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Short Communication

An asymptomatic father diagnosed with 3-methylcrotonyl-CoA carboxylase deficiency following his son newborn screening test

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ABSTRACT

3-methylcrotonyl-CoA carboxylase deficiency (3MCCD) is a hereditary disorder of leucine catabolism caused by pathogenetic variants in the *MCCC1* or *MCCC2* genes. Typically diagnosed through newborn screening (NBS), 3MCCD is characterized by elevation of 3-hydroxyisovalerylcarnitine (C5OH) in blood as well as increased excretion of 3-methylcrotonylglycine (3-MCG) in urine. While most diagnosed children remain asymptomatic, data on adults are scarce. To date, only 39 molecularly confirmed adult individuals have been reported, all being mothers diagnosed subsequent to their child NBS results. Herein, we present a 36-year-old asymptomatic man who was incidentally diagnosed with 3MCCD following his son NBS recall. Molecular analysis revealed compound heterozygosity for two pathogenic variants in the *MCCC1* gene. This is the first molecularly confirmed adult man with 3MCCD reported. This case highlights the need for additional longitudinal follow-up data on individuals with 3MCCD to clarify the clinical significance of this condition and guide clinical practice, including NBS strategy.

1. Introduction

3-methylcrotonyl-carboxylase (3MCC) deficiency (3MCCD) is an inherited disorder of leucine catabolism caused by pathogenetic variants in the *MCCC1* (#MIM210200) or *MCCC2* (#MIM210210) genes encoding the alpha and beta subunit of the 3MCC, respectively [1]. 3MCCD is characterized by elevation of 3-hydroxyisovalerylcarnitine (C5OH) in blood and as well as increased excretion of 3-methylcrotonylglycine (3-MCG) in urine [2,3].

C5OH elevation being easily detected in dried blood spot (DBS) by tandem mass spectrometry (MS/MS), 3MCCD is included in several newborn screening (NBS) programs worldwide [4]. Over the past years, 3MCCD has risen as one of the most common inherited metabolic diseases (IMDs) diagnosed by NBS with a prevalence ranging from 1:2400 to 1:68000 [2]. This frequency appears much higher than the one predicted in the pre-NBS era [1,6,7].

>90% of individuals diagnosed with 3MCCD by NBS appear to remain asymptomatic [8]. Currently, the majority of reported individuals with 3MCCD are newborn and infants. Besides affected children, healthy mothers have also been diagnosed with 3MCCD after their baby NBS recall [9–11]. To date, only 39 molecularly confirmed adult individuals have been reported all being mothers diagnosed subsequent to their child NBS results. Three additional individuals (two males and one female) were identified, for whom molecular diagnosis was not available [1,12].

Hence, additional information on long-surviving patients is warranted. We herein report on an asymptomatic 36-year-old male who was molecularly diagnosed with 3MCCD following his son NBS recall.

2. Case report

A 14-day-old boy was referred to our Metabolic Clinic due to C5OH

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elevation on NBS DBS (0.51 μmol/l, reference range 0.03–0.40). The newborn was the first baby of his parents and was born at a gestational age of 39 weeks by vaginal delivery with a birth weight of 3.230 kg (0 SDS). Uneventful perinatal events and immediate postnatal course were recorded. There was no specific medical history in his family. His physical exam was unremarkable. Oral supplementation with L-Carnitine (100 mg/kg/day) and biotin (10 mg/day) was started. Complete blood count, electrolytes, renal and liver function tests, blood gases, ammonia, biotinidase activity, serum amino acid and acylcarnitine profiles were within range. Urine organic acid profile revealed detectable 3-MCG. Hence, biotin supplementation was discontinued and molecular testing for IMDs associated with increased C5OH levels was requested. The heterozygous variant c.2079del (p.Val694Ter), known as pathogenetic, was identified in the *MCCC1* gene. In order to dissect the clinical relevance of such variant, molecular testing of *MCCC1* was requested in his parents, by searching variants in all *MCCC1* exons and flanking regions. No variants were detected in the mother. Surprisingly two variants in the *MCCC1* gene were found in the infant's father: c.2079del (p.Val694Ter), shared with his son and c.715 T > C (p.Phe239Leu) which is herein reported for the first time and is predicted to be likely pathogenetic, according to ACMG criteria (Fig. 1). Biochemical testing was subsequently ordered for the infant's father, revealing C5OH elevation in blood as well as detectable 3-MCG in urine (Table 1). Hence, the man was diagnosed with 3MCCD and was included in a follow-up program in our hospital.

Retrospective collection of the man's clinical history revealed no acute illness during infancy or adulthood. No signs or symptoms indicating possible metabolic decompensation were recorded. He displayed regular cognitive and motor development and was enrolled in an undergraduate Program in Business and Management. He regularly performed physical activity including anaerobic exercise. He developed Obsessive-compulsive disorder (OCD) at 32 years of age requiring treatment with fluvoxamine. At the time of the clinical evaluation, he was 36-year-old. No abnormal findings were detected at physical examination. Weight was 80.5 Kg (+0.8 SD), height was 169.5 cm (−1 SD), BMI was 28 (+1.4 SD) and head circumference was 57 cm (+1.3 SD). The opportunity to start L-carnitine supplementation was discussed with the patient who was considering this treatment option.

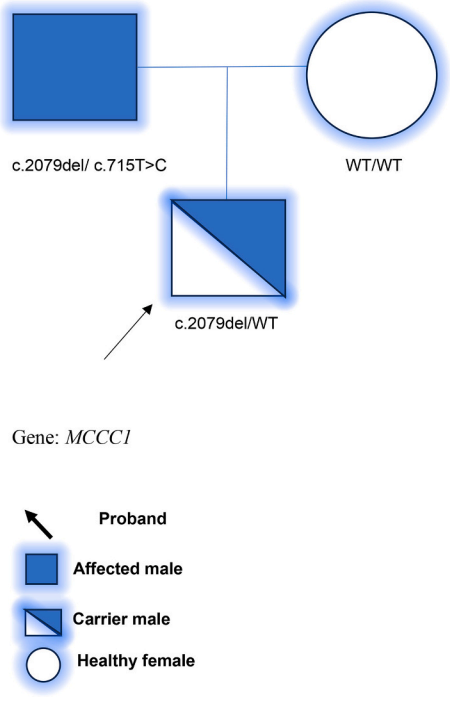

Fig. 1. Pedigree chart. For all individuals *MCCC1* genotype is shown. Proband is highlighted. WT: wild type.

Table 1
Serum acylcarnitine and urine organic acids profiles in the case study.

Serum acylcarnitine	μmol/l	Reference range
C0	36.1	(10–44.7)
C2	7.2	(3.5–15.4)
C3	0.45	(0.07–0.65)
C4	0.28	(0.12–0.42)
C5:1	0.04	(0.03–0.12)
C5	0.14	(0.05–0.24)
C6	0.09	(0.04–0.18)
C5OH	0.19	(0.01–0.13)
C8	0.15	(0.07–0.25)
C3OC	0.08	(0.04–0.15)
C10:1	0.14	(0.08–0.32)
C10	0.18	(0.09–0.43)
C4DC	0.07	(0.04–0.15)
C5DC	0.09	(0.03–0.17)
C12:1	0.05	(0.04–020)
C12	0.05	(0.04–0.21)
C6DC	0.05	(0.02–0.11)
C14:2	0.05	(0.02–0.16)
C14:1	0.07	(0.02–0.20)
C14	0.07	(0.03–0.15)
C16:1	0.04	(0.01–0.07)
C16	0.13	(0.01–0.23)
C16OH	0.02	(0.01–0.05)
C18:1	0.16	(0.02–0.34)
C18	0.05	(0.01–0.18)
C18:1OH	0.04	(0.01–0.07)
C4OH	0.04	(0.01–0.16)

Urine organic acid	mmol/mol Crea	Reference Range
Lactic acid	15	1–25
beta-hydroxybutyric acid	3	0–2
Glycolic acid	13	18–55
Glyoxylic acid	6	n.a.
3-hydroxypropionic acid	2	0–20
Piruvic acid	16	2.6–7.9
3-hydroxyisobutyric acid	6	4.1–19
3-hydroxyisovaleric acid	7	6.9–25
succinic acid	5	0.5–16
3-methylglutaconic acid	2	0–9
pyroglutamic acid	5	0–12
3-methylcrotonylglycine	1	<1
alpha-ketoglutaric acid	9	4–74
p-hydroxyphenylacetic acid	12	6–28
cis-aconitic acid	7	2.7–44
Ippuric acid	174	170–390
Citric acid	7	70–226
Palmitic acid	8	6–23
Stearic acid	3	1.6–6.6

C0:Free carnitine; C2: Acetylcarnitine; C3: Propionylcarnitine; C3DC: Malonylcarnitine; C4: Butyrylcarnitine; C4DC: Methylmalonylcarnitine; C5: Isovalerylcarnitine; C5DC: Glutarylcarnitine; C5:1: Tiglylcarnitine; C5OH: 3OH-Isovalerylcarnitine; C6: Hexanoylcarnitine; C6DC: Adipylcarnitine; C8: Octanoylcarnitine; C8:1: Octeneylcarnitine; C10: Decanoylcarnitine; C10:1: Decenoylcarnitine; C12: Dodecanoylcarnitine; C12:1: Dodecenoylcarnitine; C14: Miristoylcarnitine; C14:1: Tetradecenoylcarnitine; C14:2: Tetradecadienoylcarnitine; C16: Palmitoylcarnitine; C16:1: Esadecenoylcarnitine: C16OH: 3OH-Esadecanoylcarnitine; C18: Stearoylcarnitine: C18:1: Oleylcarnitine; C18:1OH: 3OH-Oleycarnitine. n.a.: not available.

3. Discussion

3MCCD is an IMD of leucine catabolism resulting in C5OH elevation in blood as well as increased excretion of 3-MCG in urine [2,3]. The majority of individuals reported with 3MCCD have been shown not to develop any clinical symptoms [1]. However, available information mostly refers to infants and children, warranting additional long-term follow-up data. To the best of our knowledge this is the first report on

an adult male individual with molecularly confirmed 3MCCD. This case also represents the oldest man with 3MCCD reported. The patient herein presented carried two variants in the *MCCC1* gene, of which the c.715 T > C (p.Phe239Leu) is herein reported for the first time. Despite being diagnosed with 3MCCD at 36 years of age, the patient was asymptomatic, and his medical history was unremarkable.

With the introduction of NBS using MS/MS in the 1990s, the detection rate of IMDs soon after birth has dramatically increased [5]. Alongside presymptomatic diagnosis of infants who would have developed metabolic decompensation, NBS has allowed the identification of individuals with milder phenotypes. This observation is particularly relevant to 3MCCD, which has become one of the most common IMDs diagnosed through NBS [2]. However, a number of unwanted false positive cases and/or heterozygous carriers are still identified warrating the implementation of expanded second-tier test panels [13]. In the pre-NBS time, 3MCCD was identified in children referred for various symptoms, including developmental delay, failure to thrive, hypotonia, seizures, cardiomyopathy, or metabolic disturbances such as hypoglycemia, hyperammonemia, ketoacidosis, or Reye syndrome [1,5–7,12]. Conversely, most children diagnosed with 3MCCD through NBS, appear to remain asymptomatic [8]. This finding supports a growing body of evidence arguing against the clinical relevance of this condition [14]. Nevertheless, adult individuals displaying muscular symptoms have also been reported [9,10]. In the largest cohort of individuals with 3-MCCD described [1], 69% of the 36 subjects identified by NBS have stayed completely asymptomatic until at least 3 years of age, while the remainder (31% =11) developed clinical symptoms including neurologic and muscle symptoms. At least one acute metabolic decompensation was recorded in 5 children.

Among reported adult individuals, 39 mothers have also been identified with 3MCCD following their infant NBS recall [1,5,9–11,14,15]. Among them, 34 mothers were asymptomatic while 5 exhibited various clinical symptoms such as fatigue, weakness, myopathy, increased liver enzymes, and hepatosteatosis. One mother had complaints of muscle weakness during childhood and her complaints improved upon L-carnitine supplementation [14]. Additionally, two asymptomatic male patients with 3MCCD were reported for whom molecular diagnosis was not available [1,12]. Collectively, these observations indicate that a subgroup of individuals with 3MCCD may still develop symptoms despite NBS. The mechanism explaining why only some individuals with 3MCCD develop symptoms remains unresolved. The study by Grunert et al. failed to identify a correlation between the biochemical markers (e.g. circulating C5OH levels) and the clinical phenotype [1]. Developing novel biomarkers able to predict the disease course is worthy.

Various treatment approaches have been attempted in 3MCCD, including (combination of) oral L-carnitine supplementation [1], protein-restricted or leucine-restricted diet [1], oral biotin supplementation [1] and oral glycine supplementation [1]. For many of these approaches, efficacy remains insufficiently proven. A Delphi-based consensus clinical practice panel has previously generated treatment recommendations [16]. In the management of acute illness the following actions are recommended: 1) prevention of fasting stress by providing patients with emergency letters, similar to IMDs associated with fasting intolerance [17], and 2) maintenance of adequate caloric intake and intravenous glucose administration. L-carnitine supplementation is only recommended in symptomatic individuals and pregnant women with 3MCCD [16]. Conversely, glycine supplementation and leucine-restricted diets have not demonstrated clinical efficacy and are not recommended in asymptomatic patients. Based on rarely reported efficacy in 3MCCD, a trial with biotin may be considered in symptomatic individuals [16].

In conclusion, we presented a 36-year-old man who was incidentally diagnosed with 3MCCD following his son's NBS results and remained asymptomatic. This is the first report on an adult male individual with molecularly confirmed 3MCCD pointing in favor of the limited clinical relevance of 3MCCD in most persons. Indeed, there is an ongoing debate about whether 3MCCD should be included in NBS programs [14]. This debate is mainly based on the observation that most children diagnosed with 3MCCD through NBS remain asymptomatic [8]. At least in theory even asymptomatic individuals may still develop symptoms under catabolic conditions. In this respect, NBS may allow (in)direct identification of these individuals who can be provided with appropriate instructions to prevent catabolism. Additional follow-up data on adult individuals with 3MCCD are necessary to clarify the clinical relevance of this condition, and possibly elucidate any genotype-phenotype correlation. Such data are expected to enable a critical revision of NBS strategy for this condition in the foreseeable future.

CRediT authorship contribution statement

Rosamaria Terracciano: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Margherita Ruoppolo:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Ferdinando Barretta:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Lucia Albano:** Writing – original draft, Methodology, Formal analysis, Data curation. **Daniela Crisci:** Writing – original draft, Methodology, Investigation, Data curation. **Giovanna Gallo:** Writing – original draft, Methodology, Investigation, Data curation. **Fabiana Uomo:** Writing – original draft, Methodology, Investigation, Formal analysis. **Pietro Strisciuglio:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Giancarlo Parenti:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Giulia Frisso:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Alessandro Rossi:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization, Data curation.

Declaration of competing interest

The authors declare that there is no potential conflict of interest related to this work.

Data availability

Data will be made available on request.

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