Creatine Deficiency Syndromes

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Creatine Synthesis and Transport

Creatine is synthesised by two enzymatic reactions: (1) transfer of the amidino group from arginine to glycine, yielding guanidinoacetate and catalysed by L-arginine:glycine amidinotransferase (AGAT); (2) methylation of the amidino group in the guanidinoacetate molecule by S-adenosyl-l-methionine:N-guanidinoacetate methyltransferase (GAMT) (Fig. 16.1). Creatine synthesis occurs primarily in the kidney and pancreas, which have high AGAT activity, and in liver, which has high GAMT activity. From these organs of synthesis, creatine is transported via the bloodstream to the organs of utilisation (mainly muscle and brain), where both the endogenous creatine and that derived from dietary sources are taken up by a sodium- and chloride-dependent creatine transporter (CRTR, SLC6A8). Some of the intracellular creatine is reversibly converted into the high-energy compound creatine phosphate by the action of creatine kinase (CK). Three cytosolic isoforms, brain type (BB-CK), muscle-type (MM-CK) and the MB-CK heterodimer, and two mitochondrial isoforms exist. Creatine and creatine phosphate are nonenzymatically converted into creatinine, with a constant daily turnover of 1.5 % of body creatine. Creatinine is mainly excreted in urine, and its daily excretion is directly proportional to total-body creatine, and in particular to muscle mass (20-25 mg/kg/24 h in children and adults, and lower in infants younger than 2 years).

Fig. 16.1. Metabolic pathway of creatine/creatine phosphate, which mainly occurs in the organs indicated. ADP, adenosine diphosphate; ATP, adenosine triphosphate; AGAT, arginine-glycine amidinotransferase; CK, creatine kinase; CRTR, creatine transporter (SLC6A8); GAMT, guanidinoacetate methyltransferase
Creatine deficiency syndromes (CDS) are a group of inborn errors of creatine synthesis and transport and include autosomal recessive arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) deficiencies, and deficiency of the X-linked creatine transporter (SLC6A8). In all these disorders the common clinical hallmark is mental retardation, speech delay and epilepsy. Additional frequent manifestations include failure to thrive, growth retardation, muscular hypotonia and movement disorder (mainly extrapyramidal). The common biochemical hallmark is cerebral creatine deficiency as detected by proton magnetic resonance spectroscopy (H-MRS). Increased levels of guanidinoacetate in body fluids are pathognomonic for GAMT deficiency, whereas these levels are reduced in AGAT deficiency. Increased urinary creatine/creatinine ratio is associated with SLC6A8 deficiency. Oral supplementation of creatine leads to partial restoration of the cerebral creatine pool and improvement of clinical symptoms in GAMT and AGAT deficiency. Reduction of guanidinoacetate by additional dietary restriction of arginine (and supplementation of ornithine) appears to be of additional benefit for the long-term outcome of GAMT-deficient patients. For SLC6A8-deficient patients no effective treatment is currently available. CDS may account for a considerable fraction of children and adults with mental retardation of unknown cause, and screening for these disorders (by urinary/plasma metabolites, brain H-MRS and/or a DNA approach) should therefore be included in the investigation of this population.

Secondary changes in creatine metabolism have been described mainly in disorders affecting arginine and ornithine metabolism, such as ornithine aminotransferase (OAT) deficiency and deficiency of argininosuccinate synthase and argininosuccinate lyase.

16.1 Clinical Presentation

Common clinical hallmarks of CDS are mental retardation, speech delay and epilepsy. Mental retardation ranges from mild to severe and is characteristically associated with hyperactive behaviour and autistic features [1, 2]. Movement disorder, mainly extrapyramidal, and basal ganglia changes have been observed as additional features in GAMT deficiency.

16.1.1 Guanidinoacetate Methyltransferase Deficiency

The first patient with GAMT deficiency was described in 1994 [3, 4]. This boy was considered to be normal until 4 months of age, when he was noted to have developmental arrest, hypotonia, hyperkinetic extrapyramidal movements and head nodding. His electroencephalogram (EEG) showed slow background activity and multifocal spike slow waves. Magnetic resonance imaging (MRI) revealed bilateral abnormalities of the globus pallidus consisting of hypointensities in T1-weighted images and as hyperintensities in T2-weighted images.

To date, more than 50 patients are known to the authors, and many of them have been published as single or groups of cases. An overview of 27 cases showed a broad clinical spectrum from mild to severe mental retardation, occasional to drug-resistant seizures and, in the most severe cases, extrapyramidal movement disorder and pathological signal intensities in the basal ganglia [5]. Findings were widely confirmed in a more recent report of a series including 8 new patients [6]. Presentations masquerading as Leigh-like syndrome and mitochondrial disease [7] or late-onset ballistic and dystonic movement disorder [8] have been reported.

16.1.2 Arginine: Glycine Amidinotransferase Deficiency

So far patients from only three unrelated families have been identified with AGAT deficiency. The first reported family includes three siblings and their cousin. Clinical features included developmental delay/intellectual disability, speech delay, autistic behaviour, occasional seizures and brain creatine deficiency that was reversible upon creatine supplementation [9-11]. One sibling diagnosed at birth and treated with creatine within the first few weeks remained asymptomatic until the age of 18 months [12]. The second family includes a 14-month-old American girl of Chinese descent, who presented with psychomotor delay, severe language impairment, failure to thrive and autistic behaviour [13]. Recently a 21- and 14-year-old pair of siblings belonging to a Yemenite Jewish family has been reported with AGAT deficiency. Both presented with a history of developmental delay, fatigability and poor weight gain. They had moderate and mild intellectual disability (IQ 47 and 60), proximal muscle weakness, moderately elevated CK levels (500-600 U/l) and myopathic electromyography. Muscle biopsy showed tubular aggregates and decreased activities of mitochondrial encoded respiratory chain enzymes [14].

16.1.3 SLC6A8 Deficiency

The first patient with SLC6A8 deficiency was reported in 2001 [15, 16]. He had mental retardation, autistic behav-
behaviour and speech delay. Since then at least 78 families with a total of about 170 patients (including affected males/ heterozygous females) have been diagnosed [17] (www.LOVD.nl/SLC6A8). Mental retardation, speech delay, autistic behaviour and hyperactive attention deficit are the leading clinical features [18, 19]. Additional features can include muscular hypotonia, hyperextensible joints, movement disorder, short stature, and brain atrophy, discrete facial dysmorphic features and intestinal manifestations [19-21]. Neurological and psychiatric problems can be progressive in adulthood [22]. Cardiac arrhythmia, including multiple premature ventricular contractions, has also been observed in association with SLC6A8 deficiency [23]. Epilepsy is frequently present [24], and the spectrum ranges from occasional, drug-responsive seizures [19] to frequent generalised tonic clonic seizures [25] and therapy-resistant frontal lobe epilepsy [26].

Females heterozygous for the family mutation in SLC6A8 can have learning problems/mild mental retardation [27] The most severe phenotype has been reported in a girl with mild intellectual disability, behavioural problems and intractable epilepsy [26].

The prevalence of SLC6A8 deficiency is relatively high and may be responsible for about 2% of males with X-linked mental retardation [28-32] and for 1.4% of males with sporadic mental retardation [32].

16.2 Metabolic Derangement

CDS are caused by three gene defects involved in either synthesis or transport of creatine. All three defects result in an almost complete lack of cerebral creatine (Fig. 16.2). The prominent CNS involvement in all CDS patients indicates that creatine is essential for proper brain function. Apart from its role in energy storage and transmission, creatine may have an additional role as a neuromodulator [2, 33]. Although creatine plays an important metabolic role in muscle tissue, creatine levels are only slightly reduced in skeletal muscle of patients with CDS [34, 35]. Creatine has not been measured in heart muscle, but none of the reported patients had cardiomyopathy. Plasma creatine levels are largely influenced by individual nutritional facts. Thus, normal plasma creatine levels do not exclude the presence of CDS. Urinary creatine excretion is expected to be low in patients with defects in creatine synthesis, but in SLC6A8 deficiency the urinary creatine-to-creatinine ratio is high [36]. Impaired function of SLC6A8 in renal tubular cells and subsequent reduced tubular (re)uptake of creatine is the most likely reason for this. Moreover, low intracellular creatine and creatine phosphate results in reduced production of creatinines. Thus, plasma creatinine concentrations, and in particular urinary creatinine excretion, are low in patients with CDS [37].

Guandinoacetate is the second metabolite that plays a role in CDS. In GAMT deficiency, guandinoacetate accumulates in tissues and body fluids [2, 5]. In the CSF levels up to more than 100-fold normal are found [5]. In AGAT deficiency guandinoacetate is low [2, 9, 11]. In SLC6A8 deficiency, guanidinoacetic acid levels are normal [36, 37].

S-Adenosylmethionine is methyl donor for creatine synthesis. Although one might speculate that S-adenosylmethionine accumulates in creatine synthesis defects, S-adenosylmethionine and S-adenosylhomocysteine levels were unremarkable in one patient with GAMT deficiency (unpublished).

Secondary (cerebral) creatine deficiencies have been observed in argininosuccinate lyase deficiency (argininosuccinic aciduria), argininosuccinate synthase deficiency (argininosuccinate synthase deficiency [Fig. 16.2 a, b. In vivo proton magnetic resonance spectroscopy (1H MRS) of the brain of a patient with cerebral creatine deficiency due to GAMT deficiency. a Complete lack of creatine resonance; b partial normalisation of creatine spectrum after 6 months of treatment with oral creatine monohydrate}
(citrullinaemia type 1) [38], and ornithine aminotransferase (OAT) deficiency (gyrate atrophy of the choroid and retina) [39]. Secondary changes in creatine metabolism seem also to occur in disorders of remethylation, such as in cobalamin C deficiency [40].

16.3 Genetics

The genes encoding for GAMT and AGAT (GAMT and GATM) are mapped on chromosome 19p13.3 and 15q15.3, respectively. Both disorders are inherited autosomally recessively, and many of the reported patients are the products of consanguineous marriages [5, 10].

The SLC6A8 gene has been mapped to Xq28. As SLC6A8 deficiency is an X-linked disorder, males are mainly affected, while according to the X-inactivation pattern heterozygous females may have a variable clinical phenotype [27]. Moreover, it should be noted that the X-linked pattern of inheritance will not be observed in the case of a de novo mutation. Therefore, diagnostic screening of males with sporadic mental retardation (prevalence of 1.4%; CI: 0-3.30) [31] and females (prevalence data unknown) should include screening for SLC6A8 deficiency [41].

To date, in the GAMT and SLC6A8 genes many different mutations have been identified, including nonsense, missense, splice error, insertion, deletion and frameshift mutations without a clear phenotype-genotype correlation [2, 5, 6, 42]. In the GATM gene three mutations have been found in three unrelated families; a nonsense mutation [9, 11], a splice error [12] and single nucleotide insertion [13]. There is no evidence for a hotspot region in any of these genes; however, certain mutations appear to occur more frequently. In GAMT, c.327G>A and c.59G>A occur in more than 50% of alleles. While c.327G>A occurs in all ethnicities [5, 6], c.59G>A has been found in patients from Southern Europe and Turkey only. This mutation is particularly prevalent in Portugal [43]; in the SLC6A8 gene, c.319_321delCTT, and c.1221_1223delTTC are the most frequently found [2], and at present 36 different pathogenic mutations have been reported [17] (www.LOVD.nl/SLC6A8).

16.4 Diagnostic Tests

16.4.1 MRS of Brain

Inborn errors of creatine metabolism (biosynthesis and transport) can be recognised by the marked reduction of the creatine signal in H-MRS of the brain. However, metabolite screening and molecular analysis remain necessary, to unravel the underlying defect in particular. Moreover, MRS in infants and children often requires general anaesthesia and is not generally available as a routine method. Therefore, H-MRS of the brain is not a convenient primary screening tool, even though it is increasingly becoming a part of current practice for investigating mental retardation and neurological syndromes.

16.4.2 Metabolite Screening

Analysis of urinary guanidinoacetate and the creatine-to-creatinine ratio is an important screening test for all CDS [37]. Various methods have been developed for the determination of these compounds [44]. Stable isotope gas chromatography-mass spectrometry (SID GC–MS) is a highly sensitive technique suitable for detection of low guanidinoacetate levels characteristic of AGAT deficiency and those required for analysis of this compound in the CSF. Liquid chromatography tandem mass spectrometry (LC-MS-MS) allows the rapid, simultaneous determination of urinary analytes including guanidinoacetate, creatine and creatinine [45, 46]. High guanidinoacetate levels characteristic of GAMT deficiency can be detected with all these methods.

In patients with SLC6A8 deficiency, the increase of urinary creatine excretion together with the inherently low urinary creatinine excretion results in an elevation of the urinary creatine-to-creatinine ratio, which serves as a valuable diagnostic marker in males [36, 47]. In heterozygous females this biochemical trait is not sufficiently sensitive to serve as a diagnostic marker [27]. Even in asymptomatic patients the creatine-to-creatinine ratio can be within the control range [26].

Variation of these compounds during the day is not significant, indicating that a random urine sample is sufficient for the diagnosis of CDS [36]. On the other hand, false-positive values can be detected as the result of a protein-rich diet [32].

Age-dependent normal values have been established [36, 47, 48]. The urinary creatine-to-creatinine ratio decreases after the age of 3 years, while it increases during the first 3 years of life [47]. It is of note that in metabolic urine screening, an overall increased concentration of amino acids and organic acids, expressed as millimoles per mole of creatinine, may be a result of a decreased creatinine excretion and thus represent a suggestive hint for the presence of CDS [37].

Guanidinoacetate can also be measured in dried blood spots [49, 50], thus allowing newborn screening for GAMT deficiency.
16.4.3 DNA Diagnostics

Mutation analysis for the three genes (\texttt{GAMT}, \texttt{GATM} and \texttt{SLC6A8}) involved in CDS is currently used to confirm the diagnosis. Denaturing gradient gel electrophoresis (DGGE) and denaturing high-performance liquid chromatography (HPLC) methods [51, 52] were applied in the past, allowing screening of larger sample numbers. Most patients, however, have been diagnosed individually via direct gene sequencing. New technologies such as high-throughput sequencing, will allow direct gene testing as a screening tool. This might help to diagnose more patients with AGAT deficiency and SLC6A8 deficiency, for which the currently available biomarkers are not very sensitive. This is especially true for detection of heterozygous SLC6A8 females [27].

16.4.4 Functional Tests/Enzymatic Diagnostics

Functional tests and/or enzymatic diagnostics in fibroblasts and/or lymphoblasts or in expression systems facilitate confirmation of a diagnosis at a functional level, in particular if new mutations with unknown pathogenicity are detected [53-55].

16.4.5 Prenatal Diagnosis

Prenatal diagnosis and preimplantation genetic diagnosis for at-risk pregnancies require prior identification of the disease-causing mutation(s) in the family for all three creatine deficiency syndromes [1]. In addition, GAMT deficiency can be prenatally diagnosed by guanidinoacetate measurement in the amniotic fluid in pregnancies at risk for GAMT deficiency if the underlying disease-causing mutations have not been identified in the index patient [56].

16.5 Treatment and Prognosis

16.5.1 GAMT Deficiency

Oral creatine substitution has been effective in replenishing the cerebral creatine pool to approximately 70% of normal in all patients [5, 57]. Most received creatine monohydrate at 300-400 mg/kg/day in three to six divided doses. The clinical response to oral creatine supplementation alone included resolution of extrapyramidal signs and symptoms, and in most patients considerable improvement of their epilepsy [4, 5]. Additional dietary restriction of arginine helps to reduce accumulated guanidinoacetate [58], which in high concentrations is neurotoxic. Arginine is restricted to 15-25 mg/kg/day (corresponding to 0.4-0.7 g/kg/day protein intake), and supplementation with an arginine-free amino acid mixture is necessary to provide an adequate nutritional amino acid supply [59]. Supplementation with high doses of ornithine has the potential to reduce guanidinoacetate synthesis by competitive inhibition of AGAT activity in vitro; however, this approach did not result in reduction of guanidinoacetate in one patient [60]. A combination of arginine restriction and ornithine supplementation might be more effective. This approach has led to an impressive improvement of epileptic seizures, mental capabilities and behaviour in a severely affected adult patient [34]. In this patient, sodium benzoate was given in addition to prevent ammonia accumulation due to possible lack of arginine as the essential amino acid in the urea cycle.

Initiation of combined therapy at an early age, preferably in the neonatal period, might improve the long-term outcome. As an example, a sibling of one index patient was identified by positive mutation analysis in the neonatal period. Treatment was initiated at age 3 weeks in the presymptomatic stage of the disease. The child had a normal neurodevelopmental outcome at age 14 months compared in contrast to the older, symptomatic sibling at the same age [61]. This finding raises the question of adding GAA to the routine derivatised MS/MS newborn screen, as it is simple and adds little to the cost [62].

16.5.2 AGAT Deficiency

Oral creatine supplementation (300-400 mg/kg/day) has been effective in replenishing the cerebral creatine pool and in improving abnormal developmental scores [10-12]. Early diagnosis and treatment seem to be particularly efficient in improving outcomes. One child with global developmental delay at the age of 16 months had achieved normal developmental scores at the age of 40 months, after 23 months of treatment with creatine [13]. Another child diagnosed at age 2 years had a borderline IQ at age 8 years, whereas two children in the same family who started treatment at ages 7 and 5 years had moderate intellectual deficit at the ages of 13 and 11 years [11]. An additional patient from the same family was diagnosed prenatally, and creatine supplementation was started at 4 months. This child showed normal development in at age 18 months, in contrast to his siblings, who had already shown signs of retardation at this age [12].
16.5.3 SLC6A8 Deficiency

SLC6A8 deficiency appears not to be treatable by any of the approaches described above. Treatment of both males and females affected with SLC6A8 deficiency with creatine monohydrate has not proved successful [18]. Only one heterozygous female patient with learning disability and a mildly decreased creatine concentration on brain MRS showed mild improvement on neuropsychological testing after 18 weeks of treatment with creatine monohydrate (250-750 mg/kg/day) [16]. Supplementation with high doses of L-arginine and L-glycine, which are the primary substrates for creatine biosynthesis, combined with high doses of creatine monohydrate, are currently being investigated. The rationale behind this protocol is based on an increased cerebral uptake of both amino acids with the aim of enhancing intracerebral creatine synthesis [63]. Supplementation of L-arginine alone has resulted in developmental progress in one male patient [64]. In four male patients this treatment has failed to improve intellectual outcomes [65]. Additional supplementation of L-glycine might enhance the therapeutic effect. Combined treatment with creatine, arginine and glycine has resulted in a significant improvement of intractable seizures in a female patient with SLC6A8 deficiency [26]. In addition, alternative strategies may be developed that facilitate creatine transport into the brain (e.g. by modified transport via carrier peptides/molecules).

References


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