FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

RAFAEL CORREIA BARÃO

Spectrum of Ophthalmological Manifestations of Early-Onset Cobalamin C Deficiency

ARTIGO CIENTÍFICO ORIGINAL

ÁREA CIENTÍFICA DE OFTALMOLOGIA

Trabalho realizado sob a orientação de:

EDUARDO JOSÉ GIL DUARTE SILVA

MARÇO/2017

SPECTRUM OF OPHTHALMOLOGICAL MANIFESTATIONS OF EARLY-ONSET COBALAMIN C DEFICIENCY

Artigo Científico

R. Barão[1], E. Silva[2]

[1]Aluno do Mestrado Integrado em Medicina, Faculdade de Medicina, Universidade de Coimbra, Portugal

(rc_barao@hotmail.com)

[2]Professor Auxiliar de Oftalmologia, Faculdade de Medicina, Universidade de Coimbra, Portugal

(esilva2579@hotmail.com)

Trabalho final do 6º ano médico com vista à atribuição do grau de mestre no âmbito do ciclo de estudos do Mestrado Integrado em Medicina

Área científica: Oftalmologia

ABSTRACT

Introduction: Cobalamin C deficiency is an autosomal recessive disorder of intracellular vitamin B12 metabolism caused by *MMACHC* gene mutations that leads to combined methylmalonic aciduria and homocystinuria. The majority of patients have early-onset disease with severe ophthamological manifestations as well as neurological impairment and other systemic manifestations.

Purpose: To explore and detail the ophthalmological phenotype of a series of early-onset cobalamin C deficiency patients.

Design: retrospective case series.

Materials and methods: Seven patients were diagnosed at an early age using biochemical and genetic testing and followed at the Centre of Neurodevelopment and Metabolic Diseases at the Hospital Pediátrico de Coimbra and the Ophthamology Clinic of Centro Cirúrgico de Coimbra. Ophthamological examination included best-corrected visual acuity, slit-lamp and dilated fundus examination, wide-field retinal photography, ERG, OCT and FAF imaging.

Results: All patients were diagnosed in the neonatal period and were homozygous for the c.271dupA (p.R91KfsX14) mutation. All presented with neurological impairment, developmental delay and exhibited ophthalmological findings. Age at initial ocular examination ranged from 2 to 6 months. Decreased visual acuity, nystagmus, and maculopathy were universal, while strabismus, peripheral retinal pigmentary changes, optic pallor and vascular changes were frequent. All patients who were followed past two years of age developed severe macular atrophic lesions, such as bull's eye or macular coloboma-like lesion. FAF changes include macular hypoautofluorescence and variable islands of, hyper/hypoautofluorescence that tend to progress with disease severity. OCT scans showed retinal thinning and variable layer disorganization. ERG results were highly variable. Optimal

metabolic control was not achieved in any of the patients, although there were some improvements in patients receiving adequate therapy.

Conclusions: All patients suffered from early and progressive macular and/or peripheral retinal degenerative changes and neurological impairment despite early treatment and some metabolic improvement. Early-onset maculopathy, particularly when severe atrophic changes are present, may be a valuable clue to the diagnosis of cobalamin C deficiency, especially in settings where newborn screening is unavailable. Since current proposed treatment regimen is unable to prevent ocular disease and neurological manifestations, new therapeutic approaches are in need.

ABBREVIATIONS

ADHD: Attention deficit hyperactivity disorder

BCVA: Best-corrected visual acuity

CblC: Cobalamin C

CCC: Centro Cirúrgico de Coimbra

CNMD: Centre of Neurodevelopment and Metabolic Diseases

ERG: Electroretinography

FAF: Fundus autofluorescence

Hcy: Homocysteine

HPC/CHUC: Hospital Pediátrico de Coimbra/Centro Hospitalar e Universitário de Coimbra

Met: Methionine

MMA: Methylmalonic acid

MRI: Magnetic ressonance imaging

NBS: Newborn screening

OCT: Optical coherence tomography

OHCbl: Hydroxocobalamin

INTRODUCTION

Cobalamin C (cblC) deficiency (OMIM #277400) is a autosomal recessive disorder of intracellular vitamin B12 metabolism[1,2], due to mutations in the *MMACHC* gene (OMIM *609831) on chromosome 1p34.2[3], leading to impaired conversion of vitamin B12 into its active forms, adenosylcobalamin and methylcobalamin, which are co-factors for the enzymes methylmalonyl-CoA mutase and methionine synthase, respectively. This results in combined methylmalonic aciduria and homocystinuria with hypomethioninemia. Despite previous estimations of incidence of approximately 1:200,000 live births, recent newborn screening (NBS) studies revealed an incidence of 1:100,000 in New York State[4], 1:60,000 in California, 1:37,000 in Hispanic populations[5] and 1:85,000 in Portugal[6], confirming that cblC deficiency is panethnic and the most common inborn error of intracellular cobalamin metabolism, comprising around 80% of cases[2].

Although there are more than 50 known *MMACHC* gene mutations causing the disease, the most common genotype is homozygous c.271dupA mutation, prevalent in approximately 40% of cases and almost universally associated with early-onset disease (< 1 year of age), while the most prevalent mutations in late-onset cases (> 1 year of age) is the homozygous c.394C>T mutation, apparently protective from early-onset disease. In compound heterozygote genotypes, age onset is harder to predict[3,7–10]. In Portugal, the c.271dupA allele is also the most frequently found (77% of alleles)[6].

Diagnostic investigations are usually undertaken after NBS – when available – reveals elevated C3-carnitine levels, low C0-carnitine, low methionine (Met) levels and elevated C3-carnitine/C0-carnitine and C3/Met raios[4–6], or after clinical suspicion arises. An immediate analysis of urine organic acids, plasma methylmalonic acid (MMA), plasma homocysteine (Hcy) and Met levels, as well as other aminoacids and an acylcarnitine profile is usually performed[11]. The diagnosis is strongly suggested by elevated urinary or plasma MMA levels

and elevated plasma Hcy, combined with reduced plasma Met levels. Confirmation of diagnosis requires either a fibroblast complementation study or genetic testing, the latter being the preferred modality because it is more cost-effective, less time consuming and allows adequate genetic counseling[11]. Prenatal diagnosis is also feasible through metabolic measurements and genetic testing[12–14].

Clinical heterogeneity is a known characteristic of cblC deficiency. Two classical phenotypes are described, early-onset and late-onset, which differ in age of onset, systemic manifestations and neurological impairment, and also in long-term outcome and treatment. A prenatal presentation is also described, manifesting as intrauterine growth retardation, dysmorphic features, microcephaly and, ocasionally, congenital heart disease and fetal dilated cardiomyopathy[10,11,15–17]. Early-onset disease is the most common phenotype of the disease, representing 86-88% of cases[18–20]. It is often severe and typically manifests as failure to thrive, feeding difficulties, hypotonia, acidosis, microcephaly, seizures, developmental delay, cognitive impairment, behavioural disturbances (such as hyperactivity or autistic traits), ocular disease and hematological abnormalities, such as thrombocytopenia, macrocytic anemia and leukopenia[18–25].

Ophthalmologic manifestations in early-onset patients are multiple, often severe, and are present with variable frequency. These include nystagmus (45%-71%[18,26,27]), strabismus (23%-52%[18,26,27]), maculopathy (60%-72%[26,27]), pigmentary retinopathy (15-21%[18]), optic atrophy (25-68%[18,26–28]), vascular changes (64%[27]), decreased visual acuity and refractive errors[26–37]. Early signs of eye disease are nystagmus, strabismus or inability to fixate[26,27,37]. Maculopathy often starts as subtle macular pigmentary or atrophic changes which may progress to bull's eye maculopathy (a hypopigmented perimacular zone surrounded by a hyperpigmented ring) and/or macular coloboma-like lesions[27–29,37,38]. These macular changes may expand outwards to involve the mid-peripheral regions of the retina, in variable fundoscopic patterns, and can result in end-stage retinal degeneration. Fundus

autofluorescence (FAF) imaging can help better define these retinal lesions, typically showing peripheral retina sparing, rings of hyperautofluorescence surrounding areas of markedly reduced autofluorescence (atrophic areas), indicating that photoreceptor degeneration coexists with pigmentary changes[27,37]. One frequent finding of unknown significance but common to other retinal degenerative conditions is relative preservation of peripapillary autofluorescence, which is also observed in Stargardt disease. Electroretinography (ERG) frequently shows variable progressive reduction and delay of scotopic and/or photopic responses, reflecting both rod and/or cone dysfunction[27–30,32,33,35–38], which arguably better correlate with visual prognosis than fundus findings[26]. Optical coherence tomography (OCT) findings may show foveal thinning, loss of laminar structure, abnormal retinal nerve fibre layer thickening, photoreceptor loss, and severe parafoveal outer nuclear layer and ganglion cell loss[27,29,38]. Optic atrophy (25%-68%[18,26,27]) may be diffuse or predominantly temporal[27] and may be present without significant retinal disease[34,39]. Retinal vascular changes (64%[27]), when present, include increased tortuosity and/or decreased vascular caliber[27].

Other important manifestations of early-onset disease include renal thrombotic microangiopathy[40] and hemolytic-uremic syndrome[18,19,22,40,41], while demyelinating neuropathy[42], subacute degeneration of the spinal cord[43], atrophic gastritis[18,22], cardiomyopathy[15,18,22], *cor pulmonale*[15], pulmonary hypertension[40] and protein-losing enteropathy[44] are more rare.

In late-onset disease, presenting in childhood or adolescence, neurological and psychiatric disturbances are predominant[18,20,24,45–47]. Thromboembolic complications can also occur in a significant portion of cases[18,40,47,48]. Global clinical picture is less severe than in early-onset cases and ophthalmological manifestations are rare[18,19,27,33]. To our knowledge, only one case of maculopathy in late-onset disease has been described[49]. Long-term outcome in early-onset cases is unfavorable despite treatment, with significant morbidity, and mortality between 11% and 30%, while late-onset cases have more favorable outcome[18,19].

Neurorradiological findings can include cortical and corpus callosum atrophy, white matter abnormalities, basal ganglia lesions and hydrocephalus and these findings are typically being more severe in early-onset cases. Although some neurological abnormalities can affect visual performance, there appears to be no strict correlation between magnetic resonance imaging (MRI) findings and visual performance[18,25,50–52].

Treatment aims to improve manifestations and normalize Met, Hcy and MMA levels. Treatment and metabolic control reduces mortality, improves growth and clinical picture, particularly in systemic non-neurological and non-opththalmological manifestations[11,18,19], although some neurological manifestations may improve[47,53–55]. It is, however, unable prevent developmental delay, cognitive impairment[18,23] or eventual progression of retinal disease or optic atrophy[26–29,32–34,36]. Current treatment regimens consists of hydroxocobalamin (OHCbl; and not cyanocobalamin[56]), betaine, folinic acid and L-carnitine[11,57,58]. There is evidence that OHCbl dose escalation is beneficial in metabolic control[54,59] and that reducing frequency of administration to less than daily dosing leads to sub-therapeutic levels which worsens metabolic control and neurological deterioration[11,55]. A low-protein diet is not recommended if B12 levels are in the therapeutic range, because it may accentuate hypomethioninemia leading to clinical deterioration[11,16,33,60].

Follow-up includes serial measurements of plasma or urine MMA, plasma Hcy and Met levels and also plasma B12 levels in order to confirm that B12 levels achieve the therapeutic range, echocardiographic screening and regular metabolic and ophthalmological examination[4,11,26].

In this study we aim to describe the spectrum of ophthalmological manifestations and their progression over time in seven portuguese caucasian patients with early-onset cblC deficiency.

MATERIALS AND METHODS

Study design

This was a retrospective case series study which included patients from the Centre of Neurodevelopment and Metabolic Diseases (CNMD) at Hospital Pediátrico de Coimbra (HPC/CHUC). All individuals, their parents or legal guardians, included in the study were informed about its objectives and volunteered to participate. Informed consent was obtained from all subjects, their parents or legal guardians in accordance with the tenets of the declaration of Helsinki. The study was approved by the local Ethics Committee.

Study subjects

Seven patients with cblC deficiency were identified through the medical records of the CNMD at HPC/CHUC. Three patients were male and 4 were female. Two individuals (patients 6 and 7) were homozygous twins. All other individuals were unrelated.

Diagnosis was confirmed in all patients through molecular genetic testing prior to this study. Six patients underwent molecular testing after NBS suggested the disease. Patient 1 was not screened at birth because NBS for cblC deficiency was not available at the time. This patient initially underwent fibroblast complementation studies after clinical suspicion arose during the neonatal period, confirming the diagnosis, and post-mortem molecular testing once the causative genetic defect for cblC deficiency was identified.

All affected individuals had been previously screened for cardiovascular findings through echocardiography and for developmental delay and cognitive impairment using repeat testing with Griffiths, WPPSI-R and WISC-III scales, according to age. MRI scans were already available in three patients; however, neurological imaging testing is outside the scope of the present work. Metabolic assessment was performed in accordance with international guidelines and was already available in the medical records.

Study protocol

Affected individuals were followed at CNMD and the Ophthalmology Clinic of Centro Cirúrgico de Coimbra (CCC). Ophthalmological observations were requested by attending pediatricians after metabolic or molecular diagnosis of cblC deficiency. Patients who did not have a recent eye examination were invited to return for a repeat visit.

Best-corrected visual acuity (BCVA) was tested in a manner appropriate for the patient's age and developmental ability with the use of Teller Preferential Looking Cards, Modified Teller Acuity Cards, or Snellen Acuity Charts. Ophthalmological examination included assessment of BCVA after manifest or cycloplegic refraction, ocular alignment and motility, slit-lamp examination, fundus dilated examination using a non-contact 78-diopter lens or a 20-diopter lens with indirect ophthalmoscopy. Wide-field fundus images acquired in accordance with the International accepted guidelines using a Optos ultrawide-field retinal imaging device (Optos PLC, Dunfermline, Scotland, UK). Very young individuals were examined under anesthesia, whenever necessary.

Ganzfeld electroretinography (ERG) was performed in accordance with the ISCEV (International Society for Clinical Electrophysiology of Vision) guidelines. In brief, patients were dark-adapted for a period of 30 minutes followed by scotopic assessment. The ERG was then completed with recordings obtained in photopic conditions.

Optical coherence tomography (OCT) scans were performed using an OCT device (Stratus OCT; Carl Zeiss Meditec, Dublin, CA; or Spectral domain OCT, Heidelberg Engineering, Dossenheim, Germany) to obtain cross-sectional images centered in the macula, 26 with axial resolution of 10 μm or less, transversal resolution of 20 μm, and longitudinal scan range of 2 mm. With this OCT device (Stratus OCT; Carl Zeiss Meditec), six radial line scans 6 mm in length and 128 A-scans 30° apart were scanned in 1.92 seconds, and a nine-region retinal thickness map was obtained by segmenting the retina from other layers with an

algorithm detecting the edge of the retinal pigmentary epithelium (RPE) and the photoreceptor layer.

FAF imaging was performed in selected cases using the HRAII device (Heidelberg Engineering, Dossenheim, Germany) or Optos PLC (Dunfermline, Scotland, UK) in accordance with the instructions from the manufacturer.

Current proposed treatment consists of daily parenteral OHCbl (0.33mg/kg/day; in order to achieve plasma B12 levels over 1,000,000 pg/ml), daily oral betaine (250mg/kg/day divided in 2 or 3 doses), daily oral L-carnitine (50-200mg/kg/day divided in 3 doses) and daily folinic acid (5-15mg in 2 or 3 doses), without routine protein restriction diet[11]. All patients received early treatment (within 1 week from NBS findings or clinical suspicion in case of patient 1) but regimens varied widely across patients, both in daily dosage and frequency of administrations. Ocular disease progression will be assessed to evalute the individual therapeutic adequacy of regimes.

RESULTS

A total of seven patients had regular metabolic and ophthalmological consults (yearly or more frequently). Ages at most recent observation ranged from 21 months in the case of patient 1, who died at 23 months of age, to 10 years in the case of patient 2.

All patients in our group had onset of disease within the first month of life. Metabolic acidosis, hypotonia and feeding difficulties were universal findings at onset. Patient 1 was lethargic, developed seizures and had neutropenia, macrocytic anemia and thrombocytopenia. Onset of disease in patient 4 was complicated by pneumonia and sepsis. Developmental delay and failure to thrive in the first weeks of life were present in all patients. Most acute findings at onset improved rapidly with early treatment.

Neonatal screening in patients 2 through 7 revealed high C3-carnitine levels, low C0-carnitine and Met levels, and increased C3/Met and C3/C2 ratios, prompting metabolic assessment for cblC deficiency. Patient 1 underwent initial metabolic assessment after clinical suspicion arose since NBS was not available at the time. Elevated urinary MMA (average: 2183; range: 73-10000; normal: 0-6.2 μmol/mmol creatinine) and plasma Hcy levels (average: 160; range: 121-209; normal: 5-15 μmol/L), as well as low plasma Met levels (average: 5; range: 4-7; normal: 11-33 μmol/L), which strongly suggest cblC deficiency, were detected soon after onset in all patients. Molecular testing was performed in all patients, showing homozygous c.271dupA (p.R91KfsX14) mutation of *MMACHC* gene. Age at onset, genotype and summarized clinical findings for all patients are detailed in table 1.

Table 1. Age at diagnosis, genotype and summary of clinical findings.

Patient #	Genotype (exon/type of mutation)	Gender (Female/Male)	Age at diagnosis months	Age at study enrollment years/months	Clinical findings					MRI findings
					Ophthalmological*	Hematological	Neurological	CV	Other	
1	c.271dupA p.R91KfsX14	M	<1m	23m†	+	Macrocytic anemia, neutropenia and thrombocytopenia (resolved) NN Anemia	Development/Speech delay	Dysmorphic MV and TV	PLE	n/r
2		M	<1m	10y	+	-	Development/Speech delay	-	-	n/r
3		F	<1m	6y10m†	+	-	Development/Speech delay Wide-based gait Epilepsy	-	Type 1 Diabetes mellitus	WMA VW CA
4		F	<1m	5y	+	-	Development/Speech delay Poor hand-eye coordination	PFO LVH (resolved)	-	n/r
5		M	<1m	2y	+	-	Development/Speech delay Poor hand-eye coordination	ASD-OS (resolved) TAA	-	n/r
6		F	<1m	6y6m	+	-	Development/Speech delay ADHD	-	-	MD VW CCA CA
7		F	<1m	6y6m	+	-	Development/Speech delay ADHD	-	-	MD VW CCA CA
TOTAL		4F, 3M	<1m	n/a	7/7	1/7	7/7	3/7	1/7	3/7

† = deceased; n/r = not reported; CV = cardiovascular; MRI = magnetic resonance imaging; NN = normocytic and normochromic; WMA = white matter abnormalities; VW = ventricular widening; CA = cortical atrophy; MD = myelination delay; CCA = corpous callosum atrophy; MV = mitral valve; TV = tricuspid valve; PFO = patent foramen ovale; LVH = left ventricular hypertrophy; ASD-OS = atrial septum defect, ostium secundum type; TAA = tortuous aortic arch; PLE = protein-losing enteropathy; * = see text, tables 3 and 4 for detail.

12

None of the patients reached complete metabolic control (Table 2). All patients showed elevated average urinary MMA levels (average; 153; range: 48-485 µmol/mmol creatinine) and plasma Hcy levels (average: 55; range: 28-94 µmol/L). Plasma Met levels (average: 30; range: 9-56 µmol/L) responded well to therapy in patients 2-7 (range of average levels in these patients: 22-56), even exceeding the upper limit of normal in patients 5, 6 and 7. There was no significant improvement in Met levels in patient 1. Plasma B12 levels were only assessed in patients 2 and 4, both revealing levels over therapeutic objective. Average and last recorded values of metabolic parameters, as well as adequacy of therapeutic regimes, are detailed in table 2.

Table 2. Metabolic control during follow-up and last consults and adequacy of therapeutic regimen.

| Patient # | Metabolic control* | | | | | | | Therapeutic regimen adequacy§ | | | | |
| | Urine MMA, µmol/mmol Cr (N: 0-6.2) | | Plasma Hcy, µmol/L (N: 5-15) | | Plasma Met, µmol/L** (N: 11-33) | | Plasma B12, pg/ml*** | | | | | |
	Avg.	Last	Avg.	Last	Avg.	Last	Last	OHCbl	Betaine	L-Carnitine	Folinic acid	Protein restriction diet
1	151	254	39	30	9	5	n/r	-	-	-	-	Yes
2	85	184	55	39	27	**34**	> 1M	-/+	-/+	-/+	-/+	Yes/No
3	485	155	37	33	27	**42**	n/r	-/+	-/+	-/+	-/+	Yes/No
4	161	25	28	32	22	12	> 1M	+	+	+	+	No
5	48	71	39	29	**56**	104	n/r	+	+	+	+	No
6	63	72	94	40	**37**	7	n/r	+	+	+	+	No
7	78	94	90	43	29	11	n/r	+	+	+	+	No
TOTAL	Average							n/a				
	153	122	55	35	30	31						

MMA = methylmalonic acid; Cr = creatinine; Hcy = homocysteine; Met = methionine; N = normal; B12 = vitamin B12; Avg. = average M = million; OHCbl = hidroxycobalaimn; n/r = not reported; n/a = not applicable; * = shading indicates average values out of normal range indicating suboptimal metabolic control; ** = bold indicates Met values over normal range; *** = therapeutic objective is > 1M pg/ml; § = adequacy of therapeutic regimen in accordance with proposed regimen[11], considering daily dosage and/or frequency of administrations; - = inadequate; -/+ = initially inadequate but later corrected; + = adequate throughout follow-up; Yes/No = protein restriction diet initially prescribed but later discontinued.

Ophthalmological manifestations (Table 3)

All patients had their first ophthalmological observation before age 6 months (range: 2-6 months) and were regularly (yearly or more frequently) followed. BCVA was decreased in all patients. With the exception of patient 1, all individuals had BCVA for near ranging from 20/50 to 20/100, and for distance from 20/100 to 20/600 at the time of last consult. Patient 1 died at age 23 months and medical records only report that he was reactive to light stimulation and presented an overlooking pattern of fixation. Manifest nystagmus was present in all patients except patient 2 at first consult but was universal at last consult. Strabismus was present in one patient at first examination and in 4 at last examination. Patients 3, 6 and 7 had esotropia and patient 4 had exotropia.

All patients, during the course of their disease, developed visible maculopathy at fundus examination. Age of first report of maculopathy ranged from four months in patient 1 to three years in patient 3, who was the only patient in our group to develop macular changes after the first year of life. In patients 5, 6 and 7, maculopathy initially presented as pigmentary changes. In patient 5, these changes remained stable up to last consult at two years of age. In the case of patients 6 and 7 maculopathy progressed to bilateral macular coloboma-like lesions in the third year of life (Fig. 1A-D).

In patients 1, 2 and 3 maculopathy presented as atrophic changes at first report. These changes were initially mild in patient 2 but rapidly progressed to bull's eye maculopathy at 9 months of age (the only patient in our group in which we observed this lesion) and to bilateral macular coloboma-like lesions in the third year of life (Fig. 1E-F). In contrast, initial atrophic changes were marked in patients 1 and 3, with the former presenting as macular atrophy as early as age 4 months, and the latter presenting with bilateral coloboma-like lesions at first report (three years of age). Maculopathy in patient 4 presented as mixed atrophic and

pigmentary changes and also progressed to macular coloboma-like lesions in the second year of life.

Retinal pigmentary changes were visible in all patients except patient 5. All affected patients had predominantely mid-peripheral changes, exhibiting diffuse, granular or salt-and-pepper patterns, which remained stable during the course of the disease, except in patient 1, whose retinal changes worsened dramatically to a generalized depigmented appearance. Onset of retinal pigmentary changes was early for patients 1, 2 and 3 (before age 9 months), while in patients 4, 6 and 7 these changes appeared in the second year of life.

Optic atrophy was noted in 5 of 7 patients. Patients 1 and 5 presented with optic pallor at first observation, while patients 3, 6 and 7 developed optic pallor later in the disease. Pallor was diffuse in patients 1, 5, 6 and 7, and predominatly temporal in patient 3. In patients 3, 5, 6 and 7 these changes remained stable throughout the course of the disease. In contrast, optic pallor in patient 1 worsened significantly.

Retinal vascular changes were present 4 of 7 patients. Patient 1 and 2 had slight vascular caliber reduction at first consult which progressed to marked vascular thinning. Patient 6 and 7 had no vascular changes at first consult but eventually exhibited slight decrease of retinal vascular caliber.

No anterior segment abnormalities were detected.

Figure 1. Fundus photographs. **A-D**, Patient 6. Fundus photos reveal bilateral macular coloboma-like lesions and mid-peripheral pigmentary changes along with diffuse optic pallor and reduced vascular caliber. **E,F**, Patient 2. Fundus photos discloses bull's eye maculopathy and mid-peripheral pigmentary changes. Retinal vessels are slightly thinned.

Table 3. Ophthalmological findings at onset and last examination.

Patient #	Age at first ophthalmological consult, months	Age at last ophthalmological consult, years/months	Manifest nystagmus 1st	Manifest nystagmus Last	Strabismus 1st	Strabismus Last	BCVA Near and Far at last visit	Retinal changes — Maculopathy (Atrophic or Pigmentary) First reported, years/months	Degree and type of change at first report	Bull's-eye maculopathy	Coloboma like lesion 1st	Coloboma like lesion Last	Peripheral pigmentary changes First reported, years/months	Peripheral pigmentary changes 1st	Peripheral pigmentary changes Last	Optic atrophy — Degree and Temporal or Diffuse pallor 1st	Optic atrophy Last	Vascular changes — Degree of Caliber reduction (↓) 1st	Vascular changes Last
1	4m	21m	+	+	-	-	n/a LP	4m	A++	-	-	-	4m	+	++	D+	D++	→	↓↓
2	2m	10y	-	+	-	-	20/50 N 20/600 F	9m	A+	+	-	+	9m	+	+	-	-	→	↓↓
3	2m	6y	+	+	-	+ ET	20/80 N 20/400 F	3y	A++	-	-	+	6m	+	+	-	T+	-	-
4	6m	5y	+	+	-	+ XT	20/50 N 20/400 F	6m	AP+	-	-	+	18m	-	+	-	-	-	-
5	4m	2y	+	+	-	-	20/60 N 20/100 F	5m	P+	-	-	-	n/r	-	-	D+	D+	-	-
6	2m	6y6m	+	+	+ ET	+ ET	20/80 N 20/600 F	10m	P+	-	-	+	2y	-	+	-	D+	-	→
7	2m	6y6m	+	+	-	+ ET	20/100 N 20/600 F	10m	P+	-	-	+	2y	-	+	-	D+	-	→
TOTAL	Avg 3m	Avg n/a	n 6	n 7	n 1	n 4	n/a	Avg 11m	n 7	n 1	n 0	n 5	Avg 14m	n 3	n 6	n 2	n 5	n 2	n 4

+ = present and mild/moderate; ++ = present and severe; - = absent; LP = light perception; n = number; n/a = not applicable; n/r = not reported; Avg = average; ET = esotropia; XT = exotropia; shading indicates presence of abnormal finding and darker shading indicates increased severity or worsening of finding.

Retinal imaging and electrophysiology

FAF imaging, OCT and ERG were performed in all patients except patients 1 and 5. FAF results were relatively uniform among tested patients, showing central hypoautofluorescence, preserved peripapillary autofluorescence, surrounded by an hyperautofluorescent ring (island-like geographical distribution) and normal autofluorescence of peripheral retinal fields. Patients 3, 6 and 7 showed more marked central hypoautofluorescence (Fig. 2A,B), while patient 2 showed increased peripapillary hyperautofluorescence (Fig. 2C,D). OCT scans revealed retinal thinning and variable retinal layer disorganization in all patients tested. Retinal thinning and layer disorganization was particularly severe in patient 3, while patients 2 and 4 showed mainly inner segment changes and patients 6 and 7 showed moderate disorganization of both inner and outer segments and bilateral posterior staphylomas (Fig. 3B-E).

ERG findings were highly variable. Scotopic and photopic response were mildly reduced in patients 2 and 3, normal in patient 4, and moderately reduced in amplitude but with normal timing patients 6 and 7. Combined response was normal in patient 2, mildly reduced in patients 3 and 4 but moderately reduced with slight delay in patients 6 and 7. Flicker response was normal in patients 2 and 3, mildly reduced in patient 4 and only mildly reduced in amplitude in patients 6 and 7. Table 4 details morphological and functional assessment of the retina at last consult. Retinal imaging was obtained in several consecutive examinations in some patients whenever disease status and collaboration allowed.
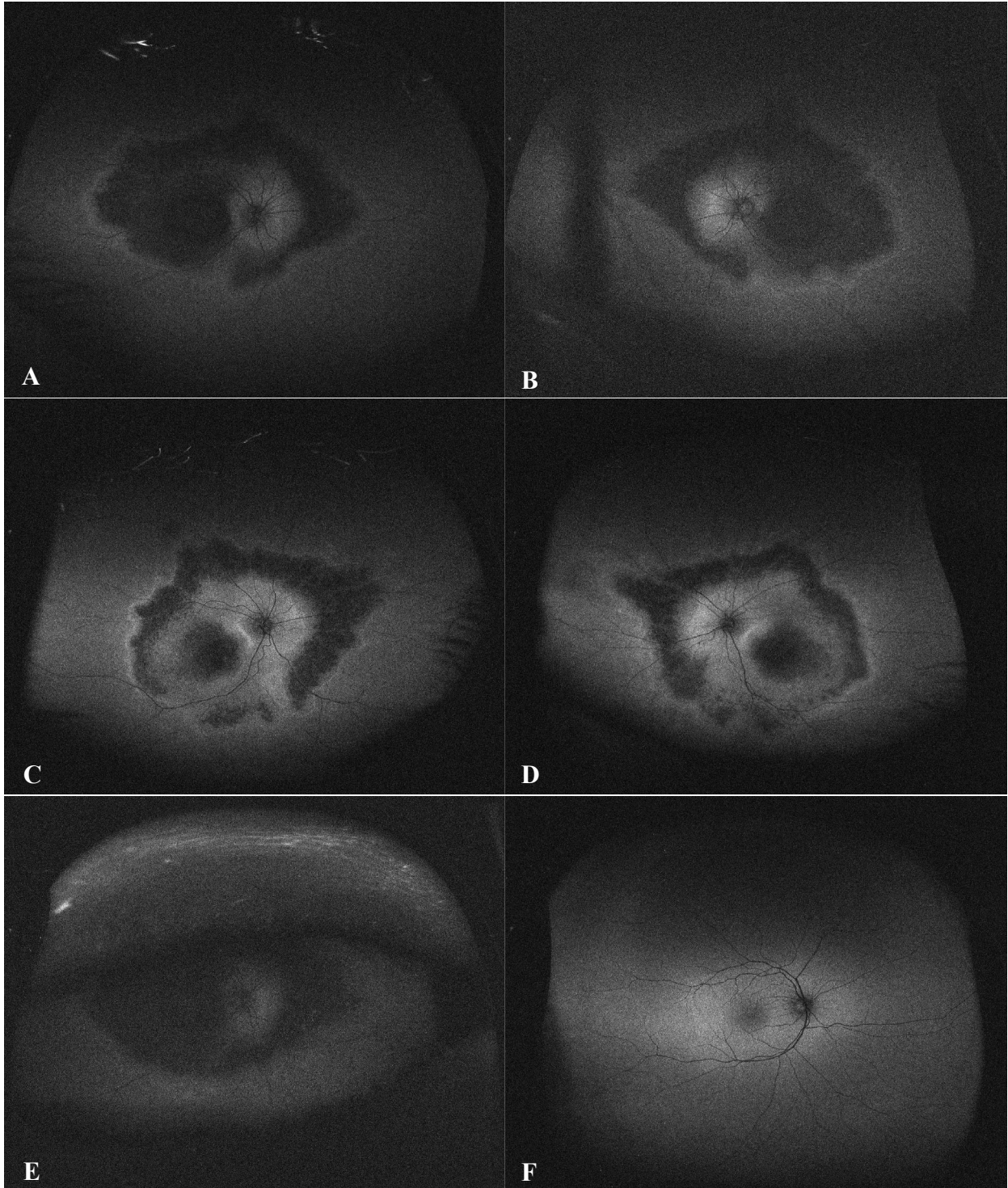
Figure 2. Fundus autofluorescence imaging. **A,B**, Patient 6. FAF reveals marked central hypoautofluorescence surrounded by a mid-peripheral ring of hyperautofluorescence of geographical distribution. Note relative preservation of peripapillary fluorescence and sparing of peripheral retinal fields. **C,D**, Patient 2. A similar pattern of autofluorescence is depicted, but with less marked central hypoautofluorescence and increased peripapillary autofluorescence. **E**, Patient 7. FAF shows overlap with images taken from patient 6 (A,B). **F**, FAF from normal age-matched subject.
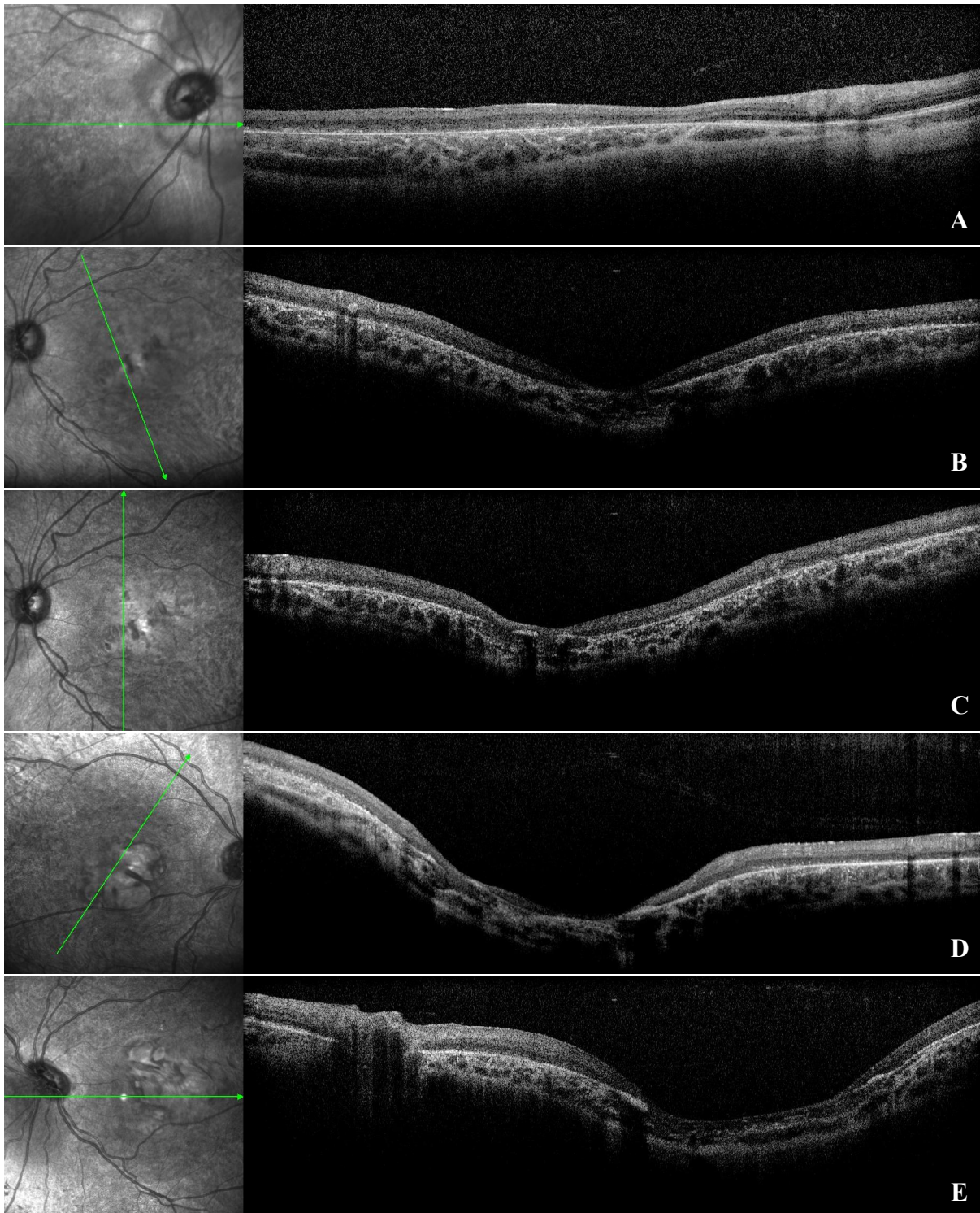
Figure 3. **A,** Patient 2. OCT shows retinal thinning and disorganization of outer layers. **B-D**, Patient 6. OCT illustrates complete neurosensory retina disorganization and depicts posterior staphyloma associated with coloboma-like lesion. **E**, Patient 7. OCT reflects a similar picture compared with Patient 6, although the retinal thickness of the papillomacular bundle seems more preserved.

Table 4. Summary of retinal imaging and ERG studies.

Patient #	FAF[1]	OCT[2]	ERG findings[3]			
			Scotopic response	Photopic response	Combined response	Flicker
1	n/a	n/a	n/a	n/a	n/a	n/a
2	Central ↓ Peripapillary ↑↑ PGD	Retinal thinning Inner segments changes	↓	↓	Normal	Normal
3	Central ↓↓ Peripapillary ↑ PGD	Retinal thinning ++ Disorganization all layers	↓	↓	↓	Normal
4	Central ↓ Peripapillary ↑ PGD	Retinal thinning Inner segments changes	Normal	Normal	↓	↓
5	n/a	n/a	n/a	n/a	n/a	n/a
6	Central ↓↓ Peripapillary ↑ PGD	Retinal thinning Disorganization of inner and outer structures Macular staphyloma	↓↓ Amplitude Normal timing	↓↓ Amplitude Slight delay	↓↓ Amplitude Slight delay	↓ Amplitude
7	Central ↓↓ Peripapillary ↑ PGD	Retinal thinning Disorganization of inner and outer structures Macular staphyloma	↓↓ Amplitude Normal timing	↓↓ Amplitude Slight delay	↓↓ Amplitude Slight delay	↓ Amplitude

1: ↓ = hypoautofluorescence; ↑ = hyperautofluorescence; the number of arrows indicate degree of change; PGD = peripheral geographic distribution; 2: ++ = severe change; 3: ↓ = mild reduction; ↓↓ = moderate reduction.

Other manifestations (Table 1)

Neurological manifestations were universal among all 7 patients. Developmental delay, cognitive impairment, speech delay and poor hand-eye coordination were the most common findings. Patient 3 displayed a wide-based gait and developed myoclonic epilepsy in the third year of life, which later progressed to overt epileptic encephalopathy. Patients 6 and 7 were diagnosed with attention deficit hyperactivity disorder (ADHD). MRI scans were performed on patients 3, 6 and 7. Periventricular white matter abnormalities, mild ventricular widening and cortical atrophy were seen patient 3, while myelination delay, lateral ventricle widening, corpus callosum atrophy and marked cortical atrophy were prominent in patients 6 and 7, with significant overlap.

Cardiovascular anomalies were detected in patients 1, 4 and 5 through prompt echocardiography screening. Patient 1 had dysmorphic mitral and tricuspid valves, mild tricuspid regurgitation and widening of aortic root. These findings were deemed not clinically significant. Patient 4 had a patent foramen ovale and hypertrophic left ventricle at 2 months of age. The latter finding resolved with treatment in a few months. Patient 5 had a restrictive atrial septal defect, detected at at 3 months of age, that closed at 17 months of age, and also a tortuous aortic arch, displaying turbulent flow, of little hemodynamic significance.

Hematological abnormalities in patient 1 at presentation were neutropenia, macrocytic anemia and thrombocytopenia. These initial abnormalities resolved rapidly with treatment but he developed normocytic normochromic anemia that was resitant to treatment. There was no record of development of cytopenias during the course of the disease of any other patient.

Patient 1 developed chronic diarrhea and hypoalbuminemia which were found to be caused by protein-losing enteropathy. Patient 3 was diagnosed with type 1 diabetes mellitus at age two.

DISCUSSION

CblC deficiency can affect multiple systems, including the visual and nervous systems, in spite of early treatment. Although significant ocular complications may be found in patients with cblC deficiency, particularly in early-onset disease, this disorder is poorly recognized by most ophthalmologists.

We present the largest cohort of early-onset cblC patients described in Portugal concerning the ophthalmological phenotype. Our study details the clinical course of seven patients aged 21 months to 10 years at last consult, summarizing clinical and biochemical data, with particular focus on the description ophthalmological manifestations over time. We were able to regularly follow the patients in our study from disease onset to apparent stabilization of ocular disease in some patients, which provides insight into the natural history and the impact of therapy in progression of ocular disease. However, some patients were too young at time of last consult to extrapolate data regarding natural history of the disease and long-term visual outcome.

All patients in our study were homozygous to the c.271dupA (p.R91KfsX14) mutation of *MMACHC* gene, which is in accordance to the high prevalence of this allele in Portugal[6]. Despite limiting our findings to the subset of patients with this genotype, we believe it strenghtens our findings as being representative of portuguese population and determines a genotypical homogeneity in our study. This mutation is known to be associated with severe ocular disease[26,27], as our study also supports.

Global clinical presentation was relatively homogeneous among patients. Onset with metabolic acidosis and acute neurological impairment was universal and, despite early diagnosis and treatment, all developed ophthalmological and neurological manifestations and some had cardiovascular abnormalities, which is in accordance to the reported incidence and prevalence of these manifestations in early-onset patients of cblC deficiency[15,18,21,22,26]. It

should be underscored that patient 1 developed chronic diarrhea and hypoalbuminemia which were found to be caused by protein-losing enteropathy and, to the best of our knowledge, this is only the second case ever reported of protein-losing enteropathy in association with cblC deficiency[44].

Ophthalmological findings are present very early in life in children with early-onset disease and, although they are variable, the progression of these ophthalmologic manifestations seems to lead to legal blindness within the first decade of life despite adequate treatment[21]. Ophthalmological manifestations were similar among patients in our study, despite their differences in therapy or metabolic control. This agrees with the claim that, although adequate treatment and metabolic control are desirable, adequate therapy has little effect in preventing ocular disease, nor does metabolic control strictly correlate with ophthalmological phenotype, as supported by the fact that some patients with relatively spared retinas are poorly controlled and other patients with controlled metabolic parameters may show generalized retinal degeneration[26–29,32–34,36].

All patients had low visual acuity, nystagmus and some form of maculopathy, and most developed strabismus and pigmentary retinopathy, with relative frequencies greater than reported in current literature[18,19,26–28,33], albeit ours is a small sample. Optic atrophy and vascular changes were also common. Maculopathy was heterogeneous and frank progression was invariably noted in patients who were followed past 2 years of age to bull's eye lesion and/or coloboma-like lesions. We believe that the fact that patients 1 and 5 did not develop these overt macular manifestations is related to the young age at last consult. Most patients who developed optic pallor did so after onset of maculopathy, and we did not observe significant progression of these findings in most patients. Vascular changes were present in over half of our patients and are of unknown significance. Those whose vascular changes worsened were the ones in which these manifestations developed early.

Whereas most maculopathies do not present until a later age, maculopathy described in patients with cblC occurs very early, likely starting antenatally[30,38] and progressing postnatally encompassing a critical period of foveal development[38]. Ophthalmological examination may be essential in suggesting the diagnosis of this systemic disease, because overt maculopathy in an infant - particularly when coloboma-like lesions are present - together with pigmentary retinopathy with or without optic atrophy, should suggest cblC deficiency, allowing for prompt treatment and avoiding unnecessary diagnostic testing. This is especially important in settings where NBS for this disease is not available.

FAF imaging showed typical findings of central hypoautofluorescence surrounded by ring of hyperautofluorescence of geographical distribution. These rings seem to behave as the progressively outwards expanding border of retinal degeneration. Also, peripapillay autofluorescence, which is initially relatively preserved, seems to fade with disease progression. OCT scans showed progressive retinal thinning and disorganization reflecting the direct negative impact of metabolite deficiency or byproduct accumulation, in retinal microanatomy. The presence of staphylomas superimposed on coloboma-like lesions, such as the ones we identified in patients 6 and 7, has only been reported once before[37], and is of unknown pathophysiological and clinical significance. ERG recording showed highly variable degree of scotopic and photopic response attenuation with no clear correlation with structural findings. Unfortunately we were unable to obtain serial ERG evaluations in most patients, which limits our ability to draw conclusions on the natural history of retinal function impairment.

OCT, serial retinal photography and FAF may prove essential in monitoring the progression of the disease and in seeking biochemical correlations which can be used to evaluate response to treatment and guide therapeutic approaches with likely implications in long-term outcome[26,38]. Electrophysiological testing, however, does not provide significant advantage in the identification of structural problems nor seems to significantly correlate with

disease progression. There seems to be a lack in functional testing, difficult to correlate with structural abnormalities. A surveillance framework for cblC deficiency patients has recently been suggested that may improve long-term outcome in these patients[26].

Management strategies for cblC deficiency, which consist mainly of parenteral OHCbl with betaine, folinic acid and L-carnitine, provide general health-benefits, are effective in lowering infantile mortality rate, and improve growth[11,18,19] and prenatal therapy may have a more significant impact in metabolic control and in improving patient outcome[14,34]. However, treatment usually fails to completely normalize Met, Hcy and MMA levels[19,20,23,54] and, as stated before, seems to have little effect in slowing the progression of ocular disease. Despite the fact that prenatal therapy may delay the progression of retinal disease[27,37], it does not prevent eventual retinal degeneration or optic atrophy[14,27,34,37].

Although all patients in our group started treatment early, only modest metabolic response was seen, with overall insufficient decreases in MMA and Hcy levels and increase of Met levels. Optimal metabolic control was not achieved in any of the patients, regardless of therapeutic regimen. However, methionine levels were demonstrably higher in patients with adequate therapy, often achieving or exceeding the upper limit of normal.

Methionine levels may be of special importance in ophthalmological outcome given that normalization of methionine levels may normalize ERG photopic responses[36]. Although the pathogenesis of retinal degeneration in cblC disease is poorly understood, one theory states that low methionine levels increases oxidative damage to the RPE due to reduced formation of reduced glutathione[35,36]. Moreover, methionine depletion may impair methylation capacity by reducing S-adenosylmethionine, resulting in myelination abnormalities that may account for optic atrophy[26]. Interestingly, patients isolated methylmalonic aciduria or homocystinuria do not present with hypomethioninemia and have no retinal or macular degeneration, which not only supports the claim that methionine levels

may paramount in retinal disease, but also makes it unlikely that high levels methylmalonic acid or homocysteine are responsible for retinal degeneration. However, other cobalamin deficiencies with low Met levels also do not present with ocular disease, which indicates that the role of methionine in retinal and macular degeneration may me more complex.

In our group, we found no clear correlation between treatment and the progression of macular and retinal disease. However, we observed that patients who were initially inadequately treated due to lack of proper guidelines, as was the case of patients 1, 2 and 3, showed a tendency to develop peripheral retinal changes more rapidly and to manifest an overall more severe ocular phenotype. This was especially noted in patient 1.

Despite there being no convincing data that correlates metabolic control with the progression of eye disease, it would be of interest to find, if possible, what Met, Hcy or MMA levels better predict a positive ocular long-term outcome, in order to tailor and titrate dosing regimens individually.

There is evidence that OHCbl dose escalation is beneficial in metabolic control[54,59] and that reducing frequency of administration to less than daily dosing leads to sub-therapeutic levels which worsens metabolic control and neurological deterioration[11,55], which suggests that more aggressive OHCbl dosing may be needed. However, since current treatment strategies have little effect on ocular disease despite improving metabolic status and systemic complications, it stands to reason that future studies will need to focus on the pathogenesis of ocular disease in cblC deficiency in order to develop new therapeutic targets. Methionine supplements, antioxidant compounds and different regimens of OHCbl and betaine may be necessary to better manage retinal and macular degeneration and improve long-term outcome of patients with early-onset cblC disease. Gene transfer strategies may play a future role in the disease, as they already have in other rare heritable neurodegenerative

disorders[26], and local ocular therapy with vitamin B12 has also been suggested as an alternative[27].

ACKNOWLEDGEMENTS

REFERENCES

1. Froese DS, Gravel RA. Genetic disorders of vitamin B12 metabolism: eight complementation groups--eight genes. Expert Rev Mol Med. 2010;12(November):e37.

2. Carrillo-Carrasco N, Adams D, Venditti CP. Disorders of Intracellular Cobalamin Metabolism. GeneReviews(R) [Internet]. 2008;1–26.

3. Lerner-Ellis JP, Tirone JC, Pawelek PD, Doré C, Atkinson JL, Watkins D, et al. Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. Nat Genet. 2006;38(1):93–100.

4. Weisfeld-Adams JD, Morrissey MA, Kirmse BM, Salveson BR, Wasserstein MP, McGuire PJ, 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. 2010;99(2):116–23.

5. Cusmano-Ozog K, Lorey F, Levine S, Martin M, Nicholas E, Packman S, et al. Cobalamin C Disease and Expanded Newborn Screening: the California Experience. J Investig Med. 2007;55(1):S90.

6. Nogueira C, Marcão A, Sousa C, Fonseca H, Valongo C, Vilarinho L. Molecular picture of cobalamin C / D defects before and after newborn screening era. J Med Screen. 2016;0(0):1–6.

7. Morel CF, Lerner-Ellis JP, Rosenblatt DS. Combined methylmalonic aciduria and homocystinuria (cblC): Phenotype-genotype correlations and ethnic-specific observations. Mol Genet Metab. 2006;88(4):315–21.

8. Lerner-Ellis JP, Anastasio N, Liu J, Coelho D, Suormala T, Stucki M, et al. Spectrum of mutations in MMACHC, allelic expression, and evidence for genotype-phenotype correlations. Hum Mutat. 2009;30(7):1072–81.

9.   Wang X, Sun W, Yang Y, Jia J, Li C. A clinical and gene analysis of late-onset combined methylmalonic aciduria and homocystinuria, cblC type, in China. J Neurol Sci. 2012;318(1–2):155–9.

10.   Nogueira C, Aiello C, Cerone R, Martins E, Caruso U, Moroni I, et al. Spectrum of MMACHC mutations in Italian and Portuguese patients with combined methylmalonic aciduria and homocystinuria, cblC type. Mol Genet Metab. 2008;93(4):475–80.

11.   Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. J Inherit Metab Dis. 2012;35(1):91–102.

12.   Morel CF, Watkins D, Scott P, Rinaldo P, Rosenblatt DS. Prenatal diagnosis for methylmalonic acidemia and inborn errors of vitamin B12 metabolism and transport. Mol Genet Metab. 2005;86(1–2):160–71.

13.   Zong Y, Liu N, Zhao Z, Kong X. Prenatal diagnosis using genetic sequencing and identification of a novel mutation in MMACHC. BMC Med Genet. 2015;16:48.

14.   Huemer M, Simma B, Fowler B, Suormala T, Bodamer OA, Sass JO. Prenatal and postnatal treatment in cobalamin C defect. J Pediatr. 2005;147(4):469–72.

15.   Profitlich LE, Kirmse B, Wasserstein MP, Diaz GA, Srivastava S. High prevalence of structural heart disease in children with cblC-type methylmalonic aciduria and homocystinuria. Mol Genet Metab. 2009;98(4):344–8.

16.   De Bie I, Nizard SDP, Mitchell GA. Fetal dilated cardiomyopathy: an unsuspected presentation of methylmalonic aciduria and hyperhomocystinuria, cblC type. Prenat Diagn. 2009;29:266–70.

17.   Cerone R, Schiaffino MC, Caruso U, Lupino S, Gatti R. Minor facial anomalies in combined methylmalonic aciduria and homocystinuria due to a defect in cobalamin

metabolism. J Inherit Metab Dis. 1999;22(3):247–50.

18.   Fischer S, Huemer M, Baumgartner M, Deodato F, Ballhausen D, Boneh A, et al. Clinical presentation and outcome in a series of 88 patients with the cblC defect. J Inherit Metab Dis. 2014;37(5):831–40.

19.   Rosenblatt DS, Aspler AL, Shevell MI, Pletcher BA, Fenton WA, Seashore MR. Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). J Inherit Metab Dis. 1997;20(4):528–38.

20.   Wang F, Han L, Yang Y, Gu X, Ye J, Qiu W, et al. Clinical, biochemical, and molecular analysis of combined methylmalonic acidemia and hyperhomocysteinemia (cblC type) in China. J Inherit Metab Dis. 2010;33(SUPPL. 3).

21.   Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. II. Complications, pathophysiology, and outcomes. J Inherit Metab Dis. 2012;35(1):103–14.

22.   Deodato F, Boenzi S, Rizzo C, Dionisi-Vici C. The clinical picture of early-onset cobalamin c defect (methylmalonic aciduria and homocystinuria). Paediatr Child Health (Oxford). 2008;18(SUPPL. 1):S57–60.

23.   Han B, Cao Z, Tian L, Zou H, Yang L, Zhu W, et al. Clinical presentation, gene analysis and outcomes in young patients with early-treated combined methylmalonic acidemia and homocysteinemia (cblC type) in Shandong province, China. Brain Dev. 2016;38(5):491–7.

24.   Martinelli D, Deodato F, Dionisi-Vici C. Cobalamin C defect: Natural history, pathophysiology, and treatment. J Inherit Metab Dis. 2011;34(1):127–35.

25.   Weisfeld-Adams JD, Bender HA, Miley-Åkerstedt A, Frempong T, Schrager NL, Patel K, et al. Neurologic and neurodevelopmental phenotypes in young children with early-

treated combined methylmalonic acidemia and homocystinuria, cobalamin C type. Mol Genet Metab. 2013;110(3):241–7.

26. Weisfeld-Adams JD, McCourt EA, Diaz GA, Oliver SC. Ocular disease in the cobalamin C defect: A review of the literature and a suggested framework for clinical surveillance. Mol Genet Metab. 2015;114(4):537–46.

27. Brooks BP, Thompson AH, Sloan JL, Manoli I, Carrillo-Carrasco N, Zein WM, et al. Ophthalmic manifestations and long-term visual outcomes in patients with cobalamin C deficiency. Ophthalmology. 2016;123(3):571–82.

28. Gizicki R, Robert MC, Gómez-López L, Orquin J, Decarie JC, Mitchell GA, et al. Long-term visual outcome of methylmalonic aciduria and homocystinuria, cobalamin C type. Ophthalmology. 2014;121(1):381–6.

29. Bonafede L, Ficicioglu CH, Serrano L, Han G, Morgan JIW, Mills MD, et al. Cobalamin C deficiency shows a rapidly progressing maculopathy with severe photoreceptor and ganglion cell loss. Investig Ophthalmol Vis Sci. 2015;56(13):7875–87.

30. Fuchs LR, Robert M, Ingster-Moati I, Couette L, Dufier JL, De Lonlay P, et al. Ocular manifestations of cobalamin C type methylmalonic aciduria with homocystinuria. J AAPOS. 2012;16(4):370–5.

31. Figueiredo A, Macedo M, Miranda V, Parreira R, Gonçalves S, Menéres P. Acidúria metilmalónica com homocistinúria, tipo cobalamina c: fenótipo oftalmológico. Oftalmologia. 2015;39(2):129–36.

32. Gaillard MC, Matthieu JM, Borruat FX. Retinal dysfunction in combined methylmalonic aciduria and homocystinuria (Cblc) disease: A spectrum of disorders. Klin Monbl Augenheilkd. 2008;225(5):491–4.

33. Gerth C, Morel CF, Feigenbaum A, Levin A V. Ocular phenotype in patients with methylmalonic aciduria and homocystinuria, cobalamin C type. J AAPOS. 2008;12(6):591–6.

34. Patton N, Beatty S, Lloyd IC, Wraith JE. Optic atrophy in association with cobalamin C (cblC) disease. Ophthalmic Genet. 2000;21(3):151–4.

35. Schimel AM, Mets MB. The natural history of retinal degeneration in association with cobalamin C (cbl C) disease. Ophthalmic Genet. 2006;27(1):9–14.

36. Tsina EK, Marsden DL, Hansen RM, Fulton AB. Maculopathy and Retinal Degeneration in Cobalamin C Methylmalonic Aciduria and Homocystinuria. Arch Ophthalmol. 2005;123(8):1143–6.

37. Ku CA, Ng JK, Karr DJ, Reznick L, Harding CO, Weleber RG, et al. Spectrum of ocular manifestations in cobalamin C and cobalamin A types of methylmalonic acidemia. Ophthalmic Genet. 2016;37(4):404–14.

38. Aleman TS, Brodie F, Garvin C, Gewaily DY, Ficicioglu CH, Mills MD, et al. Retinal Structure in Cobalamin C Disease: Mechanistic and Therapeutic Implications. Ophthalmic Genet. 2015;36(4):339–48.

39. Ricci D, Pane M, Deodato F, Vasco G, Randò T, Caviglia S, et al. Assessment of visual function in children with methylmalonic aciduria and homocystinuria. Neuropediatrics. 2005;36(3):181–5.

40. Beck BB, van Spronsen F, Diepstra A, Berger RMF, Kömhoff M. Renal thrombotic microangiopathy in patients with cblC defect : review of an under-recognized entity. Pediatr Nephrol. 2016;1–9.

41. Sharma AP, Greenberg CR, Prasad AN, Prasad C. Hemolytic uremic syndrome (HUS) secondary to cobalamin C (cblC) disorder. Pediatr Nephrol. 2007;22(12):2097–103.

42. Frattini D, Fusco C, Ucchino V, Tavazzi B, Della Giustina E. Early Onset Methylmalonic Aciduria and Homocystinuria cblC Type With Demyelinating Neuropathy. Pediatr Neurol. 2010;43(2):135–8.

43. Smith SE, Kinney HC, Swoboda KJ, Levy HL. Subacute combined degeneration of the spinal cord in cblC disorder despite treatment with B12. Mol Genet Metab. 2006;88(2):138–45.

44. Ellaway C, Christodoulou J, Kamath R, Carpenter K, Wilcken B. The association of protein-losing enteropathy with cobalamin C defect. J Inherit Metab Dis. 1998;21(1):17–22.

45. Tsai AC-H, Morel CF, Scharer G, Yang M, Lerner-Ellis JP, Rosenblatt DS, et al. Late-Onset Combined Homocystinuria and Methylmalonic Aciduria (cblC) and Neuropsychiatric Disturbance. Am J Med Genet A. 2007;143A:2430–4.

46. Roze E, Gervais D, Demeret S, Ogier de Baulny H, Zittoun J, Benoist J-F, et al. Neuropsychiatric disturbances in presumed late-onset cobalamin C disease. Arch Neurol. 2003;60(10):1457–62.

47. Thauvin-Robinet C, Roze E, Couvreur G, Horellou M-H, Sedel F, Grabli D, et al. The adolescent and adult form of cobalamin C disease: clinical and molecular spectrum. J Neurol Neurosurg Psychiatry. 2008;79(6):725–8.

48. Van Hove JLK, Van Damme-Lombaerts R, Grünewald S, Peters H, Van Damme B, Fryns JP, et al. Cobalamin disorder Cbl-C presenting with late-onset thrombotic microangiopathy. Am J Med Genet. 2002;111(2):195–201.

49. Collison FT, Xie YA, Gambin T, Jhangiani S, Muzny D, Gibbs R, et al. Whole Exome Sequencing Identifies an Adult-Onset Case of Methylmalonic Aciduria and Homocystinuria Type C (cblC) with Non-Syndromic Bull's Eye Maculopathy.

Ophthalmic Genet. 2015;36(3):270–5.

50. Rossi A, Cerone R, Biancheri R, Gatti R, Schiaffino MC, Fonda C, et al. Early-onset combined methylmalonic aciduria and homocystinuria: Neuroradiologic findings. Am J Neuroradiol. 2001;22(3):554–63.

51. Biancheri R, Cerone R, Schiaffino MC, Caruso U, Veneselli E, Perrone M V., et al. Cobalamin (Cbl) C/D deficiency: Clinical, neurophysiological and neuroradiologic findings in 14 cases. Neuropediatrics. 2001;32(1):14–22.

52. Longo D, Fariello G, Dionisi-Vici C, Cannatà V, Boenzi S, Genovese E, et al. MRI and 1H-MRS findings in early-onset cobalamin C/D defect. Neuropediatrics. 2005;36(6):366–72.

53. Ben-Omran TI, Wong H, Blaser S, Feigenbaum A. Late-onset cobalamin-C disorder: a challenging diagnosis. Am J Med Genet A. 2007 May 1;143A(9):979–84.

54. Matos IV, Castejón E, Meavilla S, O'Callaghan M, Garcia-Villoria J, López-Sala A, et al. Clinical and biochemical outcome after hydroxocobalamin dose escalation in a series of patients with cobalamin C deficiency. Mol Genet Metab. 2013;109(4):360–5.

55. Augoustides-Savvopoulou P, Mylonas I, Sewell AC, Rosenblatt DS. Reversible dementia in an adolescent with cblC disease: Clinical heterogeneity within the same family. J Inherit Metab Dis. 1999;22:756–8.

56. Andersson HC, Shapira E. Biochemical and clinical response to hydroxocobalamin versus cyanocobalamin treatment in patients with methylmalonic acidemia and homocystinuria (cblC). J Pediatr. 1998;132(1):121–4.

57. Ribes A, Briones P, Vilaseca MA, Lluch M, Rodes M, Maya A, et al. Methylmalonic aciduria with homocystinuria: Biochemical studies, treatment, and clinical course of a Cbl-C patient. Eur J Pediatr. 1990;149(6):412–5.

58. Bartholomew DW, Batshaw ML, Allen RH, Roe CR, Rosenblatt D, Valle DL, et al. Therapeutic approaches to cobalamin-C methylmalonic acidemia and homocystinuria. J Pediatr. 1988;112(1):32–9.

59. Carrillo-Carrasco N, Sloan J, Valle D, Hamosh A, Venditti CP. Hydroxocobalamin dose escalation improves metabolic control in cblC. J Inherit Metab Dis. 2009;32(6):728–31.

60. Manoli I, Myles JG, Sloan JL, Carrillo-Carrasco N, Morava E, Strauss KA, et al. A critical reappraisal of dietary practices in methylmalonic acidemia raises concerns about the safety of medical foods. Part 2: cobalamin C deficiency. Genet Med. 2015;18(4).