

# Thalassemia and Hemoglobinopathies in Thailand: A Systematic Review

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**Received** March 20, 2023  
**Accepted** May 12, 2023  
**Published** December 31, 2023

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## ABSTRACT

Thalassemia and hemoglobinopathies are inherited autosomal recessive blood diseases, that have been the major health problem in Thailand. The study on the prevalence of thalassemia and hemoglobinopathies is important for the prevention and control of thalassemia diseases. The object of this article is to provide the current prevalence of thalassemia mutations in Thailand, focusing on common  $\alpha$ - and  $\beta$ -thalassemia mutations. The prevalence of  $\alpha$ -thalassemia mutations and the data reveal that the frequency of  $\alpha^0$ -thalassemia (SEA type) is the most common throughout the country. Meanwhile,  $\alpha^+$ -thalassemia (3.7 kb deletion) is also high, followed by 4.2 kb deletion. While codon 41/42 (-TCTT) and codon 17 (A>T) make up the majority of  $\beta$ -thalassemia mutations, the frequency of these two types is high, accounting for more than 50% in the North, Northeast, and Central Thailand. While it is lower in southern Thailand, averaging about 22-32% because codon 19 (A>G) is widely distributed after codon 41/42. However, Hb E is the major  $\beta$ -hemoglobinopathy owing to its high prevalence in all regions.

**Keywords:** Thalassemia, Hemoglobinopathies, Thailand

## Introduction

Thalassemia is an inherited autosomal recessive blood disease caused by genetic defects through mutation, deletion of genes, or insertion of bases, characterized by reduced or absent synthesis of the essential globin chains of hemoglobin. A recessive condition is one where both genes of the pair associated with a condition must be changed or mutated. A carrier state can exist in which a person carries one normal and one mutated gene. There are two categories of thalassemia disease: these are thalassemia and hemoglobinopathies. Thalassemia is the term applied to the quantitative problem of having decreased or absent globin chain synthesis. At the same time, hemoglobinopathies describe the qualitative problem of having structurally abnormal globin chains or abnormal forms of hemoglobin. Thalassemia and hemoglobinopathies are the most prevalent in the Mediterranean (Greece, Italy, and Cyprus), Middle East, parts of Africa, India, and Asia regions, especially Southeast Asia [1].

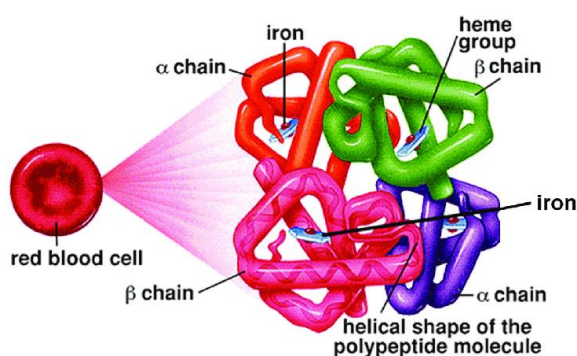
Thalassemia and hemoglobinopathies are major health problems in Thailand. Approximately 1% (620,000 people) of the Thai population has thalassemia disease and around 30-40% (18-24 million people) are

carriers of thalassemia genes. Each year, almost 50,000 pregnancies are at risk of having babies with thalassemia disease, of which approximately 12,000 results in thalassemic newborns. Among the 12,000 affected children each year, around 5,000 of them are affected by thalassemia major [2]. Thalassemia disease affects not only thalassemia health but also the socio-economic situation and mental health of their families. The Thai government spends over 5,000 million bath annually for the prevention and treatment of thalassemia disease. Thus, the disease burden for the country is huge [3]. The study on the prevalence of thalassemia and hemoglobinopathies is important for the prevention and control of thalassemia diseases. Therefore, this article aims to describe the prevalence of thalassemia mutations in Thailand that were studied from 2002 to 2022. These include common  $\alpha$ - and  $\beta$ -thalassemia mutations, severe thalassemia diseases, and strategies for the prevention and control of thalassemia.

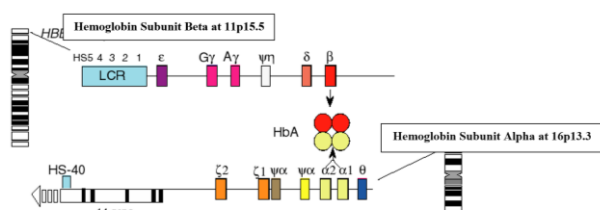
## Hemoglobin synthesis

The hemoglobin molecule within red blood cells transports oxygen from the lungs to all parts of the body. Hemoglobin is a tetrameric-structured protein that is made up of 2  $\alpha$ -like and 2  $\beta$ -like globin chains. Each

subunit of hemoglobin has a heme group made up of iron and porphyrin rings. At various developmental stages,  $\beta$ -like globin chains are combined with  $\alpha$ -like globin chains to form a variety of hemoglobin tetramers [4]. Hemoglobin synthesis is controlled by two multiple-gene clusters, one on chromosome 11p.15.5 encodes  $\beta$ -like globin chains including  $\epsilon$ ,  $G\gamma$ ,  $A\gamma$ ,  $\delta$  and  $\beta$ -globin chains and another on chromosome 16p.13.3 encoding  $\alpha$ -like globin chains including  $\zeta$  and  $\alpha$ -globin chains. In normal adults, 95-97% of total hemoglobin is HbA ( $\alpha_2\beta_2$ ), while 2-3% is HbA<sub>2</sub> ( $\alpha_2\delta_2$ ) and less than 1% is HbF ( $\alpha_2\gamma_2$ ) [4]. Thalassemia syndrome can be caused by variations in the genes encoding  $\alpha$ - and  $\beta$ -globin chains, which can lead to improper hemoglobin function.



**Figure 1** Tetrameric structure of hemoglobin that contains 2 $\alpha$ -like and 2 $\beta$ -like globin chains [5]



**Figure 2** Schematic representation of the human  $\beta$ -globin gene cluster on chromosome 11p15.5 and the  $\alpha$ -globin gene cluster on 16p13.3 [6]

### Classification of thalassemia

Thalassemia syndrome can be classified according to the particular globin chains responsible for  $\alpha$ ,  $\beta$ ,  $\delta\beta$ , and  $\epsilon\gamma\beta$  thalassemia's inefficient synthesis. However, the most frequently found thalassemia worldwide are  $\alpha$ - and  $\beta$ -thalassemia.

### $\alpha$ -thalassemias and $\alpha$ -hemoglobinopathies

$\alpha$ -thalassemia results in reduced or absent production of the  $\alpha$ -globin chain. Anemia is caused by decreasing levels of  $\alpha$ -globin chains that contain hemoglobins, HbA ( $\alpha_2\beta_2$ ), HbA<sub>2</sub> ( $\alpha_2\delta_2$ ), and HbF ( $\alpha_2\gamma_2$ ). Under-production of  $\alpha$ -globin chains leads to excess production of  $\gamma$ -globin chains resulting in the formation of  $\gamma_4$ -tetramers (Hb Bart's) and  $\beta_4$ -tetramers (HbH). Therefore, the diagnostic marker for  $\alpha$ -thalassemia is the presence of Hb Bart's and Hb H in the blood [7].

$\alpha$ -thalassemia is caused by either deletion or non-deletion of the  $\alpha$ -globin gene such as a point mutation. The deletional type is the major form of  $\alpha$ -thalassemia, which involves the deletion of one or two  $\alpha$ -globin genes on the cis chromosome and gives rise to two types of deletional  $\alpha$ -thalassemia. The first type is  $\alpha$ -thalassemia 2 ( $\alpha^+$ -thalassemia) in which one cis  $\alpha$ -globin gene is deleted leaving the other cis  $\alpha$ -globin gene intact.  $\alpha^+$ -thalassemias consists of two forms,  $\alpha^+$ -thalassemia caused by the rightward deletion (3.7 kb deletion) and leftward deletion (4.2 kb deletion), etc. The other non-deletional types are caused by mutations i.e., Hb Constant Spring (Hb CS) and Hb Pakse, that are included in  $\alpha$ -hemoglobinopathies. The second type is  $\alpha$ -thalassemia 1 ( $\alpha^0$ -thalassemia), in which both cis  $\alpha$ -globin genes are deleted. Homozygotes for  $\alpha^0$ -thalassemia can lead to Hb Bart's hydrops fetalis [1, 8-10].

### $\beta$ -thalassemias and $\beta$ -hemoglobinopathies

$\beta$ -thalassemias are a group of blood disorders characterized by reduced or absent synthesis of  $\beta$ -globin chains. The major molecular defect in most cases of  $\beta$ -thalassemia is a point, whereas few cases are caused by deletion. The two types of  $\beta$ -thalassemia are  $\beta^0$  which shows a complete absence of the  $\beta$ -globin chain, and  $\beta^+$ -thalassemia where reduced levels of the  $\beta$ -globin chain are found [11-12].

At present, there are more than 150 mutations in  $\beta$ -globin genes that cause  $\beta^0$  and  $\beta^+$ -thalassemia [13]. In Thailand, the 4bp (-TCTT) deletion at codon 41/42 ( $\beta^{41/42}$ ) and A>T substitution at codon 17 of the  $\beta$ -globin genes are the predominant mutations observed in  $\beta^0$ -thalassemia patients. In  $\beta^+$ -thalassemia, the A>G substitution at nucleotide -28 in the promoter region of the  $\beta$ -globin gene ( $\beta^{-28}$ ) is the major molecular defect found in Thailand. Within the group of  $\beta$ -hemoglobinopathies in Thailand, Hb E is the most important hemoglobin variant due to its high prevalence across the country [14]. Hb E is caused by G>A substitution at codon 26 of the  $\beta$ -globin gene and is named the  $\beta^E$ -allele. This mutation changes the triplet codon for glutamic acid to that for lysine. In addition, this G>A mutation activates a cryptic splice site at codon 25, which results in reduced synthesis of the  $\beta^E$ -globin chain and leads to the  $\beta^+$ -thalassemia phenotype [9, 14-20].

### Prevalence of thalassemia and hemoglobinopathies in Thailand

Globally, the carrier frequency of  $\beta$ -thalassemia falls between 1% and 20%, whereas that of  $\alpha$ -thalassemia is much higher, with a range of 10-20%, or maybe as high as 40% in some Middle Eastern and Indian populations. The distribution of  $\alpha^0$ -thalassemia is restricted; it is more common in regions of Southeast Asia. In Thailand,  $\alpha$  and  $\beta$  thalassemias are the most common types of thalassemias. As previously reported,

before 2000, approximately 20-30% of the Thai population had  $\alpha$ -thalassemia genes, while 3-9% had  $\beta$ -thalassemia genes. Hb Constant Spring (Hb CS) was the major  $\alpha$ -hemoglobinopathy found in Thailand, (1-8%) and Hb E was the major  $\beta$ -hemoglobinopathy (8-60%). These vary depending on each region [8, 21-25].

From 2002 to 2022, in Thailand, there was modest regional variation in the prevalence of common thalassemia mutations. In the north, the frequencies were 5.2-21.0% for  $\alpha^0$ -thalassemia (SEA type),  $\alpha^+$ -thalassemia (3.7 kb deletion and 4.2 kb deletion) 19.9%, and 4.5%, 14.9-37.3% for Hb E, 0.3-4.2% for Hb CS and 0.5% for Hb Pakse, respectively [26-32]. In the northeast,  $\alpha^0$ -thalassemia (SEA type) was 3.1-13.6%,  $\alpha^+$ -thalassemia (3.7 kb deletion and 4.2 kb deletion) were 25.1 and 0.8%, Hb CS was 5.4%, Hb Pakse was 1.4% and Hb E was 39.1-(50-60)%, respectively [33-34]. While in the Central region, we found 4.6% of  $\alpha^0$ -thalassemia (SEA type),  $\alpha^+$ -thalassemia (3.7 kb deletion plus 4.2 kb deletion) was 17.2%, Hb CS 5.6%, Hb Pakse 0.5% and 22.7% of Hb E respectively [35]. Finally, in the South region, frequencies were 4.8% of  $\alpha^0$ -thalassemia (uncharacterized) and 16% of Hb E [36-38].

At present, there are more than 20  $\alpha$ -thalassemia mutations in Thailand. The predominant genotype of  $\alpha^0$ -thalassemia among Thai people is SEA type. Whereas with the  $\alpha^+$ -thalassemia gene, the 3.7-kb deletion ( $-\alpha^{3.7}$ ) is the major genotype, and the 4.2-kb deletion ( $-\alpha^{4.2}$ ) is the major one [26-29,32,34,36-38]. There are approximately 30 types of  $\beta$ -thalassemia that have been identified in Thailand. The frequency of  $\beta$ -thalassemia varies modestly. The 4 bp deletion at codon 41/42 ( $-TCTT$ ) or  $\beta^{41/42}$  is the most common, followed by nonsense A>T mutation at codon 17 (A>T). Hb E is the major  $\beta$ -hemoglobinopathy due to its widespread throughout the country [26-31, 36-37, 39-40].

### Severe thalassemia diseases in Thailand

Severe thalassemia diseases in Thailand comprise homozygous  $\alpha^0$ -thalassemia (Hb Bart's hydrops fetalis), homozygous  $\beta$ -thalassemia, and  $\beta$ -thal/Hb E. In Thailand, the most common type of  $\alpha$ -thalassemia 1 is the Southeast Asian type ( $--^{SEA}$ ), which involves the deletion of approximately 20 kb of DNA, removing  $\psi\alpha 2$ ,  $\psi\alpha 1$ ,  $\alpha 2$ ,  $\alpha 1$ ,  $\theta$  globin genes [23]. 5.2% to 21% of the Thai population carry this deletion [26-28,33-34]. Homozygosity of  $\alpha$ -thalassemia 1 (SEA type) can cause Hb Bart's hydrops fetalis. The other  $\alpha$ -thalassemia 1 deletion that can result in hydrops fetalis is the compound heterozygosity of  $--^{SEA}/--^{THAI}$ . In 2019, new cases of Hb Bart's hydrops fetalis in lower northern Thailand were found to be caused by the compound heterozygous mutations Southeast Asian type and Chiang Rai deletion type ( $--^{SEA}/--^{CR}$ ). THAI deletion is caused by the deletion of approximately 34.5 kb, resulting in the removal of the entire  $\alpha$ -globin gene family. The most recently described cause of severe thalassemia, the Chiang Rai deletion type, was found in

2019 and causes an approximately 44.6 kb  $\alpha^0$ -thalassemia deletion on chromosome 16 [41-42].

Hb Bart's hydrops fetalis is the most severe form of  $\alpha$ -thalassemia, a lethal form of thalassemia. Most of the affected fetuses died before labor began, during labor and delivery, or shortly after delivery. The prevalence of this disease varies from 1:200 to 1:2,000 in Southeast Asia [43]. Fetuses with Hb Bart's hydrops fetalis, where all 4  $\alpha$ -genes were deleted, had non-functional hemoglobin homotetramers ( $\gamma_4$  and  $\beta_4$ ).  $\gamma_4$  homotetramers have a high affinity for oxygen, living only a small amount of embryonic Hb Portland ( $\delta_2\gamma_2$ ) to deliver and liberate oxygen to the tissues of the fetus. Fetuses with this disease suffered severe anemia in utero, resulting in severe tissue hypoxia, heart failure, and abnormalities such as hepatosplenomegaly and urogenital overload, resulting in edema over the entire body, leading to the designation hydrops fetalis. Risks for the mother include preeclampsia, polyhydramnios or oligohydramnios, antepartum hemorrhage, and premature delivery. Pregnant women and carrier couples are at risk of having a fetus with Hb Bart's fetalis and because of fetal and maternal complications, termination is usually advised [44]. Other severe forms consist of  $\beta$ -thalassemia major including homozygous  $\beta$ -thalassemia and some cases of  $\beta$ -thal/Hb E. These two forms affected children, presenting symptoms within the first 2 years of birth, often 3 to 6 months after birth and hemoglobin levels in untreated patients were 3-6 g/dL. People with  $\beta$ -thalassemia major require life-long blood transfusions and need monitoring for complications and treatment for iron overload. The child with these severe forms can be pale, have a poor appetite, and present with many infections during the first 1-2 years of life. Without treatment, it can cause an enlarged liver and spleen and the bones become thin and brittle, with the presence of a prominent forehead, frontal bossing, full cheekbones, and a depressed bridge of the nose. In teenagers and adults, complications with iron overload commonly occur, which may result in heart failure, liver cirrhosis, hypoparathyroidism, hypogonadism, etc. [45].

### Thalassemia and hemoglobinopathies in northern Thailand

A study in 2004 [29] among 516 pregnant women who attended the Antenatal Clinic of Maharaj Nakorn Chiangmai Hospital. Most of these women resided in Chiangmai, while others resided in other northern provinces. The percentage of pregnant women who were carriers of  $\alpha$ -thal 1 (SEA type) was 6.6%,  $\beta$ -thalassemia heterozygote 3.7%, Hb E heterozygote 11.6%, combination of  $\alpha$ -thal 1 (SEA type) and  $\beta$ -thal heterozygote 1.2%, combination of  $\alpha$ -thal 1 (SEA type) and Hb E heterozygote 1.5%, homozygous Hb E 0.8% and  $\beta$ -thal/Hb E disease 0.2% [29].

A study in 2005 [26] among 500 people from Uttaradit, Phetchabun, and Phitsanulok provinces in northern Thailand found that 40.4% of the population

carried a thalassemia gene with the following distribution, 7.8% heterozygous  $\alpha$ -thalassemia 1 (SEA type), 2.2%  $\beta$ -thalassemia trait, 26.6% Hb E heterozygous, and 3.8% homozygous Hb E. A study of pregnant women and their partners [27] who attended Buddachinnaraj provincial hospital and 8 community hospitals in Phitsanulok between 2002 and 2003, found that 4.8% were heterozygous for  $\alpha$ -thalassemia 1, 1.6% for the  $\beta$ -thalassemia trait, 12.4% were heterozygous for Hb E, 2.7% were homozygous for Hb E and 0.25% for Hb CS [27]. A study in 2013 [30] among 50 persons who attended hospitals in Phitsanulok province and were diagnosed with  $\beta$ -thalassemia. The distribution of codon 41/42 (-TCTT) was highest at 48%, followed by codon 17 (A>T) at 30%, nt-28 (A>G) at 6%, IVS-I-1 (G>T) 6%, -87 (C>A) at 4%, codon 35 (C>A) at 2%, codon 71/72 (+A) at 2% and IVS-II-654 (C>T) at 2% [30].

Parameeyong, et al [32] reported that people who attended 7 hospitals in Phayao province and found that the percentage of carriers with  $\alpha$ -thalassemia 1 (SEA type) was 9.6% [32]. Pharephan, et al [28] reported that  $\alpha$ -thalassemias in 638 pregnant women who attended the Antenatal Clinic at Maharaj Nakorn Chiangmai Hospital, came from 15 provinces in northern Thailand, such as Chiangmai, Chiangrai, Kamphaeng Phet, Lampang, Lamphun, Mae Hong Son, Nan, Nakorn Sawan, Phayao, Phitsanulok, Phrae, Sukhothai, Tak, Utthai Thani and Uttaradit. They noted that thalassemia distribution of heterozygous  $\alpha^+$ -thalassemia ( $-\alpha^{3.7}$ ) was high, about 15.52%, followed by heterozygous  $\alpha^0$ -thalassemia (SEA type) 12.23%, heterozygous Hb CS 4.23%, deletional disease ( $--^{SEA}/-\alpha^{3.7}$ ) 2.82%, homozygous  $\alpha^+$ -thalassemia ( $-\alpha^{3.7}$ ) 0.78% and heterozygous  $\alpha^+$ -thalassemia ( $-\alpha^{4.2}$ ) about 0.31%.

The prevalence of thalassemia genes was also studied by Limveerapajak [31], who investigated at-risk couples who attended the Antenatal Care clinic, Sawanpracharak Hospital, Nakorn Sawan province, between 2012 to 2017. He found the percentage of Hb E heterozygote was high, about 17.5%, followed by  $\beta$ -thalassemia heterozygote (15.7%), SEA  $\alpha$ -thal 1 heterozygote (13.9%), double heterozygote SEA/E (6.3%), homozygous Hb E (4.4%), Hb E/ $\beta$ -thalassemia (2.1%) and Hb H disease (1.3%).

#### Thalassemia and hemoglobinopathies in northeastern Thailand

A large study in Northeastern Thailand looking at the prevalence of thalassemia genes amongst a population was done by Vimolsarte, et al. [34]. They studied 18,461 subjects in patients, pregnant women, and their partners, who attended Health Service Area 7 of Thailand during 2011-2012. The distribution was as follows:  $\alpha$ -thalassemia 1 (SEA type) was 13.6%, while THAI deletion type was 0.11%. Hb E was the most common for  $\beta$ -hemoglobinopathies about 50-60%; the  $\beta$ -thalassemia mutation found codon 41/42 (-TCTT) at

43.1%, codon 17 (A>T) at 21.5%, nt-28 (A>G) at 21.5%, codon 71/72 (+A) at 7.6%, IVS-II-654 (C>T) at 1.5%.

Chaibunruang, et al. [33] reported that among 350 newborns delivered at Maternal and Child Hospital, Regional Health Promotion Center 7, Khon Kaen, Northeastern Thailand. The distribution of thalassemia genes showed that heterozygous Hb E was highest at about 17.1%, followed by heterozygous  $\alpha^+$ -thalassemia (3.7 kb deletion) at 11.4%, a combination of 3.7 kb deletion and Hb E at 5.7%, heterozygous Hb CS at 3.4%, homozygous Hb E at 2.9%, combination of  $\alpha^0$ -thalassemia (SEA type) and Hb E at 1.4%, homozygous  $\alpha^+$ -thalassemia (3.7 kb deletion) and heterozygous  $\alpha^0$ -thalassemia (SEA type) at 1.1%.

#### Thalassemia and hemoglobinopathies in central Thailand

Studies from central Thailand found that the prevalence of Hb E was highest at about 22.7%, followed by  $\alpha^+$ -thalassemia (3.7 kb deletion) plus (4.2 kb deletion) at about 17.2%, Hb CS at 5.6%,  $\alpha^0$ -thalassemia (SEA type) at 4.6%, Hb Pakse at 0.5% and  $\beta$ -thalassemia at 0.7% [33].

A study conducted 80 pediatric patients who were followed in the Phramongkutkha Hospital, Central Thailand [38] and were carrying  $\beta$ -thalassemia mutations. Most patients resided in Bangkok while some resided in other provinces in central Thailand. The genotypes of  $\beta$ -thalassemia encountered were alleles of codon 41/42 (-TCTT) 37.5%, followed by codon 17 (A>T) 26.1%, IVS-I-5 (G>C) 8%, IVS-II-654 (C>T) 6.8%, IVS-I-1 (G>T) 4.5% and codon 71/72 (+A) 2.3% and codon 19 (A>G) 1.1%. Panichchob, et al [40] reported that the distribution of  $\beta$ -thalassemia mutations in eastern Thailand. The data showed that six common  $\beta$ -thalassemia mutations were identified as codon 41/42 (-TCTT) at 35.6%, codon 17 (A>T) at 18.9%, nt-28 (A>G) at 15.7%, IVS-II-654 (C>T) at 6.3%, IVS-I-1 (G>T) at 5.8% and codon 19 (A>G) at 4.7%.

#### Thalassemia and hemoglobinopathies in southern Thailand

In southern Thailand,  $\alpha$ -thalassemia mutations were rarely reported. A 2006 study by Tienthavorn V et al. investigated [35] the prevalence of thalassemia carriers in Nakorn Si Thammarat and found that carriers for  $\alpha^0$ -thalassemia were 4.8%. Panyasai, et al [37] found the frequency of Hb Pakse in Nakorn Si Thammarat to be 0.4%. Nopparatana, et al. [36] studied at-risk couples who resided in 14 provinces in southern Thailand and found heterozygosity for  $\beta$ -thalassemia was 5.6%,  $\alpha^0$ -thalassemia was 4.5% and Hb E mutation was 16.0%. The proportion of  $\alpha^0$ -thalassemia (SEA type) was 98% while the THAI type was 2%. The seven most common mutations causing  $\beta$ -thalassemia were, codon 19 (A>G) 18.6%, followed by codon 41/42 (-TCTT) 14.4%, IVS-I-5 (G>C) 13.2%, 3.5 kb deletion 9.2%, codon 17 (A>T)

7.7%, nt-28 (A>G) 7.3% and IVS-I-1 (G>T) 7.1% [36]. Additionally, Nuinoon, et al. [39] studied the frequency of  $\beta$ -thalassemia mutations in 181  $\beta$ -thalassemia patients and found that codon 41/42 (-TCTT) was

23.9%, codon 17 (A>T) was 14.1%, codon 19 (A>G) was 6.3%, nt-28 (A>G) was 4.4% and IVS II-654 (C>T) was 4.4%.

### Prevalence of common thalassemia mutations in Thailand

**Table 1** Prevalence of common  $\alpha$ -thalassemia mutations in the North, Northeast, Central and South of Thailand, studied from 2002 to 2022.

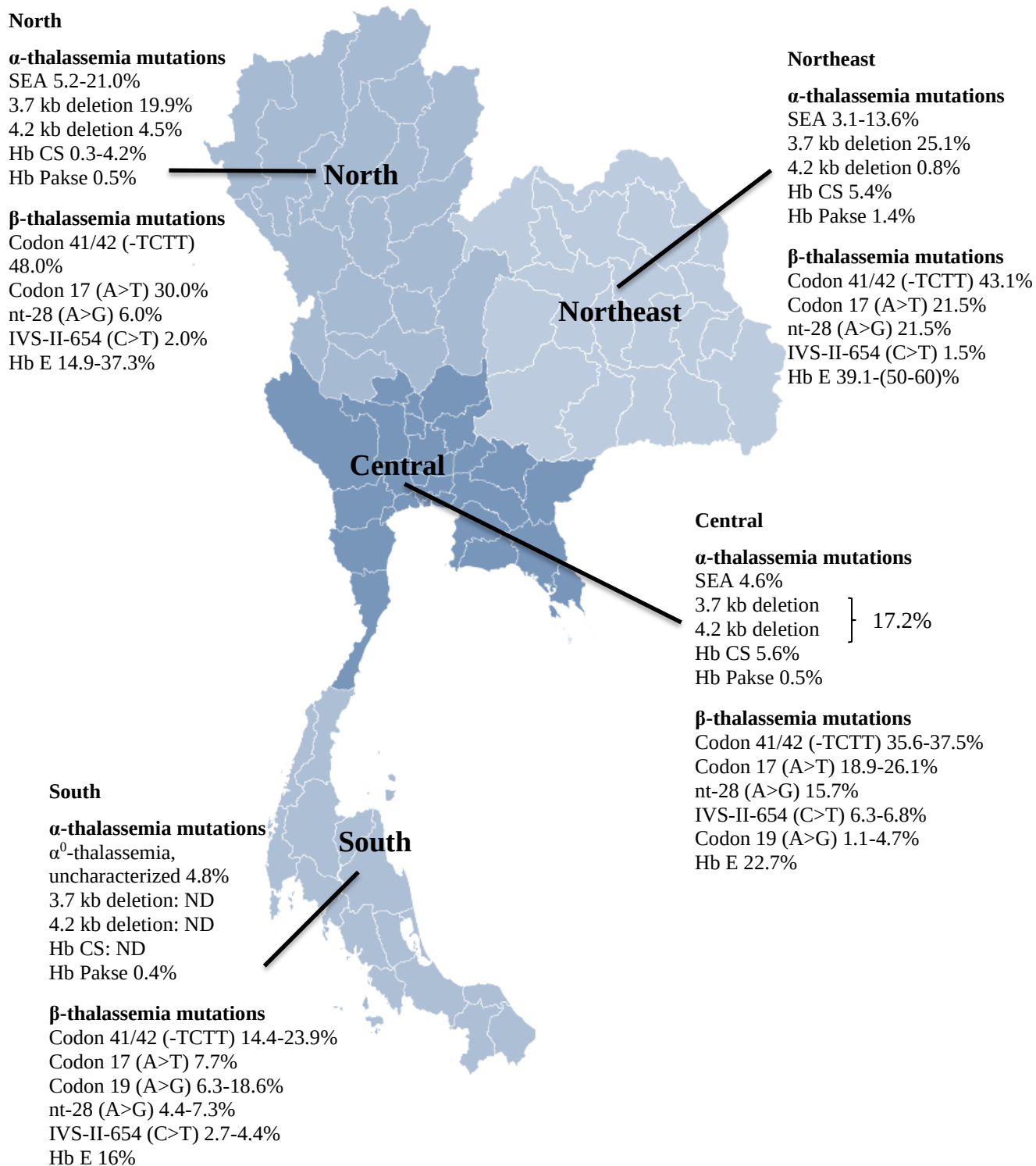
| $\alpha$ -thalassemia mutations | North<br>%<br>[26-29,31-32] | Northeast<br>%<br>[33-34] | Central<br>%<br>[33] | South<br>%<br>[35,37] |
|---------------------------------|-----------------------------|---------------------------|----------------------|-----------------------|
| SEA type                        | 5.2-21.0                    | 3.1-13.6                  | 4.6                  |                       |
| $\alpha^0$ -thalassemia         |                             |                           |                      | 4.8                   |
| uncharacterized                 |                             |                           |                      |                       |
| 3.7 kb deletion                 | 19.9                        | 25.1                      | 17.2                 | ND                    |
| 4.2 kb deletion                 | 4.5                         | 0.8                       |                      |                       |
| Hb CS                           | 0.3-4.2                     | 5.4                       | 5.6                  | ND                    |
| Hb Pakse                        | 0.5                         | 1.4                       | 0.5                  | 0.4                   |

Note that there was rarely reported in the South

\* ND; not determined

**Table 2** Prevalence of common  $\beta$ -thalassemia mutation in the North, Northeast, Central and South of Thailand, studied from 2002 to 2022.

| $\beta$ -thalassemia mutations | North<br>%<br>[26-27, 29-31] | Northeast<br>%<br>[34] | Central<br>%<br>[33, 38, 40] | South<br>%<br>[36, 39] |
|--------------------------------|------------------------------|------------------------|------------------------------|------------------------|
| Codon 41/42 (-TCTT)            | 48.0                         | 43.1                   | 35.6-37.5                    | 14.4-23.9              |
| Codon 17 (A>T)                 | 30.0                         | 21.5                   | 18.9-26.1                    | 7.7                    |
| Codon 19 (A>G)                 | -                            | -                      | 1.1-4.7                      | 6.3-18.6               |
| nt-28 (A>G)                    | 6.0                          | 21.5                   | 15.7                         | 4.4-7.3                |
| IVS-II-654 (C>T)               | 2.0                          | 1.5                    | 6.3-6.8                      | 2.7-4.4                |
| Hb E                           | 14.9-37.3                    | 39.1-(50-60)           | 22.7                         | 16                     |



**Figure 3** Map representation for common thalassemia mutations in Thailand, studied from 2002 to 2022. ND; not determined

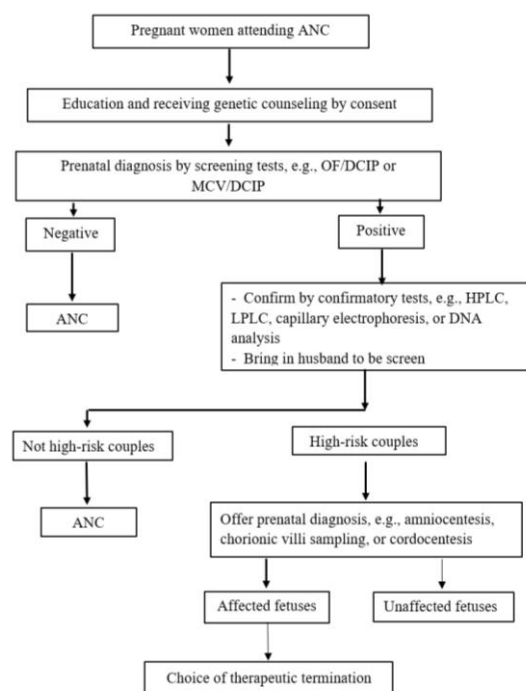
## Clinical symptoms of thalassemia and hemoglobinopathies

The clinical symptoms of thalassemia can range from asymptomatic carriers to severe anemia with these complications. When left with adequate treatment, thalassemia can cause severe anemia, extramedullary hematopoiesis, growth deficits, and iron overload, all of which can substantially reduce life expectancy. The quantity of lost globin chains is correlated with the severity of clinical symptoms. In clinics, three types of thalassemia syndrome are typically seen, including i) thalassemia major: is the most severe group of thalassemia, consisting of Hb Bart's hydrops fetalis in those who are homozygous for  $\alpha$ -thalassemia 1 and  $\beta$ -thalassemia major, who are homozygous or a compound heterozygote of severe types of  $\beta$ -globin gene mutation. People with thalassemia major manifest clinically between the ages of 6 and 12 months. Hemoglobin levels in untreated patients are as low as 3 to 4 g/dL, which makes the patient transfusion dependent, ii) thalassemia intermediate: this group of thalassemia is clinically milder than the major form of the disease. This type of thalassemia includes Hb H disease which refers to a compound heterozygote form of  $\alpha$ -thalassemia 1 and  $\alpha$ -thalassemia 2 and some cases of homozygous  $\beta$ -thalassemia or compound heterozygous of Hb E and  $\beta$ -thalassemia. When compared to patients with thalassemia major, patients with intermediate thalassemia typically develop clinical symptoms later in life. With hemoglobin levels between 7-9 g/dL and late disease onset, patients with intermediate types thalassemia typically require only infrequent or no blood transfusion; and iii) thalassemia minor: this type of thalassemia is seen in a carrier, heterozygote trait states. No obvious clinical symptoms are seen for this type. Hemoglobin level is usually equal to or more than 9 g/dL [45-48].

## Strategies for the prevention and control of thalassemia diseases in Thailand

Thailand's national-wide program for the prevention and control of severe thalassemia which includes Hb Bart's hydrops fetalis, homozygous  $\beta$ -thalassemia, and  $\beta$ -thalassemia/Hb E was established by the Ministry of Public Health in 1997. The two main principles of this program were: 1) to offer the best treatments and improve patients' quality of life 2) prevent or minimize new births affected by severe thalassemia through screening, counseling and prenatal diagnosis under the policy (choose partner, select pregnancy, select delivery) [49]. In addition, laboratory staff members received varying degrees of training on how to identify high-risk couples and thalassemia carriers. The first simple screening included the osmotic fragility test (OF test) and dichlorophenol-indophenol precipitation test (DCIP test), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin). Secondly, if a couple had positive screening such as reduced MCV (<80 fL cutoff value) and MCH (<27 pg

cutoff value) or positive OF/DCIP will be further investigated by a confirmatory test such as high-performance liquid chromatography (HPLC), low performance liquid chromatography (LPLC), capillary electrophoresis or using DNA analysis that is an important technique for the diagnosis of  $\alpha$ -thalassemias [50]. The national policy to promote the prevention and control of severe thalassemia in Thailand was established in 2005 as follows [49]; (i) Every pregnant woman should receive genetic counseling for thalassemia; (ii) Every pregnant woman should receive thalassemia screening by consent. If the result of the pregnant woman is found to be positive for thalassemia or hemoglobinopathies, then the husband will also be screen; (iii) If the pregnant woman and her husband both have a positive thalassemia screen, they should undergo confirmatory tests to be sure that they are appropriately categorized as a couple at high-risk of having an offspring with severe thalassemia; (iv) If the pregnant woman and her husband are found to be a high-risk couple, they should be offered a prenatal diagnosis; (v) Every health care unit should establish a quality service system to promote/prevention/control of thalassemia. If any service exceeds the capacity of the unit, the case should be referred to a service network.; and (vi) Students, women of child bearing age and married couples will be extensively informed about thalassemia.



**Figure 4** The schematic of the prenatal prevention program for severe thalassemia in Thailand: ANC; Antenatal Care Clinic, OF; osmotic fragility test, DCIP; dichlorophenol-indophenol precipitation test, MCV; mean corpuscular volume, HPLC; high performance liquid chromatography, LPLC; low performance liquid chromatography

## Conclusion

This article described the current prevalence of  $\alpha$ - and  $\beta$ -thalassemia mutations and focused on common mutations in the North, Northeast, Central and South of Thailand. For the prevalence of  $\alpha$ -thalassemia mutations, the data reveals that the frequency of  $\alpha^0$ -thalassemia (SEA type) is highest throughout the country, while,  $\alpha^+$ -thalassemia (3.7 kb deletion) is also high, followed by 4.2 kb deletion. For the prevalence of  $\beta$ -thalassemia mutations, the frequency of codon 41/42 (-TCTT) is the most frequent mutation and codon 17 (A>T) is the major one. These two types accounted for more than 50% in North, Northeast and Central Thailand, while in the South, the frequency is lower, averaging about 22-32%, because of codon 19 (A>G) which is widely distributed after codon 41/42. However, Hb E is the major  $\beta$ -hemoglobinopathies owing to its high prevalence in all regions.

## Acknowledgements

The author thanks Dr. Joseph William Sullivan, Oregon Medical Director, Medical Teams International, Portland, Oregon, USA, for proofreading the English of the manuscript.

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