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REVIEW
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Creatine Deficiency Disorders: Phenotypes, Genotypes, Diagnosis, and Treatment Outcomes

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ABSTRACT

Creatine is synthetized from arginine and glycine. There are two enzymes in the synthesis: L-arginine:glycine amidinotransferase and guanidinoacetate methyltransferase. After the synthesis, it is taken up by high-energy-requiring organs using creatine transporter. Biallelic pathogenic variants in *GAMT* result in guanidinoacetate methyltransferase deficiency and biallelic pathogenic variants in *GATM* result in L-arginine:glycine amidinotransferase deficiency. Hemizygous pathogenic variant in males and heterozygous pathogenic variant in females in *SLC6A8* result in creatine transporter deficiency. Patients with these disorders present with a wide range of symptoms, including developmental delay, seizures, movement disorder, behavioral problems, and hypotonia. The diagnosis can be suspected by elevated guanidinoacetate and low creatine levels in body fluids in guanidinoacetate methyltransferase deficiency, low guanidinoacetate and low creatine levels in body fluids in L-arginine:glycine amidinotransferase deficiency, and elevated creatine-to-creatinine ratio in urine in creatine transporter deficiency in males as well as absent or significantly decreased creatine level in brain proton magnetic resonance spectroscopy. Genetic investigations such as targeted next-generation sequencing panel or exome sequencing can also identify these disorders; however, metabolite measurements and creatine in proton magnetic resonance spectroscopy are crucial to confirm the diagnosis. While all 3 disorders are currently treated with creatine supplementation, guanidinoacetate methyltransferase deficiency is also treated with ornithine supplementation and a protein- or arginine-restricted diet, and creatine transporter deficiency is treated with arginine and glycine supplementation (with no proven improvements).

Keywords: Creatine, guanidinoacetate, guanidinoacetate methyltransferase deficiency, l-arginine, glycine amidinotransferase deficiency, creatine transporter deficiency, *SLC6A8*, *GAMT*, *GATM*, neurodevelopmental disorders

INTRODUCTION

Creatine, also called *N*-aminoiminomethyl-*N*-methyl glycine, is necessary for human energy metabolism.[1] It is stored in tissues with high-energy demands, including skeletal muscle, brain, heart, as well as hepatic, renal, and other tissues.[2] About half of the body's creatine is endogenously synthetized, while the other half comes from diet, primarily meat and fish.[3] Creatine biosynthesis involves 2 amino acids, arginine and glycine, and 2 enzymes, L-arginine:glycine amidinotransferase (AGAT) (EC 2.1.4.1) and guanidinoacetate methyltransferase (GAMT) (EC 2.1.1.2).[4,5] Creatine synthesis begins with AGAT enzyme catalyzing the transfer of the amidino group of arginine to glycine; this reaction yields L-ornithine and guanidinoacetate. Guanidinoacetate is then methylated from *S*-adenosylmethionine by GAMT at the amidino group which produces creatine. After synthesis, creatine must be transported into the cells; through the bloodstream, creatine travels to different areas, primarily muscle and brain. Once it reaches the respective organs, sodium/chloride-dependent creatine transporter (CRTR) is responsible for its uptake.[1] Lastly, creatine is converted into creatinine non-enzymatically for excretion through urine (Figure 1).[2] The GAMT, AGAT, and CRTR deficiencies

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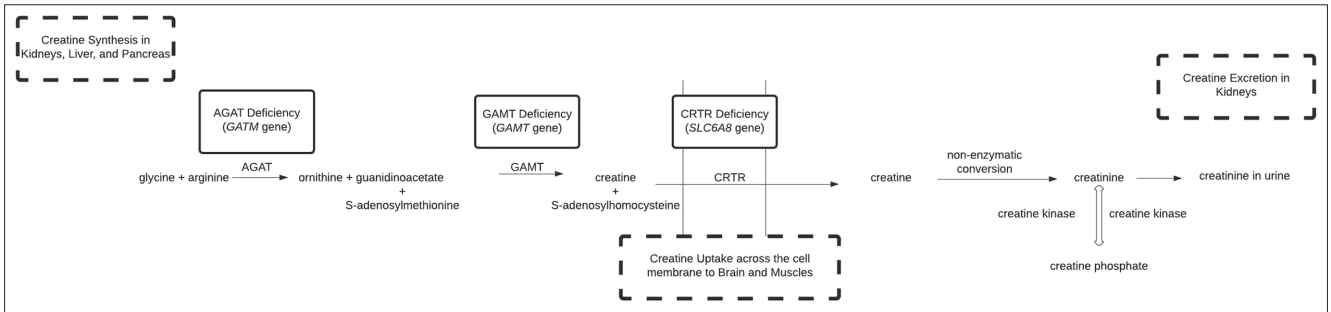

Figure 1. Creatine biosynthesis, transport, conversion from ADP to ATP, as well as nonenzymatic conversion to creatinine. ATP, adenosine triphosphate; ADP, adenosine diphosphate.

are called creatine deficiency disorders (CDD) and are inborn errors of creatine biosynthesis and transport.

Biallelic pathogenic variants in *GATM* result in AGAT deficiency and biallelic pathogenic variants in *GAMT* result in GAMT deficiency.[2] Hemizygous pathogenic variants in males and heterozygous pathogenic variants in some females in *SLC6A8* result in CRTR deficiency. The CDD are ultrarare disorders that were reported by <500 cases worldwide in the medical literature since their first description in 1994 including <150 cases with GAMT deficiency, <200 cases with CRTR deficiency, and <20 cases with AGAT deficiency.[2] All CDD patients present with neurodevelopmental disorders. The biochemical hallmark of CDD is creatine deficiency in the brain for all these disorders and accumulation of guanidinoacetate in GAMT deficiency and lack of guanidinoacetate in AGAT deficiency. The clinical and biochemical features and treatments of CDD are summarized in Table 1.

The purpose of this review article was to discuss the current level of knowledge surrounding CDD, including types of disorders, their clinical and biochemical features, current treatments, as well as secondary CDDs.

CREATINE DEFICIENCY DISORDERS

L-Arginine:Glycine Amidinotransferase Deficiency

First reported in 2001, AGAT deficiency (OMIM #612718) has the lowest number of documented cases of the CDD, having been reported in <20 patients.[3,4] AGAT deficiency is due to AGAT (EC 2.1.4.1) deficiency, encoded by *GATM* (OMIM #602360) on chromosome 15q21.1.[4] AGAT is the first enzyme in the creatine biosynthesis pathway. It is an autosomal recessive disorder due to biallelic pathogenic variants in *GATM*. Its estimated carrier frequency is 0.077% in the general population.[5] Parents, who carry a heterozygous pathogenic variant in *GATM*, are reported to be asymptomatic such that the family history is unremarkable for most families especially when there is no history of consanguinity in parents or grandparents or positive family history of AGAT deficiency.[2,6,7]

L-Arginine:glycine amidinotransferase deficiency is suspected in patients with global developmental delay (GDD), cognitive dysfunction or intellectual disability, and muscle weakness. Unfortunately, these clinical features are nonspecific and can be suggestive of many other neurometabolic or neurogenetic diseases. All affected patients with AGAT deficiency present with GDD and cognitive dysfunction or intellectual disability. The severity ranges from mild to moderate in 80% of affected patients. Fifty percent of the affected patients present with muscle weakness and myopathy. Different types of behavioral disorders are present in 25% of the affected patients. Single seizure is reported in 10% of the affected patients.[2]

AGAT deficiency can be diagnosed using characteristic biomarkers, including low guanidinoacetate levels and low or low-normal creatine and creatinine levels in urine, plasma, and cerebrospinal fluid (CSF) and absent or markedly decreased creatine peak in proton magnetic resonance spectroscopy (^{1}H-MRS).[2] Low plasma guanidinoacetate and creatine levels suggest suspected AGAT deficiency, which needs to be confirmed by absent or markedly decreased creatine peak in ^{1}H-MRS as well as biallelic pathogenic variants in *GATM*. Currently, the diagnosis of AGAT deficiency can be confirmed using targeted next-generation sequencing panels for intellectual disability, autism spectrum disorder, epilepsy, or exome sequencing. The biochemical investigations can be performed if there are 2 variants in *GATM*. If a single pathogenic variant or variants of unknown significance in *GATM* are identified, AGAT activity can be measured in fibroblasts[8] to confirm the diagnosis of AGAT deficiency. Sometimes, low urine and plasma guanidinoacetate levels may be identified, but normal creatine peak in ^{1}H-MRS excludes AGAT deficiency. These findings suggest a decreased protein intake or failure to thrive.

Molecular genetic and genomic tests can identify variants in *GATM*. The molecular genetic confirmation is important for the confirmation of the diagnosis of AGAT deficiency. The identification of pathogenic variants in *GATM* can provide diagnostics to other family members at risk. Prenatal diagnosis can be applied to identify whether fetuses are affected with AGAT deficiency. If the genomic test, such as targeted next-generation sequencing panel for epilepsy or intellectual disability or exome sequencing, was the first-line diagnostic investigation, the pathogenicity of variants should be confirmed by urine and plasma guanidinoacetate measurement and brain ^{1}H-MRS. There were 9 different variants in *GATM* from 8 different families. The missense and nonsense variants were the most common variants (44% of variants, respectively).[3] All variants are listed in Leiden Open Variation Database (LOVD3) (https://databases.lovd.nl/shared/genes/GATM, accessed January 29, 2023) with their pathogenicity.

The current treatment of AGAT deficiency is oral supplementation of creatine. This treatment results in improvements in

Table 1. Clinical and Biochemical Features as well as Treatments of CDD

Features	AGAT Deficiency (Percentages)	GAMT Deficiency (Percentages)	CRTR Deficiency in Males (Percentages)	CRTR Deficiency in Females
Clinical features	GDD, cognitive dysfunction, ID (100%)	GDD, cognitive dysfunction, ID (100%)	GDD, cognitive dysfunction, ID (100%)	From asymptomatic to like males
	Muscle weakness, myopathy (50%)	Muscle weakness, myopathy (0%)	Muscle weakness, myopathy (0%)	Muscle weakness, myopathy (0%)
	Single seizure (10%)	Seizures (70%)	Seizures (59%)	From asymptomatic to like males
	Behavior disorder (25%)	Behavior disorder (75%)	Behavior disorder (85%)	From asymptomatic to like males
	Movement disorders (0%)	Movement disorders (30%)	Movement disorders (11%)	From asymptomatic to like males
	Other: speech language disorder	Other: none	Other: hypotonia (40%), spasticity (26%), slender build (45%), gastrointestinal features (35%), and cardiac features (39%)	From asymptomatic to like males
Biochemical features	Low GAA in urine, plasma, and CSF	Elevated GAA in urine, plasma, and CSF	Normal GAA in urine, plasma, and CSF	Normal GAA in urine, plasma, and CSF
	Low Cr in urine, plasma, and CSF	Low Cr in urine, plasma, and CSF	Elevated urine creatine (to creatinine ratio)	Normal to mildly elevated
	Absent or markedly decreased Cr in brain ^{1}H-MRS	Absent or markedly decreased Cr in brain ^{1}H-MRS	Absent or markedly decreased Cr in brain ^{1}H-MRS	Normal to mildly decreased
Treatments	Creatine (400-800 mg/kg/day, divided in 4 doses)	Creatine (400-800 mg/kg/day, divided in 4 doses)	Creatine (100-200 mg/kg/day, divided in 3 doses)	Creatine (100-200 mg/kg/day, divided in 3 doses)
		Ornithine (400-800 mg/kg/day, divided in 4 doses)	Arginine (400 mg/kg/day divided in 3 doses)	If symptomatic same as males
		Protein (0.4-0.8 g/kg/day) or arginine-restricted diet with essential amino acid supplements	Glycine (150 mg/kg/day divided in 3 doses)	If symptomatic same as males
		Other: lysine (400-800 mg/kg/day, divided in 3-4 doses)	Others: *S*-adenosylmethionine (100 mg/kg/day, maximum 1500 mg/day)	If symptomatic same as males

^{1}H-MRS, proton magnetic resonance spectroscopy; AGAT, L-arginine:glycine amidinotransferase; Cr, creatine; CRTR, creatine transporter; CSF, cerebrospinal fluid; GAA, guanidinoacetate; GAMT, guanidinoacetate methyltransferase; GDD, global developmental delay; ID, intellectual disability.

cognitive dysfunction in some patients and improvements of muscle weakness in all patients. Treatment effectiveness was influenced by age at administration with cognitive function restored when patients were less than 2 years old at the time of treatment initiation. Unfortunately for patients who received treatment after 10 years old, their cognitive function or intellectual disability did not improve.[3] If the treatment is initiated in the first few months of life prior to symptom onset, those patients diagnosed prenatally or neonatally achieved normal neurodevelopment.[3,9,10]

A proposed alternative to creatine supplementation is guanidinoacetate supplementation due to its deficiency in addition to creatine deficiency.[11] Guanidinoacetate can cross blood–brain barrier by diffusion or using gamma-aminobutyric acid, taurine, or CRTRs. These transporters may allow higher creatine levels in the brain, as guanidinoacetate is converted to creatine by GAMT in the brain which may be a more effective therapy than creatine supplementation.[11] As creatine is transported by CRTR, only that can be oversaturated due to high creatine intake, and creatine uptake into the brain may be limited. Additionally, there are some potential risks of guanidinoacetate supplementation, including methyl group depletion and guanidinoacetate-driven hyperhomocysteinemia.[11,12]

Guanidinoacetate Methyltransferase Deficiency

The second enzyme defect in the creatine biosynthesis is GAMT deficiency (OMIM #612736), which is an autosomal recessively inherited disease that was first reported in 1994.[13] Guanidinoacetate methyltransferase deficiency is due to biallelic pathogenic variants in *GAMT* (OMIM #601240), located on chromosome 19p13.3 which encodes glycine GAMT (EC 2.1.1.2). This is the first discovered CDD in 1994.[13] Treatment outcome of creatine supplementation of the first case was reported in 1996.[14]

All patients with GAMT deficiency present with GDD, cognitive dysfunction, or intellectual disability (ranging from severe to mild).[15] About two-thirds of the patients manifest with seizures. The seizure types are myoclonic, head nodding, atonic, partial complex, and generalized tonic–clonic seizures in patients with GAMT deficiency. [15,16] About three-quarters of the patients with GAMT deficiency have different types of behavior disorders

ranging from attention-deficit to self-injurious and aggressive behaviors.[15] The less common clinical manifestations include movement disorders, present in about one-third of the affected patients.[15,17] The movement disorders associated with GAMT deficiency include chorea, athetosis, dystonia, and ataxia.[15]

Guanidinoacetate methyltransferase deficiency can be suspected through biochemical and ^{1}H-MRS findings. Urine, plasma, and CSF guanidinoacetate levels are elevated. Brain ^{1}H-MRS reveals markedly decreased or absent creatine peak.[6] Brain magnetic resonance imaging (MRI) can be normal or may show increased T2 signal intensity in basal ganglia, dorsal pons, or cortical atrophy in patients with GAMT deficiency.[15,18]

Molecular genetic and genomic tests can identify variants in *GAMT.* The molecular genetic confirmation is important for the confirmation of the diagnosis of GAMT deficiency. The identification of pathogenic variants in *GAMT* can provide diagnostics to other family members at risk. Prenatal diagnosis can be applied to identify whether fetuses are affected with GAMT deficiency. If the genomic test, such as targeted next-generation sequencing panel for epilepsy or intellectual disability or exome sequencing, was the first-line diagnostic investigation, the pathogenicity of variants should be confirmed by urine and plasma guanidinoacetate measurement and brain ^{1}H-MRS. There were more than 80 different variants in *GAMT*. The most common variant was c.327G>A (p.Lys109Lys) splice site variant in 48 patients (36.5% of all alleles) which is a panethnic variant. The second most common variant was c.59G>C (p.Trp20Ser), which is a common variant in Mediterranean countries (e.g., Portugal, Spain, and Turkey).[19] All variants are listed in LOVD3 (https://databases.lovd.nl/shared/genes/GAMT, accessed January 29, 2023) with their pathogenicity. The treatment of GAMT deficiency consists of oral creatine monohydrate and ornithine supplementation that competitively inhibit AGAT activity and decrease guanidinoacetate synthesis. Protein- or arginine-restricted diet and sodium benzoate (by conjugating glycine) further decrease the synthesis of guanidinoacetate by decreasing arginine and glycine for the synthesis of guanidinoacetate.[2,14,15,19,20] The current treatment resulted in seizure freedom in more than half of the patients and movement disorders in about half of the patients with GAMT deficiency.[15] Improvements were reported in behavior, language, and daily living skills in the majority of the patients with GAT deficiency.[19] Creatine supplementation results in significant increases of creatine level in ^{1}H-MRS as well as reversal of basal ganglia increased signal intensities.[15,19] Pre-symptomatic treatment of GAMT deficiency in the neonatal or early infantile age with good treatment compliance was reported to result in normal neurodevelopmental outcomes in few patients.[19,21,22]

Normal neurodevelopmental outcomes of pre-symptomatic patients with GAMT deficiency pave the way for newborn screening of GAMT deficiency. There are different algorithms applied for newborn screening of GAMT deficiency, ranging from guanidinoacetate and creatine measurements in dried blood dot spot to sequencing of *GAMT.*[22-27] Guanidinoacetate methyltransferase deficiency was recommended to be added to the Recommended Uniform Screening Panel in 2022 (https://www.hrsa.gov/sites/default/files/hrsa/advisory-committees/heritable-disorders/rusp/gamt-deficiency-consumer-summary.pdf).

Creatine Transporter Deficiency

The third CDD is CRTR deficiency (OMIM #300352), which was reported for the first time in 2001. The disorder is caused by pathogenic hemizygous variants in males and pathogenic heterozygous variants in some females in *SLC6A8* (OMIM #300036) on chromosome Xq28, which encodes CRTR protein.[28,29]

All males with CRTR deficiency have GDD and cognitive dysfunction or intellectual disability. Interestingly, 85% of males with CRTR deficiency, who were younger than 4 years old, have mild-to-moderate intellectual disability, whereas 75% of adult males with CRTR deficiency have severe intellectual disability.[30] Speech delay was reported in all males, ranging from no speech development (14%) to ability to speak in sentences (31%).[30] The prevalence of behavior disorders was 85% in males affected with CRTR deficiency. The most prevalent behavior disorder was the attention-deficit and/or hyperactivity disorder (55%), which was followed by autistic features (41%). About one-third of males reported to have impulsive behavior, anxiety, stereotypic behavior, and aggressive behavior, whereas self-injurious behavior was reported in 10% of males with CRTR deficiency.[30] Seizures were reported in 59% of males with CRTR deficiency. Seizure types included simple or complex partial seizures, generalized tonic–clonic, absence, and myoclonic seizures.[31] Movement disorders were reported in 11% of males with CRTR deficiency, including athetosis and ataxia.[30] A variety of other neurological clinical features were reported in males with CRTR deficiency, including hypotonia (40%), spasticity (26%), hearing loss, strabismus or bilateral abducens nerve palsy, myopathic face, ptosis, joint laxity, and decreased muscle bulk.[31] Aside from these neurological clinical manifestations, affected males also present with non-neurological clinical features, including dysmorphic features (e.g., broad forehead, deeply set eyes, midface hypoplasia, short nose, prominent nasal bridge, microcephaly, high palate, under-folded helices, large ears, cupped ears, and fifth finger clinodactyly), gastrointestinal features (e.g., vomiting, and gastroesophageal reflux disease), and cardiac features (e.g., long QT syndrome). Heterozygous pathogenic variants in *SLC6A8* in females can be asymptomatic or have phenotypes similar to males with CRTR deficiency depending on their X-inactivation in different tissues and organs.[32]

Creatine transporter deficiency is associated with elevated creatine-to-creatinine ratio in urine in males, which is a characteristic biomarker for this disease. However, affected females with CRTR deficiency may have mildly elevated or normal creatine-to-creatinine ratio in urine.[6,7] Brain ^{1}H-MRS reveals markedly decreased or absent creatine peak in males with CRTR deficiency. However, affected females do not always have a depleted creatine level, and if they do have depleted creatine level in brain ^{1}H-MRS, the creatine peak is only partially depleted.[6,32]

Molecular genetic and genomic tests can identify variants in *SLC6A8.* The molecular genetic confirmation is important for the confirmation of the diagnosis of CRTR deficiency. The identification of pathogenic variant in *SLC6A8* can provide diagnostics to other family members at risk. Prenatal diagnosis can be applied to identify whether fetuses are affected with CRTR deficiency. If the genomic test, such as targeted

next-generation sequencing panel for epilepsy or intellectual disability or exome sequencing, was the first-line diagnostic investigation, the pathogenicity of variants should be confirmed by urine creatine-to-creatinine measurement and brain ^{1}H-MRS. There were more than 60 different variants in 64 families. The most common variant type was missense (31% of families), followed by 3 base pair deletion (24% of families) and frameshift variants (23% of families).[30] All variants are listed in LOVD3 (https://databases.lovd.nl/shared/genes/SLC6A8, accessed January 29, 2023) with their pathogenicity.

Creatine transporter deficiency treatments are not as advanced as GAMT and AGAT deficiency treatments; in fact, to date, CRTR deficiency does not have an effective treatment to improve neurodevelopmental and seizure outcomes. Unlike GAMT and AGAT deficiency, oral creatine supplementation was reported to be ineffective as CRTR is the only transporter for creatine to cross the blood–brain barrier.[33] The path for creatine to effectively reach the brain is quite complicated as creatine must cross 3 to 5 membranes from microcapillary endothelial cells at the blood–brain barrier to brain cells within the central nervous system parenchyma.[34] Recommendations of creatine, arginine, and glycine supplementations might have stopped disease progression; however, there are no randomized controlled trials to assess the effectiveness of these supplements.[2,6,35-38]

Secondary Creatine Deficiency Disorders

Partial creatine deficiency in brain ^{1}H-MRS has been reported in some of the inherited metabolic disorders.[39] Those patients have decreased creatine synthesis either due to low arginine levels (decrease in creatine synthesis due to arginine deficiency as a substrate) or high ornithine levels (suppression of AGAT, first enzyme in the creatine synthesis). Patients with these disorders have normal creatine-to-creatinine ratio in urine and normal or low normal levels of guanidinoacetate in urine, plasma, or CSF. Creatine supplementation may improve some of the clinical features, but to the best of our knowledge, this has not been tried. These disorders are listed below with some of their key features.[2]

Argininosuccinate lyase (ASL) deficiency is due to biallelic pathogenic variants in *ASL* (OMIM #608310). This is one of the urea cycle disorders. There are 2 phenotypes: (1) severe neonatal-onset ASL deficiency and (2) late-onset ASL deficiency. Neonatal-onset ASL deficiency presents with tachypnea, vomiting, refusing to feed, somnolence, respiratory alkalosis, and increasing lethargy in the first few days of life due to accumulation of ammonia, while late-onset ASL deficiency can present with episodic hyperammonemia, behavior disorder, learning disabilities, and/or cognitive dysfunction. The biochemical features are elevated ammonia, mild to moderately elevated citrulline and glutamine, and low arginine in plasma amino acid analysis and elevated argininosuccinic acid in urine amino acid analysis.[40-42]

Argininosuccinate synthetase deficiency is due to biallelic pathogenic variants in *ASS1* (OMIM #603470). This is one of the urea cycle disorders. Affected patients present with GDD, hepatic cirrhosis, failure to thrive, hyperammonemic encephalopathy, ataxia, and seizures.[2,42,43] The biochemical features are elevated ammonia, markedly elevated citrulline and glutamine, and low arginine in plasma amino acid analysis as well as significantly decreased creatine levels in plasma.[42]

Ornithine transcarbamylase (OTC) deficiency, gene *OTC* (OMIM #311250), is to date the only secondary CDD with an X-linked mode of inheritance. Affected patients may present with hyperammonemic encephalopathy, ataxia, seizures, slow growth, and developmental delay.[2,43] Low levels of guanidinoacetate and creatine in body fluids were reported in patients with OTC deficiency.[44]

Lysinuric protein intolerance is caused by biallelic pathogenic variants in *SLC7A7*. Due to the cationic amino acid transporter defect, arginine, lysine, and ornithine are excreted in urine and result in arginine, lysine, and ornithine deficiency. Affected patients present with failure to thrive, short stature, vomiting, respiratory insufficiency, hepatomegaly, osteoporosis, and diarrhea.[2,43]

Hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome is due to biallelic pathogenic variants in *SLC25A15* (OMIM #603861). Affected patients present with spasticity, hypotonia, failure to thrive, seizures, developmental delay, and hepatomegaly.[2,43] There was a decreased urine creatine excretion.[45]

Pyruvate carboxylase deficiency is due to biallelic pathogenic variants in *PC* (OMIM #608786). Affected patients present with seizures, hypotonia, hepatomegaly, lactic acidosis, spasticity, and developmental delay. It has been reported to result in decreased creatine level in brain ^{1}H-MRS.[2,46]

Ornithine aminotransferase deficiency is due to biallelic pathogenic variants in *OAT*. Affected patients present with night blindness, posterior subcapsular cataracts, progressive chorioretinal degeneration, loss of peripheral vision, myopia, and proximal muscle weakness.[2,39] Plasma amino acid analysis shows markedly elevated ornithine levels. Creatine supplementation did not show any improvements in brain MRI or electroencephalography of treated patients compared to controls.[47] Secondary creatine deficiency in brain ^{1}H-MRS was reported. There were low levels of creatine and/or guanidinoacetate in plasma and urine.[48]

Delta-1-pyrroline-5-carboxylate synthase deficiency is due to biallelic or heterozygous pathogenic variants in *ALDH18A1*. Affected patients present with developmental delay, failure to thrive, spasticity, myopathy, and dysmorphic features. Brain ^{1}H-MRS revealed decreased brain creatine level, which was normalized after arginine supplementation.[2,49]

CONCLUSION

Creatine deficiency disorders are rare inherited metabolic disorders of creatine biosynthesis and transport. There are specific treatments for each CDD which are important to start in early infancy to improve neurodevelopmental outcomes or prevent disease-related complications. For these reasons, the early diagnosis is crucial. In patients with GDD, cognitive dysfunction, with or without epilepsy, movement and behavior disorders, urine and plasma guanidinoacetate, and creatine measurements should be included into the diagnostic

investigations. When a brain MRI is performed for GDD, a brain ^{1}H-MRS should be added that can also identify absent or markedly low creatine peak to guide diagnostic investigations. These are crucial biomarkers to identify patients with CDD so that the treatments can be started to improve neurodevelopmental outcomes.

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