SEVERE ANEMIA ASSOCIATED WITH α^0-THALASSEMIA/Hb J-BUDA [α61(E10) LYS→ASN, AAG>AAT] COINHERITANCE IN A THAI GIRL: A CASE REPORT

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Abstract. Hemoglobin (Hb) J-Buda [α61(E10) Lys→Asn, AAG>AAT] is a very rare α-chain variant in Southeast Asia. To the best of our knowledge, we report the first case of severe hypochromic microcytic anemia in a 4-year-old Thai girl with coinheritance α^0-thalassemia and Hb J-Buda (36.1%). Her clinical and hematological features were likely the results of a non-deletional Hb H disease, known to be more severe than the classical form of Hb H disease. Thus, knowledge of complex inheritance is important when providing genetic counseling and planning for a thalassemia prevention program.

Keywords: anemia, coinheritance, Hb J-Buda, hemoglobinopathy, severe Hb H disease

INTRODUCTION

Hemoglobin (Hb) J-Buda [α61(E10) Lys→Asn, AAG>AAT; HBA2:c.(186G>C) (or HBA1) or 186G>T (or HBA1)] is an α-globin chain variant resulting from a point mutation at codon 61 of the α1-globin gene on chromosome 16p (Itchayanan *et al*, 1999). This hemoglobinopathy was first discovered in a Hungarian subject in association with Hb G-Pest [α74(EF3) Asp→Asn, GAC>AAC; HBA2:c.223G>A (or HBA1)] (Hollan *et al*, 1972). The patient has abnormally high Hb concentration, erythrocyte counts and packed cell volume (PCV), but normal leucocyte and platelet counts, and was diagnosed as polycythemia vera. Hb J-Buda was also reported in a 28-year-old Thai female normal values for Hb concentration (12.7 g/dl), PCV (0.40 l/l), mean corpuscular volume (MCV; 82 fl), mean corpuscular Hb (MCH; 28 pg), and mean corpuscular Hb concentration (MCHC; 340 g/l) (Itchayanan *et al*, 1999). Although heterozygote and homozygote for Hb J-Buda are clinically silent conditions, coinheritance of this α-globin variant with α^0-thalassemia might result in severe clinical symptoms. In this study, we report α^0-thalassemia/Hb J-Buda coinheritance in a Thai girl with severe anemia.

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CASE REPORT

A 4-year-old Thai girl presented at Lampang Hospital, Lampang, Thailand with high fever for the previous five days. Physical examination showed a body temperature of 39.0°C, palpebral conjunctiva but no hepatosplenomegaly nor a history of blood transfusion. A complete blood count (CBC) (ADVIA 2120i hematology analyzer; Siemens Healthcare Diagnostic, Deerfield, IL) of EDTA-blood sample revealed a low Hb concentration (7.1 g/dl) (Table 1), and was administered a transfusion of 130 ml of red blood cell (RBC) concentrate, 7.5 ml of acetaminophen liquid (160 mg/5 ml) every 4 hours to control body temperature. The patient

Table 1
Characteristics and hematological parameters of the proposita and parents.

Characteristic	Proposita		Mother	Father
	At initial presentation	Three months after blood transfusion		
Age (years)	4	4	35	35
Red blood cell counts (10^{12}/l)	5.5	5.4	6.8	5.7
Total Hb (g/dl)	7.1	10.4	10.2	14.8
PCV (l/l)	0.25	0.34	0.34	0.47
MCV (fl)	48.5	62.8	50.4	83.0
MCH (pg)	13.6	19.0	15.0	26.1
MCHC (g/l)	280	303	297	315
RDW (%)	25.2	15.7	16.2	11.9
Hb J-Buda (%)	ND	36.1	-	26.4
Hb A (%)	ND	55.5	74.8	66.3
Hb A_2/E (%)*	ND	1.7	14.8	2.0
Hb F (%)	ND	0.8	1.8	0.5
α-Globin genotype		$--^{SEA}/\alpha\alpha^{J\text{-}Buda}$	$--^{SEA}/-\alpha^{3.7}$	$-\alpha^{3.7}/\alpha\alpha^{J\text{-}Buda}$
β-Globin genotype		β/β	β/β^E	β/β
SI (μg/dl)	ND	80	141	93
TIBC (μg/dl)	ND	320	293	316
Transferrin saturation (%)	ND	25.0	48.1	29.4
Ferritin (μg/l)	ND	11	54	258

*Hb A_2 and Hb E co-elute. ND, not done. Normal range: red blood cell counts. 4.2-6.1x10^{12}/; total Hb, 12.0-18.0 g/dl; packed cell volume (PCV), 0.37-0.52 l/l; mean cell volume (MCV), 80-100 fl; mean cell Hb (MCH), 27-31 pg; mean cell Hb concentration (MCHC) 320-360 g/l; red cell distribution width (RDW), 11.5-14.5%; Hb A >85%; Hb A_2 1.5-3.5%; Hb F 0-1%; serum iron concentration (SI), 50-160 μg/dl; total iron-binding capacity (TIBC), 240-400 μg/dl; transferrin saturation, 20-55%; ferritin concentration, 10-400 μg/l.

was discharged one day following blood transfusion.

A follow-up visit three months later showed a CBC picture typical of thalassemia [MCV = 62.8 fl, MCH = 19.0 pg and red cell distribution width (RDW) = 15.7%], with serum iron (SI), total iron-binding capacity (TIBC), transferrin saturation and serum ferritin levels within reference ranges (Table 1). For thalassemia diagnosis, Hb analysis was performed using VARIANT™ β-Thalassemia Short Program HPLC (Bio-Rad Lab, Hercules, CA). In addition DNA was extracted from whole blood sample using a NucleoSpin® DNA extraction kit (Macherey-Nagel KG, Duren, Germany) and stored at -20°C until used in a SYBR Green1-based quantitative PCR equipped with a high resolution melting analyzer for diagnosis of α^0-thalassemia Southeast Asian ($--^{SEA}$) and Thai ($--^{THAI}$) deletions (Pornprasert *et al*,

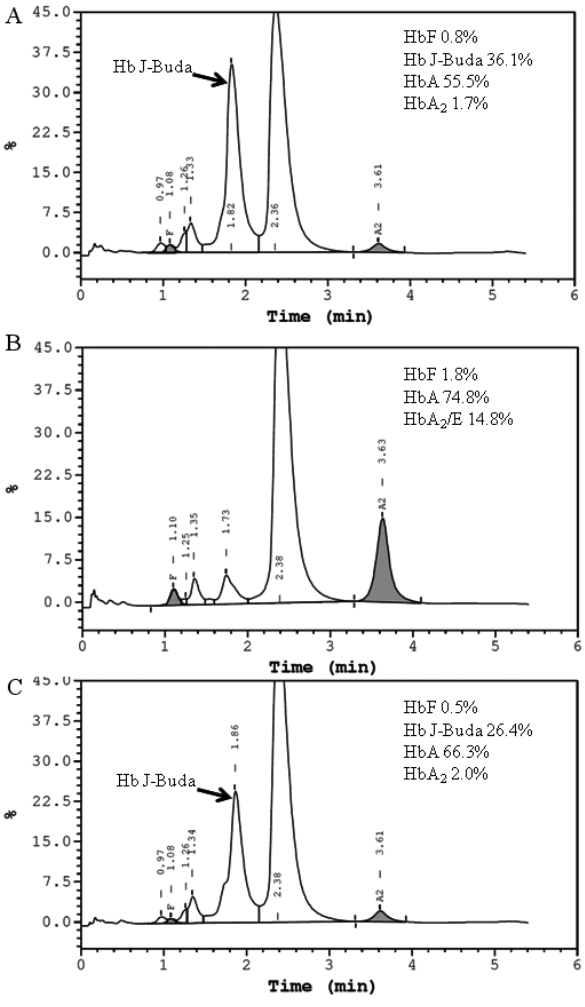

Fig 1-Representative HPLC chromatogram of proposita (A), mother (B) and father (C). Hemoglobin analysis was performed using VARIANT™ β-Thalassemia Short Program HPLC (Bio-Rad Lab, Hercules, CA).

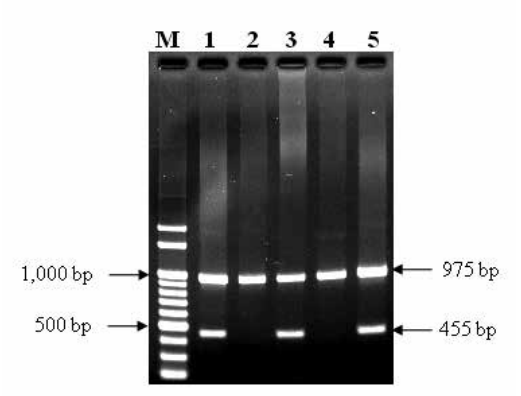

Fig 2-Allele-specific (AS)PCR for identification of Hb J-Buda allele. ASPCR was carried out as described by Panyasai *et al* (2016). Lane M, 100-bp DNA size markers; lane 1, DNA of heterozygous Hb J-Buda (positive control); lane 2, DNA of normal control; lane 3, DNA of proposita; lane 4, DNA of mother; lane 5, DNA of father.

2008) and α^+-thalassemia (-$\alpha^{3.7}$ and -$\alpha^{4.2}$) deletions (Fucharoen *et al*, 2002). On the HPLC chromatogram, an abnormal Hb peak was present ahead of Hb A with a retention time of 1.82-1.86 minutes (Fig 1A). The Hb variant usually appearing at this retention time is Hb J-Buda and this conjecture was confirmed by an allele-specific PCR, which amplified the expected 455-bp amplicon (Fig 2) (Panyasai *et al*, 2016).

The proposita was thus diagnosed as having Hb J-Buda/α^0-thalassemia (– –^{SEA}) coinheritance. The father was diagnosed of having α^+-thalassemia (-$\alpha^{3.7}$)/Hb J-Buda coinheritance and the mother EABart's disease (– –^{SEA}/–$\alpha^{3.7}$, β^A/β^E) (Fig 1B and C). The mother and the proposita had mild anemia status (Hb 10.0–10.9 g/dl) with more hypochromic microcytic red cells as compared to the father's (Table 1).

DISCUSSION

Over 1,000 abnormal Hbs resulting from alterations in the α-, β-, γ- and δ-globin chains are reported worldwide (Giardine *et al*, 2014). Although the heterozygosity of these Hb variants is clinically and hematologically normal, homozygosity or coinheritance with thalassemias and hemoglobinopathies can result in severe anemia. Thus, an accurate diagnosis of such hemoglobinopathies is essential for appropriate genetic counseling.

In the current study, we describe the hematological characteristics of a proposita with coinheritance of α^0-thalassemia/Hb J-Buda and presentation of severe anemia reminiscent of non-deletional Hb H disease, such as Hb H/Constant Spring and Hb H/Paksé (Pornprasert *et al*, 2018). These non-deletional Hb H disease genotypes are commonly associated with more severe clinical and hematological features, especially at younger age and often requiring blood transfusion (Charoenkwan *et al*, 2005). Previous studies reported patients with EABart's disease have comparable clinical manifestations to classical Hb H disease (Boonsa *et al*, 2004; Charoenkwan *et al*, 2005), and the presence of Hb E (in the mother) did not appear to ameliorate the clinical and hematological features. The anemia features of the father could be attributed to coinheritance of α^+-thalassemia with Hb J-Buda, consistent with previous reports in heterozygosity of deletional and non-deletional α^+-thalassemias (Fucharoen and Winichagoon, 2011). Therefore, Hb J-Buda might play a similar role to that of Hb Constant Spring or Hb Paksé in severe Hb H disease (Pornprasert *et al*, 2018). However, the actual mechanism of the role of Hb J-Buda in severe Hb H disease remains to be elucidated.

In summary, α^0-thalassemia/Hb J-Buda coinheritance causes severe anemia with hypochromic microcytic red cells.

Knowledge of such complex inheritance is important when providing appropriate genetic counseling and planning for a thalassemia prevention program.

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