Are cerebral creatine deficiency syndromes on the radar screen?

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Cerebral creatine deficiency syndromes (CCDS) are responsible for a considerable proportion of the population affected with mental retardation. CCDS are caused by either an inborn error of the proteins involved in creatine biosynthesis or in the creatine transporter. Besides mental retardation, the clinical characteristics of CCDS are speech and language delay, epilepsy and features of autism. CCDS can be diagnosed by proton magnetic resonance spectroscopy of the brain and/or by biochemical and molecular analysis. Treatment of the defects in creatine biosynthesis has yielded favorable outcomes, while treatments for creatine transporter deficiency are still under investigation at this time. The relatively large contribution of the CCDS to the monogenic causes of mental retardation emphasizes the importance of including CCDS in the differential diagnosis of mental retardation of unknown etiology. Pathophysiology is not yet unravelled, although it is known that creatine plays an important role in energy storage and transmission. Moreover, *in vitro* data indicate that creatine acts as a neuromodulator in the brain.

In the last decade, a novel group of inborn errors of proteins involved in creatine biosynthesis and its transporter has been identified. The high prevalence of these defects within the mentally retarded population and the promising treatment possibilities argue for the inclusion of cerebral creatine deficiency syndromes (CCDS) in the differential diagnosis of mental retardation of unknown etiology. CCDS arise by mutations in either one of the autosomal genes *AGAT* and *GAMT*, which encode the two enzymes (arginine:glycine amidinotransferase and guanidinoacetate methyltransferase, respectively) [1,2] necessary for creatine biosynthesis, or by mutations in the X chromosomal creatine transporter gene (solute carrier family 6 member 8 [*SLC6A8*]) [3]. Until recently, this group of syndromes has been termed creatine deficiency syndromes (CDS). However, in bodily fluids, no creatine deficiency exists in creatine transporter-deficient patients, thus, this term may be misleading. We therefore prefer to use the term CCDS, which also correlates better to the main clinical hallmarks that are related to CNS involvement.

In the general population, the incidence of mental retardation is estimated to be approximately 1% (profound, severe and moderate cases) to 3% if mild cases are included (IQ 50–70). Within the mentally retarded population, the frequency of X-linked mental retardation (XLMR) is estimated at 5–12% [4]. This number, and the fact that a large group of XLMR genes are already known [5], indicates that each monogenic cause of XLMR only accounts for a small percentage of

mental retardation in males [6,7]. For the fragile X mental retardation gene (*FMR1*), the Aristaless-related homeobox gene (*ARX*) and, to a lesser extent, for the creatine transporter gene (*SLC6A8*), this seems to be different, as they account for a larger proportion of the XLMR subgroup [7].

The prevalence of SLC6A8 deficiency has been studied in three different patient groups. In the first panel, consisting of males with a strong predilection to having XLMR, a prevalence of 2.1% (six out of 288) was found. Considering the estimation that only 10% of the patients with mental retardation are affected with an X-linked defect, the prevalence of 2.1% in XLMR would translate to approximately 0.2% in the general mental retardation population of unknown etiology [8]. However, the prevalence in the two panels with mental retardation (four out of 478 = 0.8%, [9]) and global development delay (two out of 92 = 2.2%, [10]) was somewhat higher than predicted. This latter group was investigated by proton magnetic resonance spectroscopy (MRS), whereas the other groups were studied by DNA sequence analysis.

The prevalence of AGAT and GAMT deficiencies are not expected to be high, since they are autosomal recessive disorders. Indeed, only 29 GAMT- and five AGAT-deficient patients have been reported [11–15]. However, awareness of GAMT deficiency may be of utmost importance in Mediterranean countries, because a high carrier rate of a pathogenic *GAMT* mutation exists [16,17], and most of the GAMT-deficient patients are from this region.

future
medicine

The relatively high prevalence of CCDS highlights the importance of CCDS inclusion in the differential diagnosis of mental retardation of unknown etiology.

Inborn errors of proteins involved in creatine biosynthesis & its transporter

Arginine:glycine amidinotransferase deficiency

The initial and rate-limiting step in creatine biosynthesis is catalyzed by the AGAT enzyme, which forms guanidinoacetic acid, the precursor of creatine (Figure 1). In 2000, the first siblings with a defect in this enzyme were recognized [18]. These sisters (4- and 6-years old) presented with mental retardation and severe language delay. Routine blood and urine analyses were normal and further investigations did not suggest a

neurometabolic disorder. Magnetic resonance imaging (MRI) of the brain was normal, but proton MRS revealed the absence of the creatine–phosphocreatine signal, suggesting a defect in the proteins involved in creatine biosynthesis or in the creatine transporter. In plasma, concentrations of creatine and guanidinoacetic acid were within normal values, which ruled out a deficiency of GAMT. The patients were reinvestigated in 2001, and analysis of urine revealed consistently decreased levels of guanidinoacetic acid [19]. The diagnosis of AGAT deficiency was confirmed by impaired AGAT activity in cultured cells and the detection of a pathogenic mutation in exon 3 of the *AGAT* gene [19].

Presently, five AGAT-deficient patients are known from two unrelated families, in whom reduced levels of GAA in body fluids have been detected [14,15]. The general clinical and biochemical findings are listed in Table 1.

Guanidinoacetate methyltransferase deficiency

The second step in creatine biosynthesis is mediated by the GAMT enzyme (Figure 1). In 1994, the first GAMT-deficient patient, a 22-month-old male was identified by Stöckler and collaborators [20,21]. The onset of symptoms started at 5 months with developmental arrest. The patient was hypotonic, unable to sit or roll over, showed uncoordinated swallowing and developed a severe extrapyramidal disorder. He had no organomegaly and his head circumference, hearing and vision appeared normal. Electrocardiogram and cardiac ultrasound examinations were normal; electroencephalogram showed low background activity and multifocal spikes. Nonspecific biochemical elevations were reported due to decreased creatinine levels. Magnetic resonance studies were performed for the first time at the age of 12 months: MRI revealed bilateral abnormalities in the globus pallidus. Proton MRS showed a spectrum lacking a creatine signal and an elevated guanidinoacetic acid peak. Treatment with arginine, the substrate of the AGAT enzyme, did not result in restoration of brain creatine. Moreover, guanidinoacetic acid in the brain, measured by proton MRS, remained increased, indicating that the defect was caused by a block in GAMT activity [20]. Indeed, in 1996, impaired GAMT activity in cultured cells and pathogenic mutations in the *GAMT* gene were identified [21]. Furthermore, a GAMT knockout mouse model has been developed, mimicking the biochemical characteristics of GAMT deficiency in humans [22–24].

Figure 1. Creatine biosynthesis.
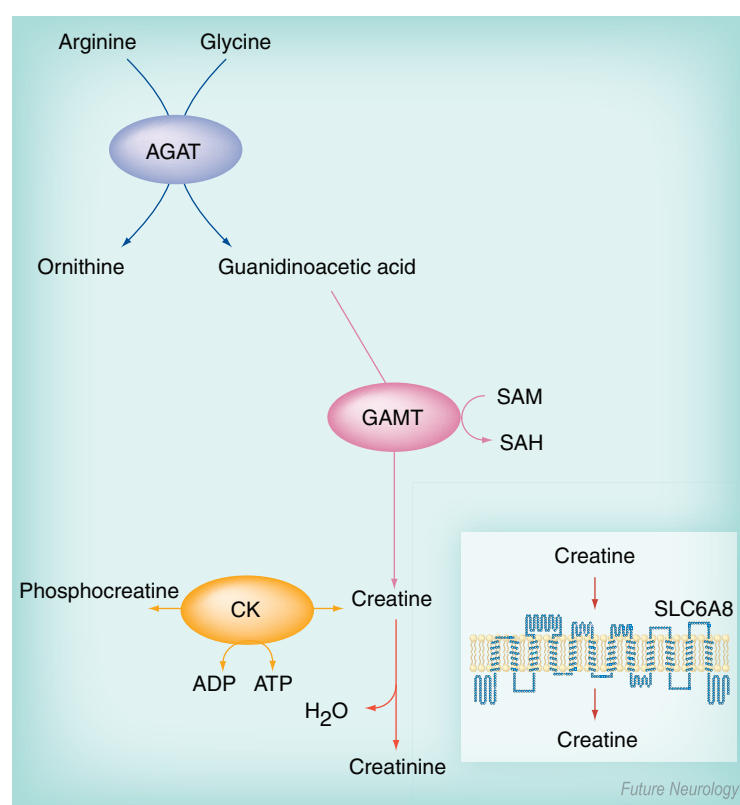
Creatine biosynthesis involves a two-step reaction: the first is catalyzed by arginine:glycine amidinotransferase (AGAT, EC 2.1.4.1) and the second by guanidinoacetate methyltransferase (GAMT, EC 2.1.1.2). Creatine is transported via the bloodstream and taken up by tissues with high CK activity, such as muscle and brain, via a creatine transporter (SLC6A8). CK catalyzes the phosphorylation and dephosphorylation of creatine and phosphocreatine, respectively, thus providing a high-energy phosphate buffering system during ATP release and use.
ADP: Adenosine diphosphate; ATP: Adenosine triphosphate; CK: Creatine kinase.

Table 1. Overview of the patients described to date.

Cerebral creatine deficiency	AGAT (n = 5)[§]	GAMT (n = 29)[¶]	SLC6A8 (n = 24)[#]
Clinical traits:			
Age at diagnosis	0–5	0–26	2–66 years
Developmental delay	5/5	27/29	24/24
Speech and language delay	5/5	21/29	24/24
Mental retardation	5/5	27/29	24/24
Hypotonia	2/5	27/29	11/16
Behavior disorder	1/5	21/29	14/19
Movement disorder	NR	15/29	10/19
Seizures	NR[‡]	25/29	16/24
Mild phenotype*	NA	3/29	NA
Intermediate phenotype*	NA	12/29	NA
Severe phenotype*	NA	12/29	NA
H-MRS of the brain: absence/reduction Cr	5/5	27/29	12/12
Biochemical findings:			
Urinary creatine:creatinine			Increase in 17/17
Urinary guanidinoacetic acid	Decrease in 5/5	Increase in 29/29	Increase in 2/4
Plasmatic guanidinoacetic acid	Decrease in 5/5	Increase in 28/28	Increase in 1/2
Plasmatic creatine	Decrease in 5/5	Decrease in 28/28	NR
Treatment available	Yes	Yes	?

*Based on [11].
‡Febrile seizures were reported in one of the first patients to be described [18].
§ [14,15,19]; ¶ [11,12,13]; #[9,53,66,67].
AGAT: Arginine–Glycine amidinotransferase; GAMT: Guanidinoacetate methyltransferase; NA: Not applicable; NR: Not reported.

To date, 29 patients have been described, varying from neonate to 29 years of age [11–13]. Owing to heterogeneous clinical presentation, GAMT-deficient patients can be classified as having a mild, moderate or severe phenotype, based on the severity grade of the main clinical characteristics (i.e., mental retardation, epilepsy and movement disorder). An overview of the clinical characteristics reported so far is presented in Table 1.

Creatine transporter deficiency

The creatine transporter, encoded by the *SLC6A8* gene, is essential for creatine uptake into cells. In 2001, the first patient with SLC6A8 deficiency was described. The patient presented with mild developmental delay at the age of 7 months in combination with central hypotonia. Prenatal and perinatal histories were unremarkable [25]. There was a history of learning disabilities and mental
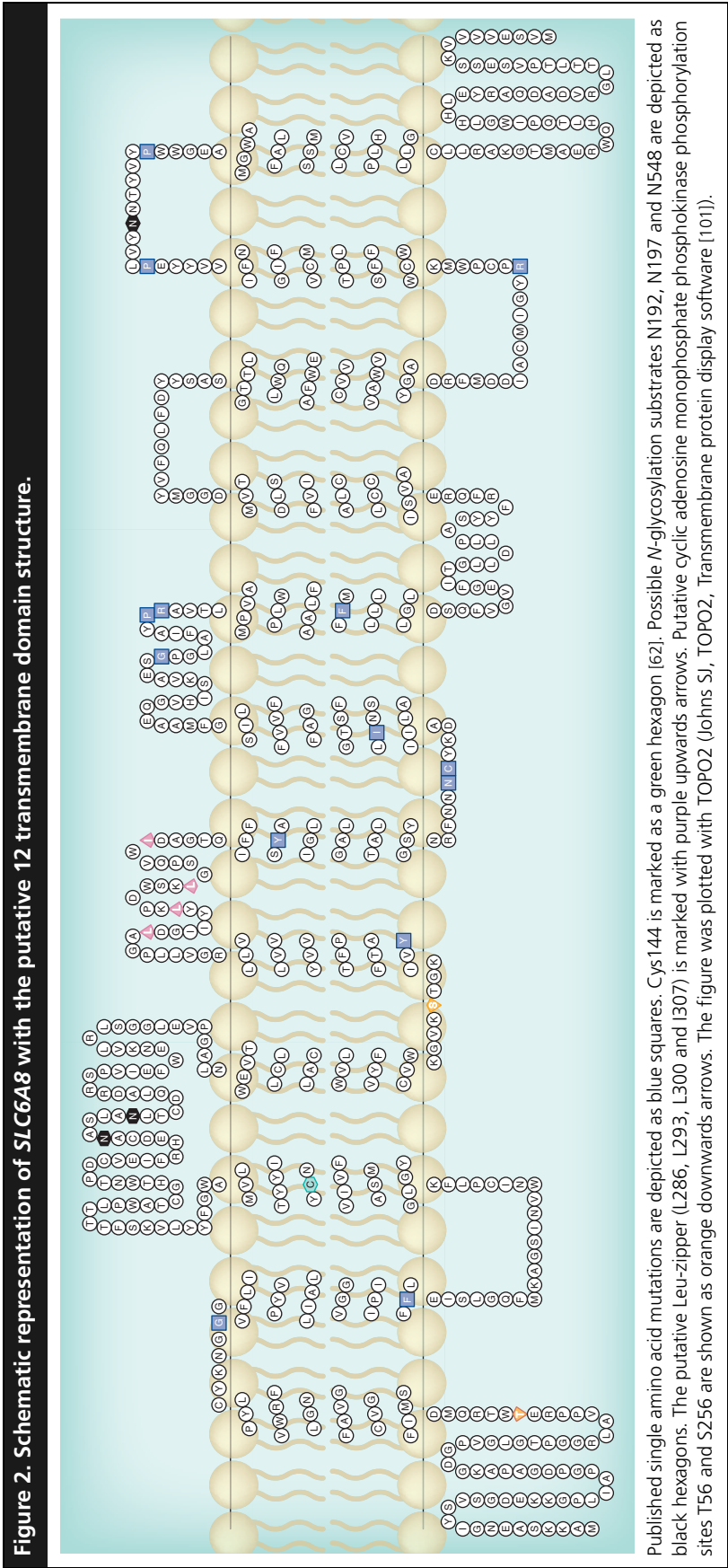
retardation in the family, compatible with an X-linked disorder. At 2 years of age the patient was admitted to hospital owing to a partial status epilepticus. Examination at 6 years of age showed a severe delay in speech and language development. A progressive increase of the head circumference (75th percentile to 95th percentile) prompted MRI and proton MRS. Proton MRS highlighted an almost complete loss of the creatine and phosphocreatine signal, similar to that observed in patients with AGAT and GAMT deficiency [26]. Consequently, oral creatine supplementation was commenced. However, after 4 months no restoration of cerebral creatine concentration was observed, which was in line with the lack of clinical improvement. Therefore, oral creatine supplementation was discontinued.

This, and the biochemical findings, ruled out a creatine biosynthesis defect. Moreover, high urinary creatine concentrations suggested a defect in cellular creatine transport. The inheritance pattern suggestive for X-linked disease and the fact that the gene encoding the creatine transporter, *SLC6A8*, is mapped to Xq28 [27], strengthened this hypothesis. This transporter is a member of the Na^+/Cl^--dependent neurotransmitter transporter family (Figure 2). Indeed, sequence analysis of *SLC6A8* revealed a hemizygous nonsense mutation. Furthermore, impaired creatine uptake in cultured fibroblasts was demonstrated [28]. The carrier status for this mutation was confirmed in the female relatives.

So far, more than 34 patients have been reported with SLC6A8 deficiency. The clinical characteristics of 24 male patients are listed in Table 1. In females, a very heterogeneous clinical phenotype is expected due to skewed X-inactivation, varying from learning disabilities to mental retardation.

How to diagnose cerebral creatine deficiency syndromes?

A marked reduction of cerebral creatine measured by proton MRS is highly indicative of a primary creatine deficiency syndrome. Workup by metabolite, molecular and functional studies is required to identify the underlying defect (i.e., AGAT, GAMT or SLC6A8 deficiency). Additionally, in families where individuals have previously been diagnosed with CCDS, prenatal diagnosis can be performed at the molecular level in chorion villus sampling or amniotic cells [12,15]. In case of GAMT deficiency, prenatal diagnosis can also be performed from the amniotic fluid at the metabolite level [12]. At present, the primary choice for

Figure 2. Schematic representation of *SLC6A8* with the putative 12 transmembrane domain structure.

Published single amino acid mutations are depicted as blue squares. Cys144 is marked as a green hexagon [62]. Possible *N*-glycosylation substrates N192, N197 and N548 are depicted as black hexagons. The putative Leu-zipper (L286, L293, L300 and I307) is marked with purple upwards arrows. Putative cyclic adenosine monophosphate phosphokinase phosphorylation sites T56 and S256 are shown as orange downwards arrows. The figure was plotted with TOPO2 (Johns SJ, TOPO2, Transmembrane protein display software [101]).

screening of CCDS in most institutes is measuring creatine, creatine:creatinine ratio and guanidinoacetic acid in body fluids or performing proton MRS of the brain. In contrast with metabolite analysis, proton MRS is expensive, not widely available and sedation is usually required.

Metabolite analysis

Various methods to measure creatine and guanidinoacetic acid in bodily fluids are available [29]. In diagnostic settings, the techniques generally used are gas chromatography-mass spectrometry [30,31], high-performance liquid chromatography (HPLC) [32] and tandem mass spectrometry [33–35]. Increased guanidinoacetic acid levels in bodily fluids are pathognomonic for GAMT deficiency, whereas reduced levels are found in AGAT deficiency. Creatine is usually reduced in the bodily fluids of patients with a biosynthesis defect, whereas an increased urinary creatine:creatinine ratio is found in males with SLC6A8 deficiency [30]. In the majority of females with a heterozygous *SLC6A8* mutation, this ratio is not informative.

Molecular diagnosis

Molecular diagnosis is usually performed by direct sequencing of the open reading frame of the respective gene or by mutation scanning using DHPLC [36] followed by DNA sequence analysis in case of aberrant heteroduplexes.

- The *AGAT* gene (Gene ID 2628, official nomenclature: *GATM*) has been mapped to chromosome 15q15.3, is 16.8 Kb in size and contains nine exons, which encode a protein of 424 amino acids. There are two mutations described (**Figure 3**);

- The *GAMT* gene (Gene ID 2593) has been mapped to chromosome 19p13.3, its size is 4.46 Kb and it contains six exons, which encode a protein of 237 amino acids. There are 15 pathogenic mutations described throughout the gene (**Figure 4**);

- The creatine transporter gene – *SLC6A8* (Gene ID 6535) has been mapped to Xq28 [37–39]. The *SLC6A8* gene spans 8.4 Kb consisting of 13 exons, which encode a protein of 635 amino acids. To date, 20 mutations have been described throughout *SLC6A8* (**Figure 5**).

Enzyme analysis

Several assays have been reported to assess both AGAT and GAMT activity [29]. The first methods to be reported used radioactive-labeled

Figure 3. Schematic representation of the *AGAT* gene and its reported mutations.
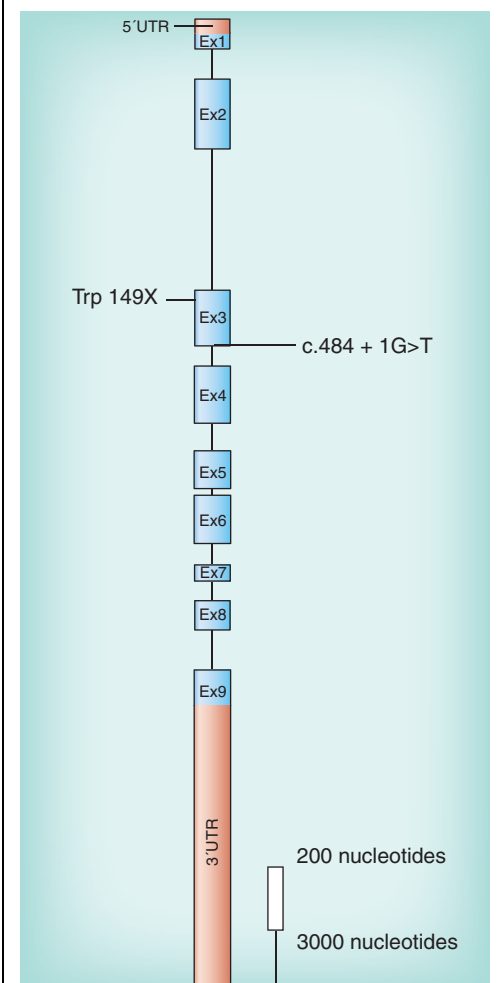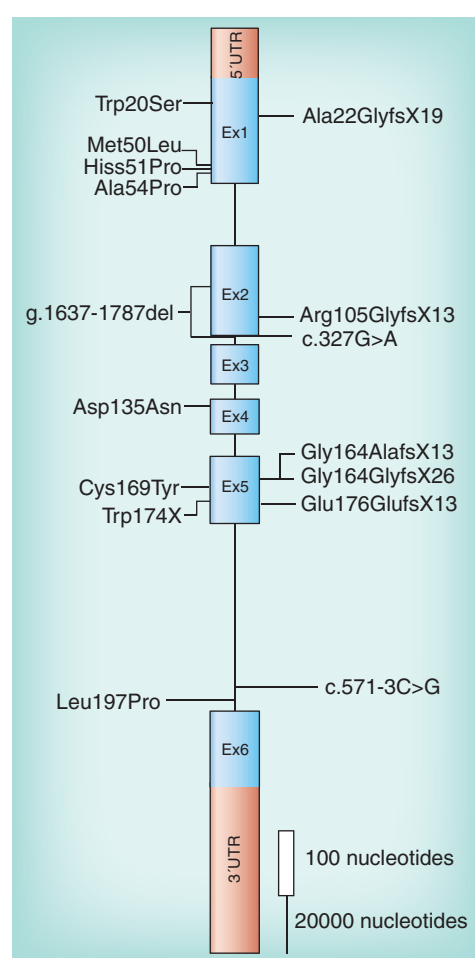
Exons (numbered boxes) and introns (black lines) are drawn to scale. To date, only two mutations have been reported (c.446G>A, p.Trp149X [19]; c.484 +1G>T, IVS3+1G>T [15]).

substrates [19,21,40,41]. In 2003, two methods using stable isotope labeled substrates were developed for both AGAT and GAMT [42,43]. The use of stable isotopes increases the sensitivity of the methods and reduces the amount of biological material required.

To study SLC6A8 deficiency, the creatine transporter function can be tested by a functional assay in fibroblasts [28].

Diagnostic pitfalls

In the last decade, only two families with AGAT deficiency were reported, in comparison with 22 families affected with GAMT deficiency. AGAT-deficient patients could remain unnoticed

Figure 4. Schematic representation of the *GAMT* gene and its reported mutations.

Exons (numbered boxes) and introns (black lines) are drawn to scale. Splice-site and frameshift mutations are indicated on the right side; missense mutations are indicated on the left side; mutations are indicated in terms of protein. References for the mutations: c.59G > C, p.Trp20Ser; c.152A > C, p.His51Pro; c.160G > C, Ala54Pro; c.64dupG, p.Ala22fsX19; c.299_311dupGGGACTGGGCCCC, p.Arg105GlyfsX13; c.491delG, p.Gly164fsX13; c.521G > A, p.Trp174X; c.526dupG, p.Glu176GlufsX13; c.327G > A, IVS2+1G > A; g.1637_1787del [11]; c.506G > A, p.Cys169Tyr [16]; c.148A > C, p.Met50Leu [12]; c.491dupG, p.Gly164fsX26; c.571–3G > C, IVS5–3G > C [63]; c.590T > C, p.Leu197Pro [64].

in metabolic screening due to techniques that are neither sensitive or specific enough to detect decreased metabolite levels. Furthermore, intake of nutrition containing high concentrations of creatine (i.e., meat and fish) may hamper diagnosis of AGAT deficiency [44].

In approximately 10% of the males with mental retardation in whom elevated urinary creatine:creatinine ratio is found, no *SLC6A8* mutation is detected. They are likely to represent false-positive outcomes. Elevated urinary creatine:creatinine ratio may also be the secondary result of other (neuro)muscular disorders, such as Duchenne muscular dystrophy and Becker muscular dystrophy [45]. In rare cases, promoter mutations or deep intronic mutations may have been missed.

Although three female SLC6A8-deficient patients were found with an elevated urinary creatine:creatinine ratio, this is not the rule for the majority of heterozygous females [29]. Proton MRS of the brain in females may be restricted by difficulties in detecting minimal reductions in brain creatine and sedation is often required. Currently, in females, molecular screening is probably the most sensitive method for the detection of SLC6A8 deficiency, and thus urinary creatine:creatinine ratio is not the primary choice. In molecular diagnostic screening, care should be taken, since a paralogous gene, *SLC6A10*, which is mapped at chromosome 16p11, is highly identical to *SLC6A8* [46].

Regarding DNA sequence analysis, it should be noted that, in rare cases, unique sequence variations are detected, both in coding and noncoding regions in the *SLC6A8*, *GAMT* or *AGAT* gene, which were not encountered in controls [8,9,16]. Thus, it is important to evaluate the nature of the variants carefully, before classifying them as pathogenic mutations or polymorphisms.

Treatment of creatine biosynthesis defects & creatine transporter defects

Treatment approaches of CCDS aim to restore creatine in the brain by creatine supplementation, with success for the biosynthesis defects [20,47,48]. For AGAT deficiency, the highest success rate is expected because there is no accumulation of substrates. In fact, if started in a presymptomatic phase, creatine supplementation in AGAT and GAMT-deficient patients leads to an impressive restoration of cerebral creatine levels, and a favorable clinical response. In an AGAT-deficient infant, treatment initiated at 4 months of age showed normal development by 18 months of age, and the restoration of creatine levels in bodily fluids and the brain were almost complete [49]. Similar success was observed with a GAMT-deficient patient in whom presymptomatic treatment was initiated at 22 days of age [13]. This suggests that creatine supplementation in early life prevents the neurological sequelae.

Figure 5. Schematic representation of the *SLC6A8* gene and its reported mutations.
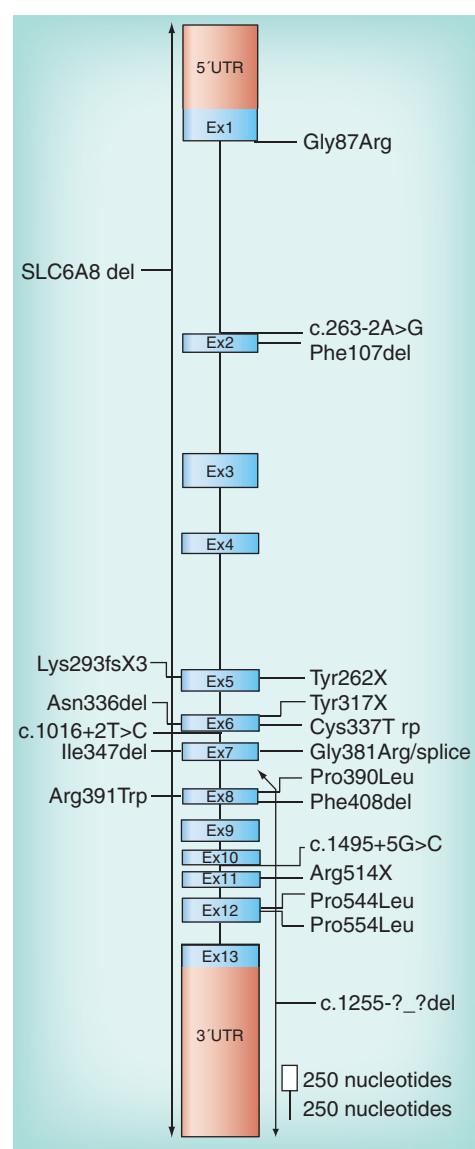
Exons (numbered boxes) and introns (black lines) are drawn to scale. References for the mutations: c.259G > A, p.Gly87Arg; c.319_321delCTT, p.Phe107del; c.950_951insA, p.Tyr317X; c.1011C > G, p.Cys337Trp; c.1169C > T, p.Pro390Leu; c.1661C > T, p.Pro554Leu [8]; c.263–2A > G, IVS2–2A > G [65]; c.319_321delCTT, p.Phe107del; c.786C > G, p.Tyr262X; c.1221_1223delCTT, p.Phe408del; c.1141G > C, p.Gly381Arg/splice; c.1540C > T, p.Arg514X [66]; c.878_879delTC, p.Lys293fsX3; c.1221_1223delCTT, p.Phe408del; [53]; c.1006_1008delAAC, p.Asn336del; c.1016+2T > C, IVS6+2T > C; c.1059_1061delCTT, p.Ile347del; c.1171C > T, p.Arg391Trp [9]; c.1255?_?del; *SLC6A8* del [67].

Currently, the most extensive experience has been obtained with the treatment of GAMT-deficient patients. The aim of the treatment is to increase the creatine and decrease the guanidinoacetic acid in brain. The severe phenotype observed in some cases in GAMT-deficient patients might be due to both the lack of creatine, but also due to guanidinoacetic acid accumulation, since guanidino compounds are known for their neurotoxic and epileptogenic effects [50]. In order to lower guanidinoacetic acid levels, AGAT activity needs to be reduced. This can be partly achieved by two different strategies:

• Substrate deprivation of AGAT by dietary restriction of arginine intake;

• With supplementation of high concentrations of ornithine and creatine.

With this therapeutic approach, levels of guanidinoacetic acid are lowered by 50% in bodily fluids [51]. This led to an additional clinical improvement as compared with treatment with creatine alone.

No successful treatment has currently been reported for SLC6A8 deficiency. Attempts with creatine supplementation in males showed no marked improvement [25,28,52,53]. Current trials are aiming at stimulation of creatine biosynthesis in the brain by supplementation with high doses of arginine and glycine, combined with high doses of creatine (Mancini GM, van der Knaap MS, Salomons GS, Unpublished Data). However, despite the expression of biosynthesis proteins in the brain, the spatial distribution of AGAT, GAMT and SLC6A8 may pose problems for this approach [54,55].

Creatine: a novel neuromodulator?

Whilst the function of creatine in energy metabolism has been addressed extensively, only a limited number of studies have focused on its role in the brain. Creatine synthesis has been observed in the CNS [56]. *In situ* hybridization studies also found *AGAT* and *GAMT* expression in almost all CNS cell types, in addition to its expression at the blood–brain barrier level, whereas SLC6A8 mRNA was only found in neurons, oligodendrocytes and brain capillary endothelial cells [54,55,57]. These data support the recent postulations that the brain is able to synthesize creatine.

Creatine seems to be essential for brain function, as patients suffering from a CCDS have mental retardation, speech and language disorders, autistic features and may have extrapyramidal movement disorders and epileptic seizures (see above). Additionally, several guanidino

compounds are neurotoxic, presumably by altering the functioning of inhibitory and excitatory amino-acid receptors [58]. Considering the effects of creatine on central neurotransmission processes, it has been shown that guanidino compounds, including creatine, may affect γ-aminobutyric acid (GABA)-ergic neurotransmission as (partial) agonists for $GABA_A$ receptors [58–60]. Indeed, it was shown that creatine is released from central neurons in a manner similar to that of classical neurotransmitters, specifically, involving an exocytotic release mechanism [61]. In view of this *in vitro* data, it was hypothesized that creatine represents a cotransmitter in the brain that modulates the functioning of postsynaptic receptors for neurotransmitters, such as GABA (**Figure 6**) [61].

Conclusions

In the last decade, a novel group of inborn errors of metabolism has been identified: the cerebral creatine deficiency syndromes. The importance of considering CCDS in the differential diagnosis of mental retardation is emphasized by the relative high frequency of SLC6A8 deficiency, and the promising results of treatment of GAMT- and AGAT-deficient patients. The diagnostic tests to detect these syndromes are readily

Figure 6. Proposed model of action for cerebral creatine.
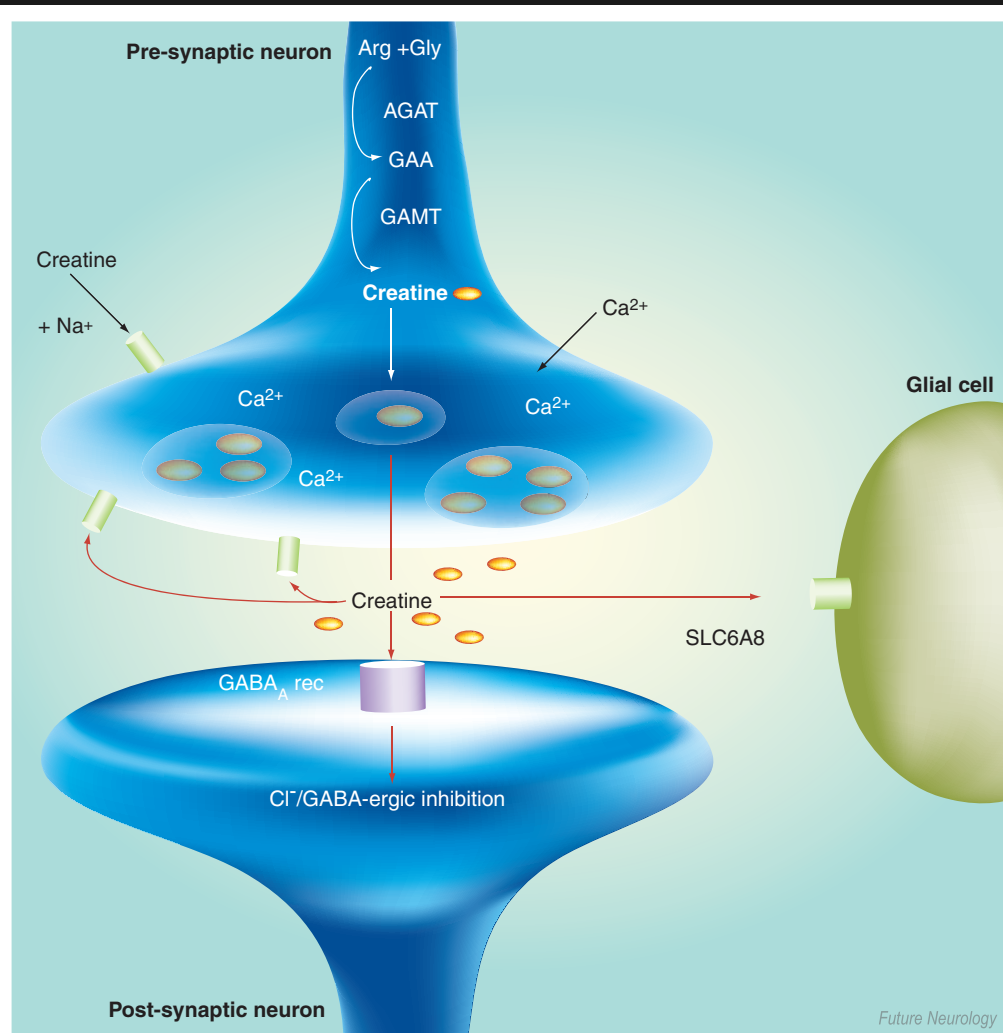
Creatine is synthesized in neurons, by the enzymes AGAT and GAMT. Upon synthesis creatine is released in a Ca^{2+} dependent (exocytotic/vesicular) manner into the synaptic cleft interacting with the $GABA_A$ receptors of the postsynaptic neuron. Creatine may subsequently be taken up from the cleft via the creatine transporter into the neurons or glial cells [61].
AGAT: Arginine:glycine amidinotransferase; GAA: guanidinoacetic acid; GABA: γ-amino butyric acid; GAMT: Guanidinoacetate methyltransferase.

available (e.g., metabolite and molecular analysis and, to a lesser extent, proton MRS), which makes this feasible for any institution that has access to at least one of these techniques. Moreover, proton MRS/MRI in particular enables the detection of multiple diseases. The recent findings that creatine acts as a neuromodulator opens up new research areas, which may be worthwhile for the elucidation of the pathophysiology of CCDS.

Future perspectives

Within the next 5–10 years in the Western world, every mentally retarded patient (male/female) will be screened for CCDS. This could be achieved primarily by proton MRS, which will certainly become more widely available, with the advantage of disclosing additional diseases. For the screening of CCDS, proton MRS is adequate, as a marked reduction or absence of the creatine signal is diagnostic for primary creatine deficiency. Currently, molecular analysis is performed by direct DNA sequencing. However, current developments in the field of microarrays allow the analysis of multiple genes, or even full genomes, in a single assay, which opens up a new world in both research and clinical applications. This technology may lead to more insights into the pathophysiology. The development of sequencing chips (i.e., resequencing arrays) also discloses new perspectives in the diagnosis and screening of CCDS,

although its sensitivity is not yet acceptable for clinical diagnostics.

Treatment of SLC6A8 deficiency is one of the big challenges. Clinical improvement has been observed in the patients with biosynthesis defects upon treatment, with almost complete restoration of creatine in the brain. This proves that cerebral creatine restoration is essential. When a vehicle for creatine uptake in the brain is found, treatment should also be successful for SLC6A8 deficiency. Elucidation of the creatine biosynthesis pathway in the brain, in addition to the clarification of the function of creatine in the brain, may increase the success rate of treatment.

Tandem mass spectrometry has the potential for simultaneous multidisease screening and has recently been applied to neonatal screening programs. In case measurement of guanidinoacetic acid in dried blood spots proves to be specific and sensitive enough for detection/exclusion of GAMT deficiency, this disorder will be included in neonatal screening [32,33,13]. AGAT and SLC6A8 deficiencies are not yet eligible for neonatal screening since creatine and creatinine do not seem to be informative in the neonatal period.

Executive summary

Inborn errors of proteins involved in creatine biosynthesis & its transporter

- Three novel disorders in creatine metabolism have been identified. Two autosomal recessive creatine biosynthesis defects (*AGAT* and *GAMT* deficiency) and one X-linked creatine transporter defect (*SLC6A8* deficiency). This group is defined as cerebral creatine deficiency syndromes (CCDS).
- The common hallmarks of CCDS are the absence, or marked reduction of the creatine signal in the proton magnetic resonance spectroscopy (MRS) of the brain, mental retardation and speech and language delay (Table 1).
- Worldwide, 29 patients with *GAMT* deficiency, five with *AGAT* deficiency and over 34 with *SLC6A8* deficiency have been reported.

High prevalence of cerebral creatine deficiency syndromes in the mental retardation population

- The prevalence of *SLC6A8* deficiency in patients with mental retardation is relatively high (~1%) compared with most monogenic causes of mental retardation.
- The prevalence of the autosomal recessive disorders AGAT- and GAMT-deficiency, is not high, however, a high carrier rate of a *GAMT* (founder) mutation exists in Mediterranean countries.
- The relatively high prevalence of CCDS highlights the importance of including them in the differential diagnosis of mental retardation of unknown etiology.

How to diagnose cerebral creatine deficiency syndromes?

- CCDS may be found either by proton MRS, metabolite screening and/or molecular investigations.
- Biochemically, AGAT-deficiency is characterized by low creatine and guanidinoacetic acid levels in bodily fluids, whereas GAMT-deficiency is characterized by elevated levels of guanidinoacetic acid in bodily fluids.
- Elevated urinary creatine:creatinine ratios are found in males affected with SLC6A8 deficiency.
- In females, the urinary creatine:creatinine ratio is not usually informative in this X-linked disorder, thus mutation analysis of *SLC6A8* is currently the primary choice.
- Prenatal diagnosis of CCDS is possible in families with affected individuals.

Executive summary

Diagnostic pitfalls

- AGAT deficiency is likely under-diagnosed because the biochemical tests may not be sensitive or specific enough to detect decreased metabolite levels.
- In approximately 10% of males with mental retardation in whom elevated urinary creatine:creatinine is found, no *SLC6A8* mutation is detected.
- False-negative biochemical findings (i.e., normal urinary creatine:creatinine ratio) are found frequently in females with heterozygous *SLC6A8* mutations.
- In molecular diagnostic screening, proper criteria should be used to classify novel sequence variants in the CCDS genes as pathogenic mutations.

Treatment of creatine biosynthesis defects & creatine transporter defect

- Treatment approaches of CCDS aim to restore cerebral creatine levels.
- The successful treatment of creatine biosynthesis defects consists primarily of daily creatine supplementation, which preferably should be initiated early in life. In addition, in GAMT deficiency, guanidinoacetic acid levels can be reduced by arginine restriction and ornithine supplementation.
- Current trials for the treatment of SLC6A8 deficiency aim at the stimulation of cerebral creatine biosynthesis by supplementation with high dosage of the substrates of the AGAT enzyme (i.e., arginine and glycine).

Creatine: a novel neuromodulator?

- Cerebral creatine biosynthesis has been proven by the demonstration of *AGAT* and *GAMT* mRNA expression in the brain.
- *In vitro* data indicate that creatine represents a novel cotransmitter in the brain.

Conclusions

- Cerebral creatine deficiency syndromes are, in part, treatable disorders.
- Diagnostic tests for the detection of cerebral creatine deficiency syndromes are widely available.
- Cerebral creatine deficiency syndromes should be included in the differential diagnosis of mental retardation, owing to their high frequency.

Future perspectives

- In the Western world, every mentally retarded patient (male/female) will be screened for CCDS, primarily by proton MRS.
- Novel techniques will increase the number of patients detected with CCDS.
- By identifying a vehicle for creatine uptake in the brain, treatment will also become available for patients with SLC6A8 deficiency.
- GAMT deficiency will be included in neonatal screening programs as guanidinoacetic acid seems to provide a suitable marker, as measured in blood spots by tandem mass spectroscopy.

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

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