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.

Acknowledgements: The authors express gratitude to all the technical and clinical staff involved in the patient's management and laboratory diagnosis.

Conflicts of interest and sources of funding: This work was supported by institutional funds and Department of Biotechnology, Ministry of Science and Technology, New Delhi (BT/PR12682/MED/12/689/2015). MJ and AA received fellowship from Indian Council of Medical Research (ICMR), New Delhi. The authors state that there are no conflicts of interest to disclose.

Manu Jamwal[1], Anu Aggarwal[1], Prashant Sharma[1], Deepak Bansal[2], Amita Trehan[2], Arindam Maitra[3], Reena Das[1]

[1]Department of Hematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India; [2]Hematology-Oncology Unit, Department of Pediatrics, Postgraduate Institute of Medical Education and Research, Chandigarh, India; [3]National Institute of Biomedical Genomics, Kalyani, West Bengal, India

Contact Dr Reena Das.
E-mail: reenadaspgi@hotmail.com

1. Zavadáková P, Fowler B, Suormala T, *et al*. cblE type of homocystinuria due to methionine synthase reductase deficiency: functional correction by minigene expression. *Hum Mutat* 2005; 25: 239−47.
2. Vilaseca MA, Vilarinho L, Zavadakova P, *et al*. CblE type of homocystinuria: mild clinical phenotype in two patients homozygous for a novel mutation in the MTRR gene. *J Inherit Metab Dis* 2003; 26: 361−9.
3. Jamwal M, Aggarwal A, Sachdeva MUS, *et al*. Overhydrated stomatocytosis associated with a complex RHAG genotype including a novel de novo mutation. *J Clin Pathol* 2018; 71: 648−52.
4. Wolthers KR, Lou X, Toogood HS, *et al*. Mechanism of coenzyme binding to human methionine synthase reductase revealed through the crystal structure of the FNR-like module and isothermal titration calorimetry. *Biochemistry* 2007; 46: 11833−44.
5. Bosco P, Guéant-Rodriguez R-M, Anello G, *et al*. Methionine synthase (MTR) 2756 (A−> G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. *Am J Med Genet A* 2003; 121A: 219−24.
6. Coppedè F. The genetics of folate metabolism and maternal risk of birth of a child with Down syndrome and associated congenital heart defects. *Front Genet* 2015; 6: 223.
7. Ahrens-Nicklas RC, Serdaroglu E, Muraresku C, *et al*. Cobalamin C disease missed by newborn screening in a patient with low carnitine level. *JIMD Rep* 2015; 23: 71−5.
8. Fowler B, Schutgens RBH, Rosenblatt DS, *et al*. Folate-responsive homocystinuria and megaloblastic anaemia in a female patient with functional methionine synthase deficiency (cblE disease). *J Inherit Metab Dis* 1997; 20: 731−41.
9. Narang N, Kotru M, Rao K, *et al*. Megaloblastic anemia with ring sideroblasts is not always myelodysplastic syndrome. *Turkish J Haematol* 2016; 33: 356−8.
10. Bharath V, Hsia CC. Vitamin B12 deficiency presenting as pancytopenia with ring sideroblasts. *Blood* 2015; 126: 2255.
11. Yoshimoto S, Takeuchi M, Tada A, *et al*. Post-gastrectomized vitamin B12 deficient anemia with marked leukoerythroblastosis and ringed sideroblasts. *Rinsho Ketsueki* 1992; 33: 688−93.
12. Khambatta S, Nguyen DL, Wittich CM. 38-year-old woman with increasing fatigue and dyspnea. *Mayo Clin Proc* 2010; 85: 392−5.

DOI: https://doi.org/10.1016/j.pathol.2018.07.008

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

Table 1 Characteristics of CML cases presenting with isolated thrombocytosis

Age (years)	Gender	Hb (g/L)	WCC ($\times 10^9$/L)	Plt ($\times 10^9$/L)	Morphology	Break-point	Additional cytogenetic abnormalities
66	M	150	10	539	Left shift	B2A2	Nil
35	F	107	5	637	Large platelet; occasional blasts seen	B2A2	Variant translocation
80	M	104	6	499	Myelocytes and rare dysplastic myeloid precursors; occasional blasts seen	B3A2	-Y

Hb, haemoglobin; WCC, white cell count; Plt, platelets.

Abelson murine leukaemia viral oncogene homolog 1 (ABL) gene on chromosome 22, resulting in a BCR-ABL fusion gene, which encodes an unregulated tyrosine kinase that allows the cells to proliferate without being regulated by cytokines.[1]

CML can rarely present as isolated thrombocytosis. Historically, atypical presentations of CML with marked thrombocytosis unaccompanied by significantly elevated granulocytes are usually associated with blast crisis or lymphoblastic transformation.[2,3] Since 2000, few case reports have described patients who presented initially with thrombocytosis without leukocytosis.[4–6] In two of these case reports, the cytogenetic studies were initially negative for the fusion gene in one case[5] while the molecular studies for BCR-ABL transcript were initially negative in the other.[6]

At the molecular level, co-expression of p190 and p210 is associated with high platelet counts, marked splenomegaly and additional chromosomal abnormalities.[7] The μ-BCR breakpoint associated with p230 BCR-ABL protein can also present with thrombocytosis.[8]

We undertook a retrospective review of 345 cases diagnosed in Western Australia from 2005 to 2016, with cytogenetic and molecular data available. Of these, we found 130 cases (38%) presented with thrombocytosis with a platelet count $>450 \times 10^9$/L, accompanied with elevated white cell count $>11 \times 10^9$/L. Only three cases (0.9%) presented with thrombocytosis and normal white cell counts (Table 1). All cases had abnormal blood films: left shift, red cell and platelet anisocytosis, and presence of occasional blasts are features described in the blood film morphology review. However, basophilia, typical of CML, was not observed.

At the genetic level, all three cases had major breakpoints, and no major route abnormalities were observed on conventional karyotype. In all three cases, we were able to detect the Ph chromosome by conventional fluorescence *in situ* hybridisation (FISH) either on the bone marrow or peripheral blood specimens. Thrombocytosis resolved in all three cases when BCR-ABL transcript levels declined with the commencement of tyrosine kinase inhibitors.

While CML should be considered in the majority of unexplained leukocytosis with accompanying thrombocytosis, CML presenting with isolated thrombocytosis is rare. The approach recommended by some authors of screening all patients with unexplained persistent thrombocytosis[9] may not be economically justifiable, given the cost of molecular or FISH testing. Rather, our data support the approach of reserving CML screening for cases where other myeloproliferative neoplams have been excluded, unless leukocytosis or atypical morphology is noted on the blood film. We recommend bone marrow examination with cytogenetic analysis to further investigate unexplained isolated thrombocytosis that is negative for JAK2, CALR and MPL mutations, in order to exclude CML and other rare causes such as 5q-syndrome. This approach will avoid missing atypical presentation of CML in the current era where targeted therapy significantly reduces transformation risks and improves survival. This approach is also in line with the British recommended diagnostic approach of unexplained thrombocytosis.[10]

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Teng Fong Ng[1], Matthew Wright[2], Rebecca De Kraa[2]

[1]Royal Perth Hospital and PathWest, Perth, WA, Australia; [2]Fiona Stanley Hospital and PathWest, Murdoch, WA, Australia

Contact: Dr Teng Fong Ng.
E-mail: ngtengfong.haem@gmail.com

1. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973; 243: 290–3.
2. Peterson LC, Bloomfield CD, Brunning RD. Blast crisis as an initial or terminal manifestation of chronic myeloid leukemia. A study of 28 patients. *Am J Med* 1976; 60: 209–20.
3. Anastasi J, Feng J, Dickstein JI, *et al*. Lineage involvement by BCR/ABL in Ph+ lymphoblastic leukemias: chronic myelogenous leukemia presenting in lymphoid blast vs Ph+ acute lymphoblastic leukemia. *Leukemia* 1996; 10: 795–802.
4. Rice L, Popat U. Every case of essential thrombocythemia should be tested for the Philadelphia chromosome. *Am J Hematol* 2005; 78: 71–3.
5. Cesar JM, Cabello P, Ferro T, *et al*. Emergence of chronic myelogenous leukemia in a patient with primary thrombocythemia and absence of BCR/ABL rearrangement. *Cancer Genet Cytogenet* 2006; 167: 74–7.
6. Weng WH, Shih LY. Occurrence of BCR-ABL1-positive chronic myeloid leukemia following essential thrombocythemia. *Acta Haematol* 2011; 126: 220–3.
7. Arana-Trejo RM, Ruíz Sánchez E, Ignacio-Ibarra G, *et al*. BCR/ABL p210, p190 and p230 fusion genes in 250 Mexican patients with chronic myeloid leukaemia (CML). *Clin Lab Haematol* 2002; 24: 145–50.
8. Melo JV. BCR-ABL gene variants. *Baillieres Clin Haematol* 1997; 10: 203–22.
9. Passamonti F, Maffioli M. Update from the latest WHO classification of MPNs: a user's manual. *Hematol Am Soc Hematol Educ Program* 2016; 2016: 534–42.
10. Harrison CN, Butt N, Campbell P, *et al*. Modification of British Committee for Standards in Haematology diagnostic criteria for essential thrombocythaemia. *Br J Haematol* 2014; 167: 421–3.

DOI: https://doi.org/10.1016/j.pathol.2018.09.059