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CASE REPORT

A novel mutation of the transcobalamin II gene in an infant presenting with hemophagocytic lymphohistiocytosis

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Abstract Transcobalamin II (TC II) deficiency is a rare disorder of cobalamin (CBL, vitamin B12) metabolism that occurs due to mutations in transcobalamin gene (TCN2). Hemophagocytic lymphohistiocytosis (HLH) in contrast is a syndrome characterized by uncontrolled immune response with hyperinflammation. A 2-month-old male baby was admitted with complaints of fever, cough, diarrhea, and respiratory distress. The parents were first cousins. The baby exhibited five of the eight diagnostic criteria for HLH-2004 and was diagnosed as HLH. A second bone marrow aspiration demonstrated megaloblastic changes in the erythroid series. The patient's vitamin B12 level was normal; however, hyperhomocysteinemia was present. A genetic deficiency of TC II was suspected. The patient and his parents were tested for TCN2 mutation. He had a homozygote mutation that was not included in Human 'Gene Mutation Database Cardiff'. The patient was treated with intramuscular vitamin B12, which was followed by improvement in both clinical and laboratory findings. He was 12 months old at the time of this report, with normal physical and neuromotor development. In this case presenting with the clinical and laboratory findings of HLH, TC II deficiency was diagnosed. A new mutation was found that was not reported before. Potential causative mechanisms of HLH induced by defects of cobalamin synthesis merit further investigation.

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Introduction

Transcobalamin II (TC II) is a serum transporter of cobalamin (Cbl, vitamin B_{12}) needed for cellular uptake of Cbl throughout the body. Mutations in transcobalamin gene (*TCN2)* produce deficiency of TC II and disturbances of cobalamin metabolism. Mucosal ulceration, vomiting, diarrhea, lethargy, irritability and growth retardation and immunological dysfunctions are observed in TC II deficiency that is inherited autosomal recessively. Laboratory findings include pancytopenia, macrocytic anemia, neutropenia, reticulocytopenia, megaloblastic bone marrow changes, methylmalonic aciduria and homocystinuria [1]. Cbl is the specific coenzyme of two intracellular enzymes, methylmalonyl CoA mutase and methionine synthetase. Above findings result from a lack of intracellular Cbl, whereas serum Cbl is usually in the normal range due to the fact that the major part of Cbl is bound to haptocorrin in the plasma.

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome characterized by uncontrolled immune response with hyperinflammation. Hyperactivation of T cells, hypercytokinemia and hemophagocytosis of blood cells by monocytes and macrophages are observed in HLH. The disease is classified as primary (genetic) and secondary (acquired) according to underlying pathogenesis. Primary HLH arises de novo without a trigger, and has an autosomal recessive inheritance pattern. In secondary forms, hemophagocytosis develops as a result of infections, drugs, malignancy, autoimmunity and metabolic reasons. Common diagnostic markers of both forms of HLH are fever, bicytopenia or pancytopenia, hyperferritinemia, hypertriglyceridemia

and/or hypofibrinogenemia, hemophagocytosis, high free interleukin-2 receptor (CD25) levels in blood, reduction or loss of CD8+ T and NK cell activity, and splenomegaly [2].

Case report

Two-month-old, male baby was admitted with complaints of fever, cough, diarrhea and respiratory distress. He was first child of his parents who were first cousins.

In physical examination, height and weight were below third percentile, hepato-splenomegaly, and rales in both lungs were noted. Hemoglobin was 10.4 g/dl, MCV was 88.8 fl, white blood cells were 3670/μl, absolute neutrophil count was 1100/μl, and platelets were 81000/μl. Hyperferritinemia (3287 ng/ml) and hypertriglyceridemia (224 mg/dl) were reported in serum with normal fibrinogen levels (255 mg/dl) in plasma. Urinalysis was normal. No proteinuria was detected. Two hemophagocytic cells were seen in bone marrow. He had 5 of 8 diagnostic criteria of HLH-2004 [3] and a diagnosis of HLH was established. Ceftriaxone had been administered to the patient as a nonspecific treatment until the underlying etiology of fever would be understood.

Due to persistence of clinical and hematological findings for a week, the patient had a second bone marrow aspiration. Megaloblastic changes were prominent in the erythroid series. Pancytopenia of the patient is characterized by Hb of 7.4 g/dl, WBC count of 3200/μl and platelet count of 61000/μl. MCV was 88 fl. Simultaneous B_{12} level was normal, however, there was hyperhomocysteinemia (23 μmol/l).

TC II deficiency was suspected. The patient and his parents were investigated for *TCN2* gene mutation. The patient had a homozygote mutation that was not included in Human 'Gene Mutation Database Cardiff'. This 5304-bp deletion began 1516 bp into intron 7 and ended 1231 bp into intron 8. The deletion included all of exon 8 and caused a frame shift to produce premature stop four codons into new reading frame (counting ATG as 1) (Fig. 1). Parents had heterozygote mutations.

The patient had been treated with intramuscular vitamin B_{12} with a dose of 1000 μg/day for first week, the same dose for three times in the following week, and once a week afterwards. His follow-up showed improvement in both clinical and laboratory findings. He is 12 months old now with normal physical and neuromotor development.

Written informed consent was obtained from the parents. The physical examination, laboratory findings and course of the patient were summarized in Fig. 2.

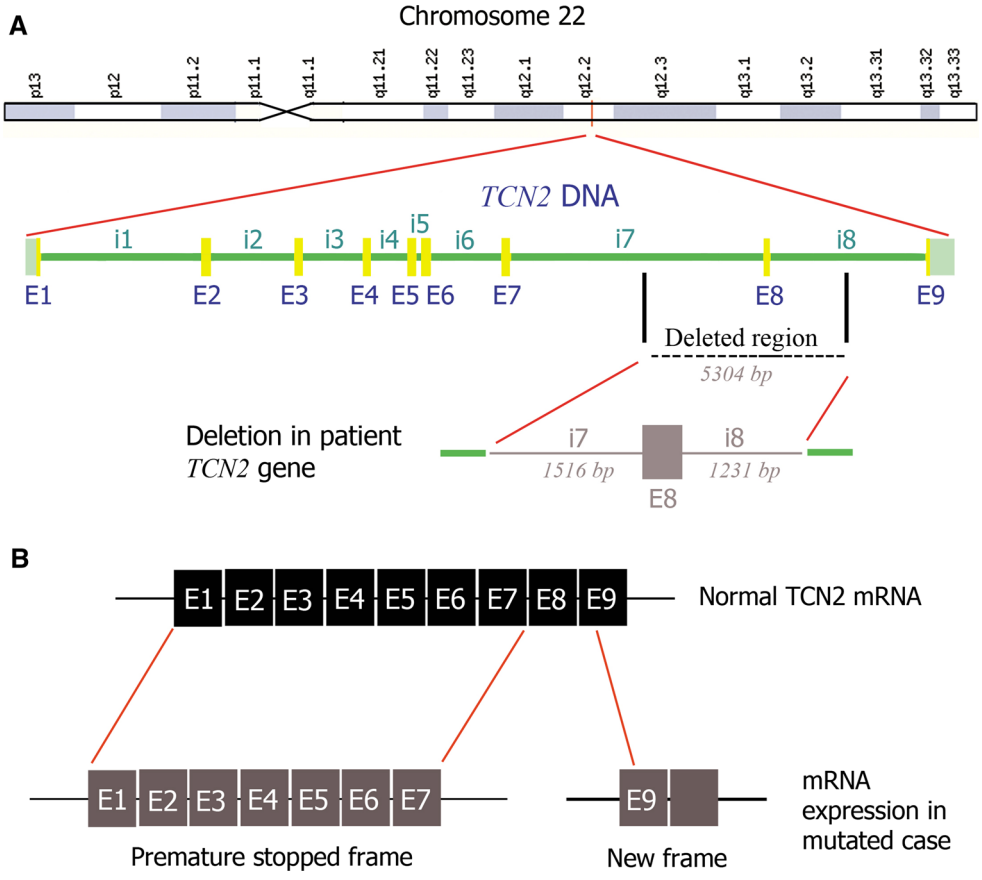

Fig. 1 There was a 5304-bp deletion beginning 1516 bp into intron 7 and ending 1231 bp into intron 8 (**a**). The deletion included all of exon 8 and caused a frame shift to produce premature stop four codons into new reading frame (counting ATG as 1). mRNA expression in the mutated case was shown in **b**

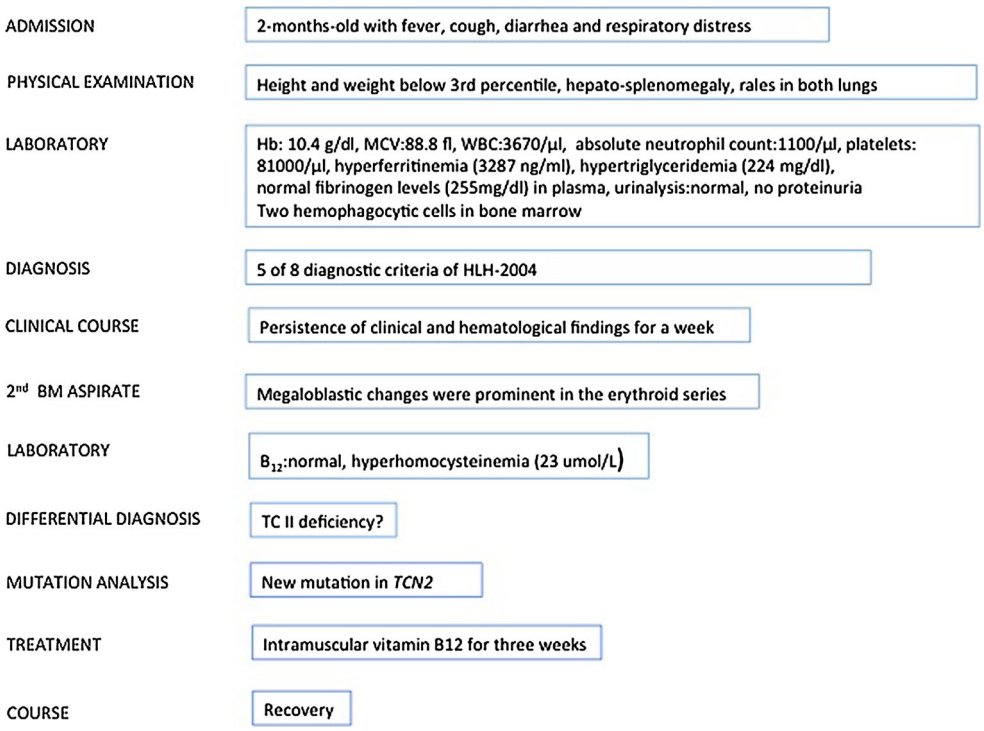

Fig. 2 Chart summarizing the clinical course of the patient

Discussion

A new mutation in TC II gene was reported here in this case of defective vitamin B_{12} metabolism in addition to HLH. Although vitamin B_{12} deficiency often develops due to nutritional disorders, rarely metabolic defects concerning vitamin B_{12} such as TC II deficiency should be considered in the differential diagnosis. TC II deficiency presents usually in the early infancy with inability to grow, diarrhea, vomiting, glossitis, neurological disorders, megaloblastic anemia and pancytopenia. It is inherited autosomal recessively. TC II is totally absent in most patients and found defective in some patients [1].

The gene encoding TC II, *TCN*, was discovered in 1994 [4] and pathogenic mutations had been reported since then [5–11]. There is a study reporting 6 different mutations that were not defined previously in exon 4 and 8 [12]. These mutations impair the interaction of TC with either cobalamin or TC receptor.

HLH can be either genetic or nongenetic. Viral infections due to agents like EBV and CMV can cause nongenetic HLH [13]. HLH is associated rarely with congenital metabolic disorders. Accumulation of metabolites in lysinuric protein intolerance and multiple sulfatase deficiency resulted with HLH [14]. Wu et al. [15] reported 'Cobalamin C disease' in a 4-month-old HLH patient with homocystinuria, methylmalonic aciduria, increased methylcitric acid in urine and increased propionylcarnitine and homocysteine in serum. Defects in cobalamin metabolism were suggested to lead HLH due to defects in DNA synthesis, secondarily NK cell functions and immune regulation [15]. Hypogammaglobulinemia, lymphopenia, and neutrophil dysfunction have been reported in TC II deficiency. Therefore, we think that immune changes caused by a lack of TC II in our patient led to the development of secondary HLH.

On the other hand, there are difficulties in discriminating pancytopenia of TCII deficiency from that of HLH. However, our patient had hyperferritinemia and hypertriglyceridemia completing a picture of HLH rather than a distinct TCII deficiency. We cannot disregard a diagnosis of HLH in this patient. The response of the patient to vitamin B_{12} therapy and recovery from HLH makes the connection between these two clinical conditions to occur together in our patient.

In a recent report about hemophagocytic syndromes (HPS) in patients with unexplained cytopenia, the major findings in the microscopy of the bone marrow specimens of the patients with HPS were megaloblastic anemia ($N = 2$), chronic granulomatous disease ($N = 1$), and bone marrow sea-blue histiocyte syndrome ($N = 1$) [16]. Therefore, megaloblastic anemia may accompany a diagnosis of HLH as in this recent report. However, it is really difficult to differentiate if megaloblastic anemia caused HLH or accompanied HLH. In our case, there is a genetic defect of B_{12} utilization by the cell. It is found as *TCN II* mutation. B_{12} treatment improved the symptoms of the patient with full recovery.

In this case presenting with the clinical and laboratory findings of HLH, TC II deficiency was also diagnosed. We report for the first time a new mutation in *TCN 2* gene being a 5304 base pair deletion starting at intron 7 and ending at intron 8. We suggest our patient as the first case of TC II deficiency presenting with HLH.

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