CASE REPORT

Detection of Co-inheritance of Hb Hope and Hb Constant Spring in Three Thai Samples by Capillary Electrophoresis

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Abstract The diagnosis of co-inheritance of Hb Hope [β 136(H14)Gly $\rightarrow$ Asp, GGT $>$ GAT] and Hb constant spring [Hb CS; α 142, Term $\rightarrow$ Gln (TAA $>$ CAA IN α 2)] by high performance liquid chromatography (HPLC) is difficult because Hb Hope has a HPLC elution pattern similar to that of Hb Pyrgos, Hb New York, Hb Kodaira, and Hb Phimai. Moreover, the Hb CS mRNA, as well as the gene product, are unstable and present at a low level in peripheral blood. We report the use of a capillary electrophoresis (CE) for diagnosis of co-inheritance of Hb Hope and Hb CS in 3 Thai females who had mild anemia with Hb and Hct varying from 91–114 g/L to 0.28–0.36 L/L, respectively. Hb Hope eluted with a retention time of 125–140 s (Zone 10) of CE electrophoregram. Furthermore, the peak of Hb CS at the retention time of 245–250 s (Zone 2) was observed in these samples. In addition, the manual analysis by taking the non-black area under both peaks of HbA and Hb Hope (inverted V) into account provided the corrected Hb CS levels which are useful in screening of heterozygote or homozygote for Hb CS. Thus, the CE method provides an accurate diagnosis of Hb Hope and Hb CS which is useful in genetic counseling, prevention and control programs for these hemoglobinopathies.

Keywords Co-inheritance · Capillary electrophoresis · Hb constant spring · Hb Hope · High performance liquid chromatography

Introduction

More than 220 mutation variants of β -thalassemia and more than 100 genetic forms of α -thalassemia are reported in the world with many community/region-specific mutations [1, 2]. Barring a few conditions like methemoglobinemic or hyperunstable hemoglobin (Hb) variants that can be symptomatic even in the heterozygous state; the most heterozygotes for hemoglobinopathies are clinically and hematologically normal. However, the homozygous form or co-inheritance together with other thalassemia and hemoglobinopathies can result in severe disease. Therefore, understanding of a prevalence of hemoglobinopathy and its genetic diversity is essential to define policies aimed at reducing the long-term health burden of hemoglobinopathy, allowing for precise diagnosis, and providing adequate genetic counseling [2]. Hb Hope [β 136 (H14) Gly $\rightarrow$ Asp, GGT $>$ GAT] is another unstable hemoglobin variant of the β -globin chain which is frequently found in the Thai population [3]. The co-inheritance of Hb Hope with α -thalassemia, β -thalassemia or Hb E [β 26 (B8) Glu $\rightarrow$ Lys, GAG $>$ AAG] in Thai patients had been reported previously [4–6].

The capillary electrophoresis (CE) has been developed for Hb fraction separation and quantification of Hb variants. This system could clearly demonstrate the presence of hemoglobinopathies. Whether the CE, which has proven superior to the HPLC in the detection of Hb constant spring [Hb CS; α 142, Term $\rightarrow$ Gln (TAA $>$ CAA IN α 2)] trait [7], could diagnose the co-inheritance of Hb Hope and Hb

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CS is unclear. In this study, we reported the CE electrophoregram of the samples with co-inheritance of Hb Hope and Hb CS. A detailed knowledge is essential for providing an accurate diagnosis, genetic counseling, prevention and control programs of these hemoglobinopathies.

Materials and Methods

Cases and Hematological Analysis

The blood samples were collected from 3 Thai females at the Associated Medical Sciences Clinical Service Center (AMS-CSC), Chiang Mai University, Chiang Mai, Thailand for their annual checkup. Hematological parameters including, red blood cell counts (RBCs), Hb, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were measured using an automated blood counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan). Quantification of HbA₂ (for detection of β -thalassemia) and identification of hemoglobinopathies were performed using a high performance liquid chromatography (HPLC, VARIANT II, β -thalassemia Short Program, Bio-Rad Laboratories, Hercules, California, USA). The hemoglobin analysis by using capillary electrophoresis (CE, CapillarysTM 2 Flex Piercing, Sebia, Norcross, Georgia, USA) was also performed in these samples.

Molecular Analysis for Diagnosis of Thalassemia and Hemoglobinopathy

The genomic DNA was extracted from the blood sample by using the NucleoSpin[®] kit (Macherey–Nagel, KG., Duren, Germany) according to manufacturers' instructions. The DNA was stored at -20°C until used. The α -thalassemia-1 SEA and Thai type deletions were detected by using real-time PCR with SYBR Green1 high resolution melting (HRM) analysis as previously described [8]. Moreover, the molecular confirmation tests for Hb Hope and Hb CS were performed by using the amplification refractory mutation system (ARMS)-PCR analysis as reported elsewhere [6, 9].

Results

Based on ARMS-PCR analysis, 2 samples were diagnosed as double heterozygote for Hb Hope and Hb CS trait because the 333 bp amplified fragment from Hb Hope allele (Fig. 1) and the 180 bp amplified fragments from both Hb CS and wide type alleles (Fig. 2) were found in these cases. The other sample was diagnosed as co-

Fig. 1 Amplification refractory mutation system (ARMS) for identifying of Hb Hope. The amplified fragments were separated by 2.0 % agarose gel electrophoresis and visualized under UV-light after ethidium bromide staining. M represents λ /Hind III size markers. The 578 bp control fragment and the 333 bp amplified fragment from Hb Hope allele are indicated

inheritance of Hb Hope and homozygote for Hb CS because the 333 bp amplified fragments from Hb Hope allele (Fig. 1) and the 180 bp amplified fragments from only Hb CS allele but not from wide type allele (Fig. 2) were observed. Their characteristics and hematological parameters are shown in Table 1. The mean age of three cases was 32.7 years (29–39 year). All samples had mild anemia with Hb and Hct varied from 91–114 g/L to 0.28–0.36 L/L, respectively. The examples of HPLC chromatograms and CE electrophoregrams of these samples are shown in Fig. 3. On HPLC chromatogram, the peak of Hb Hope was presented at the retention time of 1.44–1.49 min (P2-window) (Fig. 3a, d) which was similar to that of Hb Pyrgos, Hb New York, Hb Kodaira, Hb J-Bangkok, and Hb Phimai. The HPLC chromatogram of Hb CS revealed at least one peak at the retention time of 4.76, 5.03 or 5.25 min and these peaks were easily seen in the subject with homozygote for Hb CS (Fig. 3d) while they were difficult to visually evaluate in the subject with heterozygote for Hb CS (Fig. 3a). On CE electrophoregram, the peak of Hb Hope was presented at the retention time of 125–140 s (Zone 10) and the area of mixing HbA and Hb Hope (inverted V) was also observed (Fig. 3b, e). Furthermore, the peak of Hb CS at the retention time of

Fig. 2 Amplification refractory mutation system (ARMS) for identifying of heterozygote and homozygote for Hb CS. The amplified fragments were separated by 2.0 % agarose gel electrophoresis and visualized under UV-light after ethidium bromide staining. M represents 100 bp DNA ladder. The 391 bp control fragment, 180 bp amplified fragment from wide type allele (*lanes 1, 3, 5, 7, 9 and 11*) and 180 bp amplified fragment from Hb CS allele (*lanes 2, 4, 6, 8, 10 and 12*) are indicated

245–250 s (Zone 2) was observed in all samples and the Hb CS levels of homozygote for Hb CS was higher than that of heterozygote for Hb CS (Table 1; Fig. 3b, e). The elevated HbA₂ and Hb CS were manually corrected by taking the non-black area under both peaks of HbA and Hb Hope (inverted V, the area of mixing HbA and Hb Hope) into account of the analysis system. The HbA₂ and Hb CS levels were decreased from 5.5 to 3.5 % and 4.5 to 2.9 %, respectively in samples with homozygote for Hb CS. In 2 samples with heterozygote for Hb CS, the HbA₂ levels were decreased from 4.0 to 2.7 % and 5.3 to 3.5 % while the Hb CS levels were decreased from 0.5 to 0.3 % and 0.8 to 0.5 % (Table 1; Fig. 3c, f).

Discussion

Since the prevalence of Hb Hope and Hb CS are high in the Thai population, chances of finding a person who co-inherits these two hemoglobinopathies are high. The heterozygote for Hb Hope and Hb CS are clinically and hematologically normal. In current study, all samples had Hb and Hct levels lower than the normal values (Table 1) thus the co-inheritance of these hemoglobinopathies might be a cause of anemia. The differential diagnosis between Hb Hope, Hb Pyrgos, Hb New York,

Hb Kodaira, Hb J-Bangkok, and Hb Phimai is difficult because these hemoglobinopathies are β -globin chain variants which have similar HPLC elution pattern [10]. However, they can be classified by the CE system. Although Hb Hope has the CE elution pattern which is quite similar to that of Hb Phimai [10], Hb Hope, but not for Hb Phimai, has the area of mixing with HbA. Hb CS is often missed by routine laboratory testing, especially in the heterozygote for Hb CS because its mRNA as well as gene product are unstable and presented at a low level in peripheral blood [11]. In current study, Hb CS levels could be measured in all samples. Whereas the tiny peaks of Hb CS of sample with heterozygote for Hb CS are difficult to visually evaluate on the HPLC chromatogram. Consistency with our previous study showed that the CE is proved useful for screening of Hb CS in samples with β -thalassemia trait or Hb E trait where the expression of β -globin chain is reduced [12]. The manual analysis by taking the non-black area under both peaks of HbA and Hb Hope (inverted V) into account provided the corrected levels of both HbA₂ and Hb CS. The corrected Hb CS levels could be used for screening of heterozygote or homozygote for Hb CS. Consistency with the previous study indicated that the level of Hb CS quantified by CE proved useful in screening of heterozygote and homozygote for Hb CS [9].

Table 1 Characteristics and hematological parameters of the subjects

Characteristics and parameters	No. 1	No. 2	No. 3
Gender	Female	Female	Female
Age (years)	39	29	30
Red blood cell counts ($10^{12}/L$)	4.4	3.3	4.3
Hemoglobin (g/L)	114	91	106
Hematocrit (L/L)	0.36	0.28	0.35
MCV (fL)	82	85	82
MCH (pg)	26.0	27.5	24.8
MCHC (g/L)	367	327	302
RDW	15.0	15.4	16.0
<i>Hb analysis by HPLC</i>			
Hb Hope (%)	41.9	42.4	36.9
HbA (%)	49.8	52.3	53.0
HbA ₂ (%)	2.6	3.6	2.0
HbF (%)	0.8	0.4	3.0
<i>Hb analysis by CE: before correction/after correction</i>			
Hb Hope/Hb Hope + HbA + common non-black area (%)	39.5/96.4	36.2/95.7	30.3/90.9
HbA (%)	55.1	57.3	54.8
HbA ₂ (%)	4.0/2.7	5.3/3.5	5.5/3.5
HbF (%)	0.9/0.6	0.4/0.3	4.2/2.7
Hb CS (%)	0.5/0.3	0.8/0.5	4.5/2.9
β -globin genotype	$\beta/\beta^{\text{hope}}$	$\beta/\beta^{\text{hope}}$	$\beta/\beta^{\text{hope}}$
α -globin genotype	$\alpha\alpha/\alpha^{\text{CS}}\alpha$	$\alpha\alpha/\alpha^{\text{CS}}\alpha$	$\alpha^{\text{CS}}\alpha/\alpha^{\text{CS}}\alpha$

Normal ranges for the measurements elaborated in the table are as follows: Red blood cell counts $4.2\text{--}6.1 \times 10^{12}/L$; hemoglobin 120–180 g/L; hematocrit 0.37–0.52 L/L; MCV 80–100 fL; MCH 27–31 pg; MCHC 320–360 g/L; RDW 11.5–14.5 %; HbA > 85 %; HbA₂ 1.5–3.5 %; HbF 0–1 %

Fig. 3 Representatives of the HPLC chromatogram (a, d) and CE electrophoregram before correction (b, e) and after correction (c, f)

In conclusion, the co-inheritance of Hb Hope and heterozygote or homozygote for Hb CS can be found in the Thai population. The CE method has proved useful in diagnosis of these hemoglobinopathies. The corrected Hb CS levels could be also used for screening of heterozygote or homozygote for Hb CS. Thus, this method provides an accurate diagnosis which is useful in genetic counseling, prevention and control programs for these hemoglobinopathies.

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Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest.

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