



Case Report

Creatine transporter deficiency: Novel mutations and functional studies



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ABSTRACT

X-linked cerebral creatine deficiency (MIM 300036) is caused by deficiency of the creatine transporter encoded by the *SLC6A8* gene. Here we report three patients with this condition from Israel. These unrelated patients were evaluated for global developmental delays and language apraxia. Borderline microcephaly was noted in one of them. Diagnosis was prompted by brain magnetic resonance imaging and spectroscopy which revealed normal white matter distribution, but absence of the creatine peak in all three patients. Biochemical testing indicated normal plasma levels of creatine and guanidinoacetate, but an increased urine creatine/creatinine ratio. The diagnosis was confirmed by demonstrating absent ¹⁴C-creatinine transport in fibroblasts. Molecular studies indicated that the first patient is hemizygous for a single nucleotide change substituting a single amino acid (c.619 C>T, p.R207W). Expression studies in HeLa cells confirmed the causative role of the R207W substitution. The second patient had a three base pair deletion in the *SLC6A8* gene (c.1222_1224delTTC, p.F408del) as well as a single base change (c.1254+1G>A) at a splicing site in the intron-exon junction of exon 8, the latter occurring *de novo*. The third patient, had a three base pair deletion (c.1006_1008delAAC, p.N336del) previously reported in other patients with creatine transporter deficiency. These three patients are the first reported cases of creatine transporter deficiency in Israel.

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1. Introduction

Creatine is essential for the utilization of ATP at sites of high energy requirement such as muscle, brain and heart [1,2]. In adult humans, about half of the creatine needed is obtained by dietary intake, mostly from meat and dairy products, and the other half is synthesized in the kidney and liver [3]. Creatine and its phosphorylated form, phosphocreatine, spontaneously break down to creatinine that is excreted in the urine [3]. The loss of creatine (as creatinine) is restored by new enzymatic synthesis and by dietary intake.

Creatine deficiency syndromes are a group of rare diseases that include two autosomal disorders of creatine biosynthesis (arginine: glycine amidinotransferase (AGAT) deficiency, OMIM 612718; and guanidinoacetate methyltransferase (GAMT) deficiency, OMIM 612736) and the X-linked creatine transporter deficiency (OMIM 300352) [4–7].

The *SLC6A8* gene encodes the creatine transporter 1 (CT1 or CRTR), maps to Xq28 and is expressed in most human tissues, with highest levels found in skeletal muscle and kidney [8]. In the brain, the creatine transporter is strongly expressed, especially in the large projection neurons of the brain and spinal cord [9]. Defects in this transporter results in brain creatine deficiency that, in hemizygous males, is characterized by moderate to severe intellectual disability, delays in language and speech, autistic-like behavior, seizures in about 50% of cases, and mid-facial hypoplasia and short stature in some patients [10–13].

The diagnosis of brain creatine deficiency is suspected based on clinical presentation with usually increased creatine/creatinine ratio and/or reduced brain creatine content by magnetic resonance spectroscopy, and confirmed by measurement of long-term creatine accumulation in fibroblasts and/or DNA sequencing of the *SLC6A8* gene [10,13–15].

According to the LOVD website (Updated May 02, 2016), there are currently 141 unique DNA variants of the *SLC6A8* gene (https://grenada.lumc.nl/LOVD2/vumc/home.php?select_db=SLC6A8). The prevalence of *SLC6A8* mutations in males with X-linked intellectual disability is between 1 and 5.4% [10,12,16–22]. In this report, we describe three male patients with creatine transporter deficiency from Israel.

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2. Case reports

Patient 1 is a male who presented at 10 months of age with global developmental delay. He had failure to thrive, borderline microcephaly and significant hypotonia without dysmorphism. An initial MRI/MRS at 2 years of age identified non-specific changes in the white matter, but the lack of the creatine peak was not correctly identified at that time. A repeated MRI/MRS at 3 years of age demonstrated the lack of the creatine peak (Fig. 1A). Biochemical testing indicated increased urine creatine of 2.9 mmol/mol creatinine (normal 0.006–1.2 mmol/mol creatinine) with normal guanidinoacetate. At 8.5 years of age he has mild to moderate intellectual disability, significant language dyspraxia, and attended a special education school in special educational setting. The mother was low performing and has borderline microcephaly. She completed high school, but it is unknown whether she received a diploma. Her biochemical testing indicated normal urine creatine of 0.089 mmol/mol creatinine (normal range: 0.011–0.24 mmol/mol creatinine) and guanidinoacetate of 29.3 mmol/mol creatinine (normal range: 3–78 mmol/mol creatinine).

Patient 2, a male, was referred at 11 months of age for global developmental delay, axial hypotonia of trunk with hypertonicity of extremities. His MRI at 1.5 years of age was normal, but MRS demonstrated no creatine peak (Fig. 1B). Urine creatine was 3 mmol/mol creatinine (normal 0.006–1 mmol/mol creatinine) with normal guanidinoacetate. At 5.5 years of age, the patient has severe intellectual disability, 5–10 purposeful words, and walks independently with wide-based gait. The mother seemed to have functional and intellectual difficulties (with no microcephaly), but completed high school. It is unclear whether she received a diploma. She did not go through a formal evaluation, but MRS in the mother demonstrated a brain creatine peak lower than normal (Fig. 1C).

Patient 3, a male, presented at one year and 10 months of age with severe motor and language delays. The mother has normal intelligence and is normocephalic. Over time, the child acquired mild microcephaly. At 7.5 years he has speech apraxia, borderline intelligence with significant difference between verbal IQ (VIQ 48) to performance IQ (PIQ 102), and ADHD treated with methylphenidate. Urine creatine was 3 mmol/mol creatinine (normal 0.006–1.2 mmol/mol creatinine) with normal guanidinoacetate and subsequent brain MRS showed the absence of creatine peak (Fig. 1D).

3. Materials and methods

3.1. Biochemical testing

Measurement of plasma and urine guanidinoacetate, creatine and creatinine was performed using gas chromatography mass spectrometry

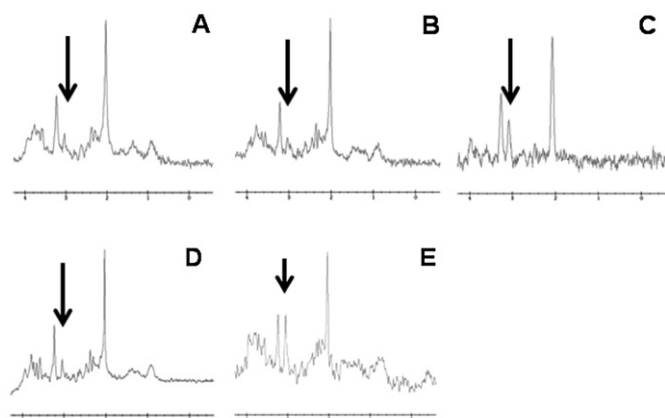


Fig. 1. Magnetic Resonance Spectroscopy (MRS) of the brain in patients with creatine transporter deficiency. A. Patient 1; B. Patient 2; C. Mother of patient 2; D. Patient 3; E. Normal control. The arrow indicates the creatine peak. Note diminished creatine peak in all patients with creatine transporter deficiency and mildly reduced creatine peak in the mother of patient 2.

in a hospital laboratory (Sheba Medical Center, Tel Hashomer, Israel) according to standard procedures [23].

3.2. Patients/samples

Fibroblasts from the three pediatric patients as well as the mother of patient 2 and DNA samples from blood of the parents of patient 1 were collected according to an Institutional Review Board-approved protocol. Fibroblasts were cultured in DMEM supplemented with 15% fetal bovine serum and 1% L-glutamine. HELA cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% L-glutamine.

3.3. Creatine transports

Creatine transport was measured at 37 °C with the cluster-tray method as previously described [22]. Briefly, cells were grown to confluence in 24-well plates (Costar) and depleted of intracellular amino acids by incubation for 60 min in Earle's balanced salt solution containing 5.5 mM D-glucose and supplemented with 0.5% bovine serum albumin. Cells were then incubated with [¹⁴C]-creatine 0.1 μCi/ml (Moravak Biochemicals, Brea, CA) containing the indicated concentration of cold creatine (2 μM) for 60 min. Non-saturable creatine transport was measured in the presence of 2 mM unlabeled substrate. The transport reaction was stopped by rapidly washing the cells 4 times with ice-cold 0.1 M MgCl₂. Intracellular creatine was extracted from the cells with 0.5 ml of ice-cold ethanol and added to 5 ml of scintillation fluid for counting. Intracellular creatine was normalized for the protein content of each well and intracellular water content and expressed as nmol/ml cell water/h. Saturable creatine transport was calculated by subtracting transport in the presence of excess (2 mM) cold substrate from total transport and values are reported as means ± SE of 3–6 independent determinations.

3.4. DNA sequencing

DNA was extracted from fibroblast cultures using MagNAPure Compact instrument (Roche Applied Science, Indianapolis, IN). The *SLC6A8* gene was sequenced using primers flanking each exon as previously described [16]. Sequence analysis was performed using BigDye v3.1 terminator and an ABI 3130xl (Applied Biosystems). Sequences were analyzed using the Mutation Surveyor software package (Softgenetics, State College, PA, USA).

3.5. HELA cell transfection

HELA cells were transfected using Lipofectamine-2000 (Invitrogen) and selected for 3 weeks with 400 mg/ml of G418 (Invitrogen). Stable transfectants were used for transport experiments.

Site Directed Mutagenesis was performed using QuikChange (Agilent) with a pCMV6-AC-GFP containing the cDNA of *SLC6A8* as a template (Origene, RG209828).

4. Results and discussion

4.1. Creatine transport by human fibroblasts

The clinical presentation and the laboratory results of plasma and urine guanidinoacetate, creatine and creatinine measurements suggested a defect in the creatine transporter. For this reason, a skin biopsy was obtained from each patient as well as the mother of the 2nd patient to confirm a defect in cellular creatine transport. Net creatine uptake in cells from all three patients was <10% of normal fibroblasts (Fig. 2). The fibroblasts from the mother of the second patient had normal creatine uptake (Fig. 2). These latter results show that in a population of cells with random X-inactivation the results of an inactivating mutation might not result in significant changes in creatine transport. A similar phenomenon might happen in the kidney, explaining why urinary

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