International Journal of
Neonatal Screening

MDPI

Case Report

A Rare Case of Malonic Aciduria Diagnosed by Newborn Screening in Qatar

Mamatha Ramaswamy [1,*], Victor Anthony Skrinska [1], Ghassan Abdoh [2], Laila Mahmoud Ahmed [3], Rola Fayez Mitri [1] and Ravi Joshi [1]

1 Metabolic Laboratory, Department of Pathology and Laboratory Medicine, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar; vaskrinska@gmail.com (V.A.S.); RMitri@hamad.qa (R.F.M.); RJoshi@hamad.qa (R.J.)
2 Newborn Screening Unit, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar; Gabdoh@hamad.qa
3 Department of Paediatrics, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar; LMahmoud4@hamad.qa
* Correspondence: mamadeep@gmail.com; Tel.: +974-4439-7780

Academic Editor: Harvey L. Levy
Received: 30 December 2016; Accepted: 3 March 2017; Published: 9 March 2017

Abstract: Malonic aciduria is a rare autosomal recessive organic acid disorder. With the widespread use of tandem mass spectrometry for analysis of the amino acid/acylcarnitine profile on dried blood spots for newborn screening (NBS), this condition can be readily diagnosed and can be included in the organic acid screen in NBS programs. In Qatar, we report the first case of an asymptomatic baby screened and diagnosed with malonic aciduria through NBS. This patient has a genetic variant of malonyl-CoA decarboxylase that has not been previously reported in the literature. This condition should be differentiated from a similar disorder, combined malonic and methylmalonic aciduria. The clinical phenotype of malonic aciduria is variable and the pathophysiology is not fully understood. There is no established guidance or recommendations regarding the appropriate treatment regimen, dietary therapy or regular follow-up of these patients. Most available evidence for treatment is based on a single study or case report.

Keywords: malonic aciduria; malonyl-CoA decarboxylase; *MLYCD*; newborn screening; malonic acid; methylmalonic acid; MCD; malonylcarnitine; C3DC

1. Introduction

Malonic aciduria is caused by malonyl-CoA decarboxylase (MCD) deficiency. It is a rare autosomal recessive disorder and until now fewer than 30 cases have been reported in the literature [1]. With the widespread use of tandem mass spectrometry methods for amino acid/acylcarnitine (AA/AC) screening on dried blood spots (DBS) by many newborn screening laboratories around the world, we are likely to be faced with the challenges of diagnosing such rare inborn errors of metabolism in our NBS programs. Access to confirmatory tests for plasma acylcarnitines, urine organic acids and molecular genetics is the key in the differentiation and confirmation of these disorders.

Malonyl-CoA decarboxylase is expressed by the *MLYCD* gene on chromosome 16 and catalyzes the decarboxylation of malonyl-CoA to acetyl-CoA. MCD activity has been described in a wide array of organisms, including prokaryotes, birds, and mammals [2–4]. In mammals, cytoplasmic malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase (CPT1) and, thus, of mitochondrial fatty acid oxidation [5]. The balance of malonyl-CoA synthesis by acetyl-CoA carboxylase and degradation by MCD is likely to have an important regulatory role in fatty acid metabolism. Increased malonyl-CoA levels potently inhibit long-chain acylcarnitine acyltransferases [5] and thus decrease the β-oxidation of fatty acids in both mitochondria and peroxisomes. The *MLYCD* gene has a tissue-specific expression

pattern [6,7] with the highest message levels in the heart muscle, then the skeletal muscle, followed by the brain, small intestine, liver, kidney and pancreas. The characteristic phenotype is variable, but may include developmental delays in early childhood, seizures, hypotonia, diarrhea, vomiting, metabolic acidosis, hypoglycemia, ketosis, abnormal urinary compounds, lactic acidemia, and hypertrophic cardiomyopathy [8].

2. Diagnosis and Clinical Presentation

Most cases of MCD deficiency reported in the literature were diagnosed after clinical presentation. Here we report a case diagnosed upon newborn screening. The DBS for newborn screening in Qatar is collected at 36 hours after birth. The AA/AC profile by tandem mass spectrometry as butyl esters showed raised malonylcarnitine (C3DC). The related ratios C3DC/C10 and C5DC/C3DC were also abnormal. Injection analysis of butyl esters by tandem mass spectrometry cannot differentiate between C3DC and C8OH. Consequently, the C3DC result may include a contribution from C8OH which can be increased in some medium-chain metabolic disorders. Additional confirmatory tests are essential for diagnosis. We performed second-tier testing on DBS by liquid chromatography/tandem mass spectrometry for methylmalonic acid which was mildly elevated. The results for this patient and the reference ranges are displayed in Table 1. The AA/AC results for this patient were uploaded into the Region 4 Genetics Collaborative (R4GC) database for comparison with previously reported MCD deficiency data. The graph in Figure 1 clearly displays the primary marker (C3DC) and secondary markers (ratios) in the abnormal range and is highly suggestive of a diagnosis of malonic aciduria.

Table 1. Patient results of newborn screening and confirmatory tests.

Analyte	Result	Reference Range
Amino acid/Acylcarnitine profile on DBS by tandem mass spectrometry		
C3DC (malonylcarnitine)	1.99	<0.25 µmol/L
C3DC/C10	23.09	<3.00
C5DC/C3DC	0.057	0.3–6.00
Second tier test on DBS by liquid chromatography/tandem mass spectrometry		
Methylmalonic acid	9.30	<5.00 µmol/L
Urine organic acid analysis *		
Malonic acid	1036	<14 mmol/molKrea
Methylmalonic acid	59	<18 mmol/molKrea

* Performed by University Clinics Heidelberg.

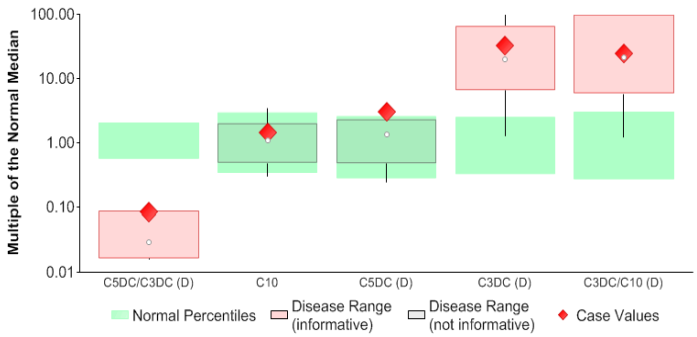

Figure 1. Cumulative normal percentile and MA* (Malonic Aciduria) disease range overlap plot.

The baby was asymptomatic at birth and discharged from the hospital as usual. Based on the NBS results raising the suspicion of an organic acid disorder, the child was recalled into the hospital for assessment and further investigations. Apart from physiological jaundice, the baby had no clinical features of organic acidemia such as hypoglycemia or metabolic acidosis. Apart from the mildly raised total bilirubin, the blood results for glucose, kidney function tests, electrolytes, liver function tests, lactate, ketone bodies and blood gases were entirely within the normal limits. Samples for the confirmatory tests were collected and sent to the University Clinics Heidelberg for the analysis. The plasma acylcarnitines analysis confirmed the raised malonylcarnitine. Urine organic acids showed markedly elevated malonic acid with a mild increase in methylmalonic acid. Molecular testing was performed by Centogene and *MLYCD* gene analysis showed homozygosity for the *MLYCD* mutation c.1213dup (p.Tyr405Leufs*74). This duplication creates a frame shift starting at codon Tyr405. The new reading frame ends at a stop codon 73 position downstream. This variant is reported in the Exome Aggregation Consortium with a frequency of 0.000025, i.e., in three among 120,544 alleles (ExAC database). This is the first time this genetic variant has been detected and it is so far not listed in CentroMD 3.0. It is classified as likely pathogenic (class 2) according to the recommendations of Centogene and American College of Medical Genetics (ACMG). No large deletions or duplications within or including the *MLYCD* gene were detected by MLPA analysis. Pathogenic variants in the *MLYCD* gene are causative for malonyl-CoA decarboxylase deficiency. The pathogenic alleles identified and reported so far in all the patients of malonyl-CoA decarboxylase deficiency are documented in the Human MLYCD allelic variant database [9].

The diagnosis was made in a full-term baby with a normal birth weight born to a 21-year-old Pakistani mother. The parents of the child had consanguineous marriage. The child is nine months old now with regular follow-ups in the metabolic clinic. He has normal growth and developmental milestones. He is receiving L-Carnitine supplements 340 mg orally three times a day. The child is under the care of the dietitian who has started him on a low long-chain triglyceride (LCT) restricted formula three times a day. The cardiologists are regularly following up the child for hypertrophic cardiomyopathy. His first echocardiogram performed at nine months of age showed moderate left ventricular dilatation (fractional shortening 23% and left ventricular end diastolic diameter 35 mm). The child will have a follow-up echocardiogram after a month and if the left ventricular dilatation persists he will be initiated on an angiotensin-converting enzyme inhibitor.

3. Discussion

Malonyl-CoA decarboxylase deficiency is a rare organic acid disorder. NBS laboratories can detect rare inborn errors of metabolism by including the related primary and secondary markers in the AA/AC screen on DBS. In patients with malonic aciduria, the C3DC is clearly elevated on the AA/AC screen and abnormal levels of the related markers C3DC/C10 and C5DC/C3DC also compliment the suspected diagnosis. For the NBS laboratories participating in the Region 4 Genetics Collaborative (R4GC) or the Mayo Clinic Collaborative Laboratory Integrated Reports (CLIR), the AA/AC profile results can be uploaded and compared to normal populations and previously reported abnormal results for specific metabolic disorders to further support the suspicion of a specific metabolic disorder. In addition, the analysis of a successive DBS helps to confirm the findings of the initial DBS. Confirmatory tests such as plasma acylcarnitines, urine organic acids, and, if possible, molecular testing are essential to confirm the diagnosis and differentiate the disorder from other related disorders or possible interferences (in this case C8OH).

While reporting malonic aciduria, one should consider differential diagnosis of combined malonic and methylmalonic aciduria (CMAMMA). In the latter condition, methylmalonic acid is also markedly elevated in addition to the malonic acid. Urine organic acid analysis shows much larger amounts of methylmalonic acid than malonic acid [10]. Confirmation of this condition is done by molecular testing showing mutation in the ACSF3 gene on chromosome 16q24, which encodes for the malonyl-CoA synthetase enzyme. This enzyme converts methylmalonic acid to

methylmalonyl- CoA. The condition is inherited as autosomal recessive and clinically may present with hypotonia, developmental delay, failure to thrive, hypoglycemia and coma. Some affected children have microcephaly. Other people with CMAMMA do not develop signs and symptoms until adulthood and these individuals usually have neurological problems, such as seizures, loss of memory, a decline in thinking ability, or psychiatric diseases.

Malonyl-CoA decarboxylase breaks down malonyl-CoA (a fatty acid precursor and a fatty acid oxidation blocker) into acetyl-CoA and carbon dioxide. The enzyme deficiency leads to high concentrations of malonyl-CoA in the cytoplasm and this will inhibit β-oxidation of fatty acids through deactivating the carrier of the fatty acyl group CPT1, and thus blocking fatty acids from going into the mitochondrial matrix for oxidation [5]. Without the enzymatic activity of MCD, cellular malonyl-CoA increases so dramatically that at the end it is instead broken down by an unspecific short-chain acyl-CoA hydrolase, which produces malonic acid and CoA. Malonic acid is a Krebs cycle inhibitor, preventing the cells from making ATP through oxidation. In this condition, the cells, to make ATP, are forced to increase glycolysis, which produces lactic acid as a by-product. The increase of lactic and malonic acid causes metabolic acidosis. L-Carnitine supplementation has been shown to stimulate CPT1 in the liver of the aged rats [11]. Wightman et al. [12] in their study found that their fourth patient, who was six days old, clinically presented with metabolic decompensation. He was placed on L-Carnitine supplements along with a low protein and low fat diet, and has been well since that time with normal growth and development and no subsequent episodes of metabolic decompensation. Gordon B et al. [13] demonstrated in their two case reports that the levels of malonic acid in the urine dramatically reduced after L-Carnitine load (100 mg/Kg body weight). Based on these studies, we speculate that L-Carnitine supplementation is a key component in the treatment of malonic aciduria patients, to stimulate beta-oxidation of the fatty acids through CPT1 and reduce the levels of malonyl-CoA and thus prevent episodes of metabolic decompensation such as metabolic acidosis and hypoglycemia. Our patient diagnosed upon newborn screening, although asymptomatic, is on L-Carnitine supplements. He has normal growth and developmental milestones, and has not developed any episodes of metabolic decompensation to date.

Currently, there are no established dietary recommendations for MCD deficiency or related disorders (CMAMMA). MacPhee et al. [13] described two patients with MCD deficiency and agreed with Brown et al. [14] and Haan et al. [15] that a low fat, high carbohydrate diet would lead to near normalization of the urinary organic acid excretion with no further hypoglycemia episodes. Hospital admission was recommended during periods of infection as well as during febrile illness as the harmful effects of the disorder became apparent. Hypertrophic cardiomyopathy has been reported in up to 40% of patients with MCD deficiency [16,17]. Fitzpatrick et al. [18] found two of their seven patients with chronic malonic aciduria developed cardiomyopathy, in contrast to the other patients, whose urine malonic acid is elevated only during acute illness. So they concluded that cardiomyopathy may occur only after prolonged exposure to high levels of malonic acid. These patients should be regularly screened for cardiomyopathy even when they are asymptomatic in order to ensure early therapy [19]. Footitt et al. [20] described a baby with MA and cardiomyopathy showing improvement in cardiac function attributable to a long chain triglyceride (LCT) restricted/medium chain triglyceride (MCT) supplemented diet (1.9% energy from LCT, 25% from MCT and the remainder carbohydrate) along with an angiotensin-converting enzyme inhibitor (ACE) therapy. Moderate Left ventricular dilatation was detected by the first echocardiogram in our patient at nine months of age. Based on these findings, he is being initiated on a LCT-restricted diet for now. He will be started on an ACE inhibitor if necessary after a month's review.

4. Conclusions

Malonic aciduria can be readily diagnosed by NBS laboratories with tandem mass spectrometry analysis of amino acids/acylcarnitines on dried blood spots. In this case report, a new allelic variant of malonyl-CoA decarboxylase deficiency is identified which has not been reported in the literature

before. Early diagnosis and initiation of treatment help reduce parental anxiety and may help reduce the risk of complications. Based on the available evidence, L-Carnitine supplementation and LCT-restricted/MCT-supplemented diet help promote the normal growth and development of the child, prevent episodes of metabolic decompensation and reduce the risk of hypertrophic cardiomyopathy in the long term.

Acknowledgments: We would like to thank Professor Georg Hoffmann and his team, University Clinics Heidelberg for the analysis of plasma acylcarnitines and urine organic acids, and also Centogene for performing the genetic analysis.

Author Contributions: Mamatha Ramaswamy and Victor Skrinska were involved in the interpretation of the results and reporting of this case, review of the literature and write up of the article. Ghassan Abdoh and Laila Mahmoud are clinicians involved in the patient management. Rola Mitri and Ravi Joshi were involved in sample analysis. All authors have contributed to the final revision of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

NBS	newborn screening
MA	malonic aciduria
DBS	dried blood spot
MCD	malonyl-CoA decarboxylase
CoA	coenzyme A
CPT1	carnitine palmitoyl transferase 1
AA/AC	amino acid/acylcarnitine
C3DC	malonylcarnitine
LCT	long chain triglycerides
MCT	medium chain triglycerides
CMAMMA	combined malonic and methylmalonic aciduria
R4GC	Region 4 Genetics Collaborative
CLIR	Collaborative Laboratory Integrated Reports
ACMG	American College of Medical Genetics
MLPA	multiplex ligation dependant probe amplification
CentoMD	Centogene Comprehensive Mutation Database

References

1. Genetics Home Reference. Available online: https://ghr.nlm.nih.gov/condition/malonyl-coa-decarboxylase-deficiency (accessed on 16 December 2016).
2. Kim, Y.S.; Kolattukudy, P.E. Purification and properties of malonyl-CoA decarboxylase from rat liver mitochondria and its immunological comparison with the enzymes from rat brain, heart, and mammary gland. *Arch. Biochem. Biophys.* **1978**, *190*, 234–246. [CrossRef]
3. Kim, Y.S.; Kolattukudy, P.E. Malonyl-CoA decarboxylase from the uropygial gland of waterfowl: Purification, properties, immunological comparison, and role in regulating the synthesis of multimethyl-branched fatty acids. *Arch. Biochem. Biophys.* **1978**, *190*, 585–597. [CrossRef]
4. An, J.H.; Kim, Y.S. A gene cluster encoding malonyl-CoA decarboxylase (MatA), malonyl-CoA synthetase (MatB) and a putative dicarboxylate carrier protein (MatC) in *Rhizobium trifolii* clining, sequencing, and expression of the enzymes in *Escherichia coli*. *Eur. J. Biochem.* **1998**, *257*, 395–402. [CrossRef] [PubMed]
5. McGarry, J.D.; Brown, N.F. The mitochondrial carnitine plamitoyltransferase system. From concept to molecular analysis. *Eur. J. Biochem.* **1997**, *244*, 1–14. [CrossRef] [PubMed]
6. Voilley, N.; Roduit, R.; Vicaretti, R.; Bonny, C.; Waeber, G.; Dyck, J.R.; Lopaschuk, G.D.; Prenti, M. Cloning and exprsession of rat pancreatic beta-cell malonyl-CoA decarboxylase. *Biochem. J.* **1999**, *340*, 213–217. [CrossRef] [PubMed]
7. Sacksteder, K.A.; Morell, J.C.; Wanders, R.J.; Matalon, R.; Gould, S.J. MCD encodes peroxisomal and cytoplasmic forms of malonyl-CoA decarboxylase and is mutated in malonyl-CoA decarboxylase deficiency. *J. Biol. Chem.* **1999**, *274*, 24461–24468. [CrossRef] [PubMed]

8. Sweetman, L.; Williams, J.C. Branched chain organic acidurias. In *The Metabolic and Molecular Basis of Inherited Disease*, 8th ed.; Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D., Eds.; McGraw-Hill: New York, NY, USA, 2001; Volume II, pp. 2155–2157.
9. Human MLYCD Allelic Variant Database. Available online: http://mlycd.hgu.mrc.ac.uk (accessed on 7 February 2017).
10. Gregg, A.R.; Warman, A.W.; Thirburn, D.R.; OBrien, W.E. Combined malonic and methylmalonic aciduria with normal malonyl-CoA decarboxylase activity; a case supporting multiple aetiologies. *J. Inherit. Metab. Dis.* **1998**, *21*, 382–390. [CrossRef] [PubMed]
11. Karlic, H.; Lohninger, S.; Koeck, T.; Lohninger, A. Dietary L-carnitine stimulates carnitine acyltransferases in the liver of aged rats. *J. Histochem. Cytochem.* **2002**, *50*, 205–212. [CrossRef] [PubMed]
12. Wightman, P.J.; Santer, R.; Ribes, A.; Dougherty, F.; McGill, N.; Thorburn, D.R.; Fitzpatrick, D.R. MLYCD mutation analysis: Evidence for protein mistargeting as a cause of MLYCD deficiency. *Hum. Mutat.* **2003**, *22*, 288–300. [CrossRef] [PubMed]
13. MacPhee, G.B.; Logan, R.W.; Mitchell, J.S.; Howells, D.W.; Tsotsis, E.; Thornburn, D.R. Malonyl coenzyme A decarboxylase deficiency. *Arch. Dis. Child.* **1993**, *69*, 433–436. [CrossRef] [PubMed]
14. Brown, G.K.; Scholem, R.D.; Bankier, A.; Danks, D.M. Malonyl coenzyme A decarboxylase deficiency. *J. Inherit. Metab. Dis.* **1984**, *7*, 21–26. [CrossRef] [PubMed]
15. Haan, E.A.; Scholem, R.D.; Croll, H.B.; Brown, G.K. Malonyl coenzyme A decarboxylase deficiency: Clinical and biochemical findings in a second child with a more severe enzyme defect. *Eur. J. Pediatr.* **1986**, *144*, 567–570. [CrossRef] [PubMed]
16. Salomons, G.S.; Jakobs, C.; Pope, L.L.; Errami, A.; Potter, M.; Nowaczyk, M.; Olpin, S.; Manning, N.; Raiman, J.A.; Slade, T.; et al. Clinical, enzymatic and molecular characterization of nine new patients with malonyl-coenzyme A decarboxylase deficiency. *J. Inherit. Metab. Dis.* **2007**, *30*, 23–28. [CrossRef] [PubMed]
17. Yano, S.; Sweetman, L.; Thorburn, D.R.; Mofidi, S.; Williams, J.C. A new case of malonyl coenzyme A decarboxylase deficiency presenting with cardiomyopathy. *Eur. J. Pediatr.* **1997**, *156*, 382–383. [CrossRef] [PubMed]
18. Fitzpatrick, D.R.; Hill, A.; Tolmie, J.L.; Thorburn, D.R.; Christodoulou, J. The molecular basis of malonyl-CoA decarboxylase deficiency. *Am. J. Hum. Genet.* **1999**, *65*, 318–326. [CrossRef] [PubMed]
19. Baertling, F.; Mayatepek, E.; Thimm, E.; Schlune, A.; Kovacevic, A.; Distelmaier, F.; Salomons, G.S.; Meissner, T. Malonic aciduria: Long-term follow-up of new patients detected by newborn screening. *Eur. J. Pediatr.* **2014**, *173*, 1719–1722. [CrossRef] [PubMed]
20. Footitt, E.J.; Stafford, J.; Dixon, M.; Burch, M.; Jakobs, C.; Salomons, G.S.; Cleary, M.A. Use of a long-chain triglyceride-restricted/medium-chain triglyceride-supplemented diet in a case of malonyl-CoA decarboxylase deficiency with cardiomyopathy. *J. Inherit. Metab. Dis.* **2010**, *33*, 253–256. [CrossRef] [PubMed]