

25A7601071

→R

Patient Data

Sample ID: 1K2754558  
Patient ID:  
Name:  
Physician:  
Sex:  
DOB:  
Comments:

Analysis Data

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Run Number: 4  
Rack ID: 0001  
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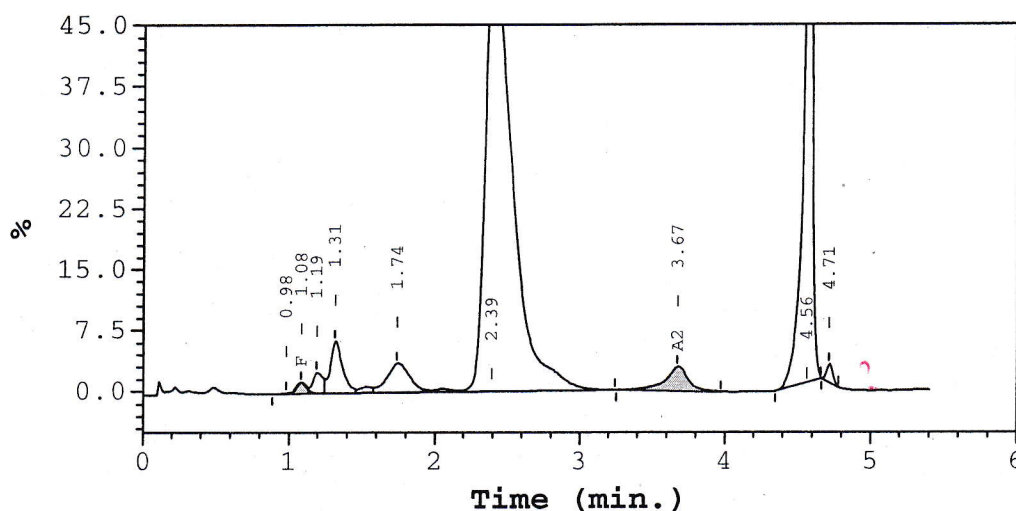
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P2	---	3.3	1.31	81182
P3	---	3.4	1.74	85799
Ao	---	61.5	2.39	1532421
A2	3.0	---	3.67	74106
S-window	---	26.5	4.56	661116
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Total Area: 2,493,594

F Concentration = 0.6 %

A2 Concentration = 3.0 %

Analysis comments:

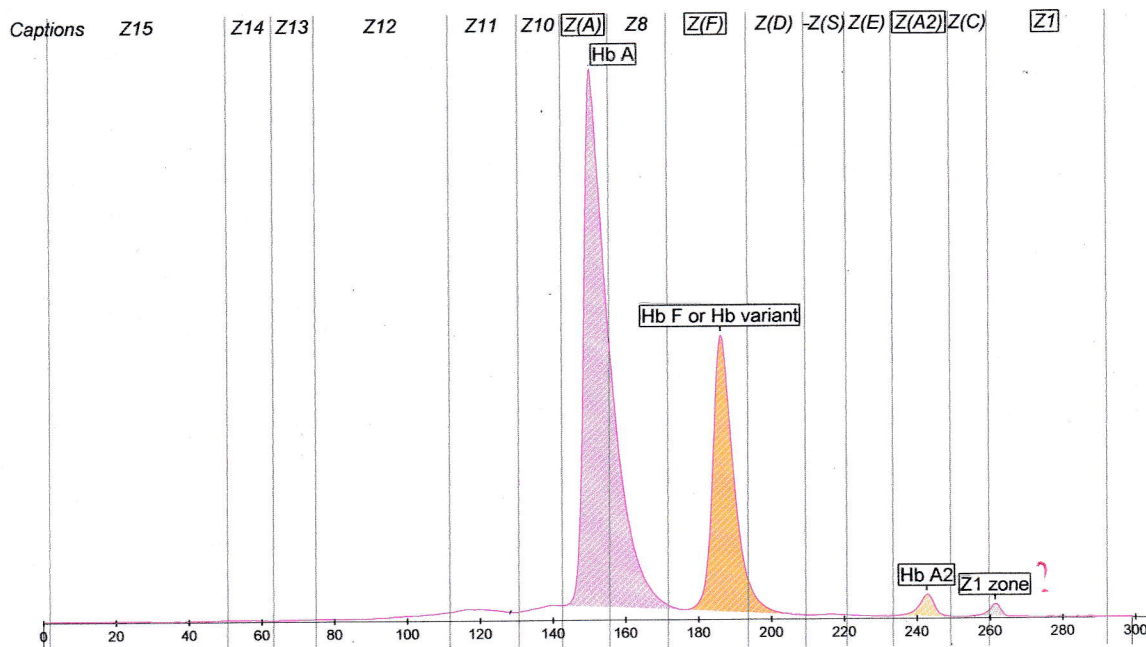
~~HB Hasharon?~~

Sample # : **11** Date : **11/20/2025**

ID : **1K2754558**

Depart. :

Birth. :



## Haemoglobin Electrophoresis

Name	%	Normal Values %
Hb A	68.4 /	
Hb F or Hb variant	29.3 /	
Hb A2	1.7 /	
Z1 zone	0.6 /	

*Hb Hasharon?*

Signature

MD

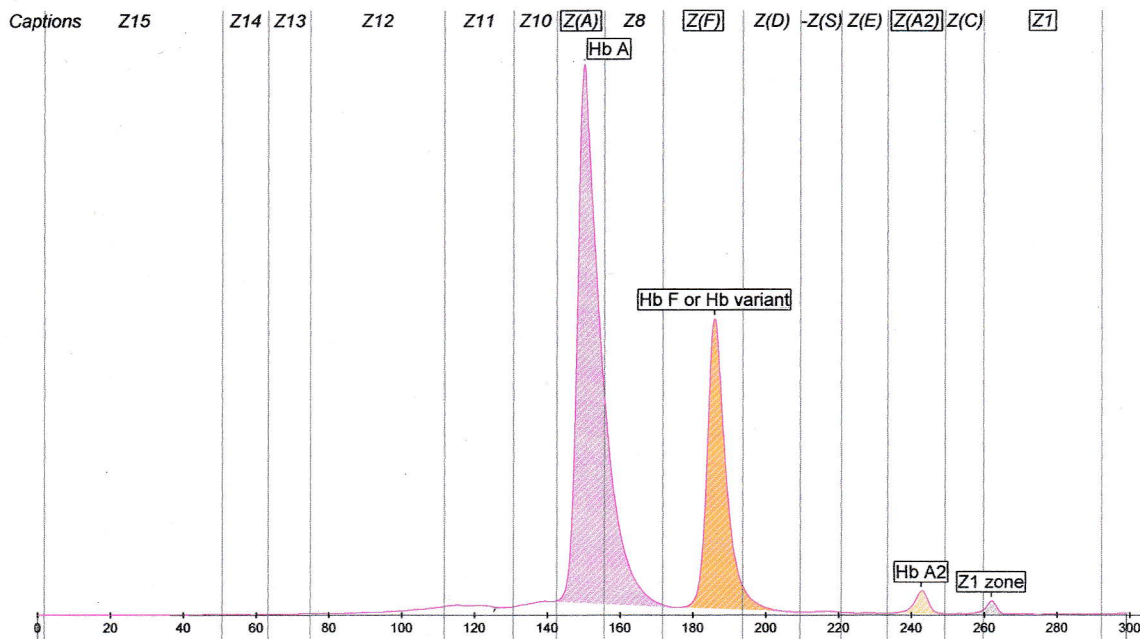
Sample # : 33 Date : 11/20/2025

*manual dilution*

ID : 1K2754558

Depart. :

Birth. :

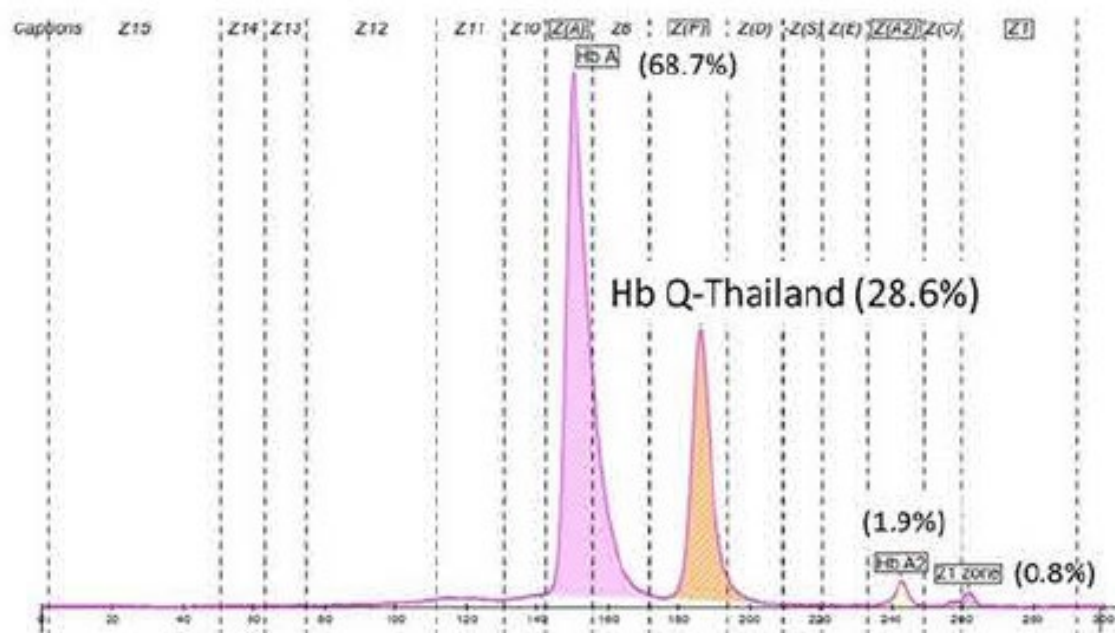


## Haemoglobin Electrophoresis

Name	%	Normal Values %
Hb A	68.2 /	
Hb F or Hb variant	29.4 /	
Hb A2	1.7 /	
Z1 zone	0.7 /	

Signature

## Suspect HbQ-Thailand



z(F): HbQ-Thailand ( $\alpha^0_2\beta_2$ )

z1: HbQA2 ( $\alpha^0_2\delta_2$ )



OPEN

# Molecular epidemiology and hematological profiles of hemoglobin variants in southern Thailand

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Data on hemoglobin (Hb) variants in southern Thailand are lacking. This study aimed to reassess the frequency of Hb variants and the clinical aspects of compound heterozygous Hb variant with other hemoglobinopathies. We enrolled 13,391 participants from ten provinces in southern Thailand during 2015–2022. Hb analysis was performed using capillary electrophoresis, and mutations in the *HBA* and *HBB* genes were identified using PCR or DNA sequencing. Hb variants were identified in 337 (2.5%) unrelated subjects. Nine  $\beta$ -chain variants, namely Hb Malay (76.9%), Hb C (10.1%), Hb D-Punjab (2.9%), Hb G-Makassar (2.3%), Hb Dhonburi (2.3%), Hb Tak (1.4%), Hb J-Bangkok (1.4%), Hb New York (0.3%), and Hb Hope (0.3%), and four  $\alpha$ -chain variants—Hb G-Georgia (*HBA1*) (0.9%), Hb G-Georgia (*HBA2*) (0.3%), Hb Q-Thailand (0.6%), and Hb St. Luke's-Thailand (0.3%)—were identified. The southern population exhibited a distinct spectrum of Hb variants compared to that observed in the populations from other areas. Several compound heterozygous genotypes were also identified. Combining Hb Malay with Hb E or high Hb F determinants did not require a blood transfusion. This study provides essential information for genetic counseling in thalassemia prevention and control programs in this region.

Hemoglobin (Hb) variants, or abnormal Hb, are hemoglobinopathies resulting from an abnormal structure of the globin chain in the hemoglobin molecule. Several Hb variants, such as Hb E (*HBB*:c.79G>A) and Hb Malay (*HBB*:c.59A>G), termed as “thalassemic Hb variants” can lead to reduced Hb variant levels. In Thailand, over 30 types of Hb variants have been reported, with a prevalence rate of 2.4%. However, Hb variants exhibit variations across populations and countries<sup>1</sup>. The interaction between Hb variants and thalassemia typically manifests as either no or mild clinical phenotypes<sup>2,3</sup>. Nevertheless, this co-inheritance may result in the misinterpretation of Hb analysis within thalassemia prevention and control programs. For example, certain Hb variants co-migrate within the Hb F zone of capillary electrophoresis (CE), leading to potential misdiagnosis as  $\beta$ -thalassemia disease or high Hb F determinants until molecular diagnosis confirms the specific mutation type<sup>4</sup>. In addition, the interaction of thalassemic Hb variants with thalassemia can contribute to moderate to severe thalassemia phenotypes, as seen in Hb H with Hb Constant Spring (CS, *HBA2*:c.427T>C) disease and Hb E/ $\beta$ -thalassemia disease, which are commonly observed in the southeast Asian population<sup>5,6</sup>. Eight Hb variants in 58 southern populations were previously identified using high-performance liquid chromatography (HPLC) and DNA sequencing<sup>7</sup>. However, the report did not include Hb Malay, a common Hb variant in southern Thailand and lacked hematological profiles of the combination of Hb Malay with other Hb variants. Up-to-date CE technique is an Hb analysis routinely performed in most laboratories in Thailand. Our center started using this method in 2015. Moreover, there is limited information on the spectrum, prevalence, and clinical phenotypes of Hb variants when co-inherited with other abnormalities in southern Thailand using the CE method. Therefore, this study aimed to reassess the molecular epidemiology of Hb variants and the clinical phenotypes of patients with co-inherited Hb variants and other thalassemia or hemoglobinopathies in the southern population.

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Results  
Genotypic and phenotypic spectra of Hb variants in the southern Thai population

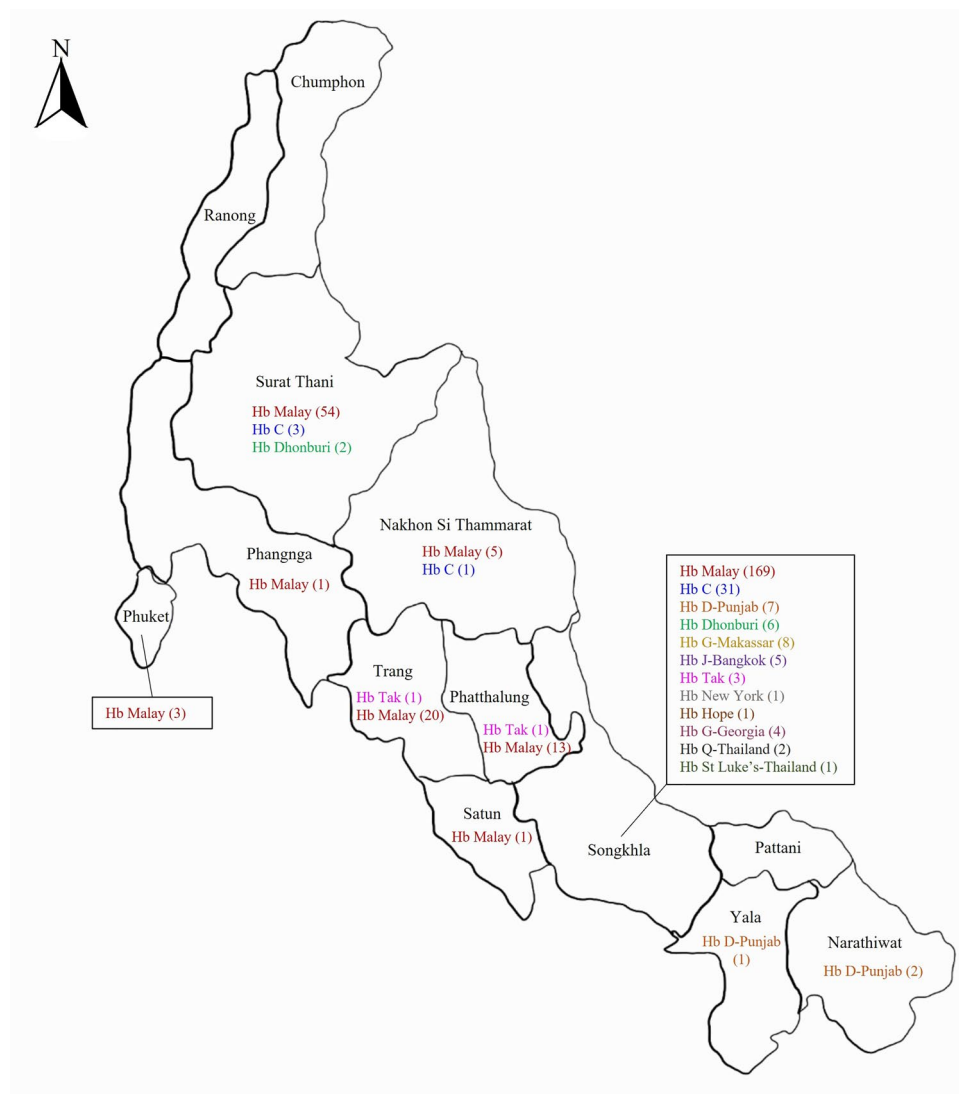
Our reference center received 13,391 samples for thalassemia and hemoglobinopathy diagnoses from 10 provinces in southern Thailand, namely Songkhla, Surat Thani, Nakhon Si Thammarat, Phatthalung, Trang, Phuket, Phangnga, Satun, Yala, and Narathiwat, spanning the period from 2015 to 2022. Routine molecular diagnosis has revealed Hb Malay and Hb Dhonburi mutations and DNA sequencing has revealed 11 distinct Hb variant mutations in 337 (2.5%) unrelated participants, corresponding to 346 chromosomes. The  $\beta$ -chain variant, with nine different mutations, was identified in 338 alleles, and the two most common mutations were Hb Malay ( $n = 266$ , 76.9%) and Hb C ( $HBB:c.19G>A$ ) ( $n = 35$ , 10.1%). The remaining seven mutations were Hb D-Punjab ( $HBB:c.364G>C$ ) ( $n = 10$ , 2.9%), Hb G-Makassar ( $HBB:c.20A>C$ ) ( $n = 8$ , 2.3%), Hb Dhonburi ( $HBB:c.380T>G$ ) ( $n = 8$ , 2.3%), Hb Tak ( $HBB:c.441_442insAC$ ) ( $n = 5$ , 1.4%), Hb J-Bangkok ( $HBB:c.170G>A$ ) ( $n = 5$ , 1.4%), Hb New York ( $HBB:c.341T>A$ ) ( $n = 1$ , 0.3%), and Hb Hope ( $HBB:c.410G>A$ ) ( $n = 1$ , 0.3%). In addition, four  $\alpha$ -chain variants, namely Hb G-Georgia ( $HBA1$ ) ( $HBA1:c.287C>T$ ) ( $n = 3$ , 0.9%), Hb G-Georgia ( $HBA2$ ) ( $HBA2:c.287C>T$ ) ( $n = 1$ , 0.3%), Hb Q-Thailand ( $HBA1:c.223G>C$ ) ( $n = 2$ , 0.6%), and Hb St. Luke's-Thailand ( $HBA2:c.287C>G$ ) ( $n = 1$ , 0.3%), were identified in seven alleles (Table 1). The distribution of Hb variants among the southern population from ten provinces is shown in Fig. 1. The hematological parameters of 263 (78.0%) subjects with heterozygous Hb variant genotypes are shown in Table 2. Hb analysis showed that Hb Malay and Hb Dhonburi migrated along Hb A (zone 9). Furthermore, three Hb variants—HbTak, Hb Q-Thailand, and Hb G-Georgia—migrated to the Hb F zone (zone 7). Herein, we report Hb G-Georgia ( $HBA1$ ) for the first time in Thailand (Fig. 2). Hb G-Georgia ( $HBA1$ ) exhibited lower levels than Hb G-Georgia ( $HBA2$ ), at 10.3–10.6% vs. 17.0%. Two Hb variants, Hb D-Punjab and Hb St. Luke's-Thailand, were migrated in zone 6. Notably, the levels of Hb D-Punjab in a case with compound heterozygous  $\alpha^0$ -thalassemia/ $\alpha^+$ -thalassemia ( $--/-\alpha$ ) were lower than those in Hb D-Punjab carriers with the normal  $HBA$  gene ( $\alpha\alpha/\alpha\alpha$ ) or heterozygous  $\alpha^+$ -thalassemia ( $-\alpha/\alpha\alpha$ ), at 23.6% vs. 32.6–39.3%. Moreover, Hb G-Makassar migrated to the Hb S ( $HBB:c.20A>T$ ) zone (zone 5). Hb C, Hb Hope, Hb New York, and Hb J-Bangkok were detected in zones 2, 10, 11, and 12, respectively (Fig. 3).

Hematological profiles of patients with homozygous or compound heterozygous Hb variants

The hematological parameters of 61 (18.1%) patients with homozygous or compound heterozygous Hb variant genotypes without blood transfusion are shown in Table 3. The results showed that eight patients with homozygous Hb Malay and 27 patients with compound heterozygous Hb Malay with Hb E displayed mild-to-moderate anemia without a history of blood transfusion. Two patients with Hb Malay and  $\beta^+$ -thalassemia (NT-28 ( $A>G$ ),  $HBB:c.-78A>G$ ) exhibited nontransfusion-dependent thalassemia and moderate anemia. In addition, five cases of compound heterozygous Hb Malay with high Hb F determinant mutations, such as HPFH6 (NG\_000007.3:g.45595\_124872del),  $\delta\beta^0$ -thalassemia (12.5 kb deletion) (NG\_000007.3:g.64383\_76994del), Indian del-inv  $\gamma\delta\beta^0$ -thalassemia (NG\_000007.3:g.48400\_49245del;49246\_64567inv;64568\_72051del), and Thai del-inv-ins  $\gamma\delta\beta^0$ -thalassemia (NG\_000007.3:g.47449\_165744del;168412\_168590invins;insAAGAAGA), along with one patient with compound heterozygous Hb Malay with  $\beta^0$ -thalassemia (3.5 kb deletion, NC\_000011.10:g.5224302-5227791del3490bp), exhibited a non-transfusion-dependent thalassemia phenotype. Among nine patients with compound heterozygous Hb C with other hemoglobinopathies, all were asymptomatic or presented mild anemia. This group included six patients with Hb C/Hb E, one patient with Hb C/Hb Malay, and two patients with Hb C/ $\beta^0$ -thalassemia (3.5 kb deletion and codon 41 ( $-C$ ), ( $HBB:c.126delC$ )). Interestingly, a patient with compound heterozygous Hb C and  $\beta^0$ -thalassemia (3.5 kb deletion) displayed significantly elevated Hb A<sub>2</sub> (7.6%) and Hb

Hemoglobin variant	No.	(%)
$\beta$ -chain variants (9 mutations)		
Hb Malay	266	(76.9)
Hb C	35	(10.1)
Hb D-Punjab	10	(2.9)
Hb Dhonburi	8	(2.3)
Hb G-Makassar	8	(2.3)
Hb J-Bangkok	5	(1.4)
Hb Tak	5	(1.4)
Hb New York	1	(0.3)
Hb Hope	1	(0.3)
$\alpha$ -chain variants (4 mutations)		
Hb G-Georgia ( <i>HBA1</i> )	3	(0.9)
Hb G-Georgia ( <i>HBA2</i> )	1	(0.3)
Hb Q-Thailand	2	(0.6)
Hb St. Luke's-Thailand	1	(0.3)

**Table 1.** Mutation frequencies of hemoglobin (Hb) variants in a total of 346 chromosomes among 337 participants from each province of southern Thailand during 2015 to 2022. The number represents the allelic count of Hb variants.



**Figure 1.** Distribution of Hb variants among 346 chromosomes of 337 participants across ten provinces of southern Thailand. The number represents the allelic count of Hb variants.

F (11.6%) levels. In contrast, a patient with compound heterozygous Hb C and  $\beta^0$ -thalassemia (codon 41 (– C)) exhibited Hb A<sub>2</sub> (3.7%) without detectable Hb F levels. This study reports several Hb variants co-inherited with Hb E in the southern population, including two cases of Hb D-Punjab/Hb E, two cases of Hb G-Makassar/Hb E, one case of Hb J-Bangkok/Hb E, and one case of Hb Hope/Hb E, all of which showed no clinical symptoms. Additionally, two patients with compound heterozygous Hb J-Bangkok with  $\beta^+$ -thalassemia [IVS1-5 (G>C), (HBB:c.126\_129delCTTT), IVS1-1 (G>T) (HBB:c.92 + 1G>T), IVS1-5 (G>C), and IVS2-654 (C>T) (HBB:c.316-197C>T)], were identified as transfusion-dependent thalassemia cases who need regular blood transfusion to manage their clinical complications and survival. The frequency of blood transfusion, clinical history, and hematological profiles in these patients is shown in Table 4.

Furthermore, 13 patients with compound heterozygous Hb Malay, harboring various  $\beta$ -thalassemia point mutations [e.g., NT-28 (A>G), codon 17 (A>T) (HBB:c.52A>T), codon 41 (– C), codons 41/42 (– TTCT) (HBB:c.126\_129delCTTT), IVS1-1 (G>T) (HBB:c.92 + 1G>T), IVS1-5 (G>C), and IVS2-654 (C>T) (HBB:c.316-197C>T)], were identified as transfusion-dependent thalassemia cases who need regular blood transfusion to manage their clinical complications and survival. The frequency of blood transfusion, clinical history, and hematological profiles in these patients is shown in Table 4.

Table 5 displays a comparative analysis of the spectrum of Hb variants in the population of southern Thailand and the populations from other areas. The findings indicate distinct prevalence patterns of Hb variants across various parts of the country. Hb Malay and Hb C were the predominant variants in the southern population, whereas Hb Hope, Hb Q-Thailand, and Hb J-Bangkok were frequently found in populations from the northern and central regions. The northeastern population presented the prevalence of four common mutations—Hb

Hemoglobin variants	No. of cases	Gender (n)	Age range (years)	α or β genotype	Hematological parameter					Hemoglobin analysis				
					Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	RDW (%)	Hb A <sub>2</sub> (%)	Hb F (%)	Hb variant (%)	Hb variant zone	Hb pattern
β-chain variants														
Hb Malay	178	F (84)	17–43	αα/αα	11.0±0.9	34.3±3.5	71.5±4.0	23.1±1.5	15.4±1.1	4.5±0.4	0.1±0.7		9	A <sub>2</sub> A <sup>a</sup>
		M (94)	16–60		13.4±1.1	41.9±3.6	71.7±3.5	23.0±1.5	15.4±1.9	4.5±0.4	0.1±0.6			
	20	F (10)	17–35	−α/αα	11.3±1.3	35.0±4.2	75.0±2.5	24.3±0.5	14.9±0.8	4.6±0.4	0			
		M (10)	18–41		13.7±0.6	41.9±1.6	76.3±2.5	24.7±0.7	14.6±1.0	4.3±0.3	0			
	3	F (1)	22	−−/αα	9.9	31.1	70	22.3	16.9	4.6	0			
		M (2)	21, 27		13.5, 11.5	39.3, 37.3	64.0, 70.0	21.8, 21.6	19.0, 15.4	4.3, 4.3	0			
Hb C	25	F (17)	3–75	αα/αα	11.8±0.9	33.7±4.0	78.2±6.7	27.4±1.9	14.0±0.9	3.5±0.2	0.9±1.2	34.1±1.6	2	A <sub>2</sub> A with Hb Var
		M (8)	1–65		14.5±1.5	41.0±5.0	79.0±5.5	28.2±2.2	13.6±1.1	3.4±0.3	2.0±2.5	34.2±1.3		
	1	F	33	−α/αα	11.1	35.1	65.5	20.7	NA	3.1	0	31.1		
Hb Dhonburi	8	F (4)	18–39	αα/αα	10.4±1.4	32.3±4.3	72.8±4.2	22.9±1.2	16.3±1.9	4.2±0.4	0		9	A <sub>2</sub> A <sup>a</sup>
		M (4)	22–36		13.7±0.9	42.0±3.4	76.5±0.6	25.0±0.4	13.2±0.6	4.1±0.3	0			
Hb D-Punjab	5	F (1)	26	αα/αα	11.0	34.0	81.1	26.3	12.4	3.2		39.3	6	A <sub>2</sub> A with Hb Var
		M (4)	15–55		11.1±2.3	34.1±4.8	68.5±13.3	22.6±5.8	15.8±3.5	2.9±0.3		35.2±2.6		
	1	F	55	−α/αα	9.5	26.8	76.0	26.8	14.6	3.1		36.6		
	1	M	34	α <sup>CS</sup> α/αα	13.2	39.3	78.0	26.3	12.8	3.0		37.7, (CS=0.5)	6, 2	A <sub>2</sub> ACS with Hb Var
	1	M	23	−−/−α	NA	NA	NA	NA	NA	3.2		23.6	6	A <sub>2</sub> A with Hb Var
Hb G-Makassar	5	F (2)	22, 56	αα/αα	12.1, 13.3	34.6, 37.3	76.0, 78.0	26.5, 27.7	13.8, 13.6	3.1, 3.3		43.5, 42.2	5	A <sub>2</sub> A with Hb Var
		M (3)	15–30		14.8±0.3	43.7±2.1	76.7±2.1	27.2±1.6	13.4±1.2	3.4±1.4		41.5±0.9		
	1	F	22	−α/αα	10.8	34.5	63.0	19.7	NA	2.3		39.1		
Hb Tak	4	F (1)	35	αα/αα	11.7	35.2	83.0	27.7	18.2	3.5	31.4		7	A <sub>2</sub> F <sup>b</sup> A
		M (3)	22–39		17.8±2.5	50.8±6.1	82.3±4.5	30.0±1.9	17.7±1.2	3.5±0.2	32.2±3.6			
Hb J-Bangkok	1	F	36	−α/αα	13.2	41.9	77.0	24.1	14.1	2.6	0.8	49.3	12	A <sub>2</sub> A with Hb Var
	1	M	57	−−/αα	7.7	25.4	70.0	24.4	18.0	2.3	0.4	48.4		
Hb New York	1	F	46	αα/αα	10.3	31.1	89.6	29.7	18.9	3		46.5	11	A <sub>2</sub> A with Hb Var
α-chain variants														
Hb G-Georgia (HBA1)	3	F (2)	18, 38	β/β	11.4, 12.0	36.1, 35.6	92.0, 82.0	29.0, 27.8	12.0, 12.6	2.3, 2.3	10.5, 10.3		7	A <sub>2</sub> F <sup>b</sup> A
		M (1)	40		13.9	41.3	88.0	29.4	13.1	2.8	10.6			
Hb G-Georgia (HBA2)	1	F	33	β <sup>E</sup> /β	NA	NA	72.0	NA	NA	3.6	17.0	(E=20)	7, 4	EF <sup>b</sup> A
Hb Q-Thailand	1	M	38	β <sup>CD41/42</sup> /β	12.7	41.3	71.0	21.7	13.4	4.4	15.8		7	A <sub>2</sub> F <sup>b</sup> A
	1	F	33	β/β	11.7	36.2	79.0	25.5	13.3	1.9	28.6		7	
Hb St. Luke's-Thailand	1	M	29	β/β	16.8	49.5	94.0	31.8	11.3	1.8		18.9	6	A <sub>2</sub> A with Hb Var

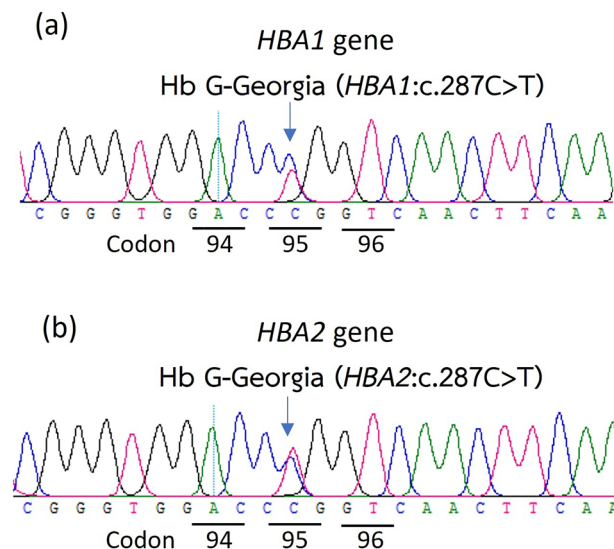
**Table 2.** Hematological characteristics of 263 Hb variant carriers. <sup>a</sup>Hb variant co-migrated with Hb A, <sup>b</sup>Hb variant co-migrated with Hb F, NA not available, *Hb Var* hemoglobin variant, CS Constant Spring, *F* female, *M* male, *CD* codon.

Q-Thailand, Hb J-Bangkok, Hb Pyrgos (*HBB*:c.251G>A), and Hb Hope. Moreover, northeastern Thailand populations displayed notable variability in the distribution of Hb variants.

Discussion

This study revisited the molecular spectrum of Hb variants in the southern population across ten provinces through a large-scale survey of specimens received by our center over eight years. Among 13,391 individuals, 337 (2.5%) carried Hb variants, with 263 (78%) identified as Hb variant carriers. The predominant Hb variant was Hb Malay, followed by Hb C. Herein, we report rare Hb variants identified in zone 7, similar to Hb F, including Hb Q-Thailand, Hb Tak, and Hb G-Georgia. To our knowledge, this study is the first to report Hb G-Georgia on the *HBA1* gene in Thailand. Hb G-Georgia (*HBA1*) heterozygote showed lower levels (10.3–10.6%) than





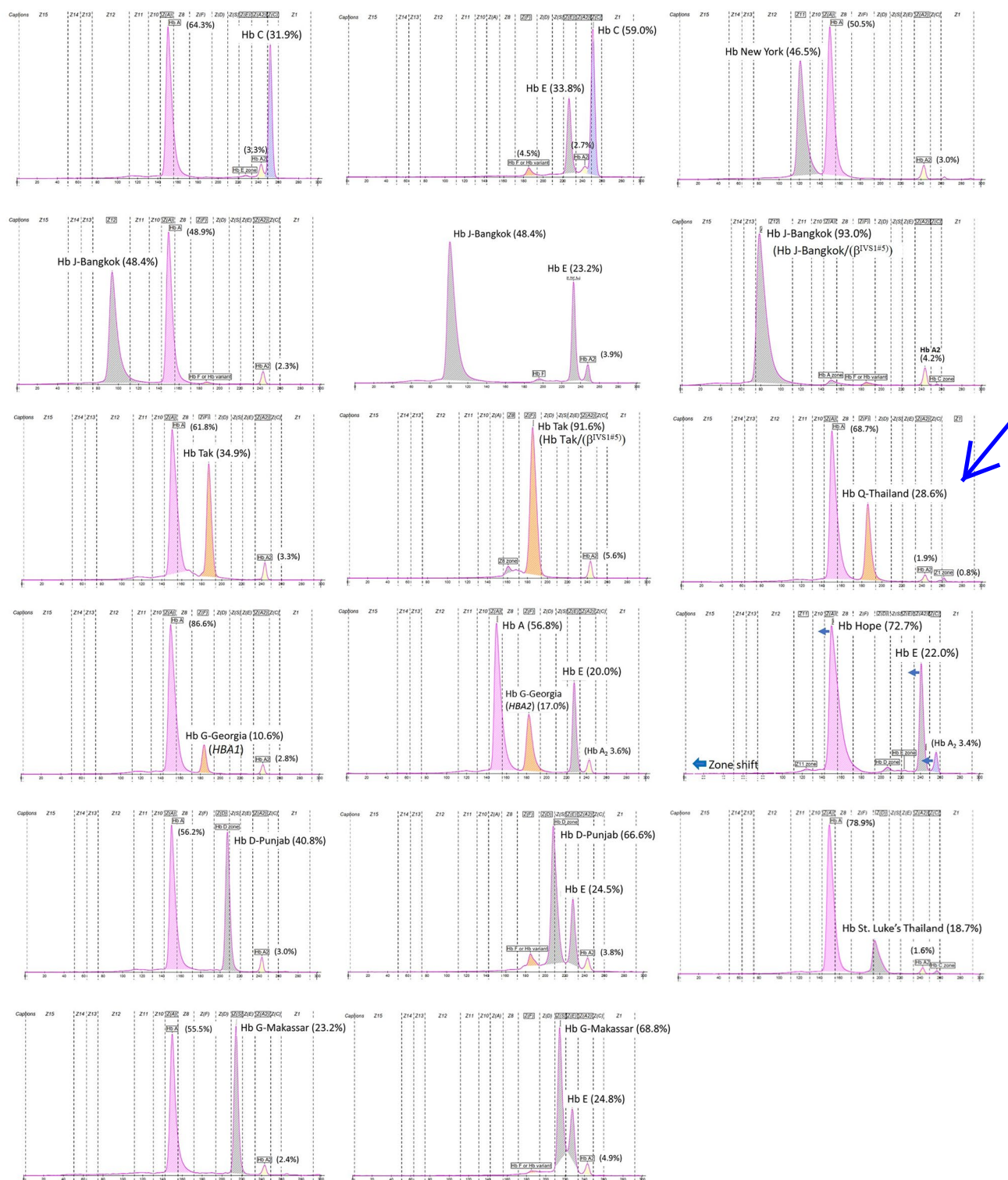
**Figure 2.** Sanger DNA sequencing results for Hb G-Georgia (*HBA1*) and (*HBA2*).

Hb G-Georgia (*HBA2*), 17.0% in a double heterozygote Hb G-Georgia and Hb E in this study and 23.4% in Hb G-Georgia heterozygote reported in a previous study<sup>8</sup>, which may be explained by approximately 2–3 times lower expression of *HBA1* gene than the *HBA2* gene<sup>9</sup>. Interestingly, Hb G-Georgia did not present any Hb A<sub>2</sub> variant peak in hemoglobin analysis using the CE technique, observed in both heterozygote Hb G-Georgia and double heterozygote Hb G-Georgia and Hb E. Thus, this could be misconstrued as  $\beta^+/\beta^+$  or  $\beta^+/\beta^0$ -thalassemia disease or  $\beta^+$ -thalassemia with Hb E disease based on the Hb pattern, reflecting A<sub>2</sub>FA or EFA. However, the three patients with heterozygous Hb G-Georgia in our study exhibited no clinical symptoms and normal RDW levels. Furthermore, an alkaline denaturation test yielded negative results. Therefore, we performed DNA sequencing to identify this Hb variant. A patient harboring compound heterozygous Hb G-Georgia with  $\alpha^0$ -thalassemia did not develop Hb H disease<sup>4</sup>, indicating that Hb G-Georgia is not classified as an  $\alpha$ -thalassemia mutation. However, rapid molecular diagnosis is required for proper genetic counseling. Thus, we developed an allele-specific PCR (AS-PCR) for detecting Hb G-Georgia in both *HBA1* and *HBA2* genes for the first time (Fig. 4). Unlike PCR-RFLP, this technique is simple, rapid, inexpensive, and does not require restriction enzymes<sup>4</sup>.

The clinical phenotype of Hb Tak often includes erythrocytosis in patients with compound heterozygous Hb Tak with  $\beta$ -thalassemia, homozygous Hb Tak, and Hb Tak with  $\delta\beta^0$ -thalassemia<sup>10–12</sup>. However, a patient with Hb Tak and  $\beta^+$ -thalassemia (IVS1-5 (G>C)) showed no symptomatic erythrocytosis (Hb 11 g/dL, Hct 37.5%), which might be explained by the underlying disease with an atrial septal defect and failure to thrive.

Thalassemia mutations are common and heterogeneous in southern populations<sup>13</sup>. We reported the interaction of Hb Malay with other abnormalities resulted in diverse genotypes in 57 (16.9%) patients. The most common genotype was compound heterozygous Hb Malay with Hb E patients (n = 27), manifesting a thalassemia intermedia phenotype without blood transfusion, similar to those reported previously<sup>14,15</sup>. Accordingly, prenatal diagnosis is deemed unnecessary for couples at risk of developing Hb Malay with Hb E disease to reduce the risk of miscarriage. Nevertheless, postnatal diagnosis and appropriate genetic counseling are imperative. This study showed that Hb Malay with  $\beta^0$ -thalassemia, including codon 17 (A>T), codon 41 (– C), codons 41/42 (– TTCT), IVS1-1 (G>T), or  $\beta^+$ -thalassemia, including IVS1-5 (G>C) and IVS2-654 (C>T), led to severe anemia, wherein patients required regular blood transfusion. Prenatal diagnosis is thus essential for families with this combination. Conversely, a patient with Hb Malay and  $\beta^0$ -thalassemia (3.5 kb deletion) presented with moderate anemia (Hb 9.1 g/dL) without the need for blood transfusion. This milder clinical manifestation could be due to co-inheritance with heterozygous  $\alpha^0$ -thalassemia, ameliorating clinical severity by balancing the levels of  $\alpha$ - and  $\beta$ -globin chains<sup>16–19</sup>. Accordingly, this study supports a previous recommendation proposing the inclusion of  $\alpha^0$ -thalassemia analysis in prenatal diagnosis for fetuses affected with thalassemia disease to make appropriate decisions<sup>20</sup>. Previous studies reported that  $\beta^0$ -thalassemia (3.5 kb deletion) carriers usually exhibit higher Hb A<sub>2</sub> and Hb F levels than other  $\beta$ -thalassemia carriers due to point mutations<sup>21,22</sup>. The positive result in reverse dot blot (RDB) hybridization indicating a homozygous Hb Malay genotype in the Hb Malay with  $\beta^0$ -thalassemia (3.5 kb deletion) case, alongside hematological profiles resembling thalassemia intermedia, raises the possibility of misdiagnosis as homozygous Hb Malay. However, the elevated Hb A<sub>2</sub> levels (8.7%) compared to those of homozygous Hb Malay (4.5–5.4%) underscored the need for further laboratory investigation into  $\beta^0$ -thalassemia (3.5 kb deletion). Subsequently, the true genotype of this patient was found to be Hb Malay with  $\beta^0$ -thalassemia (3.5 kb deletion).

Interestingly, a previous study reported that Hb Malay with  $\beta^+$ -thalassemia typically manifests as a thalassemia intermedia phenotype without the need for regular blood transfusion<sup>15</sup>. However, this study reported three cases of Hb Malay with  $\beta^+$ -thalassemia (NT-28 (A>G)) exhibiting distinct phenotypes. One patient presented transfusion-dependent thalassemia and splenomegaly; this was potentially influenced by additional abnormalities. Subsequently, gap-PCR was conducted to identify  $\alpha$ -globin gene triplication ( $aaa/aa$ )<sup>23</sup>, which, if present, could



**Figure 3.** Hemoglobin (Hb) analysis results of Hb variants from southern populations using the capillary electrophoresis method.

exacerbate globin chain imbalance and escalate clinical severity<sup>24</sup>. Despite obtaining a negative result for this patient (data not shown), we propose the application of whole-exome sequencing to comprehensively determine the clinical severity.

In Thailand, the frequency of high Hb F determinants is 1.06%<sup>25</sup>. The co-occurrence of this abnormality with  $\beta$ -thalassemia can yield diverse clinical phenotypes, ranging from mild to severe anemia, depending on the  $\beta$ -thalassemia genotype<sup>25–27</sup>. However, scant information exists on the clinical phenotypes associated with high Hb F determinants in patients with Hb Malay. We present, for the first time, a case of Hb Malay with Thai

Hemoglobin variants genotype	No. of cases	Gender (n)	Age range (years)	α-genotype	Hematological parameter					Hemoglobin analysis					
					Hb	Hct	MCV	MCH	RDW	Hb A <sub>2</sub>	Hb E	Hb F	Hb variant	Hb variant	Hb pattern
					(g/dL)	(%)	(fL)	(pg)	(%)	(%)	(%)	(%)	zone		
Homozygous genotype															
Hb Malay/Hb Malay	8	F (2)	9, 14	αα/αα	8.7, 8.7	27.0, 28.8	50.3, 51.7	16.2, 15.6	29.8, 26.5	5.4, 4.5		42.7, 45.3		9	A <sub>2</sub> FA <sup>a</sup>
		M (6)	20–33		10.0±1.7	30.6±5.1	58.2±5.7	19.7±0.7	24.1±0.9	5.2±1.0		37.6±9.8			
Compound heterozygous genotype															
Hb Malay/Hb E	27	F (12)	2–35	αα/αα	9.1±0.8	27.8±2.0	61.7±12.1	18.6±1.4	21.0±2.0	5.4±0.5	48.5±4.2	10.9±4.6		9, 4	EFA <sup>a</sup>
		M (15)	2–37		9.7±1.4	30.4±4.6	54.3±5.1	17.4±1.3	20.7±2.3	5.8±0.7	47.7±5.3	8.2±7.8			
Hb Malay/β <sup>+</sup> -thal (NT-28 (T>C))	1	F	13	αα/αα	7.7	23.7	63.0	20.5	26.3	NA		NA	NA	NA	NA
	1	F	1	–α/αα	9.1	28.9	58.3	18.3	27.9	NA		NA	NA	NA	NA
Hb Malay/Hb C	1	F	1	αα/αα	10.3	31.4	58.0	19.0	14.8	4.6		8.3	65.7	9, 2	A <sub>2</sub> A <sup>a</sup> with Hb Var
Hb Malay/δβ <sup>0</sup> -thal (12.5 kb deletion)	3	F (2)	2–44	αα/αα	9.4, 10.0	28.0, 29.8	68.0, 61.1	21.0, 20.6	20.5, 22.4	2.6, 2.3		70.0, 72.9		9	A <sub>2</sub> FA <sup>a</sup>
		M (1)			10.5	33.4	68.0	21.3	20.7	2.4		61.4			
Hb Malay/Indian del-inv <sup>Δ</sup> γδβ <sup>0</sup> -thal	1	F	11	αα/αα	9.7	30	55	17.8	25.7	3.1		62.2		9	A <sub>2</sub> FA <sup>a</sup>
Hb Malay/Thai del-inv-ins <sup>Δ</sup> γδβ <sup>0</sup> -thal	1	M	29	αα/αα	13.7	43.0	63.0	20.0	22.1	2.6		68.0		9	A <sub>2</sub> FA <sup>a</sup>
Hb Malay/β <sup>0</sup> -thal (3.5 kb deletion)	1	F	38	––/αα	9.1	27.3	61.9	20.6	27.2	8.7		52.6		9	A <sub>2</sub> FA <sup>a</sup>
Hb C/Hb E	4	F (2)	2–79	αα/αα	11.1, 10.8	30.8, 30.2	62.7, 65.0	22.6, 23.2	15.3, 16.2	4.1, 4.0	30.6, 32.0	3.1, 3.5	54.7, 56.0	2, 4	E with Hb Var
		M (2)			11.7, 15.0	31.8, 45.0	59.0, 74.0	21.7, 25.1	17.2, 16.4	3.6, 4.1	33.0, 34.0	4.0, 2.0	54.9, 53.7		
	1	M	53	α <sup>CS</sup> α/αα	9.5	26.7	71.0	25.3	15.2	4.2	34.7	5.5	55.3	2, 4	ECS with Hb Var
	1	M	22	––/αα	12.6	35.8	57.0	19.9	18.0	4.4	35.6	1.7	58.3	2, 4	E with Hb Var
Hb C/β <sup>0</sup> -thal (3.5 kb deletion)	1	F	60	αα/αα	10.7	32.3	53.5	17.7	22.9	7.6		11.6	79.2	2	A <sub>2</sub> F with Hb Var
Hb C/β <sup>0</sup> -thal (codon41 (– C))	1	M	24	αα/αα	12.4	37.7	58.9	19.4	20.0	3.7		0	91.2	2	A <sub>2</sub> with Hb Var
Hb D-Punjab/ Hb E	2	F, M	28, 29	–α/αα	15.1, 12.0	44, 33.2	79.0, 69.0	27.0, 24.9	14.0, 15.6	3.8, 3.7	24.5, 29.0	5.1, 0.0	66.6, 65.6	6, 4	E with Hb Var
Hb G-Makassar/ Hb E	2	M	38, 74	αα/αα	15.7, 12.3	44.6, 35.4	71.0, 64.7	24.9, 22.5	14.1, 15.5	5.2, 4.9	23.3, 24.8		69.2, 68.8	5	E with Hb Var
Hb J-Bangkok/ β <sup>+</sup> -thal (IVS1-5 (G>C))	2	M, F	30, 56	αα/αα	10.1, 10.6	33.6, 35.6	62.0, 67.9	18.6, 20.2	17.5, 15.0	4.8, 4.2		2.6, 0.0	89.5, 93.0	12	A <sub>2</sub> A with Hb Var
Hb J-Bangkok/ Hb E	1	F	39	αα/αα	11.0	33.6	72.0	23.6	13.1	3.9	23.2		72.0	12, 4	E with Hb Var
Hb Tak/β <sup>+</sup> -thal (IVS1-5 (G>C))	1	F	4	αα/αα	11.8	37.5	69.7	21.9	29.5	5.6		91.6		7	A <sub>2</sub> F <sup>b</sup>
Hb Hope/Hb E	1	F	47	αα/αα	11.0	34.4	80.4	25.7	14.1	3.4	22.0		72.7	10, 4	E with Hb Var

**Table 3.** Hematological characteristics of 61 patients with homozygous or compound heterozygous Hb variants and hemoglobinopathies who were not dependent on transfusion. <sup>a</sup>Hb variant co-migrated with Hb A, <sup>b</sup>Hb variant co-migrated with Hb E, NA not available, *Hb Var* hemoglobin variant, *thal* thalassemia, *F* female, *M* male, *NT* nucleotide, *IVS* intervening sequence.

Hemoglobin variants genotype	No. of cases	Gender (n)	Age	Frequency of blood transfusion	Splenectomy	Underlying condition	$\alpha$ -genotype	Hematological parameter after regular blood transfusion				
			(years)		(yes/no)			Hb	Hct	MCV	MCH	RDW
								(g/dL)	(%)	(fL)	(pg)	(%)
Hb Malay/ $\beta^+$ -thal (NT-28 (T>C))	1	M	13,	Every two months	No	Splenomegaly	$\alpha\alpha/\alpha\alpha$	5.3,	19.1	80.0	22.1	27.5,
Hb Malay/ $\beta^0$ -thal (codon 17 (A>T))	2	M, F	10, 33	Every two months	No, yes	NA, Secondary hemochromatosis and secondary pulmonary hypertension	$\alpha\alpha/\alpha\alpha$	7.6, 4.4	23.3, 16.6	78.0, 73.0	25.3, 22.0	23.4, 25.6
Hb Malay/ $\beta^0$ -thal (codon 41 (-C))	1	M	24	Once a month	Yes	Secondary hemochromatosis	$\alpha\alpha/\alpha\alpha$	6.8	20.2	77.0	26.1	18.7
Hb Malay/ $\beta^0$ -thal (codons 41/42 (-TTCT))	3	F (2)	2, 14	Once a month, every two months	No	NA	$\alpha\alpha/\alpha\alpha$	4.4, 8.7	13.9, 27.2	52.7, 72.0	16.7, 22.5	33.0, 20.9
		M (1)	29	Once a month	No	Hepatosplenomegaly		NA	NA	NA	NA	NA
Hb Malay/ $\beta^0$ -thal (IVS1-1 (G>T))	2	M	1, 3	Once or twice a month	No	NA	$\alpha\alpha/\alpha\alpha$	7.3, 6.0	22.2, 19.6	66.0, 65.0	21.6, 19.8	35.4, 26.5
Hb Malay/ $\beta^+$ -thal (IVS1-5 (G>C))	3	F (1)	11	Every two months	Yes	NA	$\alpha\alpha/\alpha\alpha$	6.5	23.0	64.0	18.6	24.4
		M (2)	24, 26	Once a month, twice a month	No	NA, Chronic renal disease		6.5, 5.7	21.9, 17.9	73.0, 65.0	25.5, 26.5	22.1, 17.9
Hb Malay/ $\beta^+$ -thal (IVS2-654 (C>T))	1	M	2	twice a month	No	NA	$\alpha\alpha/\alpha\alpha$	7.5	23.0	64.0	20.8	30.1

**Table 4.** Hematological characteristics of 13 patients with compound heterozygous Hb variants and hemoglobinopathies who were dependent on transfusion. NA not available, *thal* thalassemia, *F* female, *M* male, *NT* nucleotide, *IVS* intervening sequence.

del-inv-ins  $\Delta\gamma\delta\beta^0$ -thalassemia exhibiting no clinical symptoms. Moreover, combinations of Hb Malay with  $\delta\beta^0$ -thalassemia (12.5 kb deletion), Indian del-inv  $\Delta\gamma\delta\beta^0$ -thalassemia, or HPFH6 also presented only thalassemia intermedia phenotype without the need for blood transfusion. These results suggest that prenatal diagnosis might be unnecessary for couples carrying Hb Malay with high Hb F determinants. However, a postnatal diagnosis should be performed for proper genetic counseling.

Hb J-Bangkok is a  $\beta$ -chain variant occasionally reported in Thailand. A carrier usually presents with normal hematological parameters, with Hb J-Bangkok levels of  $44.5 \pm 4.7\%$ <sup>28</sup>. However, we report a case of Hb J-Bangkok carrier with moderate anemia (Hb 7.7 g/dL), potentially affected by an underlying disease but unconfirmed patient-specific condition. Interestingly, we report two cases of Hb J-Bangkok with  $\beta^+$ -thalassemia (IVS1-5 (G>C)) for the first time. Elevated Hb J-Bangkok levels of 89.5% and 93% in patients with mild anemia (Hb 10.1 and 10.6 g/dL) might suggest the presence of homozygous Hb J-Bangkok. However, this rare variant is infrequently reported in southern populations. Thus, these patients are preferably linked to co-inheritance with  $\beta$ -thalassemia mutation, and molecular diagnosis of  $\beta$ -thalassemia is subsequently performed in these cases.

The levels of Hb E or Hb C in pure Hb E or pure Hb C heterozygotes were higher than those genotypes co-inherited with  $\alpha^0$ -thalassemia because the  $\alpha$ -globin chain prefers to form dimerization with  $\beta$ -globin chain than  $\beta^E$  or  $\beta^C$ -globin chain. Thus, reduced  $\alpha$ -globin chain production in  $\alpha^0$ -thalassemia contributes to lower Hb E or Hb C levels. However, Hb E levels in compound heterozygous Hb C/Hb E are higher than in Hb E heterozygote. The previous studies reported that the Hb E levels in compound heterozygous Hb C/Hb E could be presented in a wide range from 32.0 to 39.7%<sup>14,29</sup>. This study reported four cases with compound heterozygous Hb C/Hb E without co-inherited  $\alpha$ -thalassemia with Hb E levels ranging from 30.6 to 34.0%, while a compound heterozygous Hb C/Hb E co-inherited  $\alpha^0$ -thalassemia presented with Hb E levels of 35.6%. Hence, lower levels of Hb E were not observed in a compound heterozygous Hb C/Hb E co-inherited with  $\alpha^0$ -thalassemia. It might be due to both Hb E and Hb C are positively charged Hb variants, which might have a similar ability to interact with the  $\alpha$ -globin chain. Moreover, the decrease of  $\alpha\beta^C$  dimer formation leading to an indirect increase in the  $\alpha\beta^E$  dimer formation<sup>29</sup> as the same as that presented in compound heterozygous Hb S/Hb E disease<sup>30</sup>.

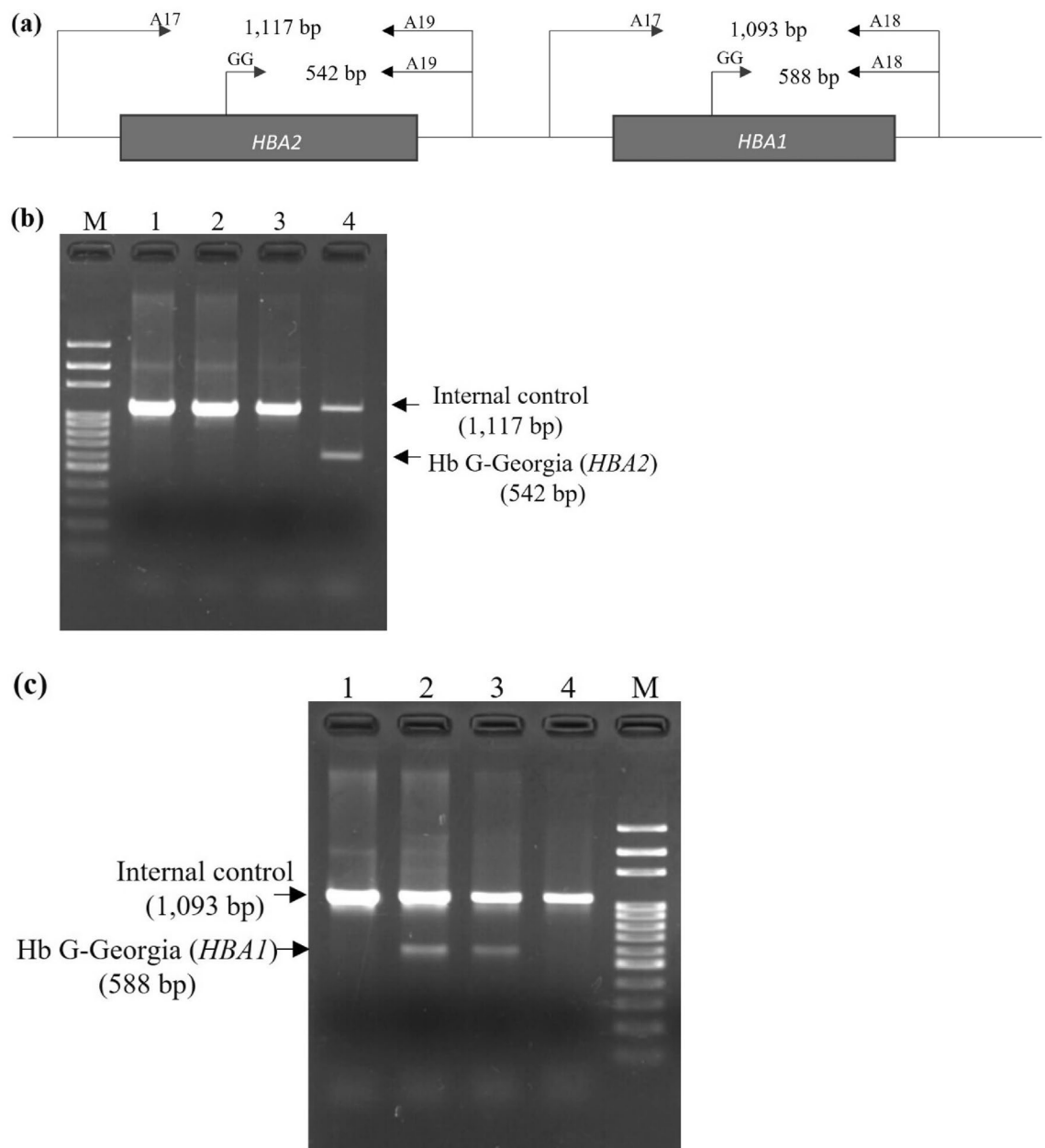
For two cases of compound heterozygous Hb D-Punjab/Hb E co-inherited with  $\alpha^+$ -thalassemia, the Hb D-Punjab value (66.6% and 65.6%) is elevated while the Hb E value (24.5% and 29.0%) is the same as the Hb E heterozygote. Hb D-Punjab mutation results in structural protein changes but does not affect the value of Hb D-Punjab production. However, Hb E mutation creates abnormal mRNA splicing, resulting in low output of Hb E. Thus, lower Hb E levels than Hb D-Punjab levels could be observed in the compound heterozygous Hb D-Punjab/Hb E cases. Moreover, co-inherited  $\alpha^+$ -thalassemia in compound heterozygous Hb D-Punjab/Hb E cases might not much affect the lower production of Hb E levels when compared to Hb E levels of compound heterozygous Hb D-Punjab/Hb E with normal  $\alpha$ -globin chain cases in a previous report (24.5% and 29.0% vs 28.4% and 29.3%)<sup>31</sup>.

Hemoglobin variants	HGVS nomenclature	South	South	South	Center	Northeast	North
		This study	7	1	1	1	2
		(%)	(%)	(%)	(%)	(%)	(%)
β-chain variants							
Hb Malay	HBB:c.59A>G	76.6	ND	29.3	3.3	4.6	ND
Hb C	HBB:c.19G>A	10.1	50.0	17.4	3.0		0.5
Hb D-Punjab	HBB:c.364G>C	3.0	21.4	23.9	4.1	2.3	
Hb G-Makassar	HBB:c.20A>C	2.4	7.1				0.5
Hb Dhonburi	HBB:c.380T>G	2.4				3.7	ND
Hb J-Bangkok	HBB:c.170G>A	1.5		1.1	8.6	8.7	1.5
Hb Tak	HBB:c.441_442insAC	1.5	12.5	13.0	14.5	16.9	14.6
Hb New York	HBB:c.341T>A	0.3					
Hb Hope	HBB:c.410G>A	0.3	1.8	2.2	32.0	12.3	55.8
Hb S	HBB:c.20A>T			1.1			1.5
Hb Korle-Bu	HBB:c.220G>A			1.1	3.3	4.1	0.5
Hb Pyrgos	HBB:c.251G>A				5.2	12.8	
Hb Cook	HBB:c.398A>C				0.7	3.7	
Hb Dhofar	HBB:c.176C>G				0.7		
Hb J-Kaohsiung	HBB:c.179A>C					1.4	
Hb Phimai	HBB:c.218G>C					0.9	
Hb Raleigh	HBB:c.5T>C					0.5	
Hb Khon Kaen	HBB:c.370_378delACCCACCA						
Hb E-Saskatoon	HBB:c.67G>A					0.5	
α-chain variants							
Hb G-Georgia (HBA1)	HBA1:c.287C>T	0.9					
Hb G-Georgia (HBA2)	HBA2:c.287C>T	0.3					
Hb Q-Thailand	HBA1:c.223G>C	0.6	3.6	5.4	20.4	18.3	19.4
Hb St. Luke's- Thailand	HBA2:c.287C>G	0.3				0.9	
Hb O-Indonesia	HBA1:c.349G>A		3.6				
Hb Queens	HBA1:c.104T>G			5.4	0.4		
Hb Siam	HBA2:c.46G>C(or HBA1)				1.9	1.4	1.0
Hb Beijing	HBA2:c.51G>C(or HBA1)				0.4		0.5
Hb Nakhon Ratchasima	HBA2:c.191C>T				0.7	1.4	
Hb G-Honolulu	HBA2:c.91G>C(or HBA1)				0.4		
Hb J-Wenchang-Wuming	HBA2:c.34A>C(or HBA1)				0.4		
Hb Hekinan	HBA1:c.84G>C					2.3	3.9
Hb Dunn	HBA2:c.19G>A(or HBA1)					1.4	
Hb Thailand	HBA1:c.170A>C					1.4	
Hb Q-India	HBA1:c.193G>C					0.5	
Hb Phnom Penh	HBA1:c.354_355insATC					0.5	
Hb Kawachi	HBA2:c.134C>G(or HBA1)						0.5
Total cases		337	56	92	269	219	206

**Table 5.** Comparison of the molecular spectrum of Hb variants among 337 participants from southern Thailand and that reported in previous studies in populations from different parts of the country. The common Hb variants in each part are presented in bold. ND not done.

The Hb variant spectra in Thailand were compared. Three common Hb variants—Hb Hope, Hb Q-Thailand, and Hb Tak—have been observed in many populations from northern, central, and northeastern Thailand<sup>1</sup>. However, the southern population showed different common Hb variants, especially compared to the northern population. It could be explained by differing ethnic backgrounds of populations between the north and south. In Thailand, most people belong to the Thai ethnicity. However, each part of the country has different minority ethnic groups. Minor ethnic groups were observed in the northern population, including Lawa, Mon, Shan, Yuan, Khuen, Lue, and Yong<sup>32</sup>. By contrast, the minority ethnic groups in the southern population are Thai Muslims, Maniq, Moken, Moklen, and Urak Lawoi<sup>33</sup>. Furthermore, the mitochondrial phylogenetic analysis revealed that the population from the northern area has distinct haplotype groups compared to those of the southern population<sup>34</sup>. This divergence may be explained by the proximity of southern Thailand to the sea, leading to populations of diverse nationalities due to human migration from neighboring countries such as Malaysia and India, where Hb Malay and Hb D-Punjab are prevalent, respectively<sup>35,36</sup>. In addition, Hb C is commonly found





**Figure 4.** Schematic illustrating the primer orientation for the newly developed allele-specific PCR (a). Agarose gel electrophoresis results for the detection of hemoglobin (Hb) G-Georgia (*HBA2*) (b) and Hb G-Georgia (*HBA1*) (c). M; 100 bp DNA marker, 1; negative for Hb G-Georgia (*HBA2* and *HBA1*), 2 and 3; positive for Hb G-Georgia (*HBA1*), 4; positive for Hb G-Georgia (*HBA2*).

in West African populations<sup>37</sup> and is occasionally reported in Southeast Asian populations of different origins<sup>29</sup>. Carriers of Hb C are immune to malarial infections<sup>38</sup>. Accordingly, Hb C is predominantly observed in southern Thailand, where malaria is endemic. Moreover, Hb G-Makassar is frequently observed in the southern population, similar to that in the Malaysian population<sup>39</sup>. Hb G-Makassar comigrated at the same retention time as that for Hb S, as determined using the CE technique. Thus, molecular testing is essential for differential diagnosis. Furthermore, we reported two cases of Hb G-Makassar with Hb E, presenting mild clinical phenotypes similar to that in a previous report<sup>39</sup>.

In conclusion, this study demonstrates a distinct spectrum of Hb variants in Thailand. In addition, we describe the clinical aspects of Hb variants in combination with thalassemia or hemoglobinopathies. This information is essential for determining the need to perform prenatal diagnosis in the prevention and control program for thalassemia in this region.



## Materials and methods

All laboratory methods were performed following the national guidelines of Thailand for laboratory diagnosis of thalassemia and hemoglobinopathy<sup>40</sup>. The study protocol was approved by the Human Research Ethics Unit (HREU) of the Faculty of Medicine, Prince of Songkla University (REC 63-458-5-2). Consent was obtained from all 337 participants with Hb variants. Participants who visited or had their blood samples collected were referred to Songklanagarind Hospital from 2015 to 2020. Informed consent was obtained via telephone, followed by sending the documents via the post office. For patients who visited Songklanagarind Hospital from 2021 to 2022, informed consent was obtained when they visited for follow-up.

Each hospital provided hematological profiles and recorded the history of blood transfusion data. The hematological profiles and history of blood transfusion data of the patients from ten provinces were collected from the laboratory requesting program of Songklanagarind Hospital. We collected hematological data, history of blood transfusion, routine molecular diagnosis results, and DNA samples of patients referred to Songklanagarind Hospital from January 2015 to December 2022 for diagnosing thalassemia.

## Samples

A total of 337 DNA specimens were obtained from molecular diagnosis at the thalassemia unit at the Department of Pathology, Faculty of Medicine, Prince of Songkla University, southern Thailand.

## Hematological analysis

The hematological profiles were obtained from each hospital in the ten provinces. In our center, hematological parameters were obtained from an automated blood cell counter (Sysmex XN 3000; Sysmex, Japan). Hemoglobin analysis of all referred samples was performed using CE technique (Capillarys 2; Sebia, Lisses, France) at our laboratory, and a thorough review of blood transfusion history was conducted.

## Molecular analysis

Routine molecular diagnosis was performed to identify  $\beta$ -thalassemia,  $\alpha$ -thalassemia, Hb Hb CS, Hb Paksé (PS, *HBA2*:c.429A>T), and high Hb F determinants using PCR-based techniques. Analysis of point mutations in  $\beta$ -thalassemia involved the examination of Hb Malay, Hb Dhonburi, and  $\beta$ -thalassemia 19 common mutations in southern Thailand were performed using RDB hybridization<sup>13</sup>. Analysis of  $\beta$ -thalassemia deletion (3.5 kb and 45 kb deletion (NG\_000007.3:g.66258\_184734del118477)) was carried out through melt-curve analysis<sup>41</sup>. High Hb F determinants, including  $\delta\beta^0$ -thalassemia (12.5 kb deletion), Indian del-inv  $\gamma\delta\beta^0$ -thalassemia, HPFH6, and Thai del-inv-ins  $\gamma\delta\beta^0$ -thalassemia (or Siriraj deletion), were identified using multiplex gap-PCR<sup>42</sup>. Nine  $\alpha$ -thalassemia deletion mutations, such as  $-\text{SEA}$  (NC\_000016.10:g.165397\_184700),  $-\text{THAI}$  (NC\_000016.10:g.149863\_183312),  $-\text{SA}$  (NG\_000006.1:g.19464\_43064del23601),  $-\text{CR}$  (NC\_000016.10:g.144,215\_188,841),  $-\text{FIL}$  (NG\_000006.1:g.11684\_43534del31851),  $-\text{MED}$  (NG\_000006.1:g.24664\_41064del16401),  $-(\alpha)^{20.5}$ ,  $-\alpha^{3.7}$  (NG\_000006.1:g.34164\_37967del3804) and  $-\alpha^{4.2}$  ((NC\_000016.10:g.149863\_183312), were identified using multiplex gap-PCR<sup>43</sup>. Allele-specific PCR was performed to identify Hb CS and Hb PS<sup>44</sup>. Additional investigation for unidentified Hb variants in the *HBA* and *HBB* genes among cases with Hb variant peak by CE method was confirmed by Sanger DNA sequencing using an ABI PRISM™ 3130xl analyzer (Applied Biosystems, Foster City, CA, USA) or by performing barcode-tagged sequencing based on next-generation sequencing on the Illumina MiSeq (Illumina, Inc., San Diego, CA).

## Development of allele-specific PCR for identification of Hb G-Georgia in the *HBA1* and *HBA2* genes

Two AS-PCR conditions were developed for the differential diagnosis of Hb G-Georgia. To identify Hb G-Georgia in *HBA1*, a 1093 bp fragment generated from primers A17 (5'-GCTCCGCGCCAGCCAATGAG-3') and A18 (5'-CTGGACTTCGCGGTGGCTC-3') was used as an internal control. A 588 bp fragment specific to Hb G-Georgia in *HBA1* was amplified using primer GG (5'-ACAAGCTTCGGGTGGACCT-3') and primer A18. For the identification of Hb G-Georgia in *HBA2*, a 1,117 bp fragment generated from primers A17 and A19 (5'-GCAGGCCTGGCACCTCTCAG-3') was used as an internal control. A 542 bp fragment specific to Hb G-Georgia in *HBA2* was amplified using primers GG and A19 (Fig. 4). Each PCR reaction (25  $\mu$ L) comprised 50–200 ng genomic DNA, 0.32 pmol of primers A17 and GG, 0.48 pmol of primers A18 or A19, 200  $\mu$ M dNTPs, 1 M Betaine, 1.75 mM  $\text{MgCl}_2$ , 1.25% DMSO, and 0.5 units of *Taq* DNA polymerase (Vivantis Technologies, Selangor Darul Ehsan, Malaysia) in 16 mM  $(\text{NH}_4)_2\text{SO}_4$  and 50 mM Tris-HCl (pH 9.2) buffer, and 0.1% Triton™ X-100. The cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 65 °C for 45 s, and 72 °C for 1 min 20 s, with a final extension at 72 °C for 5 min on a SimpliAmp™ thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA). PCR products were separated via 1.5% agarose gel electrophoresis for 30 min. The amplified fragments were detected under UV light after staining with ethidium bromide (Supplementary Figure S1).

## Data availability

The datasets generated and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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## Author contributions

W.T. acquired grants, wrote the main manuscript, and contributed to all aspects of the investigation, including conceptualization, data collection, laboratory investigation, data analysis, and revision of the final manuscript. S.K. and K.S. were involved in sample preparation and molecular analysis. C.W., C.N., C.K., and S.B. were involved in sample collection and hemoglobin analysis. T.P. and P.S. were involved in conceptualization, data collection, and data analysis. All the authors approved the final version of the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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# Complex Interaction of Hb Q-Thailand with $\alpha$ and $\beta$ Thalassemia in a Hakka Family

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## Research

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# Abstract

**Background** HbQ-Thailand is an  $\alpha$ -globin chain variant that results from a point mutation at codon 74 of the  $\alpha 1$ -globin gene on chromosome 16p. It commonly appears with a leftward single  $\alpha$ -globin gene deletion ( $-\alpha 4.2$ ). There have been few reports regarding the interaction between HbQ-Thailand and other globin gene disorders. Here we found and diagnosed it in the Hakka population of the Fujian Province, China. The study provides an important reference for the clinic diagnose and genetic counseling of thalassemia and hemoglobin diseases.

**Methods** Fresh peripheral blood samples were collected from the proband and her family members testing hematological parameters, hemoglobin components, thalassemia gene, and hemoglobin variants.

**Results** The proband (II1) and her sister (II5) manifested in the obvious microcytic hypochromic anaemia. The CE electropherogram of II1 showed an abnormal band in the migration time at 185 s, which was confirmed as HbQ-Thailand. Another exception band appeared at 250 s of migration time and was proved to be HbE by sequence analysis method. The CE electropherogram of I1 and II3 showed an anomalous band HbE. The mother of the proband (I2) and the III4 and III5 of the family members showed a HbQ-Thailand. The gene results showed that the father (I1) also carried  $\alpha$ - and  $\beta$ -thalassemia genes. His genotype was  $-\text{SEA}$  and  $\beta$  codons26 ;  $-\text{SEA}$  was inherited to II1, II 3, II5, III 1, and III2, and  $\beta$  codons26 was inherited to II1 and II3. The mother (I2) carries the  $-\alpha 4.2$  gene, which was inherited to II1, II5, III4, and III5.

**Conclusion** It was complex to diagnose when the thalassemia combined with several abnormal haemoglobin disorders, and we may use various methods to mutual confirmation. Here we found and diagnosed a rare hemoglobin disease in the Hakka population of the Fujian Province. The study provides an important reference for the clinic diagnose and genetic counseling.

## Introduction

Hemoglobinopathies are a group of inherited autosomal recessive disorders, including the alteration of hemoglobin quantity (thalassemias) and quality (Hb variants)[1]. They are widely distributed in Mediterranean countries, the Middle East, Africa, and Southeast Asia, including southern China[2–4]. HbQ-Thailand [ $\alpha 74(\text{EF3})\text{Asp} \rightarrow \text{His}(\alpha 1), \text{GAC} \rightarrow \text{CAC}, \text{HBA1:C.223G} \rightarrow \text{C}$ ], also known as Hb G-Taichung, Mahidol, Kurashiki-I, and Asabara[5], is an  $\alpha$ -globin chain variant that results from a point mutation at codon 74 of the  $\alpha 1$ -globin gene on chromosome 16p. It commonly appears with a leftward single  $\alpha$ -globin gene deletion ( $-\alpha^{4.2}$ ). The heterozygotes of HbQ-Thailand may show minor clinical symptoms or may be asymptomatic, whereas the double heterozygosity of it and thalassemia may lead to several clinical diseases[6, 7]. However, there have been few reports regarding the interaction between HbQ-Thailand and other globin gene disorders. Furthermore, the association among these disorders has important implications in clinical manifestation, laboratory diagnosis, and genetic counseling.

The Hakka people are intriguing Han Chinese populations that primarily reside in southern China, including Guangdong, Fujian, Jiangxi, and Taiwan. However, their cultural background, lifestyle, and customs are different from those of the southern Hans, and they have unique localisms[8]. Here we found a rare family of Hakka people who carry the genes of thalassemia/HbE (HBB:c.79G→A) and HbQ-Thailand, which is an under described condition at present.

## Results

The proband, a 35-year-old Chinese female from Longyan City of Fujian Province, displayed anemia with the following hematological parameters: RBC  $6.27 \times 10^{12}/L$ , Hb 93 g/L, MCV 45.9 fl, MCH 148 pg, and MCHC 323 g/L. The automated capillary electrophoresis (CE) (capillarys 2; Sebia, France) showed three dominant peaks, which revealed Hb variants and thalassemia. Most notable observation was that CE revealed a lack of adult hemoglobin, which is the main component in normal adult hemoglobin. Therefore, the proband and her twelve family members were referred to our laboratory for further investigation after obtaining informed consent. They are all Hakkas from Longyan City of Fujian Province. The pedigree of this family is shown in Fig. 1.

The 13 family members had no history of blood transfusion. Fresh peripheral blood samples were collected from all family members using a automated blood cell analyzer for hematological parameters analysis (Bc-5390; Mindray, China), and the results are shown in Table 1. The proband (Ⅰ1) and her sister (Ⅰ5) manifested in the obvious microcytic hypochromic anaemia. Analysis of hemoglobin components and variants was performed using a CE hemoglobin analyzer (capillary2™; Sebia, France). The results are shown in Table 1, and the electropherogram is shown in Fig. 2. The CE electropherogram of Ⅰ1 [Fig. 2 (a)] did not show a normal HbA band, however, there was an abnormal band in the migration time at 185 s, which was confirmed as HbQ-Thailand[9]; the corresponding HbQA<sub>2</sub> appeared in the migration time of 260 s. Another exception band appeared at 250 s of migration time and was proved to be HbE[10] inherited from his father. The CE electropherogram of Ⅰ1 [Fig. 2 (b)] shows an anomalous band HbE in the Z4 area. The peak type of family member Ⅱ3 is similar to Ⅰ1. The HbQ-Thailand of the proband originated from his mother (Ⅱ2), and her (Ⅱ2) CE electropherogram [Fig. 2 (c)] shows a HbQ-Thailand appearing in the Z7 region and HbQA<sub>2</sub> in the Z1 region. The Ⅱ4 and Ⅱ5 of family members are similar to the peak patterns. The sister (Ⅱ5) also has no normal HbA band (the same as the proband), and in her CE electropherogram [Fig. 2 (d)] in addition to HbQ-Thailand and HbQA<sub>2</sub>; HbQF peak appeared in the migration time 215 s, along with a fast swimming bands of HbH and Hb-Bart's. Figure 2 (e) is the CE electropherogram of the husband of the proband, and the result indicates that there is no presence of abnormal hemoglobin.



Table 1  
The hematological and molecular data of the family members under study.

Family members	sex-age (years)	Hb (g/L)	RBC (10 <sup>12</sup> /L)	MCV (fL)	MCH (pg)	HbA (%)	HbA <sub>2</sub> (%)	HbE (%)	HbQ-T (%)	HbQ-A2 (%)	αGenotype	βGenotype
Hb: hemoglobin; RBC: red blood cell counts; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; Hb Q-T: HbQ-Thailand												
Ⅰ <sub>1</sub>	M-61	121	5.14	75.9	23.6	80.0	3.9	16.1	0	0	– <sup>SEA</sup> /α	β <sup>codons26</sup> /β <sup>A</sup>
Ⅰ <sub>2</sub>	F-57	148	5.39	99.3	27.5	67.6	1.8	0	29.7	0.9	α/–α <sup>4.2-Q-Thailand</sup>	β <sup>A</sup> /β <sup>A</sup>
Ⅱ <sub>1</sub>	F-35	93	6.27	45.9	14.8	0	0	11.3	85.2	3.3	– <sup>SEA</sup> /–α <sup>4.2-Q-Thailand</sup>	β <sup>codons26</sup> /β <sup>A</sup>
Ⅱ <sub>2</sub>	M-36	152	5.07	94.3	30.0	97.2	2.8	0	0	0	α/α	β <sup>A</sup> /β <sup>A</sup>
Ⅱ <sub>3</sub>	M-34	130	5.71	72.6	22.7	80.6	4.0	15.4	0	0	– <sup>SEA</sup> /α	β <sup>codons26</sup> /β <sup>A</sup>
Ⅱ <sub>4</sub>	F-33	132	5.12	85.3	30.1	97.0	3.0	0	0	0	α/α	β <sup>A</sup> /β <sup>A</sup>
Ⅱ <sub>5</sub>	F-29	85	4.26	77.2	20.0	0	0	0	81.1	0.6	– <sup>SEA</sup> /–α <sup>4.2-Q-Thailand</sup>	β <sup>A</sup> /β <sup>A</sup>
Ⅱ <sub>6</sub>	M-35	158	5.08	93.1	30.8	97.4	2.6	0	0	0	α/α	β <sup>A</sup> /β <sup>A</sup>
Ⅲ <sub>1</sub>	M-11	118	6.17	61.9	19.1	97.6	2.4	0	0	0	– <sup>SEA</sup> /α	β <sup>A</sup> /β <sup>A</sup>
Ⅲ <sub>2</sub>	F-9	121	6.07	65.8	20.3	97.7	2.3	0	0	0	– <sup>SEA</sup> /α	β <sup>A</sup> /β <sup>A</sup>
Ⅲ <sub>3</sub>	M-5	119	4.33	92.6	27.5	97.1	2.9	0	0	0	α/α	β <sup>A</sup> /β <sup>A</sup>
Ⅲ <sub>4</sub>	F-12	128	5.05	88.9	27.3	68.5	1.9	0	28.8	0.8	α/–α <sup>4.2-Q-Thailand</sup>	β <sup>A</sup> /β <sup>A</sup>
Ⅲ <sub>5</sub>	M-6	112	4.51	87.1	24.8	68.2	2.0	0	29.0	0.8	α/–α <sup>4.2-Q-Thailand</sup>	β <sup>A</sup> /β <sup>A</sup>

The α-thalassemia deletions (–<sup>SEA</sup>/, –α<sup>4.2</sup>/, and –α<sup>3.7</sup>/) commonly found in Chinese populations were typed by gap-PCR. The three non deletional mutations (α<sup>CS</sup>α/, α<sup>QS</sup>α/, and αα<sup>WS</sup>/) and 17 species of β-mutant genes were performed by RDB assay in this subject. The results showed that the father (Ⅰ<sub>1</sub>) also carried α- and β-thalassemia genes. His genotype was –<sup>SEA</sup> and β<sup>codons26</sup>; –<sup>SEA</sup> was inherited to Ⅲ<sub>1</sub>, Ⅲ<sub>3</sub>, Ⅲ<sub>5</sub>, Ⅲ<sub>1</sub>, and Ⅲ<sub>2</sub>, and β<sup>codons26</sup> was inherited to Ⅲ<sub>1</sub> and Ⅲ<sub>3</sub>. The mother (Ⅰ<sub>2</sub>) carries the –α<sup>4.2</sup> gene, which was inherited to Ⅲ<sub>1</sub>, Ⅲ<sub>5</sub>, Ⅲ<sub>4</sub>, and Ⅲ<sub>5</sub>. The hemoglobin variant was identified by sequence analysis method. Ⅲ<sub>1</sub> and Ⅲ<sub>5</sub> were HbQ-Thailand homozygotes; Ⅲ<sub>2</sub>, Ⅲ<sub>4</sub> and Ⅲ<sub>5</sub> were HbQ-Thailand heterozygotes, and the sequencing results are shown in Fig. 3. The figures (a) was the father of the proband (Ⅲ<sub>1</sub>) sequencing diagram, in the α1-globin gene, the 74th base of G, is the normal sequence. The sequence diagram (b) was the result of the mother of the proband (Ⅲ<sub>2</sub>), showing the 74th base of G > C of the α1-globin gene, and the peak type was shown as heterozygote. The sequence diagram (c) was the result of the proband (Ⅲ<sub>1</sub>), showing the 74th

base of G > C of the  $\alpha 1$ - globin gene. It was shown as homozygote. We have summarized the genotypes of all family members with the results of hemoglobin analysis, the genetic tests, and generation sequencing in Table 1.

## Discussion

HbQ-Thailand is a hemoglobin variant caused by a point mutation in the  $\alpha$ -chain of hemoglobin, commonly associated with the left side deletion of the  $-\alpha^{4.2}$  [9]. This is verified by the presence of the HbQ-Thailand in the 5 family members carrying the deletion of  $-\alpha^{4.2}$ . HbQ-Thailand is generally asymptomatic, or the presence of the deletion of  $-\alpha^{4.2}$  may cause microcytic hypochromic anaemia [11]. Family members  $\#2$ ,  $\#4$ , and  $\#5$  carry HbQ-Thailand variant, accompanied with the deletion of  $-\alpha^{4.2}$ .  $\#5$  has slight anemia, whereas  $\#2$  and  $\#4$  are not characterized by anemia, with hematological parameters in the normal range. The proband ( $\#1$ ) and her sister ( $\#5$ ) were the coinheritance of HbQ-Thailand with  $-\alpha^{4.2}$  and  $-\text{SEA}$  deletion, they cannot synthesize normal alpha globin, owing to the absence of a normal alpha gene. They presented microcytic hypochromic anemia. Because of the deletion of 3  $\alpha$ -globin gene ( $-\text{SEA}/-\alpha^{4.2}$ ) and the HbQ-Thailand point mutation, only a small number of mutated alpha-globin chains were synthesized, creating a relative surplus of  $\beta$ -globin category, forming HbH ( $\beta_4$ ) and the Hb-Bart's ( $\gamma_4$ ); thus, the CE electropherogram shows two fast electrophoresis bands. In contrast, the proband ( $\#1$ ) is also carrying the  $\beta^{\text{codons}26}$  point mutation. In this individual, the protein  $\alpha$ -chain synthesis decreased and simultaneously, the  $\beta$ -chain synthesis decreased; hence, her anemia severity was less than that of the younger sister ( $\#5$ ). Thus, the combination of  $\alpha$ - and  $\beta$ -thalassemia, leads to more mildly anemia than a  $\alpha$ -thalassemia.

In general, HbQ-Thailand ( $\alpha^Q_2\beta_2$ ) is shown as a special peak presented at the Z7 region in the CE electropherogram, while HbQA<sub>2</sub> ( $\alpha^Q_2\delta_2$ ) is shown as a special peak presented at the zone1 region [Figure 2 (c)]. If the HbA and the zone7 band appear simultaneously, the individual is likely to be heterozygous for HbQ-Thailand, while the homozygous is missing the HbA. In this family, the CE electropherogram of  $\#2$ ,  $\#4$ , and  $\#5$  showed two peaks, HbA and HbQ-Thailand, which were manifestations of heterozygous mutations. The CE electropherogram of  $\#1$  and  $\#5$  did not show HbA peak, and only a high and sharp peak in a migration time of 185 s. Due to the absence of HbA and HbA<sub>2</sub> peaks, it was difficult to determine the specific zone corresponding to the anomaly in the original electropherogram; hence, the two specimens were mixed with the quality control products for testing to determine the zone position [12]; the obtained results were then combined with the results of genetic testing, to determine the genotype of  $-\text{SEA}/-\alpha^{4.2}$ -Q-Thailand. The absence of normal alpha globin chains in their erythrocytes result in the absence of HbA and HbA<sub>2</sub>. All specimens were tested by sequencing to verify the existence of mutations in HbQ-Thailand, of which  $\#2$ ,  $\#4$ , and  $\#5$  showed double peaks.  $\#1$  and  $\#5$  samples had single peaks. It verified the results of the CE electropherogram. It is noteworthy that when the alpha chain is mutated, the mutated alpha chain binds to the  $\delta$  chain, forming a HbQA<sub>2</sub> ( $\alpha^Q_2\delta_2$ ), which may cause false reduction of HbA<sub>2</sub> ( $\alpha_2\delta_2$ ), resulting in an  $\alpha$ -thalassemia misdiagnosis or  $\beta$ -thalassemia missed diagnosis. Therefore, in the CE

electropherogram, HbA<sub>2</sub> and HbQA<sub>2</sub> should be superimposed for the evaluation of the thalassemia. In general, in  $\alpha$ -thalassemia patients with decreased  $\alpha$ -chain synthesis, hemoglobin analysis showed a decline in HbA<sub>2</sub> ( $\alpha_2\delta_2$ ).  $\beta$ -thalassemia may lead to an increasing in the level of HbA<sub>2</sub> ( $\alpha_2\delta_2$ ), because of the reduction of  $\beta$ -chain and the increasing of  $\delta$ -chain and  $\gamma$ -chain accordingly. However, when  $\beta$ -thalassemia was combined with  $\alpha$ -thalassemia and/or  $\alpha$ -chain variant, the compound of  $\alpha$ -chain and  $\beta$ -chain was reduced concurrently, which could lead to a normal HbA<sub>2</sub> ratio. It may cause potential pitfalls for thalassemia. So, when screening of the thalassemia by hemoglobin analysis, we must pay attention, even if it is a normal HbA<sub>2</sub>, we cannot completely eliminate the possibility of thalassemia. The results of hemoglobin analysis should be evaluated with the results of assessment of erythrocyte parameters and the clinical characteristics of the patients, and a systematic diagnosis of thalassemia and hemoglobin diseases should be performed using gene testing and sequencing.

All the members of the family who participated in the study were from Longyan, Fujian Province (2 was from Changting County, 4 was from Liancheng County, and the rest were from Yongding County); all the participants were Hakkas. Hakkas in Guangdong, Fujian, Jiangxi, and Taiwan, being the southern ancient Han immigrant groups, regardless of factors such as language, folklore, and lifestyle, appear particularly mysterious. Here we found and diagnosed a rare hemoglobin disease in the Hakka population of the Fujian Province. This study provides an important reference for the clinic diagnose and genetic counseling of thalassemia and hemoglobin diseases in Hakka communities.

## Conclusion

It was complex to diagnose when the thalassemia combined with several abnormal haemoglobin disorders, and we may use various methods to mutual confirmation. Here we found and diagnosed a rare hemoglobin disease in the Hakka population of the Fujian Province. The study provides an important reference for the clinic diagnose and genetic counseling.

## Materials And Methods

Fresh peripheral blood samples were collected from the proband and her family members using a automated blood cell analyzer for hematological parameters analysis (Bc-5390; Mindray, China). Analysis of hemoglobin components and variants was performed using a CE hemoglobin analyzer (capillary2™; Sebia, France). The  $\alpha$ -thalassemia deletions ( $-\text{SEA}/$ ,  $-\alpha^{4.2}/$ , and  $-\alpha^{3.7}/$ ) commonly found in Chinese populations were typed by gap-PCR. The three non deletional mutations ( $\alpha^{\text{CS}}\alpha/$ ,  $\alpha^{\text{QS}}\alpha/$ , and  $\alpha\alpha^{\text{WS}}/$ ) and 17 species of  $\beta$ -mutant genes were performed by RDB assay in this subject. And the hemoglobin variants were identified by sequence analysis method.

## Abbreviations

Hb

hemoglobin; RBC:red blood cell; MCV:Mean Corpuscular Volume; MCH:Mean Corpuscular Hemoglobin; MCHC:Mean Corpuscular Hemoglobin Concentration; CE:capillary electrophoresis; RDB:reverse dot blot;

## **Declarations**

### **Acknowledgements**

Not applicable.

### **Authors' contributions**

Lin Zheng, Hailong Huang and Xiaoqing Wu prepared the main manuscript; Lin Zheng, Qingmei Shen and Meihuan Chen prepared the experiment. All authors have read and approved the final article.

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### **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Ethics approval and consent to participate**

The present study was approved by the Protection of Human Ethics Committee of Fujian Maternity and Child Health Hospital, affiliated hospital of Fujian Medical University (NO.2016-101).

### **Consent for publication**

Written informed consents were obtained from the patients for publication of this manuscript.

### **Competing interests**

The authors declare that they have no competing interests.

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Figures

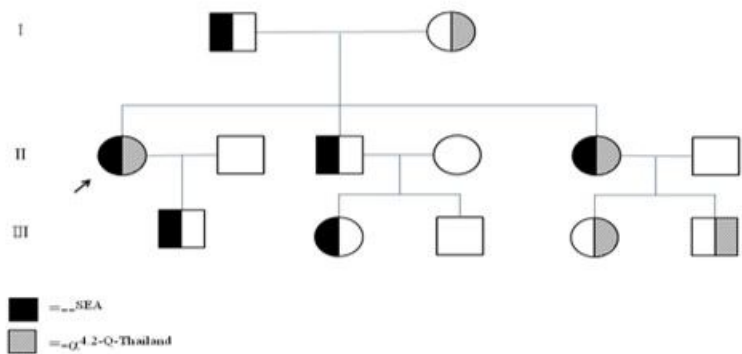
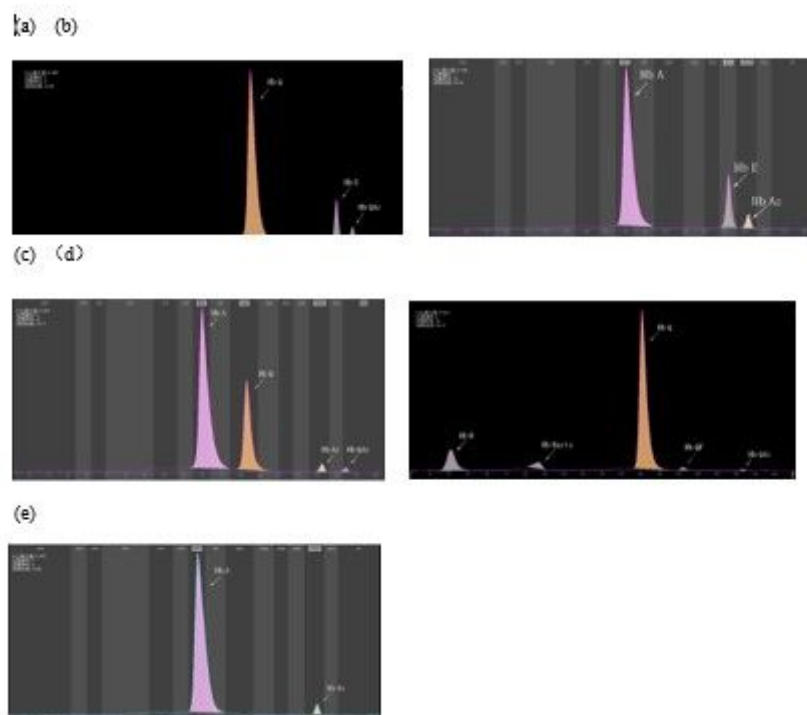


Figure1. Pedigree of the family

Figure 1

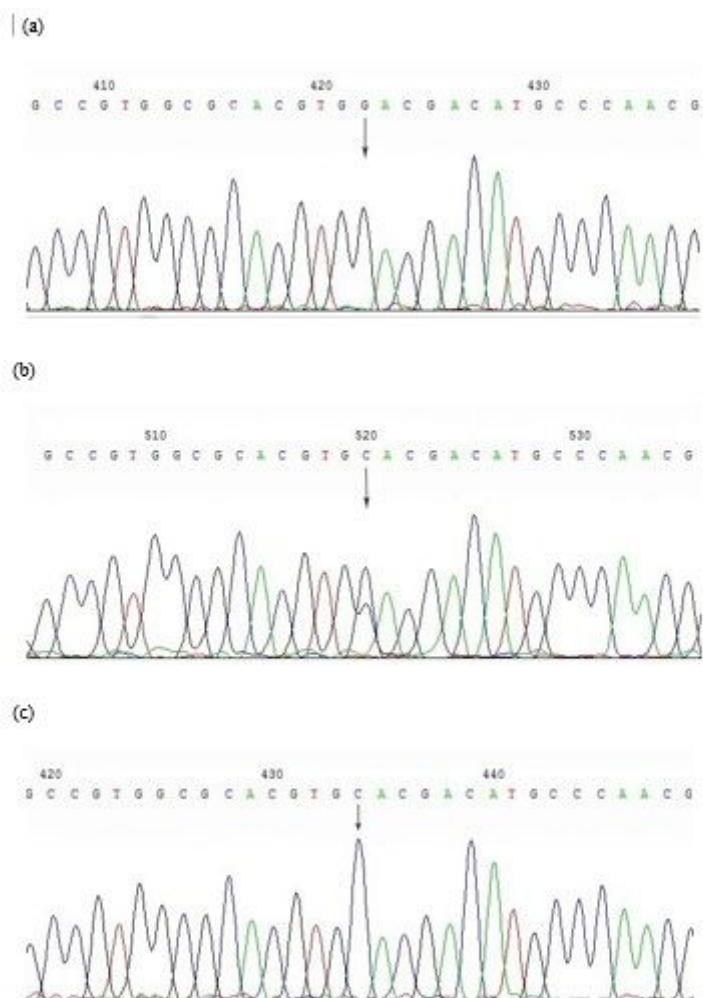
Pedigree of the family





**Figure 2**

Hemoglobin analysis of the proband and family members using CE. (a) The proband; (b) the father; (c) the mother; (d) the sister; (e) the husband. HbA, HbQ-Thailand, HbA2, HbQA2, HbQF, HbE, HbH, and HB-Bart's are indicated by arrows.



**Figure 3**

DNA sequence analysis of the amplified  $\alpha 1$ -globin gene from the proband and her family members. (a) The father; (b) the mother; (c) the proband. The downward arrow indicated the G  $\rightarrow$  C substitution at codon 74 of the  $\alpha 1$ -globin gene.