A review of X-linked creatine transporter deficiency: clinical aspects and pathophysiology

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Chapter 4  Pathogenesis

ABSTRACT

Creatine transporter deficiency was discovered in 2001 as an X-linked cause of intellectual disability characterized by cerebral creatine deficiency. This review describes the current knowledge regarding creatine metabolism, the creatine transporter and the clinical aspects of creatine transporter deficiency. It discusses the possible mechanisms leading to cerebral creatine deficiency while other organs like the muscle are relatively spared. Understanding of these mechanisms is of paramount importance in the development of an effective treatment which is up to now not available.
INTRODUCTION

X-linked creatine transporter deficiency (CRTR-D) causes intellectual disability characterized by cerebral creatine deficiency. More than 10 years after its discovery we have gained much knowledge regarding the clinical consequences of this disorder. Remarkably, CRTR-D mainly affects the brain while other creatine requiring organs, such as the muscles, are relatively spared.

In the same period, fundamental research has led to new insights regarding creatine metabolism in the brain. Now there is strong evidence that the brain ensures part of its creatine needs by endogenous synthesis, leading to the intriguing question of why cerebral creatine is deficient in CRTR-D.

Unraveling the pathogenesis of this condition is important in the development of treatment. Despite several attempts, no effective treatment is available yet.

Here we give a comprehensive overview of the current clinical and (patho)physiological insights regarding the creatine transporter and its deficiency, placed in the context of the other cerebral creatine deficiency syndromes (CDS), the creatine biosynthesis defects arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) deficiency.

CREATINE

Function

Creatine is mainly known for its essential role in energy metabolism. Creatine kinase (CK) catalyzes the reversible conversion of creatine and ATP to phosphocreatine and ADP. The creatine-phosphocreatine system serves as a cytosolic buffer for the regeneration of ATP and as a shuttle of high-energy phosphates between mitochondrial sites of production to cytosolic sites of utilization.\(^1\), \(^2\) In addition, creatine might have anti-oxidant and anti-apoptotic effects\(^3\)-\(^4\) and acts as an osmolyte.\(^5\)-\(^7\) Importantly, creatine might also have a neuromodulatory role.\(^8\) Creatine has become a major nutritional supplement in sports medicine and is intensively investigated as a therapeutic agent in psychiatric disorders\(^9\) and various neurological conditions (mitochondrial encephalopathy, stroke, traumatic neurological injury, neurodegenerative and muscular disorders).\(^3\), \(^4\), \(^10\)
Metabolism (Figure 1)
A 70-kg man contains about 120 g of total creatine of which >90% is found in skeletal muscle. Heart, brain, spermatozoa, and retina also contain high levels of creatine while kidney and liver contain relatively little.1, 11-13 Creatine and phosphocreatine are non-enzymatically converted into creatinine, which diffuses out of the cells and is excreted by the kidneys into the urine.1 In this way, about 1.7% of the total creatine pool is lost daily and needs to be replaced.1, 14, 15

Creatine is obtained from the diet and de novo synthesis. Creatine is mainly present in meat and fish and to a lesser degree in dairy milk and products.16 A typical Western diet supplies about one-half of the daily creatine need.4, 16, 17 However, vegetarians depend almost completely on de novo synthesis.16

Creatine is synthesized from arginine and glycine in two steps, using S-adenosylmethionine (SAM) as methyl group donor. AGAT catalyzes the reversible transamidination of the guanidino group from arginine to glycine, yielding guanidinoacetate and ornithine. GAMT subsequently catalyzes SAM-dependent methylation of guanidinoacetate, yielding creatine and S-adenosylhomocysteine. Creatine synthesis places a major burden on metabolism by consuming approximately 40%16 of all the labile methyl groups provided by SAM.4, 16, 18 The demand for creatine synthesis was found to significantly influence plasma homocysteine levels in rats.19

De novo creatine synthesis is strongly repressed by exogenous creatine1, 18, 20 at the level of AGAT expression and activity.21, 22 Increased availability of arginine and, to a lesser extent, glycine induces creatine synthesis.18, 22 Methionine does not appear to be a rate-limiting precursor.18

The kidneys efficiently salvage creatine from urine and excrete very little creatine except under high dietary creatine intake, fasting or muscle-mass reducing conditions.18

THE CREATINE TRANSPORTER

Function
The creatine transporter (CRTR) is responsible for the saturable Na⁺-and Cl⁻- dependent uptake of creatine into cells against a large concentration gradient. Uptake is inhibited by the competing creatine analogs β-guanidinopropionate (β-GPA) and γ-guanidinobutyrate and to a lesser extent by guanidinoacetate.23, 24 The CRTR is saturable with $K_m$ between 15-110uM25 and might be working close to saturation as plasma creatine concentrations in humans are between 15-118uM.26, 27
In addition to creatine uptake by CRTR, a low-velocity, low-capacity, Na⁺-independent, not-saturable or saturable with a high $K_m$, creatine uptake has been described, which seems irrelevant for creatine uptake in vivo and might represent diffusion. 1, 28, 29

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Figure 1. Creatine metabolism. AGAT, arginine:glycine amidinotransferase; Arg, arginine; CK, creatine kinase; Cr, creatine; Crn, creatinine; CRTR, creatine transporter; Gaa, guanidinoacetate; GAMT, guanidinoaceteate methyltransferase; Gly, glycine; Hcy, homocysteine; MCT12?, monocarboxylate transporter 12 or unknown transporter; Met, methionine; Orn, ornithine; PCr, phosphocreatine; SAH, S-adenosylmethionine; SAM, S-adenosylmethionine.

Figure 2. CRTR structure based on LeuT model with location of mutations found in CRTR-D patients.
Gene and protein

The CRTR is encoded by the \textit{SLC6A8} gene which was first sequenced in the 90s in both humans\textsuperscript{24, 30, 31} and other species.\textsuperscript{32-34} It contains 13 exons and is, in humans, located on Xq28. The predicted protein consists of 635 amino acids and has a molecular mass of 70.5kDa.\textsuperscript{23} It is a member of the SLC6 family of Na\textsuperscript{+}- and Cl\textsuperscript{-}- dependent plasma membrane neurotransmitter transporters together with transporters for GABA, norepinephrine, dopamine, serotonin, glycine, taurine, proline, and betaine\textsuperscript{35, 36} and is most closely related to the taurine transporter (52% homology) and GABA/betaine transporters (48-50% homology).\textsuperscript{23} In common with the other SLC6 family members, the CRTR consists of twelve transmembrane (TM) domains with a large extracellular loop between TM3 and TM4, containing sites for N-glycosylation, and N- en C-termini facing the cytoplasmic side of the membrane.\textsuperscript{34, 37} The CRTR also contains several phosphorylation sites.\textsuperscript{24, 25, 30, 34} A putative leucine zipper consisting of repeated leucine residues at every seventh position in the third extracellular loop might be involved in oligomerization,\textsuperscript{30} which is common among other members of the SLC transporter family and might be essential for transport from the endoplasmic reticulum to the plasma membrane.\textsuperscript{38} In 2005, the crystal structure of a prokaryotic homologue, the leucine transporter (LeuT) was resolved,\textsuperscript{39} which is used as a model for CRTR (Figure 2) and other SLC6 transporters\textsuperscript{36} and has helped to identify amino acid residues involved in the substrate selectivity of the CRTR.\textsuperscript{40}

Two evolutionary conserved splice variants have been detected in various tissues; \textit{SLC6A8C}, containing intron 4 and exons 5-13,\textsuperscript{41} and \textit{SLC6A8D} which is identical to \textit{SLC6A8C} except for an in-frame deletion of exon 9 (Ndika et al, manuscript in preparation). They are unable to transport creatine but might regulate CRTR activity by oligomerization to full-length CRTR and thereby affecting its trafficking and stability.\textsuperscript{41} A previously detected splice variant in hippocampal mRNA\textsuperscript{42} was not confirmed.\textsuperscript{41}

Paralogous creatine transporter gene

A 26.5kb region of Xq28 containing the entire \textit{SLC6A8} gene is duplicated to 16p11.1.\textsuperscript{43} In fact, there are two adjacent duplications according to the UCSC genome browser, one on the reserve strand and one on the forward strand.\textsuperscript{44} Iyer et al. found expression of the paralogous gene, which lacks the termination codon extending its open reading frame by 50 amino acids, exclusively in testis and suggested that the gene product was critical for creatine uptake into sperm cells lacking an X chromosome.\textsuperscript{45} However, the paralogous gene contains a premature stop codon in exon 4,\textsuperscript{43} and thus very probably does not
encode a protein capable of creatine transport. It is therefore considered a pseudogene, now known as \textit{SLC6A10AP}. In support of this, the promoter region of \textit{SLC6A10P}, in contrast to the promoter region of \textit{SLC6A8}, is highly methylated in all tissues except in testis, where it is probably transiently demethylated during spermatogenesis.\textsuperscript{46} On the other hand, the \textit{SLC6A10P} promoter, after cloning in luciferase assays, appears to have a stronger activity than the \textit{SLC6A8} promoter, which does suggest a functional role for \textit{SLC6A10P}, perhaps as a buffer of miRNA that would otherwise down regulate \textit{SLC6A8} expression.\textsuperscript{47} In contrast to previous studies,\textsuperscript{43, 45} Bayou et al. described expression of the \textit{SLC6A10P} in brain and suggested that the gene was involved in the autism of a patient with a de novo translocation disrupting this gene. However, MRS revealed normal cerebral creatine in this patient.\textsuperscript{48}

\textbf{Tissue expression}

In humans, CRTR is expressed predominantly in muscle, kidney and heart, but also in many other tissues including brain\textsuperscript{24, 30, 45} and retina.\textsuperscript{49} This pattern agrees with the high levels of creatine in muscle and heart and the salvage of creatine from urine in the kidney.\textsuperscript{23}

The CRTR is present in the apical (brush border) membrane of the rat renal proximal tubule cells,\textsuperscript{50, 51} increasing with renal maturation,\textsuperscript{52} and probably mediates the first step of renal creatine reabsorption. However, very little CRTR is localized at the basolateral membrane and as the Na\textsuperscript{+} Cl\textsuperscript{−} gradient is inwardly directed, the CRTR would move creatine into the kidney epithelial cells instead of from the epithelial cells to the blood.\textsuperscript{50} Therefore, to complete the transepithelial creatine reabsorption, a still unknown basolateral membrane creatine transporter is required (Figure 1).\textsuperscript{50}

The CRTR is also present in colon and small intestine in humans\textsuperscript{24, 45} and rat,\textsuperscript{53-57} and is again largely restricted to the brush border membrane.\textsuperscript{53, 55, 57} The CRTR might be involved in intestinal transepithelial absorption of creatine, necessitating the existence of a basolateral membrane creatine transporter. Alternatively, intestinal absorption of creatine might occur via paracellular movement by solvent drag transport while CRTR mainly supplies creatine for enterocyte motility (Figure 1).\textsuperscript{58}

The brain CRTR expression pattern has been studied in detail in the rat.\textsuperscript{33, 34, 54, 59-62} Expression is widespread but prominent in the olfactory bulb, cerebellum, hippocampus, cortex (layers III and V) and several brainstem nuclei but low in basal ganglia. In white matter tracts strong mRNA expression was found\textsuperscript{33, 34, 59, 61} but no CRTR protein.\textsuperscript{62} Mak et al. concluded that the CRTR is located principally in regions involved in motor and
sensory processing and in learning, memory and limbic functions.\textsuperscript{62}

CRTR is only expressed in neurons and oligodendrocytes and not in astrocytes.\textsuperscript{59, 62, 63} In contrast, Möller et al.\textsuperscript{64} measured creatine uptake in cultures of astrocytes but not of neurons and Carducci et al.\textsuperscript{65} detected uptake in both astrocytes and cerebellar granule cell (neuron) cultures. Some of these differences might be explained by in vitro culturing effects.\textsuperscript{66} In neurons, studied in hippocampal cultures of rat embryos, the CRTR was mostly located in dendrites but also in some axons and axon terminals.\textsuperscript{67} Peral et al. proved the presence of CRTR activity and protein in the synaptosomal membrane where it would allow rapid reuptake if creatine were released as a neurotransmitter.\textsuperscript{68}

Overall, the pattern of CRTR expression in the brain is compatible with a role of creatine as neurotransmitter or neuromodulator.

Regulation

Intracellular and extracellular creatine levels down regulate creatine uptake activity, decreasing the $V_{\text{max}}$ in muscle cells, HEK293 cells, and cardiomyocytes.\textsuperscript{28, 69, 70} Whether this is caused by reduced numbers of CRTR remains undecided because studies used CRTR antibodies with limited specificity\textsuperscript{71, 72} and showed conflicting results.\textsuperscript{73-76} There appears to be a maximum to creatine accumulation in the creatine requiring tissues. Creatine supplementation in healthy humans led to a maximal 5-15\% increase of total creatine levels in the brain\textsuperscript{77} and a mean increase of 20\% to maximal 140-160 mmol/kg dry muscle in skeletal muscle with the largest increases in subjects with a low initial total creatine content.\textsuperscript{78} Also, creatine supplementation in various animal species increased the presupplemental high creatine content in brain and muscle tissue to a limited extent, while large increases were found in liver and kidney, which have a low initial creatine content.\textsuperscript{11} Down regulation of creatine uptake might prevent the accumulation of excessive intracellular creatine, which might deplete the ATP stores.\textsuperscript{28} Indeed, overexpression of the myocardial CRTR in a transgenic mouse model increased myocardial creatine content resulting in increased free ADP levels and left ventricular hypertrophy and dysfunction.\textsuperscript{79}

CRTR activity might be under hormonal influences. Growth hormone and thyroid hormone affect SLC6A8 transcription in heart.\textsuperscript{80, 81} Catecholamines (through $\beta_2$ receptors), thyroid hormone, amylin, insulin (at supraphysiological concentrations), and insulin-like growth factor I increase creatine uptake,\textsuperscript{1, 25, 82, 83} probably mainly indirectly by stimulating Na$^+$/K$^+$-ATPase and therefore increasing the sodium gradient across the cell membrane which drives the CRTR.\textsuperscript{82} Regulation of the CRTR might involve its phosphorylation
by several signal molecules. Mammalian target of rapamycin (mTOR), serum and glucocorticoid inducible kinases SGK1 and SGK3, and the mammalian phosphatidylinositol-3-phosphate-5-kinase PIKfyve, probably constituting a pathway, increase CRTR activity while Janus-activated kinase-2 (JAK2) and protein kinase C (PKC) inhibit CRTR activity. AMP-activated protein kinase (AMPK), which regulates energy homeostasis by switching off energy consuming pathways in favor of energy generating processes, inhibited CRTR activity and expression in the apical membrane of kidney proximal tubule cells, possibly indirectly via inhibition of mTOR, but increased CRTR activity and expression in cultured cardiomyocytes.

Hyperammonemia induced CRTR expression in brain capillary endothelial cells and astrocytes possibly increasing the blood-brain barrier (BBB) permeability for creatine, but repressed it in oligodendrocytes.

These data suggest that creatine levels in skeletal muscle, heart, and brain are tightly regulated and maintained within a narrow normal range which might vary with their metabolic state. CRTR activity might be differentially regulated depending on the creatine needs of the tissue.

**CREATINE TRANSPORTER DEFICIENCY**

**Creatine transporter deficiency as a cause of cerebral creatine deficiency**

Proton magnetic-resonance spectroscopy (1H-MRS) has made it possible to detect cerebral creatine deficiency and this has led to the discovery of the three CDS, all characterized by intellectual disability. GAMT-deficiency (GAMT-D) was first described in 1994, followed by the discovery of both CRTR-D and AGAT deficiency (AGAT-D) in 2001.

While GAMT-D and AGAT-D are autosomal recessive conditions impairing creatine biosynthesis, CRTR-D is an X-linked condition affecting the cellular creatine uptake.

**Prevalence**

Shortly after the discovery of CRTR-D, more patients were identified within the same metropolitan area. This suggested that CRTR-D might be a relatively common cause of X-linked intellectual disability. Combining several studies (Table 1), the prevalence in males with intellectual disability can now be estimated between 0.4 and 1.4% (depending on the strictness of cohort definition) and about 2% in males with X-linked intellectual disability. No patients were detected in a cohort of 100 males with autism.
Table 1. Prevalence studies of CRTR-D.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Screening method</th>
<th>Positive/ tested</th>
<th>Prevalence % (95% CI)</th>
<th>Reference</th>
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<tr>
<td>XLID</td>
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<td>European XLID families</td>
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<td>2.1 (0.4-3.7)</td>
<td>94</td>
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<tr>
<td>Estonian XLID families</td>
<td>Urine, confirmed by</td>
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<td>2.0 (0.0-6.0)</td>
<td>97</td>
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<tr>
<td>DNA sequencing</td>
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<tr>
<td>Total XLID</td>
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<td>2.0 (0.6-3.3)</td>
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<td>3.5 (0.1-6.9)</td>
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<td>ID and other cohorts</td>
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<td>males with ID/autism (Spain)</td>
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<td>0.3 (0.0-0.5)</td>
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<td>males with neurological disease (France)</td>
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<td>0.4 (0.2-0.5)</td>
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CI, confidence interval; ID, intellectual disability; XL-ID, X-linked intellectual disability.

\(^a\) at risk: one or more symptoms of intellectual disability/developmental delay associated with speech delay, seizures, autistic behavior.

\(^b\) In one patient DNA analysis was rejected by parents and diagnosis was confirmed by \(^1\)H-MRS and creatine uptake deficiency.
A review: clinical aspects and pathophysiology

Phenotype and genotype in male patients

Clinical features

The hallmarks of CRTR-D are intellectual disability with severe speech delay (100%), behavioral abnormalities (85%), and seizures (59%). The intellectual disability becomes more pronounced with age and most adult patients have severe intellectual disability. Mild intellectual disability has so far been reported in only one adult patient. Cognitive regression might occur in older patients but a decline in IQ scores has only been ascertained in one patient. Although patients are especially delayed in speech development, most patients develop some speech. The speech defect has been well described by Mancini et al. and is characterized by dysarthria, oral dyspraxia, and semantic-pragmatic language disorder with many repetitions and echolalia. Patients often have a happy nature. However, the behavioral problems, mainly consisting of hyperactivity, attention deficit and/or autistic features, can be very invalidating for the patient and the caregivers. Seizures mainly concern generalized tonic-clonic and simple or complex partial seizures but other types also occur. Seizures are mostly infrequent and easily controlled and include febrile-induced seizures. However, patients can also present with status epilepticus and a few patients have severe refractory epilepsy. The motor development is only mildly delayed with independent walking at the mean age of 2 years. However, central hypotonia with normal or brisk deep tendon reflexes is frequently noticed, especially in the first years and (young) patients often show a stiff, wide based unstable gait with elevated arms. Extrapyramidal movement disorders, including (subtle) abnormal hand movements, intermittent dystonic posturing of the hands/wrists during walking and choreathetoid movements occur in some patients. Muscle weakness has only rarely been described. Gastrointestinal problems are relatively frequent in CRTR-D. Early in life patients can present with feeding difficulties, frequent vomiting, and failure to thrive. Later in life, some patients develop severe constipation which can even be complicated by ileus necessitating surgery. Bladder voiding dysfunction or instability might also occur more frequently. Only four reported patients presented cardiac symptoms consisting of mild cardiomyopathy in two brothers and premature ventricular contractions and long QT in one patient each. Strabism occurs in some patients but retinal anomalies have only been described in one patient with a contiguous gene deletion of PNCK, SLC6A8, and BCAP31. Patients can show subtle dysmorphism such as a broad/prominent forehead.
and myopathic facies. The most striking physical feature is the slender build and poorly developed muscular mass. However, this might not be obvious in all patients, especially not in the older patients. Height is usually below average but rarely below -3 SD. Although a progressive increase in head circumference has led to the discovery of the CRTR-D in the first patient, patients usually have a head circumference in the normal range.

GAMT-D and AGAT-D are also associated with intellectual disability with severe speech delay and behavioral abnormalities. However, in general, GAMT-D is more severe with frequently intractable seizures, extrapyramidal movement abnormalities, and sometimes self-injurious behavior although milder presentations with mild epilepsy and without specific neurological findings have been described. Seizures rarely occur in AGAT-D. Remarkably, AGAT-D is frequently associated with progressive muscle weakness and myopathic electromyography, occurring either early or later in life.

Brain MRI and MRS
Brain MRI in CRTR-D may be unremarkable or show mild abnormalities, including mildly delayed myelination, (T2-) hyperintensities, thin corpus callosum, mildly enlarged ventricles/extracerebral spaces, and cerebral/cerebellar atrophy. Progressive cerebral atrophy was reported in two brothers. Brain MRS reveals the severe reduction of creatine that is characteristic of all CDS. Creatine deficiency is apparent in all brain regions but creatine levels are significantly higher in basal ganglia and cerebellum than in gray matter and white matter. These regional differences are comparable to those in healthy subjects.

Muscle creatine
$^1$H- and $^3$P-MRS showed the presence of creatine in skeletal muscle of a CRTR-D patient and even normal creatine levels were found in a muscle biopsy of another patient. In contrast, creatine was low in a muscle biopsy of the first GAMT-D patient and $^3$P-MRS showed reduced muscle creatine in two other GAMT-D patients although this reduction was less pronounced than in the brain. $^3$P-MRS showed creatine depletion in skeletal muscle of two AGAT-D patients although, surprisingly, no changes were noted during creatine supplementation.

These, unfortunately very limited, data suggest that creatine levels in skeletal muscle are relatively preserved in CRTR-D while in GAMT-D and AGAT-D they are reduced, although less severely than in brain.
4.2 Biochemical features

The three CDS can be distinguished based on creatine, creatinine, and guanidinoacetate measurements in urine and plasma (Table 2).

Reference values are age-related. In urine, the creatine/creatinine (Cr/Crn) ratio and guanidinoacetate levels decrease with age. In plasma, creatine levels also decrease with age but guanidinoacetate levels increase. Joncquel-Chevalier et al. also found gender differences with significant higher Cr/Crn and guanidinoacetate concentrations in females in urine but not in plasma.

CRTR-D is characterized by elevated Cr/Crn in urine, probably due to reduced renal reabsorption of creatine in combination with reduced creatinine excretion. Creatine levels in plasma are normal and remarkably, creatine levels in cerebrospinal fluid (CSF) are also normal or even mildly elevated while they are severely reduced in GAMT-D.

Reduced creatinine levels in plasma and urine in CDS probably parallel reduced intramuscular creatine pools, for which the 24h urinary creatinine excretion is considered the most reliable predictor. Reduced plasma creatinine or generalized increases of urinary metabolites expressed relative to creatinine can suggest CDS. The reduced creatinine levels in CSF of CRTR-D patients might reflect reduced intracellular cerebral creatine stores.
A low serum CK level was found in one CRTR-D patient and one GAMT-D patient and was suggested as another non-specific marker for CDS. CK levels are unknown in most CRTR-D patients. However, the first described CRTR-D patient had a normal CK level.

Notably, some patients with CRTR-D, GAMT-D, or AGAT-D were initially suspected of or even diagnosed with mitochondrial encephalopathy based on mildly increased plasma lactate, increased urinary 3-methylglutaconic acid and/or ethylmalonic acid and tricarboxylic acid cycle intermediates and/or secondary decreases of variable respiratory chain complexes in muscle biopsy. Therefore, CDS can be associated with secondary respiratory chain abnormalities and should be considered in the differential diagnosis of mitochondrial encephalopathies.

**Diagnosis**

The diagnostic mainstay of CRTR-D is based on testing of Cr/Cr in urine, brain $^1$H-MRS, and SLC6A8 sequencing.

The urinary Cr/Cr ratio is becoming a more widely available screening test and is in a growing number of metabolic laboratories part of the metabolic screening in intellectual disability. It is a 100% sensitive test in CRTR-D males. However, false positives are regularly detected and a repeat morning urine sample collected after an one-day meat and fish free diet increases the test specificity.

$^1$H-MRS is also a 100% sensitive screening method but unavailable in many centers and requires general anesthesia. Further biochemical or molecular genetic tests are necessary to differentiate CRTR-D from other CDS.

The diagnosis is usually confirmed by molecular analysis of the SLC6A8 gene but the creatine transporter can also be functionally tested by creatine uptake studies in cultured fibroblasts of the patient. Cells of CRTR-D patients show no uptake when incubated with 25 μmol/l creatine. At incubation with 500 μmol/l creatine some uptake is seen which is however almost unaffected by addition of guadininoproprionate, an inhibitor of the creatine transporter, suggesting another mechanism than the CRTR, possibly diffusion or another transporter.

With the rapid development of next generation sequencing, molecular genetic screening for CRTR-D will become more common. Novel unclassified variants will be a frequent finding and in these cases the diagnosis should be confirmed by creatine uptake studies in patient fibroblasts and/or by functional characterization of the novel variant.
Genotype

SLC6A8 mutations in CRTR-D mostly concern missense mutations and one-amino acid (3 bp) deletions, which are concentrated in TM7 and TM8 (Figure 2), but also frameshift mutations, nonsense mutations, splice error mutations (intronic or synonymous variants with aberrant splicing), a translation initiation site mutation, and multi-exon deletions (LOVD database [http://www.LOVD.nl/SLC6A8]). There are a few recurrent mutations but most mutations are unique.\textsuperscript{106} Novel missense variants should be characterized by creatine uptake studies in CRTR deficient fibroblasts after transfection with SLC6A8 cDNA in which the variant has been introduced by site-directed mutagenesis.\textsuperscript{158, 159} Alternatively, the consequences of mutations on electrogenic and creatine transport activities can be studied in X.leavis oocytes.\textsuperscript{160} The pathogenicity of neutral or intronic variants should be confirmed by bioinformatic analysis and mRNA analysis.\textsuperscript{161}

Genotype-phenotype correlations

So far, four missense mutations, c.1190C>T; p.(Pro397Leu) c.1271G>A; p.(Gly424Asp), c.1661C>T; p.(Pro544Leu), and c.1699T>C; p.(Ser567Pro) were found to have residual CRTR activity.\textsuperscript{106, 158} These mutations might be associated with a milder and more variable phenotype\textsuperscript{106} as illustrated by one family in which two of the three affected brothers had a remarkably mild intellectual disability while the third brother had a more typical moderate delay.\textsuperscript{97} However, another patient with a missense mutation with residual activity had severe refractory epilepsy.\textsuperscript{106, 162}

Multi-exon deletions of the SLC6A8 gene, extending beyond the 3’end of SLC6A8 are associated with a very severe phenotype with severe hypotonia and motor delay, extrapiramidal movement disorder, and childhood mortality.\textsuperscript{106, 112, 163} (This thesis chapter 2.2) In some patients, this can be explained by involvement of the neighboring BCAP31 gene in the deletion because isolated BCAP31 defects were recently described as a cause of profound developmental delay with dystonia, and deafness.\textsuperscript{164} However, a patient with an isolated deletion of the 3’end of SLC6A8 had a similar phenotype pointing to disturbance of a regulatory element between SLC6A8 and BCAP31. (This thesis chapter 2.2)

Inheritance

CRTR-D is an X-linked condition. The mutation occurs de novo in about one third of the index boys.\textsuperscript{106} Somatic mosaicism has been detected in several mothers, including a mother with two affected sons.\textsuperscript{96, 106} Because low levels of somatic mosaicism and germline
mosaicism cannot be excluded with DNA sequencing in the mother, prenatal diagnosis in further pregnancies should always be offered.

**Female heterozygotes**

Learning difficulties and mild intellectual disability have been reported in several but not all heterozygous female family members. Female heterozygotes can also present as index patients with mild-moderate intellectual disability, behavioral problems, and seizures. The seizures can even be severe and intractable. The diagnosis might be frequently missed because the diagnosis in females is not straightforward.

The urinary Cr/Crn ratio was normal in more than half of the heterozygous female family members and although they had relatively low cerebral creatine levels on $^{1}$H-MRS there was overlap with normal controls. Also female index patients can have urinary Cr/Crn ratios in the normal range. Creatine uptake studies in cultured fibroblasts of female heterozygotes is either deficient or normal, due to severe skewing (unrelated to SLC6A8 deficiency) arising during culturing of fibroblasts. DNA analysis of the SLC6A8 gene is probably the only reliable option for screening for CRTR-D in females presenting with (mild) intellectual disability.

Skewed X-inactivation is not common in peripheral blood leukocytes, hairs, and saliva of heterozygous females, indicating that there is no selection against the mutation. Therefore, the phenotype in females is expected to vary from normal to abnormal depending on the chance variation of the X-inactivation from favorably to unfavorably skewed. However, the X-inactivation pattern in blood cells cannot be used to predict the phenotype.

**TOWARDS AN UNDERSTANDING OF CREATINE TRANSPORTER DEFICIENCY**

**Creatine synthesis versus uptake**

It was originally thought and is still often stated that guanidinoacetate is formed in the kidney and methylated to creatine in the liver after which creatine is transported through the blood and taken up by the CRTR in creatine requiring tissues. However, this might not be generally true as there are pronounced species differences in organ expressions of AGAT and GAMT. In the rat, the first step of creatine synthesis indeed takes place mainly in the kidney and the second step in the liver. However, in humans the renal guanidinoacetate production is insufficient for the daily creatine synthesis and both
human pancreas and human liver synthesize creatine from arginine and glycine.\textsuperscript{18} Even more importantly, in all species AGAT and GAMT are expressed in many tissues besides kidney, pancreas, and liver,\textsuperscript{1,171} suggesting that other tissues can also synthesize creatine. Two studies evaluated the expression of AGAT and GAMT in various human tissues. Cullen et al.\textsuperscript{174} found the highest AGAT mRNA expression in kidney, high expression in liver and brain and intermediate expression in skeletal muscle and heart. Schmidt et al.\textsuperscript{175} found GAMT mRNA and protein mostly in skeletal muscle and liver, high in heart and kidney and in smaller amounts in brain. AGAT and GAMT expression and creatine synthesis has also been detected in the Müller cells of the retina.\textsuperscript{176} These findings suggest that the creatine content of creatine requiring tissues depends on contributions of both endogenous creatine synthesis and creatine uptake (Figure 1). This is very relevant in the pathogenesis of CRTR-D because creatine synthesis might (partly) compensate for the loss of creatine uptake. Residual creatine resulting from endogenous synthesis might explain the absence of evident muscular, cardiac and retinal involvement in CRTR-D.

**Endogenous creatine synthesis in the brain**

It is now commonly accepted that brain cells synthesize creatine. Creatine synthesis has been demonstrated in rat brain in various studies both in vitro cultures of astroglial cells\textsuperscript{63, 65, 89, 177} and neurons\textsuperscript{63, 65, 89} as in vivo.\textsuperscript{178} AGAT and GAMT expression in brain has been extensively confirmed in rat\textsuperscript{59, 60, 63} and mouse\textsuperscript{29, 175, 179} but is also present in human brain.\textsuperscript{171, 174, 175} Furthermore, uptake of creatine into the brain from the periphery seems limited. Although the CRTR is present in brain capillary endothelial cells and transports creatine across the BBB from the blood to the brain,\textsuperscript{180-182} this creatine uptake was found to add only little to the cerebral creatine content.\textsuperscript{183} In creatine synthesis defects, supplementation with creatine is able to restore cerebral creatine, confirming that creatine crosses the BBB. However, restoration takes months and high doses of creatine are needed,\textsuperscript{115-117, 124, 129, 130, 142, 184} consistent with a limited permeability of the BBB for creatine. This might be explained by the absence of CRTR from astrocytes,\textsuperscript{59, 62} which feet cover more than 98% of the microcapillary endothelial cells in the BBB.\textsuperscript{185}

These data suggest that under physiological conditions, the brain mainly depends on its endogenous creatine synthesis. In contrast, the embryonic brain might depend more on extracerebral supply of creatine. Because GAMT expression is low during rat embryogenesis while CRTR is highly expressed in the choroid plexus, creatine might mainly be supplied from blood to CSF through the choroid plexus.\textsuperscript{186}
Cerebral creatine deficiency in CRTR-D

The finding of cerebral creatine synthesis is at odds with the lack of creatine in the brain of patients with CRTR-D. Various models have been proposed to explain this paradox.

The neuron-glial relationship hypothesis followed from the finding of preferential expression of GAMT in oligodendrocytes and implies that creatine synthesis occurs in glial cells and creatine must be transferred to neurons, requiring a functional CRTR. However, AGAT and GAMT are found in all brain cell types and creatine synthesis was also demonstrated in neuron cultures.

The dissociation model suggests that despite expression in all brain cell types, AGAT and GAMT rarely co-express in the same cell so that the intermediate guanidinoacetate must be transported between AGAT- and GAMT-containing cells via CRTR to ensure creatine synthesis. In this model, a similar guanidinoacetate accumulation as in GAMT-D would be expected in CRTR-D. Although 1H-MRS detected a cerebral guanidinoacetate signal in one CRTR-D patient, this is usually not observed and guanidinoacetate in CSF is not elevated in CRTR-D as it is in GAMT-D. Furthermore, guanidinoacetate is a poor substrate for the CRTR while guanidinoacetate levels in CSF are several 100-fold lower than creatine, and guanidinoacetate transport also occurs via the taurine transporter and possibly GATs.

The remarkable finding of normal to slightly elevated creatine levels in CSF of CRTR-D patients led to the hypothesis that in CRTR-D creatine is lost in CSF due to reuptake failure. A striking observation was made in mice models of defects of serotonin, dopamine, and norepinephrine neurotransmitter transporters, which facilitate reuptake of released neurotransmitter from the synaptic cleft and to which CRTR exhibits considerable homology. They all manifest severe intracellular depletion in addition to extracellular neurotransmitter elevations, confirming the importance of reuptake for maintenance of intracellular neurotransmitter stores and indicating a negligible contribution of de novo synthesis. In a similar way, cerebral creatine deficiency in CRTR-D could derive from defective creatine recycling following release. Indeed, hippocampal neurons were found to release 30-40% of accumulated creatine in 1-2 hours while HEK293-CRT cells were able to maintain high levels of accumulated creatine for long periods. Creatine might be released by reversal of the CRTR due to an altered electrochemical gradient for Na+ during excitation. Alternatively, neuronal creatine release in an action-potential dependent exocytotic manner has also been reported. This fits with a role of creatine as neuromodulator or neurotransmitter which is further supported by the presence of
CRTR activity in the synaptosomal membrane.\textsuperscript{68}

Recently, Carducci et al. found that although both astrocytes and neurons have the capability to synthesize creatine, their creatine content is to a much greater extent due to uptake.\textsuperscript{65} This suggested that cerebral creatine synthesis might be limited after all.\textsuperscript{65} However, their findings could also agree with the reuptake failure hypothesis wherein the role of creatine synthesis in maintaining creatine stores is negligible compared to reuptake.

**A second creatine transporter**

Recently, a second creatine transporter was discovered. The monocarboxylate transporter 12 (MCT12) encoded by the SLC16A12 gene was found to transport creatine.\textsuperscript{191} In contrast to CRTR, which requires Na\textsuperscript{+} and Cl\textsuperscript{−} and works against a concentration gradient, MCT12 performs facilitated transport of creatine, likely along a concentration gradient and has a much higher $K_m$ of 567.4\textmu M.\textsuperscript{191} Therefore, MCT12 might mainly facilitate creatine efflux from cells. MCT12 is highly expressed in human kidney, retina, lung, and testis, weakly in heart, muscle and lens and very weakly in brain and liver.\textsuperscript{191, 192} MCT12 is predominantly located at the basolateral membrane of the lens.\textsuperscript{193} With these characteristics, MCT12 could very well be the required transporter at the basolateral membrane of renal and intestinal epithelial cells, cooperating with CRTR in the transepithelial transport of creatine (Figure 1).\textsuperscript{191}

A nonsense mutation in SLC16A12 has been associated with autosomal dominant juvenile cataract, microcornea, and glucosuria in one family\textsuperscript{192, 193} and variants in this gene might contribute to age-related cataract.\textsuperscript{191, 194} SLC16A12 knockout rats do not develop cataract or glucosuria but have significantly increased creatine levels in urine.\textsuperscript{191}

Further studies should explore the role of MCT12 in creatine metabolism.

**Lessons from mouse models**

Male ubiquitous CRTR knockout\textsuperscript{195, 196} and brain specific CRTR knockout\textsuperscript{197} mice lack creatine in the brain and have learning and memory deficits\textsuperscript{195, 197} resembling the key features of human CRTR-D. Hyperactivity was only noted in the brain specific CRTR knockout mouse.\textsuperscript{197} Female ubiquitous CRTR knockout mice also show reduced cerebral creatine, hyperactivity, and mild cognitive defects.\textsuperscript{196} Alterations of the serotonergic system with intracerebral increases of 5-HT and 5-HIAA were found in male ubiquitous CRTR knockout mice.\textsuperscript{195} Information regarding serotonin in human CRTR-D patients is
lacking, except for the observation of slightly decreased 5-HIAA in CSF of one CRTR-D patient.\textsuperscript{108} In contrast to the human phenotype, ubiquitous CRTR knockout mice lack creatine in muscle and have severely reduced serum creatine.\textsuperscript{195} CRTR-knockout mice might be less able to compensate decreased renal creatine reabsorption and decreased creatine uptake into muscle by increasing creatine synthesis. Unfortunately, muscular metabolism and function has not been studied in the CRTR-knockout mouse.

The muscle phenotype has been extensively studied in AGAT and GAMT-knockout mice. In fact, the AGAT-knockout mouse on a creatine free diet is probably the most reliable model for total creatine depletion\textsuperscript{198} because phosphoguanidinoacetate might compensate for loss of phosphocreatine in GAMT-knockout mice\textsuperscript{199, 200} and creatine synthesis might occur in CRTR-knockout mice. AGAT-knockout mice display severe muscle atrophy, reduced grip strength, and hypotonia and several metabolic changes in muscle including reduced ATP and increased inorganic phosphate levels\textsuperscript{15} which were all reversible by creatine supplementation. GAMT-knockout mice show several similar, but less pronounced, muscle features\textsuperscript{15, 175, 199} Surprisingly, Lygate et al. found unaltered maximal exercise capacity and response to chronic myocardial infarction in GAMT-knockout mice, which made the authors question the paradigm that creatine is essential for high workload and stress responses in heart and skeletal muscle.\textsuperscript{201, 202} GAMT-knockout mice have mild cognitive impairment\textsuperscript{203} but the cognitive phenotype of the AGAT-knockout mouse still remains to be studied.

The ubiquitous CRTR-, GAMT-and AGAT-knockout mice all have reduced weight and reduced fat.\textsuperscript{195, 200, 204} The brain specific CRTR knockout have just reduced fat.\textsuperscript{197} Furthermore, total creatine depletion in AGAT-knockout mice leads to complete protection from diet-induced obesity and enhanced glucose tolerance with chronic activation of AMPK, which stimulates catabolic pathways and probably underlies this metabolic phenotype.\textsuperscript{204} Because AMPK seems to inhibit CRTR in the kidney\textsuperscript{50} but increase CRTR in the heart\textsuperscript{70} this might increase renal creatine loss but protect the heart.

Male AGAT- and GAMT-knockout mice are sub/infertile due to impaired spermatogenesis.\textsuperscript{175, 204} Also, male CRTR-knockout mice failed to reproduce.\textsuperscript{196} Therefore, creatine might affect spermatogenesis, which is consistent with the high levels of creatine in testis under physiological circumstances.\textsuperscript{1, 13} Reduced fertility might be expected in CDS patients. No polymorphisms in GAMT and SLC6A8 were found in 64 infertile men.\textsuperscript{205}
In AGAT-D and GAMT-D, creatine supplementation has led to a (partial) restoration of the cerebral creatine content and attenuation of the symptoms. In CRTR-D, high plasma creatine levels might result in some cellular creatine uptake via alternative mechanisms or residual activity of CRTR. CRTR-deficient fibroblasts take up creatine when incubated in high creatine concentrations. However, creatine monotherapy has not proved successful in patients with CRTR-D.

Since the brain is capable of endogenous creatine synthesis, supplementation with the creatine precursors L-arginine and glycine seemed a promising treatment in CRTR-D. Provision of arginine increased guanidinoacetate and creatine synthesis in astroglial cells, the rat kidney, and in human CRTR-deficient lymphoblasts. Furthermore, arginine supplementation in the first GAMT-D patient increased cerebral guanidinoacetate. However, supplementation with L-arginine with or without glycine in CRTR-D patients, reported in 22 males and 3 females (Table 3), was discouraging. Although clinical improvements were reported in some patients, cerebral creatine was not restored suggesting that supplementation does not increase cerebral creatine synthesis. This highlights the importance of the central question why CRTR-D leads to cerebral creatine deficiency while the brain is capable of creatine synthesis. Arginine supplementation might have an effect outside the brain, for instance on the muscle function. Increased urinary guanidinoacetate excretion in several patients during arginine supplementation suggests increased peripheral synthesis. Increased methylation demands of the GAMT reaction might explain the increased homocysteine and decreased folate levels found during arginine and glycine supplementation in CRTR-D patients. Therefore, folate supplementation seems advisable to prevent hyperhomocysteinemia but also to enhance creatine synthesis. SAM supplementation might also strengthen the cerebral creatine synthesis as it crosses the blood-brain barrier and increases cerebral phosphocreatine.

Lipophilic creatine analogs are considered as alternative treatment as they might cross the BBB and cell membranes independent of CRTR after which they might be metabolized to creatine. Creatine-benzyl-ester, phosphocreatine-Mg-complex acetate, creatine-ethyl-ester, and dodecyl-creatine-ester led to variable increases of creatine in CRTR-blocked mouse brain slices or human CRTR-deficient fibroblasts. Unfortunately, the creatine esters are subject to rapid degradation to creatinine and no effect of treatment with creatine-ethyl-ester was found in CRTR-D patients.
Cyclocreatine treatment in the brain-specific Slc6a8 knockout mouse showed uptake of cyclocreatine in the brain and improved cognition.\textsuperscript{197} These are promising results but warrant further studies, for instance in the ubiquitous Slc6a8 knockout mouse which provides a better model for the BBB. In mouse hippocampal slices cyclocreatine uptake was largely CRTR dependent although a small amount still entered the cells after blockage of the CRTR.\textsuperscript{217} Cyclocreatine was not converted to creatine after uptake.\textsuperscript{217} Cyclocreatine is a very good substrate for CK, although still a 6-fold less efficient than creatine.\textsuperscript{18} Importantly, cyclocreatine-phosphate is in contrast a very poor substrate for CK, donating phosphate at a rate of 1% of the rate of phosphocreatine and is therefore able to maintain phosphate pools for a longer period of time.\textsuperscript{18} Cyclocreatine(-phosphate) might protect against ischemic damage.\textsuperscript{1, 18} However, it remains to be seen whether cyclocreatine with these different properties can compensate for the cerebral creatine deficiency in CRTR-D.

**CONCLUSIONS**

Since the discovery of the CRTR-D in 2001, many patients have been diagnosed and the clinical presentation has been well defined. In the same period the previous model of peripheral creatine synthesis followed by uptake through the CRTR into the creatine requiring tissues (brain, muscle, heart, and retina) has been questioned. Now there is strong evidence that the brain ensures (part of) its creatine needs by endogenous synthesis. Also other tissues like skeletal muscle, heart, and retina express the enzymes AGAT and GAMT required for endogenous creatine synthesis and might synthesize creatine in addition to creatine uptake from the blood. Creatine tissue levels appear tightly and differentially regulated.

CRTR-D mainly affects the brain while creatine is, under physiological circumstances, predominantly concentrated in skeletal muscle and high creatine levels are also present in heart and retina. Complete systemic creatine depletion in the AGAT knockout mouse on a creatine free diet leads to severe muscular changes suggesting that creatine does play an important role in skeletal muscle. Furthermore, myopathy occurs frequently in AGAT-D patients. Limited data showed the presence and even normal levels of creatine in the muscle of CRTR-D patients. Creatine synthesis might compensate for the loss of creatine uptake in the muscle. More studies on muscular creatine levels and function in CRTR-D are needed.
**Table 3.** Treatment trials with creatine precursors L-arginine and glycine.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>M/F</td>
<td>Age (years)</td>
<td>Supplements</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>8.5</td>
<td>L-arginine 300mg/kg/d</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>9-16</td>
<td>L-arginine 400mg/kg/d</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>6</td>
<td>Creatine 300mg/kg/d + L-arginine 450mg/kg/d + glycine 150mg/kg/d</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>2-5</td>
<td>1. Creatine 400mg/kg/d</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>14-16</td>
<td>2. Creatine + L-arginine 200 mg/kg/d + glycine 200mg/kg/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. L-arginine 200 mg/kg/d + glycine 200mg/kg/d</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>0-10</td>
<td>Creatine 400mg/kg/d + L-arginine 400mg/kg/d (+glycine 150mg/kg/d)</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>5-17</td>
<td>L-arginine 300mg/kg/d</td>
</tr>
</tbody>
</table>

Cr, creatine; F, female; M, male; N, number; NPA, neuropsychological assessment; PCr, phosphocreatine.

* same patient included.  
* glycine from start in two, added after 15-21 months in five and not added at all in two patients.
Another central question is why CRTR-D causes cerebral creatine deficiency if the brain synthesizes creatine. The normal to slightly elevated creatine levels in CSF of CRTR-D patients might suggest that the cerebral creatine deficiency derives from reuptake failure and defective creatine recycling following release. This is compatible with a role of creatine as neuromodulator or neurotransmitter. Further characterization of the creatine transporter knockout mouse might bring new insights. Unraveling the pathogenesis of CRTR-D and understanding the role of creatine in the brain is essential in the development of an effective treatment.

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REFERENCES


A review: clinical aspects and pathophysiology


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198. Snow RJ. AGAT knockout mice provide an opportunity to titrate tissue creatine content. J Physiol 2013 Jan 15;591(Pt 2):393.


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