

Laboratory diagnosis of creatine deficiency syndromes: a technical standard and guideline of the American College of Medical Genetics and Genomics

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Cerebral creatine deficiency syndromes are neurometabolic conditions characterized by intellectual disability, seizures, speech delay, and behavioral abnormalities. Several laboratory methods are available for preliminary and confirmatory diagnosis of these conditions, including measurement of creatine and related metabolites in biofluids using liquid chromatography–tandem mass spectrometry or gas chromatography–mass spectrometry, enzyme activity assays in cultured cells, and DNA sequence analysis. These guidelines are intended to standardize these procedures to help optimize

the diagnosis of creatine deficiency syndromes. While biochemical methods are emphasized, considerations for confirmatory molecular testing are also discussed, along with variables that influence test results and interpretation.

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OVERVIEW

Cerebral creatine deficiency syndromes (CDSs) consist of three neurodevelopmental disorders caused by dysfunctional creatine biosynthesis or transport. Deficiency of the two biosynthetic enzymes exhibit autosomal recessive inheritance, while creatine transport deficiency is an X-linked trait. All three conditions are characterized by muscle hypotonia, speech delay, seizures, intellectual impairment, and behavioral abnormalities (including autistic and autoaggressive behaviors) associated with absent or nearly absent levels of creatine in the central nervous system. In vivo diagnosis of these conditions may be accomplished using proton magnetic resonance spectroscopy, which detects reduced levels of creatine in the brain of an affected individual. However, more practical and specific preliminary diagnostic evaluation

is possible by measuring creatine and related metabolites (guanidinoacetate and creatinine) in blood and/or urine, using liquid chromatography–tandem mass spectrometry (LC-MS/MS) or gas chromatography–mass spectrometry (GC-MS). Confirmatory diagnostic methods include enzymatic or uptake studies in cultured cells or DNA sequence analysis. In general, disorders of creatine biosynthesis can be treated with oral creatine supplementation and dietary manipulations, which seem more effective when started early in life.^{1–3} Guanidinoacetate methyltransferase (GAMT) deficiency requires additional treatment with ornithine and benzoate, aimed at reducing the toxic overproduction of guanidinoacetate.^{4,5} Creatine transport deficiency is not currently amenable to therapeutic intervention, although several creatine derivatives have been investigated for treatment efficacy.^{6–12}

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BACKGROUND

Sources of creatine

Creatine (2-methyl-guanidinoethanoic acid) is a nitrogenous organic acid best known for its important role in supplementing cellular adenosine triphosphate (ATP) production, particularly in skeletal muscle and brain. The majority (~95%) of creatine reserves are located in skeletal muscle,¹³ although this is not an important biosynthetic tissue. While approximately half of the creatine found in the body is obtained from dietary consumption of meat and fish, this substance is also produced endogenously in several human tissues.

Endogenous creatine production in humans (**Figure 1**) requires the coordinated activities of two enzymes, L-arginine:glycine amidinotransferase (AGAT; EC:2.1.4.1) and GAMT (EC:2.1.1.2). AGAT catalyzes the transfer of an amidino group from arginine to glycine, forming guanidinoacetate, which is then methylated by GAMT to form creatine.

Circulatory creatine is taken up by tissues and reabsorbed in the renal tubules via the creatine transporter (CRTR), encoded by *SLC6A8*. Stores of creatine must be continuously replenished from endogenous and exogenous sources, as creatine spontaneously converts to creatinine at a constant rate of approximately 2%/day.¹⁴ Chemically stable under physiological conditions, creatinine is excreted in the urine and can be used to calculate the glomerular filtration rate, a widely utilized measure of kidney function.¹⁵

Functions of creatine

The primary physiological role of creatine is to act as a temporal and spatial buffer for ATP.^{16,17} Isoforms of creatine kinase catalyze a reversible reaction in which creatine and ATP interconvert with phosphocreatine and adenosine diphosphate (**Figure 1**). Phosphocreatine is stored in muscle, which provides a rapidly available source of high-energy phosphate for ATP regeneration. This process maintains optimal ATP-to-adenosine diphosphate ratios, thereby facilitating myosin and Ca²⁺ ATPase functions required for contractile burst energy. In the central nervous system, the precise role(s) of creatine is less well defined but likely involves multiple ATP-dependent processes underlying brain function and development.¹⁸

Creatine may also have several important “secondary” physiological functions. In particular, creatine might act as a neuromodulator or even neurotransmitter.¹⁹ In support of this assertion, neuronal creatine is released in an action potential-dependent exocytotic manner,¹⁸ and the presence of CRTR in the synaptosomal membrane could facilitate creatine reuptake.²⁰ In addition, creatine possesses antioxidant activity, protects cell membranes, promotes bone and muscle growth, and has neuroprotective properties.¹⁶

Biochemical and molecular characteristics of AGAT, GAMT, and CRTR

AGAT is encoded by *GATM* (NM_001482.2) on 15q21.1 and is primarily expressed in the kidneys and pancreas. The enzyme functions as a dimer, and two slightly different isoforms have

been localized to the cytosol (391 amino acid monomer) and the mitochondrial intermembrane space (386 amino acids),²¹ respectively. AGAT generates both guanidinoacetate and ornithine via transfer of the amidino group from arginine to glycine. This reaction represents the rate-limiting step of creatine biosynthesis and is negatively regulated by both creatine and ornithine.²¹ AGAT deficiency appears to be the rarest of the creatine deficiency syndromes, with a small number of patients reported thus far.²²

The *GAMT* gene (NM_138924.2) maps to chromosome 19p13.3. The monomeric protein is 236 amino acids in size and is mainly found in the cytosol of liver, pancreas, and kidney cells. GAMT synthesizes creatine via methylation of guanidinoacetate, concomitant with conversion of the methyl donor S-adenosylmethionine to S-adenosylhomocysteine. At least 50 different pathogenic *GAMT* variants have been reported, including nonsense and missense variants, insertions, deletions, frameshifts, and splice errors.^{4,23} The most common pathogenic variants are c.327G>A (p.K109K, splice site exon 2) and c.59G>C (p.W20S).⁴

AGAT and GAMT are also expressed in neurons and microglia, suggesting that central nervous system creatine synthesis might be necessary to compensate for the limited passage of creatine across the blood–brain barrier.²⁴

Uptake and storage of both endogenously synthesized and dietary creatine is facilitated by CRTR, a sodium-dependent, solute carrier-type protein of 635 amino acids encoded by *SLC6A8* (NM_001142805.1) on Xq28.^{25,26} While the majority of creatine is stored in skeletal muscle, CRTR is also found in the plasma membrane of kidney, brain, heart, colon, testis, and prostate cells.²⁷ CRTR deficiency is the most prevalent

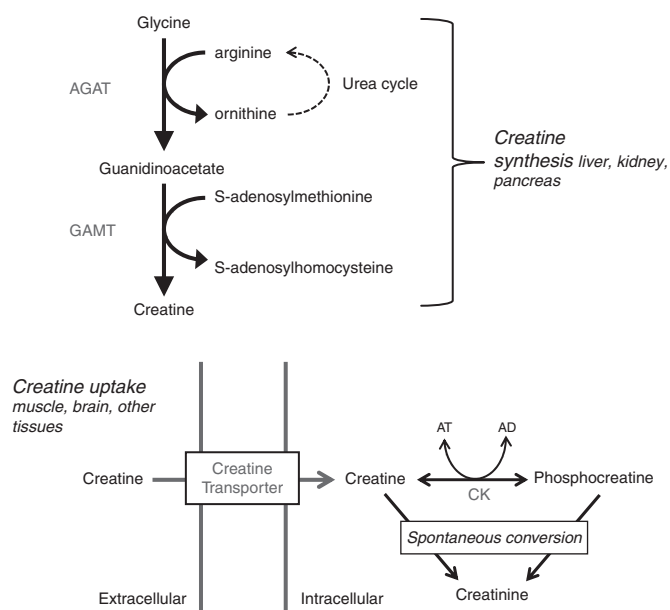


Figure 1 Creatine metabolic pathway, including biosynthesis, uptake by cells, phosphotransfer, and conversion to creatinine. AGAT: arginine:glycine amidinotransferase; GAMT: guanidinoacetate methyltransferase; CK: creatine kinase.

disorder of creatine metabolism, and approximately 65 pathogenic *SLC6A8* variants have been described to date.^{28,29}

Clinical description of CDSs

AGAT (OMIM 612718), GAMT (OMIM 612736), and CRTR (OMIM 300352) deficiencies cause a continuum of clinical phenotypes known as cerebral CDSs. Neurological manifestations dominate the CDS phenotype, demonstrating the important physiological role of creatine in the central nervous system. Symptoms typically appear during infancy to early childhood and include cognitive impairment, developmental and speech delays, muscle hypotonia, seizures, movement disorders, and behavioral abnormalities, including autism spectrum and autoaggressive behaviors. Patients with AGAT deficiency may also have a prominent myopathy.³⁰ GAMT deficiency often shows a more complex phenotype (intractable seizures, extrapyramidal movement abnormalities, and dysfunction of the basal ganglia), which may be attributed to the specific toxicity of accumulating guanidinoacetate.^{5,24} Females heterozygous for *SLC6A8* pathogenic variants may be asymptomatic or present with signs and symptoms of variable severity, ranging from learning disabilities to seizures.^{31–33}

Treatment

Deficiency of both AGAT and GAMT can be treated with oral supplementation of creatine monohydrate. In addition, GAMT deficiency requires arginine restriction through a low-protein diet, and ornithine supplementation and benzoate (reduces glycine levels via the formation and excretion of hippuric acid) to reduce guanidinoacetate synthesis and its associated toxicity (creatine supplementation also partially inhibits guanidinoacetate formation^{4,5}). Treatment for CRTR deficiency is not currently available, but studies of cyclocreatine in an *SLC6A8* knockout mouse model have generated promising results.¹⁰

Biochemical testing for CDSs

Cerebral creatine deficiency can be detected in vivo using proton magnetic resonance spectroscopy, but this approach is expensive, requires sedation and/or anesthesia in children, and fails to specify the CDS. Therefore, the initial diagnosis is typically established by measuring creatine, guanidinoacetate, and creatinine via LC-MS/MS or GC-MS in plasma, urine, cerebrospinal fluid (CSF), or dried blood spots (Table 1). These tests can effectively differentiate creatine-deficient patients with AGAT deficiency (characterized by significantly reduced guanidinoacetate and creatine concentrations) from those with GAMT deficiency (elevated guanidinoacetate concentrations with low creatine levels), and can identify hemizygous males

with X-linked creatine transporter deficiency (increased urinary excretion of creatine, which is associated with an increased creatine-to-creatinine ratio). Females heterozygous for creatine transporter deficiency may present with ambiguously elevated urinary creatine levels, but this approach is not considered reliable for this particular population. Measurements of creatine and guanidinoacetate in plasma are recommended for the diagnosis of AGAT and GAMT deficiency. Measurements of creatine, creatinine, and guanidinoacetate in urine identify male patients with creatine transporter deficiency and patients with GAMT deficiency after the neonatal period. Patients with AGAT deficiency may be missed by the study of urine creatine and guanidinoacetate alone because of inherently low concentrations of these compounds in some unaffected individuals. Additionally, patients with GAMT deficiency in the newborn period may have normal guanidinoacetate-to-creatinine ratios in urine;^{34,35} hence, it is important to evaluate plasma in this age group. In the event of an abnormal metabolite test result, repeat analysis is recommended because of the relatively low specificity of elevated urinary creatine in the diagnosis of creatine transporter deficiency, and low guanidinoacetate and creatine concentrations in AGAT deficiency.^{36,37}

Definitive confirmation of the diagnosis requires DNA sequencing of the appropriate gene and (if molecular analysis is ambiguous) measurement of AGAT or GAMT enzyme activity or of CRTR-mediated transport. Confirmation by these methods is important because other conditions (see below) may cause altered concentrations of creatine and guanidinoacetate in plasma and urine. Determination of AGAT and GAMT activity requires specific isotope-labeled substrates,³⁸ which may not be commercially available. CRTR-mediated uptake of creatine into fibroblasts can be measured by isotope dilution GC-MS³⁸ or by uptake of radiolabeled creatine.³⁹ Molecular analysis is based on sequencing of all coding regions, exon/intron boundaries, and promoter regions where applicable. In addition, molecular analysis may be used for carrier identification as well as prenatal and preimplantation testing. While an in-depth description of *GATM*, *GAMT*, and *SLC6A8* molecular sequence analysis is beyond the scope of these guidelines, it should be noted that analysis of *SLC6A8* is complicated by the presence of pseudogenes and relatively high GC content.⁴⁰

Prevalence

CDSs produce a relatively nonspecific phenotype and are likely underdiagnosed. Nevertheless, these disorders are among the most important known metabolic causes of intellectual impairment and autistic behaviors. CRTR deficiency

Table 1 Characteristic metabolite patterns for creatine deficiency disorders

Condition	Plasma			Urine	CSF	
	GA	Creatine	GA/creatinine	Creatine/creatinine	GA	Creatine
AGAT	Low	Low	Low	Normal	*Low	Low
GAMT	Elevated	Low	Elevated	Normal	Elevated	Low
CRTR	Normal	Normal	Normal	*Elevated	*Normal	*Normal

*inconsistent data (variable urinary creatine/creatinine in females with CRTR deficiency).

is the most common creatine deficiency syndrome, with over 150 patients reported,^{29,41} accounting for approximately 2% of males with nonsyndromic, X-linked intellectual disability (after fragile X has been ruled out^{47–49}) and 0.3 to 2.2% of males with an IQ less than 70.^{36,50} GAMT deficiency is the second most common creatine disorder, with approximately 110 patients reported worldwide.⁴¹ In Utah, the minimum frequency for this disorder was estimated to be 1 in 114,000⁵, while in the Netherlands a pilot newborn screening study estimated an incidence of 1 in 250,000.⁴¹ AGAT deficiency appears to be much rarer, with fewer than 20 patients reported to date.^{3,30,42–46}

Modes of inheritance

CDS due to AGAT or GAMT deficiencies are inherited as autosomal recessive traits, while deficiency of CRTR is inherited as an X-linked trait.

PREANALYTICAL REQUIREMENTS

Sample types

Quantitative analysis of creatine, guanidinoacetate, and creatinine can be reliably performed in urine, plasma, CSF, and dried blood spots.⁵¹ The low metabolite concentrations in CSF require methods with high sensitivity. For the diagnosis of AGAT or GAMT deficiency, either plasma or urine can be used, although testing sensitivity may increase if both sample types are analyzed concurrently. Urine is the sample of choice for diagnosing creatine transporter deficiency in males, as plasma creatine concentrations are typically normal in this condition. Fasting samples are preferred, since dietary intake of creatine may increase urinary excretion.³⁶

In vitro analysis of AGAT and GAMT enzyme activities can be carried out in Epstein-Barr virus–transformed lymphoblasts, while GAMT activity and CRTR-mediated creatine transport can be measured in cultured fibroblasts.^{38,39}

Molecular genetic studies (including carrier and prenatal testing) can be performed on DNA isolated from leukocytes, fibroblasts, or dried blood spots. Sequence analysis of the *SLC6A8* gene is recommended for diagnosing manifesting heterozygote females with a creatine transporter deficiency, as urinary creatine concentration, creatine uptake, and brain magnetic resonance spectroscopy studies may be inconclusive in these patients.

Sample requirements

Specific requirements for sample type(s), collection volumes, and sample shipping and handling should be established by the individual laboratory, and this information should be made readily available to referring physicians. In general, 1–2 ml of serum or plasma from whole blood collected in a sodium heparin (green top) or EDTA (purple top) tube, frozen urine, or CSF (free of blood contamination) are sufficient for LC-MS/MS- or GC-MS-based metabolite studies. A fibroblast culture can be established from a 3-mm skin punch, typically taken from the lateral upper thigh under sterile conditions. Fibroblasts can

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be cultured according to standard protocols. Leukocytes are extracted from 5–10 ml of blood collected in a sodium heparin (green top) or acid citrate dextrose (yellow top) tube and transformed into lymphoblasts according to standard protocols. Transformed lymphoblastoid cell lines should be used fresh and not stored. Isolation and analysis of DNA for molecular studies generally requires 1–2 ml of whole blood in an EDTA tube (purple top).

Conditions of sample shipping, handling, and storage

Plasma, urine, and CSF should be stored frozen (minimum –20 °C) and shipped on dry ice. Shipping of whole blood for biochemical analysis is not recommended. Blood for DNA analysis should be shipped to the laboratory at an ambient temperature within 24 h, or at 4 °C on cool packs if transport is delayed longer than 24 h after collection. Leukocyte pellets for DNA analysis should be stored frozen and shipped frozen on dry ice. Fibroblasts for enzyme or creatine transport measurements should be shipped in T-25 flasks filled with medium at room temperature. Whole blood collected for lymphoblast preparation should be transported at ambient temperature and, whenever possible, an overnight courier service should be used.

METHOD VALIDATION

As with any clinical diagnostic test, assay performance should be validated by each individual laboratory to ensure reliability and to compensate for interlaboratory variations. As stipulated by the CLIA, these performance characteristics should be documented and verified on a regular basis.

LC-MS/MS and GC-MS are the most commonly employed techniques for measuring creatine metabolites. Calibration and quantification generally involve the use of isotope-labeled internal standards and purified synthetic standards for generating calibration curves. Products suitable for these purposes are commercially available, but reagents should be carefully prepared and validated prior to use. Note that creatine and creatinine undergo spontaneous interconversion over time; therefore, it is important to take steps to minimize and correct for this process to ensure accuracy. In general, the validation process for any quantitative instrumental method should include evaluation of assay performance in terms of linearity and the dynamic range of the assay (including lower and upper limits of quantification), sensitivity, specificity, accuracy, precision, reproducibility, stability, recovery, carryover, and any potential sources of interference. When performing mass spectrometry-based analyses of complex (i.e., unpurified) biological samples, it is important to evaluate the potential effects of ion suppression and cross-analyte interference.

As with metabolite-based methods, confirmatory enzymatic and molecular assays should also be validated to ensure acceptable test performance. Also see General Policies C7, “Levels of Development of a Diagnostic Test,” and C8, “Test Validation,” in the ACMG Standards and Guidelines for Clinical Genetics Laboratories, 2008 Edition, revised 02/2007 (<https://www.acmg.net>).

acmg.net/acmg/Publications/Standards___Guidelines/General_Policies.aspx).

Reference ranges

For quantitative metabolite studies, it is recommended that laboratory-specific reference ranges for creatine, guanidinoacetate, and creatinine be determined for each sample type (plasma, urine, and/or CSF), using guidelines as defined by the laboratory's policy and procedure for method validation (e.g., Clinical and Laboratory Standards Institute document EP28-A3c, "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition"). Use of anonymized samples from a general patient population is also acceptable if those patients with an identified diagnosis are excluded. These ranges should be categorized by age, as appropriate, and updated on a regular basis. Establishing laboratory-specific reference ranges for affected individuals is more challenging because of the scarcity of patients, particularly in the case of AGAT deficiency, although it may be possible to obtain patient samples from other laboratories. An alternate approach to defining discrete, age-specific reference intervals is to use a continuous reference interval by age.⁵² While not optimal, in the absence of laboratory-specific data, several published reports describe ranges for affected individuals.^{38,53}

Laboratory-specific ranges for normal and affected individuals (where available) should also be established for AGAT, GAMT, and CRTR enzyme activity assays. Again, samples from a suitably large control population should be analyzed to establish normative values, and samples from affected controls should be included in each assay for quality control (QC) purposes.

Testing personnel

Laboratory personnel who are performing tests for screening or diagnosis of creatine synthesis or transport disorders should be documented as having received appropriate training and as demonstrating competency in the performance of these methods. In addition, laboratory personnel should satisfy CLIA requirements for high-complexity testing and have, at a minimum, an associate degree in a laboratory science or medical laboratory technology from an accredited institution. More comprehensive requirements apply in some states.⁵⁴ See General Policies B, "Personnel Policies," in the ACMG Standards and Guidelines for Clinical Genetics Laboratories, 2008 Edition, Revised 02/2007 (https://www.acmg.net/acmg/Publications/Standards___Guidelines/Personnel_Policies.aspx).

TESTING FOR CDSs

This section describes the diagnosis of CDSs by metabolite analysis, enzyme activity testing, and molecular sequencing.

Analysis of creatine, guanidinoacetate, and creatinine

Several methods have been described for the analysis of creatine, guanidinoacetate, and creatinine in plasma, urine, and CSF. Methods using stable isotope dilution mass spectrometry

coupled with liquid or gas chromatography are the most commonly used and typically have a high degree of sensitivity and specificity. LC-MS/MS-based measurement of creatine and guanidinoacetate can be performed with^{55,56} or without derivatization.^{56,57} Although slightly less sensitive, the underivatized method allows for more rapid preparation of urine and plasma samples, and simultaneous detection of creatinine. Samples are first combined with a stable isotope-labeled internal standard; for plasma, this may be prepared in an organic solvent (e.g., acetonitrile) to facilitate deproteinization, or an organic solvent may be added sequentially after the internal standards. Clarified samples are then concentrated by drying under nitrogen and resuspended in the appropriate solvent for LC-MS/MS analysis. Urine generally does not require deproteinization *per se*; however, it is good practice to clarify these samples (via centrifugation and/or filtration) after addition of organic solvents to remove precipitated proteins in the case of proteinuria and other potential contaminants. For the derivatized method, samples are mixed with an organic solvent, dried, and then reconstituted in butanolic HCl and incubated at 65 °C for approximately 15 min. After a second nitrogen-drying step, the sample is reconstituted in a solvent matrix prior to analysis. For both preparation methods, the additional precaution of microfiltrating the samples before analysis by electrospray ionization–LC-MS/MS is recommended. Separation by hydrophilic or reverse-phase chromatography has been used to enhance the sensitivity and specificity of LC-MS/MS methods. Typically, analytes and the corresponding isotope-labeled internal standards are detected in the positive ion mode by the mass spectrometer using selected reaction monitoring, which also confers increased sensitivity and specificity.

Quantitative GC-MS analysis of creatine and guanidinoacetate is also a sensitive and specific approach but requires significantly more preparation time than does tandem mass spectrometry.^{56,58,59} A number of methods have been developed that utilize various derivatives and gas chromatography capillary columns of differing polarities, electron impact, or negative chemical ionization, and selected ion monitoring. Electron impact methods appear to have a sensitivity similar to that of older LC-MS/MS systems but may be less sensitive compared with more modern, higher-performing LC-MS/MS instruments. The electron impact methods use a two-step derivatization procedure using hexafluoroacetylacetone that stabilizes the guanidine group, followed by derivatization with *N*-methyl-*N*-tert-butyltrimethylsilyltrifluoroacetamide to produce di(*tert*-butyldimethylsilyl) derivatives of the carboxylic acid moiety. These derivatives are analyzed using nonpolar columns (e.g., Supelco SPB-1). The negative chemical ionization method has greater sensitivity than electron impact methods and can measure the low concentrations of guanidinoacetate found in CSF.⁵⁸ In this procedure, samples are also subjected to a two-step derivatization procedure with hexafluoroacetylacetone and pentafluorobenzoylbromide, and are analyzed on polar columns such as an SGE BPX-70 (70% cyanopropyl polysilphenylene-siloxane; Trajan Scientific).

Analyte concentrations are calculated by extrapolation from calibration curves constructed from the peak area ratio of the analyte to that of the internal standard plotted against the calibrator concentrations. Creatine and guanidinoacetate in urine are normalized to the creatinine concentration, which may be concurrently analyzed using underivatized LC-MS/MS methods or determined separately using routine methods. In this context, it is important to note that the Jaffé method, in which creatinine reacts with alkaline picrate to form a complex absorbing at 480–520 nm, is still commonly used to determine creatinine, despite well-recognized interference by bilirubin, protein, ketones, ketoacids, fatty acids, and some drugs.⁶⁰ Therefore, the Jaffé method can give false-negative results in plasma when investigating low creatinine levels, which provides a diagnostic clue for AGAT and GAMT deficiencies.⁶¹ Thus, creatinine determination in plasma should not be relied on as a screening method for these disorders.

QC for analyte assays

Comprehensive QC guidelines for quantitative assays are available from the Clinical and Laboratory Standards Institute. In general, each batch of patient tests should include at least two levels of quality control, ideally consisting of the appropriate sample type (plasma, urine, or CSF). A target concentration for each analyte and tolerances for error should be established by analysis of replicate QC samples (ideally 20 or more) over multiple days, ideally by more than one operator. For example, in order to obtain appropriate normal and pathologic concentrations of creatine and related metabolites, one approach would be to utilize a low-level QC at approximately twice the concentrations of the lowest calibrator, and a high-level QC at 75% of the upper calibrator. Examples of other recommended QC options include samples with concentrations at approximately the midpoint of the calibration range or, if a dilution protocol is used, an identical dilution of the high QC using the same protocol.

Measurement of AGAT, GAMT, and CRTR activities

AGAT enzyme activity can be determined in lymphoblasts by measuring the formation of guanidinoacetate from isotopically labeled glycine and arginine.³⁸

The activity of GAMT can be determined in fibroblasts, lymphoblasts, and amniocytes via the formation of creatine from radioactive or stable isotope-labeled guanidinoacetate.^{38,62} For CRTR activity, the transport of radioactive isotope-labeled creatine is measured in fibroblasts. Affected patients have <10% normal activity. This assay may not detect female carriers since transport activity depends on the random inactivation of the X chromosome carrying the pathogenic variant or normal allele.³⁹ As is typical for enzyme assays, positive (affected) and normal (unaffected) controls should be included in each batch as a QC measure.

Molecular sequence analysis of *GATM*, *GAMT*, and *SLC6A8* genes

General recommendations regarding sample collection, analysis, interpretation, and QC of molecular studies is provided by

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the American College of Medical Genetics Standards Guidelines for Clinical Genetics Laboratories, Part G (Clinical Molecular Genetics), available at https://www.acmg.net/StaticContent/SGs/Section_G_2010.pdf.

Proficiency testing

Documented participation in ongoing proficiency testing activities is an important element of any laboratory quality assurance program. For relatively rare tests, like those for CDSs, a regular interlaboratory comparison program can be helpful in terms of both qualitative and quantitative performance. In addition, the European Research Network for Evaluation and Improvement of Screening, Diagnosis and Treatment of Inherited Disorders of Metabolism provides a commercially available, external proficiency testing service for the quantitative analysis of creatine, guanidinoacetate, and creatinine in serum and urine.

TEST INTERPRETATION AND REPORTING

Interpretation

Test results, including raw analytical data, should be reviewed carefully and in the appropriate clinical context (when available). True CDS cases (particularly biosynthesis disorders) are generally recognizable based on significant and unique deviations in analyte levels (especially guanidinoacetate and, to a lesser extent, creatine) compared with controls (**Table 1**). Samples from patients with AGAT deficiency typically have extremely low, almost undetectable concentrations of guanidinoacetate. Cases of GAMT deficiency are generally characterized by greatly increased guanidinoacetate concentrations (20–30 times that of controls),⁶³ although these levels may vary; hence, repeat analysis is recommended in situations involving moderate elevations (unpublished observations). Note that guanidinoacetate concentrations tend to be significantly lower in patients taking creatine and ornithine supplements, consistent with the negative regulatory effect imposed by these compounds on AGAT activity. Consistent with magnetic resonance spectroscopy findings, CSF creatine concentrations have been reported to be low in GAMT deficiency,^{64–66} and are expected to be low in AGAT deficiency. In CRTR deficiency, creatine concentrations in CSF are normal to mildly elevated, suggesting that creatine in the brain is lost because of a reuptake failure.²⁹ In general, specific enzyme activity assays and/or DNA testing should be used to confirm biochemical (metabolite) findings.

CRTR deficiency is the most common CDS and can be the most challenging to diagnose. Plasma creatine levels are generally unreliable indicators of CRTR status, as they may be elevated or normal in affected patients. Male patients with this disorder typically have significantly increased ratios of creatine to creatinine in urine (often >2; normal is generally <1).⁶⁷ However, this ratio may be elevated or normal in heterozygous CRTR females. Individuals with myopathy and/or low muscle mass may also have increased urinary creatine levels,^{27,68} while inconsistent findings have been reported for patients with various kidney disorders.²⁷ Urinary creatine levels can also be significantly elevated in postprandial samples, particularly after consumption

of meat or fish. Creatine is also a popular and readily available dietary supplement, although this consideration is obviously more important in older patients. Confounders for CDS disorders include ornithine aminotransferase deficiency and the hyperammonemia, hyperornithinemia, homocitrullinuria syndrome, which have been reported to cause secondary creatine deficiency.³³ Guanidinoacetate can be elevated in patients with urea cycle defects such as arginase deficiency, although creatine concentrations tend to be normal or mildly elevated in this situation.^{69,70} While not a CDS indicator, it is worth noting that elevated creatine in plasma has been suggested as a potential biomarker for mitochondrial disorders.^{71,72}

In general, the use of creatinine as a biomarker for the diagnosis of CDSs (aside from the creatine-to-creatinine ratio for CRTR deficiency) is of limited value. While levels in plasma and urine may be low in AGAT or GAMT deficiency, they may also be normal and are usually low in patients with low muscle mass. Regardless of the cause, low urinary creatinine concentrations are sometimes revealed due to inaccurate normalization for urinary organic or amino acid analysis, which results in apparent generalized elevations of these compounds.

Reporting

Reports should contain appropriate patient and specimen information, as described in the American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories, Sections 2.4, 2.41, and 2.42 (https://www.acmg.net/acmg/Publications/Standards_Guidelines/General_Policies.aspx) and as specified by CLIA. Written reports should provide units of measure; age-dependent, laboratory-specific reference ranges; and an interpretation. When abnormal results are detected, the interpretation should include an overview of the results and their significance, a correlation to any available clinical information, elements of a differential diagnosis, recommendations for additional biochemical testing and any available confirmatory studies (e.g., enzyme assay, molecular analysis), and a phone number to reach the reporting laboratory for additional questions. Recommendations for follow-up evaluation, including referral to a metabolic specialist, should also be included when appropriate.

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