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Regular Article

Clinical, biochemical and molecular characteristics of malonyl-CoA decarboxylase deficiency and long-term follow-up of nine patients

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1. Introduction

Malonyl-CoA decarboxylase (E.C.4.1.1.9) deficiency (MLYCDD, OMIM 248360) is a rare autosomal recessive inborn error of metabolism MLYCD is encoded by a *MLYCD* gene located on chromosome 16q23.3 and consists of 5 exons [1,2]. The expression of the protein is highest in the cardiac muscle, followed by the skeletal muscle, brain, small intestine, liver, pancreas and kidney [1–3]. The substrate for MLYCD is malonyl-CoA, which has been recognized as a key player in fatty acid synthesis and oxidation in relation to its dual function: 1) as an intermediate in fatty acid biosynthesis (the formation of malonyl-CoA by acetyl-CoA carboxylase is the first step in fatty acid biosynthesis); and 2) as a regulatory effector of fatty acid oxidation through inhibition of carnitine palmitoyl transferase 1 (CPT1) [4,5]. CPT1 exists in 3 isoforms – CPT1A, expressed in the liver [6]; CPT1B, expressed predominantly in the muscular/cardiac tissue [6]; and CPT1C, found recently in brain [3]. It has been suggested that malonyl-CoA differentially inhibits these isoforms. CPT1B (expressed predominantly in muscle tissues, where the synthesis of fatty acids is negligible) is 100-fold more sensitive to malonyl-CoA concentration [7–10]. These findings argue for predominantly regulatory rather than synthetic role of the malonyl-CoA in the skeletal and heart muscle - slight alterations in malonyl-CoA concentration linked to loss of MLYCD activity may have profound effects on substrate consumption and energy supply in heart and muscle and probably can account for the phenotypic similarities of mitochondrial fatty acid oxidation disorders seen in individuals with MLYCDD. Additionally, accumulation of cytoplasmic malonyl-CoA inhibits long-chain acylcarnitine acetyltansferases, resulting in impaired fatty acid uptake and beta oxidation in both mitochondria and peroxisomes [11,12].

The diagnosis of malonic aciduria is based on detection of elevated levels of malonylcarnitine (C3DC) in blood acylcarnitine profiles [13,14]. Urine organic acid analysis shows high levels of malonic acid. In some patients, this is accompanied by mild elevations of methylmalonic acid, especially during the initial presentation, which may resemble combined malonic and methylmalonic aciduria, due to mutations in the *ACSF3* gene.

In some cases, ketotic dicarboxylic aciduria can be detected by urine organic acid analysis, a finding not typical for most fatty acid oxidation disorders [15]. The diagnosis can be confirmed by molecular testing or detection of reduced enzyme activity in fibroblasts.

Individuals with MLYCDD present with a variable phenotype which includes developmental delay, seizures, hypotonia, metabolic acidosis, hypoglycemia, ketosis and cardiomyopathy. Developmental delay is the most prominent feature, and cardiomyopathy is the leading cause of morbidity and mortality. Brain abnormalities characterized by malformation in cortical development and white matter involvement have been reported in some patients [16–18].

If not detected by newborn screening, most cases of MLYCDD present with metabolic decompensation characterized by severe metabolic acidosis and hypoglycemia, associated with poor prognosis. As a result of expanded newborn screening, more patients with MLYCDD have been identified prior to the onset of symptoms, allowing for early initiation of treatment and potential prevention of complications such as hypoglycemia, cognitive impairment and cardiomyopathy.

Currently, there are no specific treatment guidelines for MLYCDD. Similar to fatty acid oxidation disorders, a high carbohydrate and low-fat diet has been suggested [2,13,14,19] and medications such as ACE

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inhibitors and beta-blockers have been used for treatment of cardiomyopathy. In addition, carnitine supplementation may be useful to correct secondary carnitine deficiency and to potentially improve cardiomyopathy and muscle weakness. Since its first description in 1984, at least 49 patients with MLYCDD have been reported in the literature, mostly as single case reports. Therefore, not much is known about the natural history, effect of treatment, benefit of interventions before the onset of symptoms, long term outcomes and genotype-phenotype correlation.

In this case series we report the clinical, biochemical, molecular and long-term follow-up, including a systematic review of brain MRI findings, in eight previously unreported cases with MLYCDD and update on one previously published patient [15]. We provide information about dietary management, long term outcomes and expand on the knowledge regarding the molecular defects associated with this disorder.

2. Methods

This study is a multi-site collaboration between metabolic centers at CHOC Children's Hospital (4 patients), Children's National Medical Center (1 patient), University of Missouri (2 patients), Saint Peter's University Hospital (1 patient) and Children's Hospital of Wisconsin (1 patient). CHOC Children's and Mayo Clinic functioned as the coordinating investigation sites. IRB approval (CHOC IRB # 180423) was obtained and collaborating institutions signed a letter of agreement to share their patient's information. All patients had biochemical diagnosis of MLYCDD. Enzyme activity in fibroblasts (5 patients) was performed under research protocol using a modification of the method of Scholte [20]. Mutation analysis by Sanger sequencing and deletion analysis by MLPA were performed by routine clinical testing [21,22]. *In silico* analysis of the missense mutations and the effect of splice site alterations was performed utilizing Alamut Visual software (Alamut Visual version 2.11 -Interactive Biosoftware, Rouen, France). This package includes the predictive algorithms Mutation taster, SIFT, Polyphen-2 and Align GVGD. The splice site variants were evaluated by 4 different programs (MaxEntScan, NNSPLICE, GeneSplicer and SpliceSiteFinder-like). In addition, minor allele frequency (MAF, when available) and conservation of particular amino acid residue in comparison with orthologues were also taken into account. Retrospective chart review of the patients was performed. De-identified data was collected through a standardized form by collaborating Institutions at participating sites. Patient data included demographics, family history, and detailed information about clinical / biochemical presentation, treatment and long-term outcomes. Additionally, available brain MRI images (n = 5) and reports (n = 2) were reviewed by the same pediatric neurologist. Newborn screening (NBS) and confirmatory testing were performed as mandated by the State where each patient was born. Data regarding cut-off values and secondary markers reflect those used at the time of NBS sample collection. Participating institutions provided nutritional treatment information, and all data was reviewed by the same metabolic dietitian. Longitudinal laboratory data obtained after treatment initiation was analyzed in the 4 patients that were followed at the same Institution, using the same laboratory. Only data obtained in ambulatory setting, while patients were not acutely ill was included. One way Anova was used to determine statistically significant differences between the C3DC means of the different patients and Pearson correlation coefficient was used to assess correlation between the C3DC and free carnitine values.

3. Results

Table 1 summarizes the initial presentation and diagnostic evaluation of each patient. Four out of 9 patients had neonatal presentation which included hypoglycemia, metabolic acidosis, respiratory distress, hypotonia and seizures. The remaining patients were asymptomatic at the time of diagnosis. Patient 1 was born before expanded newborn

Table 1
Initial presentation and diagnostic evaluation of each patient. Normal values are in parenthesis. NA: Not available. Bold: homozygous.

	Sex	Ethnicity	Age at diagnosis	Initial clinical presentation	Initial NBS C3DC µmol/L	Secondary marker (C3DC/C10)	Organic acids	C3DC umol/L	Total carnitine umol/L	Free carnitine umol/L	Enzyme activity nmoles/mg protein	MLYCD mutations
1	M	Caucasian	8 d	Respiratory distress metabolic acidosis hypoglycemia hypotonia seizures	n/a	n/a	↑↑ MA	1.15[a]	NA	NA	0.6 (16.2 ± 1.8)	Del of exon 1 and 5'UTR del of exon 1 and 5'UTR
2	M	Mexican	8 d	Asymptomatic	4.08 (< 0.30)	68 (< 5.00)	↑↑MA ↑ MMA	1.44 (< 0.11)	14.9 (17–59)	6.00 (12–46.2)	0.38 (16.2 ± 1.8)	c.640_641 + 2del c.640_641 + 2del
3	F	Mexican	6 d	Respiratory distress hypotonia	3.30 (< 0.30)	47.14 (< 5.00)	↑↑MA ↑ MMA	1.32 (< 0.11)	28.9 (17–59)	14.4 (12–46.2)	0.19 (16.2 ± 1.8)	Del of exon 5 Del of exon 5
4	M	Caucasian	8 d	Asymptomatic	2.70 (< 0.33)	n/a	↑↑ MA	0.80 (< 0.13)	16.0 (17–41)	10.0 (10 – 21)	NA	c.365T > C c.1152G > T
5	M	Mexican	6 m	Respiratory distress hypotonia metabolic acidosis	0.49 (< 0.40)	4.45 (< 5.00)	↑↑MA ↑ MMA	2.33 (< 0.13)	20.0 (26–66)	10.0 (21–53)	NA	Sibling of patient 9
6	M	Caucasian -native American -Mexican	21 d	Hypoglycemia metabolic acidosis	3.36 (< 0.30)	n/a	NA	↑	NA	NA	NA	c.641 + 4_641 + 7del c.641 + 4_641 + 7del
7	M	Nigerian	5 d	Asymptomatic	3.7 (< 0.19)	43.28 (< 3.67)	NA	NA	NA	NA	NA	c.23_24ins13 c.799-3C > G
8	F	Middle Eastern[b]	5 d	Asymptomatic	1.60 (< 0.30)	27.65 (< 6.00)	NA	NA	NA	NA	NA	c.929G > C c.929G > C
9	M	Mexican	17 d	Asymptomatic	0.44 (< 0.30)	6.28 (< 5.00)	↑↑MA ↑ MMA	1.94 (< 0.13)	24.9 (17–59)	15.0 (12–46.2)	0.27 (16.2 ± 1.8)	Del of exon 5 Del of exon 5

[a] Normal range not provided for C3DC.
[b] Consanguinity.

screening (NBS) was available. All patients who had NBS testing had elevated C3DC, while C3DC/C10 ratio was used as a secondary marker by some centers. The state of California (CA) was not using secondary markers for MLYCDD at the time patients 2, 3 and 9 were born; the values reflected on the table were calculated retrospectively using the raw data from the NBS report. Based on those calculations, patients 2, 3 and 9 would have been positive for the secondary marker as well. In contrast, patient 5 was born after CA NBS program had implemented C3DC/C10 ratio as secondary marker, and his result was below the cut-off of 5.00 established at the time. Therefore, he was not flagged by the NBS program, which at the time only flagged patients who had elevations in both primary and secondary markers for MLYCDD, and was diagnosed at 6 months of age after presenting with respiratory distress and metabolic acidosis secondary to cardiomyopathy.

Confirmatory testing was available for 5 out of the 7 patients detected by NBS. All of them had elevated C3DC and/or abnormal urine organic acids, diagnostic of MLYCDD. Free carnitine levels tended to be low, being below the normal range in 2 of the patients. Four out of the 9 patients had enzymatic analysis in skin fibroblasts with extremely low enzyme levels.

Almost all types of molecular changes were observed in this cohort. Even though consanguinity was only reported in one family, seven out of nine patients had homozygous mutations. Most of the mutations were large deletions including the entire exon, or splice site variants leading to a frame shift and termination codon downstream. Two of the variants were previously described. Patient one has a large homozygous deletion including 5′ UTR and parts of exon one. However, the boundaries of this deletion were not identified by long range PCR [15]. Both parents were carriers as identified by MLPA analysis. The homozygous mutation in patient two was also previously described [1], this deletion leads to frameshift and stop codon downstream [c.640_641 + 2del; p.(Glu214Glyfs*2)]. The rest of the variants were novel. Deletion of the entire exon 5 was the most frequently encountered alteration, found in three individuals including two siblings – patients 5 and 9. A duplication of 13 nucleotides was observed in patient seven [(c.22_34dup, p.(Arg12Leufs*200))] leading to frameshift and stop codon further downstream. In this patient, another variant was found at splice position c.799-3C > G in IVS3 potentially leading to loss of an acceptor splice site as predicted by the splice site algorithms. Another deletion (c.641 + 4_641 + 7del) in IVS2 is also predicted to alter donor splice site. Three missense variants were identified in this study. The c.365T > C (p.Leu122Pro) variant has not been previously observed, and it affects a highly conserved amino acid (up to *C. elegans*, considering 12 species). All used predictor algorithms identified the variant as potentially damaging or disease causing (SIFT score 0; MutationTaster – disease causing; Polyphne-2 score 0.999; and Align GVGD – C25). The other two variants (c.1125G > T, p.Trp384Cys and c.929G > C, p.Arg31Pro) are known validated dbSNP variants. c.929G > C has MAF of 0.001 and 0 homozygotes. All four algorithms predict damaging or disease causing effect (SIFT - 0.01; MutationTaster – disease causing; Polyphne-2 -1.0; and Align GVGD – C35) and the amino acid at that position is conserved to Tetraodon (12 species considered). This variant has been reported twice in ClinVar (RCV000764082.1 and RCV000482691.1) and classified as variant of uncertain significance (VUS). In gnomAD (2.1) overall MAF was 0.0016% and in NFE – 0.0035%. The variant c.1152G > T (p.Trp384Cys) is known to dbSNP (rs1177070767). It affects a highly conserved amino acid (down to Tetraodon, considering 12 species). All four algorithms predict damaging or disease causing effect (SIFT - 0.03; MutationTaster – disease causing; Polyphne-2 -1.0; and Align GVGD – C25).

Table 2 summarizes the patient outcomes at the time of their last evaluation. One patient died (patient 9) of cardiac failure at 9 months. The remaining 8 patients have a median age of 6.5 y (ranged from 1.3 to 14). Only patient 9 had severe failure to thrive.

Routine follow-up over the years, clearly demonstrated that there is some degree of neurodevelopmental disability in patients with MLYCDD. Most patients had hypotonia that was frequently associated with gross motor delays. In addition, most patients had speech and fine motor delays as well as microcephaly. Intellectual disabilities were detected in most school aged children.

Three patients in our cohort had seizures. Patient 1 presented with seizures on day of life 4 possibly related to hypoglycemic episode. After the correction of the glucose levels he was treated with phenobarbital, phenytoin and lorazepam which were discontinued over time. Patient 6 presented with seizures at 6 months of age. Initial EEG showed intermittent focal slowing and occasional potentially epileptiform discharges. He was not treated with anti-seizure medications and repeat EEG at 9 months of age was normal. Patient 9 was diagnosed with seizures at 4 months of age. His EEG showed mild-to-moderate encephalopathic trace with no epileptiform discharges. He was initially started on fosphenytoin and later switched to levetiracetam until the time of his death.

Seven out of 9 patients had a brain MRI, and 4 of them had abnormal findings, including T2 hyperintensities in periventricular white matter and basal ganglia (Fig. 1).

Patient 5, who was not detected by NBS, presented with dilated cardiomyopathy. All other patients had routine echocardiograms that lead to the detection of dilated cardiomyopathy in 4 additional patients and left ventricular non-compaction in one. In most cases, cardiomyopathies improved or resolved with specific treatment.

Care of children with MLYCDD required several hospitalizations for initial evaluations and treatment, acute metabolic decompensations (acidosis/hypoglycemia), prevention of metabolic crisis during intercurrent illness and/or management of cardiomyopathy. Seven out of the 9 patients had a total of 23 admissions with an average of 28 hospital-days per admission. Hospital treatment included high glucose infusion (10% dextrose) and carnitine (50–300 mg/kg/day), as well as management of cardiomyopathy and the intercurrent illness.

It has been recommended that dietary management for MLYCDD patients mimics that of patients with long-chain fatty acid oxidation disorders such as VLCAD [14]. Therefore, our patients were treated with modified diets to restrict the amount of long -chain fats and supplemented with MCT. Various modalities were used by the different centers.

Table 3 summarizes the treatment during the first 6 months of age, before the introduction of solid foods. Five out of 9 patients were prescribed a special medical formula restricted in long-chain fats, with high MCT content, with or without the use of glucose polymers or additional oil to meet caloric and essential fatty acids (EFA) requirements. In 3 patients breast milk was used in combination with MCT and/or other formulas, and only 1 patient was exclusively breast fed. Four out of 9 patients required tube feedings, due to poor muscle tone.

Nutritional analysis shows that total fat as a percentage of total calories was mildly restricted from 22% to 36% in 4 patients, while the remaining 5 met the DRI requirements for age (40–55%). The intake of long-chain fat as percentage of total calories was restricted in 8 out of the 9 patients. Three of them (patients 6, 8 and 9) received < 10% while the remainder received 14–23%. Accordingly, the percentage of calories provided as MCT ranged from 14 to 38%. Only the patient who was exclusively breast fed had no long-chain fat restriction or MCT supplementation. No essential fatty acid deficiencies were reported.

Table 4 summarizes treatment at the time of last evaluation. Patient 9 was not included in this table as he died at 9 months of age. All patients were prescribed a diet restricted in long chain fats with MCT supplementation. For all patients total fat intake was close to the DRI for age. Out of the 3 patients that required G-tube feedings, only patient 5 was still using it.

All patients, including patient 1 who was not following dietary treatment initially, were restricted in long chain fats ranging from 8 to 18% total calories, with exception of patient 5 who was following a very restricted long chain fat intake. MCT supplementation ranged from

Table 2
Patient outcomes at the time of their last evaluation.

	Age	Weight percentile	Height percentile	Microcephaly	Developmental delays	Intellectual disability	Hypotonia	Seizures	Brain MRI	History of cardiomyopathy	History of cardiac medications	Hospital admissions (total # of days)
1	14 y	82	95	Y	Speech fine motor	Y	N	Y	7 d: Supratentorial cortical/subcortical acute-subacute ischemia in non-vascular distribution. 7 m: Microcephaly, delayed myelination. 21 m: Increase in myelination, pattern remains delayed. Increased T2/FLAIR signal in the white matter of the corona radiata. Thinning of the corpus callosum. 13 y: Worsening of increased T2 signal involving bifrontal, biparietal and bioccipital lobes	Dilated	Enalapril (DC), digoxin, furosemide	2 (6)
2	10 y	17	33	Y	Speech gross motor fine motor	Y	Y	N	13 m: Normal. 10y: Normal	No	None	3 (3)
3	9 y	> 95	64	N	Speech gross motor Fine motor	Y	Y	N	13 d: Normal. 9 y: Bilateral, symmetric mild increased in T2/FLAIR signal in the periventricular and deep white matter.Cystic lesions in BG	Dilated	Furosemide (DC)	1 (28)
4	7 y	> 95	> 95	N	N	N	N	N	7 y: Retrocerebellar arachnoid cyst	No	None	None
5	6 y	13	18	Y	Speech gross motor fine motor	y	Y	N	6 y: White matter and putaminal T2 hyperintensties	Dilated	Epinephrine (DC), Milrinone (DC), Chlorothiazide (DC), Aspirin (DC), Furosemide (DC), Carvedilol, Captopril	3 (57)
6	5 y	16	37	NA	Speech gross motor fine motor	Y	Y	Y	6 m: Delayed myelination. 19 m: Bilateral T2 medial putaminal hyperintensities	Dilated	Captopril (DC), Enalapril (DC)	5 (51)
7	28 m	> 95	> 95	N	Speech	NA	Y	N	ND	Left ventricular non-compaction	Enalapril (DC), Digoxin	2 (5)
8	16 m	29	89	Y	N	NA	N	N	ND	No	None	None
9	9 m[a]	ZS: -4.2 SD	ZS:-3.1 SD	Y	Gross motor Fine Motor	NA	Y	Y	20 d: Normal	Dilated	Captopril, Furosemide, Carvedilol, Milrinone	7 (44)

NA: not applicable. ND: not done. DC: discontinued.
Z-Score was calculated when weight/height were < 10%ile.
[a] Deceased.

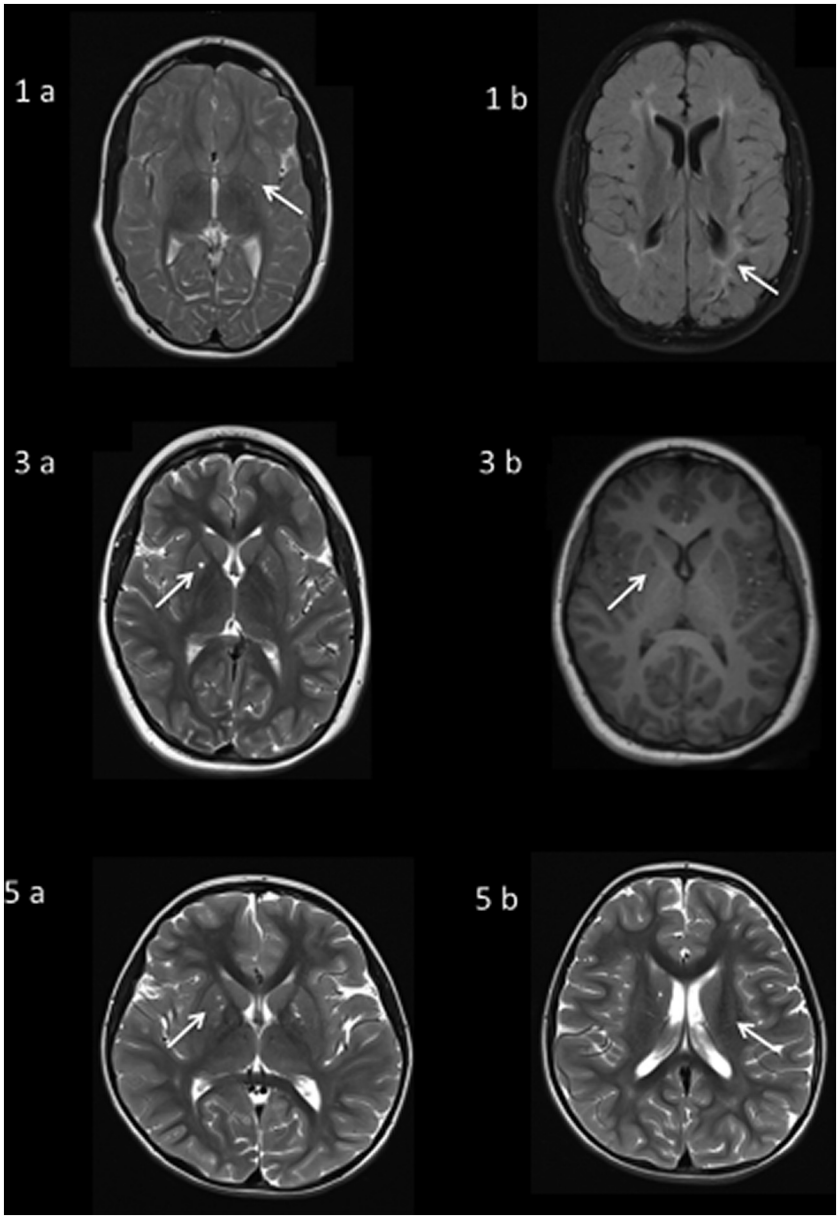

Fig. 1. MRI of the brain, axial cuts. Patient 1: At 13 years of age. 1a: T2: basal ganglia hyperintensities. 1b: T2: white matter hyperintensities. Patient 3: At 9 years of age. 3a: T2: periventricular white matter and bilateral basal ganglia hyperintensities. 3b: T1: corresponding basal ganglia hypointensities. Patient 5: At 6 years of age. 5a: T2: bilateral symmetric basal ganglia hyperintensities. 5b:T2: periventricular white matter hyperintensities.

Table 3
Treatment during the first 6 months of age, before the introduction of solid foods.

	Nutritional source	Tube feedings	Total fat (% of total calories)	Long chain fats (% of total calories)	MCT (% of total calories)
1	BM	N	55	52.2	3.8
2	P + PL	N	34	15.0	19.0
3	P + PL	G-tube	36	14.0	22.0
4	P	N	49	23.0	25.0
5	BM + MCT	G-tube	40	15.6	24.7
6	M + PL + MCT + O	G-tube	22	7.7	14.3
7	E + BM + O	N	50	20.0	30.0
8	BM + MCT	N	30	8.0	22.0
9	E	NG-tube	45	7.0	38.0

BM: Breastmilk. PL: Polycose ®. P: Pregestimil ®. E: Enfaport ®. M: Monogen ®. O: walnut, safflower or flaxseed oil to provide EFAs.

15 to 26% of the total calories, with exception of patient 3 who was prescribed 13% but was unable to follow recommendations and her MCT intake was only 2% of total calories.

In order to assess if long term biochemical data correlated with clinical outcomes, we analyzed the available laboratory data obtained after treatment initiation in the 4 patients that were followed at the same Institution, using the same laboratory. C3DC levels tended to be higher (up to 8.66 μMol) during illness. Therefore, only data obtained in ambulatory setting, while patients were not acutely ill was included. Unfortunately, urine organic acids or MMA levels in blood were not routinely followed. Liver enzymes and CK remained within normal limits. C3DC levels varied in the same patient over time and among the different patients (Table 5). Patient 2 had the lowest mean C3DC levels (1.31 μMol) and has a milder clinical course, with no cardiomyopathy or brain MRI abnormalities. Statistical analysis showed that his levels were significantly different from those of patient 5 and 9, who had a more severe clinical course (Fig. 2a). While the above could suggest a

Table 4
Dietary treatment at the time of last evaluation.

	Age	Tube feedings	Total fat (% of total calories)	Long chain fats (% of total calories)	Supplemental MCT (% of total calories)	Supplemental MCT (g/kg/day)
1	14 y	N	30.0	15.0	15.0	0.7
2	10 y	N	30.0	10.0	20.0	1.5
3	9 y	N	20.0	18.0	2 [a]	1.5
4	7 y	N	25.0	10.0	15.0	0.6
5	6 y	G-tube	31.0	4.1	26.5	2–2.5
6	5 y	N	37.5	11.5	26.0	2.3
7	28 m	N	35.0	14.0	21.0	NA
8	16 m	N	30.0	8.0	22.0	2.3

Patient 9 not included in this table as he died at 9 months of age.
[a] Prescribed MCT but patient was not compliant. Sources of MCT included: Liquigen ®, Monogen ®, MCT oil Nestle ®.

Table 5
Longitudinal C3DC levels.

Patient	Average	SD	SEM	Max	MIN	N
2	1.31	0.65	0.09	2.98	0.33	47
3	2.01	1.07	0.26	4.03	0.58	17
5	3.77	1.74	0.33	7.98	1.49	28
9	2.59	0.50	0.10	3.66	1.97	7

SD: standard deviation. SEM: standard error of mean. MAX: maximum level. MIN: minimum level. N: number of values measured.

correlation between C3DC levels and disease severity, levels of C3DC in patient 3, who has same mutations and similar severe clinical course as patients 5 and 9, were not significantly different from patient 2. To assess for the possibility that C3DC levels could be influenced by free carnitine, we compared all free carnitine values (n = 87) with the C3DC levels that were obtained at the same time. We found that there was a weak but significant correlation between these 2 parameters (r2 0.134, $p < 0.0005$) (Fig. 2b).

4. Discussion

MLYCDD is a severe and rare inborn error of metabolism.

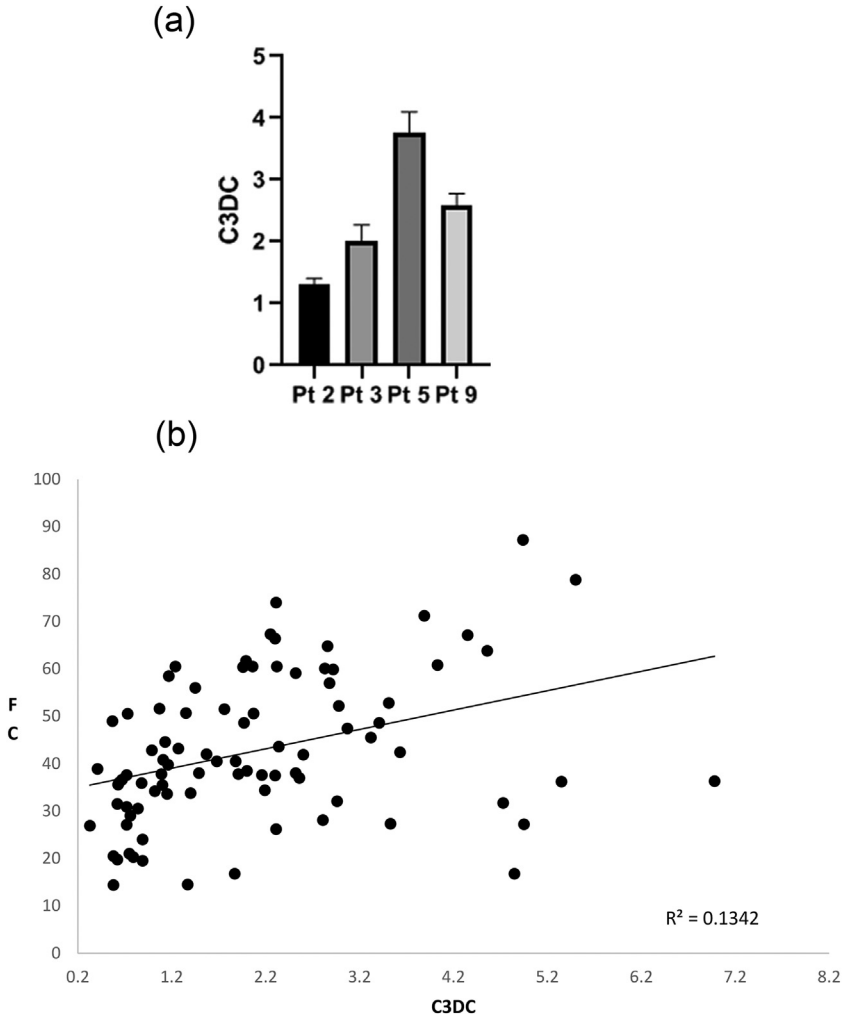

Fig. 2. a) Mean C3DC values on treatment. b) Linear regression analysis of C3DC levels relative to free carnitine levels. N = 87.

Implementation of NBS programs has allowed for early identification and reporting of more patients, however, birth prevalence is still very low.

Our findings significantly expand the number of reported cases and molecular spectrum of MLYCDD. Additionally, we provide important information about long term manifestations and response to treatment.

While NBS can detect most MLYCDD cases, newborns can be symptomatic prior to the availability of NBS results, high level of suspicion is required if clinical presentation is suggestive (hypoglycemia, respiratory distress etc.).

All patients who had NBS testing had elevated C3DC. A secondary marker, C3DC/C10 was used by some programs and appeared to be useful, except for patient 5 who had borderline low levels.

Most recently, the Collaborative Laboratory Integrated Reports (CLIR) post-analytical interpretive tools have been available to laboratories and clinicians to assist in the interpretation of initial NBS results [23]. The clinical utility of these tools is based on the replacement of traditional cutoff values with covariate-adjusted reference and disease ranges and the integration of informative markers with all possible permutation of calculated ratios. Single condition tools integrate all relevant results into a single score [23]. To evaluate if a tool for the condition under study would have been useful in our population, we entered NBS values for patients 2, 3, 5 and 9, for whom all required information, including birthweight and hours of age at specimen collection, was available. For patients 2 and 3 the tool predicted the diagnosis of MLYCD to be very likely, while the diagnosis was predicted as likely for patients 5 and 9. Interestingly, these 2 patients were siblings, had the lowest C3DC values, and one of them (patient 5) was not detected by the NBS program as his C3DC/C10 ratio was below the chosen cutoff value. The use of CLIR could have prompted a referral and confirmatory testing in this case. Therefore, the routine use of this tool may facilitate the correct diagnosis of MLYCDD even when NBS results are deemed inconclusive by a conventional approach. This is particularly important considering that patients 5 and 9 had severe disease manifestations including death, and initial C3DC levels do not appear to correlate with disease severity or outcome.

Most of our families were of Mexican descent, seven families had homozygous mutations however, consanguinity was only reported in one family. All but two mutations have not been previously reported.

No correlation between mutation and enzyme activity could be established given that the measured enzyme activity was significantly low in all patients and it is difficult to make comparisons regarding residual enzyme activity. In addition to the typical clinical presentation, biochemical abnormalities and, in some cases, the determined low enzyme activity, the predicted *in silico* outcome of the identified variants argue that they are disease causing mutations. Since crystal structure of malonyl-CoA decarboxylase has not been reported, *in vitro* studies are needed to further determine the effect on molecular level.

Patients 3, 5 and 9 have the same homozygous mutations, however their clinical presentations and outcomes are different. Patients 5 and 9 are siblings, and while the latter was picked up by NBS and had the benefit of early treatment, he died of severe cardiomyopathy before the first year of life. On the other hand, his brother started treatment late, after presenting with cardiomyopathy at 6 months of age. He is currently 6 years old, has stable cardiomyopathy and mild neurodevelopmental delays. Unlike patients 5 and 9, patient 3 had a severe neonatal presentation however; she is also doing well at 9 years of age with stable cardiomyopathy and mild delays. It is possible that additional factors such as intercurrent illnesses, or a second underlying disorder in consanguineous families, may play a role in the severity of the clinical presentations and outcomes.

As previously reported, most common manifestations were related to cardiomyopathy and neurological involvement. However, clinical expression was variable, ranging from asymptomatic to severe cardiomyopathy and death. It is possible that some of the observed clinical heterogeneity might be due to variable expression of the *MLYCD* gene in different cell types [24,25].

Neurodevelopmental delay was the most prevalent finding in our patient cohort, followed by microcephaly and hypotonia. While seizures are an expected complication in patients with MLYCDD [24], only 3 patients in our cohort had a history of seizures. Based on our data and previous reports [5,15,16,26,27] there doesn't seem to be a specific seizure type or EEG pattern. Of note, the 3 patients who had seizures presented during infancy and the 2 who are still alive have been seizure free and off antiepileptic treatment at ages 5 and 14 years. As in the case of patient 1, the seizure episode could have been related to severe hypoglycemia.

The etiology of neurodevelopmental delays or seizures in MLYCDD is not clear, although it is possible that hypoglycemia could be a predisposing factor.

Consistent with previous reports [16], brain MRI abnormalities were found in 4 out of 7 patients. The most common abnormalities observed were T2 hyperintensities in the white matter and basal ganglia (Fig. 1), which are not uncommon manifestations of organic acidemias [28,29].

Interestingly, different from previous reports, brain atrophy was not observed in our patient cohort [5,12,17,24,30]. In most of the reported patients, atrophy was detected in infancy; therefore, the lack of brain atrophy in our cohort cannot be attributed to their age. Early delayed myelination observed in patients 1 and 7 is likely to be related to factors other than MLYCDD given that they resolved over time.

The etiology of MRI abnormalities in patients with MLYCDD is unclear but could be related to episodes of acute metabolic decompensation or hypoxia related to cardiac insufficiency. Another mechanism could be the persistently elevated levels of malonyl-CoA in the brain, with secondary inhibition of CPT-1 expressed in the brain [3].

One out of the 9 patients in our series died, which is comparable to the outcome reported by Celato et al. in larger cohort [31]. The cause of death in our patient, as well as in those previously reported, was secondary to severe cardiomyopathy. He also had severe feeding intolerance and failure to thrive. NG tube feedings were attempted during the last month of life. It is possible that a more aggressive nutritional management could have improved the outcome.

The most common cardiac abnormality in our cohort was dilated cardiomyopathy, while previous reports have described both, hypertrophic [1,15,32,33] as well as dilated cardiomyopathies [14,26,34,35]. Patient 7, had left ventricular non-compaction (LVNC); making him the third reported MLCYD patient with this finding [36,37]. Therefore, LVNC together with hypertrophic and dilated cardiomyopathy are common manifestations in patients with MLYCDD.

The cause of the cardiac findings in MLCYDD, is not fully understood. Dilated cardiomyopathy is commonly seen in patients with long chain fatty acid oxidation disorders (FAOD) and could be expected based on the inhibitory effect of malonyl-CoA on CPT1. However, LVNC or hypertrophy cardiomyopathies are not common in FAOD and could be related to other mechanisms, including mitochondrial dysfunction due to accumulation of potentially toxic organic acids [4,36,38].

Of note, most of our patients were asymptomatic at the time of diagnosis and cardiomyopathy was identified during routine echocardiogram. All patients with cardiomyopathy, with exception of the patient who died, exhibited improvement in cardiac function after initiation of treatment with cardiac medications and/or dietary modifications (Table 2).

Due to the known inhibition of mitochondrial FAO by malonyl-CoA, historically patients with MLYCDD have been treated with a fat restricted diet [13,14,35]. Currently there are no specific dietary guidelines for MLYCDD, therefore dietary recommendations for VLCAD are usually followed. According to GMDI Management Guidelines [39] patients should receive the dietary reference intake (DRI) for age for total fat, however the composition of fat intake should be modified by restricting long chain fats and adding MCT. All our patients followed similar dietary recommendations with some variations (Tables 3 and 4).

Due to the limited number of reported patients at the time, Patient 1, who has a severe mutation and had neonatal presentation, was started on modified diet supplemented with MCT oil at 6 months of age. The combination of dietary treatment and treatment with angiotensin-converting enzyme (ACE) inhibitor led to resolution of cardiomyopathy (EF > 30%; normal for age < 30%) and the ACE inhibitor was discontinued. Similarly, patient 5 did not start dietary treatment until 6 months of age. Interestingly, long term clinical course for these 2 patients is not different from the rest of our cohort. Patient 9 died of severe cardiomyopathy despite receiving the most significant long-chain fat restriction and higher amount of MCT. This dietary intervention does not appear to have contributed to his death, given that patients 6 and 8 were following similar restriction in long chain-fat and are currently doing well.

Based on our findings, current treatment modalities appear to be beneficial to treat / prevent acute metabolic crises. However, as previously reported, cardiomyopathy cannot be prevented by fasting precautions or dietary interventions alone. However, a combination of a long chain fat restriction, MCT supplementation and ACE inhibitor therapy improves cardiac outcome [36]. Considering that patient 9 died despite receiving the above interventions, it is possible that MLYCDD patients with severe cardiomyopathy may benefit from new therapies that appear to be beneficial for patients with long chain FAOD and severe cardiac disease [40].

Longitudinal biochemical markers were reviewed in 4 patients. C3DC values varied over time and although increases were seen at the time of acute illness, statistical analysis did not support a correlation between C3DC mean values and prognosis. It is possible that our analysis may be influenced by the different number of samples available for each subject and/or the carnitine levels. Despite the variations noted over time, we observed that C3DC values tended to be higher when patients were on MCT formulas and appeared to decrease with age, irrespective of dietary intervention or compliance. It is possible that measuring malonic acid levels in blood or urine in these patients could have been useful to monitor treatment or predict disease severity.

5. Conclusions

Our findings significantly expand the number of reported cases and molecular spectrum of MLYCDD. While NBS can detect most MLYCDD cases, clinicians must be aware of this condition as newborns can be symptomatic prior to the availability of NBS results. Early implementation of a diet restricted in long-chain fat and high MCT in combination with cardiac medications improve the outcome of cardiac disease, however, these interventions may not completely prevent other disease complications such as neurodevelopmental disabilities and brain MRI abnormalities.

Based on our experience, monitoring for CNS disease (neurodevelopmental testing/brain MRI) and cardiomyopathy (serial echocardiograms) should be implemented as standard of care for this disorder.

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