.47 (1.6)	1.54 (0.6)

Discussion: The first MR spectroscopic examination did not revealed a signal of guanidinoacetate in the spectra and because of changes only in the male's spectra and his clinical status, a hypothesis of presumed CRTR deficiency was made. Based on spectroscopic findings, treatment using creatine supplementation was initiated only in the male and enzymatic tests were requested for both siblings. The repeated MR examination one year later together with unsuccessful treatment supported the hypothesis of CRTR deficiency. The final diagnosis of CRTR deficiency was later confirmed in both patients using genetic tests. Sequence analysis revealed an as yet undiscovered mutation in exon 1 of the *SLC6A8* gene.
We can presume that only two examinations using ^{1}H MR spectroscopy performed before and during creatine supplementation treatment are sufficient for the differential diagnosis of CRTR deficiency. Our data point out that although the Cr signal can be almost invisible in MR spectra from the qualitative point of view, the calculated concentration still remains above 1 mM. Based on MR spectroscopy, we assume that in the male the function of the Cr transporter is probably substituted by other mechanisms.
Spectra of the female patient reflect almost normal brain development. Marginal Cr concentration can probably indicate hidden disturbances in creatine metabolism and it is in line with the mild phenotype expected in heterozygotes.

Conclusion: We described two siblings with confirmed CRTR deficiency due to a novel mutation of the *SLC6A8* gene. We conclude that proton MR spectroscopy performed before and after treatment by creatine supplementation in suspected patients can, together with clinical symptoms, unambiguously distinguish CRTR deficiency.

References: **1**. Salomons GS, et al. (2003) J Inher Metab Dis 26(2-3), 309-318;
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