Homozygous Transcobalamin II Deficiency Maintained on Oral Hydroxocobalamin

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A case of transcobalamin II (TCII) deficiency in which a total absence of TCII was demonstrated both functionally and immunologically is reported. Unlike previously described patients, this child has been maintained on oral hydroxocobalamin, 2 mg daily, without any parenteral supplementation for the last five years. At the age of six years her development is normal and her health is good. Plasma cobalamin levels are in the range of 3,000 ng/L and most of this appears to be bound to a molecule, which on gel filtration, elutes with albumin. In an extended family study, a clear separation of heterozygotes from both the proposi-
tus and from normal subjects suggests that the underlying defect in this condition is confined to a single gene.

TRANSCOBALAMIN II (TCII) is an important plasma cobalamin (Cbl, vitamin B12) transport protein consisting of a single polypeptide chain with a mol wt of 38,000. It has a short plasma half-life; it appears to be synthesized at multiple sites in the body including bone marrow, liver, and the ileal enterocyte. Plasma TCII is largely unsaturated, carrying only 10% to 20% of the total plasma Cbl, most of which is bound to transcobalamin I, which together with transcobalamin III are collectively known as R-binder. However, in contrast to R-binder, TCII is crucial for the delivery of Cbl to the tissues and has been shown in vitro to facilitate its uptake into cells. It is also thought to play a role in normal gastrointestinal Cbl absorption.

To date, five persons with total absence of TCII have been described in detail. Typically, they have first been examined in the first weeks of life and have had severe megaloblastosis and infection responding rapidly to massive and frequent doses of parenteral Cbl. We report the case of a child, now 6 years of age, in good health and developing normally, who has been successfully maintained on oral hydroxocobalamin alone.

MATERIALS AND METHODS

Case report. K. C. was delivered normally at term following an uneventful pregnancy; her birth weight was 3.4 kg. Her parents are unrelated; the mother is English, and the father is of Danish extraction. She appeared to be a healthy infant and was formula-fed from birth. She was well for the first ten days after birth, when she developed diarrhea and vomiting that required hospital admission. Her hemoglobin at this time was 13.5 g/dL. She was discharged on an oral multivitamin preparation, Ketovite (Pabym Laboratories, Greenford, Middlesex, England), which included cyanocobalamin (CNCbl) 12.5 μg daily. She was given this preparation intermittently over the following months. Each time the ketovite was stopped, she became lethargic within two to three weeks, lacking in appetite, reluctant to suck, and had a sore tongue, regurgitation, and vomiting. Despite these symptoms, her early developmental milestones were normal. At the age of seven months, however, she was clearly failing to thrive and was admitted for investigation. She was pale, mildly pyrexic (38 °C), and her weight was below the third percentile for her age (3.53 kg). The only other physical sign was a smooth red tongue with some buccal ulceration. Investigations revealed a megaloblastic anemia with a hemoglobin of 8.5 g/dL; mean corpuscular volume (MCV) 108; WBC 6.3 × 109/L (neutrophils 0.5 × 109/L); platelet count 124 × 109/L; and the presence of megaloblasts and hypersegmented neutrophils in the peripheral blood film. Serum Cbl was 170 ng/L (repeat 420 ng/L), serum folate 17.2 μg/L, red cell folate 690 μg/L, serum iron 18 μmol/L, and total iron binding capacity 48 μmol/L. Other investigations were normal; in particular, serum immunoglobulins were normal.

There was no proteinuria, homocystinuria, or orotic aciduria, and x-ray studies of chest and abdomen were normal.

She was treated initially with a single intramuscular injection of hydroxocobalamin (OHCbl) 100 μg and daily folic acid 2.5 mg, together with the multivitamin preparation containing CNCbl 12.5 μg. After five days of treatment, she was dramatically better, alert, happy, and eager to feed. This was paralleled by a good reticulocyte response followed by a rise in hemoglobin, WBC, and platelets (Fig 1). However, over the next few months, she suffered frequent upper respiratory tract infections and two weeks after an injection of OHCbl or stopping the oral vitamin preparation, the feeding difficulties recurred, together with a sore tongue. Hematological indices remained normal, but in March 1979, four weeks after withdrawing all therapy for the purpose of Cbl absorption studies, she became macrocytic with a hemoglobin of 8.8 g/dL and again as when she was first seen, she had normal or high serum Cbl, folate, and red cell folate. This time she was treated solely with intramuscular OHCbl 200 μg, to which she made a brisk clinical and hematological response (Fig 1). Over the next two months, she was given 200 μg of OHCbl at two weekly intervals, increasing to 250 μg weekly and finally to 1,000 μg weekly. During this time, K. C. was much improved, but was still rather slow to feed; her tongue remained smooth and red, and her weight remained well below the tenth percentile. She also appeared to be slower in recovering from common upper respiratory infections as compared with her brothers. Hematological indices remained normal, although macrocytes and poikilocytes persisted in the peripheral blood film. It was not until June 1979, when K. C. was 13 months of age, that the suspected diagnosis of hereditary homozygous transcobalamin II deficiency was finally confirmed by the family study.

At this time, parenteral therapy was replaced entirely by oral OHCbl 1 mg daily. Following this, she became more robust, her tongue became normal, her appetite improved, she gained weight—reaching the tenth percentile—and at 14 months of age, she was walking. She recovered from upper respiratory tract infections quickly “like her brothers” and at the age of 18 months had measles from which she recovered normally with the production of specific IgG antibodies.

At the age of 2 1/2 years, while she was generally well, her skin was noted to be dry, and it was not possible to elic it tendon reflexes in her lower limbs. Her weight had gradually drifted from the tenth percentile to the third percentile (height remained on 25th percen-
and total WBC × 10\(^9\)/L, hemoglobin g/dL. Estimating plasma vitamin B\(_2\) binding capacities was taken into percentage of reticulocytes. Hematological blood counts in relation to treatment: fluoride/EDTA unless otherwise stated. Care was taken to avoid repeated freezing and thawing of samples. Total unsaturated B\(_2\) binding capacity (UBBC) was determined by labeling the plasma with \(^{57}\)Co-labeled CNCbl in 0.5 g CNCbl. Normal reference values for unsaturated binding capacities were derived in the same way from 12 healthy individuals. The distribution of endogenous Cbl in the serum of K. C. was determined at the time of treatment. Vitamin B\(_2\) profiles were carried out repeatedly on K. C., and at least once on members of her family in an extended study spanning three generations (Table 1). Hematological indices were obtained by standard procedures. Serum Cbl, serum folate, and red cell folate were measured by radioisotope dilution. Blood for estimating plasma vitamin B\(_2\) binding capacities was taken into fluoride/EDTA unless otherwise stated. Care was taken to avoid repeated freezing and thawing of samples. Total unsaturated B\(_2\) binding capacity (UBBC) was determined by labeling the plasma with \(^{57}\)Co-labeled CNCbl, 1.1 kBq per 100 \(\mu\)L of plasma, followed by absorption of unbound CNCbl with albumin-coated charcoal. The unsaturated binding capacity of individual transcobalamins was assayed using \(^{57}\)Co CNCbl-labeled plasma. This was achieved by both diethylaminoethanol (DEAE) cellulose chromatography, which separates plasma transcobalamins into three components, and also by Sephadex G-200 gel filtration, which is a more sensitive procedure for detecting small amounts of TCII but which does not divide the R-binder. Normal reference values for unsaturated binding capacities were derived in the same way from 12 healthy individuals. Total TCII was measured immunologically by radioimmunoprecipitation (RIA) (Morelli et al.) and normal values were obtained from 20 healthy individuals. The distribution of endogenous Cbl in the serum of K. C. after treatment was studied by tracing the serum with \(^{57}\)CoCbl followed by fractionation on Sephadex G-200, the fractions being analyzed for total protein, radioactivity, and endogenous cobalamin.

Fig 1. Hematology chart showing peripheral blood counts in relation to treatment: percentage of reticulocytes, -○-○-○-○-; hemoglobin g/dL, -●-; platelets × 10\(^9\)/L, -△-△-△-. A test of gastrointestinal absorption of Cbl in K. C. was attempted in March 1979, primarily to exclude an intrinsic factor deficiency. Treatment was stopped for two weeks prior to giving an oral dose of 3.7 kBq \(^{57}\)Co-labeled CNCbl in a total dose of 0.5 \(\mu\)g CNCbl followed by counting over the liver seven days later. The test was repeated with intrinsic factor. A similar absorption study was carried out on K. C.’s mother after an oral dose of 20.7 kBq of \(^{57}\)Co-labeled CNCbl in 0.5 \(\mu\)g CNCbl.

RESULTS

The clinical and hematological response to therapy with B\(_{12}\) (Fig 1) indicated a tissue deficiency of this vitamin despite a normal total plasma level. An extended family study (Fig 2; Table 1) was carried out in which Cbl transport proteins were determined functionally as unsaturated B\(_2\) binding capacities together with total TCII levels determined by RIA. Total plasma UBBC in the patient determined at the time of relapse when she had been off all treatment for five weeks (March 1979) was 31 ng/L (normal range 600 to 1,400 ng/L); after DEAE chromatography, this was distributed among the three transcobalamin fractions as follows: TCII UBBC 6.5 ng/L (normal 787 ± 180 ng/L), TCI UBBC 10 ng/L (normal 99 ± 29 ng/L), and TCIII 14.5 ng/L (normal 117 ± 24 ng/L). Similar values were obtained when she was in complete remission on oral OHCbl 1 mg daily in August 1979 (Table 1), and a comparable level for TCII UBBC was obtained from Professor Hoffbrand’s laboratory (London) on a serum sample in January 1979. Half-normal levels of TCII UBBC were found in both parents, in three of four siblings, and in other members in the extended family (mean 313 ± 70) demonstrating a homozygous TCII deficiency in K. C. (Fig 2 and Table 1). Heterozygotes were easily separated from both the propositus and normal family members (mean 674 ± 128).
Table 1. Vitamin B12 "Profile" on Family Members

| Test          | Normal Range or ± SD | III.6  | III.6  | III.6  | II.3   | II.4   | III.3  | III.4  | III.5  | I.1    | I.2    | II.1   | II.2   | III.1  | III.2  | I.3    | I.4    | II.*5  | II.*6  | III.*7 | III.*8 |
|---------------|----------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Serum Cbl     | 200–900 ng/L         | 1380   | 2400   | 3600   | 595    | 370    | 510    | 490    | 460    | 355    | 560    | 475    | 365    | 360    | 350    | 255    | 240    | 330    | 240    | 370    | 550    |
| Serum folate  | 2.6–14 µg/L          | 17.2   | 3.2    | 8.2    | 6.9    | 6.8    | 8.6    | 8.2    | 8.9    | 6.7    | 5.3    | 5.2    | 6.2    | 5.5    | 5.1    | 4.9    | 2.5    | 10.1   | 14.2   | 9.6    | 8.8    |
| RBC folate    | 130–600 µg/L         | 690    | 320    | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      | —      |
| Total UBBC    | 600–1,400 ng/L       | 31     | 43     | —      | 510    | 410    | 495    | 1028   | 520    | 440    | 1120   | 1105   | 590    | 1235   | 800    | 910    | 570    | 945    | 1160   | 1310   | 1271   |
| TCI UBBC      | 99 ± 29 ng/L         | 10     | 8      | —      | 110    | 74     | 75     | 160    | 66     | 65     | 105    | 183    | 75     | 373    | 180    | 164    | 90     | 310    | 130    | 131    | 205    |
| TCI IUBB      | 117 ± 24 ng/L        | 14.5   | 14     | —      | 140    | 130    | 149    | 240    | 126    | 130    | 210    | 311    | 135    | 325    | 240    | 196    | 140    | 230    | 390    | 264    | 359    |
| TCI IUBB      | 787 ± 180 ng/L       | 6.5    | 21     | —      | 260    | 205    | 277    | 628    | 328    | 245    | 805    | 611    | 380    | 537    | 380    | 550    | 340    | 405    | 640    | 910    | 707    |
| TCI IUBB      | 971 ± 227 ng/L       | —      | ~50    | —      | 160    | 320    | 410    | 950    | 450    | 340    | 950    | 570    | 300    | 1000   | 520    | 950    | 570    | —      | —      | —      | —      | —      |

UBBC, total unsaturated B12 binding capacity; RIA, radioimmunoassay.

Notation of family members refers to Fig 2 (III.6, propositus; II.3, father; II.4 mother).

*Estimations performed on freshly separated serum as opposed to fluoride EDTA plasma giving rise to slightly higher R-binder values.
in the assay made no difference, thus supporting the diagnosis of a total absence of TCII. The RIA levels in family members correlated well with functional measurements. When K. C. was 16 months of age and in full remission on oral OHCbl 1 mg daily, a study of the distribution of endogenous Cbl in the serum was undertaken in C.A. Hall's laboratory. All of the radioactive tracer cobalamin added to the serum eluted as free cobalamin, indicating the lack of free binding sites on any Cbl binder in K. C.'s serum. Most of the endogenous cobalamin eluted with albumin, and a small amount eluted with R binder. There was no TCII-bound Cbl, and there was no indication of a high mol wt complex such as was observed in the serum of a Swiss child with TCII deficiency when on treatment.

A Cbl absorption test made by counting over the liver a week after giving an oral dose of 3.7 kBq 58Co-labeled CNCbl gave results suggesting a Cbl absorption of 5.8% ± 3.1%. This picture was unchanged when the test was repeated with the addition of intrinsic factor. A similar test carried out on K. C.'s mother demonstrated a normal Cbl absorption of 70%.

**DISCUSSION**

TCII deficiency is a rare cause of megaloblastic anemia in infancy. It is, however, distinguished by its response to cobalamin occurring in conjunction with normal total plasma Cbl and folate levels and, if recognized early, treatment with massive, frequent doses of Cbl should ensure normal development. However, the early confirmation of Cbl deficiency is difficult. Our patient, typically, was seen at the age of ten days; she had diarrhea, but partial treatment with a multivitamin preparation delayed the onset of hemopoietic megaloblastosis until she was seven months of age. This was similar to the case described by Hitzig. The combined administration of Cbl and folate when megaloblastic anemia occurs is unavoidable, because folate deficiency is a more likely cause of megaloblastic anemia in this age group. Further delay in reaching the diagnosis occurred through difficulty in interpreting the low plasma Cbl binding capacities of all three transcobalamin which are consistent with administration of pharmacological doses of Cbl as well as being characteristic of TCII deficiency, regardless of therapy. The diagnosis was established by determining the TCII UBBCs in members of the immediate family. The immunological measurement of TCII is useful in distinguishing between a true absence of TCII as in this patient and the presence of an abnormal molecule such as has been described in two patients with functional TCII deficiency.

In TCII-deficient infants, symptoms are referable to rapidly dividing tissues, eg, the gut, immune system, and hemopoietic system, and within this group the requirements for Cbl appear variable. A Swiss child had hypogammaglobulinemia and a granulocyte-killing defect while apparently in hematological remission; both resolved with adequate therapy. Our patient had normal immunoglobulin level but suffered frequently from what appeared to be viral infections and was slow to recover from them at times when her hematological indices were normal and her Cbl therapy ranged from 12.5 µg daily to 1,000 µg intramuscularly weekly. It was only when our patient received 1,000 µg OHCbl daily by mouth that she became completely well. This implies that not only the dose but the frequency of administration is important in order to maintain a constant supply of available Cbl to cells that turn over quickly.

Early developmental milestones were not delayed in our patient despite the fact that she was failing to thrive in the first months of life. This is consistent with the absence of neurological abnormalities in the other three children in whom early diagnosis and treatment was instituted. Unlike previous patients with TCII deficiency, our patient had normal immunoglobulin level but suffered frequently from what appeared to be viral infections and was slow to recover from them at times when her hematological indices were normal and her Cbl therapy ranged from 12.5 µg daily to 1,000 µg intramuscularly weekly. It was only when our patient received 1,000 µg OHCbl daily by mouth that she became completely well. This implies that not only the dose but the frequency of administration is important in order to maintain a constant supply of available Cbl to cells that turn over quickly.

Unlike previous patients with TCII deficiency, our patient is being successfully maintained on oral OHCbl in comparable dosage but without any parental supplementation. The
role, if any, of TCII in normal Cbl absorption is still unclear. Newly absorbed Cbl is found bound to TCII in the circulation\(^4\) and there is indirect evidence to suggest that this TCII may actually originate from the ileal enterocyte during the process of absorption.\(^4\) Evidence for the importance of TCII in Cbl absorption is largely based on abnormal results of Cbl absorption tests in the reported cases of TCII deficiency. However, these results are difficult to interpret. Tests that depend on normal Cbl transport and uptake to tissues such as counting over the liver are clearly unsuitable for studying Cbl absorption in known TCII deficiency and when Schilling tests have been used, it is quite possible that the patients were not on enough Cbl therapy to ensure normal Cbl status in the gut.\(^5,14,15\) The mother of our patient who has half-normal levels of TCII UBBC appears to absorb Cbl normally; moreover, the Schilling test was reported to be normal in two patients with an abnormal TCII; one who has half-normal levels of a TCII that fails to bind Cbl\(^2\) and one who has TCII Cardiza, which binds Cbl but fails to facilitate its cellular uptake.\(^2\) It follows that if TCII does participate in normal Cbl absorption, it not only does so at half-normal levels, but this action would appear also to be independent of both its Cbl binding properties and its ability to deliver Cbl to the tissues. Although massive pharmacological doses of oral hydroxocobalamin presumably bypass the normal intrinsic factor/ileal cell route of absorption it is noteworthy that initially a daily dose of 12.5 \(\mu\)g CNCbl orally in our patient was sufficient to prevent the development of megaloblastic anemia and to produce plasma levels of Cbl >1,000 ng/L.

During complete remission while on oral OHCbl 2mg daily, K. C. has plasma levels of Cbl that range from 3,000 to 4,000 ng/L. Most of this is bound to protein of similar mol wt to albumin.\(^21\) TCII has been shown in vitro to enhance greatly the uptake of Cbl into Hela cells,\(^6\) human reticulocytes,\(^7\) human fibroblasts,\(^8\) human amnion cells,\(^7\) and human kidney cells\(^7\) by means of specific receptors. However, free Cbl will enter cells and, once inside the cell, function normally, ie, is converted to its functional forms adenosyl and methyl Cbl.\(^22\) The Cbl binding to “albumin” may be so loose that Cbl is actually taken up as if it were free. This notion is supported by the in vitro observation that OHCBl bound in the albumin component dissociates from that component at 37 °C (J.A. Begley and C.A. Hall, unpublished observations). In healthy subjects, there is much greater binding of OHCbl than CNCbl to a serum protein of the mol wt of albumin following intramuscular injection\(^23\); this may explain why OHCbl is better retained in the body. It would seem reasonable to assume that Cbl binding to “albumin” is beneficial in TCII deficiency by providing a circulating and readily available store of free Cbl. Hydroxocobalamine would therefore be the preparation of choice in the treatment of this condition.

To date, this is the largest family study in TCII deficiency; the clear separation of heterozygotes from both the propotitus and from normal individuals suggests that the underlying defect in this condition is confined to a single gene.

In conclusion, we would like to make the following suggestions for the early detection and management of TCII deficiency: first, an infant with megaloblastic anemia responding to Cbl and folate, despite normal serum levels of these vitamins and a normal red cell folate, should be started on treatment with large and frequent doses of Cbl before the diagnosis of TCII deficiency is confirmed and regardless of whether the response to Cbl alone has been established. In establishing the diagnosis, the finding of half-normal levels of TCII UBBC in the parents, who are obligatory heterozygotes, provides useful confirmation of the diagnosis, particularly if pretreatment serum or plasma from the patient is unavailable. (It is, of course, essential that the normal reference values are obtained by the precise same method.) Finally, once the diagnosis has been made, we feel it is worthwhile to attempt maintenance therapy with oral hydroxocobalamin commencing at a dose of 1 to 2 mg on at least a daily basis, if not more frequently.

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