I. INTRODUCTION

Homozygous β -thalassemia is the recessive inherited disorder which results in a severe anemia requiring regular blood transfusion from early infancy. The lifelong transfusional treatment is neccessitated for these patients to sustain life and relieve the symptoms of anemia (1). Therefore homozygous β -thalassemia becomes a huge public health problem in Thailand. The past studies have suggested that β-thalassemia genes are born in the Thai population at estimated frequencies of 3-9% (2). If one considers the size of population and the increasing rate of birth, it is quite clear that an increasing number of β -thalassemic infants will be born every year. At this time prenatal diagnosis is one approach to reduce this problem.

It is now recognized that α -thalassemia is common in many countries in which β -thalassemia is also common. The occurence of α -thalassemia may ameliorate the clinical severity of homozygous β -thalassemia (3-6). This is the factor that restores globin chain imbalance and leads to less severe phenotypic manifestation. The development of a technique for analysis of the globin genes now enables one to test this hypothesis directly. In the present study, the α -globin gene deletions were investigated in a group of homozygous β -thalassemia patients in Chiang Mai Thailand.

The objectives of this study include:

1). The analysis by DNA mapping of the α-globin gene deletion in a group of homozygous β-thalassemia patients with different levels of severity

2). The analysis of the phenotypic expression, clinical symptoms and hematologic data of the subjects with normal and deleted α-globin genes identified in 1.

I.1 The structure and expression of globin genes

A. Structure

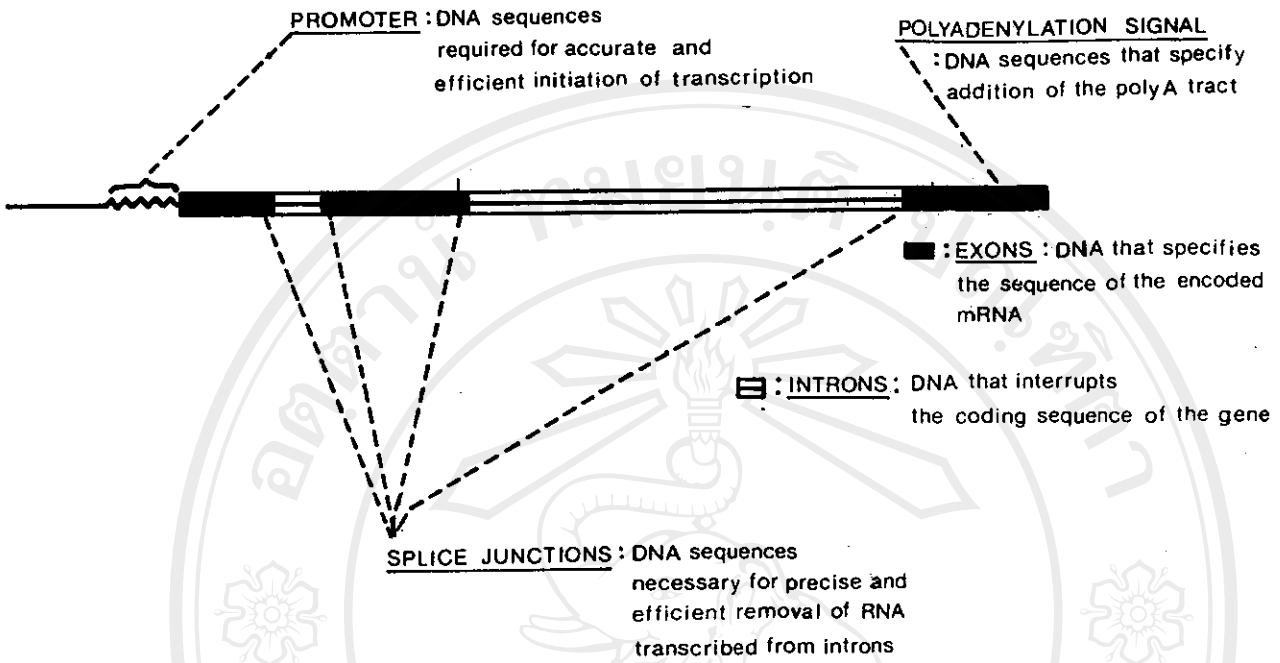
The expressed portion of each globin gene is contained in three coding sequences which are called exons seperated by two intervening sequences (IVS) or introns. The arrangement of codons that specify the 141 or 146 amino acid sequence of the α -like or β -like globin chains are shown in Figure I.1.

B. Expression

The series of expression steps from gene to globin peptide are shown in Figure I.2. The transcription is initiated at the site of DNA double helix corresponding to the 5' end of a globin mRNA. Then the 5' end of this newly synthesized RNA molecule is modified by a series of methylations which are called "Capping". After splicing and addition of poly A tract at the 3' end, the transcripted mRNA is transported to the cytoplasm for use as a template in protein synthesis. The translation initiates from a triplet codon (AUG) that encodes for methionine. Then each triplet codon is read sequentially to give a protein of correct sequence (Figure I.3).

C. Organization

The human globin genes are organized into two clusters (7,8) (Figure I.3). The α -globin gene cluster resides on the short arm of chromosome 16 in a 30 kb (9). There are three functional genes that



	EXON 1 Codons	INTRON 1 Length	EXON 2 Codons	INTRON 2 Length	EXON 3 Codons
α-Like Genes	1–31	117 bp ($\alpha_1\alpha_2$) 1265 bp (ζ)	32–99	140 149 or ($\alpha_1\alpha_2$) 341 (ζ)	100–141
β-Like Genes	1–30	122–130 bp	31–104	850–904	105–146

Figure I.1 Functional elements of a globin gene.

The size of exons and introns given in the lower part of the figure are those of the human globin genes (15).

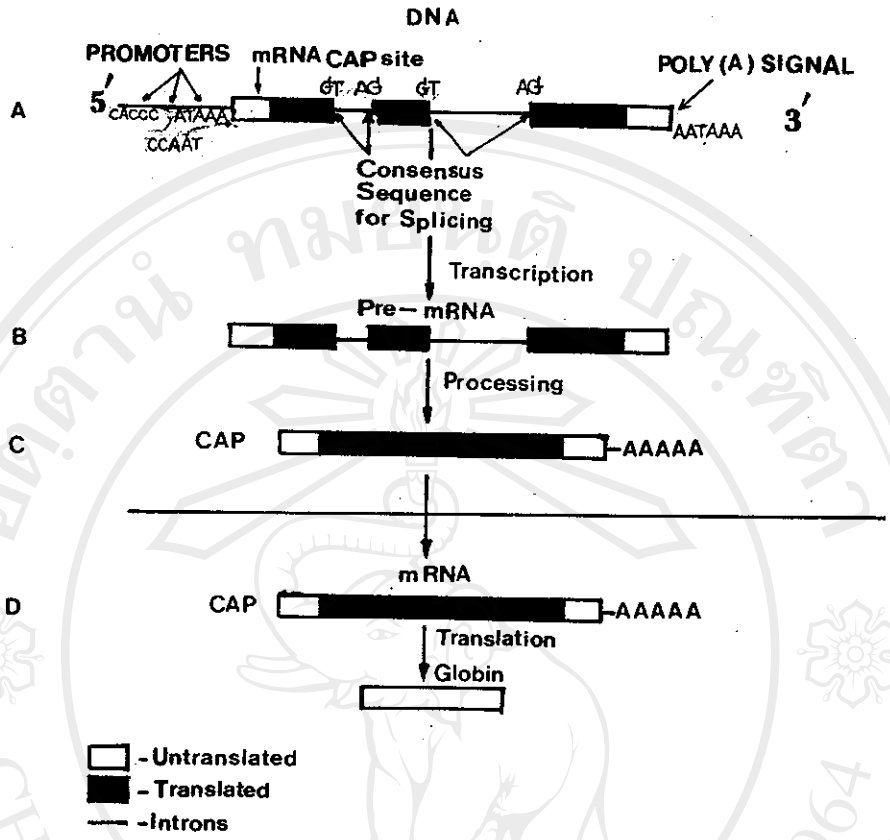


Figure I.2 Molecular aspects of globin biosynthesis. (A) The globin gene containing three blocks of exons interrupted by two introns. Promotors are located at of 5' to the gene and represented here by the CCAAT, ATA box. The invariant nucleotides, embedded in consensus sequences. The poly (A) signal is instrumental in 3' end processing after transcription ending. (B) The entire gene is transcribed into a pre-mRNA. (C) mRNA is processed by the excision of introns and ligation of exons. (D) The mRNA is then exported to cytoplasm for translation to globin peptide (19).

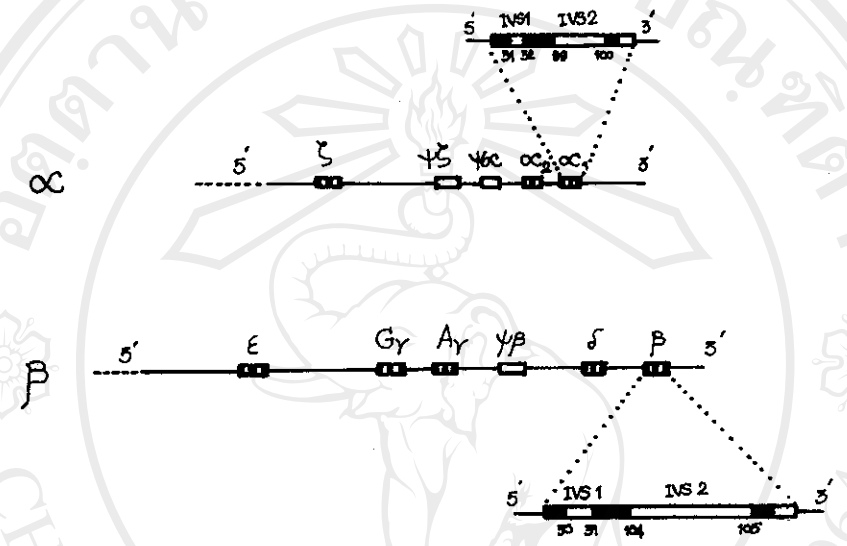


Figure I.3 Arrangement of α- and β-globin gene complex.

For every gene, black boxes represent coding regions (exons), white boxes represent intervening sequences (introns, IVS) and hatched boxes are the 5' and 3' untranslated regions. The number of the codons separated by each intron is also shown under the area of the coding sequences.

code for the ζ and α proteins. The two genes (α_2 and α_1) differ slightly in sequence, but their protein-coding regions are identical. The α_2 gene has been demonstrated to be the major locus that encodes 2-3 fold more protein than the α_1 gene (10). In addition, two pseudogenes ($\psi\alpha$ and $\psi\zeta$) are found in the α gene cluster. Pseudogenes are defective in some essential component of gene structure and thus they cannot be expressed in a globin product despite their sequence homology to the functional genes. They are thought to be by-products of evolution which were previously functional genes and inactivated by mutation in coding or regulatory regions.

The β-globin gene cluster is found on the short arm of chromosome 11(11,12) in 50 kb region. It includes the functional genes, ϵ, G_γ, A_γ, δ, and β and a pseudogene ($\psi\beta$). The genes encode identical products with the exception of either glycine (G_γ) or alanine (A_γ) at position 136 on the polypeptide chain (13). The δ gene is defective in several structure elements leading to inefficient expression (14).

I.2 The structure and genetic control of human hemoglobin

The human hemoglobins are tetramers composed of four globin chains attached with a heme group. Two globin chains are encoded by a gene in the α cluster, whereas the other two are encoded by a gene in the β cluster. During the human development the ζ and ϵ genes are activated first, leading to synthesis of Hb Gower 1 ($\zeta_2\epsilon_2$) in the embryonic life, followed by the expression of α and γ genes. Hence Hb

Gower2 ($\alpha_2 \epsilon_2$), Hb Portland ($\zeta_2 \gamma_2$) and Hb F ($\alpha_2 \gamma_2$) are produced. The δ and β genes are expressed later in the perinatal period, when Hb A ($\alpha_2 \beta_2$) becomes the major Hb and HbA_2 ($\alpha_2 \delta_2$) is the minor component in the adult (15). The hemoglobin production in each stage of human development is shown in Figure I.4.

I.3. The thalassemia syndromes

The thalassemias are disorders of Hb synthesis characterized by a reduction in synthesis of one of the globin subunits of the Hb molecule. Two major thalassemic diseases are α- and β- thalassemias. In both conditions the defective synthesis of one globin chain results in a relative excess of the other. The excess chain is unstable and precipitates causing the cell membrane to be damaged and leading to a change of cell morphology and ineffective erythropoeisis (16).

The α- thalassemia is the most prevalent inherited disorder characterized by diminished or absence of chain synthesis. Two types of α- thalassemia are designated, α-thalassemia 1 and α-thlassemia 2. The α-thalassemia 1 causes a more severe reduction in α chain synthesis than α-thalassemia 2. A heterozygote for α-thalassemia 1 shows a slightly more pronouced manifestation whereas the α-thalassemia 2 heterozygote is asymptomatic. A double heterozygote for α-thalassemia 1 and α thalassemia 2 results in Hb H disease. The homozygote for α-thalassemia 1 in which there is no α chain production, results in the Hb Bart's hydrop fetalis syndrome.

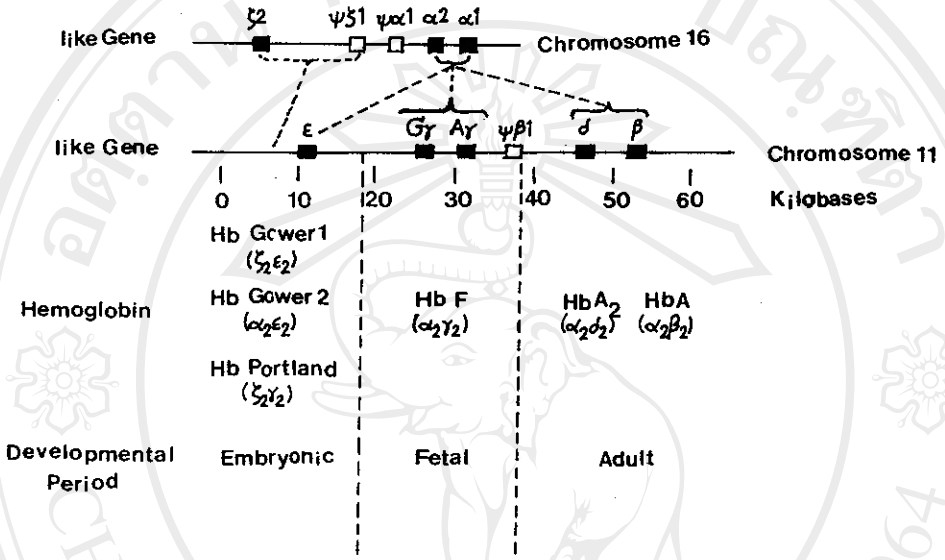


Figure I.4 Organization of the human globin genes and hemoglobins produced in each stage of human development (15).

The β-thalassemia is a disorder of β-globin gene expression in which there is a decrease in or absence of β-globin synthesis. These disorders can be divided into two groups, β^+-thalassemia which is due to a partial deficiency of β chain production and β°- thalassemia which is due to a complete absence of chain synthesis. Both β^+- and β°-thalassemia are extremely heterogeneous at molecular and phenotypic levels (17). The hemozygous state for β^+- or β°- thalassemia or the compound heterozygous states for both β^+- and β°-thalassemia are usually associated with transfusion-dependent anemia from early life. These conditions are clinically called thalassemia major. The disease commonly presents during the first year, at the time when γ chain synthesis is giving place to β chain synthesis. The severe anemia is caused by both the deficiency of normal Hb A and the production of excess α-globin chains which precipitate in cells. The β-thalassemia heterozygote is a mild condition in which there is mild anemia with elevated Hb A_2 level. In addition, some of the homozygous β-thalassemia patients show a milder course than usual. They are called the β-thalassemia intermedia.

I.4 The molecular basis of β-thalassemia

The variety of molecular defects of the β-globin gene has been studied for nearly two decades. Two conditions can be distinguished between the "simple β-thalassemia" in which only β-globin synthesis is affected, and the "complex β-thalassemia" in which the

deletion involves other genes in the β-globin gene cluster, resulting in the production of several β-like globin polypeptides(15). The molecular defects, including of the partial deletions and mutations which cause β-thalassemia, have been identified. Three deletions and 51 mutations in the β-globin gene are known (18). Most of the mutations which cause β-thalassemia are single nucleotide substitutions, involving the defects in transcription, RNA splicing, RNA modification, translation via frameshifts and nonsense codons, and producing the unstable β-globin chains.

1. Gene deletions

Since there is a lack of homology among the β-like globin genes, as compared to the α-globin genes, deletion of large portions of DNA is not a common cause of the β-thalassemia. The three partial deletions are shown in Figure I.5. Although these do not remove the entire gene, the removal region is enough for the gene inactivation (19). The first pattern is found in some Asian Indians. This deletion starts from within the second intervening sequence (IVS-2) and extends past the end of the gene, removing 619 bp of DNA (20). The other two deletions, which are larger, are found in American Black and Dutch.

2. Nondeletion forms of β-thalassemia.

The various mutations in β-thalassemia can be divided into a number of classes according to the expression process which is

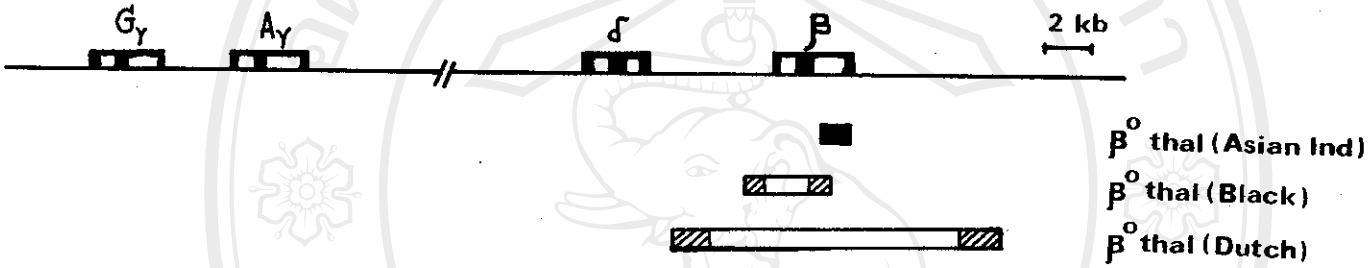


Figure I.5 Partial β-globin gene deletions in simple β-thalassemia. (19).

affected. The location of the β-thalsssemia mutations in the gene and their effect on gene expression are shown in Figure I.6 and Table I. 1.

2.1 Transcription mutants

The mutations that affect transcription are found in the conserved DNA sequence or promotor region that lie 5' to the β-globin genes. They are single nucleotide substitutions in the sequence CACCC, which is conserved upstream of the γ-globin genes and in the ATA box homology that forms part of the proximal promotor element (Figure I.7). These defects cause only a moderate impairment in globin synthesis and they are observed in β-thalassemia intermedia.

2.2 Nonfunctional messenger RNAs.

The β-thalassemia genes which contain nonsense or frameshift mutations will cause the premature chain termination during translation of β-mRNA. These mutations result in β°-thalassemia in which no β-globin can be produced. Nonsense mutations interfere directly with translation by creation of a stop signal via a single nucleotide substitution, whereas frameshift defects alter the reading frame and cause termination further downstream (Figure I.8). The nonsense mutation in amino acid codon 39 is quite common among Mediteraneans and constitutes the vast majority of thalassemia in Sardinia.

2.3 RNA splicing mutants

These mutants interfere with or alter the normal mRNA splicing. In the normal splicing process, introns must be removed and exon spliced. The critical fidelity of this process results in

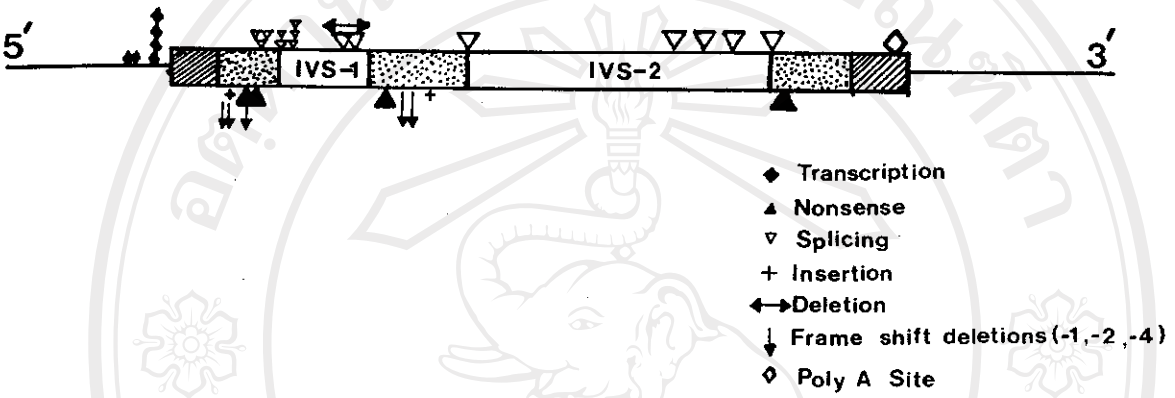


Figure I.6 The location of β-thalassemia mutations in the human β-globin gene (18).
Each of the specific mutations listed in Table I.1 is indicated along the linear map of the β-gene.

Table I.1 Mutations in β-thalassemia (18)

Mutant Class	Type	Origin
Nonfunction mRNA		
Nonsense mutants		
Codon 17 (A-T)	β°	Chinese
Codon 39 (C-T)	β°	Mediterranean
Codon 15 (G-A)	β°	Indian
Codon 121 (G-T)	β°	Polish
Frameshift mutants		
- 1 codon 6	β°	Mediterranean
- 2 codon 8	β°	Turkish
+ 1 codons 8,9	β°	Indian
- 1 codon 16	β°	Indian
- 4 codons 41,42	β°	Indian
- 1 codon 44	β°	Kurdish
+ 1 codons 71,72	β°	Chinese
RNA Processing Mutants		
Splice-junction changes		
IVS-1 position 1 (G-A)	β°	Mediterranean
IVS-1 position 1 (G-T)	β°	Indian
IVS-1 3' -end -25 bp	β°	Indian
IVS-1 3'-end -17 bp	β°	Kuwaiti
IVS-2 position 1 (G-A)	β°	Mediterranean

IVS-2 3' end (A-G)	β^{o}	Black
Consensus changes		
IVS-1 position 5 (G-T)	β^{+}	Mediterranean
IVS-1 position 5 (G-C)	β^{+}	Mediterranean
IVS-1 position 6 (T-C)	β^{+}	Mediterranean
Internal changes in IVS		
IVS-1 position 110 (G-A)	β^{+}	Mediterranean
IVS-1 position 116 (T-G)		Mediterranean
IVS-2 position 654 (C-T)	β^{o}	Chinese
IVS-2 position 705 (T-G)	β^{+}	Mediterranean
IVS-2 position 745 (C-G)	β^{+}	Mediterranean
Internal changes in coding region		
Codon 24 (T-A)	β^{+}	Black
Codon 26 (G-A)	β^{E}	Southeast Asian
Codon 27 (G-T)	$\beta^{Knossos}$	Mediterranean
Promoter mutants		
- 88 (C-T)	β^{+}	Black
- 87 (C-G)	β^{+}	Mediterranean
- 31 (A-G)	β^{+}	Japanese
- 29 (A-G)	β^{+}	Black
- 28 (A-G)	β^{+}	Chinese
- 28 (A-C)	β^{+}	Kurdish
RNA cleavage mutant.		
AATAAA-AACAAA	β^{+}	Black

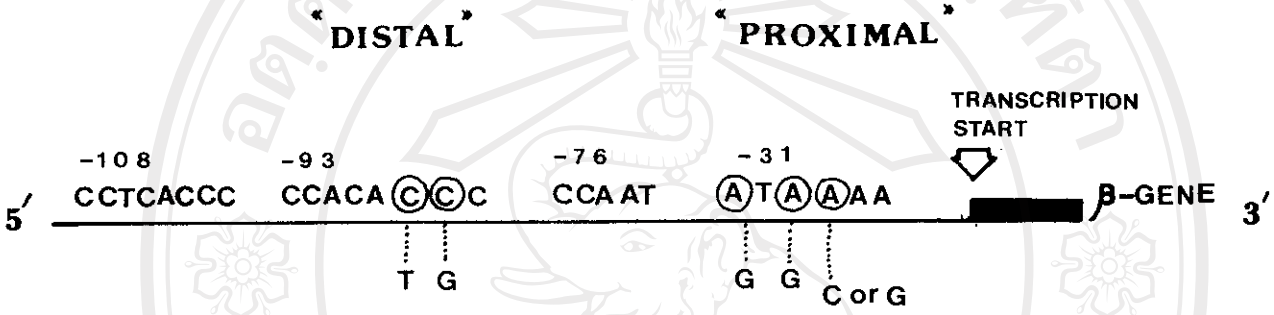


Figure I.7 Transcription mutations of the β-globin gene in β-thalassemia.

A partial sequence of the 5' flanking region of the β-gene is presented. Nucleotides are numbered with respect to the transcription start site. Nucleotides given below the line indicate the substitutions identified in β-thalassemia.

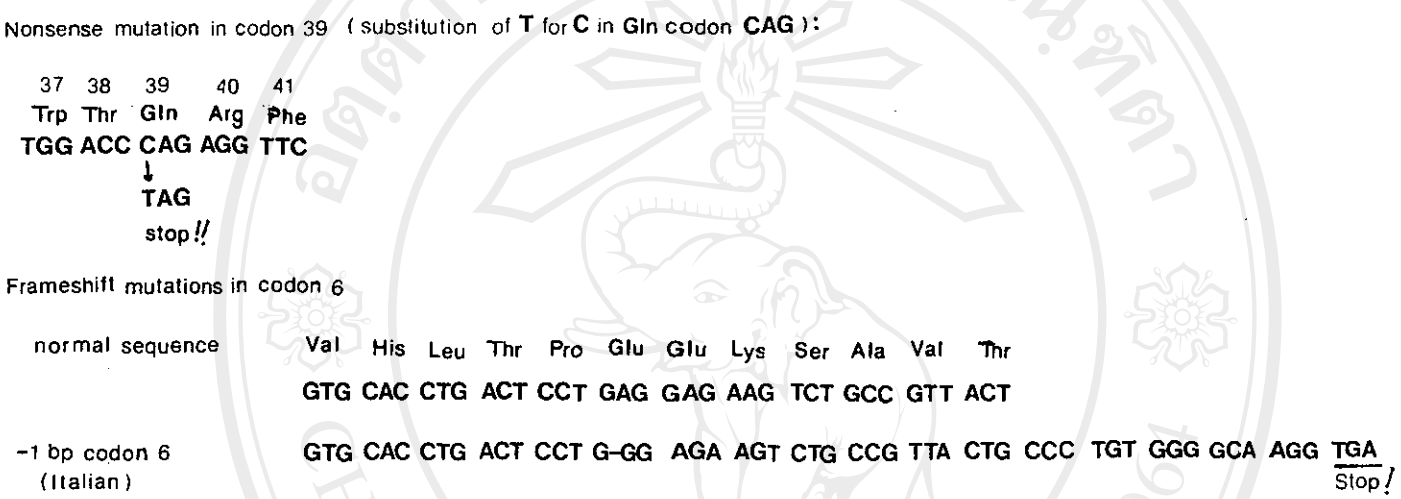


Figure I.8 Examples of nonsense and frameshift mutations in β-thalassemia (15).

specific conserved nucleotides that bind the 5' (donor) and the 3' (acceptor) splice junction which are called the invariant residues (Figure I.2). The mutations that alter or destroy the invariant residues (GT and AG) of splice sequence result in β°-thalassemia in which normal mRNA is not present. Another way, when the mutations do not involve these important residues, some normal mRNA is produced and β^{+}-thalassemia will result.

2.4 Mutation of the cap site and polyadenylation signal

The RNA modification defects are found at both the 5' end or cap site and the 3' end in the RNA cleavage and polyadenylation signal (AATAAA). The cap site mutation (A→C) affects function of RNA either by reducing transcription or slowing the 5' capping process. The mutation in the AATAAA signal at the 3' end of transcript was found to affect the efficiency of endolytic cleavage. The defect leads to an elongated mRNA molecule resulting in the cleavage and polyadenylation occurring downstream from the normal cleavage site, and the transcription can proceed at least 900 bp downstream from the human β-globin gene.

I.5 The β-thalassemia intermedia

The term β-thalassemia intermedia is used to describe the clinical and hematologic thalassemia phenotype that is similar or less severe than thalassemia major but more severe than the β-thalassemia heterozygous carrier state. The wide spectrum of clinical conditions

range from a moderate microcytic anemia to a severe anemia. The patients do not usually require a continuous transfusion, but they may become transfusion dependent or transiently dependent if the anemia is exacerbated because of the development of hypersplenism, folic acid deficiency, nutritional deficiency or intercurrent infection (16).

The differentiation of thalassemia intermedia from thalassemia major at presentation is a critical step in the clinical management of this group of disorders. Children with thalassemia major usually present the clinical picture between age 3 months and 2 years and begin a continuous transfusion program while those with thalassemia intermedia become manifest later, usually after age 18 months, with a similar but less pronounced clinical picture. The Hb level of the intermedia group are higher (mean 8.2 gm/dl) than those (mean 6.8 gm/dl) found in thalassemia major (21). The genetic mechanisms which cause individual intermedia form have been studied and three possible mechanisms are described: 1) mild defects of β-globin chain, 2). the coinheritance of α-thalassemia, and 3). unusually efficient γ-chain production.

I.5.1 Mild defects of β-globin chain

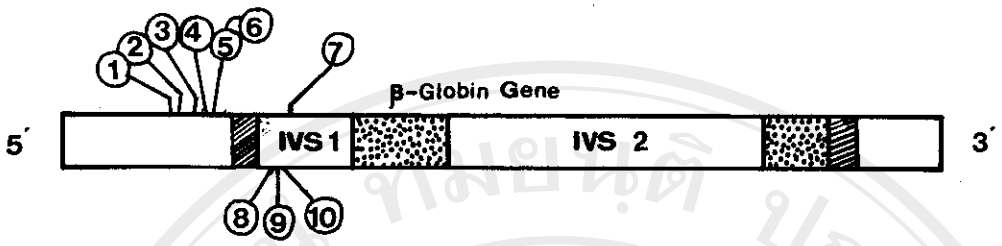
The phenotype-genotype correlations of β-thalassemia have been studied and showed a limited number of β-thalassemia mutations which are associated with relatively mild defects of β-globin chain synthesis (22). They usually result from mutation affecting gene

promotor activity or mRNA processing in which the level of functional mRNAs are reduced. A number of these mutants are summarized in Figure I.9. The homozygous state and the compound heterozygous state for these genes result in the clinical phenotype of thalassemia intermedia.

In addition, since the linkage of specific β-thalassemia mutations to restriction fragment length polymorphism (RFLP) in the β-globin gene cluster (β haplotypes) has been discovered (23), it enables the researcher to obtain a more precise conclusion of different genetic factors in modifying the clinical course of homozygous β-thalassemia. A recent study of molecular basis of β-thalassemia in Asian Indian population has shown that a particular β haplotype associated with the high level of Hb F and the inheritance of a mild β-thalassemia mutation are the major ameliorating factors of disease severity in Asian Indians (24).

I.5.2 The coinheritance of α-thalassemia

There are many individual family reports suggesting that the clinical course of homozygous β-thalassemia can be modified by the coinheritance of α-thalassemia. Wainscoat et al. (5) showed the analysis of α-globin gene in Cypriot patients with thalassemia major compared with a group of patients with thalassemia intermedia. The results indicate that coinheritance of one or two α-globin gene deletion play an important role in determining the clinical course of homozygous β-thalassemia. A different conclusion was reported when



	MUTATION	ORIGIN
	PROMOTER REGION MUTANTS	
1.	-88 C→T	AMERICAN BLACK
2	-87 C→G (ACACGC)	MEDITERRANEAN
3	-31 A→G	JAPANESE
4	-29 A→G (CATGAAA)	AMERICAN BLACK
5	-28 A→C (CATACAA)	KURDISH
6	-28 A→G	CHINESE
	RNA SPLICING MUTANTS	
7	IVS 1 POSITION 6 T→C	MEDITERRANEAN
8	24 (T→A)	AMERICAN BLACK
9	26 (G→A)	SOUTH EAST ASIAN
10	27 (G→T) (Hb KNOSSOS)	MEDITERRANEAN

Figure I.9 Mild β^+-thalassemia alleles producing thalassemia intermedia (55).

the investigation was carried out in the Sardinian population (3,25). They found no significant difference in the incidence of one α-globin gene deletion between two groups of β-thalassemia homozygotes, one with the clinical phenotype of thalassemia major and the other with the intermediate forms. However, it seems that the deletion of two α-globin genes may convert the clinical picture of homozygous β-thalassemia to milder forms.

Another analysis of α-globin genotypes and β-haplotypes (RFLP) associated with β-thalassemia mutations was performed again in the Cypriot population (26). The results suggested that the majority of mild forms of homozygous β-thalassemia results from the coinheritance of α-thalassemia or from the presence of an unusually mild form of β-thalassemia mutations.

However, it is apparent that the co-existence of α-thalassemia and/or the presence of relatively mild β-thalassemia mutations cannot explain all cases of the β-thalassemia intermedia.

I.5.3 Unusually efficient γ-chain production

The genetic determinant that has been analysed as a possible candidate for ameliorating homozygous β-thalassemia is the inherited condition called hereditary persistence of fetal hemoglobin (HPFH). This condition is characterized by an elevated level of Hb F. The abnormality produced by this HPFH gene appears to represent the β-chain deficit, associated with a continuing synthesis of γ-chains in

adult life. The relatively large number of γ-chains combine with excess α-chains to produce Hb F and hence the clinical phenotype of β-thalassemia intermedia results.

I.6 The molecular basis of α- thalassemia

The most common mechanism that produces α-thalassemia is gene deletion that removes one or both α-globin genes from a single chromosome. However, the less common defects that lead to the impairment of α-globin production and do not involve gross deletion are associated as the nondeletion-type of α-thalassemia (27).

I.6.1 Deletion-type α-thalassemia

The detailed analysis of the α_1 and α_2 loci using DNA cloning and sequencing techniques revealed that the two α-globin genes are embeded within two homologous segments of DNA, each approximately 4 kb long. The areas could be divided into three regions, X, Y, and Z (28) as demonstrated in Figure I.10. The gene deletions occur as a result of crossover between these highly homologous regions. The crossover deletes a segment of DNA from one chromosome and adds this to the reciprocal chromosome(29). An example of some of the deletions that have been described in the α-globin gene cluster are shown in Figure I.11.

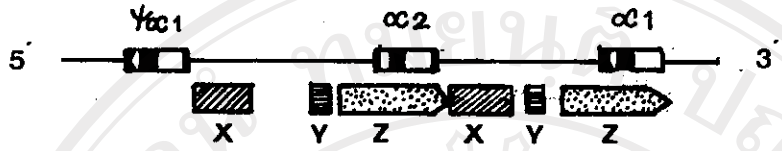


Figure I.10 The homology blocks of α-globin DNA (55)

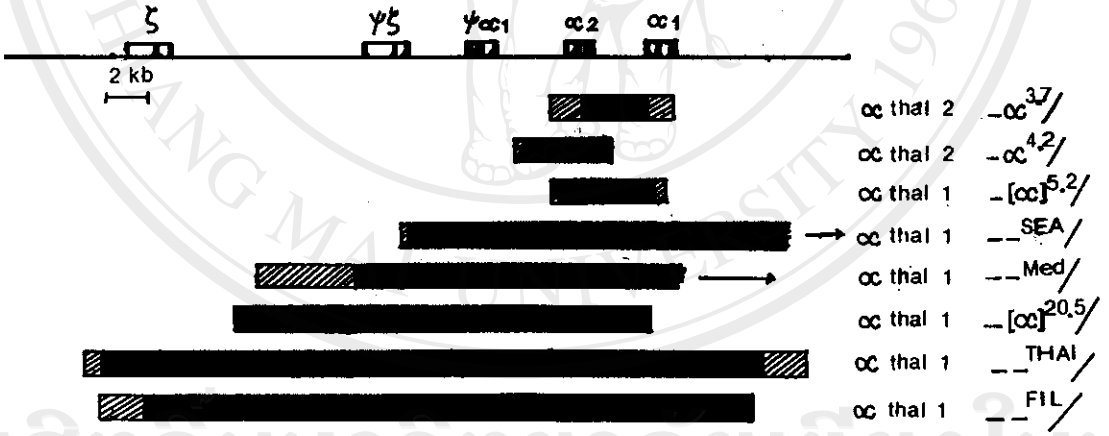


Figure I.11 Deletional mapping of the human α-globin gene cluster. (19).

I.6.1.1 α-Thalassemia 2 ($-\alpha$/haplotype)

α-Thalassemia 2 occurs from a deletion of one α-globin gene locus on the chromosome resulting in a decreased α-globin chain production(30). Two different deletions that are responsible for the α-thalassemia 2 haplotype have been characterized. One is the most common deletion, and it predominates in all ethnic groups where the α-thalassemia is present including Thai (35). It results from removal of 3.7 kb of a DNA fragment bridging the two genes; it is called the "rightward type" ($-\alpha^{3.7}$/)(31). The other is a result of a 4.2 kb deletion involving the α_2 gene. It is called the "leftward type" ($-\alpha^{4.2}$). This type is relatively common only in Asian subjects (31) and very rare in Black individuals (32).

The unequal crossover which causes the two types of α-thalassemia 2 are shown in Figure I.12. Crossing over between the Z homology boxes results in a single-gene chromosome of the "rightward type" deletion, whereas the crossing over mediated by the X homology boxes yields a "leftward type" of deletion (33).

I.6.1.2 α-Thalassemia 1 ($--$/haplotype)

α-Thalassemia 1 occurs from a deletion of both α-globin gene loci. The deletions which cause α-thalassemia 1 phenotype in Southeast Asian (SEA) including Thais involves a large segment, removing both α-globin genes. The 5' breakpoint of this deletion lies in the third exon of the $\psi\zeta$ gene and the 3' end of this deletion terminates within the hypervariable region (HVR) at the 3' end

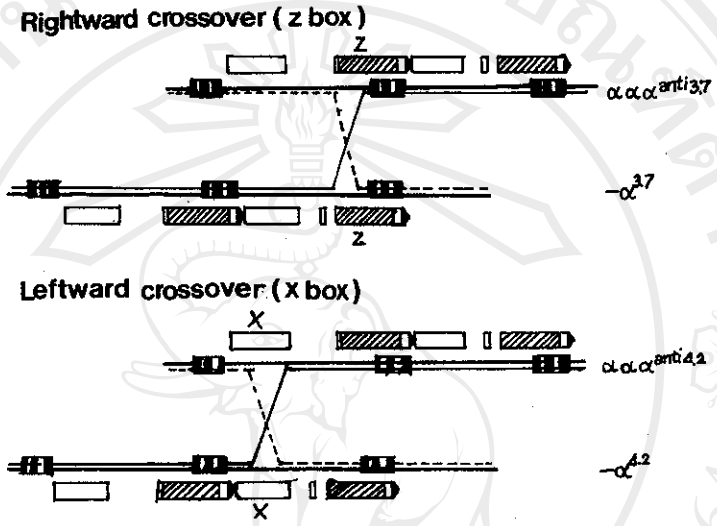


Figure I.12 Single gene and triplicated-gene complexes generated by homozygous pairing and recombination (56).

of gene cluster. It involves removing 17.5 kb of DNA and is represented as $--^{SEA}$/(34). In Mediterranean (MED) patients, the α-thalassemia 1 deletion involves both α-globin genes, the $\psi\alpha$ gene, and the $\psi\zeta$ gene (35,36). The 5' breakpoint lies upstream of the gene and the 3' end terminates at the 3' end of the α-globin gene complex. This deletion is represented as $--^{MED}$/. There are two additional α -thalassemia 1 haplotype in MED patients resulting from the deletion involving both of two α genes. One is a partial deletion of 5.2 kb of DNA which removes the entire α_2-globin gene and the 5' end of the α_1-globin gene. This deletion is represented as $-\alpha^{5.2}$/(37). The other type is shown to be a large deletion of DNA about 20.5 kb ($-\alpha^{20.5}$/). It includes the interzeta HVR up to the α_1 gene (38,39).

Two rare types of α-thalassemia 1 have been found in a single Thai subject and Philipinos. In Thai haplotype ($--^{THAI}$/), the deletion removes the entire α-globin gene cluster. The 5' end point is located further to the 5' side of ζ-globin gene and the 3' end terminates near the HVR at the 3' end of the α-globin gene complex (40). The haplotype which was found in Philipino subjects also involved a large deletion as $--^{THAI}$/ but the position of end point is different. The 5' end lies close to the 5' side of the ζ-globin gene and the 3' end terminates downstream to the α_1-globin gene(40).

I.6.2 Nondeletion-type α-thalassemia

The nondeletion α-thalassemias are the less common defects. The molecular mechanism is due to mutations that lead to absence of or greatly reduced expression of α-globin genes. These molecular lesions are summarized in Table I.2. The molecular basis of nondeletion α-thalassemia from various ethnic groups have been described with different mechanisms. In Mediteranean patients, two types of mutations have been found. The first mutation is associated with abnormal processing of precursor mRNA. It occurs from a pentanucleotide deletion within the 5' splice junction of the α_2-globin gene (41), preventing normal RNA splicing. The second mutation is associated with the nonfunctional mRNA. It is due to a single base substitution (ATG→ACG) in the initiation codon for translation of α_2-gene (42). This change abolished translation of the mutant mRNA.

The other nondeletion mutation which is found in Saudi Arabian patients is associated with the abnormal polyadenylation. It is due to a point mutation resulting in alteration of the typical poly A cleavage signal in the α_2-gene. The sequence, which is changed from AATAAA to AATAAG, creates a new cleavage site, downstream of the normal poly A addition (43). This produces the elongated α_2-mRNA which is unstable and results in rapid turnover. A different type of mutation has been found in a Chinese patient. A single nucleotide substitution in codon 125 of the α_2-gene,

Table I.2 Nondeletion ∝ -thalassemia mutations (15).

	Class	Ethnic Group
1.	RNA processing mutation -5 bp IVS-1 splice junction	Mediterranean
2.	Nonfunctional RNA Initiator mutation (ATG→ACG)	Mediterranean
3.	3'-End processing defect AATAAA-AATAAG	Saudi Arabian
4.	Unstable ∝ globin Hb Quong Sze (codon 125)	Chinese
5.	Chain-termination mutations Hb Constant Spring Koya Dora. etc.	Southeast Asian and others

exchanging the encoded amino acid from leucine to proline and resulting in the synthesis of a highly unstable α-globin variant which is named Hb Quong Sze (44). Another type of nondeletion α-thalassemia is the chain-termination mutation. This mutation occurs from the single nucleotide substitutions within the termination codon in the α_2-gene, leading to the continued translation into the 3'-untranslated region of mRNA and producing elongated α-globin variants(45). The first Hb variant which was described was Hb Constant Spring (Hb CS). The 31 additional amino acids were found at the C-terminus of the α-globin chain giving a globin with 172 amino acids. Other types of chain termination variants have been identified : Hb Icaria, Koya Dora, and Seal Rock. However, the interesting observation is that no chain-termination mutations of the α_1-globin gene have been identified to date (46).