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Coexistence of Megaconial Congenital Muscular Dystrophy and Cystinuria: Mimicking Hypotonia-Cystinuria Syndrome

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Established Facts

- Megaconial congenital muscular dystrophy is an autosomal recessive disorder. Nearly 40 patients have been identified in the literature to date, the majority being of Turkish origin.
- The most common manifestations are neonatal/infantile-onset hypotonia, progressive generalized muscle weakness, autistic behavior, intellectual disability, ichthyosis-like skin manifestations, and cardiomyopathy.

Novel Insights

- We present a case with clinical findings of hypotonia-cystinuria syndrome, who was diagnosed with coexisting cystinuria and megaconial congenital muscular dystrophy following whole-exome sequencing analysis.
- A novel homozygous NM_005198.5:c.225-2A>T pathogenic variant in the first intron of the *CHKB* gene was identified with whole-exome sequencing analysis.

Keywords
Cystinuria · Hypotonia-cystinuria syndrome · Megaconial congenital muscular dystrophy · Whole-exome sequencing · Coexistence

Abstract

Introduction: Hypotonia-cystinuria syndrome is a contiguous gene deletion syndrome that is characterized by hypotonia, developmental delay, and cystinuria type A. We present a male patient who was admitted to our center with clinical findings of hypotonia-cystinuria syndrome and diagnosed with megaconial congenital muscular dystrophy and cystinuria. ***Case Presentation:*** A 16-month-old male patient was admitted with complaints of restlessness and body laxity. It was stated that the patient had hypotonia and growth retardation at the age of 2 months. Physical examination revealed mild hypotonia, growth retardation, and development delay, while laboratory examinations identified elevated serum creatine kinase and elevated dibasic amino

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acid in urine analysis. Because of the findings of hypotonia, growth retardation, developmental delay, and cystinuria, hypotonia-cystinuria syndrome was considered as a differential diagnosis. However, by chromosomal microarray no contiguous deletion in region 2p21 was found, while a novel homozygous c.225-2A>T pathogenic variant in the *CHKB* gene and a c.1266_1267delGT heterozygous variant in the *SLC7A9* gene inherited from the mother were identified with whole-exome sequencing. The co-occurrence of megaconial congenital muscular dystrophy and cystinuria, mimicking hypotonia-cystinuria syndrome, was confirmed. ***Conclusion:*** This case suggests that in countries with a high frequency of consanguineous marriage, even if the molecular genetic analysis results are not compatible with the clinical findings, it should be kept in mind that different genetic diseases may coexist.

Introduction

Hypotonia-cystinuria syndrome (HCS) is an autosomal recessive contiguous gene deletion syndrome caused by deletions of different sizes in chromosome 2p21, which contain the *PREPL* and *SLC3A1* genes [Eggermann et al., 2012a]. The syndrome is characterized by marked hypotonia-nutritional problems in the neonatal and infantile period, developmental delay, growth hormone deficiency, short stature, and cystinuria type A (cystine stones). In late childhood, overweight, hyperphagia, normal/mild cognitive problems, delayed puberty, and hypergonadotropic hypogonadism may occur. In adults, nasal dysarthria, facial weakness, ptosis, neck flexor weakness, and proximal weakness may be observed [Eggermann et al., 2012a; Kilic et al., 2018].

Cystinuria is a cystine metabolism disorder caused by *SLC3A1* (chromosome 2p21) or *SLC7A9* (chromosome 19q12) gene mutations that lead to cystine stone formation. These genes encode the renal amino acid transporters that are required for the transport and absorption of dibasic amino acids in the intestine and renal proximal tubules. Deficiencies in these transporters and defective reabsorption lead to cystine accumulation over time, recurrent stone production in the urinary tract, and renal disease [Eggermann et al., 2012b; Taroni et al., 2019].

Megaconial congenital muscular dystrophy (CMD) is characterized by mildly elevated serum creatine kinase (CK) levels, proximal weakness, early-onset hypotonia, muscle wastage, intellectual disability, delay in gross motor developmental milestones, ichthyosis-like skin changes, some cardiac problems, hearing loss, mental retardation, seizures, and behavioral problems, and is the result of a mutation in the choline kinase beta gene (*CHKB*), which encodes the choline kinase [Kutluk et al., 2020].

Here, we present a case with clinical findings of HCS, but who was diagnosed with coexisting cystinuria and megaconial CMD following a whole-exome sequencing (WES) analysis.

Case Report

A 16-month-old male patient was admitted to our center with complaints of restlessness (sleep disturbance, crying episodes, feeding difficulty/reluctance), body laxity, and growth retardation.

The patient was born to consanguineous parents at a gestational age of 34 weeks with birth weight 2,500 g (89.8 percentile, SDS 1.27) and birth length 47 cm (82.1 percentile, SDS 0.92) (Fig. 1a). It was stated that hypotonia and growth retardation were detected at the age of 2 months, and that there was a family history of nephrolithiasis. His mother has a history of kidney stones, but a characterization of the stones was not available.

Physical examination revealed growth retardation (weight 8,100 g, SDS −2.54; length 70 cm, SDS −3.25; head circumference 46 cm, 3rd–10th percentile). The patient was hypotonic and could sit unaided for a short time. He had a slightly long facial appearance, deep sunken eyes, and slightly drooping ears. Brief eye contact was possible. He had global developmental delay. An ophthalmological examination and a hearing test were both normal, and other system examinations were unremarkable.

Laboratory examination revealed a complete blood count, serum electrolytes, and liver, renal and thyroid function tests to be within the normal range. Serum CK was moderately high (335 U/L [normal <171]). Tandem mass spectrometry, plasma amino acid and lactate levels were normal. While urine organic acid analysis was unremarkable, quantitative urine amino acid analysis revealed elevated dibasic amino acids (cystine: 2,611 μmol/crea [normal <160], ornithine: 435.5 μmol/crea [normal <250], arginine: 304.2 μmol/crea [normal <100], lysine: 3,710 μmol/crea [normal <200]).

Urinary system ultrasonography carried out for the evaluation of nephrolithiasis produced normal results, and brain magnetic resonance imaging and echocardiography performed for hypotonia and development delay were both normal.

Based on the findings of hypotonia, growth retardation, developmental delay, and cystinuria, HCS was considered as a differential diagnosis, and genetic analysis was performed.

Genetic Analysis

These genetic analyses were for clinical diagnosis only and did not require the institution's ethics committee's approval. Written informed consent was obtained from the parents of the patient. Chromosomal microarray analyses (CMA) revealed no deletion in 2p21. The Infinium CytoSNP-850K v1.2 BeadChip (Illumina, Inc., San Diego, CA, USA) was used to perform CMA. A data analysis was carried out using the BlueFuse Multi Software. WES was performed. An IDT xGen Exome Research Panel v2 was performed using the Nextseq 550 next-generation sequencing platform (Illumina) in accordance with the manufacturer's instructions for the WES.

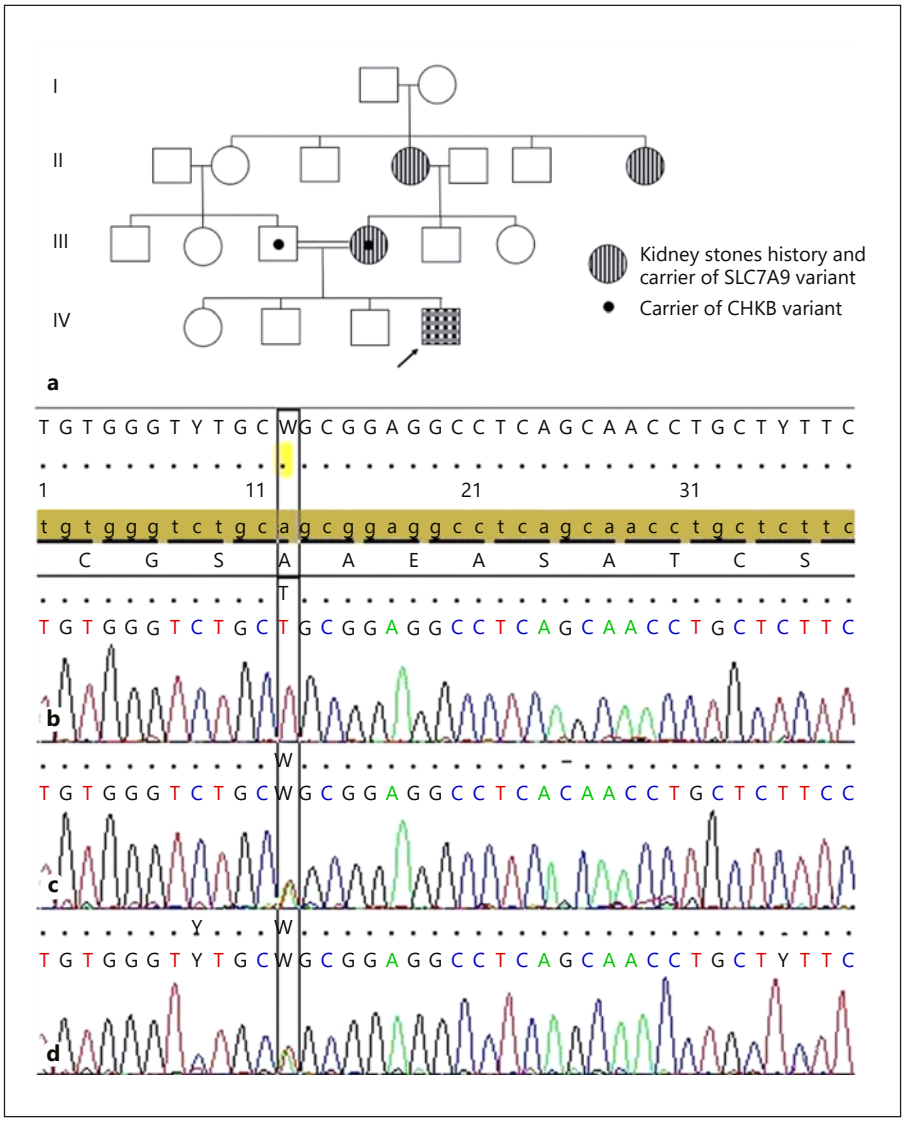

Fig. 1. a Pedigree of the family. **b–d** A novel homozygous NM_005198.5:c.225–2A>T variant was detected in the first intron of the *CHKB* gene in the patient (**b**), and heterozygous variations in the mother (**c**) and father (**d**) of the patient.

FASTQ files were analyzed on QCIAU1.6 and the annotation of VCF files was completed with Qiagen Ingenuity Variant Analysis and Clinical Insight Interpretation. A novel homozygous NM_005198.5:c.225-2A>T variant was detected in the first intron in *CHKB,* and a heterozygous NM_001243036.2:c.1266_1267delGT, p.(Leu424Glyfs*63) variant was detected in the 12th exon of the *SLC7A9* gene (Fig. 1b). The variations were confirmed with segregation analysis of the family (Fig. 1c, d). The variant was interpreted as "pathogenic" according to American College of Medical Genetics and Genomics (ACMG) guidelines. The clinical and laboratory findings of the patient were consistent with the coexistence of megaconial CMD and cystinuria. In the family investigation, heterozygous c.225-2A>T variants in the *CHKB* gene were detected in the patient's mother and father. A heterozygous c.1266_1267delGT variant in the *SLC7A9* gene was detected in the mother (Fig. 1a).

The patient's last physical examination at the age of 4 years revealed progressive hypotonia. His weight and height were 12 kg (SDS –3.01) and 93 cm (SDS –2.95), respectively. He has been followed up with a diagnosis of autism spectrum disorder for 2 years, and was undertaking an individualized education program. He did not meet the language developmental milestones for his age, and has been walking with support for 4 months. (He walks with broad steps, supporting the body by utilizing the strength of the hip flexors, the posterior structures of the knee joint and the ankle plantar flexors.)

Discussion

HCS is a quite rare disease (estimated incidence 1/1,000,000) with the main clinical findings of hypotonia-nutritional problems, developmental delay, growth hormone deficiency, dysmorphic facial findings (such as myopathic face, narrow bitemporal diameter, ptosis) and

Table 1. Clinical and laboratory findings in HCS, megaconial CMD, cystinuria, and in our patient

Clinical and laboratory findings	HCS	Megaconial CMD	Cystinuria	Our patient
Neonatal/infantile-onset hypotonia	+	+	–	+
Developmental delay	+	+	–	+
Elevated serum lactate concentrations	+	–	–	–
Neuromotor retardation	+	+	–	+
Progressive generalized muscle weakness	–	+	–	+
Cardiomyopathy	+/–	+/–	–	–
Neonatal seizures	+/–	+/–	–	–
Mildly elevated serum CK	–	+	–	+
Ichthyotic skin changes	–	+	–	–
Urinary excretion of dibasic amino acids	+	–	+	+

CK, creatine kinase; CMD, congenital muscular dystrophy; HCS, hypotonia-cystinuria syndrome.

cystine stones (cystinuria type A) [Eggermann et al., 2012a; Kilic et al., 2018]. In our case, clinical and laboratory findings were compatible with HCS, aside from the mildly elevated CK level.

HCS is caused by deletion of 2 contiguous genes (*SLC3A1* and *PREPL*) localized in chromosome 2p21. The clinical spectrum of the disorder depends on the size of the deletion. While deletions in *PREPL*, *SLC3A1*, and *C2orf34* genes or in *C2orf34* or *PREPL* genes present as atypical HCS, severe developmental delay, neonatal seizures, elevated serum lactate concentrations, and reduced activity of the respiratory chain complexes (I-III-IV-V) are observed in 2p21 deletion syndrome (up to 179 kb) which includes deletions in the *PPM1B*, *C2orf34*, *SLC3A1*, and *PREPL* genes [Chabrol et al., 2009]. Our patient had mild developmental delay and mild neuromotor retardation, while no seizures or elevated serum lactate levels were determined. Based on these findings, atypical HCS was suspected, however, no deletion was detected in the *SLC3A1*, *PREPL*, *C2orf34,* or *PPM1B* genes via CMA. Therefore, WES analysis was carried out which revealed a novel homozygous c.225-2A>T pathogenic variant in the *CHKB* gene and a heterozygous c.1266_1267delGT variant in the *SLC7A9* gene. The c.225-2A>T mutation in *CHKB* is located in the splice region and is not reported in the gnomAD (v2.1.1) and in-house databases, but it is listed as a "disease causing" mutation in several in silico databases such as Mutation Taster, DANN, Human Splicing Finder (HSF). The c.1266_1267delGT variant in the *SLC7A9* gene was previously reported [Font-Llitjos et al., 2005]. We detected the coexistence of megaconial CMD and cystinuria, compatible with the clinical and laboratory findings of our case.

Megaconial CMD is an autosomal recessive disorder, nearly 40 patients of which have been identified in the literature to date, with the majority being of Turkish origin [Haliloglu et al., 2015]. The most common manifestations are neonatal/infantile-onset hypotonia, progressive generalized muscle weakness, autistic behavior, intellectual disability, ichthyosis-like skin manifestations, and cardiomyopathy [Kutluk et al., 2020; Bardhan et al., 2021]. Most patients have mildly elevated serum CK levels [Kutluk et al., 2020], while rare manifestations include seizures, severe mental retardation, hearing loss, dysmorphic facial features (long facial appearance, upward-curved palpebral space, hypertelorism, flattening above the eye, deep sunken eyes, nasal bridge, low-set ears, wide and protruding lower lip, low hairline) and microcephaly [Haliloglu et al., 2015]. Our case had clinical signs of early-onset hypotonia, developmental delay, fatigue, and mildly elevated serum CK laboratory findings. In the clinical follow-up, progressive motor retardation was observed. All of the clinical and laboratory findings were related with megaconial CMD.

Cystinuria is a metabolic disease with an incidence that varies according to the population, with an estimated neonatal prevalence of 1/7,000 worldwide. Approximately 40% of patients diagnosed with cystinuria have a family history of stones. Due to cystine accumulation in the urinary tract, recurrent stone production can progress to final stage kidney disease over time [Taroni et al., 2019]. The diagnosis of cystinuria is made when cystine crystals and high levels of urinary cystine are detected, with different types of cystinuria identified with specific genetic testing [Eggermann et al., 2012b; Taroni et al., 2019]. Cystinuria is usually considered to be an autosomal recessive disorder related to variants in *SLC3A1* or *SLC7A9* genes.

Three phenotypes of cystinuria have traditionally been described on the basis of the urinary excretion of cystine and dibasic amino acids of the obligate heterozygous parent as type I, II, and III. Obligate heterozygous relatives (*SLC3A1* and *SLC7A9* carriers) of patients with type I cystinuria have normal aminoaciduria, while obligate heterozygous relatives (*SLC7A9* carriers) of individuals with type II and III cystinuria have high or moderate hyperexcretion of cystine and dibasic amino acids, respectively [Strologo et al., 2002; Chillarón et al., 2010]. Overall, some mutations in both genes can be inherited in an autosomal dominant mode. In our case, there was a kidney stone history in his mother. In the urinary analysis, high levels of cystine and dibasic amino acids were revealed. Furthermore, the diagnosis was confirmed with the presence of a heterozygous c.1266_1267delGT variant in the *SLC7A9* gene which has already been published [Font-Llitjos et al., 2005].

More than one rare genetic disease can occur in the same patient, and especially in countries where consanguineous marriage is common, multiple genetic diseases can be seen in patients, albeit rarely [Zlotogora, 2007]. With the growing access to WES testing and increased experience, the identification of coexisting rare diseases has become more frequent. Posey et al. [2017] reported that 2 or more molecular diagnoses are detected in 4.9% of patients with WES. Accordingly, an expanded genetic study is required if the findings of a disease do not exactly explain the clinical features of the considered disease in the patient [Kim et al., 2021]. Interestingly, in our case the clinical and laboratory findings of 2 rare diseases (megaconial CMD and cystinuria) could both be explained by HCS. Clinical and laboratory findings of HCS and megaconial CMD and the findings of our patient are presented in Table 1.

It was concluded in the present report that, if the clinical findings are compatible but no genetic mutation is detected, and/or if not all the findings of the patient match the disease considered in the pre-diagnosis, different genetic diseases may coexist, especially in countries where consanguineous marriages are common. In such situations, WES can be considered an effective diagnostic approach.

Acknowledgement

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Statement of Ethics

This study was exempt from ethical approval procedures, being a case report of a single patient whose parents provided verbal consent to participate in the study and gave written consent to have the case published. Informed consent for genetic analysis and publication of clinical reports and photographs were obtained from the patient's parents in compliance with the national ethics regulation. There is no name or number indicating the patient's identity.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All authors contributed equally to this study. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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