HEMOGLOBIN AND THALASSEMIA

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Summary
Hemoglobins play an very important role in red blood cells, they carry oxygen and carbon dioxide throughout the body. Hemoglobin molecule consists of four polypeptide subunits, two identical α-globin chains and two identical β-globin chains, covalently bound with a heme prostetic pigments. The β-like globin genes located on chromosome 11p15.5 and α-like globin genes located on chromosome 16p13.3 are responsible for synthesis of globin chains of Hemoglobins. Mutations of these genes cause hypochromic microcytic hemolytic anemia including α-thalassemia, β-thalassemia and some other types.Thalassemia is among the most common inherited diseases worldwide, it has been estimated that 7% of the world's population are carriers for different inherited disorders of hemoglobin . There are certain mutations responsible for >90% thalassemia cases and about 10% mutations which are unknown or considered as very rare ones. Molecular defects of α-thalassemia are mainly associated with deletions or mutations of one or more of the α-globin genes, of which now more than 50 known deletional mutations have been discovered. In β-thalassemia, more than 200 point mutations and, rarely deletions, have been reported. The list of Hb mutations is updated at http://globin.bx.psu.edu/hbvar/
Key words*: Hemoglobin, thalassemia.*

Thalassemia is one of the major hemoglobinopathies among the population all around the world. It is a single gene hereditary hemoglobin disorder in human. It has been reported nowadays approximately 1 out of 14 peoples are carriers for different subtypes of thalassemia. Each year about 400,000 infants born with serious hemoglopinopathies and carrier frequency is about 270 million. Thalassemia is mainly caused due to decreased or absent amount of globin chain of hemoglobin. Thalassemia is mainly prevalent in populations in the Mediterranean area, South-East Asia, the Middle East, Transcaucasus, Central Asia, the Indian subcontinent, and the Far East. However, they are also quite common prevalent in populations of African heritage. Because of population migration and inter racial marriage system, nowadays thalassemia is also common in Northern Europe, North Central and South America, and Australia. There are certain mutations responsible for > 90% of thalassemia cases and about 10% mutations which are still unknown or considered as very rare ones [2][8].

STRUCTURE AND GENETICS OF HEMOGLOBIN

Hemoglobins (Hb) are globular proteins having the fundamental role to carry oxygen (O_2) molecules and carbon dioxide (CO_2) molecules throughout the body, and it serves also to destroy the physiologically important nitric oxide molecule [10]. It has evolved to perform its transport functions in a highly efficient manner: a) The oxygen affinity of Hb allows nearly complete saturation with oxygen in the lungs, as well as efficient oxygen unloading in the tissues; b) Its affinity increases with oxygenation, resulting in the sigmoid shape of the oxygen dissociation curve; and c) Deoxy-Hb binds protons and oxy-Hb releases protons. This last property, which is known as the "alkaline Bohr effect", also facilitates

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oxygen loading in the lungs and unloading in the tissues. The Perutz models of oxygenated and deoxygenated Hb provide important insights into the structural basis of these three major features of the equilibria of oxygen with Hb [1].

The roles of different parts of the Hb molecule in its equilibria have been deduced from its amino acid sequence, its helical conformation, models derived from x-ray crystallography studies of the kinetics of reactions of Hb with ligands and observations utilizing nuclear magnetic resonance [4]. The concentration of Hb within human red cells is extraordinarily high (14 g/dl), and its efficiency as an oxygen carrier is enhanced by its packaging in flexible cells of optimal shape for the diffusion of gases [3][6].

As shown in Fig. 1, Hb molecule consists of four polypeptide subunits, two identical α-globin chains and two identical β-globin chains, covalently bound with a heme prostetic pigments, one in each of the subunits, which are held together by ionic bonds, hydrogen bonds, hydrophobic interactions, and van der Waals forces [10]. The heme group contains a positively-charged iron (Fe^{2+}) molecules (Fig. 2) which can reversibly bind to oxygen molecules to be transported to the various areas of the body. As the heme groups bind or release their oxygen loads, the overall Hb undergoes conformational changes which alters their affinity for oxygen.

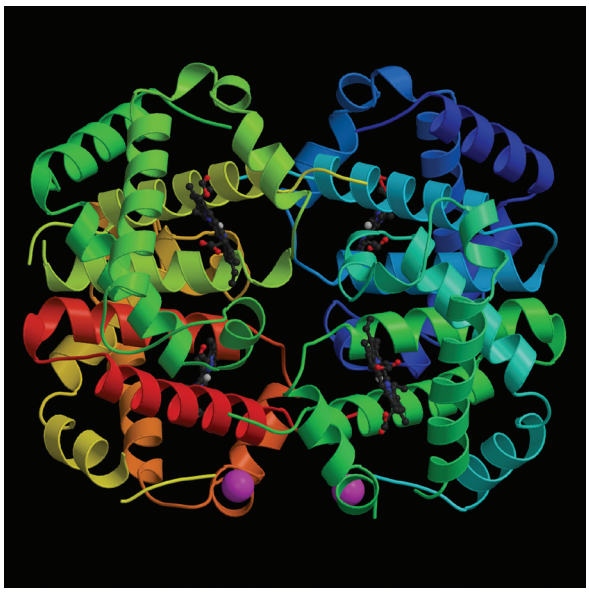

Figure 1. Structure of Hb [10]

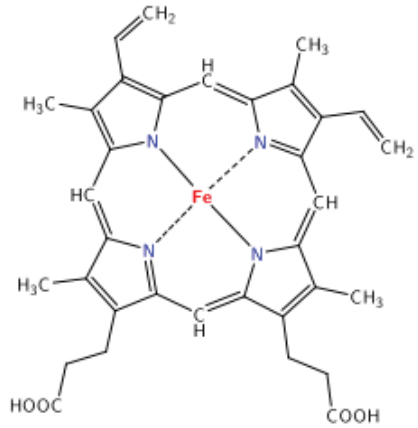

Figure 2. The heme group [10]

The heme consists of a ring structure, protoporphyrin IX, which binds an iron atom in its Fe^{2+} oxydation state. The Fe atom has six coordination bonds, four bonded to the flat porphyrin molecule and two perpendicular to it. Fifth and sixth coordination bonds are perpendicular to the flat cyclic ring structures; one is bound to a N atom of a His residue (the proximal His residue) whereas the other serves as the binding site for an O_2 molecule. This group is found in myoglobin, Hb, cytochromes, and many other heme proteins.

Human Hbs are tetrameric molecules encoded at two separate *loci*, the β-like globin gene cluster located on chromosome 11p15.5 (close to the olfactory receptor genes) and the α-like globin gene cluster on the terminus of chromosome 16p13.3 (close to heterochromatic gene encoding a putative RNA-binding protein) (11,12). As shown in Fig. 3 and 4, in each cluster the active genes are arrayed on the chromosome in the same order they are expressed developmentally: 5'-ε(embryonic)-$G\gamma$(fetal)-$A\gamma$(fetal)-δ(minor adult)-β(major adult)-3', and 5'-ζ(embryonic)-α_2(fetal and adult)-α_1(fetal and adult)-3', respectively. Hemoglobin production is characterized by two switches: the production of embryonic Gower1 ($\zeta_2\varepsilon_2$), Gower2 ($\alpha_2\varepsilon_2$) and Portland ($\zeta_2\gamma_2$) Hbs switches around the first two months of gestation to the production of two different fetal Hbs (HbF) ($\alpha_2{}^G\gamma_2$ and $\alpha_2{}^A\gamma_2$) followed, just before birth, to the major adult HbA ($\alpha_2\beta_2$) and minor adult HbA_2 ($\alpha_2\delta_2$) tetramers. As the result of the second switch, at birth the circulating Hb contains from 70 to 80% of HbF whereas 6 months later HbF covers less than 4-5% of the

total. The final adult Hb pattern is reached at 1 year of life (Fig. 4). At that time HbA comprises ~97%, HbA_2 ~2% and HbF ~1%. It is at this stage that mutations affecting the β gene become clinically apparent. At birth, HbF contains $^{G}\gamma$ and $^{A}\gamma$ chains in the 70:30 ratio. The switch from fetal to adult Hb production is not complete, so that in the small amounts of HbF which persist in adult life the proportion of the two chains reverses to 40:60. The switch from fetal to adult Hb is not due to changes in stem cell populations but rather to changes in programs of gene expression occurring in the progeny of a single stem cell population. All adults have residual amounts of HbF, present in a subset of erythrocytes called F-cells which also contains HbA. The levels of HbF and F-cells in adults vary considerably, and are largely genetically controlled [3][4].

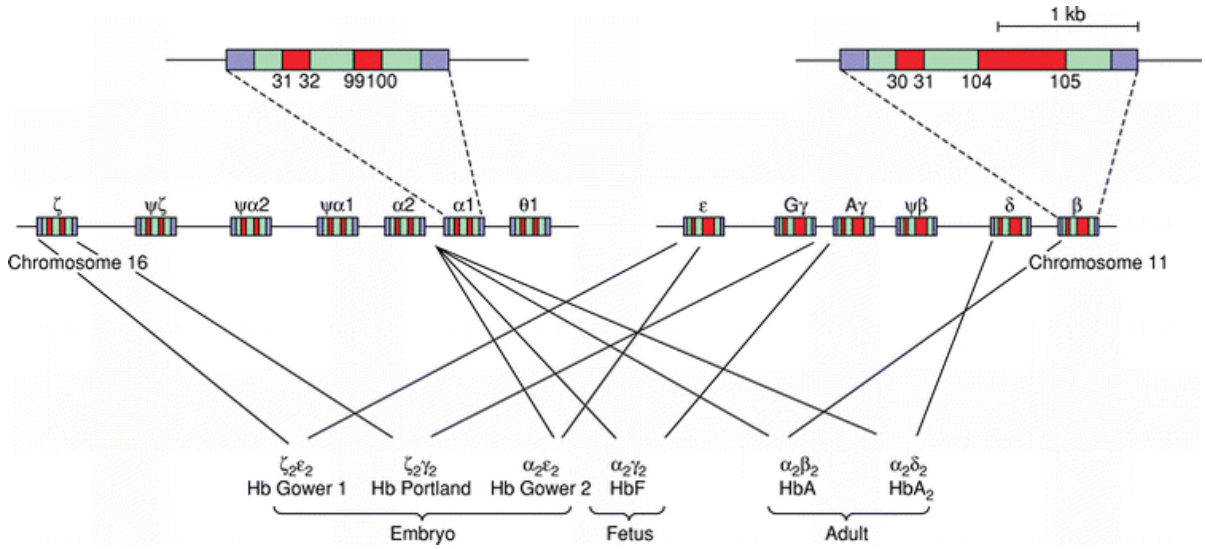

Figure 3. Basic organization of human α- and β-globin gene complexes and expression of the globin genes during ontogenesis [3][4]

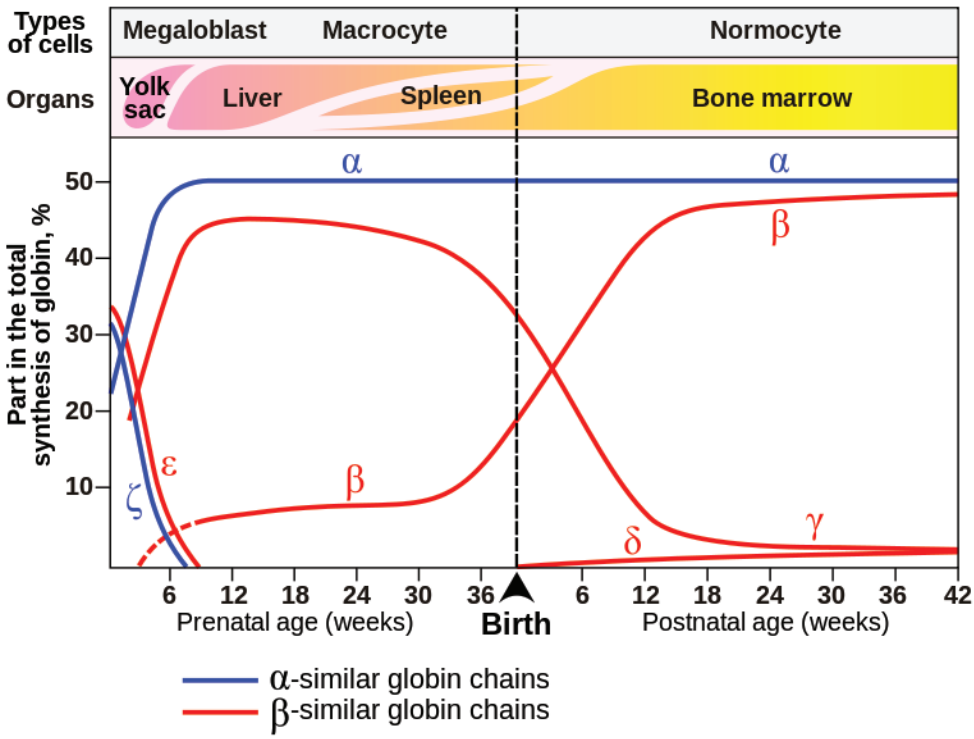

Figure 4. Globin chains production during ontigenesis [10]

The production of embryonic Gower1 ($\zeta_2\varepsilon_2$), Hb switches around the first two months of gestation to the production of HbF ($\alpha_2\gamma_2$) followed, just before birth, to the major adult HbA ($\alpha_2\beta_2$) and minor adult HbA_2 ($\alpha_2\delta_2$) tetramers.

ALPHA-THALASSEMIA

α-Thalassemia is caused due to decreased amount of α-globin chain synthesis. As there are 2 α globin genes in each haploid genome, so mutation may occur in one to four alleles.

Clinical forms

Number of functional alleles of the alpha-globin chain	Number of nonfunctional alleles of the alpha-globin chain	Name of disease	Symptoms
4	0	Healthy individual	Normal hematological profile
3	1	Alpha-thalassemia silent carrier	- Clinically asymptomatic - Blood test usually normal, hemoglobin normal - Slight changes in size of red blood cells (microcytic) - Slightly lighter color of red blood cell (hypochromic)
2	2	Alpha-thalassemia trait	- Mild anemia - Small red blood cells (microcytic) - Light, pale color of red blood cells (hypochromic)
1	3	Hemoglobin H(HbH) disease	- Moderate to severe anemia - Small red blood cells (microcytic) - Light, pale color of red blood cells (hypochromic) - Mild jaundice - Fatigue - Hepato-splenomegaly - Skeletal deformities (in some cases)
0	4	Alpha-thalassemia major or hemoglobin Barts hydrops fetalis (HbBarts) syndrome	- Intrauterine death of shortly after birth - Skeletal deformities - Cardiovascular problem - Improper brain growth - Enlarged placenta - Hepato-splenomegaly

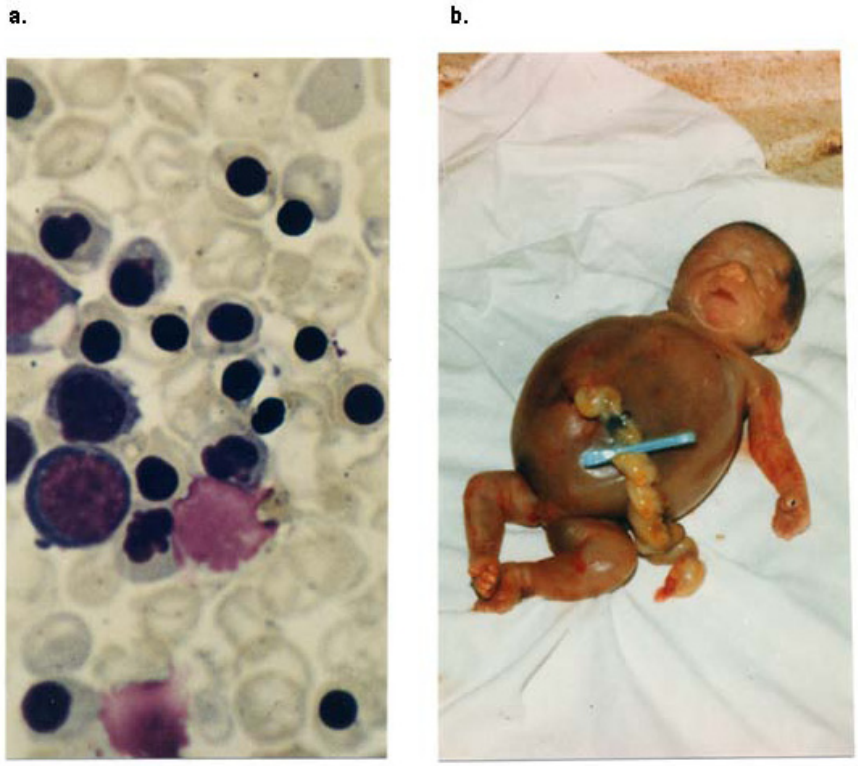

Figure 5. The Hemoglobin Bart's hydrops syndrome [5]

a. Peripheral blood film with immature red-cell precursors and hypochromic, microcytic, red cells showing anisocytosis and poikilocytosis; b. Stillborn hydropic infant

Mutations

The molecular basis of α- thalassemia is now understood in great detail. Normally, the α-like genes are arranged along chromosome 16 in the order in which they are expressed in development (telomere-ζ2-α2-α1-centromere). Furthermore, we now know that the cluster lies in a telomeric, gene-rich region of the genome, surrounded by widely expressed genes. Full expression of the α-like genes is critically dependent on the presence of a regulatory element (called HS-40), which lies 40 kb upstream of the cluster (toward the telomere). As many as 50 deletions removing one (-α/) or both (--) genes have been characterized and of these six (-α3.7/, -α4.2/, --SEA/, --MED/, -(α)20.5/ and --FIL/) represent by far the most common causes of α-thalassemia worldwide. In addition, many different point mutations affecting the structural genes have been identified, these cause the less common nondeletional forms of α-thalassemia (αTα/). This information has allowed researchers and hematologists to establish logical and robust screening programs for identifying patients with _ thalassemia. This in turn allows clinicians to provide accurate genetic counseling and prenatal diagnosis for the severe syndromes of α-thalassemia, including Hb Bart's Hydrops Fetalis and transfusion-dependent forms of HbH disease .When a mutation(s) completely abolishes expression from a chromosome this is called α0-thalassemia and when the mutation(s) only partially downregulate expression from the chromosome this is called α+-thalassemia [5][9]

α+-thalassaemia due to deletions

The α-globin genes are embedded within two highly homologous 4 kb duplication units [61-65]. One very common α-thalassaemia deletion is the rightward deletion, a 3.7 kb deletion caused by reciprocal recombination between Z segments producing a chromosome with only one functional α-gene (α-3.7 or rightward deletion) causing α-thalassaemia and an α-triplication allele without a thalassemic effect. Likewise a reciprocal recombination between mispaired X-boxes results in a 4.2 kb deletion, called leftward deletion (-α4.2). An increasing number of deletions resulting in the

loss of a single α-gene are reported due to non-homologous recombination events, most of which are rare, or highly region specific.

α+-thalassemia due to non-deletion types of α-thalassemia

Alpha-thalassemia is more frequently caused by deletion than single point mutations or nucleotide insertions and deletions involving the canonical sequences controlling gene expression. In general the non-deletion α+-thalassaemia determinants may give rise to a more severe reduction in α-chain synthesis than the -α deletion type of chromosomes. Many mutations have been described affecting mRNA processing, mRNA translation, and α-globin stability. Of these the most common non-deletional variants are the αIVSI (-5nt)α (in Mediterraneans), polyadenylation site mutations α2AATAAG, α2AATGAA and α2AATA-- (in the Mediterranean and Middle East) [71-74], termination codon mutations leading to elongated Hb variants, such as Hb Constant Spring (HbCS), Hb Icaria, Hb Koya Dora, Hb Seal Rock and Hb Pakse (middle East, Mediterranean and South East Asia) and structural mutations causing highly unstable α-globin variants; for example, Hb Quong Sze, Hb Suan Dok, Hb Petah Tikvah, Hb Adana, Hb Aghia Sophia. A regularly updated overview is provided by the HbVar web-site

α0-thalassemia due to deletions

The complete or partial deletion of both α-genes in cis results in no α-chain synthesis directed by these chromosomes *in vivo*. Homozygotes for such deletions have the Hb Bart's Hydrops Foetalis Syndrome. Many deletions were described which remove the ζ- and α-genes and although heterozygotes appear to develop normally, it is unlikely that homozygotes could survive even the early stages of gestation since neither embryonic (ζ2γ2) nor foetal (α2γ2) haemoglobins could be made. Rare deletions causing α0-thalassaemia remove the regulatory region, which lies 40-50 kb upstream of the α-globin gene cluster leaving the α-genes intact. This region composed of four multispecies conserved sequences (MCS), called MCS-R1 to R4, correspond to the previously identified erythroid-specific DNAse1 hypersensitive sites referred to as HS-48, HS-40, HS-33 and HS-10. Of these elements, only MCS-R2 (HS-40), 40 kb upstream from the ζ globin mRNA cap-site has been shown to be essential for α globin expression. Ethnic origin may guide molecular diagnosis. Knowledge of the mutations found in a specific population may allow strategic choice in laboratory diagnostics, especially in selection of the molecular techniques to be applied [5].

In addition to these common forms of α-thalassemia there are many rare and unusual molecular defects that have been identified. These are important because they provide an explanation for patients with hitherto undiagnosed anemia, and they help us to understand how the α cluster is normally regulated in vivo. Rarely, α-thalassemia is caused by deletions that remove the α-globin regulatory element (HS-40). In general these mutations have been observed outside of the "malaria belt", indicating that they are sporadic genetic events that have not been selected during evolution. These natural deletions first indicated the existence of this unexpected form of long-range control of α-globin expression. There are also two rare forms of α-thalassemia that are found in association with a variety of developmental abnormalities, and in particular with mental retardation (so-called α-thalassemia with mental retardation, ATR syndromes). The first group of patients has large (>1 Mb) deletions from the tip of chromosome 16 including the α-globin genes (ATR-16). These usually result from chromosome truncations or translocations and in fact this syndrome provided the first examples in human genetics of subcytogenetic chromosomal translocations, which are now known to underlie many cases of unexplained mental retardation. The second group of patients is now known to have mutations in a trans-acting factor (called ATRX) encoded on the X-chromosome (ATR-X syndrome). These patients have α-thalassemia with profound mental retardation, facial abnormalities, and urogenital anomalies. In this case it is thought that the X-encoded factor regulates expression of many genes, the α genes being but one target. Finally, there is a rare and unexplained form of α-thalassemia that is seen as an acquired mutation

in patients with myelodysplasia, hence called the ATMDS syndrome.

These patients inherit a normal complement of α genes (αα/αα) but later in life develop myelodysplasia and presumably acquire a clonal genetic abnormality during the course of their disease. It is interesting that the majority of these patients are elderly males who at some stage of their disease have abnormal erythropoiesis. It has recently been shown that these patients have acquired mutations in the *ATRX* gene [9]

BETA-THALASSEMIA

In β-thalassemia, there is reduction or absence of β-globin chain synthesis

Clinical forms

The β-thalassemia includes four clinical syndromes of increasing severity: two conditions are generally asymptomatic, the silent carrier state and β-thalassemia trait, and usually result from the inheritance of one mutant β-globin gene, and two require medical management, thalassemia intermedia and thalassemia major. The more severe forms most often result from homozygosity or compound heterozygosity for a mutant β-globin allele and, occasionally, from heterozygosity for dominant mutations. Homozygous or compound heterozygous β-thalassemia usually presents no diagnostic problems. The early onset of anemia, characteristic blood changes, and elevated fetal hemoglobin concentrations are found in no other condition. The diagnosis can be confirmed by the demonstration of the β-thalassemia trait in both parents.This condition is characterized by mild anemia, reduced mean cell volumes and mean cell hemoglobin concentrations, and elevated concentrations of the normal minor adult component of hemoglobin (usually exceeding 3.5 percent), hemoglobin A2 (α2δ2) [7][8].

Mutations

Nearly 200 different mutations have been described in patients with β-thalassemia and related disorders. Although most are small nucleotide substitutions within the cluster, deletions may also cause β-thalassemia. All the mutations result in either the absence of the synthesis of β-globin chains (β^o-thalassemia) or a reduction in synthesis (β^+-thalassemia) (Fig. 6)

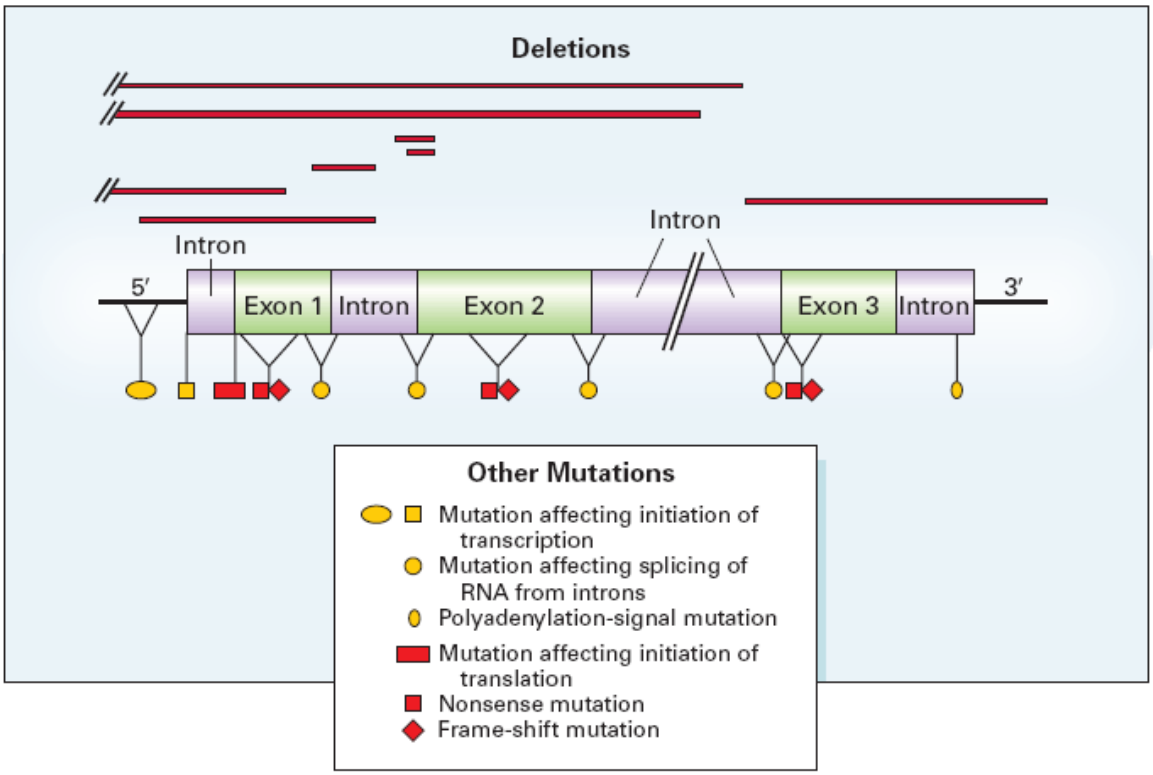

Figure 6. The Normal Structure of the β-Globin Gene and the Locations and Types of Mutations Resulting in β-Thalassemia [7]

All β-globin-like genes contain three exons and two introns between codons 30 and 31 and 104 and 105, respectively. The primary action of all the mutations is to abolish the output of β-globin chains (β^0-thalassemia; shown in red) or reduce the output (β^+-thalassemia;shown in green). The 170 different mutations that act in this way may interfere with the action of the β-globin gene at the transcriptional level, in the processing of the primary transcript, in the translation of β-globin messenger RNA, or in the post-translational stability of the β-globin gene product.

β^o Mutations			
Codon 5(-CT)	Codon 26(G-T)	Codon 51(-C)	IVSI-1(G-A)
Codon 6(-A)	Codon 27/28(+C)	Codon 67(-TG)	IVSI-1(G-T)
Codon 8(-AA)	Codon 30(AGG-ACG)	Codon 71/72(+A)	IVSI-2(T-A)
Codon 8/9(+G)	Codon 36/37(-T)	Codon 95(+A)	IVSI-2(T-C)
Codon 14/15(+G)	Codon 37(GG-GA)	Codon 106/107(+G)	IVSII-1(G-A)
Codon 15(GG-AG)	Codon 39(C-T)	Codon 121(G-T)	IVSII-849(A-G)
Codon 15(GG-GA)	Codon 41/42(-CTTT)	Codon 11(-T)	IVSII-850(G-T)
Codon 16(-C)	Codon 43(G-T)	Codon 15(-T)	
Codon 17(A-T)	Codon 44(-C)	Initiation codon (T-G)	
β^+ Mutations			
-90(C-T)	-28(A-C)	IVSI-5(G-C)	IVSII-654(C-T)
-88(C-T)	-28(A-G)	IVSI-5(G-A)	IVSII-745(C-G)
-87(C-G)	Codon 24(T-A)	IVSI-6(T-C)	Poly A(T-C)
-30(T-A)	Codon 27(G-T)	IVSI-110(G-A)	Poly A(A-G)
-29(A-G)	Codon 29(C-T)	IVSII-848(C-A)	
Hemoglobin Variants			
Hb S Codon 6(A-T)	**Hb C** Codon 6(G-A)	**Hb E** Codon 26(G-A)	**Hb D** Codon 121(G-C)

Table 2. The common β Mutations [9]

In addition, there are several forms of thalassemia or thalassemia-like disorders that are related to the β-thalassemia. They include the δβ, εγδβ and δ-thalassemias and hereditary persistence of fetal hemoglobin (HPFH)[8][9]

REFERENCES

1. Baglioni C. *The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion*. Proc Natl Acad Sci USA 1962;48:1880-6
2. Cao A, Kan Y.W. The Prevention of Thalassemia. Cold Spring Harb Perspect Med. 2012; 3: 01-15
3. Deisseroth A, Nienhuis A, Turner P, et al. *Localization of the human alpha globin structural gene to chromosome 16 in somatic cell hybrids by molecular hybridization assay*. Cell 1977;12:205-18
4. Deisseroth A, Nienhuis AW, Lawrence J, Giles RE, Turner P, and Ruddle FH. *Chromosomal localization of the human beta globin gene to human chromosome 11 in somatic cell hybrids*. Proc Nat Acad Sci, USA 1978;75:1456-60
5. Harteveld C.L, Higgs D.R. *α–Thalassemia*. Orphanet Journal of Rare Diseases. 2010; 5 (13): 1-21
6. Kendall AG, Ojwang PJ, Schroeder WA, and Huisman TH. *Hemoglobin Kenya, the product of a gamma-beta fusion gene: studies of the family*. Am J Hum Genet.1973;25:548-63
7. Olivieri N.F. *The β-Thalassemias*. The New England Journal of Medicine. 1999; 341 (2): 99-109
8. Panja A, Ghosh TK, Basu A. *Genetics of thalassemia in Indian population*. Journal of Community Nutrition & Health. 2012; 1(1): 39-46
9. Steinberg M.H, Forget B.G, Higgs D.R, Weatherall D.J. *Disorders of Hemoglobin – Genetics, Pathophysiology, and Clinical Management*. Cambrigde University Press. 2009
10. Weatherall DJ and Clegg JB. *Thalassemia Syndromes*. 3rd. ed. Oxford:Blackwell Scientific Publications.1981