Molecular Genetics and Metabolism Reports 41 (2024) 101158

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

# A case series of Cypriot patients with CblC defect: Clinical, biochemical and molecular characteristics

Theodoros Georgiou [a], Olga Grafakou [b], Anna Malekkou [a], Emilia Athanasiou [c], Ioannis Ioannou [d], Vivi Choleva [e], Maria Dionysiou [a], Gabriella Mavrikiou [a], Anthi Demetriadou [a], Violetta Anastasiadou [c], Anthi Drousiotou [a], Petros P. Petrou [a,*]

[a] Biochemical Genetics Department, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
[b] Inborn Errors of Metabolism Clinic, Archbishop Makarios III Hospital, Nicosia, Cyprus
[c] Clinical Genetics Department, Archbishop Makarios III Hospital, Nicosia, Cyprus
[d] Paediatric Neurology Clinic, Archbishop Makarios III Hospital, Nicosia, Cyprus
[e] Ophthalmology Clinic, Archbishop Makarios III Hospital, Nicosia, Cyprus

ARTICLE INFO

*Keywords:*
Cobalamin
CblC
Methylmalonic aciduria
Homocystinuria

ABSTRACT

Methylmalonic aciduria and homocystinuria, CblC type, is an inborn error of intracellular vitamin B12 (cobalamin) metabolism caused, in the majority of cases, by mutations in the *MMACHC* gene. Five Cypriot patients (four males and one female) were diagnosed with a CblC defect. Age at diagnosis ranged from 10 days to 9 months. We present here the clinical, biochemical and molecular findings of these patients. Our retrospective study indicates that all patients were carriers of the known p.Arg91LysfsTer14 variant in either a homozygous or compound heterozygous state with other known *MMACHC* pathogenic variants. Out of three patients sharing the same genotype the one diagnosed and initiated treatment in the neonatal period displayed an improved clinical outcome.

## 1. Introduction

Methylmalonic aciduria and homocystinuria, CblC type (OMIM #277400), is the most common genetic disorder of cobalamin intracellular metabolism. In the majority of cases, it is caused by mutations in the *MMACHC* gene, and is inherited in an autosomal recessive manner [1,2]. The biochemical hallmarks of CblC defect are hyperhomocysteinemia, methylmalonic aciduria and low plasma methionine.

The human *MMACHC* gene has been mapped to chromosome 1p34.1 and consists of four coding exons and one non-coding exon [3]. It encodes a polypeptide of 282 amino acids.

The clinical phenotype in early onset disease, which is the most common, includes a wide range of neurological and multisystem manifestations such as feeding difficulties, developmental delay, seizures, pancytopenia and microangiopathy causing atypical hemolytic uremic syndrome. Later onset forms may present with neuropathy/myelopathy, encephalopathic features, psychiatric symptoms, thrombotic microangiopathy and other thrombotic phenomena and seizures [4]. Early treatment with B12 (hydroxocobalamin), betaine, folate and carnitine improves survival and prevents severe organ damage [5].

The incidence of CblC disease ranges from 1:46,000 to 1:200,000 [6,7] in Europe and the United States, while in China reports indicate a higher incidence ranging from 1:3220 to 1:21,488 [8,9].

## 2. Methods

### 2.1. Patients

Patients were referred to the Archbishop Makarios III Hospital, in Nicosia which serves as a reference children's hospital in Cyprus, where patient management and monitoring was also conducted. During the period of 1990–2022, five children (four males and one female) were diagnosed with a CblC defect. Four patients were of Greek-Cypriot origin and one had a Greek father and a Greek-Cypriot mother. All families were unrelated and there was no consanguinity. All patients were defined as early onset since clinical symptoms appeared within the first year of life. Age at diagnosis ranged from 10 days to 9 months. At the time of diagnosis, the patients presented with multisystemic disease and

* Corresponding author at: Biochemical Genetics Department, The Cyprus Institute of Neurology and Genetics, P.O. Box 23462, Nicosia 1683, Cyprus.
*E-mail address:* petrosp@cing.ac.cy (P.P. Petrou).

https://doi.org/10.1016/j.ymgmr.2024.101158
Received 23 September 2024; Received in revised form 5 November 2024; Accepted 5 November 2024

symptoms including failure to thrive, hypotonia, vomiting, feeding difficulties, seizures, anaemia, vision problems and developmental delay.

### 2.2. Laboratory tests

Biochemical and genetic laboratory tests were performed at the Biochemical Genetics Department of the Cyprus Institute of Neurology and Genetics. Plasma total homocysteine was measured on an Abbott Architect i1000SR immunoanalyser and amino acid analysis was performed by ion-exchange chromatography with post-column ninhydrin detection. Urine organic acid analysis was performed by GC/MS, and plasma acylcarnitine analysis by tandem mass spectrometry. DNA analysis was carried out by PCR amplification of all exons of the *MMACHC* gene, including exon/intron boundaries, followed by Sanger Sequencing.

## 3. Results

Five children have been diagnosed with CblC defect in the last 33 years (incidence about 1:62,000 births) [10] in Cyprus, an island with a population of 920,000 and approximately 10,000 births per year. Newborn screening in Cyprus which was introduced in 1989 currently includes two conditions, phenylketonuria and congenital hypothyroidism. Thus, in all five cases the diagnosis was made during the metabolic workup of the patients following the presentation of clinical symptoms and confirmed by genetic analysis.

At diagnosis all patients displayed high levels of homocysteine, propionylcarnitine, methylmalonylcarnitine and low levels of methionine in blood. Methylmalonic acid and methylcitrate in urine were significantly elevated (Table 1).

Genetic analysis revealed three previously described variants. The most common was the c.271dupA [p.Arg91LysfsTer14] pathogenic variant and was detected in six out of ten independent alleles. The c.616C > T [p.Arg206Trp] variant was identified in three out of ten mutant alleles. The nonsense variant c.331C > T [p.Arg111Ter] was found in one allele. Three patients had the genotype [p.Arg91LysfsTer14]; p.[Arg206Trp]. The genotypes of the remaining two patients were [p.Arg91LysfsTer14]; [p.Arg91LysfsTer14] and [p.Arg91LysfsTer14]; p.[Arg111Ter].

After treatment, with B12, betaine and carnitine, blood levels of homocysteine decreased to below or around 50 μmol/L and dropped further to 21–29 μmol/L with increased dose of B12 for all patients except for patient 1 whose homocysteine levels ranged around 100 μmol/L and came down to 60 μmol/L after B12 increase. Methylmalonic aciduria persists only for patient 1. The best clinical outcome was observed for the patient for whom treatment was started on the 10th day of life (Table 1).

## 4. Discussion

In this cohort of Cypriot patients with CblC deficiency, pathogenic variants were identified in 10 out of 10 *MMACHC* alleles investigated, representing a mutation detection rate of 100 %. All parents were confirmed as heterozygous carriers of the respective mutations. The identification of the variants responsible for CblC defect in Cypriot patients facilitated carrier detection as well as post and pre-natal diagnosis.

To date, over 150 pathogenic or likely pathogenic *MMACHC* variants are reported (Clinvar database). The p.Arg91LysfsTer14 variant which is associated with early onset disease is the most common variant in Caucasians and was detected in six out of ten independent Cypriot disease alleles. The p.Arg111Ter variant, initially found in patients of Cajun and French Canadian [11,12] descent is also considered as one of the most common variants associated with early onset disease in the Caucasian population [11–15]. The p.Arg206Trp variant found in three out of ten alleles in our cohort was first described in homozygosity in a Turkish patient who presented symptoms at two days of age [11].

Many patients with early-onset severe disease have the p.Arg91LysfsTer14 or the p.Arg111Ter variant in a homozygous or compound heterozygote state [16]. The genotype [p.Arg91LysfsTer14]; p.[Arg111Ter] which was identified in one Cypriot patient has been reported to be associated with early onset disease [9,13]. Three patients in our cohort have the genotype [p.Arg91LysfsTer14; p.[Arg206Trp]. All three were born small for gestational age, and presented with hypotonia and feeding difficulties. Initial biochemical parameters were severely compromised for all three patients. Regarding patient 5, clinical picture improved and no additional symptoms appeared. On the other hand, patients 2 and 4 developed maculopathy, atypical hemolytic uremic syndrome, a known disease complication and some degree of developmental delay. Whether the better clinical outcome of patient 5 is the result of earlier diagnosis or more aggressive treatment cannot be concluded with certainty. The above variant combination has been previously reported in an Italian patient investigated at the age of seven months due to low birth weight, microcephaly, strabismus, nystagmus, hypotonia, failure to thrive as well as severe demyelinating neuropathy [17]. The patient died at the age of 12 months due to infection and severe neurologic disease. A delayed diagnosis may have contributed to the patient's severe course.

There is increasing evidence that early treatment with high B12 doses improves patients' clinical and biochemical profiles [18,19]. Our patients were gradually administered significantly higher doses than those given as a baseline. All five patients clinically improved following treatment with high B12 dosage combined with anhydrous betaine, folate and occasionally carnitine.

## 5. Conclusion

This is the first study reporting CblC defects in the Greek-Cypriot population. The best clinical outcome was observed in one of the five patients who was diagnosed and started treatment at the 10th day of life. On the other hand, patient 1, our oldest patient who was diagnosed at the age of 9 months and was not closely followed, is nowadays severely impaired.

## Research funding

No specific funding was received for this study.

## Ethics statement

All procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Informed consent was obtained for genetic studies.

## CRediT authorship contribution statement

**Theodoros Georgiou:** Writing – original draft, Conceptualization, Investigation, Writing – review & editing. **Olga Grafakou:** Writing – review & editing, Resources, Conceptualization, Data curation. **Anna Malekkou:** Writing – review & editing, Investigation. **Emilia Athanasiou:** Writing – review & editing, Resources. **Ioannis Ioannou:** Writing – review & editing, Resources. **Vivi Choleva:** Resources. **Maria Dionysiou:** Investigation. **Gabriella Mavrikiou:** Investigation. **Anthi Demetriadou:** Investigation. **Violetta Anastasiadou:** Resources. **Anthi Drousiotou:** Supervision, Resources, Writing – original draft. **Petros P. Petrou:** Writing – review & editing, Supervision, Resources, Writing – original draft.

## Declaration of competing interest

The authors declare that they have no competing interests.

**Table 1**
Clinical, biochemical and molecular characteristics of Cypriot patients with early onset methylmalonic aciduria and homocystinuria, CblC type.

| Patient | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Ethnic origin | Greek Cypriot | Greek Cypriot | Greek Cypriot | Greek Cypriot | Greek Cypriot/Greek |
| Sex | M | M | F | M | M |
| Age at first presentation | 6 months | 5 months | 1,5 months | 2 months | 7 days |
| Age at diagnosis | 9 months | 5 months | 2 months | 2 months | 10 days |
| Age today | 27 years | 8 years | 8 years | 4 years | 2 years |
| Birth weight (grams) | Not recorded | 2980 | 2750 | 2880 | 2610 |
| Presentation | Lethargy, myoclonic seizures, hypotonia, tachycardia, hypothermia, tachypnoea, anaemia, feeding difficulties, vomiting. | Nystagmus, hypotonia, failure to thrive, vision problems (poor eye fixation), fever, aHUS, vomiting. | Lethargy, seizures, hypotonia, hydrocephalus, failure to thrive, growth retardation, oedemas, vomiting. | Lethargy, hypotonia, failure to thrive, growth retardation, aHUS, anaemia, vomiting. | Hypotonia, feeding difficulties. |
| Plasma Homocysteine NR: (5-15 μmol/L) | 206 | 163 | 130 | 125 | 190 |
| Plasma Methionine NR: (10–60 μmol/L) | 5 | 10 | 9 | 4 | 3 |
| Urine: | | | | | |
| MMA | Grossly elevated | Greatly elevated | Elevated | Grossly elevated | Grossly elevated |
| Methylcitrate | Elevated | Elevated | Elevated | Elevated | Elevated |
| Plasma Acylcarnitines C3 NR: (0.11–1.25 μmol/L) | 4.89 | 9.92 | 3.41 | 7.93 | 10.90 |
| C4DC NR: (0.0–0.09 μmol/L) | 0.33 | 0.26 | 0.29 | 0.22 | 0.18 |
| *MMACHC* molecular analysis | p.Arg91LysfsTer14/p.Arg111Ter | p.Arg91LysfsTer14/p.Arg206Trp | p.Arg91LysfsTer14/p.Arg91LysfsTer14 | p.Arg91LysfsTer14/p.Arg206Trp | p.Arg91LysfsTer14/p.Arg206Trp |
| Initial B12 treatment | 1 mg/day, 5 days/week | 1 mg/day, 7 days/week | 1 mg/day, 7 days/week | 1 mg/day, 7 days/week | 1 mg/day, 7 days/week |
| Initial betaine treatment | 250 mg/Kg/day | 250 mg/Kg/day | 250 mg/Kg/day | 200 mg/Kg/day | 200 mg/Kg/day |
| Other initial treatment | Folate: 5 mg/day<br>Carnitine: 150 mg/Kg/day | Folate: 5 mg/week | Folate: 5 mg/week | Folate: 5 mg/week<br>Carnitine: 100 mg/Kg/day | Folate: 5 mg/week |
| Additional treatment | Oxcarbazepine, risperidone, melatonin | | Lamotrigine, topiramate, oxcarbazepine | Eculizumab | |
| Clinical course | Global delay, microcephaly, able to walk at 4 years, able to use some words, normal eating, sphincter control. At the age of 15, difficulty in walking due to spasticity, tendon surgery, possible stroke (imaging not performed), wheelchair bound, no sensory perception below waist, constipation. Post-surgery epileptic seizures, behavior disorder, aggressiveness, sleep disorder. | Global delay, able to walk before 3 years, no sphincter control. Sensory deficits, vomits easily, eats only pureed foods, unable to swallow small pieces. Ataxia, dysmetria. Dysarthria. Axial hypotonia, peripheral hypertonia. Attention deficits | Global delay, epilepsy, axial hypertonia, peripheral hypertonia, no walking, no sphincter control. Normal eating. Very limited verbal communication. | Slight global delay, Transient heart hypertrophy, Normal muscle tone and tendon reflexes. Transient hypertransaminasemia | Normal growth and development. Slight upper arm hypertonia resolved with physiotherapy. Transient hypertransaminasemia |
| Ophthalmologic examination just before B12 dose increase | Extremely limited cooperation. He can fix and follow. No strabismus, no nystagmus, no maculopathy. | Reduced vision, horizontal pendular nystagmus, bilateral maculopathy. | Reduced vision, strabismus, nystagmus, bilateral maculopathy. | Limited cooperation. Alternating variable esotropia, small horizontal nystagmus, macular changes in both eyes. | Normal |
| Age at gradual dose increase | 25 years | 7.5 years | 5.5 years | 9 months | 4 months |
| Current B12 dose (in brackets is the total weekly dose divided per weight and day) | 0.14 mg/Kg/day, 5 days/week<br>(0.10 mg/Kg/day) | 0.46 mg/Kg/day, 6 days/week<br>(0.39 mg/Kg/day) | 0.45 mg/Kg/day, 6 days/week<br>(0.39 mg/Kg/day) | 0.81 mg/Kg/day, 6 days/week<br>(0.69 mg/Kg/day) | 0.65 mg/Kg/day, 7 days/week<br>(0.65 mg/Kg/day) |
| Current maximum daily B12 dose | 10 mg | 10 mg | 10 mg | 10 mg | 6 mg |
| Current betaine dose | 84 mg/Kg/day | 250 mg/Kg/day | 250 mg/Kg/day | 206 mg/Kg/day | 213 mg/Kg/day |
| Perceived improvement after treatment intensification | Better sleep and calmer, happier disposition is reported. | Increased stability and movement control. Improvement of ataxia. Improved speech. | Improved axial tone, able to walk a few steps. Better coordination and ability to express herself is reported. No epilepsy is reported. | Good progress in motor and mental abilities is reported. Able to walk at 18 months. | Normal development. Able to walk at the age if 15 months. |

(*continued on next page*)

**Table 1** (*continued*)

| Patient | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Ethnic origin | Greek Cypriot | Greek Cypriot | Greek Cypriot | Greek Cypriot | Greek Cypriot/Greek |
| Cognitive functions | Increased concentration span. | Increased concentration span. He can make long sentences, tell a story, make and respond to jokes. | She attends special education school. Increased vocabulary. She does not form phrases. Short concentration span. | Improved speech and vocabulary. Articulation problems. Increased concentration span. He could make a two-word phrase at 2.5 years. He can tell a story. | Able to speak more than 10 words at the stage of 18 months. Not able to make a two-word phrase. |
| Opththalmologic examination after B12 dose increase | Not performed | Stable findings | Stable findings | Stable findings | Normal examination. Normal visual evoked potentials. |

Abbreviations: B12: hydroxocobalamin [1 mg/1 ml Accord Healthcare (UK), used for doses up to 2 mg; Megamibedoce 10 mg/2 ml AristoPharma Iberia S. L. (Spain), used for doses from 3 to 10 mg], NR: normal range, C3: propionylcarnitine, C4DC: methylmalonylcarnitine, aHUS: atypical hemolytic uremic syndrome.

## Data availability

Data will be made available on request.

## References


[1] M. Huemer, D. Diodato, D. Martinelli, et al., Phenotype, treatment practice and outcome in the cobalamin-dependent remethylation disorders and MTHFR deficiency: data from the E-HOD registry, J. Inherit. Metab. Dis. 42 (2) (2019) 333–352.

[2] D. Watkins, D.S. Rosenblatt, Inborn errors of cobalamin absorption and metabolism, Am. J. Med. Genet. C: Semin. Med. Genet. 157C (1) (2011) 33–44.

[3] J.P. Lerner-Ellis, J.C. Tirone, P.D. Pawelek, et al., Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type, Nat. Genet. 38 (1) (2006) 93–100.

[4] L. Arhip, N. Brox-Torrecilla, I. Romero, et al., Late-onset methylmalonic acidemia and homocysteinemia (cblC disease): systematic review, Orphanet J. Rare Dis. 19 (1) (2024) 20.

[5] H.C. Andersson, M. Marble, E. Shapira, Long-term outcome in treated combined methylmalonic acidemia and homocystinemia, Genet. Med. 1 (4) (1999) 146–150.

[6] A.N. Adhikari, R.C. Gallagher, Y. Wang, et al., The role of exome sequencing in newborn screening for inborn errors of metabolism, Nat. Med. 26 (9) (2020) 1392–1397.

[7] J.D. Weisfeld-Adams, M.A. Morrissey, B.M. Kirmse, et al., Newborn screening and early biochemical follow-up in combined methylmalonic aciduria and homocystinuria, cblC type, and utility of methionine as a secondary screening analyte, Mol. Genet. Metab. 99 (2) (2010) 116–123.

[8] K. Guo, X. Zhou, X. Chen, et al., Expanded newborn screening for inborn errors of metabolism and genetic characteristics in a Chinese population, Front. Genet. 9 (2018) 122.

[9] W. Zhou, H. Li, C. Wang, et al., Newborn screening for Methylmalonic Acidemia in a Chinese population: molecular genetic confirmation and genotype phenotype correlations, Front. Genet. 9 (2018) 726.

[10] T. Georgiou, P.P. Petrou, A. Malekkou, et al., Inherited metabolic disorders in Cyprus, Mol. Genet. Metab. Rep. 39 (2024) 101083.

[11] J.P. Lerner-Ellis, N. Anastasio, J. Liu, et al., Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations, Hum. Mutat. 30 (7) (2009) 1072–1081.

[12] C. Nogueira, C. Aiello, R. Cerone, et al., Spectrum of MMACHC mutations in Italian and Portuguese patients with combined methylmalonic aciduria and homocystinuria, cblC type, Mol. Genet. Metab. 93 (4) (2008) 475–480.

[13] S. Fischer, M. Huemer, M. Baumgartner, et al., Clinical presentation and outcome in a series of 88 patients with the cblC defect, J. Inherit. Metab. Dis. 37 (5) (2014) 831–840.

[14] C.F. Morel, J.P. Lerner-Ellis, D.S. Rosenblatt, Combined methylmalonic aciduria and homocystinuria (cblC): phenotype-genotype correlations and ethnic-specific observations, Mol. Genet. Metab. 88 (4) (2006) 315–321.

[15] E. Richard, A. Jorge-Finnigan, J. Garcia-Villoria, et al., Genetic and cellular studies of oxidative stress in methylmalonic aciduria (MMA) cobalamin deficiency type C (cblC) with homocystinuria (MMACHC), Hum. Mutat. 30 (11) (2009) 1558–1566.

[16] N. Carrillo-Carrasco, R.J. Chandler, C.P. Venditti, Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management, J. Inherit. Metab. Dis. 35 (1) (2012) 91–102.

[17] D. Frattini, C. Fusco, V. Ucchino, et al., Early onset methylmalonic aciduria and homocystinuria cblC type with demyelinating neuropathy, Pediatr. Neurol. 43 (2) (2010) 135–138.

[18] G. Olivieri, B. Greco, S. Cairoli, et al., Improved biochemical and neurodevelopmental profiles with high-dose hydroxocobalamin therapy in cobalamin C defect, J. Inherit. Metab. Dis. (2024).

[19] E. Scalais, C. Geron, C. Pierron, et al., Would, early, versus late hydroxocobalamin dose intensification treatment, prevent cognitive decline, macular degeneration and ocular disease, in 5 patients with early-onset cblC deficiency? Mol. Genet. Metab. 140 (3) (2023) 107681.