Letter to the Editor
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Thrombocythemia 1 With *THPO* Variant (c.13+1G>A) Diagnosed Using Targeted Exome Sequencing: First Case in Korea

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Dear Editor,

Hereditary thrombocythemia is a very rare autosomal dominant disorder associated with polyclonal hematopoiesis of the megakaryocytic lineage [1, 2]. Thrombocythemia 1 (THCYT1), caused by *THPO* variant, has been reported in Dutch [3, 4], Japanese [5, 6], Polish [7], Italian [1], Filipino [2], and German [8] families. Here, we report the first case of a Korean boy diagnosed as having THCYT1 using next-generation sequencing (Table 1). This study was approved by the Institutional Review Board of Keimyung University Dongsan Hospital, Daegu, Korea (approval number: 2019-01-015-002). Informed consent was obtained from all individuals in this study.

In September 2017, a 25-day-old male infant was transferred to Keimyung University Dong San Hospital, following an incidental finding of an abnormal white blood cell (WBC) count. He had congenital defects of his left hand (Fig. 1A and 1B). One of his two sisters had a history of persistent thrombocythemia of unknown etiology from the age of five months, which was diagnosed at another hospital. His parents and another sister had no past medical history.

Laboratory examination at admission showed a WBC count of 34.6×10^9/L. His hemoglobin level was 134 g/L, and the platelet count was 197×10^9/L. The WBC count, determined by manual differential cell count, gradually increased to 57.61×10^9/L with 3% blasts, 45% segmented cells, 32% lymphocytes, 10% monocytes, 5% eosinophils, and 1% basophils at 39 days of age. Bone marrow analysis at 42 days old showed an increase in the myeloid series with some myeloblasts (5.1%). Megakaryocytes were adequate in number and morphologically normal. We initially suspected juvenile myelomonocytic leukemia; however, clinical and genetic evaluations were inadequate to confirm this diagnosis.

At four months of age, the leukocytosis resolved spontaneously; however, the platelet count increased to $>1{,}000\times10^9$/L and was sustained at this level (Fig. 1C). There was no evidence of infection, tissue damage, allergic disease, or autoimmune inflammation associated with secondary thrombocythemia. A second bone marrow analysis revealed slightly increased cellularity with an adequate myeloid:erythroid ratio (2.38:1) and no abnormal finding except for increased megakaryocytes (Fig. 1D). Genetic evaluation for essential thrombocythemia was performed for *JAK2, JAK2 V617F, MPL*, and *CALR*, and no pathogenic variant was observed. Treatment with low-dose acetyl salicylic acid (3 mg/kg) was initiated at four months of age.

Targeted exome sequencing was performed to determine the genetic cause of the thrombocythemia in the absence of *JAK2/MPL/CALR* mutation. Library preparation was performed using

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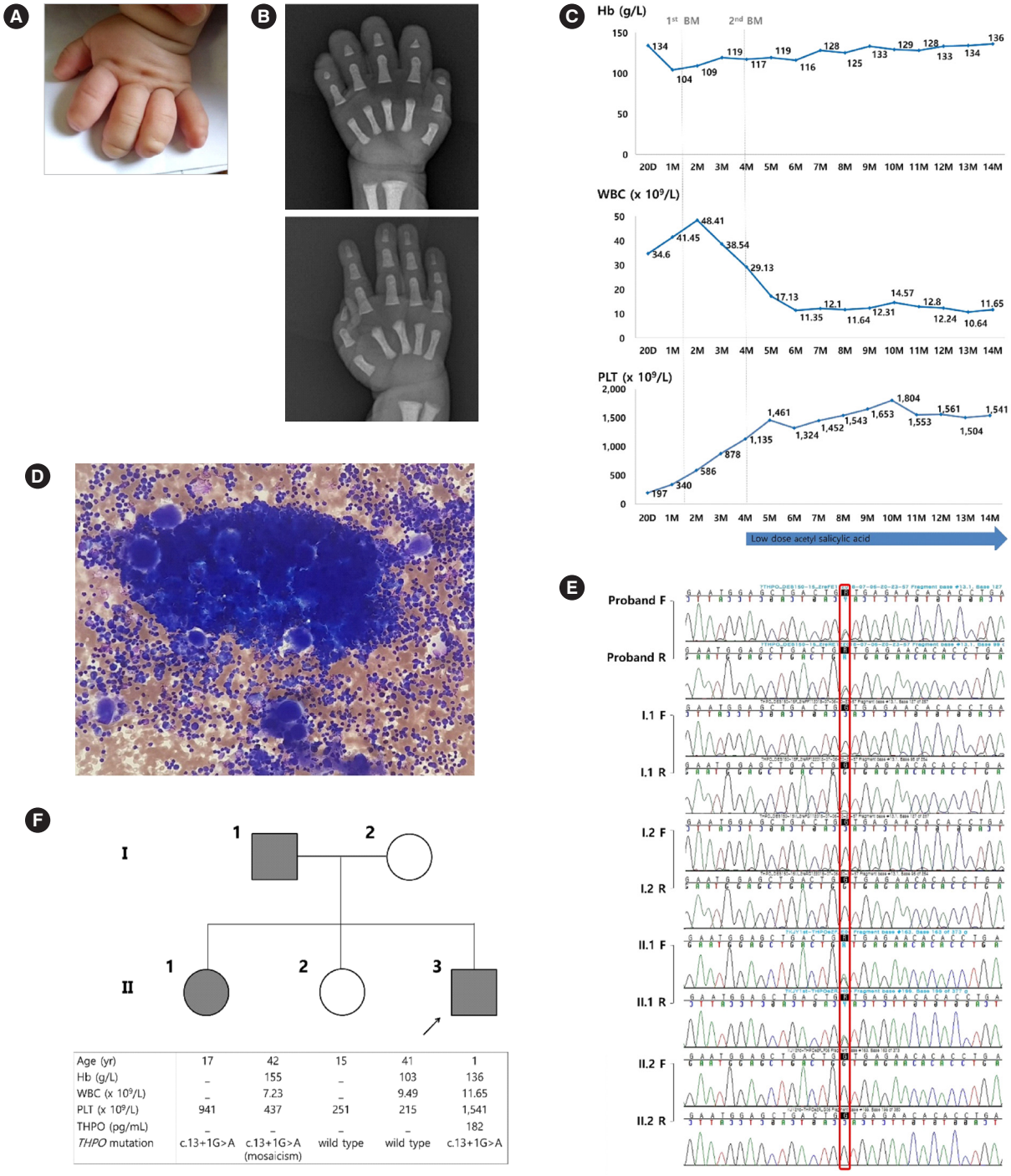
Table 1. Clinical manifestations of reported thrombocythemia 1 families with *THPO* variant

Published cases	*THPO* variant	Classification*	Complete blood count	THPO serum concentration	Clinical manifestations
Present case	c.13+1G>A	Likely pathogenic	Leukocytosis in infancy thrombocythemia	Increased	Congenital limb defects: absent distal phalanges of the left hand
Schlemper, *et al.*, 1994 [3] and Wiestner, *et al.*, 1998 [4]	IVS3, G>C, +1	Likely pathogenic	thrombocythemia	Increased	Thrombotic complications: tip paresthesia, erythromelalgia, acrocyanosis, gangrene, transient ischemic attack, carotid artery stenosis, leg claudication, angina pectoris, stillbirth
Kondo, *et al.*, 1998 [5]	del3252G	Likely pathogenic	thrombocythemia	Increased	-
Ghilardi, *et al.*, 1999 [6]	516G>T	Likely pathogenic	thrombocythemia	Increased	Hematologic malignancy: cutaneous malignant lymphoma at 4 years of age
Liu, *et al.*, 2008 [7]	IVS3, G>C, +1	Likely pathogenic	thrombocythemia	Increased	Thrombotic complications: Raynaud's phenomenon, transient ischemic attack, miscarriage, superficial vein thrombosis, Buerger's disease
Graziano, *et al.*, 2009 [1]	516G>T	Likely pathogenic	thrombocythemia	Increased	Congenital limb defects: absence of the right foot (absence of the calcaneus and astragalus), unilateral congenital transverse defect of the right upper (absence of forearm and hand) and right lower (absence of foot) limbs, milder lower limb defect in one family member, absence of the last phalange of digit 2 and the last two phalanges of digits 3–5, and left foot defect in one family member
Zhang, *et al.*, 2011 [2]	IVS2, T>C, +2	Likely pathogenic	thrombocythemia	Increased	-
Stockklausner, *et al.*, 2012 [8]**	c.13+1G>C	Likely pathogenic	thrombocythemia	Increased	Congenital limb defects: absent proximal, middle, and distal phalanges at digits 3–5; a dysplastic proximal phalanx at digit 2 with absent middle and distal phalanx and shortened metacarpal bones at digits 3 and 4; carpal bones partly fused to metacarpal bones at digits 2–5.
	c.13+1G>C	Likely pathogenic	thrombocythemia	Increased	Hematologic malignancy: early-onset multiple myeloma at 39 years of age

*The variants were classified according to the 2015 ACMG/AMP guidelines based on the descriptions in each study.
**These two families were described together in the same report [8].
Abbreviation: THPO, thrombopoietin.

the TruSight One sequencing panel (Illumina, San Diego, CA, USA). Massively parallel sequencing was conducted using the NextSeq platform (Illumina, San Diego, CA, USA). Sanger sequencing was performed using the primers F-5´-TCAGGACCC-AGACCTGAAAC-3´ and R-5´-CCTACTCTGCCCAGAAGTGC-3´. Sanger sequencing of samples from the patient and his family members confirmed heterozygosity of a splicing pathogenic variant, NM_000460.2: c.13+1G of *THPO* for the patient, his father, and the sister with a history of thrombocythemia. His father was presumed to have mosaicism due to a low heterozygous peak. His mother and the other sister possessed the wild-type sequence at c.13+1 of *THPO* (Fig. 1E). Finally, at 11 months of age, the patient was diagnosed as having THCYT1 with *THPO* variant (c.13+1G>A). The pedigree suggested that the patient's disease was inherited in an autosomal dominant manner (Fig. 1F). The patient also showed elevated THPO serum concentration (182 pg/mL, reference value 7–99 pg/mL) in a manual immunoassay (Quest Diagnostics, Valencia, CA, USA). No thrombotic or hemorrhagic events occurred during the 20-month follow-up period.

THPO is an essential cytokine associated with platelet production and plays an important role in the maintenance of early myeloid progenitors [7]. The multiple upstream AUG codons of the 5´-untranslated region (UTR) in *THPO* interrupt translation and prevent overproduction of this cytokine [7]. Variants at position +1 of the *THPO* intron 3 splice donor site result in the skipping of exon 3 and loss of the inhibitory 5´-UTR sequence, thereby disrupting the translational regulation and resulting in increased THPO production and thrombocythemia [4, 7, 8]. A possible mechanism for the early leukocytosis in patients with thrombocythemia can be related to the additional role of THPO in regulating hematopoietic stem cell viability [9, 10]. Several studies



Age (yr)	17	42	15	41	1
Hb (g/L)	–	155	–	103	136
WBC (x 10⁹/L)	–	7.23	–	9.49	11.65
PLT (x 10⁹/L)	941	437	251	215	1,541
THPO (pg/mL)	–	–	–	–	182
THPO mutation	c.13+1G>A	c.13+1G>A (mosaicism)	wild type	wild type	c.13+1G>A

Fig. 1. Clinical features of the thrombocythemia 1 patient. (A) Constricted left hand. (B) Radiographs showing the limb defects of his left hand (missing distal phalanges at digits 2–5) and his normal right hand. (C) Change in the CBC over time. (D) Bone marrow aspirate showing increased megakaryocytes in active form (Wright-Giemsa stain, ×100). (E) Sanger sequencing of *THPO* in the patients and family members. The red square indicates the position of NM_000460.2: c.13+1G. (F) Pedigree of the family with thrombocythemia 1 and laboratory results. The patient is indicated with an arrow. The filled symbols represent individuals with *THPO* gene variant. Open symbols represent normal individuals. The individuals are indicated above the corresponding lanes. Age, CBC, serum THPO level (reference value, 7 – 99 pg/mL), and *THPO* variant are shown.

Abbreviations: CBC, complete blood count; THPO, thrombopoietin; F, forward strand; R, reverse strand; Hb, hemoglobin; WBC, white blood cells; PLT, platelets.

have reported patients with THCYT1 and coexisting congenital distal limb defects, suggesting that THPO is involved in vasculogenesis, as a regulator of hemangioblast [1, 8]. Hemorrhagic or thrombotic complications have also been reported occasionally [3, 7]. Although our patient has been successfully treated with low-dose acetyl salicylic acid, an optimal management strategy for hereditary thrombocythemia is yet to be established.

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AUTHOR CONTRIBUTIONS

Conceptualization: YJS. Methodology and validation: JSH and DHK. Investigation and data curation: YJS and NJ. Writing – original draft preparation: NJ Writing – review & editing: YJS. Supervision: YJS, DHK, and JSH. Approval of final manuscript: all authors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Graziano C, Carone S, Panza E, Marino F, Magini P, Romeo G, et al. Association of hereditary thrombocythemia and distal limb defects with a thrombopoietin gene mutation. Blood 2009;114:1655-7.
2. Zhang B, Ng D, Jones C, Oh ST, Nolan GP, Salehi S, et al. A novel splice donor mutation in the thrombopoietin gene leads to exon 2 skipping in a Filipino family with hereditary thrombocythemia. Blood 2011;118:6988-90.
3. Schlemper RJ, van der Maas AP, Eikenboom JC. Familial essential thrombocythemia: clinical characteristics of 11 cases in one family. Ann Hematol 1994;68:153-8.
4. Wiestner A, Schlemper RJ, van der Maas AP, Skoda RC. An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. Nat Genet 1998;18:49-52.
5. Kondo T, Okabe M, Sanada M, Kurosawa M, Suzuki S, Kobayashi M, et al. Familial essential thrombocythemia associated with one-base deletion in the 5'-untranslated region of the thrombopoietin gene. Blood 1998;92:1091-6.
6. 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;107:310-6.
7. Liu K, Kralovics R, Rudzki Z, Grabowska B, Buser AS, Olcaydu D, et al. A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. Haematologica 2008;93:706-14.
8. Stockklausner C, Echner N, Klotter AC, Hegenbart U, Dreger P, Kulozik AE. Hereditary thrombocythemia caused by a thrombopoietin (THPO) gain-of-function mutation associated with multiple myeloma and congenital limb defects. Ann Hematol 2012;91:1129-33.
9. Hitchcock IS and Kaushansky K. Thrombopoietin from beginning to end. Br J Haematol 2014;165:259-68.
10. Kuter DJ. The biology of thrombopoietin and thrombopoietin receptor agonists. Int J Hematol 2013;98:10-23.