

## **A case of Creatine Transporter Deficiency in a Young Child with Choreoathetosis**

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### **Abstract**

**We report a case of creatine transporter deficiency in a young boy who has a constellation of symptoms and signs that are similar to what have been reported with the exception of his severe movement disorder. The child had besides developmental motor and cognitive delay very marked choreoathetosis that has been refractory to all treatment. The child presented at an early age with developmental delay and while being investigated he developed involuntary dystonic movements. The child was investigated thoroughly and all other possible causes have been ruled out. The diagnosis was reached through urine creatine:creatinine ratio, decreased creatine uptake in fibroblasts culture, decreased creatine peak on the proton magnetic resonance spectroscopy (MRS) and by molecular DNA testing.**

### **Introduction**

Creatine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to muscle. In humans, about half of the daily creatine is biosynthesized from three different amino acids - arginine, glycine, and methionine usually in the liver, kidney, pancreas and the central nervous system (CNS), the rest is taken in by alimentary sources. Ninety-five percent of creatine is later stored in the skeletal muscles [1-3]. Creatine biosynthesis involves two mitochondrial enzymes; L-arginine:glycine amidinotransferase (AGAT), which is responsible for catalyzing arginine and glycine into the intermediate guanidinoacetate (GAA), and guanidinoacetate methyltransferase (GAMT) which converts GAA to creatine [4-6]. In the 1920s it was discovered that creatine played a crucial part in the structure and function of adenosine triphosphate (ATP), the body's prime energy source [4]. In the CNS, creatine has many functions including; role in the growth cone migration and dendritic and axonal elongation, role in Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, maintenance of membrane potential, Ca<sup>2+</sup> homeostasis and the restoration of ion gradients [4,7]. Creatine was also recently hypothesized to act as a central neuromodulator, and particularly as co-transmitter on gamma aminobutyric acids GABA postsynaptic receptors [6]. Finally, creatine has been proposed to regulate appetite and weight by acting on specific hypothalamic nuclei [8]. There are at least two specific creatine transporter systems (SLC6A8) re-

sponsible for delivering creatine to different tissues where it is utilized by the creatine kinase (CK) system to generate cellular energy; the plasma membrane creatine transporter; and the mitochondrial creatine transporter. One of the body's most critical creatine transporter systems is responsible for delivering creatine from the blood stream to the brain cells [3, 8-10], however it seems that this is limited to the neurons but not to the astrocytes [11]. In contrast to the absence of SLC6A8 in astrocytes, microcapillary endothelial cells from the blood-brain barrier BBB and the blood-retina barrier do express SLC6A8 and are able to take up creatine [3,11,12]. SLC6A8 is also expressed by the choroid plexus and the ependymal epithelia [3]. Once inside the cytoplasm of the neurons and glia cells creatine is stored where it is readily available to be trans-phosphorylated by the CK system into phosphocreatine (PCr) for energy consumption inside the mitochondria [3,13]. To mediate this process, the mitochondrial creatine transporter, which sits on the inner mitochondrial membrane, is called upon to transport the creatine from the cell's cytoplasm to the mitochondria.

This system maintains constant supply of energy to the brain and therefore significantly contributes to brain function. Constant supply and delivery of creatine therefore can be interrupted at three levels; if either enzymes AGAT or GAMT are deficient then the biosynthesis is absent, the third level is when the transport system SLC6A8 is missing. Creatine Transporter Defect (CTD)

recently recognized genetic defect interrupts the transport of creatine into the brain cells leading to a significant psychomotor dysfunction, however most of the reports describe a constellation of severe mental retardation, epilepsy, speech/language delay and autistic-like behavior.

In patients with defective creatine transporter system, creatine is noticeably absent from the brain. While creatine supplementation increases the body's level of creatine and phosphocreatine in the muscles, it is ineffective in treating those symptoms that result from a defective creatine transporter system.

The CT system SLC6A8 is a polypeptide that is encoded by a gene mapped to Xq28 [14].

### **Case report**

A 7 month old infant presented to the pediatric outpatient clinic at Shaikh Khalifa Medical City in Abu Dhabi, United Arab Emirates with developmental delay. The mother who is healthy 32 year old gravida 8 para 5 had an uneventful pregnancy. She had no history of significant illnesses, hypertension, diabetes or bleeding. The mother denies being exposed to any toxins or taking any medications. The delivery was spontaneous vaginal at full term with a birth weight of 3.26 KG. The newborn had no history of respiratory distress or any significant neonatal complications. At the age of 4-5 months the mother noted the infant to be different from his siblings; smiling happened at around 2 months of age, head control was still not achieved, the infant subsequently failed to reach any milestones including rolling over, reaching or transferring with his hands. Between 6-9 months the infant started to have involuntary posturing movements which was initially episodic then became continuous, the movements involved the face, the extremities and even the trunk, this progressively worsened and became quite prominent. The infant was feeding initially breast milk then switched to formula, sucking was however normal. He received all his immunizations. He was not on any medications at the time of presentation. In the family history, the parents have common great-great grand parents; the father has a brother with seizures since the age of 13 years, a sister who died in sleep at the age of 18, and another brother who died in infancy from unknown cause. The mother had 3 miscarriages. The child's physical exam at the age of 7 months revealed a fairly normal growth; the weight was 6.84 KG (5-10<sup>th</sup> percentile), the length was 68 cm (25<sup>th</sup> percentile), the head circumference was 42.8 cm (10<sup>th</sup> percentile). The vitals were unremarkable, there were no dysmorphic features, the head, nose and throat, the chest, the cardiovascular and abdominal exams were all unremarkable. The central nervous system (CNS) exam revealed an obvious delayed infant, with obvious

intact hearing and vision, symmetrical facial features, and intact cranial nerves, choreoathetoid movements, absent neck support, hypertonic extremities, hypotonic trunk, and brisk upper and lower limbs tendon reflexes, but no clonus. Muscle bulk was decreased, and there were no contractures. The infant was then assessed to have psychomotor developmental delay, and dystonia/ choreoathetoid movements. The differential diagnosis included a congenital CNS structural dysgenesis, antenatal or neonatal CNS insult, and an in born error of metabolism disease. The infant underwent extensive investigations locally and abroad. He was diagnosed and followed at the Boston Childrens' Hospital in Boston Massachusetts, USA.

The investigations collectively included hemogram, electrolytes, blood urea, creatinine, serum ammonia, thyroid stimulating and free triiodothyronine hormones, venous blood gases, serum amino acids, liver enzymes, biotinidase, copper, caeruloplasmin, lysosomal enzymes, carnitine profile (free, total, and acylcarnitine), serum lactate, pyruvate, very long chain fatty acids (VLCFA), serum and urine acylglycine, Fish chromosomal studies, urinalysis, urine organic acids, urine sulphite, urine reducing substances, urine mucopolysaccharidosis screen, cerebrospinal fluids (CSF) amino acids, neurotransmitters and lactate, all of which was either normal or not conclusive of any specific metabolic disorder. The abnormal results were intermittently elevated lactate and ammonia, low plasma creatinine, and a urine organic acid profile that was interpreted as reflecting generalized mitochondrial dysfunction because of increased excretion of the tricarboxylic acid (TCA) cycle intermediates, 2-ketoglutaric acid and citric acid, as well as mildly increased excretion of ethyl-malonic acid and 3-methylglutaconic acid. He also had head computed tomography with contrast, magnetic resonance imaging (MRI) of the brain, electroencephalogram (EEG), visual evoke potential, brain stem auditory evoke response, electromyogram (EMG), nerve conduction study, electrocardiogram (ECG) and echocardiogram. During his assessment at the age of 18 months at the Childrens' Hospital in Boston, Massachusetts, USA by the neurological and metabolic teams, he had amongst his work up a brain MRI and MRS. The former showed abnormally increased signal in the periaxial white matter on T2 weighted images with associated small corpus callosum. The latter showed complete absence of creatine peak, subsequently serum and urine levels of GAA and creatine were measured, as well urine creatine:creatinine ratio was calculated, the workup for creatine metabolism was conclusive of CTD as there was high urine creatine, high urine creatine:creatinine ratio, normal serum GAA and creatine levels and normal urine GAA level. As the CSF lactate and MRS lactate peak were normal it was concluded that the intermittently elevated serum lactate is probably factitious. Once the diagnosis of CTD was

reached the child was started on vitamin B6, coenzyme Q, carnitine and creatine, and subsequently underwent muscle and skin biopsies while a gastrostomy tube is being placed, the child was also placed on benzhexol, and lorazepam to try to control his choreoathetoid movements. The Electron microscopy of the muscle biopsy showed normal number and morphology of mitochondria however the electron transport chain studies was reported showing a low functioning complex 1, histologically there was focal predominance of type I fibers, mildly increased amounts of lipid in all fibers, and normal PAS, NADH and AT-Pase stains. Skin fibroblast culture demonstrated deficiency of creatine uptake further confirming the diagnosis. The molecular genetic study showed a large genomic deletion of exons 8 to 13 in the SLC6A8 gene at Xq28. The mother had genetic testing after identifying the mutation and found to be negative. Therefore it was concluded that the mutation is probably *de novo*, although one can't exclude the possibility that the mother could be a carrier of a germ line mutation. Subsequently the child visited the same hospital in USA twice at the age of 33 months and five and a half years during both visits a repeat MRS failed to show any evidence of the creatine peak to have increased or changed despite the continuous supplementation of creatine; further confirming the diagnosis of transporter disorder. Clinically and neurologically the child continued to be severely delayed with minimal motor functions, severe cognitive delay and severe uncontrollable choreoathetoid movements. It was not possible to look for any autistic features in this child as his level of mental retardation was quite severe, although the child did have eye contact and would smile at those who he became familiar with, a behavior that is not consistent with the autistic tendencies. The most difficult part was the extent of the choreoathetoid movements that would not respond to any anti-movements medications including levodopa, sinemet (combination of levodopa and carbidopa), lorazepam, clonazepam, risperidone, haloperidol, benzhexol, and carbamazepine, although the later may have had some modest effects.

## Discussion

Investigation supported a CTD include MRS lack of creatine peak, reduced fibroblast culture creatine uptake, and a large deletion in the SLC6A8. The crucial step in the diagnosis process was clearly the MRS scan which has the potential to be utilized more as more substances peaks can be measured.

The clinical picture of this case is unique and perhaps different from what has been reported. There were no obvious autistic features, the speech was literally absent due to mental retardation. We noticed that the child had good eye contact with the medical staff, with his relatives and

care giver, a feature that is not consistent with autism. The main and prominent feature however was the severe dystonic/choreoathetoid movements uncontrolled by anti-convulsants or anti-movement drugs. This clearly widens the phenotypical spectrum of CTD.

It seems that there was some degree of mitochondrial dysfunction evidenced by the intermittent/transient hyperammonemia and lactic acidemia, along with subtle mitochondrial respiratory chain enzyme deficiencies and mild myopathy. This can be explained by mitochondrial energy depletion and the specific importance of creatine to the mitochondrial respiratory chain [15-16].

CTD is an x-linked inherited disease which has been described about 10 years ago; the protein is encoded by a gene locus on chromosome X [14]. The polymorphism leads to absence of the protein or dysfunction. The result is disabled transport of creatine and subsequently defects in the energy production and possibly in neurotransmission. The role of creatine in the brain is probably not only related to the energy production, it has been shown in *in vitro* studies that guanidino compounds, including creatine may affect GABA neurotransmission function as a partial agonists for GABA receptors, it was shown that creatine is released in the central nervous neurons in a parallel fashion to that of the classical exocytotic one, this led to the hypothesis that creatine is a co-transmitter in the brain that modulate post synaptic transmission [7,17]. The symptoms' constellations vary; the reports so far describe motor and cognitive delays, autistic spectrum, speech and language delay, movements' disorder and seizures [18-21].

There has been 34 cases reported worldwide [22] with varying age at diagnosis, varying clinical symptoms, and diagnosis through either biochemical testing, MRS [23,24] or DNA sequence analysis [21,22].

There is no effective treatment for CTD as supplementation with large doses of creatine didn't prove effective in any of the reported cases (including ours) and failed to induce transportation of creatine inside the cell or the mitochondria [14,25-27]. There are current experimental attempts looking at stimulating the biosynthesis of creatine in the brain by supplementation with high doses of the substrates arginine and glycine [28].

CTD therefore remains one of the deferential diagnoses of developmental delay; the diagnosis is made easier now by using MRS, which then should be followed by biochemical studies, and molecular testing. The disorder has been described not long ago; the phenotype spectrum, the exact pathophysiology that is attributing to this, and the effective treatments are yet to fully unfold.

## References

1. Pisano JJ, Abraham D, Udenfriend S. Biosynthesis and disposition of gamma-guanidinobutyric acid in mammalian tissues. *Arch Biochem Biophys* 1963;100: 323-329
2. Van Pislam JF, Stephens GC, Tylor D. Distribution of creatine, guanidinoacetate and enzymes for their biosynthesis in the animal kingdom. Implications for phylogeny. *Biochem J* 1972 :126 (2): 325-345
3. Braissant o, Henry H, Loup M, Eilers B, Bachmann C. Endogenous synthesis and transport of creatine in the rat brain: an in situ hybridization study. *Brain Res Mol Brain Res* 2001: 86: 193-201
4. Daly MM. Guanidinoacetate methyltransferase activity in tissues and cultured cells. *Arch Biochem Biophys* 1985: 236 (2): 576-584
5. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev* 2000: 80: 1107-1213
6. Stokler S, Schutz PW, Salomons GS. Cerebral creatine deficiency syndromes: clinical aspects, treatment and pathophysiology. *Subcell Biochem* 2007: 46: 149-166
7. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the Fphosphocreatine circuit for cellular energy homeostasis. *Biochem J* 1992: 281: 21-40
8. Galbraith RA, Furukawa M, Li M. Possible role of creatine concentrations in the brain in regulating appetite and weight. *Brain Res* 2006: 1101: 85-91
9. Happe Happe HK, Murrin LC. In situ hybridization analysis of CHOT1, a creatine transporter, in the rat central nervous system. *J Comp Neurol* 1995: 351: 94-103
10. Schloss P, Mayser W, Betz H. The putative rat choline transporter CHOT1 transports creatine and is highly expressed in neural and muscle-rich tissues. *Biochem Biophys Res Commun* 1994: 198: 637-645
11. Acosta ML, Kalloniatis M, Christie DL. Creatine transporter localization in developing and adult retina: importance of creatine to retinal function. *Am J Physiol Cell Physiol* 2005: 289: C1015-C1023
12. Ohtsuki S, Tachikawa M, Takanaga H, et al. The blood brain barrier creatine transporter is a major pathway for supplying creatine to the brain. *J Cereb Blood Flow Metab* 2002: 22: 1327-1335
13. Tachikawa M, Fukaya M, Terasaki T, Ohtsuki S, Watanabe M. Distinct cellular expressions of creatine synthetic enzyme GAMT and creatine kinases uCK-Mi and CK-B suggest a novel neuron-glia relationship for brain energy homeostasis. *Eur J Neurosci* 2004: 20(1): 144-160
14. Salomons GS, van Dooren SJ, Verhoeven NM, et al. X-linked creatine-transporter gene (SLC6A8) defect: a new creatine-deficiency syndrome. *Am J Hum Genet* 2001: 68: 1497-1500.
15. Brustovetsky N, Brustovetsky T, Dubinsky JM. On the mechanisms of neuroprotection by creatine and phosphocreatine. *J Neurochem* 2001; 76: 425-434.
16. Tarnopolsky MA, Roy BD, MacDonald JR. A randomized, controlled trial of creatine monohydrate in patients with mitochondrial cytopathies. *Muscle Nerve* 1997: 20:1502-1509
17. Neu A, Neuhoff H, Trube G, et al. Activation of GABA(A) receptors by guanidinoacetate: a novel pathophysiological mechanism. *Neurobiol Dis* 2002: 11: 298-307.
18. Rosenberg EH, Almeida LS, Kleefstra T, et al. High prevalence of SLC6A8 deficiency in X-linked mental retardation. *Am J Hum Genet* 2004: 75: 97-105
19. deGrauw TJ, Cecil KM, Byars AW, Salomons GS, Ball WS, Jakobs C. The clinical syndrome of creatine transporter deficiency. *Mol Cell Biochem* 2003: 244: 45-48
20. Schiaffino MC, Bellini C, Castabello L, et al. X-linked creatine transporter deficiency: clinical description of a patient with a novel SLC6A8 gene mutation. *Neurogenetics* 2005: 6: 156-168
21. Hahn KA, Salomons GS, Tackels-Horne D, et al. X-linked mental retardation with seizures and carrier manifestations is caused by a mutation in the creatine-transporter gene (SLC6A8) located in Xq28. *Am J Hum Genet* 2002: 70:1349-1356
22. Almeida S, Rosenberg EH, Verhoeven M, Jakobs, C, Salomons, G. Are cerebral creatine deficiency syndromes on the radar screen? *Future Neurol* 2006: 1(5): 637-649
23. Stromberger C, Bodamer OA, Stockler-Ipsiroglu S. Clinical characteristics and diagnostic clues in inborn errors of creatine metabolism. *J Inherit Metab Dis* 2003: 26: 299-308
24. Sykut-Cegielska J, Gradowska W, Mercimek-Mahmutoglu S, Stockler-Ipsiroglu S. Biochemical and clinical characteristics of creatine deficiency syndromes. *Acta Biochim Pol* 2004: 51: 875-882
25. Bizzi A, Bugiani M, Salomons GS, et al. X-linked creatine deficiency syndrome: a novel mutation in creatine transporter gene SLC6A8. *Ann Neurol* 2002: 52: 227-231
26. Schulze A, Mayatepek E, Bachert P, Marescau B, De Deyn PP, Rating D Therapeutic trial of arginine restriction in creatine deficiency syndrome. *Eur J Pediatr* 1998: 157: 606- 607.
27. Stockler S, Schutz PW, Salomons GS. Cerebral creatine deficiency syndromes: clinical aspects, treatment and pathophysiology. *Subcell Biochem* 2007: 46: 149-166
28. Mancini GM, Catsman-Berrevoets CE, de Coo IF, et al. Two novel mutations in SLC6A8 cause creatine transporter defect and distinctive X-linked mental retardation in two unrelated Dutch families. *Am J Med Genet A* 2005:132: 288-295.

