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Thrombopoietin measurement as a key component in the evaluation of pediatric thrombocytosis

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Abstract

JAK2, *MPL*, and *CALR* mutations, which underlie essential thrombocythemia (ET) in most adults, are infrequent in children. Consequently, additional tests are needed to confirm pediatric ET diagnoses. We report a child with suspected ET and *JAK2*, *MPL*, and *CALR* analyses. Serum thrombopoietin was markedly elevated, leading to analysis of the thrombopoietin gene, *THPO*, which contains an upstream open reading frame (uORF) known to repress *THPO* translation. Sequencing revealed a *de novo*, germline stopgain mutation in the uORF, explaining the elevated thrombopoietin and thrombocytosis. This suggests that screening thrombopoietin levels and, if elevated, *THPO* 5' UTR sequencing could be diagnostic.

Keywords

thrombocytosis; essential thrombocythemia; thrombopoietin; *THPO*; upstream open reading frame

Introduction

Essential thrombocythemia (ET) is a myeloproliferative disorder characterized by persistent thrombocytosis and increased megakaryocytes in the absence of a reactive or secondary cause or other myeloid neoplasia. Approximately 85 to 90% of adults with ET have clonal disease and an acquired *JAK2*, *MPL*, or *CALR* mutation.[1] Current algorithms for the diagnosis of ET include molecular genetic studies for a pathogenic variant in one of these

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Authorship

NN performed *THPO* 5'UTR sequencing and wrote the manuscript. AM evaluated the bone marrow aspirate and biopsy. KB provided genetic counseling services. MS was responsible for enrollment of the patient and his parents on H-29892. DM conducted the clinical evaluation of the patient. AB oversaw the project. AM, KB, DM, and AB edited the manuscript. The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

genes.[2-4] ET is a rare disorder in childhood and, in striking contrast to adults, only 25 to 30% of children diagnosed with ET will have an acquired *JAK2*, *MPL*, or *CALR* mutation. [5-6] Thus, 70-75% of children are classified as having 'triple negative' disease. X chromosome inactivation studies in girls with triple negative disease argued against clonal disease.[5] However, targeted deep sequencing performed in two cohorts of children with ET revealed that 32 to 40% of those lacking *JAK2*, *MPL*, or *CALR* mutations had somatic changes in one or more other genes previously implicated in myeloproliferative neoplasms. [6-7] In these triple negative cases, any given other gene (e.g., *ASXL1* and *IRF8*) was mutated in just a few patients. Thus, the diagnosis of ET in children remains challenging and the possibility remains that other genes may underlie the thrombocytosis.

Results

Our patient is a male who was referred at 16 months of age for persistent thrombocytosis, with platelet counts averaging between 1.2 and $1.6 \times 10^6/\mu\text{L}$ and otherwise p complete blood counts. Past medical history was remarkable for recurrent otitis and mild eczema. There was no family history of thrombocytosis and both parents had p platelet counts. On physical exam, he had mild hepatosplenomegaly (liver edge and spleen tip palpable 1 and 2 cms below the right and left costal margins, respectively), but no other findings. Laboratory studies for secondary causes were p. Bone marrow examination demonstrated a mildly hypercellular marrow (95%) with megakaryocytic hyperplasia (up to 22 megakaryocytes per high powered field), no dysplasia, and no fibrosis (Figure 1). Chromosomal analysis revealed a p male karyotype and a myelodysplastic syndrome fluorescence *in situ* hybridization panel was p, showing no evidence of $-5/5q-$, $-7/7q-$, trisomy 8 or 20q-. Targeted *JAK2* V617 pyrosequencing, *CALR* amplification and sequencing, and *MPL* gene bidirectional sequencing did not detect a variant. Variants of unknown significance in genes associated with hematologic disease (*LYST*, *FANCI*, *PRF1*, *PNPLA1*, and *CD36*) were noted in the initial interpretation of clinical whole exome sequencing (Baylor Medical Genetics Laboratory), however, none were considered to be responsible for the patient's thrombocytosis nor clinically significant.

With a presumptive diagnosis of ET, the patient was managed over 2 years, during which time his platelet count ranged between $0.8 - 2.0 \times 10^6/\mu\text{L}$ off medication (mean and median $1.2 \times 10^6/\mu\text{L}$). The peripheral blood counts remained otherwise within p range.

To further explore the pathophysiology underlying this triple negative patient's disease, a serum TPO measurement (Quest Diagnostics) was obtained to determine if thrombopoiesis was being driven by elevated TPO levels. The TPO concentration was extremely elevated at > 4000 pg/mL (reference range 7-99 pg/mL). This value was significantly higher than observed in ET or reactive thrombocytosis, in which the TPO concentrations are infrequently $> 1,000$ pg/mL,[8-13] and suggested the presence of a pathogenic variant driving TPO production.

Inherited pathogenic variants in the 5' untranslated region (UTR) of *THPO*, which encodes TPO, have been reported in kinships with thrombocytosis in multiple family members, including children, with autosomal dominant transmission and elevated TPO levels.[14-17]

The 5' UTR of the various *THPO* mRNA isoforms contains 5-7 upstream open reading frames (uORFs), which regulate translation.[18] uORF₇, present in all of the *THPO* mRNAs, exerts the strongest translational inhibition, with its translation extending past the physiologic AUG (Figure 2A). Given the highly elevated TPO level in our patient, we hypothesized that he may have a pathogenic variant in *THPO* uORF₇. Polymerase chain reaction amplification of the *THPO* 5' UTR was performed on peripheral blood DNA (forward primer 5'AATGTGAGAGAATTCAGGGCTT3' and reverse primer 5' TTTGGGAGAATGGGTTCCCC3') followed by bidirectional sequencing and comparison to the *THPO* coding sequence (NM_001290028.1). This revealed a heterozygous pathogenic variant, *THPO*:c.-34C>T, resulting in a stopgain in uORF₇ (Figure 2B). Sequencing of buccal DNA indicated the variant was germline. The variant was absent in both parents, indicating that the mutation was *de novo* (Figure 2A). The variant was considered novel, based on its absence in dbSNP and in the Exome Aggregation Consortium database, which reports the variants identified in approximately 60,706 unrelated individuals subjected to exome sequencing as part of various disease-specific and population genetic studies, with efforts made to specifically exclude individuals with severe pediatric disease (<http://exac.broadinstitute.org/about>).[19]

Re-evaluation of the prior clinical whole exome sequencing also revealed the variant, which had been initially excluded from interpretation given it was neither a previously reported pathogenic variant nor, given its noncoding position, predicted to affect splicing. Similar to previously reported inherited pathogenic variants in uORF₇,[14-15] the c.-34C>T variant increases the intercistronic distance between uORF₇ and the main ORF, and eliminates extension of uORF₇ translation over the physiologic AUG, allowing increased ribosomal initiation at the *THPO* start site and markedly enhanced TPO expression (Figure 2).

Discussion

Proper diagnosis and identification of the underlying cause of ET is critical for prognosis, treatment, and genetic counseling. This case is the first description of suspected ET in childhood caused by a *de novo* germline pathogenic variant in the 5' UTR of *THPO*. It illustrates that *THPO* uORF₇ variants may underlie not just familial thrombocytosis, but also sporadic, chronic thrombocytosis masquerading as ET. Additionally, while similar mutations have been reported previously, the potential impact of novel mutations may be unappreciated during the analysis of whole exome sequencing data due to their location outside of the coding region of *THPO*. This report brings to light the fact that base substitutions in the 5' UTR may be clinically significant even in the absence of family history, and that those who analyze clinical whole exome sequencing must consider them.

While studies have previously reported elevated TPO levels in patients with ET,[8-11-13] those levels were substantially lower than those seen in our patient and in previously reported patients with inherited *THPO* uORF₇ pathogenic variants.[14-15] Determination of TPO level is not included in current algorithms for the evaluation of persistent thrombocytosis or suspected ET. However, the discovery of a markedly elevated TPO level would direct further investigations, as in our case. Given the low proportion of *JAK2*, *MPL*, and *CALR* variants and clonality in children with suspected ET,[5] we propose that

screening for an elevated TPO level be included in their initial evaluation. If found to be markedly elevated (e.g., >1000 pg/mL), evaluation for variants in the 5' UTR of *THPO* may prove diagnostic.

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Abbreviations key

ET	essential thrombocythemia
TPO	thrombopoietin
uORF	upstream open reading frame
UTR	untranslated region

References

1. Tefferi A, Wasse EA, Guglielmelli P, Gangat N, Belachew AA, Lasho TL, Finke C, Ketterling RP, Hanson CA, Pardanani A, Wolanskyj AP, Maffioli M, Casalone R, Pacilli A, Vannucchi AM, Passamonti F. Type 1 versus Type 2 calreticulin mutations in essential thrombocythemia: a collaborative study of 1027 patients. *Am J Hematol.* 2014; 89(8):E121–124. [PubMed: 24753125]
2. Alimam S, Wilkins BS, Harrison CN. How we diagnose and treat essential thrombocythaemia. *Br J Haematol.* 2015; 171(3):306–321. [PubMed: 26464262]
3. Langabeer SE, Andrikovics H, Asp J, Bellosillo B, Carillo S, Haslam K, Kjaer L, Lippert E, Mansier O, Leibundgut E, Percy MJ, Porret N, Palmqvist L, Schwarz J, McMullin MF, Schnittger S, Pallisgaard N, Hermouet S, EuroNet MP. Molecular diagnostics of myeloproliferative neoplasms. *Eur J Haematol.* 2015; 95(4):270–279. Mpn. [PubMed: 25951317]
4. Tefferi A, Pardanani A. Myeloproliferative Neoplasms: A Contemporary Review. *JAMA Oncol.* 2015; 1(1):97–105. [PubMed: 26182311]
5. Randi ML, Geranio G, Bertozzi I, Micalizzi C, Ramenghi U, Tucci F, Notarangelo LD, Ladogana S, Menna G, Giordano P, Consarino C, Farruggia P, Zanazzo GA, Fiori GM, Burnelli R, Russo G, Jankovich M, Peroni E, Duner E, Basso G, Fabris F, Putti MC. Are all cases of paediatric essential thrombocythaemia really myeloproliferative neoplasms? Analysis of a large cohort. *Br J Haematol.* 2015; 169(4):584–589. [PubMed: 25716342]
6. Karow A, Nienhold R, Lundberg P, Peroni E, Putti MC, Randi ML, Skoda RC. Mutational profile of childhood myeloproliferative neoplasms. *Leukemia.* 2015
7. Fu R, Liu D, Cao Z, Zhu S, Li H, Su H, Zhang L, Xue F, Liu X, Zhang X, Cheng T, Yang R, Zhang L. Distinct molecular abnormalities underlie unique clinical features of essential thrombocythemia in children. *Leukemia.* 2016; 30(3):746–749. [PubMed: 26118316]
8. Werynska B, Ramlau R, Podolak-Dawidziak M, Jankowska R, Prajs I, Usnarska-Zubkiewicz L, Kuliczowski K. Serum thrombopoietin levels in patients with reactive thrombocytosis due to lung cancer and in patients with essential thrombocythemia. *Neoplasma.* 2003; 50(6):447–451. [PubMed: 14689068]
9. Griesshammer M, Hornkohl A, Nichol JL, Hecht T, Raghavachar A, Heimpel H, Schrezenmeier H. High levels of thrombopoietin in sera of patients with essential thrombocythemia: cause or consequence of abnormal platelet production? *Ann Hematol.* 1998; 77(5):211–215. [PubMed: 9858146]

10. Karakus S, Ozcebe OI, Haznedaroglu IC, Goker H, Ozatli D, Kosar A, Buyukasik Y, Ertugrul D, Sayinalp N, Kirazli IS, Dundar SV. Circulating thrombopoietin in clonal versus reactive thrombocytosis. *Hematology*. 2002; 7(1):9–12. [PubMed: 12171772]
11. Hou M, Carneskov J, Mellqvist UH, Stockelberg D, Hedberg M, Wadenvik H, Kutti J. Impact of endogenous thrombopoietin levels on the differential diagnosis of essential thrombocythaemia and reactive thrombocytosis. *Eur J Haematol*. 1998; 61(2):119–122. [PubMed: 9714524]
12. Cerutti A, Custodi P, Duranti M, Noris P, Balduini CL. Thrombopoietin levels in patients with primary and reactive thrombocytosis. *Br J Haematol*. 1997; 99(2):281–284. [PubMed: 9375739]
13. Pitcher L, Taylor K, Nichol J, Selsi D, Rodwell R, Marty J, Taylor D, Wright S, Moore D, Kelly C, Rentoul A. Thrombopoietin measurement in thrombocytosis: dysregulation and lack of feedback inhibition in essential thrombocythaemia. *Br J Haematol*. 1997; 99(4):929–932. [PubMed: 9432045]
14. Kondo T, Okabe M, Sanada M, Kurosawa M, Suzuki S, Kobayashi M, Hosokawa M, Asaka M. Familial essential thrombocythemia associated with one-base deletion in the 5'-untranslated region of the thrombopoietin gene. *Blood*. 1998; 92(4):1091–1096. [PubMed: 9694695]
15. Ghilardi N, Wiestner A, Kikuchi M, Ohsaka A, Skoda RC. Hereditary thrombocythaemia in a Japanese family is caused by a novel point mutation in the thrombopoietin gene. *Br J Haematol*. 1999; 107(2):310–316. [PubMed: 10583217]
16. Wiestner A, Schlemper RJ, Maas AP, Skoda RC. An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. *Nat Genet*. 1998; 18(1):49–52. [PubMed: 9425899]
17. Liu K, Kralovics R, Rudzki Z, Grabowska B, Buser AS, Olcaydu D, Gisslinger H, Tiedt R, Frank P, Okon K, Maas AP, Skoda RC. A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. *Haematologica*. 2008; 93(5):706–714. [PubMed: 18367486]
18. Ghilardi N, Wiestner A, Skoda RC. Thrombopoietin production is inhibited by a translational mechanism. *Blood*. 1998; 92(11):4023–4030. [PubMed: 9834204]
19. January 21. In Exome Aggregation Consortium (ExAC) <exac.broadinstitute.org>. Accessed 2016 January 21

Figure 1.

Bone marrow histopathologic evaluation showing mild bone marrow hypercellularity with markedly increased megakaryocytes and p reticulin staining. A) 10X and B) 20X magnification of bone marrow biopsy. C) Reticulin stain of bone marrow biopsy under 20X magnification. D) 20X magnification of bone marrow aspirate.

Figure 2.

A *de novo*, germline, stopgain mutation within uORF₇ of *THPO*. A) Schematic of uORF₇ and translation of wild type *THPO*. Sequence chromatograms of the proband's parents are shown below. B) Schematic of the truncated uORF₇ and effects on *THPO* translation. The sequencing chromatogram of the proband blood and buccal cell DNA is shown below. Asterisks indicate two previously identified mutations, c.-47delG and c.-31G>T.[14,15]