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ORIGINAL ARTICLE

Update on transcobalamin deficiency: clinical presentation, treatment and outcome

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Abstract Transcobalamin (TC) transports cobalamin from blood into cells. TC deficiency is a rare autosomal recessive disorder usually presenting in early infancy with failure to thrive, weakness, diarrhoea, pallor, anemia, and pancytopenia or agammaglobulinemia. It can sometimes resemble neonatal leukemia or severe combined immunodeficiency disease. Diagnosis of TC deficiency is suspected based on megaloblastic anemia, elevation of total plasma homocysteine, and blood or urine methylmalonic acid. It is confirmed by studying the synthesis of TC in cultured fibroblasts, or by molecular

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analysis of the *TCN2* gene. TC deficiency is treatable with supplemental cobalamin, but the optimal type, route and frequency of cobalamin administration and long term patient outcomes are unknown. Here we present a series of 30 patients with TC deficiency, including an update on multiple previously published patients, in order to evaluate the different treatment strategies and provide information about long term outcome. Based on the data presented, current practice appears to favour treatment of individuals with TC deficiency by intramuscular injections of hydroxy- or cyanocobalamin. In most cases presented, at least weekly injections (1 mg IM) were necessary to ensure optimal treatment. Most centres adjusted the treatment regimen based on monitoring CBC, total plasma homocysteine, plasma and urine methylmalonic acid, as well as, clinical status. Finally, continuing IM treatment into adulthood appears to be beneficial.

Introduction

Cobalamin (Cbl, vitamin B_{12}) in the blood is bound to haptocorrin (HC) (previously named transcobalamin I), of unknown function, and to transcobalamin (TC) (previously named transcobalamin II) which supports endocytosis of cobalamin by cells. TC deficiency (OMIM #275350) is a rare autosomal recessive disorder characterized by elevated total plasma homocysteine as well as elevated plasma and urine methylmalonic acid concentrations. Serum cobalamin levels are not usually low because the majority of serum cobalamin is bound to haptocorrin and not to TC. Most reported mutations are deletions or insertions in the *TCN2* gene resulting in frame shifts that predict protein truncation (Watkins and Rosenblatt 2011a; Li et al 1994a; Supplemental Table). Nonsense mutations and point mutations that activate exonic cryptic splice sites have also been reported (Li et al 1994b; Namour et al 2003). A number of polymorphic variants, have also been described (Watkins and Rosenblatt 2011a; Namour et al 2001; Watkins and Rosenblatt 2011b).

Overall, patients with TC deficiency can present with variable clinical features including failure to thrive (FTT), diarrhoea, pallor, and anemia. The symptoms typically develop within the first few months of life. The anemia is usually megaloblastic and, in some cases, is seen in the context of pancytopenia or isolated erythroid hypoplasia. TC deficiency has been misdiagnosed as leukemia (Schiff et al 2010) as has B12/folate deficiency (Aitelli et al 2004). Delayed or inadequate treatment, as well as administration of folate in the absence of Cbl, can all lead to neurological deficits including developmental delay, neuropathy, myelopathy, and retinal degeneration (Hall 1992; Souied et al 2001). TC deficiency can have a presentation similar to severe combined immunodeficiency. Defective granulocyte function with both defective humoral and cellular immunity, as well as life-threatening infections, may occur (Hitzig et al 1974). In order to evaluate prognosis and optimal management, we reviewed prior reports of patients with TC deficiency and collected updated information on them. In addition, new patients were identified through a survey. We summarized the clinical evolution of this cohort and discuss these clinical findings in relation to the dose, frequency type and administration mode of cobalamin treatment.

Methods

To identify reported cases with TC deficiency, a literature review from 1974 to 2012 was performed using the terms "Transcobalamin II deficiency, TC II deficiency and vitamin B_{12} pancytopenia". These cases were summarized in Tables 1 and 2. The first and/or senior author of each report was contacted, where possible (i.e. cases in Table 1). In addition, identification of further cases of TC deficiency was queried on Metab-L, an international mailing list with several hundred members specializing in metabolic genetic diseases. All physicians who responded were asked to complete a brief questionnaire (see supplemental data) to summarize their patient(s) with TC deficiency. For cases diagnosed by biochemical testing only, *TCN2* mutation analysis was offered on a research basis (Children's Health Research Institute, London Ontario),

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Table 1 Previously reported patients with clinical updates, included in this manuscript

Patient ID #	Age at Dx	Clinical signs and symptoms at Dx	Reference
#1	1 y	Pancytopenia	Namour et al 2003 Bibi et al 1999
#2	2 week	Pancytopenia Sibling of patient #1	Namour et al 2003 Bibi et al 1999
#3	3 week	Pancytopenia Sibling of patient #1	Namour et al 2003 Bibi et al 1999
#4	3 month	Vomiting, watery diarrhoea, pancytopenia at 6 week, weight loss, slow progression until acute abdomen/protein losing enteropathy	Ratschmann et al 2009
#5	3 day	Normal Sibling of patient #4	Ratschmann et al 2009
#6	6 month	FTT, pancytopenia, neutropenic colitis. BM suggestive of MDS (sibling died at 7 month of age with suspected leukaemia)	Prasad et al 2008
#7	1st wk of life	Normal Sibling of patient #6	Prasad et al 2008
#8	1st wk of life	Normal Sibling of patient #6	Prasad et al 2008
#9	3 month	Agammaglobulinemia at birth; megaloblastic BM at 3 months	Hitzig et al 1974 Arlet et al 2002
#10	20 day	Weight loss, pallor, glossitis, irritability, pancytopenia, megaloblastic BM (Older sibling of patients #10 died in the neonatal period; presented with anorexia, diarrhoea pallor, glossitis, irritability at 20 day)	Arrabal et al 1988
#11	~20 day	Similar to sibling (patient #10)	Arrabal et al 1988
#12	11 month	At 2 week, FTT and recurrent respiratory tract infections; at 4 month pancytopenia, at 7 month low IgGs, BM suggestive of MDS	Nissen et al 2010
#13	Birth	Sibling of patient #12	Nissen et al 2010
#14	4 month	Acute gastroenteritis, glossitis, pallor, acute hematological presentation with pancytopenia, megaloblastic BM	Schiff et al 2010
#15	10 month	FTT, thrombocytopenia and neutropenia (Two siblings of patient #15 had died; Dx not confirmed)	Schiff et al 2010
#16	23 day	At 23 day repeated vomiting, pancytopenia and megaloblastic anemia. (Older sibling died at 3 month with pancytopenia; Dx not confirmed)	Schiff et al 2010
#17	7 month	At 4-7 month delayed milestones, hypotonia, myoclonic like movements, pallor, purpura, anaemia, thrombocytopenia, megaloblastosis, aplastic BM	Schiff et al 2010 Souied et al 2001
#18	4 month	Hypotonia, FTT, projectile vomiting, glossitis, megaloblastic anemia	Li et al 1994a
#19	At birth	Normal Sibling of patient # 18	Li et al 1994b

B_{12} vitamin B_{12}; *BM* bone marrow; *d* days; *DD* developmental delay; *Dx* diagnosis; *FTT* failure to thrive; *IgGs* immunoglobulin G; *m* months; *MDS* myelodysplastic syndrome; *wks* weeks; *y* years

after appropriate consent was obtained by the patient's treating physician. Complete gene sequencing, including both exons and introns, was performed as previously published (Prasad et al 2008). Deletions were mapped by sequencing with primers flanking the deletions.

Results

Tables 1 and 2 review the clinical presentation and age at diagnosis of the previously reported patients with TC deficiency, as identified in our literature review. Table 1

Table 2 Previously reported patients without clinical updates, and not included in this manuscript

Age at Dx	Clinical presentation	Reference
3 month	FTT, diarrhea, vomiting	Schiff et al 2010
3 week	FTT, diarrhea, vomiting, hypotonia, pancytopenia	Häberle et al 2009
8 week	FTT, pancytopenia	Häberle et al 2009
6-7 week	FTT, vomiting, anemia	Häberle et al 2009
"Infant"	"As infant" had low IgG, corpus callosum agenesis, multiple nevi; at 3 y had DD and epilepsy; at 14 year poorly differentiated colonic adenocarcinoma grade 3 astrocytoma; at 21 y post-mortem dx of Lynch syndrome. Identical twin and older brother of proband had low IgG	Gururangan et al 2008
6 month	At 3 month with diarrhoea, pancytopenia, weight loss; at 6 month with sepsis	Grech et al 2001
2 y	At 26 day admitted with "feeding difficulties", anemia. At 2 y repeat exam showed microcephaly, hypotonia, frequent seizures	Thomas and Hoffbrand 1997; Thomas et al 1982
9-10 month	FTT, diarrhoea, macrocytic anaemia, low IgG	Kaikov et al 1991
A few wks	FTT, BM hypoplasia	Mayes et al 1987
10 day	Diarrhoea and vomiting; resolved, discharged on multivitamin containing B_{12}; when B_{12} was discontinued had lethargy, decreased appetite, vomiting, poor suck; at 7 month admitted for FTT	Zeitlin et al 1985
4 month	At 4 month vomiting, diarrhoea, chronic oral thrush, FTT, hypotonia	Carmel and Ravindranath 1984
7 week	Pancytopenia, low IgG, mucosal ulceration, FTT	Rana et al 1983
"A few wks"	"Severe hematological abnormalities"	Burman et al 1979

B_{12} vitamin B_{12}; *BM* bone marrow; *d* days; *DD* developmental delay; *Dx* diagnosis; *FTT* failure to thrive; *IgGs* immunoglobulin G; *m* months; *MDS* myelodysplastic syndrome; *wks* weeks; *y* years

summarizes those patients whose clinical course we were able to update, while Table 2 lists those patients whose outcome could not be updated because we were unable to contact the respective corresponding authors. Table 3 summarizes the clinical presentations of new patients ascertained with TC deficiency. Table 4 describes the Cbl supplementation regimen of the new and updated patients. Table 5 summarizes the medical and developmental outcomes of these patients, as well as their *TCN2* genotypes.

Description of patients

Our cohort consisted of 30 patients diagnosed with TC deficiency, who were from 21 unrelated families. Nineteen of these were females and 11 were males. The age of these patients at the time of diagnosis and the age when the most recent follow-up occured are depicted in Fig. 1.

Figure 2 summarizes the clinical features for the 24 patients who were symptomatic at the time of diagnosis. Twenty one patients (87.5 %) had haematological complications, including anemia or pancytopenia [95 % confidence interval (CI) 74 % to 100 %]; four patients had glossitis [17 %; 95 % C.I. 2 % to 32 %]; three patients (12.5 %; CI: -0.73 % to 25.73 %) had skin manifestations, including non-specific rash, purpura and petechiae; 16 individuals had failure to thrive (66.6 %; CI 48 % to 85 %); nine patients had gastrointestinal complications, such as vomiting and diarrhoea [37.5 %; 95 % C.I. 18 %-57 %]; seven patients had neurological findings such as weakness, hypotonia, myoclonic like movements or delayed milestones [29 %; 11 % to 47 %]; four patients had immunological abnormalities such as agammaglobulinemia, low IgGs, or low T and B cell counts [17 %; 95 % C.I. 2 % to 32 %]; and two patients had recurrent infections (8 %; CI:-2.85 % to 18.85 %).

Seven patients had a bone marrow biopsy and/or aspirate done as part of their clinical evaluation, before the diagnosis of TC deficiency was considered. Three showed features suggestive of myelodyplastic syndrome (MDS)/leukemia (patients #6, #12, #24). In six patients the bone marrow exhibited megaloblastic changes which were later found to be consistent with cobalamin deficiency (patients #21, #9, #10, #14, #18, #24).

Six families in our cohort had at least one child that died before the diagnosis of TC deficiency was made (see families of patients: #7, #9, #14, #11, #16, #23; for a total of eight deaths). The diagnosis was made post-mortem using fibroblasts in one patient (#23), in the remaining patients TC deficiency was suspected based on the diagnosis in a subsequent sibling.

Clinical outcomes

Early treatment Nine cases were identified in the context of family history of TC deficiency and thus received early

Table 3 New patients identified by survey

Patient ID #	Age at Dx	Clinical signs and symptoms at Dx
#20	7 month	Fever, persistent diarrhoea, FTT, pancytopenia, low IgG, hypotonia
#21	3 month	At 2 week old vomiting, floppy, weak cry; at 6 week pale, vomiting, FTT, anemia requiring transfusion, hypercellular megaloblastic BM
#22	2 month	FTT, megaloblastic anemia, compensated metabolic acidosis with an elevated anion gap; serum Cbl normal
#23	1 month	Normal (neonatal seizure and lethargy in context of birth asphyxia, normal brain MRI at 2 month); affected sibling diagnosed post mortem with TC def. based on skin fibroblast studies
#24	3 month	At 3 month FTT, diarrhoea, generalized hypotonia, pancytopenia, DD, low IgGs, low T and B cell counts; progressively transfusion, dependent NG feeds, lactose intolerance, opportunistic infections, intubated, megaloblastic BM, MDS suspected
#25	In-utero	Normal
		Sibling of patient #24
#26	3 month	Pallor, weakness, dyspnea, tachypnea, feeding difficulty 1/6 systolic murmur
#27	4 month	Pallor, weakness, feeding difficulty, FTT, 2/6 systolic murmur, petechial rashes, hydrocele
#28	2 month	Pallor, fever, vomiting
		FTT, umbilical hernia
#29	2.5 month	Pallor, feeding difficulty, petechial rashes, tachycardia
#30	2 month	Glossitis, megaloblastic anaemia, FTT

BM bone marrow; *Cbl* cobalamin; *DD* developmental delay; *Dx* diagnosis; *FTT* failure to thrive; *IgGs* immunoglobulin G; *m* months; *NG feeds* nasogastric feeds; *MDS* myelodysplastic syndrome; *wks* weeks

treatment in the neonatal period (i.e. during the first 4 weeks of life). All of them have had an excellent outcome (patients: #2, #3, #5, #7, #8, #11, #13, #25, #19). In this group, the oldest patient is 40 years old and there are five additional patients above the age of 9 years.

Long term outcome There are 19 patients in this cohort older than 6 years old, i.e. school age or more (see patients # 1-4, 6, 7, 9-12, 16-23, 30). Ten of these patients (patients #1, #2, #9, #10, #11, #16, #17, #18, #19, #30) are older than 15 years old. Patient #16 is the only patient who is reported to have significant intellectual deficits. Specifically, at the age of 15 years and 3 months she was reported to have retinopathy with partial blindness and intellectual disability (please refer to *Mode of administration* section below for more details). Otherwise, patient #17 had some school difficulties, progressive ataxia and intermittent myoclonus, at 13 years of age. However, these responded to treatment optimization (from intramuscular cyanocobalamin injections three times per month to weekly hydroxycobalamin injections). Currently, at the age of 32 years, she is independent and has children of her own.

Complications The most common complications for patients on treatment were speech deficits (six patients) and attention deficits (four patients), which may be unrelated to TC deficiency. Other late-onset complications reversible with treatment optimization include tremors or chorea (four patients), anemia with acute disease or menses requiring transfusions (four patients) and visual problems (three patients). One of the patients with visual problems (patient #14) had reduced visual evoked potentials of non-clinical significance) and the other two (patients #16, #17) had retinopathy. Of note, patient #16 was not on IM Cbl (but rather only on PO Cbl) and patient #17 was receiving IM Cbl only three times per month and not very consistently until the time the retinopathy was diagnosed.

Molecular characterization

Of the 21 unrelated families, molecular testing was performed on 20 families. There were 17 different mutations identified. From these 20 families, there were 16 homozygotes and four compound heterozygotes. Different intragenic deletions were identified in 15 out of the 20 families who had molecular testing. Four mutations resulted in exon skipping (Namour et al 2003): two involved exon seven and one involved exon four; the fourth was a large deletion involving exons 1 to 7. No obvious genotype-phenotype correlation was noted. There appears to be a founder mutation in the patients of Turkish ancestry, as all four presumably unrelated probands of Turkish ancestry shared a novel homozygous mutation (c.1106+1516_1222+1231del).

Cbl therapy

There was marked variation in the type of Cbl and mode of administration amongst the patients, as summarized in Table 4.

OH-Cbl versus CN-Cbl As can be seen in Tables 4 and 5, multiple patients have been treated with CN-Cbl instead of OH-Cbl without documented complications in terms of clinical status or biochemical parameters. Patients #1-3, #6, #10, #11, #24-29 were reportedly on CN-Cbl IM for several years and their outcome appears grossly normal. The oldest patient in this group is 20 years of age (patient #1). In contrast, when patient #19 was switched from OH-Cbl (1 mg IM 2×/week) to CN-Cbl for 5 months, she developed severe bone marrow suppression and MMA increase. Quick clinical improvement was noted upon switching back to OH-Cbl. Moreover, patient #9, when treated with CN-Cbl required two injections per

Table 4 Cbl supplementation of patients from Tables 1 and 3, from diagnosis until the most recent evaluation

Patient ID	Age Rx started	Treatment	Monitoring for dose adjustment
#1	1 y	CN-Cbl 1 mg IM q wk and 1 mg PO QD, then switched gradually to 2 mg IM q wk and CN-Cbl 5 mg PO QD	CBC
#2 Sib of #1	2 week	CN-Cbl 1 mg IM q wk and 1 mg PO QD, then switched gradually to 2 mg IM q wk and CN-Cbl 5 mg PO QD	CBC
#3 Sib of #1	3 week	CN-Cbl 1 mg IM q wk and 1 mg PO QD, then switched gradually to 2 mg IM q wk and CN-Cbl 5 mg PO QD	CBC
#4	3 month	OH-Cbl 1 mg/day IV × 1 week; then OH-Cbl IM 1 mg 3×/week then q wk	Urine MMA Cbl, folate q y
#5 Sib of #4	1st wk	OH-Cbl IM 1 mg IM q wk	See patient #4
#6	6 month	CN-Cbl 1 mg IM 2×/week; at 10 month switched to CN-Cbl PO QD; at 11 7/12 y switched to OH-Cbl 10 mg po QD; at 11 8/12 y added OH-Cbl 3 mg IM q wk	Cbl levels >2× Normal) tHcy, UOA
#7 Sib of #6	1st wk	CN-Cbl PO; at 8 7/12 y switched to OH-Cbl 5 mg PO QD; at 8 8/12 y added OH-Cbl 2 mg IM q wk	See patient #6
#8 Sib of #6	1st wk	CN-Cbl PO; at 18 month switched to OH-Cbl 3 mg PO QD; at 2 y added OH-Cbl 1 mg IM q wk	See patient #6
#9	5.5 month	CN-Cbl 2 mg PO QD: CBC abnormal, macrocytosis recurrent oral ulcers; switched to CN-Cbl 1 mg IM 2×/week macrocytosis, ulcers; switched to OH-Cbl 2 mg IM q wk	Clinical (mouth ulcers) and CBC
#10	20 day	CN-Cbl 1 mg IM 2×/week; at 11 y switched to CN-Cbl PO QD and OH-Cbl IM q 2 week	MCV
#11 Sib of #10	~20 day	CN-Cbl IM 1 mg 2×/week; at 8 y switched to CN-Cbl PO QD and OH-Cbl IM q 2 week	MCV
#12	11 month	OH-Cbl 1 mg IM q wk and CN-Cbl 1 mg PO QD; after some months OH-Cbl 1 mg IM was switched to q 2 week; at 6 y IM switched to q 3 week; in addition, remained on CN-Cbl 1 mg PO QD	fHcy and plasma MMA
#13 Sib of #12	DOL #1	OH-Cbl 1 mg IM q wk and CN-Cbl 1 mg PO QD; after 2 months IM was switched to q 2 week; in addition, remained on CN-Cbl 1 mg PO QD	fHcy and plasma MMA
#14	4 month	OH-Cbl IM 1 mg QD; at 1 week switched to 1 mg q 4 week, betaine 500 mg BID and L-methionine 25 mg BID; at 2 y switched to OH-Cbl 1 mg q 2 week IM and betaine 500 mg PO BID	tHcy, methionine, urinary MMA
#15	5 week	OH-Cbl IM 2×/week; at 14 month switched to intranasal 2×/week; at 19 month switched to q wk; at 2 y switched to 2 mg OH-Cbl PO QD; at 30 month (poor MMA control) switched to OH-Cbl IM q wk	Plasma MMA, urine MMA
#16	23 day	OH-Cbl IM QD; at 1 y OH-Cbl IM QD PO; at 9 y switched to OH-Cbl IM 1 mg IM 4×/week	tHcy, urine MMA
#17	8 month	CN-Cbl IM 1 mg 3 week/m and PO folate; at 13 y switched to OH-Cbl 1 mg IM 3×/week; myoclonias, resolved; only slight ataxia and tremor 2 y later.	Based on clinical criteria
#18	4 month	OH-Cbl 1 mg IM 2×/week (but with periods of poor compliance in childhood, during which q wk or none were administered)	No frequent monitoring due to financial constraints
#19 Sib of #18	In utero/at birth	(Mom had OH-Cbl 2×/week 1 mg IM during pregnancy). OH-Cbl 1 mg IM 2×/week; later switched to CN-Cbl×5 month, due to insurance issues and developed severe BM pan-suppression and MMA increase so switched back to OH-Cbl and folate with uick clinical improvement; complete biochemical improvement occurred with OH-Cbl IM QD.	Plasma MMA, tHcy levels and CBC
#20	8 month	OH-Cbl 1 mg IM 3×/week; folate 10 mg PO; IgG 2 g/kg/BW IV; at 3.5 y switched to OH-Cbl PO 1 mg/day for religious reasons but developed complications (see Table 5) and was switched back to IM	clinical, MMA levels and CBC
#21	2 month	OH-Cbl 1.5 mg IM×3/week, folinic acid 15 mg QD; at 8 month moved to Australia; at 12 y switched to OH-Cbl 1 mg IM q wk and folinic acid 7.5 mg BID	CBC, tHcy, MMA, plasma amino acids
#22	2 month	OH-Cbl IM 1 mg QD; at 16 month switched to CN-Cbl 1 mg PO; at 2.5 y switched back to OH-Cbl 1 mg IM QD due to severe anemia	Hb, hematocrit, tHcy, plasma MMA
#23	5-6 week	OH-Cbl 1 mg IM qwk; (at 4 8/12 y transiently switched to q 2 week for only 3 week)	Urine MMA, C3- carnitine on blood spot and fHcy

Table 4 (continued)

Patient ID	Age Rx started	Treatment	Monitoring for dose adjustment
#24	3 month	OH-Cbl 1 mg QD IM and folate 5 mg QD; at 2 year switched to CN-Cbl 1 mg IM 3×/week and folate 1 mg 3×/week	Urine MMA, plasma MMA, UOA, PAA, tHcy, Cbl, folate, CBC
#25 Sib of #24	1st wk	OH-Cbl 1 mg QD IM; at 6 month switched to CN-Cbl 1 mg QD IM	See patient #24
#26	3 month	CN-Cbl IM 0.5 mg QD; at 10 day switched to 2×/week; at 3 week (RBC increased) switched to q wk; at 11 week (RBC decreased) switched to 2×/week; at 15 month switched to 1 mg q wk	CBC
#27	4 month	CN-Cbl 0.5 mg IM QD; at 2 day switched to 1 mg 2×/week; at 9 day switched to 1 mg/week; at 5 month switched to 1 mg q 2 week; at 8 month switched to 1 mg q wk	CBC
#28	2 month	CN-Cbl 0.5 mg IM QD; at 10 day switched to 2×/week; at 10 month (RBC increased) switched to 1 mg/week	CBC
#29	2.5 month	CN-Cbl 0.5 mg IM QD; at 2 day switched to 1 mg 2×/week; at 10 day (RBC increased) switched to 1 mg/week	CBC
#30	2.5 month	IM QD, then IM 3×/week (type of Cbl and early changes in treatment not available), now OH-Cbl 1 mg IM q wk	MCV

abN abnormal; *BID* twice per day; *BM* bone marrow; *CBC* complete blood count; *Cbl* cobalamin; *CN-Cbl* cyanocobalamin; *d* days; *DOL* day of life; *fHcy* free (plasma) homocystine; *Hb* hemoglobin; *Hcy* homocysteine; *IM* intramuscular; *IgG* immunoglobulin G; *IV* intravenous; *q wk* every week; *m* months; *MCV* mean corpuscular volume; *Meth* methionine; *MMA* methylmalonic acid; *OH-Cbl* hydroxycobalamin; *PAA* plasma amino acids; *Plt* platelets; *PO* orally; *QD* daily; *RBC* red blood cells; *Sib* sibling; *Rx* treatment; *tHcy* total homocysteine; *UOA* urine organic acids; *wks* weeks; *y* years

week in an effort to control his diarrhoea and mucositis. In contrast, since weekly OH-Cbl injections were initiated, he has not had any complications indicating a better response to OH-Cbl in this patient. Finally, patient #17 had progressive neurological deterioration (ataxia, myoclonus, bradykynesia) under CN-Cbl treatment (1 mg) 3 times per month which strikingly improved under OH-Cbl (administered 3 times per week), albeit this may be attributed to the frequency of IM injections rather than the form of Cbl used.

Mode of administration (oral vs. IM injection) There are 19 patients in this cohort older than 6 years old, i.e. school age or more, and only one patient (patient #16) was reported to have significant intellectual disability. This is the only patient who was treated with oral OH-Cbl, instead of injections, for several years. As summarized in Table 5, despite having started treatment at 23 days old, patient #16 has retinopathy with partial blindness and intellectual disability. Similarly, patients #20, #22, who were temporarily switched to oral OH-Cbl, and patients #18, #19, who were not compliant with OH-Cbl injections for a period of their lives, all developed complications, including severe anemia, chorea, and bilateral increased T2 signal in the putamen. These complications were reversible with treatment optimization.

Frequency of IM injections Overall, better patient outcomes were associated with at least weekly IM Cbl injections. Patients #1, #2, and #3 have been treated with weekly IM injection of CN-Cbl (initially, 1 mg IM q wk then switched gradually to CN-Cbl 2 mg q week) and have reportedly had an excellent outcome (at the age of 20, 16, and 11.5 years, respectively). Similarly, there are no reported concerns for patients #5, #23, and #30, who have been treated with weekly IM OH-Cbl injections (1 mg).

In contrast, patient #17 developed progressive school difficulties, neurological deterioration (myoclonus, tremor and ataxia) and maculopathy on IM CN-Cbl (1 mg) three times per month, which responded well to increasing the frequency of treatment (OH-Cbl 1 mg IM 3 times per week). Similarly, when patient #30 decreased her frequency of treatment to OH-Cbl 1 mg IM every third week, her MCV increased from 90 to 100 over a period of 6 months, which responded to increasing the frequency of injections once per week.

Finally, patient #12, whose treatment (1 mg OH-Cbl injections) was spaced to every two weeks after a few months of life and finally to every 3 weeks at the age 6 years, is reported to have slight speech delay, difficulties with social interactions, and requires extra aid at school. Of note, these findings may be unrelated to his treatment regimen and attributed to his late diagnosis at 11 months with a consequent late start of adequate therapy. The lower frequency of the injections does not appear to have affected the outcome of this patient's sister (patient #13) at the age of 4.5 years. Patient #13 was treated from birth until 2 months of age

Table 5 Outcome and TCN2 genotype of patients from Tables 1 and 3

Patient ID	Age at last visit	Medical complications	Education and autonomy	Formal intellectual evaluation	*TCN2* mutation *HGMD Accessed on 2013/02/14*
#**1**	20 year F	Blood Tx 3-4×/y during menses	College; Lives with parents	N/A	c.427+2 T>G c.427+2 T>G HGMD # CS034069
#**2** Sibling of #1	16 year F	Blood Tx 1×/y when acutely ill	High school; Lives with parents	N/A	c.427+2 T>G c.427+2 T>G HGMD # CS034069
#**3** Sibling of #1	11.5 year F	Blood Tx 1-3×/y during menses	Junior High school;	N/A	c.427+2 T>G c.427+2 T>G HGMD # CS034069
#**4**	8.5 year F	No issues	Regular school, no issues	Formal neuro-developmental test every 2 year, normal	c.330dupC c.330dupC HGMD # CI096317
#**5** Sibling of #4	5.5 year F	No issues	Kindergarten, no issues	Formal neuro-developmental test every 2 year, normal	c.330dupC c.330dupC HGMD # CI096317
#**6**	12 year M	Minor hand tremors, tingling of limbs	Grade 6, minor attention deficit	At 5 2/12 year: average Math and Non-verbal IQ; above average reading; reduced attention, variable language skills	c.1195C>T (p.R399X) c.1195C>T (p.R399X) HGMD # CM087884
#**7** Sib of #6	9 year F	Minor hand tremors, tingling of limbs	Grade 4, no deficits	Formal neuropsychological testing at 8 9/12 year : average in most domains, weak in reading comprehension	c.1195C>T (p.R399X) c.1195C>T (p.R399X) HGMD # CM087884
#**8** Sib of #6	2 year F	None	N/A	N/A	c.1195C>T (p.R399X) c.1195C>T (p.R399X) HGMD # CM087884
#**9**	40 year M	Hx of macrocytosis, resolved after IM OH-Cbl	Computer Engineer, independent, has healthy son	N/A	c.348_349delTG c.348_349delTG Novel this paper
#**10**	34 y F	Megaloblastic crisis × 1 upon decreasing Cbl. Resolved after reversing Rx change.	No issues, independent	N/A	Biochemical diagnosis, molecular analysis not performed
#**11** Sib of #10	31 year M	None	No issues, independent	N/A	Biochemical diagnosis, molecular analysis not performed
#**12**	7 year M	History of chronic asthma. No increase in infections.	Regular school, but requires aid. Difficulties in social interactions, mild speech delay	N/A	c.1106+1G>A c.1106+1G>A HGMD # CS106900
#**13** Sib of #12	4.5 year F	None	No issues	N/A	c.1106+1G>A c.1106+1G>A HGMD # CS106900
#**14**	4 year F	Reduced visual evoked potentials at 2 8/12 year	Kindergarden, requires speech therapy	N/A	c.580+1G>C c.580+1G>C HGMD # CS106930
#**15**	4.5 year F	None	Kindergarden, No issues	N/A	c.497_498delTC c.1139dupA
#**16**	15 3/12 y F			N/A	c.497_498delTC

Table 5 (continued)

Patient ID	Age at last visit	Medical complications	Education and autonomy	Formal intellectual evaluation	*TCN2* mutation *HGMD Accessed on 2013/02/14*
		Retinopathy with partial blindness, intellectual disability	School for the blind; at 15 year performs at a primary school level		c.497_498delTC HGMD # CD106933
#**17**	32 year F	Progressive blurred vision and retinopathy since 11 year; nocturnal polyuria, recurrent syncope, anhidrosis, ataxia, intermittent myoclonus, improved after Rx change at 13 year; cerebellar ataxia and tremor at 15 year; depression at 32 year	School difficulties; stopped studies after 4 year of high school, but completed professional formation. Now office worker; Independent; married, has children	At 6, 7, 8, and 13 year Wechsler scale full IQ: 62	c.501_503delCCA c.1115_1116CA
#**18**	27 year M	Hx of gross motor delay; dystonia episode at 10 year when brother had chorea, resolved; stomatitis, glossitis, petechiae, irritability when compliance was poor, adolescent onset of hearing problems	Average to poor academic performance, ADD. Dropped out of high school. Works as salesman and lives with wife and children.	N/A	c.927-930delTCTG 22q12.2 del involving exons 1 to 7 of *TC* gene
#**19** Sib of #18	22 year M	Sydenham's chorea at 5 year; residual chorea in right upper extremity	Now Junior in college, no issues, Lives independently	N/A	c.927-930delTCTG 22q12.2 del involving exons 1 to 7 of *TCN2* gene
#**20**	12 year F	None now. At 6 month after switch to PO Cbl: MRI showed bilateral increased T2 signal in the putamen, IM Cbl was restarted.	High-school, no issues	At 7 year: DQ by Griffiths Developmental Test twice. At 9 year: IQ by WISC-II: both identified mild delays	c.423delC c.937C>T (p.R313X) Novel this paper, both
#**21**	14 year F	None	Delayed speech and had speech therapy Now in junior high school with support, no issues	ADHD diagnosis based on formalneuropsychological exam	c.940+283_286delTGGA; c.940+303_1106+764del2152insCTGG c.940+283_286delTGGA; c.940+303_1106+764del2152insCTGG Novel this paper
#**22**	11 year M	Cardiac output failure due to severe anemia at 2.5 year, transfused; Ht and Wt<5%ile; ADHD	5th grade, regular school with A and B average, on methylphenidate	ADHD diagnosis based on formalneuropsychological assessment	c.497_498delTC c.497_498delTC HGMD # CD106933
#**23**	6 2/12 year M	Neonatal seizures resolved, one further seizure when Rx changed to q 2 week IM Cbl, remains on AED	1st grade	N/A	c.497_498delTC c.497_498delTC
#**24**	2 3/12 year M	Atypical febrile seizures at ~1 year -remains on AED recurrent infections	N/A Language delay (<10 words)	N/A	c.1013_1014 delinsTAA c.1013_1014 delinsTAA (p.S338IfsX27) Novel this paper
#**25** Sib of #24	7 month M	None	N/A	N/A	c.1013_1014 delinsTAA c.1013_1014 delinsTAA (p.S338IfsX27) Novel this paper
#**26**	24 month F	History of cerebral hemorrhage	N/A Developmental delay	N/A	c.1106+1516_1222+1231del5304

Table 5 (continued)

Patient ID	Age at last visit	Medical complications	Education and autonomy	Formal intellectual evaluation	*TCN2* mutation *HGMD Accessed on 2013/02/14*
					c.1106+1516_1222 +1231del5304
					Novel this paper
#**27**	25 month M	None	N/A Speech/language delay.	N/A	c.1106+1516_1222 +1231del5304
					c.1106+1516_1222 +1231del5304
					Novel this paper
#**28**	18 month F	Congenital hip dislocation	N/A No issues	N/A	c.1106+1516_1222 +1231del5304
					c.1106+1516_1222 +1231del5304
					Novel this paper
#**29**	26 month F	None	N/A Developmental delay: especially speech/language.	N/A	c.1106+1516_1222 +1231del5304
					c.1106+1516_1222 +1231del5304
					Novel this paper
#**30**	29 year F	Prolapsed disc, atypical migraine	No school issues and completed grade 12, works in an office. Lives independently, pregnant with 1st child	N/A	c.744delG
					c.744delG

AED anti-epileptic drug; *ADD* attention deficit disorder; *ADHD* attention deficit hyperactivity disorder; *Blood Tx* blood transfusions; *Cbl* cobalamin; *CN-Cbl* cyanocobalamin; *DD* developmental delay; *Dx* diagnosis; *F* Female; *HGMD* human gene mutation database; *Ht* height; *Hx* history; *IM* intramuscular; *m* months; *M* Male; *MR* mental retardation/intellectual disability; *N/A* not applicable; *Rx* treatment; *Sib* sibling; *Rx* treatment; *Wt* weight; *y* years

with IM OH-Cbl injections (1 mg). At that point her injections were spaced from weekly to every other week. This family emphasizes that clinical status should be closely monitored even when the biochemical parameters are within normal limits.

Discussion

We report on the clinical presentations, management and outcome of a series of patients with TC deficiency. Our cohort consisted of 30 patients from 21 unrelated families. Different intragenic deletions were identified in 15 out of the 20 families who had molecular testing. No obvious genotype-phenotype correlation was noted but the remarkable frequency of deletions and the paucity of missense mutations needs to be highlighted. The supplemental table lists all the cases and mutations present in our cohort or identified through our literature review.

The most common clinical presentation in our cohort consisted of anaemia (or pancytopenia), diarrhoea, failure to thrive, and hypotonia. The clinical signs were progressive; they appeared to start a few weeks after birth but they were typically not severe enough to suspect the diagnosis until after 3 months of age. Nevertheless, a delay in diagnosis can result in acute clinical deterioration necessitating regular transfusions for anemia and thrombocytopenia, nasogastric feeds for severe failure to thrive, and intubation for life-threatening opportunistic infections (as illustrated in patient #24). Most reported deaths occurred more than 10 years ago suggesting that there is now better awareness in the medical community about TC deficiency.

All nine cases picked up in the context of family history of TC deficiency received early treatment in the neonatal period and have had optimal outcomes. This information illustrates the importance of early diagnosis and raises the question of whether newborn screening (NBS) can detect TC deficiency. There is one patient in our cohort who was identified by NBS using tandem mass spectrometry suggesting that TC deficiency may be identifiable by NBS. The flagged values were C3 and C3/C2 acylcarnitine elevation (Prasad et al 2008; 2012). For reproductive counselling of parents with a previously

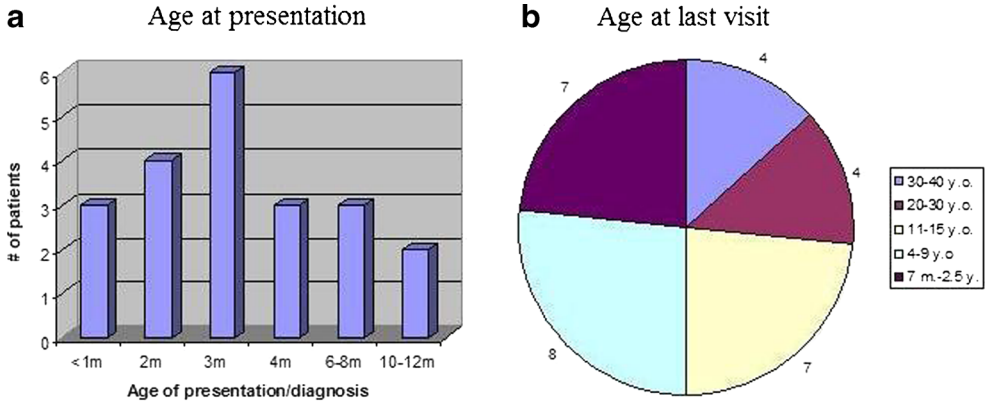

Fig. 1 A summary of the different age groups at presentation and at the time of diagnosis for the patients included in this manuscript. **1a**. In total, 12 individuals were diagnosed in the neonatal period (i.e. during the first month of life). Three of them presented with symptoms, while the remaining were diagnosed based on family history. The majority of patients (13) presented with symptoms from 2 to 4 months of age. Finally, five patients were diagnosed after the age of 6 months. **1b**. At the time of the last evaluation, four patients in our cohort were between 30 and 40 years old; four between 20 and 30 years old; seven between 11 and 15 years old; eight between 4 and 9 years old and seven below the age of 2.5 years. *Abbreviations*: FMHx: family history; m.: month(s); y.o.: years old

affected child, again the data suggest that early diagnosis and management should result in a good outcome.

Comparison of Cbl therapies

No clear correlation of the form of cobalamin used [hydroxylcobalamin (OH-Cbl) versus cyanocobalamin (CN-Cbl)] was noted in this cohort. As can be seen in Tables 4 and 5, half of the patients received CN-Cbl instead of OH-Cbl injections. Patient outcomes were similar when IM CN- or OH-Cbl was used.

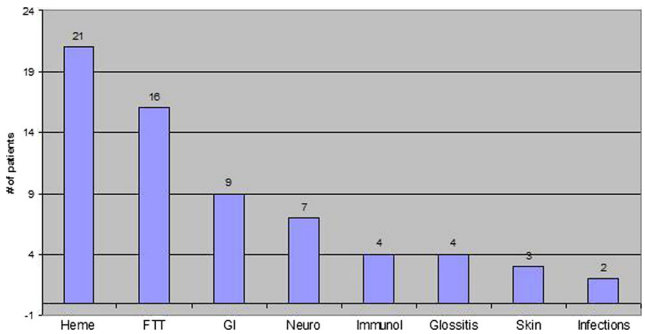

Fig. 2 Frequency of different findings in individuals included in this manuscript who were symptomatic at the time of diagnosis. Out of the 24 patients who were symptomatic at the time of diagnosis, 21 patients had haematological complications, including anemia or pancytopenia; four patients had glossitis; three patients had skin manifestations, including non-specific rash, purpura or petechiae; 16 individuals had failure to thrive; nine had gastrointestinal complications, such as vomiting and diarrhoea; seven had neurological findings such as weakness, hypotonia, myoclonic like movements or delayed milestones; four had immunological abnormalities such as agammaglobulinemia, low IgGs, or low T and B cell counts; and two patients had recurrent infections. *Abbreviations*: Heme: haematological; Neuro: neurological; GI: gastrointestinal; Immunol: immunological; FTT: failure to thrive

The collected data suggest that both the age at commencement of Cbl treatment and its mode of administration influence outcome. IM administration appears to be important since there was only one patient reported to have significant intellectual disability, the only one treated with oral OH-Cbl, instead of injections for several years. Moreover, four patients who did not receive Cbl injections for a period later in their lives, all developed complications which responded to IM injections. Interestingly, however, patients #6 and #7 (siblings aged 12 and 9 years, respectively) have not been on regular injections and their outcomes do not appear significantly compromised. Patient #6 was switched to oral Cbl at the age of 10 months while his sister (patient #7) was treated with oral Cbl from the first week of life. It is possible that the Cbl storage of patient #7 was never depleted. Minor tremors (in patients #6 and #7) and attention deficits (in patient #6) were reported in a recent evaluation. It is not clear if these symptoms are related to the mode of treatment but when IM OH-Cbl was added to their regimen total plasma homocysteine normalized, which never occurred on oral OH- or CN-Cbl.

Furthermore, some data suggest that complications can appear later in life and that IM Cbl injections should be continued for life. When patient #30 decreased her frequency of treatment to OH-Cbl 1 mg IM every third week at the age of 28 years, her MCV increased from 90 to 100 over a period of 6 months. She was subsequently restarted on weekly injections and her MCV returned to 91 within 3 months. Moreover, four patients in our cohort developed late-onset tremors, chorea or visual problems, which were reversible. For instance, patient #17 was on IM CN-Cbl (1 mg) three times per month since the age of 8 months. At the age of 13 years she had some school difficulties and was found to have a formally assessed IQ (Wechsler scale full IQ) of 62. Her progressive neurological deterioration (myoclonus, tremor and ataxia) and

maculopathy responded well to increasing the frequency of treatment (OH-Cbl 1 mg IM 3 times per week). She completed professional training and at the age of 32 years, she still has some blurry vision but is independent and has children of her own. In conclusion, the frequency of IM injections also appears to influence outcome. Six patients in our cohort were on weekly IM Cbl injections and all reportedly have normal outcome suggesting that this may be the minimum standard to ensure adequate treatment of patients with TC deficiency.

In our cohort, most physicians appear to adjust treatment in an effort to maintain haemoglobin, MCV, platelets, plasma methionine, total plasma homocysteine and MMA levels within normal limits.

Underreported complications

Speech deficits were the most common complication for patients on treatment, so close monitoring and referral to speech therapy as appropriate is advised. Late-onset tremors, myoclonus, ataxia, and late-onset retinopathy can also occur and appear to respond to regular Cbl IM injections. Epilepsy responsive to antiepileptic medication was also reported in two patients. Another example of under-reported complications is Cbl-responsive anaemia due to acute disease, menses or after spacing/decreasing treatment requiring transfusions even after infancy. Attention difficulties may also be present. Finally, severe diarrhoea, associated with a deficiency of disaccharidases (e.g. lactase) can also occur in TC deficiency. This may resolve with lactose free diet and it may be associated with severe atrophy of the intestinal mucosa (Hitzig et al 1974).

Limitations of our data

The aim of this manuscript was to provide an observational study to aid in the optimization of management in patients with TC deficiency. However, the conclusions reached should be interpreted in the context of the limitations of this work. This is a retrospective study encompassing patients from multiple institutions. The information available to us was not completed equally for all the patients included, as can be seen in Tables 4 and 5. Finally, there is a lack of metabolic data and controls which would help strengthen our conclusions. However, this is a retrospective collection of case reports, i.e. a descriptive study.

Conclusions

TC deficiency is a rare autosomal recessive inborn error of metabolism which when treated aggressively appears to be associated with an overall favourable outcome. Based on the data presented, current practice appears to favour early diagnosis and treatment of individuals with TC deficiency by intramuscular injections of OH- or CN-Cbl. In most cases presented, at least weekly injections (1 mg IM) were necessary to ensure optimal treatment. Most centres adjusted the treatment regimen based on monitoring CBC, total plasma homocysteine, plasma and urine methylmalonic acid, as well as, clinical status. Finally, some of the late-onset complications discussed were reversible with IM injections, so continuing treatment in adulthood appears to be beneficial. The benefit of using OH-Cbl over CN-Cbl was not clear in our cohort but hydroxycobalamin may aid to optimize treatment in some patients. Genetic counselling should be provided to the affected families. Newborn screening can be useful in earlier detection of this entity.

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Conflict of interest None.

References

Aitelli C, Wasson L, Page R (2004) Pernicious anemia: presentations mimicking acute leukemia. South Med J 97(3):295–297

Arlet JB, Varet B, Besson C (2002) Favorable long-term outcome of a patient with transcobalamin II deficiency. Ann Intern Med 137(8): 704–705

Arrabal MC, Villegas A, Mariscal E, Lentijo B, Luck W, Hoffbrand AV (1988) Transcobalamin II deficiency: long-term follow-up of two cases. Acta Haematol 80(3):162–166

Bibi H, Gelman-Kohan Z, Baumgartner ER, Rosenblatt DS (1999) Transcobalamin II deficiency with methylmalonic aciduria in three sisters. J Inherit Metab Dis 22(7):765–772

Burman JF, Mollin DL, Sourial NA, Sladden RA (1979) Inherited lack of transcobalamin II in serum and megaloblastic anaemia: a further patient. Br J Haematol 43(1):27–38

Carmel R, Ravindranath Y (1984) Congenital transcobalamin II deficiency presenting atypically with a low serum cobalamin level: studies demonstrating the coexistence of a circulating transcobalamin I (R binder) complex. Blood 63(3):598–605

Grech V, Vella C, Mercieca V (2001) Temporary myoclonus with treatment of congenital transcobalamin 2 deficiency. Pediatr Neurol 24(1):75–76

Gururangan S, Frankel W, Broaddus R et al (2008) Multifocal anaplastic astrocytoma in a patient with hereditary colorectal cancer, transcobalamin II deficiency, agenesis of the corpus callosum, mental retardation, and inherited PMS2 mutation. Neuro Oncol 10(1): 93–97

Häberle J, Pauli S, Berning C, Koch HG, Linnebank M (2009) TC II deficiency: avoidance of false-negative molecular genetics by RNA-based investigations. J Hum Genet 54(6):331–334

Hall CA (1992) The neurological aspects of transcobalamin II deficiency. Br J Haematol 80:117–120

Hitzig WH, Dohmann U, Pluss HJ, Vischer D (1974) Hereditary transcobalamin II deficiency: clinical findings in a new family. J Pediatr Nov 85(5):622–628

Kaikov Y, Wadsworth LD, Hall CA, Rogers PC (1991) Transcobalamin II deficiency: case report and review of the literature. Eur J Pediatr 150(12):841–843

Li N, Rosenblatt DS, Kamen BA, Seetharam S, Seetharam B (1994a) Identification of two mutant alleles of transcobalamin II in an affected family. Hum Mol Genet 3(10):1835–1840

Li N, Rosenblatt DS, Seetharam B (1994b) Nonsense mutations in human transcobalamin II deficiency. Biochem Biophys Res Comm 204: 1111–1118

Mayes JS, Say B, Marcus DL (1987) Prenatal studies in a family with transcobalamin II deficiency. Am J Hum Genet 41(4): 686–687

Namour F, Olivier J, Abdelmouttaleb I et al (2001) Transcobalamin codon 259 polymorphism in HT-29 and Caco-2 cells and in Caucasians: relation to transcobalamin and homocysteine concentration in blood. Blood 97(4):1092–1098

Namour F, Helfer AC, Quadros EV et al (2003) Transcobalamin deficiency due to activation of an intra exonic cryptic splice set. Br J Haematol 123:915–920

Nissen PH, Nordwall M, Hoffmann-Lücke E, Sorensen BS, Nexo E (2010) Transcobalamin deficiency caused by compound heterozygosity for two novel mutations in the TCN2 gene: a study of two affected siblings, their brother, and their parents. J Inherit Metab Dis 33(Suppl 3):269–274

Prasad C, Rosenblatt DS, Corley K, Cairney AE, Rupar CA (2008) Transcobalamin (TC) deficiency–potential cause of bone marrow failure in childhood. J Inherit Metab Dis 31(Suppl 2): S287–S292

Prasad C, Cairney AE, Rosenblatt DS, Rupar CA (2012) Transcobalamin (TC) deficiency and newborn screening. J Inherit Metab Dis 35(4):727

Rana SR, Colman N, Goh KO, Herbert V, Klemperer MR (1983) Transcobalamin II deficiency associated with unusual bone marrow findings and chromosomal abnormalities. Am J Hematol 14(1):89–96

Ratschmann R, Minkov M, Kis A et al (2009) Transcobalamin II deficiency at birth. Mol Genet Metab 98(3):285–288

Schiff M, Ogier de Baulny H, Bard G et al (2010) Should transcobalamin deficiency be treated aggressively? J Inherit Metab Dis 33:223–229

Souied EH, Benhamou N, Sterkers M et al (2001) Retinal degeneration associated with congenital transcobalamin II deficiency. Arch Ophthalmol 119:1076–1077

Thomas PK, Hoffbrand AV (1997) Hereditary transcobalamin II deficiency: a 22 year follow up. J Neurol Neurosurg Psychiatry 62(2): 197

Thomas PK, Hoffbrand AV, Smith IS (1982) Neurological involvement in hereditary transcobalamin II deficiency. J Neurol Neurosurg Psychiatry 45(1):74–77

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

Watkins D, Rosenblatt DS (2011) Inherited disorders of folate and cobalamin transport and metabolism, In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, eds. The Online Metabolic and Molecular Bases of Inherited Disease, 8th edn. Chap 155, on-line, McGraw-Hill, New-York

Zeitlin HC, Sheppard K, Baum JD, Bolton FG, Hall CA (1985) Homozygous transcobalamin II deficiency maintained on oral hydroxocobalamin. Blood 66(5):1022–1027