Original Article

Fourteen cases of cerebral creatine deficiency syndrome in children: a cohort study in China

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Background: This study sought to analyze the clinical characteristics, biochemical metabolic indications, treatment results, and genetic spectrum of cerebral creatine deficiency syndrome (CCDS), estimate the prevalence of CCDS in Chinese children and provide a reference to guide clinical practice.

Methods: We performed a retrospective cohort study of 3,568 children with developmental delay at Children's Hospital of Fudan University over a 6-year period (January 2017–December 2022). Metabolites in the blood/urine were detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS), and genetic testing was performed by next-generation sequencing (NGS). The patients with suspected CCDS were ultimately diagnosed by magnetic resonance spectroscopy (MRS). The patients were then treated and followed up. All the reported cases of CCDS, their gene mutations, and treatment results in China were summarized.

Results: Ultimately, 14 patients were diagnosed with CCDS. The age of onset was between 1–2 years. All the patients had developmental delay, 9 had epilepsy, and 8 had movement or behavioral disorders. A total of 17 genetic variants were identified, including 6 novel variants. c.403G>A, c.491dupG of the guanidinoacetate methyltransferase (*GAMT*) gene had a relatively high frequency. After treatment, patients with *GAMT* deficiency showed obvious improvements, and brain creatine (Cr) levels recovered to 50–80% of normal, 1 patient achieved normal neurodevelopment, and 3 patients became epilepsy free; however, 6 male patients with X-linked creatine transporter gene (*SLC6A8*) variants received Cr for 3–6 months with no effect, and 2 patients received combined therapy with few improvements.

Conclusions: The prevalence of CCDS is ~0.39% in Chinese children with developmental delay. A low-protein diet, Cr and, ornithine were useful for patients with *GAMT* deficiency. Male patients with *SLC6A8* deficiency showed only limited improvement on combined therapy.

Keywords: Cerebral creatine deficiency syndrome (CCDS); liquid chromatography-tandem mass spectrometry (LC-MS/MS); creatine (Cr); magnetic resonance spectroscopy (MRS)

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Introduction

Cerebral creatine deficiency syndromes (CCDS) are hereditary neurometabolic diseases and is characterized by reduced brain creatine (Cr) levels. There are 3 types: (I) CCDS1: creatine transporter deficiency (CTD) or SLC6A8 deficiency; and two the biosynthesis disorders; (II) CCDS2: guanidinoacetate methyltransferase (GAMT) deficiency; and (III) CCDS3: L-arginine:glycine amidinotransferase (AGAT) deficiency. Patients have mild-severe global developmental delay, characterized by intellectual impairment, hypotonia, speech delay, behavioral disorders, and epilepsy from early childhood. In 1994 (1), the first case of CCDS2 was described in a 22-month-old infant, who presented with hypotonia and movement disorders. Since then, >300 cases of CCDS have been reported in newborns, children, and adults and the results of treatment. In children, the early development delays in CCDS and other disorders are nonspecific and similar in presentation. But CCDS could be differentiated from other diseases through *in vivo* metabolites and genetic analysis (2-11).

Few cases of CCDS have been found in China due to the lack of testing equipment in many hospitals. So many clinicians know little about CCDS and have very limited treatment experience, or think that there was no treatment for CCDS, just like other genetic diseases. Several cases had not been diagnosed for a long time until they came to Children's Hospital of Fudan University, so some patients may be missed. In this study, a cohort of 3,586 patients with developmental delay, evaluated by Gesell Developmental Scale in Department of Child Care and Neurology at the Children's Hospital of Fudan University (from January 2017–December 2022) was screened for CCDS. Cr and guanidinoacetate (GAA) levels on dried blood spots (DBS), the Cr/creatinine (Cr/Crn) ratio in the urine, and Cr levels in the brain were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and magnetic resonance spectroscopy (MRS). Genetic analysis was done by next-generation sequencing (NGS). We would like to know the incidence of CCDS in Chinese children with developmental delay, and summarize our treatment results and provide some experience for clinicians. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-23-164/rc).

Highlight box

Key findings

- This study summarized the diagnosis, treatment, and follow-up of multiple cases (14 patients) with creatine deficiency syndrome (CCDS) in China.
- CCDS are rare conditions, our findings may improve understanding of children with developmental delay. The incidence of CCDS in children with developmental retardation in China is ~0.39%.

What is known and what is new?

- Many cases with CCDS have been reported and treated in North America and Europe and many patients were treatable.
- Fewer cases have been reported in children in China. This study was the first to conduct a long-term follow-up and report the outcomes of multiple cases with *CCDS*.

What is the implication, and what should change now?

- It is necessary to improve the awareness of CCDS, and metabolic/genetic analyses should be performed if CCDS is suspected.
- The long-term treatment side effects of *GAMT* deficiency require further investigation.
- New treatments of *SLC6A8* deficiency need to be explored.

Methods

Children diagnosed with developmental delay, intellectual disability, epilepsy, behavior and movement disorders, and speech and language disorders in the last 5 years at the Genetic Metabolic Diseases Laboratory and Molecular Central Laboratory, Department of Radiology of Children's Hospital were included in this study. The physicians following these patients were invited to participate in this study. This study was approved by the Medical Research Ethics Committee (No. 2015-130) at Pediatric Research Institution of Children's Hospital of Fudan University. The parents/guardians of the included patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Metabolic and genetic screening

Cr and GAA levels on DBS and the Cr/Crn ratio in the urine both were measured by LC-MS/MS (Acquity UHPLC-class Xevo TQD, Waters, USA) (8,9). The peripheral blood of the patients with suspected CCDS and their parents were obtained for next-generation sequencing (NGS). Genomic DNA was obtained from venous (whole) blood of the index patients and their parents and sequenced using an Illumina HiSeq 2500 sequencer (Illumina, San Diego, USA). All

detected mutations were confirmed by Sanger sequencing using ABI 3500 Gene Analyzer (Applied Bio-systems, USA).

Gene sequencing, variant interpretation, and reporting were performed according to the high-throughput sequencing and diagnostic protocol established by the Molecular Diagnosis Center at Pediatric Research institution (12). New variants were analyzed by Polyphen-2 (http://genetics.bwh.harvard.edu) and Mutation Taster (www.mutationtaster.org). A comprehensive assessment of each variant's pathogenicity, including the inheritance pattern, was carried out according to the American College of Medical Genetics and Genomics criteria (13).

Combined brain magnetic resonance imaging (MRI)/MRS examinations

Combined MRI/MRS examinations were performed with a 3.0T clinical scanner (Discovery TM MR 750 3.0T, GE Healthcare, USA). All patients sedated with chloral hydrate. Routine MRI scans to rule out unknown brain lesions. MRS Collection: T2WI was used to identify the basal ganglia, thalamus, and white matter as the area of interest. MRS Scanning uses a two-dimensional point resolution spectrum (2D-PRESS) sequence with horizontal position and the scanning time was 4.30 min. The original detection results of MRS were imported into the GEAW4.2 workstation and the Functool software was used for post-processing. The peak area of N-acetyl aspartate (NAA), choline (Cho) and Cr were measured by integral method.

Treatment and follow-up

The treatment of children with CCDS was based on previous reports (14-18). Six patients with the GAMT deficiency all received a low-protein diet (<20–25 g/day) with Cr (400 mg/kg/day), and ornithine (400–500 mg/kg/day) supplementation. And we added Addition nutritional (Cyclinex-2 and Pro-Phree, Abbott, USA) for patients <4 years old. Six patients with the SLC6A8 deficiency received Cr monotherapy, but this was discontinued after 3–6 months. Additionally, 1 patient received combined Cr (400 mg/kg/day), arginine (200 mg/kg/day), and glycine (100 mg/kg/day) therapy for 7 months, and 1 patient received combined Cr gluconate (300 mg/kg/day), arginine (200 mg/kg/day), and glycine (100 mg/kg/day) therapy for 2 months. Metabolites in the blood and urine were monitored after 1–2 months of treatment, and brain Cr levels were measured after 6–9 months of treatment.

Statistical analysis

For this study, Cr levels in the blood and the Cr/Crn ratio in the urine are expressed as the mean ± standard error of the mean, and differences in the clinical treatment outcomes and Cr levels in the brain were analyzed using the analysis of variance test for the continuous variables. All the statistical analyses were performed with GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). A P value <0.05 was considered statistically significant.

Results

In the last 6 years, 3,586 children were diagnosed with developmental delay, intellectual disability, epilepsy, behavior and movement disorders, and speech and language disorders by the Genetic Metabolic Diseases Laboratory and Molecular Central Laboratory, Department of Radiology at Children's Hospital. Of these patients, 148 (of whom 98 were male and 50 were female) had suspected CCDS by LC-MS/MS or genetic testing at first. Ultimately, 14 patients were confirmed with CCDS by MRS examinations, of whom 6 were diagnosed with GAMT deficiency (CCDS2) and 8 were diagnosed with SLC6A8 deficiency (CCDS1). The clinical characteristics of the 14 patients diagnosed with CCDS are summarized in *Table 1*.

Metabolic screening and genetic testing

All the patients with suspected CCDS had a Cr level from DBS below <85 μM/L on DBS. During the follow-up, the Cr level decreased in 17 patients, the GAA from DBS level increased in 6 patients, and the Cr/Crn ratio from urine increased in 8 patients by LC-MS/MS. Seven patients with suspected CCDS had compound heterozygous variants in the *GAMT* gene, suggesting the possibility of GATM deficiency. In total, 10 patients had hemizygous variants of the *SLC6A8* gene, suggesting the possibility of SLC6A8 deficiency, among whom, 6 had inherited the mutations from their mother, and 4 had spontaneous mutations.

Brain MRI/MRS examinations

The brain MRI showed that 3 patients (Patients 3, 8, 13) with suspected CCDS had a thin corpus callosum, 3 (Patients 1, 4, 13) had abnormalities in the white matter, 3 (Patients 2, 8, 10) extracerebral space enlargement, and 1 had cerebral atrophy (Patient 11). While 6 patients

Table 1 Pretreatment phenotypes and genotypes in the 14 CCDS patients

Patients No./sex/age at diagnosis/current age	Clinical features	Biochemical indications; Ref: (Cr 80–1,000 μM, GAA 0.40–3.82 μM, Cr/Crn 0.02–0.43)	Gene	Genomic variants	Brain MRI/MRS	Diagnosis
1/F/4 y/14 y	Moderate GDD, speaks 2–3 words, occasional epilepsy, hypotonia	Cr 33.26↓↓; GAA 17.25↑	*GAMT*	Known c.564G>T (p.M188I), known c.491dupG (p.Gly164Alafs*14)	Abnormal signals in the white matter/brain Cr↓↓	CCDS2
2/F/20 m/4 y	Severe GDD, mild epileptic, no speech	Cr 39.45↓↓; GAA 2.84	*GAMT*	Known c.289delC (p.Gln97Serfs*17), known c.392-1G>C	Extracerebral space enlargement/brain Cr↓↓	CCDS2
3/F/4 y/6 y	Severe GDD, epilepsy, no speech	Cr 48.32↓↓; GAA 5.46↑	*GAMT*	Novel c.328-1G>A, known c.403G>A (p.D135N)	Thin corpus callosum/brain Cr↓↓	CCDS2
4/F/6 y/9 y	Moderate GDD, refractory epilepsy, speaks 2–3 words	Cr 52.67↓; GAA 6.04↑	*GAMT*	Novel c.328-1G>A, novel c.114C>T (p.G38G)	Abnormal signals in the white matter/brain Cr↓↓	CCDS2
5/M/3 y/4 y	Moderate GDD, speaks 1–2 words	Cr 42.96↓↓; GAA 7.01↑	*GAMT*	Novel c.328-2A>G, novel c.115 A>G (p.K39E)	N/brain Cr↓↓	CCDS2
6/M/1 y/2 y	Mild GDD, speaks 1–2 words	Cr 62.62↓; GAA 6.01↑	*GAMT*		N/brain Cr↓↓	CCDS2
7/M/8 y/11 y	Severe ID, epilepsy, few teeth, speaks 2–3 words	Cr 61.23↓; Cr/Crn 1.21↑	*SLC6A8*	Known c. 626_627delCT (Pro209Argfs*87)	N/brain Cr↓↓	CCDS1
8/M/2 y/4 y	Severe ID, ADHD, laryngeal cartilage dysplasia, no language, severe epilepsy. Mother: mild ID, epileptic	Cr 67.53↓; Cr/Crn 1.38↑	*SLC6A8*	Known c.200G>A (p.G67D)	Extracerebral space enlargement, thin corpus callosum/brain Cr↓↓	CCDS1
9/M/7 y/9 y	Moderate ID, only speaks a few words, few teeth, deficit in fine motor skills	Cr 62.96↓; Cr/Crn 1.30↑	*SLC6A8*	Known c.778-2A>G	N/brain Cr↓↓	CCDS1
10/M/2 y/4 y	Severe ID, unsteady walking, inability to speak, epilepsy, constipation, ADD. Uncle: same symptoms	Cr 45.59↓↓; Cr/Crn 1.18↑	*SLC6A8*	Known c.1222_1224delTTC (p.Phe408del)	Extracerebral space enlargement/brain Cr↓↓	CCDSI
11/M/1 y/2 y	Severe ID, repeated convulsions, ADHD, epilepsy	Cr 49.59↓↓; Cr/Crn 1.24↑	*SLC6A8*	Novel c.1767+1_1767+2insA	Cerebral atrophy/brain Cr↓↓	CCDSI
12/M/3 y/5 y	Severe GDD, hypotonia, ADD, intractable epilepsy,	Cr 55.20↓; Cr/Crn 1.13↑	*SLC6A8*	Known c.321_323 del (p.F107del)	N/brain Cr↓↓	CCDS1
13/M/2 y/4 y	Severe ID, open mouth, drooling, difficulty falling asleep, crying at night, sweating, white hair	Cr 55.20↓; Cr/Crn 3.45↑	*SLC6A8*	Novel c.1496G>A (p.G499V)	Thin corpus callosum, Abnormal signals in the white matter/brain Cr↓↓	CCDS1
14/M/9 m/4 y	Moderate ID, little eye contact	Cr 47.53↓↓; Cr/Crn 2.62↑	*SLC6A8*	Novel c. 967G>C (p.A323P)	N/brain Cr ↓↓	CCDS1

↑, increased; ↓, reduced; ↓↓, significantly reduced; Patients 5 and 6 are brothers. CCDS, cerebral creatine deficiency syndrome; F, female; M, male; y, years; m, months; GDD, global developmental delay; ID, intellectual disability; ADHD, attention deficit hyperactivity disorder; ADD, attention deficit disorder; Cr, creatine; GAA, guanidinoacetic acid; Crn, creatinine; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; N, normal.

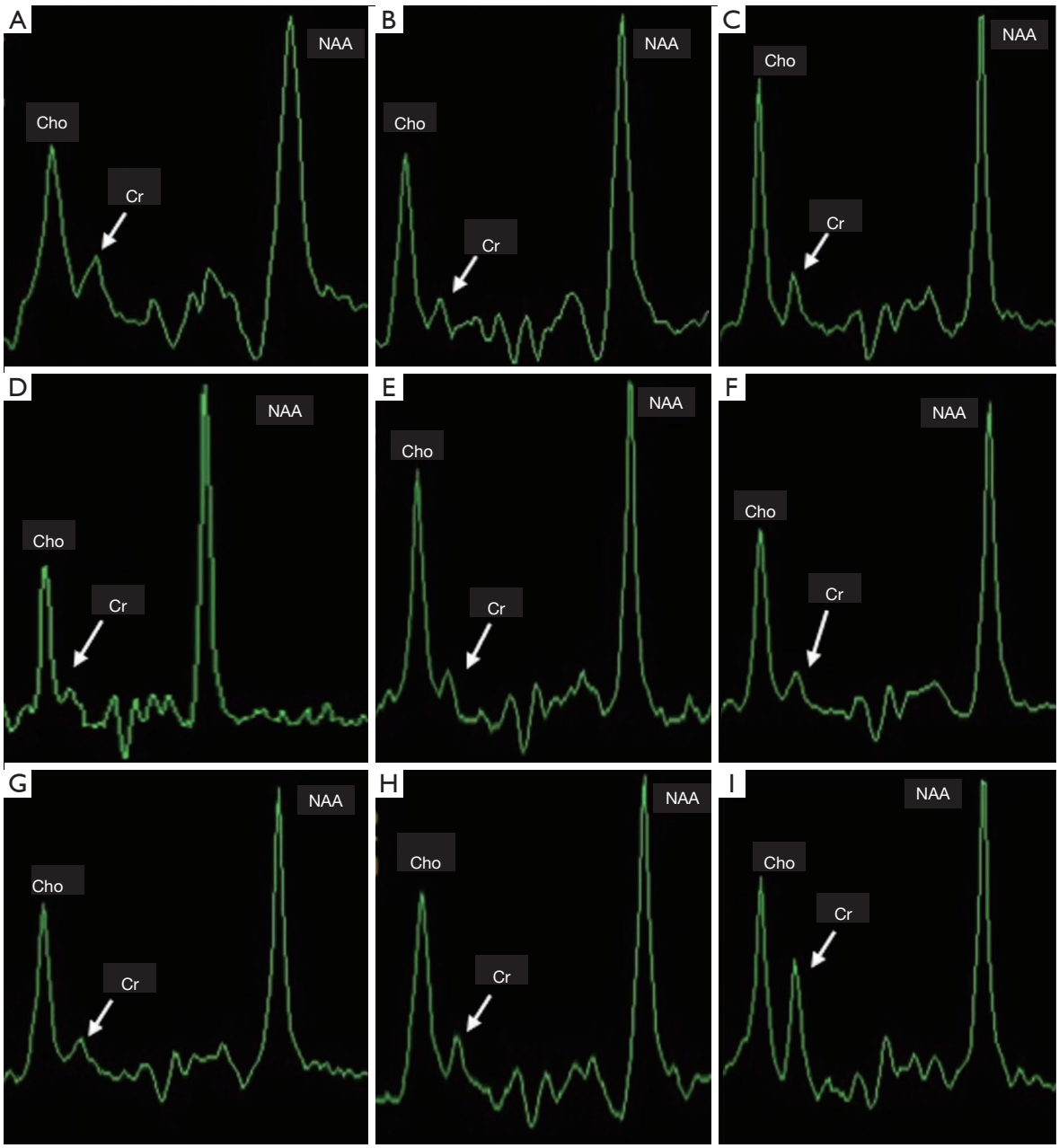

Figure 1 MRS spectra from the basal ganglia and thalamus of patients with CCDS. The Cr peak (white arrow) was significantly reduced in patients with CCDS2 [(A) Patient 3; (B) Patient 4; (C) Patient 5] and CCDS1 [(D) Patient 9; (E) Patient 10; (F) Patient 11; (G) Patient 12; (H) Patient 13]. (I) Patient 14 with CCDS1 showed a moderate reduction in the Cr peak. CCDS, cerebral creatine deficiency syndrome; MRS, magnetic resonance spectroscopy; Cr, creatine; Cho, choline; NAA, N-acetyl aspartate.

showed no abnormalities (*Table 1*). The brain MRS confirmed that the Cr peaks in the basal ganglia and thalamus were below the normative values in 13 patients and moderately reduced in 1 patient (*Figure 1*). One patient with variants in *GAMT* gene and 2 patients with variants in *SLC6A8* gene were excluded from CCDS, as brain Cr levels were normal by MRS. All cranial MRI/MRS results from Department of Radiology in Children's Hospital of Fudan University.

Treatment outcomes

The DBS results showed that the Cr and GAA levels of the patients diagnosed with CCDS2 returned to the normal range after 2–3 weeks of treatment. Brain Cr levels increased significantly 6–9 months after treatment, returning to 50–80% of normal. The clinical manifestations of a majority of the patients improved. In 3 patients (Patients 1, 3, and 4), epilepsy gradually resolved after 3–6 months of treatment, and the patients no longer required anti-epileptic drugs. Patient 1, now aged 14 years, was treated for 10 years and went to school normally, and she performed as well intellectually normal children. Patient 2 made progress in communication and simple conversations but was not able to speak long sentences after 3 years of treatment. This patient had occasional absent-mindedness and some abnormalities on electroencephalogram, which showed significant improvement with the use of anti-epileptic drugs (sodium valproate). The other 4 patients with CCDS2 were treated for 4–8 months, and their motor and cognitive skills improved (could walk, run, and climb stairs, gross motor and fine motor progress. significant progress in understanding and communication, like read children's books, start to speak), but their speech was relatively slow.

Treatment was not effective for patients diagnosed with CCDS1. Among these patients, 6 received Cr monotherapy for 6 months, and their clinical manifestations did not improve, 1 patient received Cr monotherapy for 6 months, followed by combined Cr, arginine and glycine therapy for 2 months and showed some improvements, including the ability to walk more steadily, a greater willingness to learn, and less crying, and 1 patient received combined Cr gluconate, arginine and glycine therapy for 2 months, and showed modest improvements in behavior, social interactions and strength. The treatment and outcomes of the 14 CCDS patients are summarized in *Table 2*.

Discussion

Cr/creatine-phosphate is an electrical buffer system of chemical energy in brain and muscle tissues/cells, which plays an important role in energy storage and conversion. *In vivo* Cr is synthesized mainly in the liver, pancreas and kidneys by AGAT and GAMT in two steps. The former enzymes catalyzed the formation of GAA from arginine and glycine, and the latter catalyzed the conversion of GAA into Cr. Cr is transported to the brain and muscle tissue by Cr transporters in the blood. Studies show that Cr synthesis or transporter deficiency causes severe Cr deficiency in brain and leads to a series of severe neurological disorders (1,14,17).

CCDS are hereditary metabolic diseases in which Cr synthesis or Cr transport disorders lead to low Cr levels in brain. Patients with CCDS2 have also elevated GAA levels, and patients with CCDS1 have also an elevated urinary Cr/Crn ratio. Cr is an energy-rich amino acid derivative that is produced endogenously in several human tissues. Glycine and arginine combine to form GAA, which is methylated to Cr under the catalysis of GAMT. Cr may also be obtained from the dietary consumption of meat and fish. Cr is transported through the blood-brain barrier via a Cr transporter protein. Cr maintains adenosine triphosphate (ATP) levels in the body through reversible phosphorylation. Cr and Cr-phosphate play an important role in energy homeostasis, especially in the brain. Cr deficiency causes global developmental delay, resulting in language impairment, motor and mental disorders, epilepsy, and autism (19-21). Secondary GAA increases inhibit mitochondrial respiratory chain enzymes and Cr kinase, reduces antioxidant and ATP levels, increases inflammatory factors, and results in the brain abnormalities (22,23).

Brain MRS can detect several important metabolites in the brain, including lactate, Cr, NAA, and Cho. A significant decrease in the Cr peak alone in the brain is highly suggestive of CCDS (1,10,11). MRS also provides a non-invasive tool to evaluate the degree of cerebral Cr recovery in the follow-up of children. LC-MS/MS could be used to screen blood/urine for metabolite deficiencies to identify patients with suspected CCDS (8,9). Genetic testing should be used to make a definitive diagnosis and MRS can confirm that a variant is pathogenic as a functional test. CCDS1 could also be confirmed by an *in vitro* fibrocyte Cr uptake assay (24), but this is costly, time consuming, and only available in specialized laboratories.

The outcomes of patients with CCDS2 appear to improve if they receive early treatment with Cr and ornithine supplementation and a low-protein diet. Ornithine has been shown to reduce GAA synthesis and prevents brain damage, especially in infants and young children (15,16). In 2021, CCDS2 was identified among the 116 treatable inherited metabolic disorders that cause intellectual disability (25,26). More than 150 cases of CCDS2 have been reported in the literature, mostly in infants and young children. CCDS2 in an adult patient was first described in 2003, and an estimated 25 cases have been reported since then. Children with CCDS2 should receive timely treatment to reduce the risk of developing severe

Table 2 Outcomes following combined creatine, arginine, glycine, and creatine gluconate therapy in the patients with CCDS2 and CCDS1

Cases No./ sex/age at treatment	Treatment [dose mg/kg/d]	Treatment duration	Outcome/improvement on treatment	Metabolites on treatment	Brain Cr level on treatment
1/F/4 y	Low protein diet, Cr [400], Orn [300–400], Cyclinex-2 and Pro-Phree[a]	10 y 4 m	Significant progress in movement, language, and social interactions. Epilepsy disappeared after 2 years. Normal neurodevelopmental outcome	Normal Cr and GAA levels	70–80% of normal
2/F/20 m	Low protein diet, Cr [400], Orn [300–400], Cyclinex-2 and Pro-Phree[a]	2 y 2 m	Significant progress in movement and social interactions. Ability to speak 3–4 words. Mild epilepsy that could be controlled	Normal Cr and GAA levels	70–80% of normal
3/F/4 y	Low protein diet, Cr [400], Orn [300–400]	2 y 1 m	Progress in movement and social interaction. Ability to speak 3–4 words. Epilepsy disappeared after 1 year	Normal Cr and GAA levels	50–60% of normal
4/F/6 y	Low protein diet, Cr [400], Orn [300–400]	3 y 3 m	Significant progress in movement and social interactions, Ability to speak 7–8 words. Epilepsy disappeared after 2 years	Normal Cr and GAA levels	50–60% of normal
5/M/3 y	Low protein diet, Cr [400], Orn [300–400]	7 m	Progress in movement, language, and social interactions	Normal Cr and GAA levels	50–60% of normal
6/M/1 y	Low protein diet, Cr [400], Orn [300–400]	8 m	Progress in movement, language, and social interactions	Normal Cr and GAA levels	50–60% of normal
7/M/8 y	Cr [400]	6 m	None	Cr/Crn 0.40	Not performed
8/M/2 y	Cr [400]	6 m	None	Cr/Crn 0.35	Not performed
9/M/7 y	Cr [400]	6 m	None	Cr/Crn 0.43	No change
10/M/2 y	Cr [400]	6 m	None	Cr/Crn 0.46	No change
11/M/1 y	Cr [400]	4 m	None	Cr/Crn 032	No change
12/M/3 y	Cr [400]	5 m	None	Cr/Crn 0.41	Not performed
13/M/2 y	Cr [400], Gly [150], Arg [300], creatine gluconate [400]	2 m	Improvement in some motor skills, cognition and hand behavior	Cr/Crn 0.23	Slightly increased but still very low
14/M/9 m	Cr [400], Gly [150], Arg [300], creatine gluconate [400]	3 m	Improvement in motor skills and cognition	Cr/Crn 0.37	Slightly increased but still very low

[a], when <5 years old. CCDS, cerebral creatine deficiency syndrome; F, female; M, male; y, years; m, months; d, day; Cr, creatine; Orn, ornithine; Gly, glycine; Arg, arginine; Crn, creatinine; Cr/Crn in urine, (normal 0.02–0.43); GAA, guanidinoacetate.

mental and intellectual disabilities in adulthood (5,15,16,27). In the present study, Patient 1 was diagnosed at the age of 4 years and had a normal developmental outcome after >10 years of treatment. Patients 2, 3, and 4 were treated for >1 year, and their epilepsy disappeared after 3–6 months, and they showed significant improvements in motor skills and cognition, and moderate progress in language. Patients 5 and 6 were treated for 4 months and showed noticeable progress in their global development. Patients treated displayed variable improvement, maybe it depends on the age they were treated, how long they've been treated, and how well they've followed the advice.

Approximately 20 cases of CCDS3 have been reported in the literature, but none have been reported in China. CCDS3 was first described in 2 sisters who suffered mental retardation and severe language delay (28). Early intervention with Cr supplementation has been shown to be effective in patients with CCDS3. The brain Cr level may return to 60–98% of normal if treatment begins in the neonatal period, but improvements in adult patients are limited (6,7).

CCDS1 is an X-linked Cr deficiency syndrome that was first described in a male patient with severe language delay and mild retardation (29,30). An estimated 1% of males with mental retardation of unknown etiology may have a *SLC6A8* mutation. Mutations in the *SLC6A8* gene occur *de novo* in 30% of males, and in the general population 0.024% of females are carriers. Cr supplementation may improve the phenotype in female patients with CCDS1, but treatment responses are limited in males (31-33). In the present study, Patients 13 and 14 made some progress after combined therapy. About one-third of patients could benefit by oral high-dose Cr. Studies in animals have shown that the uptake of Cr by the brain or the transport of Cr across the blood-brain barrier may be increased by pharmacochaperones, nanotechnology, changing the Cr structure, or gene therapy which has led to the identification of potential novel treatment approaches for patients with CCDS1 (34-39).

To date, 30 CCDS patients and 46 mutations of *GAMT* and *SCL6A8* have been reported in China, including the

14 cases in this study. The number of patients with CCDS1 and CCDS2 has been reported to be 50% and 50%, respectively, but the number of patients with CCDS3 has not been reported (3,4,40-43). In this study, the frequencies of *GAMT* c.403G>A (5 patients) and c.491dupG (4 patients) were relatively high in Chinese patients.

It has been reported that autism spectrum disorder is associated with inborn errors of metabolism, such as CCDS (44-46). The early stages of CCDS may be difficult to detect in childhood, and diagnosis is often missed. CCDS should be suspected in patients with developmental delay, feeding difficulties/weight gain, growth retardation, and epilepsy, especially in males (47-49). Neuroimaging plays an increasingly important role in inherited metabolic diseases and other diseases, and sometimes plays a key role in differential diagnosis, such as the diagnosis of CCDS by MRS (10,11).

Thus, in addition to clinical and biochemical profiling, neuroimaging plays a pivotal role in differentiating between metabolic disorders and other diseases, in providing a differential diagnosis, in suggesting a metabolic pathway derangement, and, on occasion, in helping to make a specific diagnosis.

Conclusions

In conclusion, we described the clinical characteristics and treatment outcomes of 14 patients with CCDS. The prevalence of CCDS is about 0.39% in Chinese children with developmental delay. Children with suspected CCDS may be early diagnosed using LC-MS/MS, genetic testing and MRS. We discovered 7 novel genetic variants, and thus expanded the spectrum of CCDS. Among the 14 patients, 6 with CCDS2 made significant progress, Patient 1 had normal neurodevelopment. Combined Cr and ornithine therapy and a low-protein diet improved seizure control, cognition, and movement disorders. The clinical characteristics of the 6 patients with CCDS1 treated with Cr monotherapy did not improve, which provides further evidence that this approach is not effective in males. Two patients with CCDS1 received combined Cr/Cr gluconate, arginine, glycine therapy and showed some improvement in motor skills, hand behaviors, and cognition. Treatment responses should be monitored by MRS, and the outcomes of combined therapy should be continually observed during the long-term follow-up.

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Footnote

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Data Sharing Statement: Available at https://tp.amegroups.com/article/view/10.21037/tp-23-164/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-23-164/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Medical Research Ethics Committee (No. 2015-130) at Pediatric Research Institution of Children's Hospital of Fudan University. The parents/guardians of the included patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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