A Highly Diverse Portrait: Heterogeneity of Neuropsychological Profiles in cblC Defect

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Abstract Cobalamin C is a rare inborn disorder of metabolism that results in multisystemic abnormalities, including progressive visual deficits. Although the cellular pathophysiology of cblC is a field of active study, little attention has been dedicated to documenting the cognitive consequences of the defect. The neuropsychological assessment of nine individuals aged between 23 months and 24 years was conducted to establish cognitive profiles. Results reveal a marked heterogeneity, with intellectual functioning ranging from extremely low to average, and cognitive difficulties (e.g., attention) evidenced even in those who are not intellectually disabled. Central nervous system abnormalities

and multisystem disease are likely to be major contributing factors to the observed cognitive impairments, with the presence of visual deficits constituting an additional impediment to normal cognitive development. This study underscores the importance of conducting in-depth neuropsychological assessments in individuals with cblC, the results of which may be particularly helpful for clinical management, guidance toward rehabilitation services, and educational/vocational planning.

Introduction

Cobalamin C defect (cblC, OMIM 277400), a clinically distinct autosomal recessive inborn error of intracellular vitamin B₁₂ metabolism, results in multiple metabolic, developmental, neurological, and ophthalmological abnormalities. The pathophysiological mechanisms of cblC are only partly understood. CblC is due to mutations in the *MMACHC* gene on chromosome 1p34.2 and results in impaired conversion of vitamin B₁₂ into two active forms, adenosylcobalamin and methylcobalamin, leading respectively to increased plasma and urine methylmalonic acid and to increased plasma homocystine and low plasma methionine levels because of impaired remethylation of homocystine to methionine (e.g., Lerner-Ellis et al. 2006; Morel et al. 2006). Impaired remethylation and oxidative stress are felt to contribute to the neurological complications of cblC and increased homocystine concentration to arterial thrombosis (McGuire et al. 2009; Richard et al. 2009).

Clinically, cblC can be divided into early- and late-onset forms, depending on whether the clinical presentation is before or after one year of age, respectively (Martinelli et al. 2011). Characteristic clinical manifestations of the early-onset type include feeding difficulties, failure to

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thrive, developmental delay, and muscular hypotonia (e.g., Fischer et al. 2014; Rosenblatt et al. 1997). Some early-onset patients develop a hemolytic-uremic-like syndrome, optic atrophy, and congenital or acquired cardiomyopathy (Martinelli et al. 2011), sometimes with noncompaction (Profitlich et al. 2009). Other clinical signs include protein-losing enteropathy, pulmonary hypertension (Iodice et al. 2013), acidosis, and hyperammonemia (Fischer et al. 2014; Martinelli et al. 2011). Although cblC is part of a group of conditions that are accompanied by an elevation of both homocystine and methylmalonic acid in blood and plasma, it is clinically unique because some features are reported only in this defect, such as the distinct maculopathy (Gizicki et al. 2014; Weisfeld-Adams et al. 2015) and hemolytic-uremic-like syndrome.

Neurological findings relative to the cblC defect include seizures, hydrocephalus, microcephaly, cerebral atrophy, and diffuse white matter edema and demyelination (Rossi et al. 2001; Weisfeld-Adams et al. 2013). More recent patient series emphasize the heterogeneous nature of magnetic resonance findings (Longo et al. 2005); common abnormalities involve white matter changes such as callosal thinning, an increased T2 FLAIR signal in the periventricular and peritrial white matter (Weisfeld-Adams et al. 2013) as well as supratentorial white matter abnormalities (Longo et al. 2005), and craniocaudal shortening of the pons (Weisfeld-Adams et al. 2013). Longo and colleagues (2005) also found that 30% of their patients had basal ganglia lesions. Concerning ophthalmological findings, progressive visual deficits such as maculopathy, retinopathy, and nystagmus, in addition to abnormal vision, are extremely common in early-onset cblC and most children will have developed these deficits before school entry, despite early and aggressive biochemical treatment (Gizicki et al. 2014; Weisfeld-Adams et al. 2015). Strabismus and optic atrophy also affect individuals with the early-onset type relatively frequently (Weisfeld-Adams et al. 2015).

Developmental delay and intellectual deficits are also frequent in cblC (Biancheri et al. 2001), and although it is clinically obvious that cognitive difficulties are a major component of cblC defect, few studies have focused on this critical feature (Rossi et al. 2001). Shinnar and Singer (1984) provided evidence of low-average intellectual functioning (IQ = 84) in a 14-year-old female treated for cblC. Beauchamp et al. (2009) reported intellectual decline and attentional-executive deficits (e.g., difficulties concerning selective and sustained attention, inhibition, emotional control, initiation, and/or goal setting) in two female patients between preschool and 12 years of age. In a group of 13

patients aged 9 to 76 months, Weisfeld-Adams et al. (2013) highlight significant and variable developmental delays in a range of adaptive skills, with a relative sparing of socialization abilities (i.e., the skills that allow for adequate communication and interaction with others, such as the modulation of one's behavior according to contextual cues). Lastly, neuropsychiatric disturbances such as personality, emotional, and behavioral changes in the presence of severe executive dysfunction are documented in adult late-onset cblC (Boxer et al. 2005; Tsai et al. 2007).

Preliminary evidence of adverse cognitive outcome despite active clinical management and treatment suggests that there is a pressing need to better identify and understand the neuropsychological repercussions of cblC. Accordingly, the goal of the present study was to describe the neuropsychological profiles of a group of individuals with cblC.

Method

Patients

Nine cblC patients aged between 23 months and 24 years were recruited through the Division of Medical Genetics, CHU Sainte-Justine, Montreal, Quebec, Canada. Five patients (63%) were initially seen following referral for increased urinary methylmalonic acid by the Québec urinary screening network. This is a voluntary program offered for newborns at 21 days of age and also after international adoption. In three patients (33%), clinical signs were present before the screening results were available and led to the diagnosis. Finally, in one case (Patient 3), diagnosis was made by family screening. The cblC patients described received once- or twice-weekly intramuscular injections of hydroxocobalamin (1 mg/injection) and supplementation with betaine (see Table 1). Dietary protein intake is currently within the normal range and total levels of homocystine are followed serially. Most also received acetylsalicylic acid (~1 mg/kg/day). The practice in our institution is to provide sufficient protein intake to ensure normal levels of plasma methionine while maintaining plasma homocystine as low as possible by the use of betaine supplementation. Lipoproteins and carotid ultrasounds are obtained at least at baseline and at 14 years of age or more frequently, if judged necessary. Patients' medical and clinical characteristics, symptomatology, *MMACHC* mutations, pretreatment metabolite levels, treatment parameters, and visual deficits are presented in Tables 1, 2, and 3.

Table 1 Pre-, peri-, and neonatal medical and diagnostic information

Patient	Delivery	Birth weight (g)	Length (cm)	Head circumference (cm)	Apgar	Pre- and neonatal particularities	
1	At term	2,863	49,5	Unknown	9-9-10	<i>Neonatal</i> : feeding difficulties	
2	At term	2,125	49	34	9-9-10	<i>Prenatal</i> : fetal pericardial hyperechogenicity (resolved at birth)	
3	At term	Unknown	Unknown	Unknown	Unknown	Normal	
4	At term	2,360	48	32	9-9-10	<i>Prenatal</i> : placental abruption and oligohydramnios (14 weeks); delivery induced at 37 weeks	
5	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	
6	At term	2,590	Unknown	Unknown	7-8-8	<i>Prenatal</i> : small placental detachment. <i>Neonatal</i> : feeding difficulties, jaundice, hypotonia; rehospitalized at 10 days for one month of investigations	
7	At term	3,190	52	31	7-9-9	<i>Prenatal</i> : insulin-treated gestational diabetes. <i>Neonatal</i> : cyanosis, weak cry, lethargy, feeding difficulties	
8	At term	3,345	48	34,5	7-9-9	Normal	
9	At term	2,750	45	33,5	9-10-10	<i>Pre- and neonatal</i> : intestinal duplication	
Patient	Age at diagnosis (months/days)	Diagnostic method	Homocysteine (μmol/L) ^a	Methionine (μmol/L) ^a	Methylmalonic acid (plasma) ^a	Methylmalonic acid (urine) ^a	Allele mutations ^b
1	1,6	Screen	135	18	23	3,690	c.388_390delTAC (p. Tyr130del)
2	1,3	Screen	Unknown	13	Unknown	4,208	c.271dupA (p.Arg91Lysfs*14)
3	58,5	Family evaluation	26	18	6	216	c.271dupA (p.Arg91Lysfs*14)
4	2,5	Screen	44	27	15	785	c.271dupA (p.Arg91Lysfs*14)
5	6,2	Screen	Unknown	Unknown	73	1290	N/A ^c
6	0,5	Medical ^d	203	10	46	1597	N/A ^c
7	1,5	Screen	234	<5	50	803	c.271dupA (p.Arg91Lysfs*14)
8	0,8	Medical ^d	166	14	Unknown	711	c.271dupA (p.Arg91Lysfs*14)
9	2,5	Medical ^d	Unknown	Unknown	Unknown	Unknown	c.388_390delTAC (p. Tyr130del)
							c.331>T (p.Arg111 Ter)
							c.271dupA (p.Arg91Lysfs*14)

(continued)

Table 1 (continued)

Patient	Age treatment onset	Betaine (mg/kg/day)	B12 (mg/day)	Complementation	Neurological conditions	Neuroimaging abnormalities	Other medical conditions
1	10 weeks	263	1	1	Febrile seizures; attention-deficit disorder	Not performed	Genu valgum
2	Unknown	392	2	1	Seizures; epilepsy (controlled); migraine	Mild cerebral and cerebellar atrophy	Arterial hypertension, osteopenia, hemolytic-uremic-like syndrome at 21 months
3	61 months	0	1	0	None	Not performed	Patent foramen ovale with mild mitral insufficiency; normal ventricular function
4	2,5 months	0	1	1	None	Not performed	Clinically normal
5	6 months	180	1	1	None	Not performed	Hypertrophic cardiomyopathy with pericardial effusion at presentation (8.5 mos), resolved; von Willebrand disease
6	1 month	201	2	1	Hypotonia	Loss of periventricular white matter, bilaterally symmetric, thin corpus callosum, unilateral carotid thrombosis and occlusion present in neonatal period	None
7	21 days	299	1	1	Seizures	Loss of periventricular white matter, T2 hypersignal, irregular ventriculomegaly particularly the atria of the lateral ventricles	None
8	1 month	224	2	1	Afebrile convulsions with multifocal activity on EEG, hydrocephaly corrected surgically; transient CVA (brief facial hemiplegia and dysarthria); migraine	Hydrocephalus (surgically corrected); loss periventricular white matter volume; thin corpus callosum; delayed myelination	Recurrent pericardial effusion and Raynaud syndrome (suspected familial Mediterranean fever); scoliosis; hyperlactatemia
9	6 weeks	199	2	1	Tonic-clonic epileptic seizures (controlled); microcephaly	Loss of periventricular white matter; thin corpus callosum	Intestinal duplication (surgically corrected), mild segmentation abnormality of thoracic spine with scoliosis; interventricular septal defect; sleep disorder; megaloblastic anemia (resolved with treatment)

^a Normal range for metabolite values: homocystine = 6.5–12.8 $\mu\text{mol/L}$; methionine = 13–25 $\mu\text{mol/L}$; methylmalonic acid (pl.) = <0.4 $\mu\text{mol/L}$; methylmalonic acid (ur.) = <10 $\mu\text{mol/L}$

^b Reference sequence used: NM_015506

^c N/A not available at the time of writing

^d Clinical signs leading to medical attention prior to age of screening. Patient 6: at 2 weeks, presence of hypotonia and somnolence. Patient 8: at 8 weeks, presence of convulsions. Patient 9: 2 days after birth, presence of intestinal duplication diagnosed prenatally and corrected surgically. The child also experienced convulsions at 4 weeks.

Table 2 Psychological aspects, rehabilitation services, and educational/occupational status

Patient	Age	Psychological	Professional services related to development ^a	Medication related to neuropsychiatric function	Schooling or work environment
1	3 years 9/12	Developmental delay, sensory integration disorder (hypersensitivity), anxiety	Npsych, OT, psych ed, psych, special ed., ST. Attends a rehabilitation center specialized in visual deficits	Methylphenidate (unknown dosage)	Specialized class for children with autism spectrum disorder
2	24 years 4/12	Developmental delay, oro-linguo-facial and ideo-motor dyspraxia, dysdiadochokinesia, hyperreflexivity, anorexia	OT, psychiatry, PT, psych, special ed, ST	Methylphenidate (ceased – headaches), amitriptyline (25 mg die)	Adult education, receptionist at family business 3 days/week
3	8 years 5/12	None	Remedial instruction	None	Regular curriculum with support from remedial teacher
4	6 years 5/12	None	None	None	Regular curriculum
5	16 years 9/12	None	None	None	Regular curriculum
6	1 year 11/12	Failure to thrive, developmental motor delay (gross and fine), dysphagia, hypotonia, difficulties in feeding (uncoordinated suction, swallowing, breathing), involuntary movements	OT, PT, psych, ST	None	At home with mother
7	9 years 1/12	Developmental delay, hypotonia	OT, PT, psych, special ed, ST	None	Special class for children with mild intellectual disability
8	12 years 11/12	Psychomotor developmental delay, bucco-lingual dyspraxia	Child psychiatry, OT, psychiatry, PT, psych, ST. Attends a rehabilitation center specialized in visual deficits	Methylphenidate (unknown dosage), amitriptyline (25 mg die – ceased), clobazam (10 mg bid)	Elementary: school for children with speech delays/disorders High school: special education class
9	14 years 1/12	Failure to thrive, oral dysphagia, oral hypersensitivity, diffuse hypotonia with hyperreflexivity, dyskinesia and hyperkinesia	Child psychiatry, OT, psychiatry, PT, psych ed, remedial instruction, ST, social work, low vision services	Melatonin (9 mg die), lorazepam (0.5 mg die)	Class for children with severe to profound intellectual disabilities (reduced teacher to student ratio)

^a Abbreviations: *PT* physiotherapy, *OT* occupational therapy, *ST* speech therapy, *psych* psychology, *Npsych* neuropsychology, *psych ed* psychoeducation, *special ed* special education

Table 3 Ophthalmic findings in patients with cbIC

Patient	Gender	Initial examination age	Follow-up (years)	Initial best corrected visual acuity (right eye to left eye)	Last best corrected visual acuity (right eye to left eye)	WHO category of visual impairment ^a	Abnormal looking retina	Electroretinogram	Nystagmus	Optic atrophy	Strabismus
1	M	6 months	2	Fx-Fx	20/150-20/150	Moderate	+	N	+	+	-
2	F	5 months	23	Fx-Fx	20/800-20/800	Blindness	+	AbN	+	-	+
3	M	6 years	0	20/20-20/20	20/20-20/20	Mild or absent	-	N/A	-	-	-
4	F	19 months	5	Fx-Fx	20/25-20/25	Mild or absent	-	N	-	-	-
5	F	9 months	13	FF-FF	20/25-20/25	Mild or absent	-	N	-	-	-
6	M	2 months	1	Fx-Fx	20/200-20/200	Moderate	+	AbN	+	-	-
7	M	2 months	7	FF-FF	FF-FF	N/A	+	AbN*	+	-	-
8	F	5 months	11	LP-LP	20/100-20/100	Moderate	-	N	+	+	-
9	M	5 years	12	LP	20/100-20/100	Moderate	+	N	+	+	+

F female, M male, Fx fixates, LP light perception, FF fixates and follows, + yes, - no, AbN abnormal, N normal, AbN* abnormal with documented deterioration, N/A not available, WHO World Health Organization. ^a World Health Organization (2003)

Neuropsychological Assessment

For patients who were able to complete intellectual functioning assessments (Patients 1, 2, 3, 4, 5), a battery of age-appropriate standardized tasks was administered. Tests were chosen to minimize visual stimuli.

General Intellectual Functioning

Developmentally appropriate scales from the Wechsler batteries (Wechsler 1997, 2002, 2003; see Table 4) were used to test intellectual functioning including verbal comprehension and perceptual reasoning. The working memory (i.e., online capacity to retain and manipulate information) and information processing speed indices were calculated when visual acuity and cognitive capacities permitted. Intellectual functioning is presented with respect to age-referenced normative data (standard score, mean = 100, SD = 15; see Table 4 for IQ classifications). The term “intellectually disabled” is used here for patients with an IQ below 70.

Visual Attention

Patients completed a selective/divided visual attention task (d2, 16+ years of age, mean = 100, SD = 15, and NEPSY-I, 3 to 12 years of age, mean = 10, SD = 3; Brickenkamp and Zillmer 1998; Korkman et al. 1998) in which target items have to be crossed out while ignoring distractors.

Auditory Attention

Patients completed the auditory attention (NEPSY-II; 3 to 16 years of age, mean = 10, SD = 3; Korkman et al. 2007) or the elevator counting task (TEA; 18 + years of age, mean = 10, SD = 3; Robertson et al. 1994). In the former, children touch a designated shape when an auditory stimulus is presented and ignoring distractors. In the latter, individuals count elevator floors, with each level increase denoted by a “beep” sound amidst auditory distractors.

Visual Memory

Immediate and delayed visual memory was evaluated using memory for designs (NEPSY-II; 3 to 16 years of age; Korkman et al. 2007), in which individuals have to memorize a configuration of abstract designs on a grid over the course of four or five learning trials and complete a 20-min delayed recall (scaled score, mean = 10, SD = 3).

Verbal Memory

A word list (CMS, 5 to 16 years of age, and WMS-III, 16+ years of age; Cohen 1997; Wechsler 1997) is presented

Table 4 Neuropsychological results on standardized cognitive tasks

	Patient				
	1	2	3	4	5
Age	3 years 9/12	24 years	8 years 5/12	6 years 8/12	16 years 9/12
<i>Intelligence [IQ (percentile)]</i>					
Verbal comprehension IQ (VIQ)	71 (2.7) Borderline	61 (0.5) Extremely low	77 (6.3) Borderline	89 (23.2) Low average	93 (32) Average
Perceptual reasoning IQ (PIQ)	67 (1.4) Extremely low	49 (<0.1) Extremely low	80 (9.1) Low average	87 (19.3) Low average	104 (60.5) Average
Working memory index (WMI)	N/A**	N/A*	91 (27.4) Average	86 (17.5) Low average	86 (17.5) Low average
Processing speed index (PSI)	N/A**	50 (<0.1) Extremely low	85 (15.9) Low average	80 (9.1) Low average	80 (9.1) Low average
Global IQ	63 Extremely low	56 Extremely low	79 Borderline	82 Low average	84 Low average
<i>Attention [SS (percentile)]</i>					
Visual	N/A*	1(0.1) Extremely low	5 (5) Borderline	7 (15) Low average	7 (17.5) Low average
Auditory	N/A*	N/A*	7 (15) Low average	8 (25) Low average	1 (0.1) Extremely low
<i>Memory [SS (percentile)]</i>					
Immediate visual recall	N/A**	N/A*	7 (15) Low average	10 (50) Average	14 (90) Superior
Delayed visual recall	N/A**	N/A*	9 (37) Average	10 (50) Average	13 (84) High average
Immediate verbal recall	N/A**	5 (5) Borderline	10 (50) Average	11 (63) Average	7 (15) Low average
Delayed verbal recall	N/A**	7 (15) Low average	9 (37) Average	10 (50) Average	8 (25) Low average
<i>Visual-motor integration [SS (percentile)]</i>					
Visual perception	N/A*	<1 (0.02) Extremely low	5 (6) Borderline	7 (16) Low average	6 (10) Borderline
Motor coordination	N/A*	<1 (0.02) Extremely low	2 (0.7) Extremely low	7 (18) Low average	8 (25) Low average
Visual-motor integration	N/A*	<1 (0.02) Extremely low	4 (2) Extremely low	8 (25) Low average	7 (14) Low average

(continued)

Table 4 (continued)

	Patient				
	1	2	3	4	5
<i>Executive functions [parental report; T score (percentile)]</i>					
Behavioral regulation index	N/A ***	46 (50) Typical	55 (72) Typical	46 (40) Typical	38 (11) Typical
Metacognition index	N/A ***	70 (90) Clinical	53 (63) Typical	47 (41) Typical	46 (41) Typical
Global executive composite	N/A ***	62 (89) Subclinical	54 (66) Typical	46 (38) Typical	42 (29) Typical
<i>Inattention/hyperactivity [parental report; T score (percentile)]</i>					
DSM-IV: inattention	63 (90) Subclinical	63 (90) Subclinical	64 (92) Subclinical	48 (42) Typical	47 (37) Typical
DSM-IV: hyperactivity/impulsivity	61 (86) Subclinical	47 (37) Typical	50 (50) Typical	59 (81) Typical	43 (23) Typical
DSM-IV: total	63 (90) Subclinical	58 (79) Typical	58 (79) Typical	53 (61) Typical	45 (30) Typical
<i>Adaptive behavior [parental report; standard score (percentile)]</i>					
Global adaptive composite	N/A ***	N/A ***	98 (45) Average	103 (58) Average	111 (77) High average

Shaded data indicate performance in the intellectually disabled or clinically significant range. N/A* = participant unable to complete the tasks due to visual and intellectual limitations; N/A** = tasks/indices do not apply given the age of the participant; N/A*** = data could not be obtained. Evaluation of intellectual functioning: (1) Wechsler Preschool and Primary Intelligence Scale-III (WPPSI-III; 2 years and 3 months to 7 years and 3 months; Wechsler, 2002) – verbal (VIQ), performance (PIQ), and full scale (FSIQ) intellectual quotient (IQ) scores. (2) Wechsler Intelligence Scale for Children-IV (WISC-IV; 6 to 16 years and 11 months of age; Wechsler, 2003) – verbal comprehension (VCI), perceptual reasoning (PRI), and full scale (FSIQ) IQ scores. Working memory (WMI) and processing speed indices (PSI) were calculated when possible. (3) Wechsler Adult Intelligence Scale-III (WAIS-III; 16+ years of age; Wechsler, 1997) – verbal (VIQ), performance (PIQ), and full scale (FSIQ) IQ scores

Legend IQ scores/composite scores: extremely low (≤ 69), borderline (70–79), low average (80–89), average (90–109), high average (110–119), superior (120–129), very superior (≥ 130)

Legend T scores: normal range (< 60), subclinical range (60–64), clinical range (≥ 65)

Table 5 Results on the Vineland Adaptive Behavior Scale for patients unable to complete standardized cognitive tasks

	Patient			
	6	7	8	9
Age	1 year 11/12	9 years 1/12	12 years 11/12	14 years 1/12
Adaptive behavior (percentile)	1	2	1	<1
Adaptive level	Mild deficit	Mild deficit	Mild deficit	Severe deficit
Communication (percentile)	3	1	1	<1
Daily living skills (percentile)	2	3	4	<1
Socialization (percentile)	4	5	<1	<1
Motor skills (percentile)	3	3	7	<1
Lowest age equivalency (subdomain)	4 months (coping skills)	3 years 2 months (expressive)	2 years 2 months (community)	10 months (expressive)
Highest age equivalency (subdomain)	1 year 2 months (receptive)	7 years 7 months (domestic)	10 years 6 months (personal)	3 years (gross)

Shaded area indicates performance in the deficient range. Domains (subdomains): communication (receptive, expressive, written), daily living skills (personal, domestic, community), socialization (interpersonal relationships, play and leisure, coping skills), and motor (gross, fine)

orally four times, and individuals must recall as many words as possible immediately after each reading and after a 30-min delay (scaled score, mean = 10, SD = 3).

Visual-Motor Integration

The Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI; full form, 2 to 100 years of age; Beery et al. 2010) consists of the following: (1) visual-motor integration in which simple and integrated geometric forms must be reproduced, (2) visual perception in which a geometric figure must be identified among distractors, and (3) motor coordination in which stimulus forms are traced with a pencil (30 items each, scaled score, mean = 10, SD = 3).

Parent Questionnaires

The Behavior Rating Inventory of Executive Function (BRIEF; 5 to 18 years of age; Gioia et al. 2000) is an 86-item parent rating of executive behavior on a three-point scale. Three summary indices are reported: behavioral regulation index (scales: inhibit, shift, emotional control), metacognition index (scales: initiate, working memory, plan/organize, organization of materials), and global executive composite (*T* scores, mean = 50, SD = 10).

The Conners Performance Rating Scale (CPRS-R:L; 6 to 18 years of age version; Conners 2000) was used to evaluate attentional difficulties and consists of 80 items rated on a four-point frequency scale. Three criteria pertaining to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) are reported (*T* scores, mean = 50, SD = 10).

Adaptive behavior, defined as the everyday practical abilities necessary to respond adequately to environmental demands such as autonomy, social interactions, and communication skills, was assessed in Patients 1–5 using the Adaptive Behavior Assessment System (ABAS-II; Harrison and Oakland 2003) which includes 211 items that must be rated according to the frequency with which children demonstrate these behaviors, without help, when necessary (standard score; mean = 100, SD = 15). The 383-item Vineland Adaptive Behavior questionnaire (Vineland-II; birth to 90 years of age; Sparrow et al. 2005) was used for patients who were unable to complete the intellectual functioning assessment (Patients 6, 7, 8, 9; see Table 5).

Neuropsychological and Statistical Analyses

Cognitive profiles were established by comparing individual performances to age-referenced normative data (interpersonal comparisons). Relative strengths and weaknesses were determined by comparing individual performances in each cognitive function to overall intellectual functioning, which serves as a baseline of expected cognitive ability (intrapersonal comparisons). A strength or weakness was considered to be present when individuals performed 1.5 to 2 standard deviations above or below their intellectual level (Lezak et al. 2004, pp. 99, 148). Fisher's exact test was conducted to determine whether there were any differences between individuals with and without intellectual disability in terms of interventions received. Additionally, Pearson correlations were performed to examine the association between age at diagnosis and age at treatment initiation and cognitive outcomes (in terms of intellectual quotient or adaptive quotient for the severely disabled individuals).

Results

Patients 3, 4, and 5 (See Table 4)

Five patients (1, 2, 3, 4, 5) completed intellectual functioning scales. Three (3, 4, 5) had an IQ within the nonintellectually disabled range (≥ 70); see supplementary Fig. 1). None presented with a *specific* deficit in terms of attentional abilities, but attention was nonetheless impaired for two of the three patients (3, 5). These latter two individuals also presented with visual-motor integration difficulties, which are the result of both poor visual perception and motor coordination. They had relative strengths in long-term verbal and visual memory, respectively. Memory was also slightly above expected levels for Patient 4, but not enough to warrant categorization as a strength.

BRIEF results were in the normal range for Patients 3, 4, and 5, suggesting intact attentional-executive abilities, as perceived by parents. This observation is supported by the results of the Conners questionnaire for Patients 4 and 5 whose parents reported no significant difficulties in terms of attentional control and hyperactivity/impulsivity. Parental reports for Patient 3 revealed clinically significant ratings for the cognitive problems/inattention and the hyperactivity dimensions, resulting in subclinical ratings on global attentional-executive measures including the Conners ADHD index and the DSM-IV inattentive index. The ABAS-II results revealed normal to slightly superior adaptive functioning compared to same-aged peers for Patients 3, 4, and 5. The intellectual and adaptive functioning scores are displayed in Table 4.

Patients 1 and 2 (See Table 4)

The other two patients (1, 2) who completed the Wechsler scales presented with severe intellectual limitations, as demonstrated by extremely low IQ scores (Table 4). Patient 2 completed all domain-specific measures except the auditory attention and the visual memory tasks, due to visual and cognitive limitations. The results reveal extremely low to borderline functioning in the areas of visual attention, short-term verbal memory, and fine motor skills. The only exception concerns a relative strength in long-term verbal memory. Both the BRIEF and Conners questionnaires reveal subclinical ratings for the global executive composite and the DSM-IV inattention index, as well as clinically significant ratings for the metacognitive index, suggesting the presence of attentional-executive difficulties. Additionally the questionnaire may underestimate difficulties in this 24-year-old patient, given it was validated only up to 18 years. Data concerning adaptive behavior (ABAS-II) could not be obtained for Patient 2.

Although Patient 1 was capable of completing the Wechsler IQ measure, severe visual and cognitive limitations precluded the completion of all domain-specific cognitive tasks. Subclinical ratings on all three indices of the Conners questionnaire suggest the presence of attentional-executive difficulties. Additional data concerning executive functions (BRIEF) and adaptive behavior (ABAS-II) could not be obtained.

Patients 6, 7, 8, and 9 (See Table 5)

An estimate of global functioning was obtained for Patients 6, 7, 8, and 9 using the Vineland-II questionnaire as they were unable to complete the Wechsler IQ scales. Adaptive deficits were reported for all of these patients (Table 5) with age equivalencies well below the individuals' chronological age.

Associated Medical and Clinical Factors

Clinical profiles and medical histories were explored in order to determine which factors may be common to those who have severe cognitive limitations. No correlations were found between age at diagnosis and age at treatment initiation and intellectual/adaptive functioning (as measured by global IQ for Patients 1, 2, 3, 4, 5 and by global adaptive behavior for Patients 6, 7, 8, 9; $r(9) = 0.29$, $p = 0.45$, $r(8) = 0.29$, $p = 0.49$).

As depicted in Tables 1, 2, and 3, all patients with intellectual disability and/or adaptive dysfunction ($IQ < 70$, Patients 1, 2, 6, 7, 8, 9) also had visual impairment (mainly retinopathy, maculopathy, and nystagmus). Many had a history of neurological complications (convulsions/epilepsy, hydrocephalus) and the involvement of other organ systems. Of note, all those who underwent clinical neuroimaging (i.e., Patients 2, 6, 7, 8, 9) had positive radiological findings, with four out of five (Patients 6, 7, 8, 9) presenting altered white matter integrity and corpus callosum atrophy, among other structural abnormalities. Globally, these individuals have extensive multisystemic involvement that requires follow-up by primary care and rehabilitation services and which has a significant impact on daily functioning, impeding them from leading independent lives. All of these patients (except Patient 6 who is too young to attend school) require specialized and adapted school curricula and services aimed at developing basic living skills and maximizing independence.

Conversely, none of the three individuals with intellectual functioning in the nondisabled range ($IQ > 70$) have visual and/or neurovisual deficits. While two of them (Patients 3 and 5) had nonneurological medical complications at a young age (e.g., mild mitral insufficiency, hypertrophic cardio-

myopathy, von Willebrand disease), these complications were not clearly associated with cblC and did not lead to chronic sequelae. All these patients are currently leading age-appropriate independent lives and follow the regular school curriculum. Only one of the three patients (Patient 3) requires remedial support. Of note, Patient 3 obtained the lowest IQ score (borderline) among those who did not have intellectual disability.

Lastly, the patients with an intellectual disability/adaptive dysfunction did not have recourse to rehabilitation services significantly more than those in the nondisabled intellectual range ($p = 0.08$).

Discussion

Cognitive Functioning and Associated Developmental, Neurobiological, and Medical Factors

IQ or adaptive skills for patients in this study ranged from extremely low to average, revealing marked cognitive differences among cblC individuals. Poorer cognitive outcome was observed in conjunction with multisystem disease, associated neurological and neuroradiological abnormalities, as well as visual deficits. In particular, most individuals with impaired functioning had corpus callosum atrophy and white matter abnormalities. Given that myelinated nerve fibers are involved in the efficient transmission of signals between neurons, facilitating speed of interhemispheric communication and information integration, white matter abnormalities may contribute to the severe cognitive limitations observed. Other potential correlates of outcome, such as metabolite levels and causal mutations, require larger cohorts to draw conclusions. To fully understand the complexity of neurocognitive profiles in cblC within a developmental context, findings must be interpreted as an interaction between child development and defect progression. When cognitive deficits are observed, genuine deterioration may be occurring, as in neurodegenerative diseases; however, it may also be that children are simply not progressing at the same rate as their peers or have reached a plateau in terms of their intellectual functioning, thereby widening the gap between their functioning and that of normally developing children, as is likely the case in cblC.

Neuropsychological Assessment in cblC Patients

Cognitive difficulties were observed in eight of the nine patients studied, regardless of their level of intellectual functioning and despite the fact that all patients received treatment including hydroxocobalamin injections. This demonstrates that current biomedical treatment of cblC does not prevent cognitive impairment, even if started at an early age in

screened, asymptomatic newborns, and indicates the potential utility of timely neuropsychological assessment. The clinical heterogeneity revealed here extends the previously reported findings (Beauchamp et al. 2009; Longo et al. 2005; Vargogli et al. 2000; Weisfeld-Adams et al. 2013). Clearly, cblC does not cause a consistent pattern of cognitive functioning. This underscores the need for individualized evaluation to understand each patient's idiosyncratic patterns of cognitive functioning. Strengths identified through such assessments can be used to compensate for potential weaknesses, as well as to determine optimal learning conditions on an individual basis. A complete neuropsychological evaluation can also identify subtle, yet significant weaknesses. For example, difficulties in visual-motor integration were observed here in patients with age-appropriate intellectual functioning and in regular school curriculums. Additionally, Patient 3 had borderline intelligence and cognitive difficulties that were not detected until middle to late primary school.

Pathophysiology, Molecular Analyses, and Treatment in Relation to Neuropsychological Outcome

The most common mutation is c.271dupA (p.Arg91Lysfs*14), a frequent protein-truncating frameshift mutation associated with severe outcome (Lerner-Ellis et al. 2009). Studies are ongoing to establish the complete mutational profile of our population of cblC patients.

As previously mentioned, the pathophysiology of cblC defect is largely unknown. The characteristically elevated metabolites of cblC, homocystine and methylmalonic acid, cannot explain many of the most important manifestations of this condition. Conversely, neither hyperhomocysteinemia nor methylmalonic acidemia can be assumed to be benign in cblC. For instance, to our knowledge, no episodes of hemolytic-uremic-like syndrome have occurred in previously asymptomatic patients during hydroxocobalamin treatment, and a rapid albeit partial decrease in homocystine occurs within weeks in most patients following the initiation of treatment. The choice of hydroxocobalamin dose has previously been discussed in the literature (Dionisi-Vici et al. 2013); our patients receive 1 mg intramuscularly once or twice weekly. Marked protein restriction is avoided because of its established negative effects on growth and anabolism. Dietary methionine provides a source of methionine that may compensate in part for the defective intracellular synthesis of methionine that follows closely from the primary defect in cblC. Although patients with cblC were traditionally restricted in protein intake, in a recent survey, none of 88 European patients received protein restriction (Fischer et al. 2014). Furthermore, recent studies of treatment in cblC found no benefit in restricting protein access and a possibly deleterious effect of using low-methionine medical foods (Manoli et al. 2015). Hyperhomocystinuria is a known risk

factor for thrombosis. General measures of prevention of thrombosis include aspirin treatment and hydration, optimization of the dose of hydroxocobalamin and avoidance of excessive methionine intake, and reduction of other cardiovascular risk factors if present, such as hypercholesterolemia and hypertension. Methylmalonic acidemia is usually considered to be a clinically minor finding in cblC, and the ketoacidic decompensations seen in classical methylmalonic aciduria resulting from methylmalonyl-coenzyme A mutase deficiency are rarely, if ever, seen in cblC defect. However, the basal ganglia lesions seen in some cblC patients (Longo et al. 2005) are reminiscent of those seen in many diseases of acyl-CoA metabolism including methylmalonyl-CoA mutase deficiency (Mitchell et al. 2008), and some role for methylmalonic acid or methylmalonyl-CoA in the pathogenesis of cblC cannot be formally excluded. From the above, it is clear that no dietary regimen can normalize the concentrations of all the metabolites that are currently considered as potentially involved in aspects of neurodevelopmental function.

Similarly, the effect of newborn screening on clinical outcome in cblC defect is unknown. Some patients detected by urine screening and for whom treatment is initiated in the first weeks of life have early-onset defect and have manifestations at birth or prenatally like cardiomyopathy (De Bie et al. 2009). Therefore, newborn screening will not correct the defect in all patients, but arguably, early detection and treatment might influence neurodevelopment favorably in some individuals and will also detect other forms of methylmalonic aciduria.

Conclusions and Future Directions

The current findings constitute the most in-depth neuropsychological evaluation to date in a large group of cblC patients. Detailed studies of cognition were previously described in only three cblC patients (Beauchamp et al. 2009; Shinnar and Singer 1984). Links with documented medical and radiological findings, despite being qualitative in nature, suggest preliminary hypotheses as to the mechanisms underlying cognitive impairment. Although visual deficits can affect cognitive performance, the deficits observed in these patients are not reducible to visual problems. Central nervous system abnormalities detected by neuroimaging as well as extensive multisystem disease are probably the main factors contributing to severe cognitive limitations. The great clinical diversity in cblC patients invites future studies of larger numbers of patients, with longitudinal neuropsychological evaluation and detailed prospective studies of *MMACHC* genotype and of dietary and pharmacological treatments.

A multicentric study could be useful in increasing the patient base and in enabling age group comparisons. This

would allow the scientific community to examine genotype and pathophysiological mechanisms in relation to clinical outcome, as well as allowing the exploration of the cognitive benefits of newborn screening. Additionally, longitudinal follow-up of patients with cblC is recommended to monitor the stability of the cognitive profile (or lack thereof) and further specify what factors promote favorable cognitive outcome. From a clinical perspective, we suggest that neuropsychological evaluation should be offered to children and adolescents with cblC defect. As well as being an essential element for accurate evaluation of the impact of treatments, neuropsychological evaluation has practical impacts for patient management. It allows for orientation to appropriate educational and remedial services, which may even benefit individuals whose difficulties are not obvious on standard pediatric evaluation. As such, early intervention, *even in children with intellectual functioning in the normal range*, may increase the chances of maintaining adequate functioning when children are faced with increasing cognitive and environmental demands at older ages.

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Synopsis

This article explores the heterogeneous nature of neuropsychological functioning in individuals with cobalamin C defect.

Compliance with Ethics Guidelines

Conflict of Interest

Jenny Bellerose, Mathilde Neugnot-Ceroli, Catherine Brunel-Guitton, Grant A. Mitchell, Luis H. Ospina, Karine Bédard, and Miriam H. Beauchamp declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Details on the Contribution of Individual Authors

Jenny Bellerose: Development of study protocol and methodology, ethics submissions, data collection, analyses, and interpretation, writing of manuscript.

Mathilde Neugnot-Ceroli: Development of study protocol and methodology, data collection and analyses, interpretation of findings, manuscript editing and review.

Luis H. Ospina: Consultant for ophthalmological aspects, interpretation of ophthalmological data, manuscript review.

Grant A. Mitchell: Development of study protocol, recruitment of patients, consultant for medical aspects, writing of manuscript.

Catherine Brunel-Guitton: Recruitment of patients, manuscript review.

Miriam H. Beauchamp (principal investigator): Development of study protocol and methodology, funding, supervision of J.B. and M.N.C., interpretation of data, writing of manuscript.

Karine Bédard: Performance and interpretation of molecular sequencing data.

Appendix: Abbreviations and Details of Neuropsychological Tests Administered

ABAS	Adaptive Behavior Assessment System Harrison PL, Oakland T. Adaptive Behavior Assessment System – Second Edition. The Psychological Corporation, San Antonio, TX, 2003
BRIEF	Behavior Rating Inventory of Executive Function Gioia GA, Isquith PK, Guy SC, Kenworth L. Behavior Rating Inventory of Executive Function. Psychological Assessment Resources, Odessa, FL, 2000
CMS	Children's Memory Scale Cohen M. Children's Memory Scale. The Psychological Corporation, San Antonio, TX, 1997
CPRS–R:L	Conners Performance Rating Scale–Revised: Long version Conners KC. Conners Performance Rating Scale - Revised: Long version. Multi-Health Systems Inc., North Tonawanda, NY, 2000
d2	d2 Test of Attention Brickenkamp R, Zillmer E. The d2 Test of Attention. Hogrefe & Huber Publishers, Seattle, WA, 1998
TEA	Test of Everyday Attention Robertson IH, Ward T, Ridgeway V, Nimmo-Smith I. The Test of Everyday Attention. The Psychological Corporation, San Antonio, TX, 1994
VMI	Beery-Buktenica Developmental Test of Visual-Motor Integration. Beery KE, Buktenica NA, Beery NA. The Beery-Buktenica Developmental Test of Visual-Motor Integration - Sixth Edition. The Psychological Corporation, San Antonio, TX, 2010

(continued)

NEPSY I-II	Neuropsychological Assessment Korkman M, Kirk U, Kemp S. NEPSY-II: A Developmental Neuropsychological Assessment – Second Edition. The Psychological Corporation, San Antonio, TX, 1998 & 2007
WMS-III	Wechsler Memory Scale Wechsler D. Wechsler Memory Scale - Third Edition. The Psychological Corporation, San Antonio, TX, 1997
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence Wechsler D. Wechsler Preschool and Primary Scale of Intelligence – Third Edition. The Psychological Corporation, San Antonio, TX, 2002
WISC-IV	Wechsler Intelligence Scale for Children Wechsler D. Wechsler Intelligence Scale for Children – Fourth Edition. The Psychological Corporation, San Antonio, TX, 2003
WAIS-III	Wechsler Adult Intelligence Scale Wechsler D. Wechsler Intelligence Scale for Children – Fourth Edition. The Psychological Corporation, San Antonio, TX, 1997
Vineland-II	Vineland Adaptive Behavior Scales Sparrow SS, Cicchetti DV, Balla DA. Vineland Adaptive Behavior Scales – Second Edition. The Psychological Corporation, San Antonio, TX, 2005

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