CORRESPONDENCE

Co-inheritance of Hb J-Kaohsiung [β 59(E3) AAG>ACG, Lys→Thr] and HbE [β 26(B8) GAG>AAG, Glu→Lys] in a Thai Woman

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To the Editor

Hemoglobin (Hb) J-Kaohsiung [β 59(E3) AAG>ACG, Lys→Thr] is the β -globin chain variant that results from a point mutation (AAG>ACG) at codon 59 of β -globin gene where the normally occurring lysine is replaced by threonine. It was found, in 1969, in the Taiwanese male resident of Kaohsiung City, Taiwan, during a population survey for hemoglobin variants [1]. The pure heterozygote of Hb J-Kaohsiung and the combination of Hb J-Kaohsiung heterozygote with α -thalassemia had been also reported in three Thai subjects [2]. We reported here for the first time the combination of Hb J-Kaohsiung heterozygote with HbE [β 26 GAG>AAG, Glu→Lys] in a 34-year-old single Thai woman. The subject was seen by a physician at Theppanya Hospital, Chiang Mai, Thailand for her annual health checkup. Upon examination, she was pale and did not have hepatosplenomegaly. She had no history of receiving a blood transfusion. The complete blood count (CBC) was analyzed by using the automated blood counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan). Laboratory findings are as follows: WBC 9.10×10^9 cells/L, RBC 4.34×10^{12} cells/L, Hb 107 g/L, PCV 0.34 L/L, MCV 78 fL, MCH 24.6 pg, MCHC 31.5 g/L, RDW 13.7% and platelet 196×10^9 /L. Thus, she was diagnosed as having mild anemia with the red blood cell microcytosis. The

blood sample was also sent to the Associated Medical Sciences–Clinical Service Center (AMS–CSC), Chiang Mai University, Chiang Mai, Thailand for the thalassemia diagnosis. The hemoglobin analysis was performed by using the high-performance liquid chromatography (HPLC, VARIANT II, β -thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). There was no peak of HbA on the HPLC chromatogram while her HbF and HbA₂/E were 2.5 and 25.9%, respectively. In addition, the abnormal Hb peak with a value of 65.3% of the total Hb was observed at the retention time of 2.06 min (Fig. 1a). The hemoglobin analysis was also performed by using the capillary electrophoresis method (CE, Capillarys™ 2 Flex Piercing, Sebia, Norcross, Georgia, USA). Her HbE and HbA₂ were 22.7 and 3.0 respectively. In addition, the abnormal Hb peak with the value of 74.3% of total Hb was also presented at a migration position of 50–80 s (Zone 13–14) (Fig. 1b). The real-time PCR with SYBR Green1 and high resolution melting (HRM) analysis for detection of the α -thalassemia-1 Southeast Asian (SEA) and Thai type deletions [3] is routinely performed at the same time the hemoglobin analysis is carried out. Furthermore, the multiplex Gap-PCR for detection of α -thalassemia-2 ($-\alpha^{3.7}$ and $-\alpha^{4.2}$ kb deletions) and the multiplex allele specific PCR for diagnosis of Hb Constant Spring (Hb CS) and Hb Pakse' [4, 5] were also performed in this case. The negative analysis results for α -thalassemia-1 and -2 deletions and Hb CS and Hb Pakse' mutations were observed. In order to identify the abnormal Hb, the PCR amplification of the β -globin gene was accomplished according to conditions described previously [5] and then the direct DNA sequencing of the amplified product was performed on an ABI PRISM™ 3130 XL analyzer (Applied Biosystems, Foster City, CA, USA). The results showed the molecular defect causing abnormal hemoglobin resulting in A–C

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Fig. 1 The HPLC chromatogram (a) and CE electrophoregram (b) of the subject

transversion at the second position of codon 59 (AAG>ACG) of β -globin gene (data not shown) that leads to a substitution of threonine for lysine as described previously for Hb J-Kaohsiung [1]. In addition, DNA sequencing revealed also a GAG>AAG transition at codon 26 of the β -globin gene (data not shown) that leads to substitution of lysine for glutamic acid as described previously for HbE. Thus, the subject was finally diagnosed as co-inheritance of Hb J-Kaohsiung and HbE trait and she refused to have parental hematologic and genetic studies.

Hb J-Kaohsiung has more negative charge than HbA thus its peak was presented ahead of HbA with the retention time of 2.06 min on HPLC chromatogram (Fig. 1a) and the migration position of 50–80 s (Zone 13–14) on CE electrophoregram (Fig. 1b). The level of Hb J-Kaohsiung was higher than that of HbE. Thus, it might be explained by

the fact that the gene expression of Hb J-Kaohsiung is higher than those of HbE. In addition, the normal α -globin chain is more likely to bind to the $\beta^{\text{J-Kaohsiung}}$ -globin chain than the β^{E} -globin chain. The heterozygote of Hb J-Kaohsiung is clinically and hematologically normal [2] whereas the co-inheritance of Hb J-Kaohsiung with HbE trait leads to mild anemia as shown in the present study. However, the study is limited by the inability to do the family study.

In conclusion, the co-inheritance of Hb J-Kaohsiung and HbE trait can be found in the Thai population. Therefore, the better understanding of HPLC chromatogram and/or CE electrophoregram patterns and clinical features of Hb J-Kaohsiung and its combination is useful for genetic counseling, prevention and control programs for thalassemia and hemoglobinopathy.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest.

Informed consent This study was approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. The informed consent was obtained from a subject included in the study.

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