

# A rare but treatable inborn error of metabolism: Arginine glycine amidinotransferase (AGAT) deficiency

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## ABSTRACT

AGAT deficiency is a rare and treatable autosomal recessive disorder. The symptoms are early-onset developmental mild to moderate intellectual disability, delayed speech acquisition, behavioral problems or proximal muscle weakness. Biochemical screening for creatine, creatinine and urinary guanidinoacetate and genetic tests are used for diagnosis. Electromyography may be normal or may have a myopathic pattern with low amplitude polyphasic waves. Muscle biopsy may show abnormalities including small myocytes. Creatine supplementation can fully prevent the neurological disability, if the treatment is started early in life; the muscular function improves irrespective of the supplementation moment.

**Keywords:** AGAT, GAMT, CRTR, creatine deficiency syndromes, intellectual disability, language delay, creatine-phosphocreatine system

## INTRODUCTION

L-arginine:glycine amidinotransferase (AGAT) deficiency, or AGAT-d, is an inborn error of metabo-

lism that, along with guanidinoacetate methyltransferase (GAMT) deficiency and creatine transporter (CRTR) deficiency, affect the creatine metabolism and are known as creatine deficiency (1,2).

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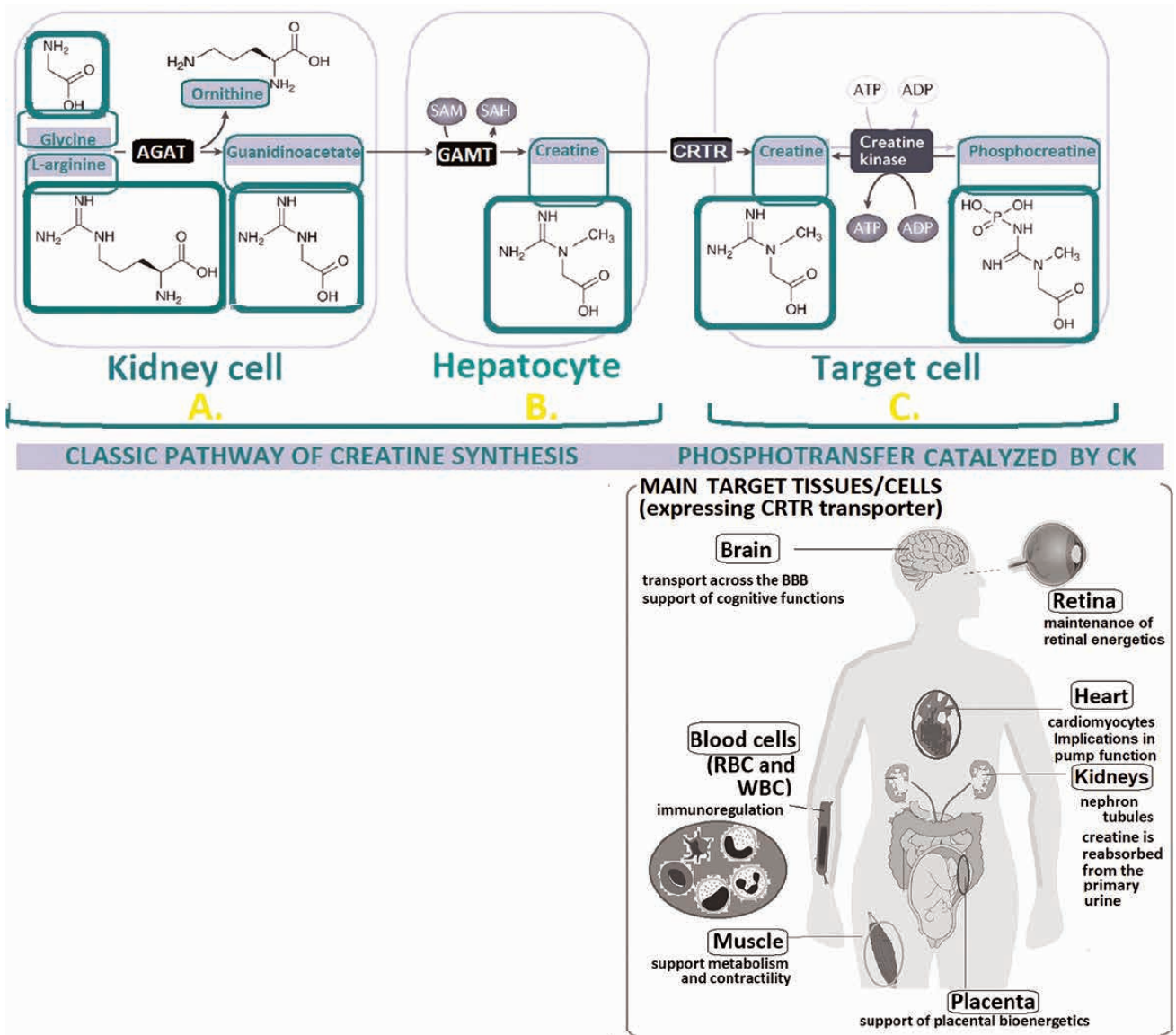
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**FIGURE 1.** Representation of the endogenous creatine synthesis and transport of creatine into the main tissues and cells incapable of self-synthesizing sufficient amounts of creatine, but expressing the CRTR transporter (i.e., brain, muscle, blood cells, skeletal muscle, retina, heart, kidney, placenta etc) – adapted after (3).

**A.** In the kidney cells, synthesis of guanidinoacetate with enzyme AGAT: arginine:glycine amidinotransferase;  
**B.** Guanidinoacetate is exported to the circulation and taken up by hepatic cells, where guanidinoacetate N-methyltransferase (GAMT) converts it to creatine. **C.** Creatine is released from hepatocytes into the circulation, and is taken by cells expressing the creatine transporter (CRTR or SLC6A8). Creatine can be phosphorylated to phosphocreatine by creatine kinase, using ATP. During times of high and fluctuating energy demand (high ATP consumption), phosphocreatine can be used in reverse by creatine kinase to buffer the ATP pool. *SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine, BBB: blood-brain barrier, RBC: red blood cells, WBC: white blood cells*

AGAT has a 5-fold pseudosymmetry of circularly arranged  $\beta\beta\alpha\beta$ -modules, with an overall structure resembling a “basket with handles” (4). The enzyme is mainly found in the cortex of the kidney, where it catalyzes the transfer of an amidino group from L-arginine to glycine, therefore producing guanidinoacetic acid (GAA) and L-ornithine (5), though L-homoarginine can also be produced (4,6). In man, AGAT is also found in pancreas, liver, muscle, brain

and possibly in heart (7,8). Sub-cellularly, it is localized in the mitochondria intermembrane space (where it has 386 aminoacids) and to a lesser extent in cytoplasm (with 391 aminoacids) (9).

AGAT catalyzes the first step in the creatine biosynthesis pathway. The resulting guanidinoacetic acid is transported to the liver, where it is methylated to creatine by GAMT (5,10). Creatine is then transported to the tissues that require it (such as

neurons, myocytes and cardiomyocytes) where the uptake is regulated by special creatine transporter (SLC6A8). There, it is involved in energy storage and transmission through its involvement in ATP (adenosine triphosphate) production (11).

In the neuron, the creatine/phosphocreatine system helps couple ATP-consuming with ATP-producing processes (12). SLC6A8 transporter allows creatine to penetrate the brain-blood barrier in modest amounts (13,14). Most brain cells express only one or none of the creatine biosynthesis pathway enzymes (13,15). In consequence, the brain is almost totally dependent on the uptake of exogenous creatine or endogenous creatine produced in other tissues.

Most creatine is found nevertheless in skeletal muscle, where it plays a similar role (11). About 1.7% of the total creatine content of a human is converted daily to creatinine and excreted in urine, i.e. two grams of creatine per day for a 70-kg man (5). When AGAT is not functional, the only option for creatine replenishment is the uptake of creatine from alimentation (16).

The usual symptoms of AGAT-d are developmental and language delay, intellectual disability, behavioral problems, myopathy or proximal muscle weakness (17). Rarely, seizures can appear. This is in contrast to GAMT deficiency (the second step in the creatine synthesis – Figure 1), where the build-up of guanidinoacetic acid has neurotoxic effects (15) and frequently is manifested with seizures (11).

## GENETICS

AGAT deficiency is an autosomal recessive disorder. The AGAT gene (official nomenclature GATM, encoding a mitochondrial enzyme that belongs to the amidinotransferase family) has been mapped to chromosom region 15q and contains nine exons. Nine different pathogenic mutations (nonsense, splice, frame shift, missense) have been reported so far (2). Both nonsense and missense mutations of the GATM gene can lead to the enzyme loss of function (17); normally this enzyme catalyzes the first step in the creatine biosynthesis pathway – Figure 1 (5,9).

Mutations in the gene coding the creatine transporter SLC6A8 or CRTR lead to CRTR deficiency, with symptoms similar to AGAT-d, but much harder to treat; in some cases, folding defects are amenable to rescue by small molecules like pharmacochaperones and/or allosteric modulators (18). AGAT deficiency might represent the most underdiagnosed disease among the creatine deficiency syndromes, and has the mildest clinical phenotype comparing with GAMT or CRTR deficiencies (2).

## PATHOPHYSIOLOGY

The lack of AGAT disrupts the entire creatine biosynthesis pathway, leading to a lack of endogenous creatine. This affects tissues with high and fluctuating energy demands which usually contain the highest levels of creatine, such as brain, skeletal and heart muscle, but also photoreceptor cells of the retina and spermatozoa, although no ophthalmological or sexual function-related symptoms of AGAT-d have been described so far (2,5).

Besides the role in the energetic metabolism, creatine is also relevant in GABAergic and glutamatergic signaling in the central nervous system, has antioxidant properties and helps water retention in muscle (9). Creatine also plays a role in certain gene transcription regulation in brain. Two genes, BCAS1 and SLC6A8, upregulated in AGAT-d mice, normalize their expression after creatine supplementation (19). Bcas1 is an important protein found in brain whose level increases after stroke, most likely as a compensatory mechanism to energy deficiency and hypoxia (20), and whose decrease is associated with hypomyelination (19). SLC6A8 encoding the creatine transmembrane transporter (CRTR- Figure 1), is upregulated in AGAT-d mice probably as a compensatory mechanism (13, 19). The expression of SLC6A8 on the brain-blood barrier (BBB) allows the entry of creatine especially in early life, when the BBB is not as tightly regulated as in maturity, partly explaining the efficacy of early, pre-symptomatic treatment (9,14). AGAT-d mice deficient behavior and spatial memory improved after creatine supplementation (21).

Creatine deficiency also alters muscle energy metabolism, where the creatine-phosphocreatine system helps couple oxidative phosphorylation with the ADP-ATP system (22,23). In AGAT-d mice, the lack of creatine was associated with muscular atrophy, smaller myocyte diameter, and an increased dependence on glycolytic ATP production, due to the dysfunction of mitochondrial respiratory chain complexes – all reversed by creatine supplementation (22). Creatine levels in muscle reached normal levels in one day, while hypothalamic or hippocampal levels took 20 days to normalize (22). Mice AGAT-d muscle also presented more than one hundred significantly regulated genes implicated in pyruvate and glucose metabolism, oxidation/reduction, and one-carbon metabolism (23). In conclusion, in the absence of creatine, skeletal muscle is severely affected (24).

Creatine is also found in spermatozoa (5) and AGAT-d mice have been found to be sterile (9). However, creatine kinase function in spermatozoa may not correlate with male fertility, since energy can also be produced by glycolysis, independent of creatine kinase (25). This calls for further research in

the relationship between creatine and reproductive system health.

Since creatine is involved in multiple metabolic processes, AGAT-d mice can exhibit abnormal metabolic phenotypes (23). Creatine-deficient mice present enhanced glucose tolerance, reduced adiposity and cholesterol levels, due to the chronic creatine-dependent activation of AMP-activated protein kinase (26). Moreover, the expression of GLUT4 is elevated, and hepatic gluconeogenesis is severely impaired (23).

Apart from guanidinoacetic acid, AGAT can also synthesize L-homoarginine by transferring the amidino group to lysine instead of glycine (4). Low levels of homoarginine are associated with heart failure increased cardiovascular risk and mortality (4, 6). In heart failure the expression and enzyme activity of AGAT are increased (8). On the other hand, homoarginine acts as a weak substrate for nitric oxide synthase (NOS) (6) and high levels of homoarginine may subsequently increase NOS activity and blood pressure (4). Homoarginine supplementation also restores the expression of *Kcnip3* and *Ivd*, two genes relevant to cerebrovascular function, down-regulated in AGAT-d mice (19). The first codes for

calsenilin, a neuronal calcium-binding protein implicated in Alzheimer's disease (27). The second codes for an enzyme implicated in L-leucine degradation, and in consequence, AGAT-d murine brains show increased leucine levels, which did not normalize with homoarginine supplementation (19). In healthy volunteers who were administered creatine, circulating homoarginine increased by 35% (28). This study could suggest long-term cardiovascular implications of daily creatine supplementation in AGAT-d patients (4).

AGAT might also be indirectly linked to gyrate atrophy of the choroid and retina, another rare inherited disease caused by the deficiency of ornithine aminotransferase, which leads to ornithine concentrations 10 to 20 times those found in normal plasma, and also to creatine deficiency (7,29). Since ornithine is an inhibitor of AGAT, increased circulating ornithine might suppress creatine synthesis through AGAT inhibition, which would lead to a lack of readily available energy, and thus atrophy (7). No ophthalmic symptoms have been reported in AGAT-d patients so far, but further research is necessary (17).

**TABLE 1.** Physiology and pathophysiology in AGAT-related creatine metabolism

System involved	Clinical and molecular features affected by AGAT-related creatine metabolism	References
Brain	Signaling in GABAergic and glutamatergic pathways in the central nervous system	(9)
	Regulations of several genes involved in compensatory mechanisms after stroke (energy deficiency and hypoxia), myelination	(19)
	In AGAT-d mice, the behavior and spatial memory is improved with creatine supplementation	(21)
	Synthesis of caldesmon, a neuronal calcium-binding protein involved in Alzheimer's disease	(27)
	Influences the expression of <i>SLC6A8</i> gene on the brain-blood barrier	(9, 14, 19)
Eye	Affect the expression of caldesmon, a neuronal calcium-binding protein implicated in Alzheimer's disease, involved in AGAT-d mice	(19, 27)
	Likely indirectly implications in gyrate atrophy of the choroid and retina ornithine due to the high ornithine levels that suppress creatine synthesis through AGAT inhibition; secondary this will cause lack of energy and atrophy	(7, 29)
Muscle	Implicated in buffering reactions in mitochondrial ADP-ATP system, affects the muscle energy metabolism	(22, 23)
	Antioxidant properties	(9)
	Stimulates water retention in muscle	(9)
	Normal development of myocytes; lack of creatine in AGAT-d mice is associated with muscular atrophy, smaller myocyte diameter, increased dependence on glycolytic ATP production	(22)
	Gene regulation in AGAT-d mice related to pyruvate and glucose metabolism, oxidation/reduction reactions, one-carbon metabolism	(23)
Metabolism	Energetic metabolism, physiology of mitochondrial respiratory chain complexes coupled with ADP-ATP system	(22, 23)
	Affects the expression of <i>GLUT4</i> gene	(23)
	Influences glucose tolerance; hepatic gluconeogenesis is severely impaired in creatine-deficient mice	(23)
	Involvement in adiposity and cholesterol levels through the chronic creatine-dependent activation of AMP-activated protein kinase	(26)
Cardio-vascular system	Implications in heart pump function through low synthesis of L-homoarginine	(4, 6)
	Affects NO (nitric oxide) metabolism and blood pressure through synthesis of L-homoarginine	(4, 6)
Reproductive system	Creatine is also found in spermatozoa; there are possible implications in sterility ins AGAT-d mice	(5, 9, 25)

## DIAGNOSTIC AND SCREENING

AGAT-d can be diagnosed by measuring blood and urine guanidinoacetate, creatine and creatinine levels. Plasma and urine guanidinoacetate in AGAT-d are undetectable, or <10% of lower range of the reference values. Plasma amino acid concentrations (excepting S-adenosyl-homocysteine, SAH) are normal in AGAT-d (17). Newborn biochemical screening for AGAT-d is challenging, as the normal range for guanidinoacetic acid is below the detection limit of assays suitable for blood spot analysis. The GATM gene can be sequenced and were identified several types of mutations (11). Brain creatine levels can be measured using proton magnetic resonance spectroscopy ( $H^1$ -MRS), and is used mostly for monitoring the treatment (2).

## CLINICAL PICTURE

The main characteristic of AGAT-d is intellectual disability, as creatine has been associated with the development of higher cognitive functions (14). Most patients had language delay (with onset during the first year of life), behavioral problems and mild to moderate mental retardation, with intelligence or developmental quotients (IQ/DQ) between 35 and 60 before treatment (2,16,17). In the absence of creatine, irreversible brain damage may occur in the first year of life (14,17,30). Oral creatine treatment marginally improves IQ/DQ scores in humans despite almost full brain creatine replenishment in older patients, but the earlier treatment was started, the greater recovery in brain function was attained (3). Cerebral MRI shows normal brain anatomy and structure, while in vivo brain  $H^1$ -MRS reveals profound cerebral creatine deficiency (2,16,17).

Myopathy, with low muscle mass and proximal muscle weakness, was reported in half of the patients with ages between 6 and 23 (17). Myopathy seems to be a symptom mostly in later age, since patients diagnosed at a young age presented no muscle weakness and developed normally following treatment (31). The electromyography (EMG)

was normal or showed a myopathic pattern with low amplitude polyphasic waves (17,23,24). Muscle biopsy may show small myocytes and other abnormalities, including mitochondrial at ultrastructural studies (17).

## TREATMENT AND PROGNOSIS

Early diagnosis and treatment may prevent intellectual disability and myopathy in patients with AGAT-d. The treatment purpose is to restore cerebral and muscular creatine levels; the recommended doses of creatine monohydrate are 100–800 mg/kg/day, and results in almost complete restoration of brain creatine levels and significant improvement of myopathy in most patients (2). Although the brain creatine levels normalizes in patients treated later, some irreversible neuronal damage occur in the first year of life (17,14,31). Muscle improvement after creatine supplementation is fast, while the neurological one is slow (22).

As some metabolic disturbances were registered (such as enhanced glucose tolerance, reduced adiposity, reduced cholesterol levels, and decreased of hepatic gluconeogenesis), the metabolic parameters should be monitored during the creatine therapy (3,23,26). Also, weight gain as a side effect of creatine supplementation should be considered in some treated AGAT-d patients (23,31).

## CONCLUSIONS

AGAT-d is a rare and treatable disorder, and should be suspected and searched for as soon as possible in infants with signs of developmental delay, muscle hypotonia and intellectual disability. Creatine supplementation can fully prevent the neurological disability, if started early in life; the muscular function improves irrespective of the supplementation moment.

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The last two authors have equal contribution.

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