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Molecular spectrum and carrier frequency of deletional hereditary persistence of fetal hemoglobin and delta-beta thalassemia in Malaysia

Faidatul Syazlin Abdul Hamid[1,2], Sabariah Md Noor[2*], Mei I Lai[2], Samsol Kamal Mohd Bahari[2], Ezalia Esa[3], Ermi Neiza Mohd Sahid[1], Norafiza Mohd Yasin[1] and Yuslina Mat Yusoff[1*]

Abstract

Purpose Thalassemia is a major public health concern in Southeast Asia, particularly in Malaysia, where a high carrier rate places significant pressure on healthcare systems. Hereditary Persistence of Fetal Hemoglobin (HPFH) and delta-beta (δβ) thalassemia are genetic conditions associated with elevated levels of fetal hemoglobin (Hb F). This study aimed to determine the frequency of common beta (β)-globin gene cluster deletions among Malaysian carriers of HPFH or δβ thalassemia, while also providing an overview of the thalassemia burden in the region.

Methods A retrospective study was conducted on 534 blood samples submitted to the Institute for Medical Research (IMR), Malaysia, for β-thalassemia genotyping between January 2017 and December 2019. Demographic data, including full blood count parameters and hemoglobin (Hb) analysis, were retrieved. Deoxyribonucleic acid (DNA) was extracted and analyzed using Multiplex Gap-Polymerase Chain Reaction (PCR) to detect large deletions in the β-globin gene cluster.

Results Seven distinct deletions were identified among the 534 heterozygous carriers. The two most common deletions were $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassemia Siriraj I (~ 118 kilobase pairs [kb]) and $\delta\beta^{0}$-thalassemia Thai (~ 12.5 kb), accounting for 30.0% and 29.8% of cases, respectively. The HPFH-6 deletion was observed in 20.0% of cases, followed by $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassemia Asian-Indian Inversion-Deletion (Inv/Del) (14.2%), $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassemia Chinese (~ 100 kb) (4.3%), HPFH-3 (0.9%), and $^{G}\gamma(^{A}\gamma\delta\beta)^{0}$-thalassemia Asian (~ 49.3 kb) (0.7%). The ethnic distribution showed a predominance among Malay patients (93.4%), with specific deletions suggesting ethnic clustering. Genotype–phenotype analysis revealed notable variations in hematological parameters: carriers of HPFH-3 had the highest Hb F levels ($25.3 \pm 3.1\%$) as measured by high-performance liquid chromatography (HPLC) and showed the least severe microcytosis, while carriers of $\delta\beta^{0}$-thalassemia Thai (~ 12.5 kb) demonstrated more pronounced hematological abnormalities. Findings were consistent with previous reports from Southeast Asia, underscoring the importance of incorporating molecular diagnostics into national screening programs. Although Multiplex Gap-PCR is robust, further studies using Next-Generation Sequencing (NGS) and Multiplex Ligation-dependent Probe Amplification (MLPA) are recommended to detect rare or undetected mutations.

*Correspondence:
Sabariah Md Noor
md_sabariah@upm.edu.my
Yuslina Mat Yusoff
yuslina.my@moh.gov.my
Full list of author information is available at the end of the article

Conclusions This study provides crucial data on the molecular spectrum of HPFH and δβ thalassemia in Malaysia, contributing to improved diagnostic strategies and genetic counselling. Future research should explore additional genetic variants to enhance national thalassemia prevention programs.

Keywords Delta, Beta thalassemia, Multiplex Gap, PCR, Hereditary Persistence of Fetal Hemoglobin, Large deletions of the β, Globin gene cluster, Polymerase chain reaction, High Hb F

Introduction

Thalassemia is a significant public health burden in Southeast Asia, particularly in Malaysia, where a high carrier rate contributes to the strain on Healthcare systems. In Malaysia, it is estimated that approximately 4.5% of the population are beta (β)-thalassemia carriers, with certain ethnic groups, such as Malays and Chinese, showing higher prevalence rates [1, 2]. The disease imposes substantial economic and healthcare burdens due to the lifelong need for blood transfusions and iron chelation therapy among patients with thalassemia major [3, 4].

To mitigate the impact of thalassemia, Malaysia has implemented several prevention strategies, including nationwide carrier screening programs, premarital screening, and genetic counselling [5–7]. The National Thalassemia Prevention and Control Program aims to reduce the number of new thalassemia major cases by promoting early detection and reproductive counselling for at-risk couples [3]. Despite these initiatives, gaps remain in the identification of less common hemoglobinopathies, such as Hereditary Persistence of Fetal Hemoglobin (HPFH) and delta-beta (δβ) thalassemia, highlighting the need for further molecular studies.

HPFH and δβ thalassemia are conditions associated with decreased β-globin gene expression, leading to compensatory increases in fetal hemoglobin (Hb F). In δβ thalassemia, large deletions within the β-globin gene cluster result in Hb F overproduction. Carriers of δβ thalassemia typically present with hypochromic, microcytic red blood cells, normal hemoglobin A_2 (Hb A_2) levels (< 3.0%), and elevated Hb F levels (5–15%) [8]. When inherited together with a β-thalassemia mutation, the condition can result in thalassemia intermedia or thalassemia major.

In contrast, carriers of HPFH generally have normal red blood cell indices and moderately elevated Hb F levels (15–30%) [8]. Individuals with homozygous HPFH typically exhibit normal clinical profiles, although those co-inheriting HPFH with β-thalassemia mutations may develop mild anemia [8].

Despite extensive research in other Southeast Asian populations, data on the molecular spectrum of these deletions in Malaysia remain limited. This study aimed to determine the frequency of common β-globin gene cluster deletions among Malaysian patients who are carriers of HPFH or δβ thalassemia, while also providing an overview of the thalassemia burden in the region.

Materials and Methods

Study background

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health (MOH), Malaysia (NMRR-19–37-46,156). Written informed consent for the use of clinical information and molecular genotyping was obtained from all participants prior to blood collection. All collected data were anonymized, and confidentiality was maintained through appropriate coding procedures.

This was a retrospective study that utilized secondary data, including patients' clinical information and stored deoxyribonucleic acid (DNA) specimens. The study included all confirmed cases of deletional δβ thalassemia referred between January 2017 and December 2019. Inclusion criteria comprised cases with hemoglobin (Hb) analysis reports suggestive of HPFH or δβ thalassemia carrier status, molecularly confirmed δβ thalassemia cases, and individuals of Malaysian nationality. Exclusion criteria included samples from individuals under two years of age, samples with incomplete clinical data, inadequate or poor-quality stored DNA, and cases with other causes of elevated Hb F levels greater than 5%, such as Hb Lepore (a hybrid δβ-chain).

Universal sampling was employed in this study, followed by backward tracing to obtain the required information. The sample list was compiled by identifying cases that had undergone molecular testing for thalassemia and hemoglobinopathy confirmation. Based on this list, the corresponding request forms and stored DNA specimens were retrieved. For samples with sufficient stored DNA, demographic information, clinical history, and hematological profiles (including full blood count parameters and Hb analysis results) were obtained from the request forms.

Molecular analysis

DNA from the samples was extracted using the QIAsymphony DSP DNA Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was measured using the NanoDrop

1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The samples were analyzed using a previously reported Multiplex Gap- Polymerase Chain Reaction (PCR) method, with minor modifications, to screen for common HPFH and δβ thalassemia deletions across three separate panels [9–11].

The first panel was designed to detect eight common β-globin gene cluster deletions found in Southeast Asia [9]. In addition to HPFH and δβ thalassemia deletions, this panel was also used to rule out β-thalassemia deletions. Specifically, it was capable of detecting the following deletions: $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kilobase pairs [kb]) deletion, δβ°-thalassemia Thai (~ 12.5 kb) deletion, HPFH-6 deletion, Hb Lepore deletion, β°-thalassemia Southeast Asian (SEA) (~ 27 kb) deletion, β°-thalassemia Filipino (~ 118 kb) deletion, β°-thalassemia (3.5 kb) deletion and β°-thalassemia (619 base pairs [bp]) deletion.

The PCR reaction for the first panel was performed in a final volume of 25 µl (µL) using the HotStarTaq® Master Mix Kit, which includes 2.5 units of HotStarTaq DNA Polymerase, 1X PCR buffer with 1.5 mM (mM) magnesium chloride ($MgCl_2$), and 200 micromolar (µM) of each deoxynucleotide triphosphate (dNTP) (QIAGEN GmbH, Hilden, Germany). The reaction also contained 0.5X Q-Solution (QIAGEN GmbH, Hilden, Germany), primers at varying concentrations (Supplementary Table S1), and 100 nanograms (ng) of genomic DNA. Amplifications were carried out using an Eppendorf Mastercycler® pro S (Eppendorf, Hamburg, Germany) under the following thermal cycling conditions: initial denaturation at 96 degrees Celsius (°C) for 15 min; followed by 30 cycles of 94 °C for 60 s, 62 °C for 120 s, 59 °C for 60 s, and 72 °C for 60 s; with a final extension at 72 °C for 10 min. PCR products were separated on 1.2% weight/volume (w/v) agarose gels for 50 min at 110 V (V) and visualized under an ultraviolet (UV) transilluminator (Supplementary Figure S1).

The second panel was used to detect the $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) deletion, HPFH-3 deletion and $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian (~ 49.3 kb) deletion [10, 11]. PCR reactions were performed in a final volume of 25 µL using the HotStarTaq® Master Mix Kit, which contains 2.5 units of HotStarTaq DNA Polymerase, 1X PCR buffer with 1.5 mM $MgCl_2$, and 200 µM of each dNTP (QIAGEN GmbH, Hilden, Germany). The reaction also included 0.5X Q-Solution (QIAGEN GmbH, Hilden, Germany), primers at varying concentrations (Supplementary Table S2), and 100 ng of genomic DNA. Amplifications were conducted using an Eppendorf Mastercycler® pro S (Eppendorf, Hamburg, Germany) under the following cycling conditions: initial denaturation at 96 °C for 15 min; followed by 38 cycles of 96 °C for 30 s, 58.7 °C for 30 s, and 72 °C for 60 s; with a final extension at 72 °C for 7 min. PCR products were separated on 1.5% (w/v) agarose gels for 50 min at 110 V and visualized under a UV transilluminator (Supplementary Figure S2).

The final panel was used to detect the $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inversion-Deletion (Inv/Del) [10]. PCR reactions were performed in a final volume of 25 µL using the HotStarTaq® Master Mix Kit, which contains 2.5 units of HotStarTaq DNA Polymerase, 1X PCR buffer with 1.5 mM $MgCl_2$, and 200 µM of each dNTP (QIAGEN GmbH, Hilden, Germany). The reaction also included 0.5X Q-Solution (QIAGEN GmbH, Hilden, Germany), primers at varying concentrations (Supplementary Table S3), and 100 ng of genomic DNA. Amplifications were carried out using an Eppendorf Mastercycler® pro S (Eppendorf, Hamburg, Germany) under the following cycling conditions: initial denaturation at 96 °C for 15 min; followed by 33 cycles of 94 °C for 60 s, 58 °C for 60 s, and 72 °C for 60 s; with a final extension at 72 °C for 7 min. PCR products were separated on 1.5% (w/v) agarose gels for 50 min at 110 V and visualized under a UV transilluminator (Supplementary Figure S3).

Results

Demographic Characteristics

A total of 534 samples meeting the inclusion and exclusion criteria were included in the final analysis. The study population demonstrated a wide age distribution ranging from 2 to 74 years, with a median age of 16 years (interquartile range: 13–19 years), reflecting the diverse screening programs from which samples were derived. The gender distribution showed a predominance of females (n = 342, 64.0%) compared to males (n = 192, 36.0%).

Ethnic distribution analysis revealed a marked predominance of Malay patients (n = 499, 93.4%), followed by Chinese patients (n = 29, 5.5%), Indian patients (n = 4, 0.7%), and Siamese and other ethnicities with one case each (0.2% respectively) (Fig. 1). This distribution largely reflects the demographic composition of Malaysia and the referral patterns to the national reference laboratory.

The main indication for thalassemia screening was high school screening programs (n = 366, 68.5%), demonstrating the effectiveness of the national school-based screening initiative. Other indications included general health screening and premarital screening (n = 64, 12.0%), antenatal screening (n = 55, 10.3%), and cascade screening of family members (n = 49, 9.2%).

Molecular Genotyping Results

Comprehensive molecular analysis using three sequential Multiplex Gap-PCR panels included detection of the following deletions: $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj

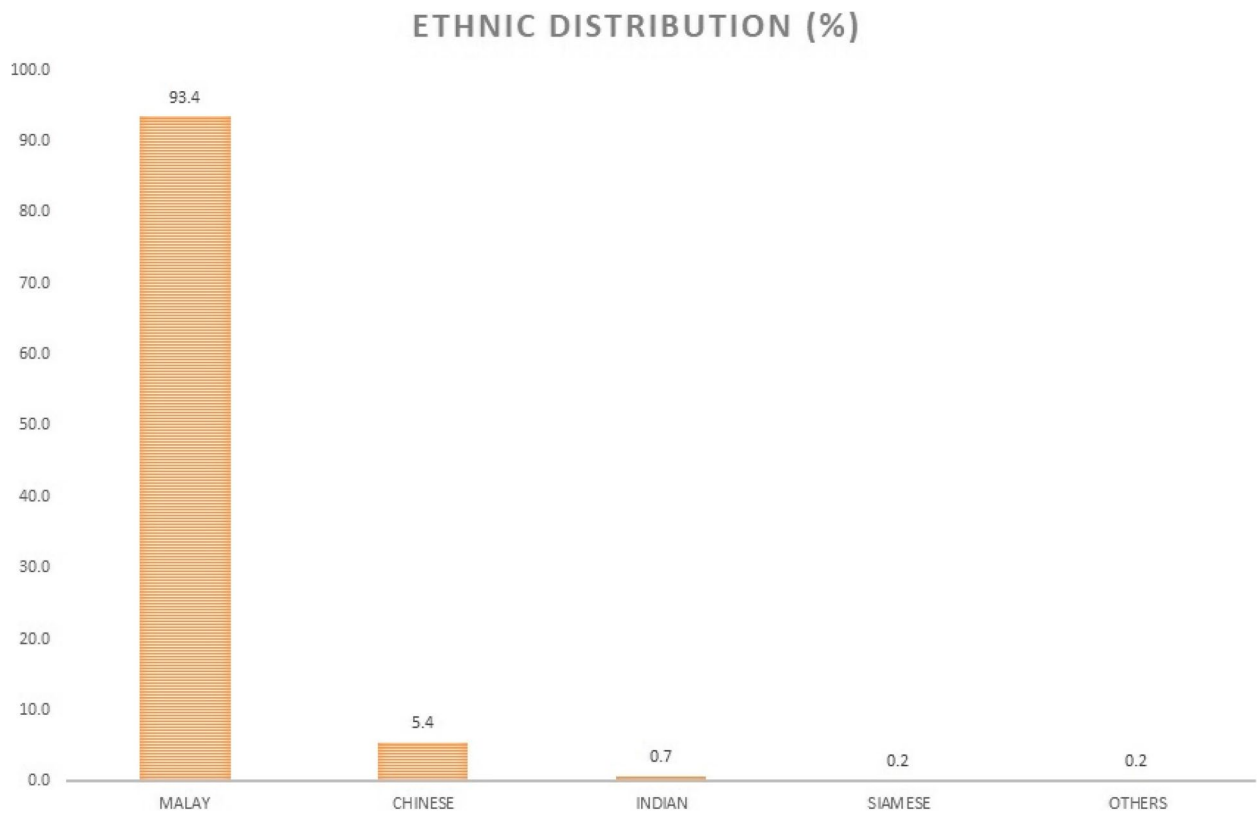

Fig. 1 The ethnic distribution of the samples in this study shows that the majority of cases were Malay, followed by Chinese, Indian, Siamese and others

Table 1 Frequency of heterozygous large deletions in the β-globin gene cluster

Type of deletion	Frequency, n (%)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb)	160 (30.0)
$(\delta\beta)^{\circ}$-thalassemia Thai (~ 12.5 kb)	159 (29.8)
HPFH-6	107 (20.0)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del	76 (14.2)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb)	23 (4.3)
HPFH-3	5 (0.9)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian (~ 49.3 kb)	4 (0.7)

n number of samples

I (~ 118 kb) deletion, δβ°-thalassemia Thai (~ 12.5 kb) deletion, HPFH-6 deletion, $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del, $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) deletion, HPFH-3 deletion, and $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian (~ 49.3 kb) deletion. Table 1 shows seven distinct types of large deletions in the β-globin gene cluster among the 534 heterozygous carriers.

The most common deletion was $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb) deletion, identified in 160 cases (30.0%). This was followed closely by δβ°-thalassemia Thai (~ 12.5 kb) deletion, observed in 159 cases (29.8%). The HPFH-6 deletion represented the third most common variant, present in 107 cases (20.0%), followed by $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del, identified in 76 cases (14.2%). Less frequently observed deletions included $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) deletion (n = 23, 4.3%), HPFH-3 deletion (n = 5, 0.9%), and $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian (~ 49.3 kb) deletion (n = 4, 0.7%).

Ethnic Distribution of Deletions

Analysis of deletion distribution by ethnicity revealed distinct patterns that reflect both population genetics and historical migration (Table 2). Among Malay individuals, the most prevalent deletions were δβ°-thalassemia Thai (~ 12.5 kb) (n = 158, 29.6%) and $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb) (n = 154, 28.8%), followed by HPFH-6 (n = 105, 19.7%) and $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del (n = 75, 14.0%). In the Chinese population, a markedly different pattern emerged, with $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) deletion being the predominant variant (n = 22, 4.1% of total cohort), representing 75.9% of all deletions identified in Chinese individuals. Meanwhile, the HPFH-3 deletion was primarily

Table 2 Frequency distribution of heterozygous HPFH and δβ thalassemia deletions by ethnicity

Type of deletion	Ethnicity, n (%)					
	Malay	Chinese	Indian	Siamese	Others	Total
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb)	154 (28.8)	4 (0.7)	1 (0.2)	1 (0.2)		160 (30.0)
$(\delta\beta)^{\circ}$-thalassemia Thai (~ 12.5 kb)	158 (29.6)	1 (0.2)				159 (29.8)
HPFH-6	105 (19.7)	2 (0.4)				107 (20.0)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del	75 (14.0)				1 (0.2)	76 (14.2)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb)	1 (0.2)	22 (4.1)				23 (4.3)
HPFH-3	2 (0.4)		3 (0.6)			5 (0.9)
$^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian (~ 49.3 kb)	4 (0.7)					4 (0.7)
Total	**499 (93.4)**	**29 (5.4)**	**4 (0.7)**	**1 (0.2)**	**1 (0.2)**	**534 (100)**

n number of samples

detected among individuals of Indian descent (n = 3, 0.6%).

Genotype–Phenotype Correlations

Comprehensive hematological analysis revealed significant variations in clinical parameters across different deletion types (Table 3). Patients with $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb) and HPFH-6 deletions showed the highest mean Hb levels (13.4 ± 1.6 g per deciliter [g/dL] and 13.4 ± 1.7 g/dL, respectively), as well as relatively higher mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values, indicating a less severe microcytic profile. Conversely, carriers of the $\delta\beta^{\circ}$-thalassemia Thai (~ 12.5 kb) and HPFH-3 deletions had lower Hb levels (12.8 ± 1.5 g/dL and 12.2 ± 2.0 g/dL, respectively) and more pronounced microcytosis, with the lowest MCV and MCH values observed in the $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) deletion group (68.8 ± 3.5 femtoliters [fL] and 22.3 ± 1.3 picograms [pg], respectively).

Hb F levels, measured using both high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), showed significant variation among genotypes. The HPFH-3 group exhibited the highest Hb F levels (25.3 ± 3.1% by HPLC; 29.6 ± 3.6% by CE), followed by HPFH-6 and $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb) deletions. In contrast, the $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) and $\delta\beta^{\circ}$-thalassemia Thai (~ 12.5 kb) groups showed relatively lower Hb F percentages (13.8 ± 3.0% and 16.7 ± 4.3% by HPLC, respectively).

Hb A_2 levels remained within or slightly below the normal range across all deletion types, which is characteristic of δβ thalassemia and HPFH. This finding is diagnostically significant, as it helps distinguish these conditions from β-thalassemia trait, which typically shows elevated Hb A_2 levels.

Discussion

This study provides valuable insights into the molecular spectrum of HPFH and δβ thalassemia deletions in Malaysia. The findings contribute crucial information on the genetic architecture of these conditions and establish baseline data that can improve diagnostic strategies and genetic counselling programs.

The identification of seven distinct deletion types among 534 patients who were carriers demonstrates the remarkable genetic diversity of HPFH and δβ thalassemia in Malaysia. The high frequency of $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb) and $\delta\beta^{\circ}$-thalassemia Thai (~ 12.5 kb) deletions, each accounting for 30.0% and 29.8% of cases, respectively, suggests that these represent the predominant deletions in the Malaysian population. As shown in Table 2, these deletions were more commonly observed in Malay patients, reinforcing findings from previous Southeast Asian studies that reported similar genetic distributions [9, 12, 13]. The HPFH-6 deletion, detected in 20.0% of cases, was the third most prevalent variant. This is consistent with prior research from Thailand, where HPFH-6 has also been reported as a common deletion in Southeast Asia [9, 12, 13]. Table 2 further illustrates the geographical distribution of HPFH-6 cases, showing a concentration in regions with high Malay populations.

Interestingly, $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del was predominantly identified in Malay individuals. This observation suggests possible genetic admixture due to historical migration patterns. The presence of $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del, typically reported in Indian populations [14], among Malay patients may reflect centuries of regional trade, intermarriage, and migration between the Indian subcontinent and the Malay Peninsula. Similarly, the $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb) deletion was found mainly in Chinese participants, corroborating

Table 3 Overall hematological parameters of heterozygous HPFH and δβ thalassemia deletions in Malaysia, according to type of deletion

Type of deletion	n	Parameter (Mean ± SD)													
		Age (years old)	RBC (10^{12}/L)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW-CV (%)	HPLC			CE		
										Hb A (%)	Hb A_2 (%)	Hb F (%)	Hb A (%)	Hb A_2 (%)	Hb F (%)
Siriraj I ~ 118 kb[1]	160	3–59	5.5 ± 0.6	13.4 ± 1.6	41.3 ± 4.6	75.0 ± 4.6	24.3 ± 1.6	32.4 ± 1.8	15.8 ± 5.2	68.7 ± 4.3	2.4 ± 0.3	20.5 ± 2.9	75.1 ± 2.5	2.1 ± 0.2	22.6 ± 2.5
Thai ~ 12.5 kb[2]	159	2–74	5.7 ± 0.7	12.8 ± 1.5	39.7 ± 4.5	70.4 ± 4.8	22.7 ± 1.9	32.2 ± 1.3	20.8 ± 3.8	71.1 ± 4.7	2.4 ± 0.3	16.7 ± 4.3	80.5 ± 5.0	2.2 ± 0.3	17.3 ± 5.0
HPFH-6	107	3–66	5.4 ± 0.7	13.4 ± 1.7	41.2 ± 5.0	76.0 ± 5.6	24.8 ± 2.2	32.7 ± 1.3	15.6 ± 2.0	65.6 ± 4.0	2.3 ± 0.2	22.3 ± 2.6	73.9 ± 3.1	2.0 ± 0.2	24.3 ± 2.9
Asian-Indian Inv/Del[3]	76	10–45	5.5 ± 0.6	12.9 ± 1.5	39.9 ± 4.5	72.8 ± 4.2	23.6 ± 1.7	32.3 ± 1.3	16.8 ± 1.8	71.5 ± 2.9	2.5 ± 0.3	16.1 ± 2.4	80.6 ± 2.6	2.3 ± 0.3	17.2 ± 2.6
Chinese ~ 100 kb[4]	23	3–55	5.9 ± 0.8	13.2 ± 1.6	40.8 ± 4.6	68.8 ± 3.5	22.3 ± 1.3	32.4 ± 1.5	18.5 ± 2.5	75.4 ± 4.6	2.9 ± 0.3	13.8 ± 3.0	82.6 ± 3.8	2.5 ± 0.2	14.8 ± 3.4
HPFH-3	5	16–50	5.1 ± 0.3	12.2 ± 2.0	37.4 ± 5.1	73.3 ± 7.0	24.0 ± 3.3	32.6 ± 1.4	16.3 ± 1.9	59.3 ± 2.5	2.1 ± 0.3	25.3 ± 3.1	67.1 ± 2.0	1.6 ± 0.4	29.6 ± 3.6
Asian ~ 49.3 kb[5]	4	16–43	5.8 ± 0.8	13.3 ± 1.4	37.2 ± 3.4	70.3 ± 7.1	23.1 ± 1.9	33.1 ± 0.2	19.3 ± 1.8	67.4 ± 0.0	2.6 ± 0.3	16.1 ± 4.5	78.9 ± 0.7	2.4 ± 0.2	19.9 ± 2.0

n number of samples, *SD* standard deviation, *RBC* red blood cells, 10^{12}/*L* trillion per liter, *Hb* haemoglobin, *g/dL* grams per deciliter, *HCT* hematocrit, *MCV* mean corpuscular volume, *fL* femtoliter, *MCH* mean corpuscular hemoglobin, *pg* picogram, *MCHC* mean corpuscular hemoglobin concentration, *RDW-CV* red cell distribution width – coefficient of variation, *HPLC* high-performance liquid chromatography, *CE* capillary electrophoresis, *Hb A* hemoglobin A, *Hb* A_2 hemoglobin A_2, *Hb F*, fetal hemoglobin/hemoglobin F

[1] δβ°-thalassemia Thai (~ 12.5 kb)

[2] $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~ 118 kb)

[3] $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian-Indian Inv/Del

[4] $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~ 100 kb)

[5] $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Asian (~ 49.3 kb)

previous reports that this mutation is prevalent among Chinese populations [15–17].

Overall, this study enhances understanding of HPFH and δβ thalassemia deletions in Malaysia, with findings that may contribute to improved diagnostic and genetic counselling strategies. The high frequency of $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~118 kb) and δβ°-thalassemia Thai (~12.5 kb) deletions aligns with findings from other Southeast Asian studies, reinforcing their genetic predominance in the region [9, 12, 13]. The presence of HPFH-6 in 20.0% of cases further suggests that this deletion is relatively common among Malaysians, as previously reported in studies conducted in Thailand [9, 12, 13].

The ethnic clustering of specific deletions provides compelling evidence for population-specific genetic architectures. The predominance of $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~100 kb) deletion among Chinese individuals (75.9% of Chinese cases) and HPFH-3 among Indian participants (75% of Indian cases) demonstrates the importance of ethnicity-guided screening strategies. These findings have immediate practical implications for targeted screening programs and risk assessment protocols.

The genotype–phenotype correlations observed in this study reveal important clinical associations that improve understanding of the pathophysiology and diagnostic implications of these conditions. The observation that carriers of HPFH-3 had the highest Hb F levels (25.3 ± 3.1% by HPLC) while maintaining relatively preserved hemoglobin levels supports the classification of this variant as a true HPFH condition rather than δβ thalassemia. The more severe microcytic changes observed in $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Chinese (~100 kb) and δβ°-thalassemia Thai (~12.5 kb) carriers, combined with their lower Hb F levels, suggest more significant impairment of globin chain balance. These findings have important implications for genetic counselling, as carriers of these deletions may be at higher risk of producing offspring with more severe phenotypes when combined with other β-globin mutations.

The consistent pattern of normal or slightly reduced Hb A_2 levels across all deletion types confirms the diagnostic utility of this parameter in differentiating HPFH and δβ thalassemia from typical β-thalassemia trait. This supports the incorporation of comprehensive hemoglobin analysis into routine screening protocols.

Despite the strengths of this study, several limitations must be acknowledged. As a retrospective analysis, the study relied on pre-existing clinical data, which may have introduced selection bias. Additionally, while Multiplex Gap-PCR is a valuable screening tool, it cannot detect all mutations affecting the β-globin gene cluster, particularly large or atypical deletions. This limitation underscores the need for complementary molecular approaches.

Next-Generation Sequencing (NGS) offers high-throughput, sequence-level resolution and can simultaneously detect point mutations, small insertions or deletions, and some copy number variations across the globin genes. However, NGS may have reduced sensitivity for certain large deletions or complex rearrangements due to its reliance on short-read sequencing, coverage variability, challenges in repetitive genomic regions, and susceptibility to GC-content bias [18–20]. In such cases, Multiplex Ligation-dependent Probe Amplification (MLPA) serves as a cost-effective and targeted alternative for detecting large deletions and duplications within the β-globin gene cluster [21]. Future studies integrating both NGS and MLPA could provide a more accurate and comprehensive assessment of the full spectrum of genetic variations.

Conclusions

This study highlights the molecular diversity of HPFH and δβ thalassemia deletions in Malaysia. The identification of seven distinct deletion types among 534 carriers, with significant ethnic clustering and phenotypic variation, underscores the complexity of hemoglobinopathy genetics in this diverse population. The predominance of $^{G}\gamma(^{A}\gamma\delta\beta)^{\circ}$-thalassemia Siriraj I (~118 kb) deletion and δβ°-thalassemia Thai (~12.5 kb) deletion, along with the significant frequency of HPFH-6, provides important guidance for the development of targeted screening strategies. The establishment of clear genotype–phenotype correlations enhances diagnostic capabilities and supports more accurate genetic counselling.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44313-025-00100-7.

Supplementary Material 1.

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Authors' contributions

Conceptualization, F.S.A.H., S.M.N., L.M.I. and Y.M.Y.; methodology, F.S.A.H. and S.K.M.B.; validation, E.N.M.S., N.M.Y., E.E. and Y.M.Y.; formal analysis, F.S.A.H.; investigation, F.S.A.H. and S.K.M.B.; resources, N.M.Y. and E.E.; writing—original draft preparation, F.S.A.H.; writ-ing—review and editing, S.M.N., L.M.I. and Y.M.Y.; visualization, F.S.A.H.; supervision, S.M.N., L.M.I. and Y.M.Y.; project

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent to participate
Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare no competing interests.

Author details
[1]Hematology Unit, Cancer Research Centre, Institute for Medical Research, National Institutes of Health, Seksyen U13 Setia Alam, Shah Alam, Selangor 40170, Malaysia. [2]Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia. [3]Virology Unit, Infection Disease Research Centre, Institute for Medical Research, National Institutes of Health, Seksyen U13 Setia Alam, Shah Alam, Selangor 40170, Malaysia.

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