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CORRESPONDENCE

# First report of homocystinuria-megaloblastic anaemia, cobalamin E complementation type, in an Indian child

Sir,

Homocystinuria-megaloblastic anaemia, cobalamin E complementation type (cblE defect, #OMIM 236270), is a rare autosomal recessive disorder of intracellular cobalamin (cbl) metabolism that results from homozygous or compound heterozygous mutations in the methionine synthase reductase (*MTRR)* gene. This enzyme plays an auxiliary role in the methionine synthase catalysed transmethylation reaction that converts homocysteine to methionine. Clinical features of the cblE defect are variable. Most cases manifest in early childhood with failure-to-thrive, developmental delay, and megaloblastic anaemia. Work-up reveals hyperhomocysteinaemia, hyperhomocystinuria, and hypomethioninaemia without methylmalonic aciduria.[1,2] Patients typically respond to cobalamin supplementation, making nutritional megaloblastosis a difficult differential diagnosis in children from impoverished backgrounds receiving a purely vegetarian diet. We describe here for the first time an *MTRR* defect in an Indian child. In summary, our patient presented at age 12 years with a good scholastic record. She had a history of recurrent anaemia and megaloblastosis, and had haemolysis, together with findings of elevated iron stores, a significant percentage of ring sideroblasts, elevated plasma total homocysteine but no methylmalonic aciduria and plasma B12 level in the middle of the reference range.

This 12-year-old north Indian female born of a non-consanguineous marriage was referred with a long history of intermittent severe anaemia that had necessitated three RBC transfusions at ages 1.5, 12 and 12.5 years, respectively. In the current episode, she reported progressive pallor and lassitude for 15 days with mild jaundice but no history of bleeding, fever, weight loss, leg ulcers or lymphadenopathy. Her scholastic achievements were above average. She took a mixed ovolactovegetarian diet, occasionally incorporating meat products. There was no similar family history; her parents and brother reported good health.

On physical examination, the severely pale child showed haemolytic facies, mild icterus, and splenohepatomegaly 7 and 5 cm below the respective costal margins. Abdominal ultrasound revealed cholelithiasis. Her haemoglobin was 41 g/L, corrected reticulocyte count 1.9%, reticulocyte production index 0.8, mean corpuscular volume 112 fL, mean corpuscular haemoglobin 39.3 pg, mean corpuscular haemoglobin concentration 35 g% and red cell distribution width (coefficient of variation) was 33%. Total leukocyte and platelet counts were normal.

Blood films showed severe red cell anisocytosis, mostly macrocytic erythrocytes with a few spherocytes, teardrop cells and polychromatophils (Fig. 1A). Hypersegmented neutrophils were not seen. Biochemical investigations revealed mild unconjugated hyperbilirubinaemia (total bilirubin 2.59 mg/dL, conjugated fraction 0.24 mg/dL) with markedly elevated lactate dehydrogenase 3376 U/L [reference range (RR) 240–480] as well as serum homocysteine (>50 µmol/L, RR 4.44–13.56). Urine methylmalonic acid was negative. Iron stores were elevated (serum iron 322 µg/dL, TIBC

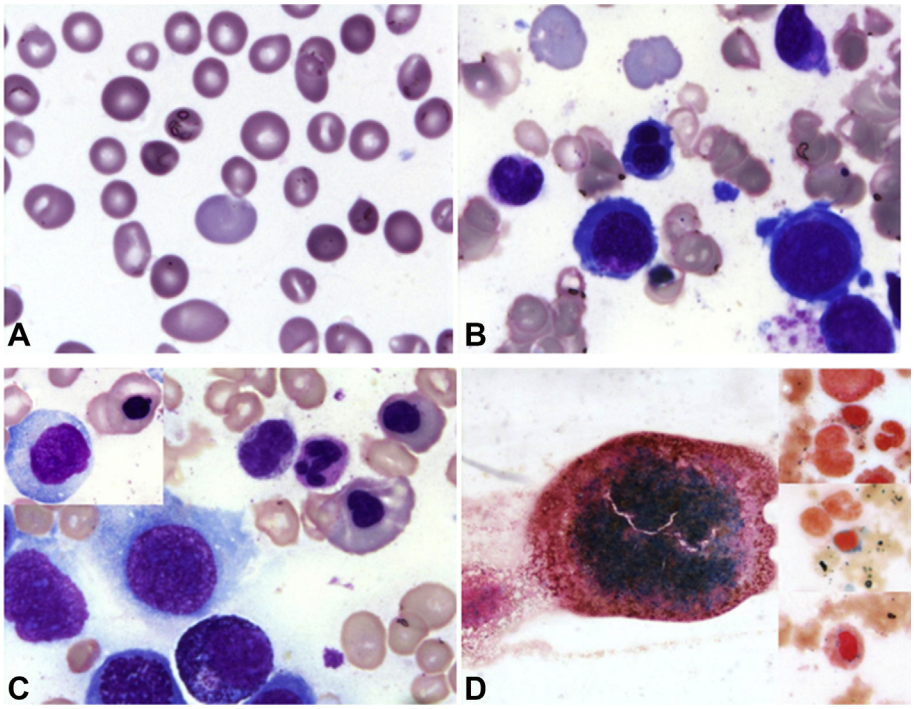


**Fig. 1** (A) Peripheral blood smear showing moderate anisocytosis with macrocytic to normocytic erythrocytes. (B) Hypercellular bone marrow showing dyserythropoiesis in the form of binucleate erythroblasts. (C) Hypercellular bone marrow showing megaloblastosis. (D) Perls' staining 5+ with ring sideroblasts.

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361 µg/dL, transferrin saturation 89%, serum ferritin 814 ng/mL), with normal serum folate (22.14 ng/dL, RR 5−22) and vitamin B12 (357 pg/mL, RR 150−900) levels using chemiluminescent methods.

Haemolytic workup comprising glucose-6-phosphate dehydrogenase deficiency screening, plasma and urine haemoglobin, direct Coombs test, flow cytometric eosin 5′ maleimide dye-binding test (control/patient mean fluorescence intensities ratio 0.97) and anti-nuclear antibodies were normal. Haemoglobin high-performance liquid chromatography revealed 80% haemoglobin $A_0$, 3% haemoglobin $A_2$ and 5% haemoglobin F (the last was possibly mildly increased due to stress erythropoiesis). Sanger sequencing of the UDP glucuronosyltransferase 1 family, polypeptide A1 (*UGT1A1*) gene promoter responsible for the Gilbert syndrome, revealed heterozygosity for the TA repeats [$TA_{6/7}$] in the patient and both her parents.

Her bone marrow was hypercellular with florid erythroid hyperplasia showing 26% dyserythropoiesis (Fig. 1B,C) with normal granulocytic and megakaryocytic lineages. Perls' stain showed markedly increased iron stores (graded 5+). In addition, 9% ringed sideroblasts were noted (Fig. 1D). Detailed history did not reveal the intake of drugs like isoniazid, chloramphenicol, linezolid, nor any alcohol intake or lead exposure.

On enquiring about the treatment history, her parents informed that she had been empirically supplemented with long term oral folic acid and intermittent parenteral vitamin B12 ever since the first anaemic episode at age 1.5 years. In the current admission, she was administered 1000 µg intramuscular vitamin B12 and started on 5 mg folic acid/day. Pallor and icterus improved in 15 days. Repeat blood count at 2 and 3 months revealed improved anaemia (haemoglobin 92 g/L) but with continued markedly elevated mean corpuscular volume and mean corpuscular haemoglobin and moderate anisopoikilocytosis with severe macrocytosis on the blood smear. Bone marrow was not repeated after the trial. At discharge, she was started on vitamin B12 injection 1000 µg/monthly which was reduced to 1000 µg every 3 months after 6 months, along with 5 mg folic acid/day awaiting a definitive diagnosis.

At the laboratory end, the differential diagnoses considered for her refractory and episodic megaloblastic anaemia were congenital dyserythropoietic anaemia, paediatric myelodysplastic syndrome, atrophic gastritis/pernicious anaemia or malabsorption syndrome, an intermittent haemolytic illness like paroxysmal nocturnal haemoglobinuria as well as rarer entities like orotic aciduria and the Imerslund−Gräsbeck and Lesch−Nyhan syndromes. Of these, pernicious anaemia was excluded by negative studies for anti-intrinsic factor and anti-parietal cell antibodies and paroxysmal nocturnal haemoglobinuria was excluded by flow cytometric demonstration of FLAER and specific GPI-linked antigens on leukocytes. The myelodysplastic syndrome was considered unlikely after the hypercellular bone marrow as well as clinically.

In view of the obscure diagnosis, the patient's DNA was subjected to targeted next-generation sequencing (NGS) using the TruSight One Sequencing Panel (Illumina, USA). Parental consent, the child's assent, and institutional ethics committee approval were obtained (vide number PGI/IEC/2015/837). DNA libraries were prepared as previously described[3] and sequenced on a MiSeq System (Illumina) using the 300 cycle's v3 chemistry. Data analysis with VariantStudio 2.0 software (Illumina) revealed a homozygous mutation NM_024010.2 (*MTRR*): c.1910C>T, (p.Ser637Leu), in exon 14 of the methionine synthase reductase (*MTRR*) gene (rs778650591) (Fig. 2). This mutation had a minor allele frequency of T=0.000008/1 in ExAC database. The MTRR enzyme helps in reductive activation of methionine synthase (MTR) enzyme which converts

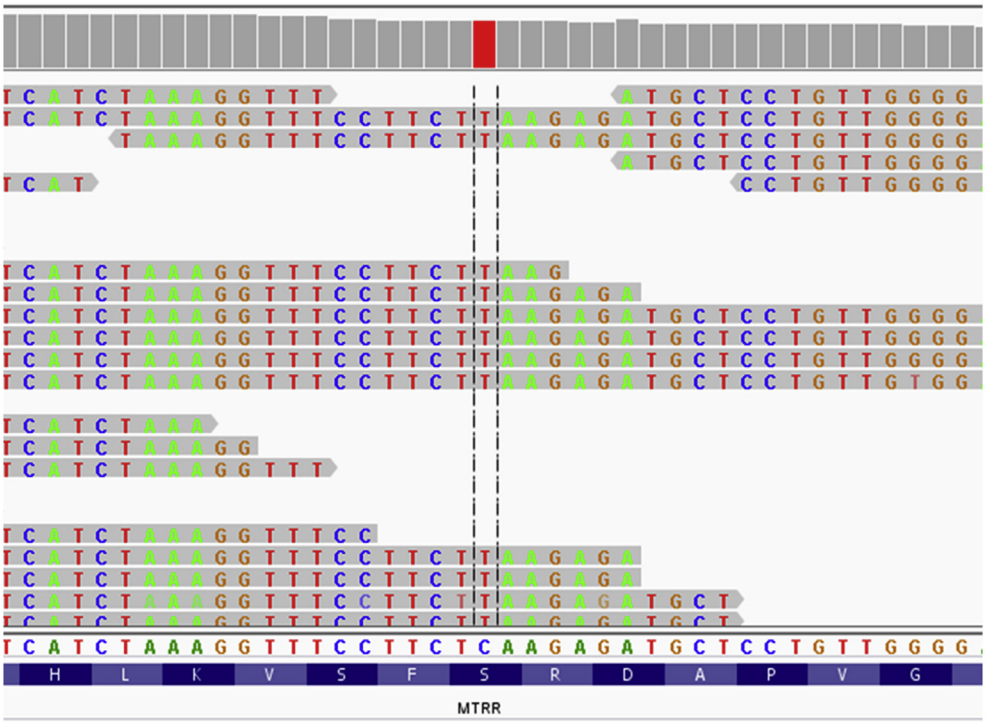


**Fig. 2** Integrated Genome Viewer showing a homozygous mutation detected at position c.1910C>T in the *MTRR* gene.

homocysteine to methionine. *In silico* analysis using PolyPhen-2, SIFT, PROVEAN, MutationTaster and MutPred software unanimously scored this mutation as probably highly deleterious/pathogenic. Subsequent Sanger sequencing revealed both her parents as well as her asymptomatic brother to be heterozygous for this mutation, confirming an autosomal recessive mode of inheritance (Fig. 3).

Amino acid positions 637−638 in MTRR protein constitutes the site for its nicotinamide adenine dinucleotide phosphate (NADP) ligand binding. These amino acids are located in the β-strands within the protein sequence (amino acid 631−639).[4] The Ser637Leu mutation likely disrupted the NADP binding, thus seriously abrogating enzyme activity. We then screened 125 unrelated individuals without anaemia or ring sideroblasts to confirm that the mutation was absent in all.

Her 9% ring sideroblasts remained unexplained; hence, we next screened her NGS data for all genes previously implicated in inherited sideroblastic anaemias (*SLC25A38, ALAS1, SF3B1, ABCB7, GLRX5, SLC19A2, PUS1, YARS2*). No probable causative mutation was found. Further analysis for other polymorphisms affecting vitamin B12 and folate metabolism,[5,6] showed the patient to be homozygous wild-type for *MTFHR* C677T (rs1801133); *MTRR* A66G (rs1801394) and heterozygous for *MTHFR* A1298C (rs1801131) and *MTR* A2756G (rs1805087). The *HFE* gene implicated in haemochromatosis was screened for the His63Asp (rs1799945) and Cys282Tyr (rs1800562) mutations in view of iron overload. Both were absent.

An inborn error of intracellular cobalamin metabolism, cblE defect, usually presents with developmental delay with laboratory tests reflecting megaloblastic anaemia and homocystinuria without methylmalonic aciduria. Our patient's normal vitamin B12 and folate levels (first measured at age 12 years) are not unexpected since inherited disorders of intracellular cobalamin metabolism far along the cobalamin metabolic pathway may have normal B12 and folate levels.[7] Her megaloblastic anaemia, hyperhomocysteinaemia without methylmalonic aciduria and family screening helped establish causality after the genetic result. The absence of neurological symptoms and normal development were possibly seen because the patient was well-supplemented with empirically administered parenteral folic acid and vitamin B12 from the first episode at the age of 1.5 years. Her family informed that she was supplemented with parenteral vitamin B12 intermittently and was on oral tablet folic acid, 5 mg/day following the very first episode. Her presentation to us at age 12 years with jaundice and severe anaemia may have been due to increased physiological demands during puberty coupled with the fact that she had been off supplementation for the previous year. Prior reports have found that the disorder responds better to hydroxocobalamin.[8] However, our patient was supplemented with cyanocobalamin and folic acid alone.

Reported phenotype cblE defect is usually severe except for two milder patients reported by Vilaseca *et al.*[2] They

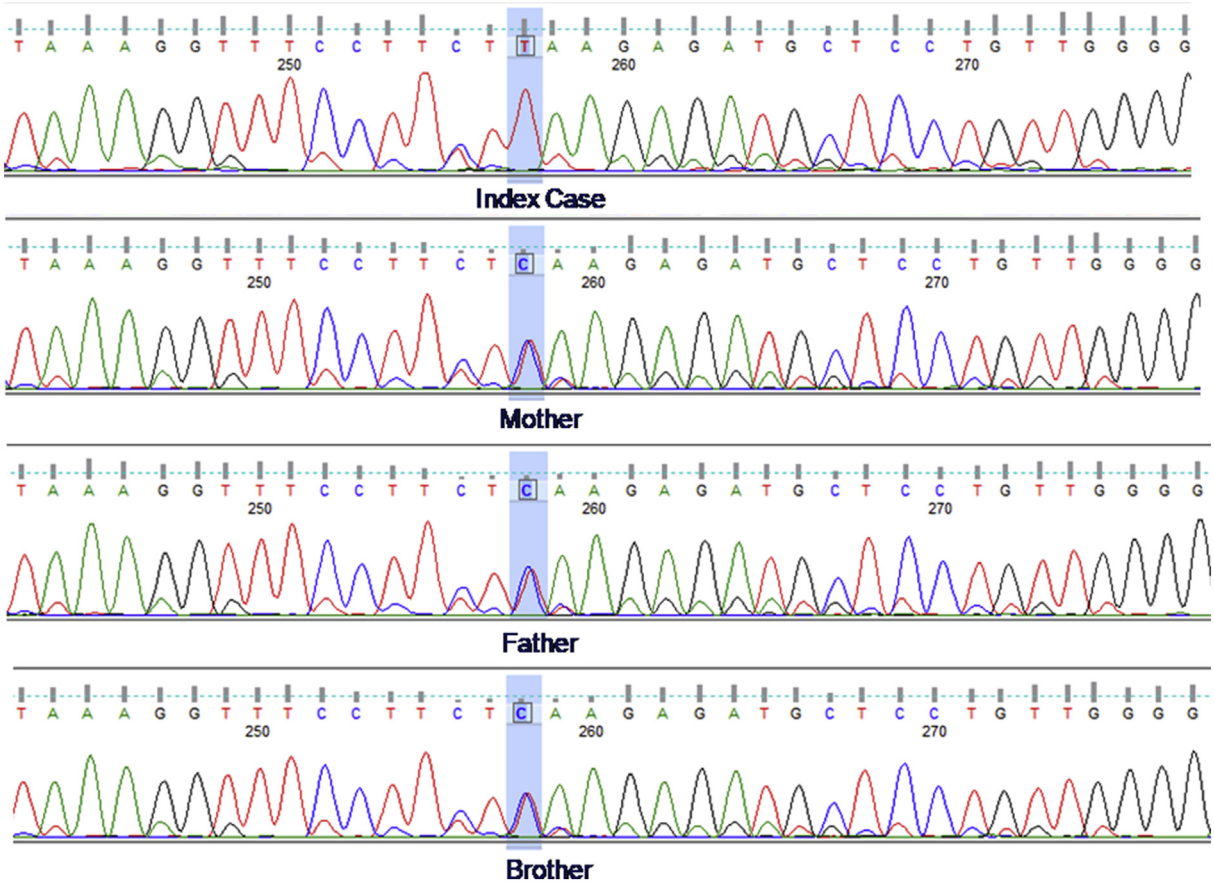


**Fig. 3** Chromatogram showing a screening of mutation Ser637Leu in index case and family members. The index case was homozygous for c.1910C>T, (p.Ser637Leu) mutation whereas symptomatic parents and sibling were heterozygous.

found c.1361C>T, p.Ser454Leu in the *MTRR* in a highly conserved flavin adenine dinucleotide (FAD) binding domain and proposed it to be associated with a milder phenotype. We also found the similar change of amino acid Ser637Leu which however binds to the NADP ligand. No apparent genotype-phenotype correlation has been recognised for this disorder except for a link between a milder, predominantly haematological presentation and homozygosity for the Ser454Leu mutation by Zavadáková *et al.*[1] Unconjugated hyperbilirubinaemia in our case may be due to the megaloblastosis, with contribution from the heterozygosity of *UGT1A1* [$TA_{6/7}$] polymorphism.

There is phenotypic heterogeneity ranging from mild to severe neurological impairments and most of the clinical and laboratory findings in cblE defect overlap with other disorders. The disorder is often initially misdiagnosed as congenital dyserythropoietic anaemia and delay in therapy may lead to severe neurological deficits. Persistent treatment refractory megaloblastosis, hyperhomocysteinaemia, increased mean corpuscular volume and mean corpuscular haemoglobin in the face of normal B12 and folate levels should lead to a suspicion of *MTRR*/*MTR* defects. The absence of neurological symptoms may indicate a milder phenotype of cblE type. Timely diagnosis can eliminate inappropriate therapeutic effects and limit irreversible damage.

The 9% ring sideroblasts in the marrow could also be a secondary manifestation of the *MTRR* defect since a few case reports describe the presence of ~10−15% ring sideroblasts and megaloblastic anaemia with vitamin B12 deficiency.[9–12] No molecular screening for the associated genes was performed in these reports. The classical presentation of congenital sideroblastic anaemia is microcytic anaemia. Targeted resequencing does not exclude the possibility of a long deletion/insertion, an intronic mutation or a mitochondrial DNA deletion in our patient.

In conclusion, we report the first Indian case of the cblE defect and the first with 9% ring sideroblasts. Given the varied aetiologies of macrocytic anaemias, NGS in refractory cases (especially in children) is a rapid and precise way to clinch the genetic diagnosis and enable genetic counselling. Data analysis focused on finding the causative mutations in genes implicated in megaloblastic anaemia is likely to yield the best results. Our case highlights that meticulously characterising the phenotype prior to NGS aids substantially in clinching the diagnosis. It also illustrates the growing importance of NGS in routine laboratory diagnosis.

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## Approximately 1% of chronic myeloid leukaemia cases present with isolated thrombocytosis and express common major breakpoints: a finding from a laboratory audit

Sir,

Chronic myeloid leukaemia (CML) is a myeloproliferative neoplasm that originates in an abnormal pluripotent bone marrow stem cell, usually presenting with leukocytosis with increased number of granulocytes (neutrophils, eosinophils and basophils) and their progenitors. CML is consistently associated with the reciprocal translocation of breakpoint cluster region protein (BCR) gene on chromosome 9 to