Pathophysiology of Compound Heterozygotes Involving Hemoglobinopathies and Thalassemias

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ABBREVIATIONS: Ala = alanine; Arg = arginine; Asn = asparagine; DNA = deoxyribonucleic acid; Gln = glutamine; Glu = glutamic acid (glutamate); Gly = glycine; Hb = hemoglobin; HPLC = high performance liquid chromatography; IVSII-745 = intervening sequence at codon 745; Leu = leucine; Lys = lysine; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; RBC = red blood cell; Term = termination codon; Thr = threonine; Val = valine.

INDEX TERMS: compound heterozygotes; hemoglobinopathies; thalassemias.

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LEARNING OBJECTIVES

1. Compare and contrast hemoglobinopathies and thalassemias.
2. Describe the most common type of mutation found in the majority of hemoglobinopathies and α-thalassemias.
3. List the five categories of mutations common in β-thalassemia.
4. Discuss why compound heterozygotes involving HbS and either a β-chain hemoglobinopathy or β^+-thalassemia are less severe than sickle cell disease but more severe than sickle cell trait.
5. Discuss why an α-thalassemia mutation occurring in a HbSS patient lessens the severity of the existing sickle cell disease.
6. List two compound heterozygotes that mimic other hemoglobinopathy and/or thalassemia conditions.

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Hemoglobinopathies and thalassemias are both hematologic diseases involving mutations in the genes that control the synthesis of globin chains that compose hemoglobin. Some hematologists use the term hemoglobinopathy to describe any hemoglobin disorder to include the hemoglobin variants (e.g., sickle cell) and thalassemia. Other hematologists use the term hemoglobinopathy to describe only the qualitative hemoglobin variants and the term thalassemia to describe disorders producing a quantitative reduction in hemoglobin synthesis. The latter approach will be used in this review.

GENETIC MUTATIONS

Single nucleotide substitutions (point mutations) are the most common types of lesions occurring in the hemoglobinopathies. To date over 900 distinct mutations have been identified that are known to cause a hemoglobinopathy.[1] In most cases the nucleotide alteration found in the hemoglobinopathies is a simple substitution causing the nucleotide sequence to remain "in frame" resulting in a single amino acid substitution that will not change the overall size of the globin protein product. Thus the defect in the globin molecule is ordinarily an amino acid substitution that changes the amino acid sequence affecting protein structure and function rather than quantity. Typical alterations in hemoglobin function include changes in oxygen binding affinity, molecular solubility, and the manner in which the individual hemoglobin molecules interact within the red blood cell (RBC). See Table 1.

In contrast, the types of mutations encountered in the thalassemias are broad and diverse and ultimately affect the quantity of protein manufactured inside the developing RBC. Although over 300 different mutations have been identified as the cause of thalassemia,[2] nearly all of the mutations can be classified into one of five categories: deletions, promoter mutations, nonsense mutations, stop codon mutations, and splice site mutations.[3] In each case, the globin defect is a quantitative reduction in the amount of globin protein produced by the defective gene, ranging from little to no protein product (designated by the symbol β^0), to a mild or moderate reduction in total protein produced (designated by the symbol β^+). The associated clinical symptoms reflect the total number of globin genes affected and the degree to which each individual gene produces the globin protein (Table 1).

Table 1. Mutation types commonly found in hemoglobinopathies and thalassemias

Disorder	Type of Mutation
Hemoglobinopathy	Point mutation → nucleotide substitution → change in nucleotide sequence of codon → no frame shift → amino acid substitution → size of protein product remains unchanged unless mutation occurs in termination codon
α-thalassemia	Gene deletion → total loss of gene product → severity dependent on number of genes deleted
β-thalassemia	Deletions, promoter mutations, nonsense mutations, stop codon mutations, splice site mutations → alters transcription efficiency or changes length of protein product → continuum of gene product concentration from none to nearly normal depending on mutation

NORMAL HUMAN GLOBIN GENES, GLOBIN CHAINS, AND HEMOGLOBIN MOLECULES

Although the types of mutations that cause hemoglobinopathies and thalassemias are very different, the genes that bear the mutation are the same, namely the globin genes and the associated regulatory genes. In the normal human genome there are six types of globin genes on each allele: zeta (ζ), epsilon (ε), gamma (γ), delta (δ), alpha (α), and beta (β). Each gene normally produces globin protein chains bearing the same name. The α and ζ genes are located on chromosome 16 and the β, γ, δ and ε genes are located on chromosome 11 (Figure 1). The α and ζ chains produced by the corresponding genes are referred to as the α-like chains and the β, γ, δ, and ε chains are called β-like chains. Normal human hemoglobin contains an identical pair of α-like chains and a pair of identical β-like chains that combine with four protoporphyrin rings each holding an iron atom. The hemoglobin molecule is described as tetrameric in that it is composed of four like subunits, each containing one globin chain, one protoporphyrin ring, and one iron atom. However, the tetrameric molecule functions more like two dimers rather than one tetramer. Each dimer is composed of an α-like and a β-like chain with the corresponding two protoporphyrin rings and the two iron atoms. Each globin chain within the dimer pair binds the partner chain with high affinity causing the two dimers pairs to function as two distinct subunits within the one hemoglobin molecule. This close association between structure and function must be maintained to produce optimal hemoglobin function.

The most abundant normal hemoglobin found in adults is called hemoglobin A (HbA) and is composed of two α and two β-globin chains. HbA makes up approximately 97% of normal adult hemoglobin. For this reason, any mutation that affects the α or β-chains, resulting in either a functional (qualitative/hemoglobin variant) or concentration (quantitative/thalassemia) change in HbA, has a dramatic affect on hemoglobin function in the adult. Therefore, hemoglobinopathies and thalassemias involving α or β-genes are more clinically significant.

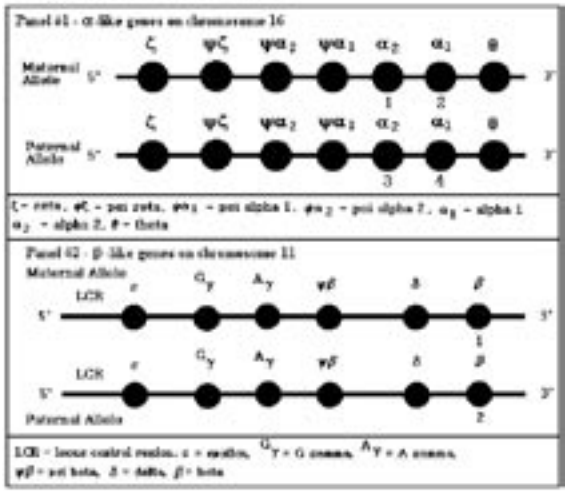
In the human genome there are two α-gene loci on each chromosome 16 and one β-gene locus on each chromosome 11 so the number of mutated genes loosely correlates with clinical severity (Figure 1). Because there are a total of 4 α-genes and 2 β-genes, α-gene mutations would expect to manifest as four levels of severity while a β-gene mutation would have two levels of severity depending on the number of genes that bear a mutation. Generally, this principle is true for both the

hemoglobinopathies and the thalassemias. However, there are many documented mutations in both the thalassemias and hemoglobinopathies that have either no effect or a subclinical effect on either the function or concentration of hemoglobin. Excluding these examples, a correlation between the number of mutated alleles and clinical severity generally holds true, particularly in the α-thalassemias and hemoglobinopathies. Since most α-thalassemias are caused by gene deletions, the number of remaining intact genes is somewhat proportional to the amount of α-chains produced and, thus, the concentration of the corresponding α–containing hemoglobins. This is less true in β-thalassemia because many of the mutations do not manifest like a gene deletion but rather result in the production of varying amounts of protein product from each mutated gene. The genetic term homozygous was created to describe a single gene defect where both alleles are affected whereas heterozygous is defined as one defective allele and one normal allele. Therefore, a homozygote is usually very ill while a heterozygote is either asymptomatic (carrier) or exhibits mild symptoms.

COMPOUND HETEROZYGOTES

A compound heterozygote results from inheriting one defective allele from one type of disorder and inheriting a second defective allele from another disorder. A person with one hemoglobinopathy mutation and one thalassemia mutation is considered a compound heterozygote, as is a person with one α-thalassemia mutation and one β-thalassemia mutation or a patient with a HbS mutation and a HbC mutation. Interestingly, compound heterozygotes do not always follow the pattern of an increase in severity with additional mutations. Some compound heterozygotes do show the expected increase in severity with each additional mutation, while some exhibit a severity intermediate between the heterozygous and homozygous levels, and others actually show an improved prognosis by virtue of an additional mutation. The remainder of this review will identify examples of compound heterozygotes in which the clinical expression of the disease is either different than expected or creates a diagnostic dilemma.

Figure 1. Human globin gene map



US prevalence of compound heterozygotes

Although many of the compound heterozygous conditions are especially problematic in countries other than the US where the diseases have a high prevalence, increasing immigration to the US makes these disorders important to the US healthcare system. Since HbS is the most common and severe hemoglobinopathy in the US, compound heterozygotes with HbS occur at the highest frequency. The prevalence of African-Americans born with sickle cell trait is approximately 1/12 while the rate born with sickle cell disease in the same population is approximately 1/375.[4] HbSC represents the compound heterozygote of highest prevalence in the US and is estimated to be between 0.04-0.13%.[4] Compare the frequency of African-American babies born with HbSC disease (1/835) to HbSS (1/375) and then to the next most prevalent compound heterozygote in the same group, HbS/β-thalassemia (1/1667). Because of the low prevalence of most of the other hemoglobinopathies and thalassemias, there is a paucity of prevalence data concerning compound heterozygotes in the US involving these disorders. Nonetheless, excluding those disorders previously described, HbS/HbD$^{\text{Los Angeles (Punjab)}}$, HbS/Hb$^{\text{Montgomery}}$, and HbS/α-thalassemia are some of the more common remaining compound heterozygotes observed in the US.

COMPOUND HETEROZYGOTES WITH HbS

Sickle cell disease

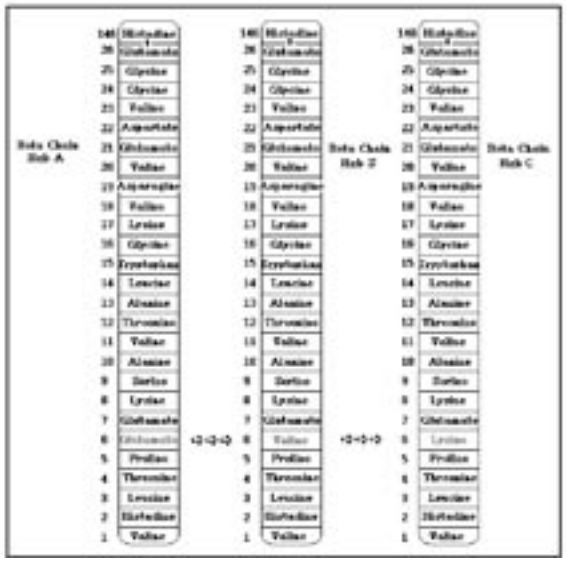
Sickle cell anemia results from a change in a single nucleotide (A→T) in the sixth codon of the β-globin gene (GAG→GTG), resulting in a single amino acid substitution of valine for glutamic acid at the sixth amino acid position of the β-globin chain (Figure 2). Compound heterozygotes involving HbS generally experience symptoms intermediate between a sickle cell homozygote (SS) and the asymptomatic heterozygote (AS). Patients who are homozygous for HbS (HbSS) inherit a life threatening disorder owing primarily to the polymerization of HbS inside the RBC producing vaso-occlusive crises

in several organ systems and the resultant pathology. HbS polymers are long and thin and stretch the RBC membrane forming the characteristic sickle cells. In contrast, heterozygous individuals with one sickle β-gene and one normal β-gene (HbAS) are mostly asymptomatic. However, most compound heterozygotes expressing one sickle cell allele and a non-sickle β-gene mutation manifest symptoms that fall between the sickle cell heterozygote and the homozygote.[4] The most common example is HbSC disease.

Hemoglobin C and HbSC disease

Like HbS, hemoglobin C (HbC) also results from a single nucleotide change at the sixth position of the β-globin gene. However, in this case the glutamic acid at the sixth position of the β-chain is replaced by a lysine (Figure 2). Lysine is slightly larger than both glutamate and valine and it has a +1 charge, altering the tertiary structure of the globin chain. HbC also polymerizes inside the RBC forming crystals but the polymers are shorter and broader than HbS polymers. Although HbC crystals are observed inside RBCs, sickle-like cells are not formed and vaso-occlusive crises do not occur. A milder hemolytic anemia is the primary manifestation of HbC disease. In the compound heterozygote, HbSC, the hemoglobin molecules polymerize into hybrid crystals that are unique, appearing longer than HbC crystals but shorter and thicker than HbS crystals. Vaso-occlusive crises can occur from HbSC crystals but the frequency and intensity is reduced. Therefore, the HbSC patient expresses symptoms more severe than a homozygous HbC (HbCC) patient, but less severe than a homozygous sickle cell (HbSS) patient.[4]

Figure 2. Etiology of Hemoglobin S (HbS) and Hemoglobin C (HbC)



HbS/β-chain variants

Co-expression of HbS with other β-chain hemoglobin variants will also reduce symptoms compared to sickle cell homozygotes. Although most abnormal β-chain variants show some level of functional abnormality, they reduce symptoms compared to a sickle cell homozygote because they do not polymerize like HbS and actually interfere with the polymerization of HbS molecules. The hemoglobin D variants are of particular importance because they migrate with HbS on alkaline electrophoresis producing a single band in the S position, resembling sickle cell homozygotes. Hb $D^{Los\ Angeles\ (Punjab)}$ (β121 Glu→Gln), $HbD^{Bushman}$ (β16 Gly→Arg), $HbD^{Ouled\ Rabah}$ (β19 Asn→Lys), $HbD^{Granada}$ (β22 Glu→Val), HbD^{Iran} (β22 Glu→Gln), HbD^{Ibadan} (β87 Thr→Lys), and HbD^{Neath} (β121 Glu→Ala) all manifest as a milder disease when inherited with HbS. This phenomenon is also observed in many other compound heterozygotes involving sickle cell to include HbO^{Arab} (β121 Glu→Lys) and $Hb^{Montgomery}$ (α48 Leu→Arg).[5]

HbS/α-thalassemia

Another example in which the inheritance of a hemoglobin abnormality with sickle cell actually reduces symptoms involves the HbS/α-thalassemia compound heterozygote. In this case the patient has inherited two HbS mutations on both β-genes along with one or more mutations/deletions on the α-gene(s). Since HbS is composed of two α-chains and two sickle chains, decreased production of α-chains from a HbS/α-thalassemia compound heterozygote will reduce the amount of HbS and the corresponding vaso-occlusive crises. It has been shown that the concentration of HbS in these compound heterozygotes is proportional to the number of α-gene deletions. If one α-gene is deleted the HbS percentage is reduced to approximately 35%. Two α-gene deletions produce 28% HbS while three deleted α-genes produce 20% HbS. By reducing the concentration of HbS inside the RBC, fewer polymerization interactions can occur that lead to vaso-occlusive events.[6] These patients experience severe anemia owing to their inability to make compensatory hemoglobins but the lessened vaso-occlusive events result in an improved outcome. HbS and $HbG^{Philadelphia}$ (α68 Asp→Lys) compound heterozygotes are usually asymptomatic since $HbG\text{-}^{Philadelphia}$ is an α-gene mutation that still allows for sufficient α-chain production to manifest like a sickle cell heterozygote.[4]

HbS/β-thalassemia

In contrast to HbS/α-thalassemia, a sickle cell/β-thalassemia compound heterozygote intensifies the clinical severity. If the β-thalassemia mutation is severe (β^0), very few to no normal β-chains are produced causing the patient with only one HbS allele to manifest similarly to a sickle cell homozygote (HbSS). The less severe β-thalassemia mutations (β^+) will manifest milder symptoms but unlike the sickle cell heterozygote (HbAS), these compound heterozygotes will usually exhibit at least mild symptoms.[7] Compound heterozygotes involving both β^0 and α-thalassemia in conjunction with the sickle cell allele closely resemble the sickle cell/ β^0-thalassemia except there is a balanced reduction in both β and α-globin chain levels with lower HbF levels due to the reduced α-chain synthesis.

HbS/Hb Hope and Hb Hope/α-thalassemia

A compound heterozygote involving the sickle cell mutation and the Hb Hope mutation also lessens the clinical severity compared to a sickle cell homozygote. Hb Hope was discovered in 1965 and shown to be caused by a single nucleotide change (GGT to GAT) at codon 136 of the β-chain that substitutes asparagine for glycine at position 136.[8] Hb Hope is clinically silent even in the homozygous form and migrates closely with HbF on alkaline electrophoresis. This migration pattern makes it difficult to differentiate neonates with HbSS from HbS/Hb Hope compound heterozygotes. Since neonates are born with high levels of HbF and Hb Hope migrates with HbF, newborns with either HbSS or HbS/Hope will show a band at the S position and a band at the F position. Hb Hope has been found in a variety of compound heterozygotes but because of its minimal reduction of function, it generally lessens the clinical symptoms compared to the homozygote counterpart.[9] However, Hb Hope has contributed to serious microcytic anemia when inherited in combination with a particular version of α-thalassemia called Hemoglobin H disease. HbH disease is a form of α-thalassemia in which three of four α-genes are mutated. Although most versions of HbH disease produce clinically significant microcytic anemia that is transfusion dependent, HbH disease ($--^{SEA}/-\alpha^{3.7}$) does not produce clinically significant anemia, thalassemia facies, hepatosplenomegaly or failure to thrive. Still, a 28-year-old woman from Lumpoon Province in Northern Thailand who was diagnosed with homozygous Hb Hope and HbH disease ($--^{SEA}/-\alpha^{3.7}$) did express a severe microcytic/hypochromic anemia (Hb=5.7g/dL, MCV=62 fL, MCH=16.8pg), elevated HbF (4.3%), Hb Hope (78.1%), with elevated Hb Bart's at birth and HbH in adulthood. Although this patient was not transfusion-dependent, she did present with thalassemia facies, marked pallor, icteric sclera, mild hepatosplenomegaly, and failure to thrive since childhood.[10]

HbS/Hb Volga

An interesting sickle cell compound heterozygote involving the unstable hemoglobin Volga produces a diagnostic dilemma. Like the other sickle compound heterozygotes, patients with HbS/Volga experience a reduction in symptoms compared to a sickle cell homozygote (SS) but are often misdiagnosed as either a sickle cell heterozygote (AS) or as a HbS/β-thalassemia compound heterozygote. Hb Volga involves a single amino acid substitution of aspartate for alanine at position 27 of the β-globin chain, resulting in a migration pattern that is indistinguishable from normal HbA on HPLC, isoelectric focusing, and alkaline agar electrophoresis. Since Hb Volga migrates in the HbA position and also produces elevated HbA_2, HbS/Volga compound heterozygotes are often misdiagnosed as either a sickle cell heterozygote (AS) or a HbS/β^+-thalassemia compound heterozygote. Careful documentation of patient history will likely reveal one parent and other members of their family as having Hb Volga by virtue of being sickle cell negative, expressing a mild hemolytic anemia with reticulocytosis, basophilic stippling and moderate poikilocytosis.[11]

THALASSEMIA COMPOUND HETEROZYGOTES

α and β-thalassemia

The thalassemias represent a complex group of disorders in which compound heterozygosity further complicates the issue. Since there are four α-globin genes in the human genome and gene deletion is the most common α-globin gene mutation, there are four severities of α-thalassemia: hydrops fetalis (four deleted genes); hemoglobin H (HbH) disease (three deleted genes); α-thalassemia minor (two deleted genes); and silent carrier (one deleted gene) (Figure 3). In the absence of any α-globin chain production (hydrops fetalis), none of the three normal adult hemoglobins can be made [HbA ($\alpha_2\beta_2$), HbA_2 ($\alpha_2\delta_2$), and HbF ($\alpha_2\gamma_2$)] resulting in spontaneous abortions, stillbirths, and death shortly after birth. Silent carrier and α-thalassemia minor are asymptomatic, leaving HbH disease as the clinically significant α-thalassemia. Although there are only two β-globin genes in the human genome, a variety of mutation types, previously listed, result in a continuum of clinical severities among the β-thalassemias. Nonetheless, all levels of severity among the β-thalassemias have been grouped into three clinical categories; β-thalassemia major (two gene mutations), β-thalassemia intermedia (two gene mutations), and β-thalassemia minor (one or two gene mutations). β-thalassemia minor is usually asymptomatic while β-thalassemia intermedia and β-thalassemia major

produce moderate to severe anemia, respectively, depending on the exact mutations present (Figure 4).

Compound heterozygotes expressing both α and β-thalassemia can either exacerbate or improve the clinical symptoms compared to the heterozygous state depending on the combination of gene mutations. HbH disease is a serious α-thalassemia in which the one remaining unmutated α-gene is only able to produce about 40% of the normal amount of α-globin chains resulting in a dramatic reduction in HbA concentration. The anemia is further exacerbated by a hemolytic process caused by splenic removal of HbH inclusion bodies composed of excess β-globin chains that form tetramers (β_4) inside the RBC. Therefore, patients with HbH disease who also inherit β-thalassemia minor produce fewer β-globin chains and fewer HbH inclusion bodies, thereby resulting in a less severe anemia from splenic removal.[4]

In contrast, patients with β-thalassemia major usually present with severe anemia of varying levels depending on the types of mutations present, but produce a more severe version when co-expressed with α-thalassemia. In β-thalassemia major, few to no β-globin chains are produced and non β-chain containing hemoglobins like HbF, HbA_2, and Hb Barts (γ_4) are synthesized in higher concentrations to compensate. Since two of these hemoglobins contain α-globin chains, a concomitant α-thalassemia will reduce the concentration of compensatory hemoglobins and exacerbate the anemia.[4]

Figure 3. Four levels of α-thalassemia based on the number of gene deletions

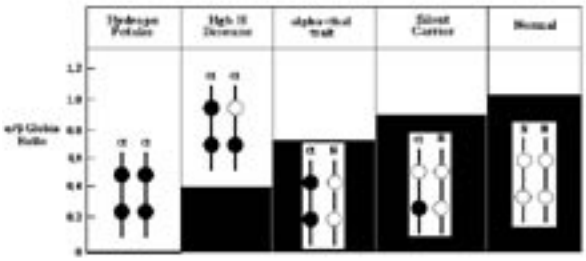
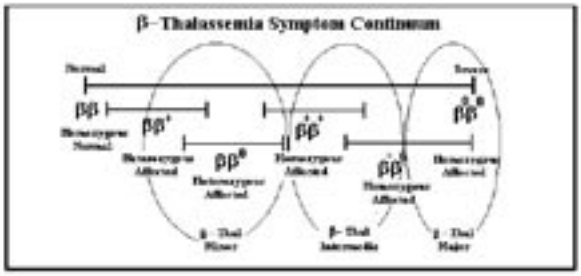


Figure 4. Relationship between gene mutations and symptoms in β-thalassemia



Another clinical concern with thalassemia compound heterozygotes involves the potential of Hb Bart's hydrops fetalis occurring in an infant born to unsuspecting parents. Parents who share a common type of thalassemia are counseled about the risks of having children with α-thalassemia hydrops fetalis, if they both have α-thalassemia minor; or having children with β-thalassemia major, if they both have β-thalassemia minor. However, most couples are not adequately counseled if one is diagnosed with α-thalassemia and the other with β-thalassemia because the risk of serious illness to the child is presumed to be very low. The concern in this situation is that individuals diagnosed with β-thalassemia may actually be a compound heterozygote with a silent α-thalassemia. Most laboratories screen for β-thalassemia by detecting an elevated HbA_2 in the context of a microcytic/ hypochromic anemia exhibiting target cells in the peripheral blood. In the absence of genetic testing many patients presumed to have only β-thalassemia minor may actually be a compound heterozygote with a concomitant α-thalassemia minor. Therefore, if a person diagnosed with α-thalassemia minor conceives with an individual diagnosed with β-thalassemia minor, who also has a silent α-thalassemia minor, they have a 25% chance of conceiving a child with hydrops fetalis. This scenario presumes that the α-thalassemia minor in both cases involves the deletion of both α-genes on the same chromosome. For example, in the Guangdong province of China where the prevalence of α^0-thalassemia ($--^{SEA}$) is 4.1% and β-thalassemia heterozygotes are 2.5%, the frequency of compound α^0/β heterozygotes is 1 in 25 patients (4.4%) among known β-thalassemia heterozygotes, causing this scenario to be a realistic threat.[12]

HbE/β-thalassemia

Compound heterozygotes involving HbE/β-thalassemia have become a diagnostic concern among Southeast Asian populations where HbE is prevalent because the co-inheritance of β-thalassemia with HbE manifests as thalassemia intermedia. HbE involves the substitution of lysine for glutamic acid at the 26th position of the β-chain and occurs with highest frequency in Thailand, Cambodia, and Laos. Homozygous

HbE patients (EE) experience a mild, asymptomatic anemia characterized by microcytosis and target cells whereas the heterozygotes (AE) are clinically normal but express a few microcytes and HbE on electrophoresis. Therefore, it is surprising for asymptomatic parents heterozygous for HbE to give birth to an infant with a moderately severe anemia, which occurs in infants inheriting HbE with β-thalassemia. In addition, the phenotypes of these patients are remarkably variable with hemoglobin levels ranging from 2.5 to 13.5g/dL.[13] The explanation for such a wide range of severities in these patients is unknown.

HbD^{Iran}/β^0-thalassemia

HbD^{Iran} in combination with β^0-thalassemia produces a moderate microcytic and hypochromic anemia that is not transfusion-dependent. HbD^{Iran} is caused by a substitution of glutamine for glutamic acid at the 22nd position on the β-globin chain.[14] The β^0-thalassemia allele fails to produce any β-globin chains allowing the variant β-globin gene to produce all the β-like globin chains. The issue is that HbD^{Iran} appears as HbSS on alkaline electrophoresis and migrates with HbA_2 and HbE using HPLC methods. In laboratories utilizing HPLC instruments, HbD^{Iran}/β^0-thalassemia compound heterozygotes are suspected of having either homozygous HbEE, if the HbD^{Iran} is misinterpreted as elevated HbE, or β-thalassemia major if the HbD^{Iran} is thought to be elevated HbA_2. Laboratories using hemoglobin electrophoresis will suspect a homozygous sickle cell patient since HbD^{Iran} migrates with HbS on alkaline electrophoresis.[15] Since HbE is found primarily in patients of Asian ethnicity, HbS in African Blacks, and HbD^{Iran} in patients of Iranian ancestry (as well as Pakistani, Jamaican Black, Indian, and Italian races), determination of the patient's ethnicity along with DNA analysis is paramount in making the correct diagnosis.

Hb Knossos/β^0-thalassemia

Some β-chain hemoglobin variants manifest as a reduction of total hemoglobin production, resulting in a thalassemia major phenotype when inherited with a β^0-thalassemia. An example is the hemoglobin variant Hb Knossos, which presents as a thalassemia major when co-inherited with the β^0-thalassemia, IVSII-745. Hb Knossos is a rare hemoglobinopathy in which alanine is replaced with serine at position 27 of the β-chain. Heterozygous Hb Knossos patients present with a mild β-thalassemia phenotype while homozygotes express thalassemia major. Interestingly, β^0-thalassemia/Hb Knossos compound heterozygotes express a normal HbA_2, rather than the elevated HbA_2 typical for patients with β-thalassemia.[16] In addition, Hb Knossos migrates with HbA on alkaline electrophoresis further complicating the diagnosis. Therefore, couples of Mediterranean descent who both present with a β-thalassemia minor, with one showing a normal HbA_2, should be investigated further to determine the possible presence of a β^0-thalassemia/Hb Knossos compound heterozygote. If so, the frequency of having a child with a β-thalassemia major phenotype increases from 25% to 50%.[17]

α-chain Hemoglobin Variants

Similarly, α-chain hemoglobin variants that reduce hemoglobin synthesis can produce the α-thalassemia, hemoglobin H disease, when co-expressed with α-thalassemia minor. Hemoglobin H disease occurs most commonly in patients from Southeast Asia, the Middle East, and countries around the Mediterranean Sea due to the deletion of three of four α-genes. However, unstable hemoglobins co-expressed with α-thalassemia minor can produce HbH disease in the same patient populations. Hemoglobin Quong Sze [α125 CTG→CCG, Leu→Pro] in Chinese,[18] Hb Constant Spring [$\alpha_2$142 TAA→CAA, Term→amino acid] in Southeast Asians,[19] Hb Paske [$\alpha_2$142 TAA→TAT, Term→Tyr] in Thai-wanese,[20] Hb Suan-Dok [α109 CTG→CGC, Leu→Arg] in Africans,[21] Hb Sallanches [α_2 104 TGC→TAC, Cys→Tyr] in Indians,[22] and Poly A mutations found in the Mediterranean and Middle Eastern areas[2] all result in a reduction of HbA production by different mechanisms. In Hb Quong Sze, the replacement of leucine with proline introduces a "kink" in the secondary structure of the α-chain disrupting the H helix creating instability of the resulting hemoglobin molecule. Hemoglobin Constant Spring and Hb Paske both affect the termination codon 142, changing it to an amino acid producing an extended protein product that becomes the target of intracellular proteolytic cleavage. Likewise, mutations in the poly-A tail will also extend the protein length creating instability and proteolysis. In Hb Sallanches the loss of cystein results in one less disulfide bond which otherwise stabilizes the protein structure.

Anti-Lepore Hong Kong/β^0-thalassemia

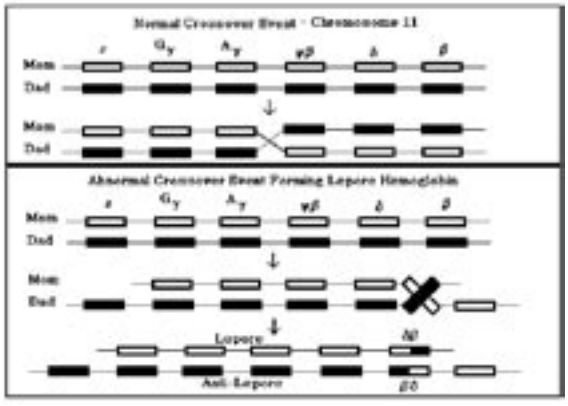
Lastly, some compound heterozygotes generate clinical and laboratory information that closely resembles other hemoglobinopathy/thalassemia combinations. For example, the compound heterozygous condition involving anti-Lepore Hong Kong with β^0-thalassemia closely resembles other compound heterozygotes causing diagnostic confusion. Anti-Lepore Hong Kong is a βδ fusion variant that occurs during meiosis from a non-homologous (unequal) crossover event[23] (Figure 5). Anti-Lepore Hong Kong produces the βδ fusion chain and causes an overexpression of the δ-globin chain,

elevating HbA_2, and a suppression of the β-gene, reducing HbA production. In addition, HbE and HbA_2 have the same mobility and retention time by HPLC methods and both anti-Lepore Hong Kong and HbE are common in Asian patients. Overexpression of δ-globin chains results in elevated HbA_2 which can reach levels of over 40% in these compound heterozygotes. Therefore, patients with anti-Lepore Hong Kong and β^0-thalassemia presenting with a microcytic/hypochromic anemia and a very heavy band at the HbE/HbA_2 region on HPLC, closely resemble HbE/β^+-thalassemia and HbE/α-thalassemia. In the first case, the elevated HbA_2 (>40%) is so high that it is misinterpreted as HbE and the β^+-thalassemia will elevate the HbA_2 to further contribute to the heavy band and account for the symptoms. HbEE alone is unlikely because the condition is asymptomatic. A diagnosis of HbE/α-thalassemia can also be considered because the α-thalassemia will inhibit globin chain production from the other β-allele causing less production of HbA and reducing the overall HbE levels to around the 40% mark.[24]

LABORATORY DIAGNOSIS

Data generated by laboratory professionals are essential in making a correct diagnosis which, in turn, is necessary to provide patients with accurate therapeutic and prognostic information. Over 1200 individual globin gene mutations have been identified resulting in either a hemoglobinopathy (hemoglobin variant) or thalassemia, creating a plethora of diagnostic and prognostic possibilities. This situation is exacerbated by the seemingly limitless number of possible compound heterozygotes. As discussed in this review, some combinations exacerbate the problem, some minimize the clinical manifestations, and others create a diagnostic dilemma. Therefore, an accurate diagnosis is critical. The combination of alkaline and acid hemoglobin electrophoresis or HPLC separation of hemoglobin types is often the first step in evaluating patients suspected of having some form of hemoglobinopathy and/or thalassemia. Diagnosing patients who appear as HbAS, HbSS, HbAC, HbCC, or HbSC on either alkaline electrophoresis or HPLC, and who express the corresponding ethnicity and phenotype, have become relatively routine. The same can be said for patients with uncomplicated α or β-thalassemia. However, the rarer hemoglobinopathies and thalassemia mutations, whether alone or in various combinations, require the analysis of DNA by the laboratory professional. Nucleic acid screening kits for the more prevalent thalassemia mutations common to particular ethnic groups have been available for decades in readout formats to include radioactivity, fluorescence, chemiluminescence, and spectrophotometric ELISA systems. A common approach to thalassemia diagnosis is to use a kit designed to screen for the most common four to eight mutations found in the ethnic group of the patient being evaluated, when available. If the mutation is not among those being screened, an economical follow-up approach is to amplify and sequence the globin gene in question and compare the sequence to a computer database. Individual nucleic acid kits are available for many thalassemia and hemoglobinopathy mutations, but the increasing availability of thermocyclers and DNA sequencers is making this approach the method of choice. The laboratory diagnosis of hemoglobinopathies and thalassemias is further evidence that clinical laboratory scientists must embrace nucleic acid testing in order to remain symbiotic with the medical technology we employ.

Figure 5. Mechanisms of equal and unequal crossover



Clin Lab Sci encourages readers to respond with thoughts, questions, or comments regarding this Focus section. Email responses to ic.ink@mchsi.com. In the subject line, please type "CLIN LAB SCI 21(4) FO ANEMIA IN SELECTED POPULATIONS". Selected responses will appear in the Dialogue and Discussion section in a future issue. Responses may be edited for length and clarity. We look forward to hearing from you.

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