Letter to the Editor
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The First Korean Hemoglobinopathy With Unique Hemoglobin Electrophoresis Results Diagnosed as Hemoglobin Boras

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Dear Editor,

Hemoglobinopathies are the most common single-gene hemoglobin (Hb) disorders, including sickle cell disease, thalassemia, and unstable hemoglobinopathies. Unstable Hb is typically generated by abnormal Hb variants, in which amino acid mutations alter the Hb structure, and is associated with hemolytic diseases owing to unstable red blood cells (RBCs) [1]. Currently, more than 200 unstable Hb variants, including Hb Boras, have been reported [2]. Hb Boras was first reported in 1969 in Swedish family members and was caused by a missense mutation in *HBB* [3]. Patients with Hb Boras show clinical features of moderate hemolytic anemia and macrocytosis with Heinz bodies [3]. The second Hb Boras case was reported in a South African woman with normal parents [4]. The third case of Hb Boras was reported in a Mexican neonate with double missense mutations [5]. Here, we report the first patient with Hb Boras in Korea and only the fourth globally, among reports of unexpected Hb electrophoresis (EP) results for the first time. This study was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (IRB No. 4-2022-1657) with waived informed consent.

A 5-year-old Korean boy presented at our hospital with anemia at the beginning of the study period for 6-years follow-up. There was no specific birth history except for neonatal jaundice without anemia. RBC transfusions were performed at the age of 4 years to resolve anemia (Hb 70 g/L), which was accompanied by a fever lasting 3 days. Additional blood transfusions were performed the following year (Hb 65 g/L). On physical examination at the time of admission, the patient had a slightly pale appearance and a 4 cm size palpable spleen below the left costal margin (Fig. 1A).

As for family history, the proband's father was diagnosed with hemolytic anemia at the age of six (Fig. 1B). At the time of diag-

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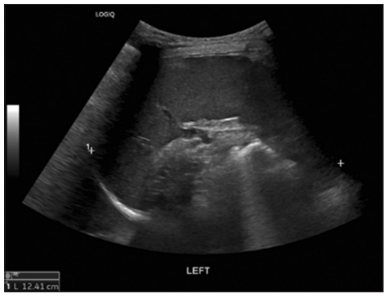

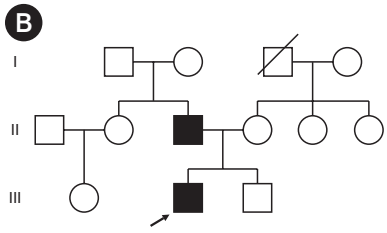

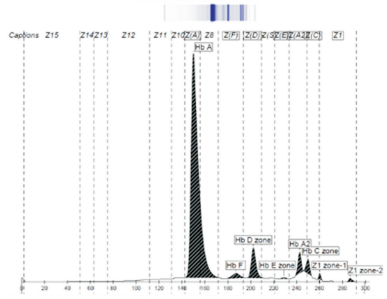

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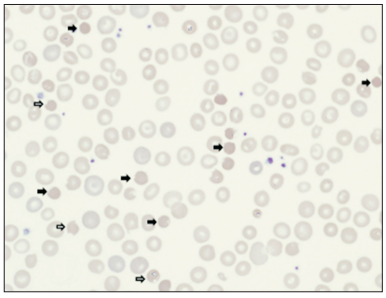
Laboratory parameter, unit	Previous result (age 4)	Current result (age 5)	Reference range
WBC, 10^9/L	8.05	9.33	5.0–14.5
RBC, 10^{12}/L	n/a	3.46	3.9–5.3
Hb, g/L	70	92	115–145
Hct, %	Not available	32.1	34–40
MCV, fL	Not available	92.8	76–90
MCH, fmol	Not available	1.65	1.55–1.86
MCHC, mmol/L	Not available	17.81	19.86–22.34
RDW, %	Not available	18.8	11.5–15.0
Platelet, 10^9/L	180	244	150–450
Reticulocyte, %	Not available	12.4	0.5–1.5
ALP, μkat/L	2.08	2.25	0.25–0.83
ALT, μkat/L	0.25	0.22	0.17–0.42
Total bilirubin, μmol/L	42.75	35.91	3.42–17.1
Direct bilirubin, μmol/L	Not available	10.26	< 5.99
LD, μkat/L	Not available	8.17	7.83–15.0
Transferrin, g/L	1.49	1.78	1.69–3.0
Ferritin, nmol/L	Not available	0.28	0.08–0.19
Iron, μmol/L	23.04	13.79	9.85–26.87
TIBC, μmol/L	43.02	40.11	44.77–55.51
Transferrin saturation, %	28	34	15–39



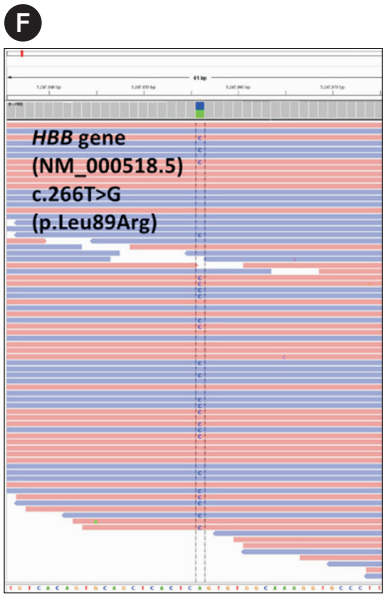


Fig. 1. Clinical phenotype, pedigree and laboratory findings in the present case of Hb Boras. (A) Upper abdomen ultrasonography of the index patient shows splenomegaly (12.4 cm). (B) The family history of the patient: There was no history of anemia or jaundice except for that observed in his father. The patient and his father had a c.266T>G (p.Leu89Arg) mutation in the *HBB* gene, and his younger brother had the wild type. His maternal grandfather died from malignant lymphoma. (C) Laboratory values at the first referral (current result) and past evaluation at another hospital one-year ago (previous result) of the index case. (D) Peripheral blood smear of the index patient shows spherocytes (black arrow) and bite cells (white arrow) (Wright-Giemsa stain, ×1000). (E) Hb electrophoresis of the index patient shows multiple irregular peaks. (F) *HBB* missense variant c.266T>G (p.Leu89Arg) identified in the index patient.

Abbreviations: ALP, alkaline phosphatase; LD, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red blood cell distribution width; TIBC, total iron-binding capacity; WBC, white blood cell.

nosis, he presented with anemia (Hb 42 g/L), bilirubinemia (total bilirubin 34.2 μmol/L), and hepatosplenomegaly. Since Hb EP and molecular testing was unavailable at the time, he was diagnosed with hemolytic anemia. He then underwent splenectomy.

The laboratory findings at the time of the patient's visit revealed normocytic hypochromic anemia with a high reticulocyte count and elevated indirect bilirubin and lactate dehydrogenase levels (Fig. 1C). Peripheral blood smear (PBS) showed mild anisopoikilocytosis with bite cells and spherocytes (Fig. 1D). Hb EP analysis was conducted using Capillaries 2 Flex Peering (Sebia, France), which presented a number of irregular variations (Fig. 1E). In the Hb EP performed using another whole blood sample after 2 months since the initial sampling, the same peak pattern was observed, despite small variations shown in the first test (Table 1). Next-generation sequencing (NGS) analysis of germline mutations in the proband revealed a heterozygous c.266T>G (p.Leu89Arg) mutation in the *HBB* gene (NM_000518.5) (Fig. 1F). After diagnosis, the patient was doing well with folic acid without additional transfusions. The patient was recommended for a follow-up by a hematologist to monitor the possibility of hemolysis.

There have been only three reports of Hb Boras since the first

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Table 1. Hb electrophoresis of the index patient

Hb type	Initial test (%)	Confirmatory test (%)	Reference range (%)
Hb A	79.2	82.3	>95
Hb A2	3.9	3.8	1.5–3.5
Hb F	1.8	2.2	<2
Hb C	8.3	2.2	Absent
Hb D zone	5.5	6.2	Absent
Hb E zone	0.2	0.7	Absent
Hb H zone	0	1.1	Absent
Z1 zone-1	0.4	0.8	Absent
Z1 zone-2	0.7	0.7	Absent

one in 1969 [3]; however, this has never been reported in East Asians. The Hb EP separates the Hb band by moving proteins, separated by high voltage, from the anode to the cathode. Modification of Hb through nucleotide sequence variation can lead to changes in electrophoretic mobility; however, the Hb EP peak pattern of Hb Boras has not yet been reported in detail. The Hb EP in our patient had a peculiarity, in which five to six types of irregular peaks were observed. This unique pattern of several peaks in different zones, such as zones D, C, and F, was simultaneously observed to be different from those observed in other Hb variants.

Anemia, caused by Hb structure or synthesis disorder, first occurs in infancy or toddlerhood. Few symptoms are recognizable immediately after birth; however, symptoms gradually develop as patients grow [6]. Most patients with unstable Hb levels experience only mild hemolytic anemia that does not require intervention. However, severely symptomatic individuals may require blood transfusion or splenectomy. As the clinical manifestations of unstable Hb are diverse, hemoglobinopathy cannot be ruled out as a cause, even without the typical findings in PBS [7]. Hb EP helps diagnose Hb variants; however, in the case of unstable Hb, it may appear normal if it undergoes rapid degeneration or presents as a portion of Hb A [8]. An accurate diagnosis of unstable Hb variants through genetic testing enables proper treatment and management of patients through family counseling [7].

In conclusion, we report the first Korean patient with Hb Boras diagnosed through comprehensive laboratory and genetic testing and unique Hb EP patterns. The possibility of Hb Boras as well as a new Hb variant should be considered on observing unique Hb EP patterns.

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AUTHOR CONTRIBUTIONS

Bae J, Ahn WK, Lyu CJ and Lim J designed the study. Bae J and Ahn WK wrote the manuscript, and Lyu CJ and Lim J supervised the study. Jang J, Jang H, Kang H and Rim JH performed diagnostic tests and interpreted the laboratory results. Hahn SM and Han JW collected clinical data and family history. All authors approved the final manuscript to be published.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

1. Williamson D. The unstable haemoglobins. Blood Rev 1993;7:146-63.
2. Giardine B, Borg J, Viennas E, Pavlidis C, Moradkhani K, Joly P, et al. Updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res 2014;42:D1063-9.
3. Hollender A, Lorkin P, Lehmann H, Svensson B. New unstable haemoglobin Borås: β88 (F4) leucine→ arginine. Nature 1969;222:953-5.
4. Bird A, Elliot T, Wilson J, Webber B, Kutlar F, Kutlar A, et al. Hb Borås or α2β288 (F4) Leu→ Arg in a South African Female. Hemoglobin 1987;11:157-60.
5. Martinez Villegas O, Mendoza-Meléndez D, Trueba-Gómez R, Rosenfeld-Mann F, Baptista-González HA, Estrada-Juárez H. Analysis of a Novel Mexican Variant of the HBB Gene Associated with β-Thalassemia Using Bioinformatic Tools. Hemoglobin 2021;45:87-93.
6. Janus J and Moerschel SK. Evaluation of anemia in children. Am Fam Physician 2010;81:1462-71.
7. Medri C, Mendez A, Hammerer-Lercher A, Rovo A, Angelillo-Scherrer A. Unstable hemoglobin Montreal II uncovered in an adult with unexplained hemolysis exacerbated by a presumed viral infection: a case report. J Med Case Rep 2022;16:145.
8. Yates AM, Mortier NA, Hyde KS, Hankins JS, Ware RE. The diagnostic dilemma of congenital unstable hemoglobinopathies. Pediatr Blood Cancer 2010;55:1393-5.