Original Article

Common Alpha Globin Deletion Mutation Spectrum in Hemoglobin H Disease Patients in the Mekong Delta, Vietnam

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Abstract

Introduction: Hemoglobin H disease is a type of α-thalassemia brought on by a shortage in the generation of hemoglobin globin chains (Hb). The patients produce a form of hemoglobin called hemoglobin H by inactivation of three α-globin genes. In this paper, we report the presence of the four deletion mutations of Southeast Asia in hemoglobin H disease patients in the Mekong Delta, Vietnam.

Methods: DNA from 50 hemoglobin H disease patients were extracted from EDTA-anticoagulated whole blood and screened for the four common α-globin deletion mutations using Gap-Polymerase Chain Reaction.

Results: The most common type of deletion was $--^{SEA}$ deletion, accounting for 73.5% of the mutant alleles, followed by the $-\alpha^{3.7}$ (rightward) deletion (19.1%) and $-\alpha^{4.2}$ (leftward) deletion (7.4%) mutation in this region. In this study, the $--^{THAI}$ mutation was not detected.

Conclusions: This study gave an overview of the prevalence of typical α-globin gene mutations in Vietnam and might act as a starting point for a further research into these genetic flaws.

Keywords: Gap-PCR, HbH disease, α-thalassemia, Mekong Delta, Vietnam

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Introduction

Thalassemias are inherited anemia diseases that are brought on by a shortage in the generation of hemoglobin globin chains (Hb). The kind of the afflicted globin chain determines the name given to the condition, thalassemia. The two most prevalent kinds of thalassemia are α-thalassemia and β-thalassemia. All conditions in which there is a shortage in the formation of hemoglobin α-globin chains are referred to the term α-thalassemia. The α-globin gene mutation is carried by about 7.0 percent of the world's populations.[1,2]

Four genes with two α-globin chains (HBA2 and HBA1) encode the α-globin. Two α-globin genes of each chromosome 16 (αα/αα) are found on the short arm of healthy people. One (-α) or both (--) α-globin genes are deleted in more than 95.0 percent of α-thalassemia mutations; the remaining mutations are brought on by non-deletions.[3]

α-thalassemia-2 (-α/αα) is a condition caused by the deletion of one of two related genes on a single chromosome. The two most prevalent variants of this deletion are types $-\alpha^{3.7}$ and $-\alpha^{4.2}$. Larger deletions are known as α-thalassemia-1 (--/αα), and they are connected to the loss of both α genes. The most prevalent form of α-thalassemia-1 in Southeast Asia is α-thalassemia-1 with $--^{SEA}$ deletion.[4] The three out of four α genes (--/-α) are deleted in hemoglobin H disorder. There are no functional α genes (--/--) in hemoglobin Bart's fetalis syndrome (--/--).[5] The most prevalent form of α-thalassemia worldwide is α-thalassemia-2, with an estimated frequency of 30 to 50 percent.[6]

Hematological and clinical problems are not always linked to having α-thalassemia-2 heterozygosity. Affected individuals with heterozygous α-thalassemia-1 or homozygous α-thalassemia-2 may have mild microcytic and hypochromic red blood cell characteristics. However, they remain clinically quiet. Since it will change the illness phenotype and become clinically significant when it interacts with other thalassemia genes, this interaction is problematic. A more severe type α-thalassemia that results from the interplay of α-thalassemia-1 and α-thalassemia-2 is hemoglobin H disease.[4,7] There is just one functional alpha globin gene, and the affected individual produces hemoglobin H, a four β-chain (β4) type of hemoglobin.

Hemoglobin H disease comes in two types: deletion and non-deletion. The first type, which is the most prevalent form of hemoglobin H disease, is brought on by compound heterozygosity, which results in a double α-globin gene deletion on one allele and a single α-globin gene deletion on the other allele (16p13.3). The second kind of hemoglobin H disease is characterized by at least one of the genetic abnormalities- non-deletion.[8] Non-deletion hemoglobin H disease is relatively rare; Usually, homozygosity for non-deletional alleles causes non-deletion hemoglobin H disease, but it may also result from compound heterozygosity for a double α-globin gene deletion on one chromosome and a point mutation of either the α1 or α2-globin gene on the other chromosome.[8] In general, the clinical manifestations of hemoglobin H disease can be variable, ranging from asymptomatic to severe, as in certain individuals receiving frequent or irregular blood transfusions. Clinical signs of non-deletion hemoglobin H disease are more severe, and patients are more anemic, more likely to develop hepatosplenomegaly, and more dependent on blood transfusions.[9,10]

Gap-Polymerase Chain Reaction (Gap-PCR) is helpful in diagnostic labs for the quick and easy identification of common α-thalassemia mutations in hematology. Gap-PCR finds deletion that DNA sequencing could miss. Primer sets are created to flank a known deletion. The foundation of gap-PCR is the fact that without a deletion connecting the flanking sequences, the primers are unable to produce a PCR result. PCR amplification will take place if a deletion is present, and the result is then evaluated by electrophoresis. Multiple target deletions can be found at once using multiplex gap-PCR. For instance, the most prevalent α-thalassemia deletions are used as numerous specific primers to diagnose Hb H disease.[11]

The Mediterranean countries, Southeast Asia, Africa, the Middle East, and the Indian subcontinent are regions where alpha thalassemia is most prevalent. Although thalassemia mostly affects developing nations, little is known about the exact prevalence and distribution of the disease there. In order to develop effective preventive and control programs and treatment plans, it is crucial to understand the prevalence of thalassemia and the frequency of the mutations that cause it.[12]

Two community-based surveys in Vietnam have produced prevalence statistics on thalassemias and hemoglobinopathies that have been reported.[13,14] The distribution of typical α-globin gene deletion mutations is seldom ever known, though. In this study, hemoglobin H disease patients in the Mekong Delta of Vietnam were examined for the incidence of four prevalent deletion mutations.

Methods

Study Design

This cross-sectional descriptive study was conducted on 50 hemoglobin H disease patients from October 2021 to June 2022.

Setting

This study was conducted at Can Tho Hematology Blood Transfusion Hospital and Can Tho University of Medicine and Pharmacy Hospital, Can Tho City, Vietnam.

Participants

The target population included patients with hemoglobin H disease diagnosis: hemoglobin electrophoresis showed increased Hb H or/and Hb Bart's.

Study Size

A total of 50 hemoglobin H disease patients diagnosed and treated at Can Tho Hematology Blood Transfusion Hospital and Can Tho University of Medicine and Pharmacy Hospital were enrolled.

Data Collection Process

The necessary permissions were obtained from the hospitals' research committees after the approval of the project in Can Tho University of Medicine and Pharmacy. The researcher then entered the research setting and briefed the eligible patients about the research objectives. The participants were selected via convenience sampling.

The primary outcome of this study was allele frequency of α-globin gene deletion mutations in HbH disease patients, and the secondary outcome was genotypes of HbH disease patients.

DNA Extraction

A whole blood sample was purified and its genomic DNA was extracted using the TopPure Blood DNA Extraction Kit (ABT, Vietnam). The DNA concentration was calculated using BioDrop uLite (BioDrop, UK). A DNA concentration of around 100ng/L was needed to carry out PCR for the deletion detection in this investigation.

Gap-Polymerase Chain Reaction

Each 50μL reaction contained 100-200ng of genomic DNA, 12 distinct primers at varying concentrations were shown in Table 1, 200μM of each dNTP, 1.5mM $MgCl_2$, 1X Q-solution, and 2.5U HotStarTaq DNA polymerase in provided reaction buffer from Qiagen, Germany. An initial 15-minute denaturation at 96°C was followed by 30 cycles of 98°C denaturation for 45 seconds, 60°C annealing for 90 seconds, and 72°C extension for 150 seconds in a C1000 Thermal Cycler (Bio-rad Laboratories, USA) used for the reactions. The reaction was completed after a final 5-minute extension at 72°C.[15]

By electrophoresis through a 1 percent agarose gel in 1X Tris-Borate-EDTA buffer at 10 volts/cm for an hour, five microliters of each amplified product were examined. Table 1 provides the anticipated amplicon sizes for each deletion junction fragment, as well as the control 2-globin gene and LIS1 gene 3' untranslated region (UTR) segments. The α2-globin gene is eliminated entirely or partially by any of the four deletions, therefore when a deletion allele is also present, the 2-globin gene's positive amplification acts as a sign of heterozygosity. For general amplification success, a different control is provided by the LIS1 gene 3' UTR region.[15]

Table 1 Primer sequences for gap-PCR and expected amplicon sizes

Name	5'➔3' sequence	GenBank ID: nucleotides	Concentration	Amplicon (size)
LIS1-F	GTCGTCACTGGCA GCGTAGATC	HSLIS10: 407➔428	0.5 µM	LIS1 3'UTR fragment (2503bp)
LIS1-R	GATTCCAGGTTGT AGACGGACTG	HSLIS10: 2909➔2887	0.5 µM	
α2/3.7-F	CCCCTCGCCAAGT CCACCC	HUMHBA4: 5676➔5694	0.2 µM	-α3.7 jxn[a] fragment (2022/2029bp)
3.7-R	AAAGCACTCTAGG GTCCAGCG	HUMHBA4: 11514➔11494	0.2 µM	
α2/3.7-F	As above	As above	-	α2 gene (1800bp)
α2-R	AGACCAGGAAGG GCCGGTG	HUMHBA: 7475➔7457	0.2 µM	
4.2-F	GGTTTACCCATGT GGTGCCTC	HUMHBA: 3064➔3084	0.5 µM	-α4.2 jxn fragment (1628bp)
4.2-R	CCCGTTGGATCTT CTCATTTCCC	HUMHBA4: 8942➔8920	0.5 µM	
SEA-F	CGATCTGGGCTCT GTGTTCTC	HSGG1: 26120➔26140	0.2 µM	--SEA jxn fragment (1349bp)
SEA-R	AGCCCACGTTGTG TTCATGGC	HSCOS12: 3817➔3797	0.2 µM	
THAI-F	GACCATTCCTCAG CGTGGGTG	HSGG1: 9592➔9612	0.3 µM	--THAI jxn fragment (1153bp)
THAI-R	CAAGTGGGCTGAG CCCTTGAG	HSCOS12: 1241➔1221	0.3 µM	

Ethical Consideration

All study processes were conducted after gaining the approval of the Ethics Committee of Can Tho University of Medicine and Pharmacy on March 30, 2021.

Statistical Methods

The data were analyzed using the SPSS for Windows (version 18). The collected data reported as frequency and ratio.

Results

Characteristics of Patients

Fifty patients diagnosed with HbH disease were enrolled in this study. Eight patients were male (16.0%) and thirty-two patients were female (64.0%). The average age was 43 years (min. = 9, max. = 81). Laboratory findings of the patients with HbH disease were analyzed and described in Table 2.

Table 2 Hematologic findings and hemoglobin typing of HbH patients

Parameters	HbH disease patients (Mean ± SD)	Normal range
RBC (x10^{12} cell/L)	3.9 ± 0.9	3.9 - 5.4
HGB (g/dL)	78.9 ± 14.5	125.0 - 145.0
MCV(fL)	74.3 ± 8.4	85.0 - 95.0
MCH (pg)	20.4 ± 2.3	28.0 - 32.0
RDW-CV (%)	25.2 ± 4.5	11.5 - 14.5
HbA (%)	87.8 ± 7.4	96.8 - 97.8
HbA2 (%)	1.4 ± 0.6	2.2 - 3.2
HbF (%)	0.2 ± 0.7	0.5 - 1.0
HbH (%)	8.2 ± 6.7	-
HbBart's (%)	0.9 ± 1.3	-
HbE (%)	0.9 ± 2.9	-
HbC (%)	0.4 ± 0.8	-
Reticulocyte count (%)	6.6 ± 1.9	0.5 - 2.5
HbH inclusion bodies (%)	23.9 ± 5.9	-
Blood film	Red cells are hypochromic, microcytic, moderately severe anisopoikilocytosis and numerous target cells present	-

RBC, red blood cell count; Hgb, hemoglobin concentration; MCV, mean cell volume; MCH, mean cell hemoglobin; RDW-CV, red cell distribution width; Hb, hemoglobin; -, not available.

Allele Frequency of α-globin Gene Deletion Mutations by Gap-PCR

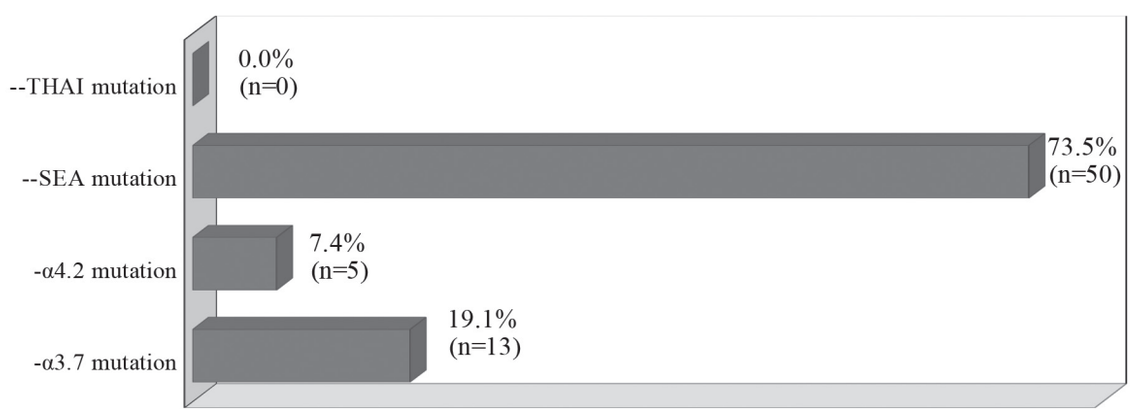

Figure 1 Allele frequency of α-globin gene deletion mutations.

Gap-PCR results were positive in all 50 cases (100%). Allele frequency of α-globin gene deletion mutations were shown in Figure 1. The --^{SEA} mutation was the most common deletion, accounting for 73.5% of the mutant alleles followed by -α$^{3.7}$ (19.1%) and -α$^{4.2}$ (7.4%) mutation. We did not detect the --^{THAI} deletion in this small number of hemoglobin H disease patients (n = 50).

Genotypic Data of HbH Disease Patients

Table 3 Genotypes of HbH disease patients

Genotypes		Frequency (n)	Ratio (%)
Deletional HbH	$--^{SEA}/-\alpha^{3.7}$	13/50	26.0
	$--^{SEA}/-\alpha^{4.2}$	5/50	10.0
Deletional/Non-deletional HbH	$--^{SEA}/\alpha^{T}\alpha$	32/50	64.0
Total		50/50	100

Genotypes of the 50 HbH disease patients were shown in Table 3: 36.0% had the deletional genotype and 64.0% had the deletional/non-deletional genotype. Among 18 patients with deletional HbH disease, ($--^{SEA}/-\alpha^{3.7}$) mutation was most commonly found in 13 patients (26.0%) followed by ($--^{SEA}/-\alpha^{4.2}$) mutation (10.0%).

Discussion

In this study, the most common type of deletion was $--^{SEA}$ deletion, accounting for 73.5% of the mutant alleles, followed by the $-\alpha^{3.7}$ (rightward) deletion (19.1%) and $-\alpha^{4.2}$ (leftward) deletion (7.4%) mutation in this region. Southeast Asia's most prevalent deletion was the $--^{SEA}$ mutation. In the study by Ngo D.N. (2018) among 97 Hb H disease patients in the northern of Vietnam, 100% of patients carried $--^{SEA}$ mutation and the study by Traivaree C. among 58 HbH disease patients in Thailand, 98.3% of patients carried $--^{SEA}$ mutation.[16,17] As a result, our report's prevalence of $--^{SEA}$ mutation was comparable to that of earlier surveys from Vietnam and Southeast Asia. The presence of $--^{THAI}$ mutation was not detected in this study, suggesting the regional specificity of $--^{THAI}$ mutation in Thailand.[18] Our finding is similar to a report showing that $--^{THAI}$ deletion is not common in Vietnam.[13]

We detected 18 of 50 patients (36.0%) were deletion HbH disease. Among 18 patients with deletion HbH disease, SEA deletion/3.7kb deletion ($--^{SEA}/-\alpha^{3.7}$) mutation was most commonly found in 13 patients (26.0%) followed by SEA deletion/4.2kb deletion ($--^{SEA}/-\alpha^{4.2}$) mutation (10.0%). In the study by Ngo D.N. in the northern of Vietnam, SEA deletion/3.7kb deletion ($--^{SEA}/-\alpha^{3.7}$) mutation was the most common (21%) followed by SEA deletion/4.2kb deletion ($--^{SEA}/-\alpha^{4.2}$) mutation (9.2%). In the study by Traivaree C. in Thailand, SEA deletion/3.7kb deletion ($--^{SEA}/-\alpha^{3.7}$) mutation was markedly high (43%) followed by SEA deletion/4.2kb deletion ($--^{SEA}/-\alpha^{4.2}$) mutation (6.8%).[16,17]

In this study, we detected 32 of 50 HbH disease patients had only one $--^{SEA}$ mutation in the genotype (64.0%). We classified these 32 patients into deletional/non-deletional HbH subgroup. In Vietnam, this subgroup compromises nearly 68.0% of HbH disease patients.[17] The shortcomings of this study was screening common deletion mutations of Southeast Asia by using Gap-PCR, so we couldn't determine the genotypes of deletional/non-deletional HbH subgroup exactly. We suggested that genotypes of HbH disease patients with non-deletional mutation in this study should be determined by DNA sequencing.

In brief, our findings showed the prevalence of frequent deletion mutations of the α-globin gene in the Vietnamese Mekong Delta. However, a further research is needed to completely comprehend the distribution of α-globin mutations in Vietnam.

Gap-PCR might be a useful initial screening technique for finding the deletions of the α-globin gene. This study gave an overview of the prevalence of typical α-globin gene mutations in Vietnam and might act as a starting point for a further research into these genetic flaws.

Ethics of Study

All study processes were conducted after gaining the approval of the Ethics Committee of Can Tho University of Medicine and Pharmacy on March 30, 2021.

Limitation

One of the shortcomings of this study was its cross-sectional descriptive design. Long-term and prospective studies are, therefore, necessary. In addition, further studies are recommended to investigate the range of typical α-globin gene variants in Vietnam.

Generalisability

In order to increase the generalisability of the findings, further studies with larger sample sizes are recommended.

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Conflict of Interest None

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Authors' Contributions

Conception and design: VTT, TPT, LTHM.

Analysis and interpretation of the data: VTT, LTHM.

Drafting of the article: VTT, TPT, TTHC.

Critical revision of the article for important intellectual content: VTT, LTHM, TTHC.

Final approval of the article: VTT, LTHM, TTHC, PTNN.

Provision of study materials or patients: PHD, NPD.

Statistical expertise: TPT, PHD, NPD.

Obtaining fund: VTT, TPT.

Administrative, technical, or logistic support: PHD, NPD.

Collection and assembly of data: VTT, TPT, PHD.

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