

CASE REPORT

FIRST IDENTIFICATION OF HEMOGLOBIN LANSING-RAMATHIBODI [α 87(F8)His \rightarrow Gln; CAC>CAG (*HBA1*: c.264C>G)] IN A THAI FAMILY WITH SPURIOUS HYPOXEMIA

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Abstract. We report, for the first time, hemoglobin (Hb) Lansing-Ramathibodi [α 87(F8)His \rightarrow Gln; CAC>CAG (*HBA1*: c.264C>G)] in four members of a Thai family presented with low measured oxygen saturation by pulse oximetry (SpO₂), with discrepancy between low SpO₂ and normal calculated oxygen saturation by arterial blood gas analysis, and no cyanosis or methemoglobinemia. The causative mutation is located in *HBA1* whereas in previous reports of Hb Lansing the mutation is on *HBA2*, including that in a Japanese individual. The index and a male sibling also co-inherited Hb Pakse, a non-deletional α -thalassemia 2, resulting in mild reticulocytosis. Correct Hb identification is crucial for genetic counselling and, thereby, avoiding unnecessary investigation and treatment for spurious hypoxemia.

Keywords: Hb Lansing-Ramathibodi, *HBA1*, oxygen saturation, pulse oximetry, Asians

INTRODUCTION

Pulse oximeter is a commonly used instrument to rapidly measure blood oxygen saturation (Sinex, 1999). A “saturation gap” or difference between low

oxygen saturation measured by a pulse oximeter (SpO₂) and oxygen saturation (SaO₂) determined by arterial blood gas analysis indicates, in general, an underlying condition of dyshemoglobinemia or hemoglobinopathy (Verhovsek *et al*, 2010). As a number of Hb variants have low oxygen affinity or interfere with pulse oximeter measurement, low SpO₂ values might be observed in such situations (Zur *et al*, 2012).

Hb Lansing [α 87(F8)His \rightarrow Gln; CAC>CAG (*HBA2*: c.264C>G)] is one of the Hb variants that gives rise to low SpO₂

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Table 1
Hematologic, blood chemistry and co-oximeter values, Hb typing and α -thalassemia analysis of index case and siblings.

Parameter	Index case	Sibling 1	Sibling 2	Offspring of Sibling 1
Sex/Age	Female/40	Male/48	Male/44	Male/17
Hb (g/dl) (normal range: 12-16 in female, 13-18 in male)	11	15.5	13.3	14.5
Hematocrit (%) (normal range: 36-48 in female, 40-54 in male)	35	46.9	42.3	46.3
Mean corpuscular volume (fl) (normal range: 80-99 fl)	81.8	84.7	77.9	78.7
Mean corpuscular Hb (pg) (normal range: 27-31 pg)	25.7	28	24.5	24.6
Mean corpuscular Hb concentration (g/dl) (normal range: 33-37 g/dl)	31.4	33	31.4	31.2
Red cell distribution width (%) (normal range: 11.5-14.5%)	14.0	13.8	12.0	13.7
Reticulocyte count (%) (normal range: 0.9-2.1)	4.8	2.1	5.0	1.8
Total bilirubin (mg/dl) (normal range: 0-1 mg/dl)	0.5	0.7	1.1	0.8
Direct bilirubin (mg/dl) (normal range: 0-0.3 mg/dl)	0.2	0.3	0.4	0.3
Lactate dehydrogenase (U/l) (normal range: 100-190 U/l)	171	168	264	192
MetHb (%)	0.8	0.3	0.1	-
OxyHb (%)	69.1	69.3	64.1	-
DeoxyHb (%)	30	34.7	34.7	-
Hb A (%)	80.6	74.8	76	-
Hb A2 (%)	2.3	2	1.7	-
Hb F (%)	0.6	0.8	2.1	-
α -thalassemia genotype	$\alpha^{\text{Pakse}}\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$\alpha^{\text{Pakse}}\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$

(Sarikonda *et al*, 2009; Ishitsuka *et al*, 2012; Akar *et al*, 2014; Hassan *et al*, 2015). Here, we report an index case presenting with the oxygen saturation gap and subsequently was diagnosed as a carrier of Hb Lansing [α 87(F8)His \rightarrow Gln; CAC>CAG (*HBA1*: c.264C>G)], *ie*, the causative mutation located in α 1-globin gene. This is the

first report, to the best of our knowledge, of such a mutation causing Hb Lansing.

CASE REPORT

A 38-year-old female presented with fatigue and dizziness over a number of days. The *proposita* had no previous

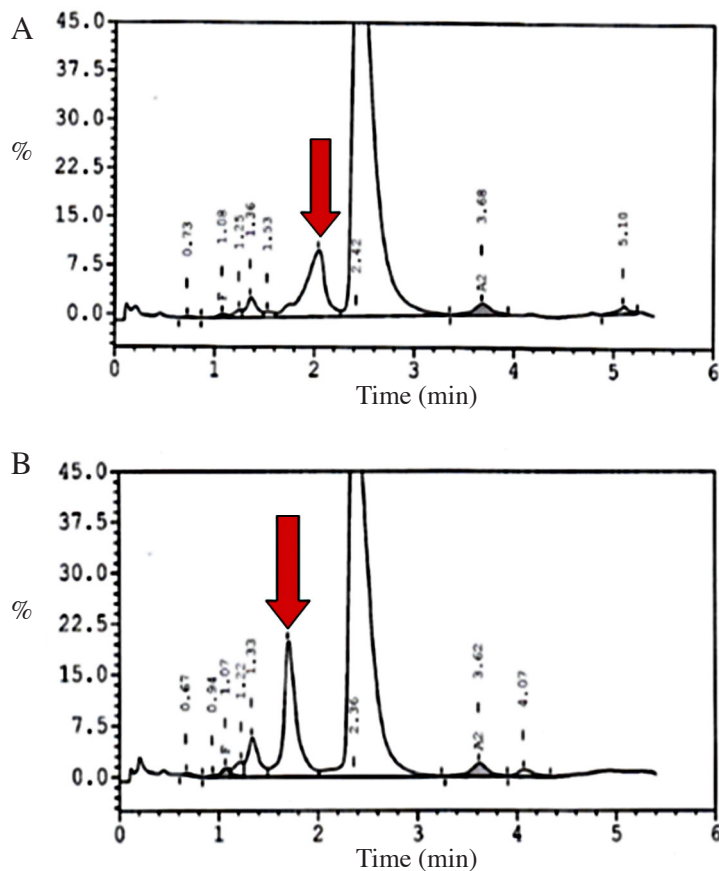


Fig 1—High performance liquid chromatography of red blood cell lysate from index case (A) and sibling 1 (B). Red blood cell lysate was separated using Variant-II HPLC instrument (Bio-Rad, France). Abnormal peak was identified at retention time of 1.70-2.03 minutes (arrow).

history of illness and had not or was not presently taking any herbal medicine or prescription drug. Vital signs and physical examinations were normal, except for SpO₂ reading of 84% under room air, with mild improvement (89%) following oxygen cannula (5 liters/minute). The proposita had no obvious cyanosis and no signs of clubbing. Following admission, measurement of arterial blood gas (on mask delivering 10 liters/minute O₂) showed a pH of 7.4, a partial pressure of O₂ (Pa O₂) of 385 mmHg, a partial

pressure of CO₂ (PaCO₂) of 37 mmHg and SaO₂ of 100%. Chest X-ray, echocardiogram and computed tomography angiography of the chest were all unremarkable. Methemoglobin level was 0.8%, and co-oximetry revealed low oxyhemoglobin level (69.1%), normal carboxyhemoglobin (0.1%) and high deoxyhemoglobin (30%) levels. After receiving supportive treatment, the proposita completely recovered and all symptoms were no longer apparent, leaving only low SpO₂ reading. The proposita was discharged but asked to return, with family, for further investigations into the possible cause of persistent low SpO₂.

The index case has three children with normal SpO₂ values but her two (elder) brothers showed low SpO₂ (88% and 71%) readings as well as her nephew (90%). All subjects

did not have any history of hemolysis or of receiving blood transfusion, looked healthy with no obvious cyanosis. The index case, two sibling and nephew blood pictures were normal as were LDH, total bilirubin, direct bilirubin, and methemoglobin levels (Table 1), but measurement of sulfhemoglobin level was unavailable.

High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) were performed using Variant-II HPLC instrument (Bio-Rad, Marnes-la-Coquettes, France), and Capillarys-2 (Se-

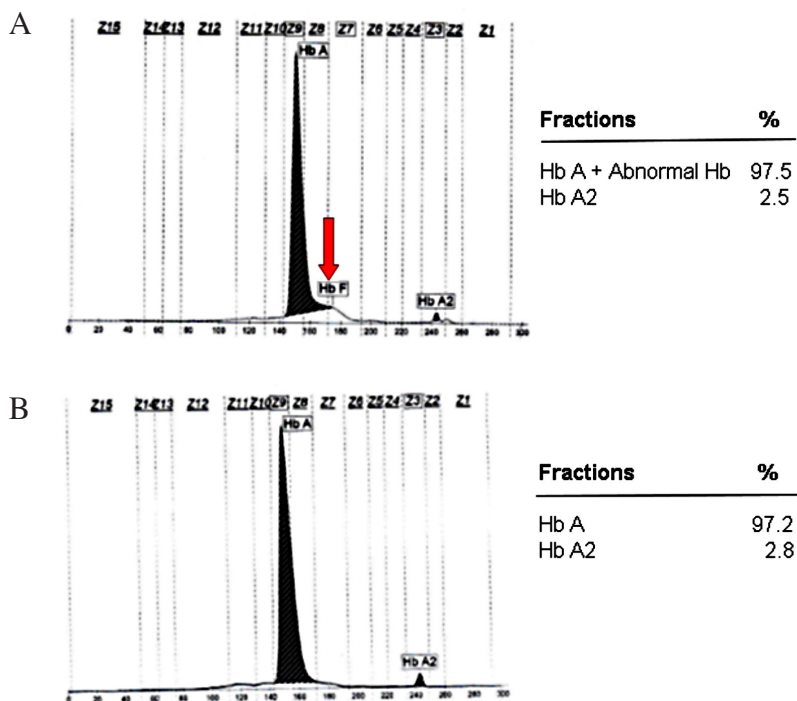


Fig 2—Capillary electrophoresis of red blood cell lysate from index case. Red blood cell lysate was separated using Capillarys-2 (Sebia, France). A peak with a shoulder in the trailing edge between Hb A and Hb F window (arrow) was identified in the index case who carries Hb Lansing-Ramathibodi and Hb Pakse (A), whereas sibling 1 carrying only Hb Lansing-Ramathibodi without Hb Pakse exhibits no abnormal peak (B).

bia, Lisses, France), respectively according to manufacturers' instructions. DNA was extracted from peripheral blood leukocytes using phenol-chloroform method (Loparev *et al*, 1991) and characterized for suspected mutation by PCR using allele-specific primers (Mastercycler[®] pro, Little Rock, AR) and amplicons directly sequenced in an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). The entire coding and flanking splice sites of *HBA1* and *HBA2* were sequenced to identify seven common deletional and two common non-deletional α -thalassemia 2 were performed as previously described (Ayala *et al*, 1996; Sura *et al*,

2007). Identification of Hb variant of interest in 100 unrelated individuals was conducted by PCR-based restriction fragment length polymorphism with *PstI* (New England Biolabs, Ipswich, MA).

Hb typing by HPLC and CE together with α -thalassemia DNA analysis of all cases with low SpO₂ were performed. DNA analysis for thalassemia traits indicated that the index case and one sibling are heterozygotes of Hb Pakse [α 142 Ter m \rightarrow Tyr ; TAA>TAT (*HBA2*: c.429A>T)] (data not shown). HPLC of hemolysate from a sibling without Hb Pakse revealed an abnormal peak (12.8%) at retention

time of 2.0 minutes for index case and an abnormal peak (16.5%) at retention time of 1.7 minutes (Fig 1). CE showed, in the index case, a peak with a shoulder in the trailing edge at the window of Hb F (Fig 2A) similar to that of her brother who also has Hb Pakse, while the other brother without Hb Pakse showed a normal profile (Fig 2B).

DNA sequence analysis of α -globin gene cluster indicates the presence of heterozygous c.264C>G of *HBA1* resulting in a substitution of His by Gln in codon 87 of the index case, her two brothers and a nephew (Fig 3). This mutation corresponds to Hb Lansing, where the mutation lies in

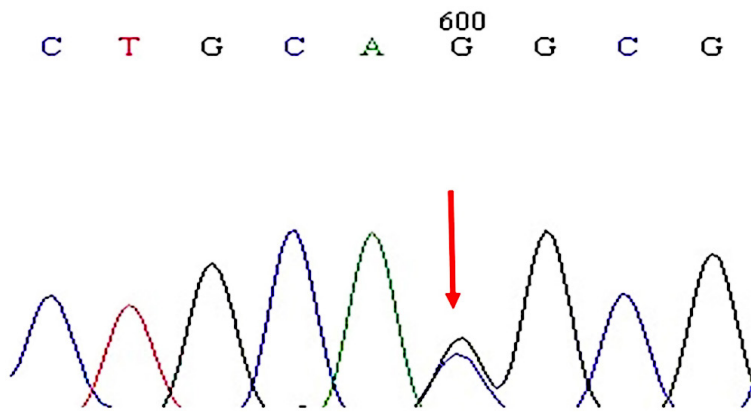


Fig 3–Nucleotide sequencing of region surrounding codon 87 of Hb Lansing-Ramathibodi. Arrow indicates presence of C and G at nucleotide 264.

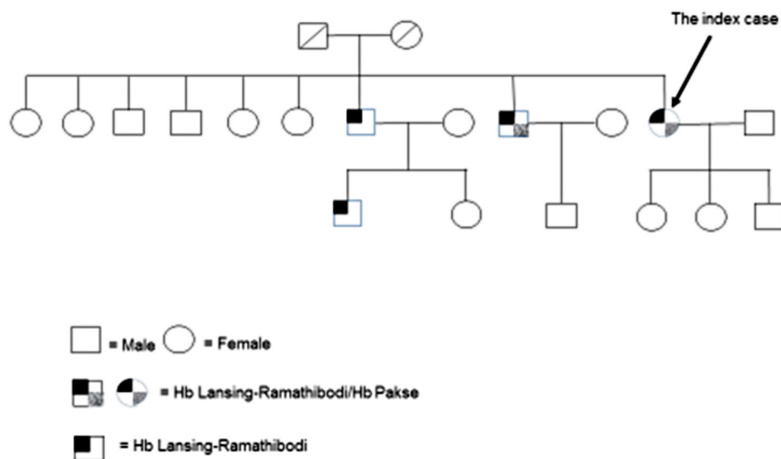


Fig 4–Pedigree of propisita carrying Hb Lansing-Ramathibodi.

HBA2. We propose to name this type of Hb variant as “Hb Lansing-Ramathibodi” [$\alpha 87(\text{F8})\text{His} \rightarrow \text{Gln}; \text{CAC} > \text{CAG}$ (*HBA1*: c.264C>G)]. The pedigree of the index case family is shown in Fig 4.

Genomic testing for Hb Lansing-Ramathibodi in 100 Thai unrelated

individuals using donated samples from Ramathibodi Hospital DNA bank failed to discover this Hb variant, indicating this variant α -globin allele (probably) is not polymorphic in the Thai population.

DISCUSSION

We report an index case together with two male siblings and a nephew presenting with low SpO₂, discordant with normal oxygenation of arterial blood gas, who are heterozygous Hb Lansing-Ramathibodi [$\alpha 87(\text{F8})\text{His} \rightarrow \text{Gln}; \text{CAC} > \text{CAG}$ (*HBA1*: c.264C>G)]. To the best of our knowledge, there has been no report of Hb Lansing in which the causative mutation lies in *HBA1*. A previous report of Hb Lansing in an Asian (Japanese), the mutation was located in *HBA2* (Ishitsuka *et al*, 2012).

Although no physiological studies of oxygen affinity and

Bohr Effect were conducted on Lansing-Ramathibodi, we surmise that it has the same properties of Hb Lansing (Sarikonda *et al*, 2009; Ishitsuka *et al*, 2012; Akar *et al*, 2014; Hassan *et al*, 2015). Interestingly, the index case and one of her sibling are compound heterozygotes

of Hb Lansing-Ramathibodi and Hb Pakse (Fig 4). Co-inheritance of Hb Pakse provides confirmatory evidence for the existence of Hb Lansing-Ramathibodi as α^{Pakse} -globin gene is located in *HBA2* (Viprakasit *et al*, 2002); therefore, Hb Pakse is present in *trans*. The other explanation to support this conjecture is that one of her siblings had only Hb Lansing-Ramathibodi without Hb Pakse. Hb Pakse is produced in very small amount and is thus considered as a non-deletional α -thalassemia 2, and carriers of deletional or non-deletional α -thalassemia 2 chromosome have no clinical symptom (Viprakasit *et al*, 2002; Fucharoen and Viprakasit, 2009). However, co-inheritance of Hb Lansing-Ramathibodi and Hb Pakse may produce mild hemolytic anemia as the index case is mildly anemic and both the index and pertinent sibling have increased reticulocyte counts. It is worth noting that the level of transcription from *HBA2* is 2-3 times higher than from *HBA1* (Galanello and Cao, 2011).

Hb variant similar to Hb Lansing-Ramathibodi is Hb Bonn [$\alpha 87 \text{ His} \rightarrow \text{Asp}$; CAC>GAC (*HBA1*: c.262C>G)] (Zur *et al*, 2008; So *et al*, 2010; Zur *et al*, 2013). Carriers of Hb Bonn show no apparent anemia but have low SpO₂.

Hb Lansing-Ramathibodi could not be detected by CE method but generated an abnormal peak on HPLC. Compound heterozygous Hb Lansing-Ramathibodi and Hb Pakse sample produced an abnormal shape peak on CE. However, without further analysis, identity of these abnormal peaks must remain speculative. The first report of Hb Lansing was demonstrated by the presence of an abnormal peak by both HPLC and CE methods (Sarikonda *et al*, 2009).

In conclusion, this is the first report of Hb Lansing in which the causative

mutation lies in *HBA1*, in contrast to all previous reports of Hb Lansing, in which the mutation is located in *HBA2*. We propose to name this Hb variant Hb Lansing-Ramathibodi. Although carriers of Hb Lansing have no apparent anemia other than the characteristic low SpO₂, co-inheritance with Hb Pakse, equivalent to a non-deletional α -thalassemia 2, produces mild anemia and low level reticulocytosis. Correct and prompt Hb identification is crucial for patients' reassurance, genetic counselling and, moreover, for avoiding unnecessary investigation and treatment to uncover the cause of spurious hypoxemia. In addition, continuing research is required for better understanding of hemoglobinopathies arising from interaction of different Hb variants, especially those highly prevalent in the Southeast Asian region, not to mention the thalassemias.

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