79 Hemoglobinopathies: The How, Why and What

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This session will focus on two aspects of hemoglobinopathies: (1) describing the methodology; and (2) presenting various case studies, including interaction with the audience on how to proceed in the work-up of the case.

- The major methodologies used in the diagnosis of hemoglobin disorders will be reviewed. The advantages and limitations of each will be reviewed.
- A series of case studies will be utilized to illustrate the appropriate work-up of hemoglobin disorders. The extent of the work-up needed based on the complexity of the case will be emphasized. Audience participation is encouraged.

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Hemoglobinopathies: The How, Why, and What

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I. Structure

The full hemoglobin molecule is composed of 4 globin chains linked to 4 porphyrin rings. Normal Hb A is composed of 2 α and 2 β chains ($\alpha_2\beta_2$). There is a minor component in adults called Hb A_2 which is composed of 2 α chains and 2 δ chains ($\alpha_2\delta_2$). Fetal hemoglobin is composed of 2 α chains and 2 γ chains ($\alpha_2\gamma_2$). The 4 globin subunits are held together by several weak, noncovalent bonds that occur at the interface points between the α and β subunits. With progressive oxygenation of the molecule, there are several conformational changes that occur at the interface between the subunits that produce a cooperative binding effect. That is, as each porphyrin ring obtains an oxygen molecule, it becomes progressively easier for the remaining porphyrin units to obtain an oxygen molecule as well. This is the reason for the sigmoidal shape of the oxygen dissociation curve.

There are 3 embryonic hemoglobins seen in the early embryonic period (Hb Portland I ($\zeta_2\gamma_2$), Hb Gower I ($\zeta_2\varepsilon_2$), Hb Gower II ($\alpha_2\varepsilon_2$). Later in fetal life, there is a switch to the production of α and γ chains, resulting in Hb F. At birth, there is an additional switch from the production of γ chains to the production of β and δ chains, resulting in Hb A and Hb A_2.

The structure of all globin genes is similar, being composed of a promoter region, 3 exons with 2 intervening introns. The α globin gene complex is located on chromosome 16, whereas the β globin gene complex is located on chromosome 11. The α globin gene complex consists of 2 functional α globin genes (α 1 and α 2) as well as the embryonic zeta chain and several pseudo genes. The beta globin gene complex consists of 2 γ chains, the δ globin gene, the β globin gene, the embryonic epsilon gene and 1 pseudo gene.

II. Terminology

A. Thalassemias. These are characterized by decreased production of the affected globin chains. These are primarily grouped into the α or β thalassemias. The globin chains that are produced are structurally normal, however there is an imbalance in the α to β globin chain ratio which is the cause of the manifestations seen.

B. Hemoglobinopathies. These are a group of disorders in which there is a structural abnormality of either the α, β or δ chains. The majority of these are due to a single amino acid substitution caused by a point mutation in the globin chain DNA.

C. Hereditary persistence of fetal hemoglobin and δ β thalassemia. These are a group of disorders which are characterized by persistent elevation of fetal hemoglobin (HB F) into adulthood.

III. Epidemiology

Many hemoglobin variants are associated with a distinct geographic distribution. For example, Hb S is typically associated with areas in both eastern and western Africa as well as areas in Saudi Arabia and India, and in localized pockets all around the Mediterranean Sea. Hb C originated in a specific area in western Africa, corresponding to the modern-day countries of Ivory Coast, Ghana, Togo, and Benin. Hb E is associated almost exclusively with heritage from Southeast Asia. Hb E is found in highest prevalence in Laos, Cambodia, and Thailand, although it is also found in other countries such as Malaysia, the Philippines, and over into Burma and Bangladesh. However, it is found in very low prevalence in ethnic Vietnamese and Chinese.

IV. Laboratory Methods.

A. Sickle Solubility Test.
An RBC lysate is placed in a high phosphate buffer solution, and dithionate (a reducing agent) is added to lower oxygen tension of the solution. This creates an in vitro situation which promotes the sickling phenomenon. Hb S, if present, with precipitate to form a cloudy solution. It is important to note that this test does not distinguish sickle cell trait from homozygous S or Hb S in combination with other hemoglobin variants. False negative tests can be obtained if the percentage of Hb S is <10%, such as in newborns or in heavily transfused patients. False positive tests can be obtained if there are large numbers of nucleated red blood cells in the peripheral blood. Patients with hyperlipidemia or a paraproteinemia could also potentially have a false positive sickle solubility test.

B. Electrophoretic Methods.

The methods primarily used for the diagnosis of hemoglobin variants are various electrophoretic methods.

1. Cellulose acetate electrophoresis.

This also called alkaline electrophoresis, as it is performed at a pH of 8.6. At this pH, the hemoglobin molecule is negatively charged, and so when placed in electric field, it will move toward the positive pole (anode). With hemoglobin variants, if there is an amino acid substitution that alters the overall charge of the molecule (due to a charge difference in the new amino acid side change), then it will have an electrophoretic mobility different from Hb A as observed on cellulose acetate electrophoresis.

2. Citrate Agar Electrophoresis.

   Also called acid electrophoresis, as it is performed at a pH of 6.2. This method is not based on charge differences alone. There is a compound within the agar termed "**agaropectin**" that combines some hemoglobin variants (usually those with a substitution at the surface of the molecule) to alter its electrophoretic mobility relative to Hb A. This method is very useful for the confirmation of Hb S, Hb C, and Hb E.

3. Isoelectric Focusing.

   This electrophoretic method utilizes carrier ampholytes, small proteins which are able to carry both current and pH. These compounds have molecular weights of 300-1000 and are used in mixtures of 50-100 individual compounds. The ampholytes are incorporated into the support medium (usually agarose). When a current is applied to the support medium, these ampholytes will gradually establish a pH gradient throughout the medium (for example, a pH range of 6-8 for hemoglobin analysis). High voltages must be used because the carrier ampholytes are present in high concentrations. Samples then placed on the gel will travel to their isoelectric point (the point at which they carry a net zero charge), where migration stops. Unlike alkaline electrophoresis and other electrophoretic methods, there is no danger of running the gel too long. Due to minor differences in isoelectric points of various hemoglobin variants, isoelectric focusing gives better separation of hemoglobin variants that show similar mobilities on alkaline electrophoresis. The bands present are much sharper than that seen on alkaline electrophoresis. Some hemoglobin variants, such as Hb Malmö, show separation from Hb A, which is not seen on alkaline electrophoresis. Additionally, minor bands (such as Hb H, Barts and δ-chain variants) are easily seen. However, minor bands (due to glycosylated hemoglobins) and aging bands (methemoglobin, glycerated hemoglobin) are also seen and may cause confusion in interpretation. Some typical patterns obtained with IEF are shown in Figure 1.

4. Capillary electrophoresis

   This is a relatively new method that has been gaining in popularity over recent years. As the name implies, it utilizes a very long, thin capillary tube which has a very high surface area. This property enables it to effectively dissipate the heat that is generated when a voltage is applied to the system. Thus, very high voltages (10,000 V) can be used. This serves to shorten run times significantly. The capillary tube is made of silica and has a negative charge on its internal surface. When a buffer is placed within the system, an electro-osmotic force (EOF) is created. When a current is applied, this EOF moves toward the cathode. The electric current applied will serve to separate hemoglobin fractions (similar to other methods), but because of the EOF all fractions are still carried toward the cathode. Thus, the pattern of migration is the exact opposite of other methods such as cellulose acetate electrophoresis or isoelectric focusing, i.e., Hb A_2 is detected first and Hbs H and Barts are the last variants detected. This method, of all methods, has the fewest

number of variants that can be confused with the three most common (Hbs S, C, E). However, like other methods, there is a significant number of variants that will not show distinct separation from Hb A.

C. High performance liquid chromatography

HPLC instruments have become widely available that are compact, user-friendly, and dedicated to the detection of hemoglobins and their variants. Run lengths have been shortened from over 20 minutes to 6-7 minutes. These instruments are FDA approved for the measurements of Hbs A_2 and F, but also give useful information for other hemoglobin variants that may be present.

These instruments generally utilize a weak cation exchange column. Increasing the ionic strength of the eluting solution causes the hemoglobin protein to come off the column at a particular retention time. Amino acid substitutions that are present in the hemoglobin variant will alter the retention time relative to Hb A. There is some analogy between the retention times obtained by HPLC and the pattern seen on alkaline electrophoresis. Amino acid substitutions at alkaline pH that give the molecule an overall more negative charge will run faster than Hb A. Similarly, these same substitutions usually result in a shorter retention time than Hb A on the HPLC column. Conversely, those amino acid substitutions that would results in a more positively charged molecule on alkaline electrophoresis will usually result in a longer retention time than Hb A on HPLC. This method also has the advantage that Hb C does not co-elute with Hb A_2 and so Hb A_2 can be measured in the presence of Hb C. Hbs E and O-Arab, however, still co-elute with Hb A_2 by this method.

D. Mass spectrometry

Mass spectrometry systems have been present in clinical laboratories for at least 35-40 years but only more recently have been applied to the analysis of protein. Mass spectrometry instruments are expensive and require a high degree of expertise and thus are not utilized in many laboratories in the United States. However, in the future these instruments may become more user friendly. MS has the ability to provide very precise identification of even rare variants and can decrease the need for identification of these variants by other methods such as amino acid or DNA sequencing. This method may also be a useful screen to detect those variants which will not separate from Hb A on other methods due to a neutral-charge substitution.

E. Molecular Methods

1. DNA sequencing

These procedures are undertaken by very few laboratories due to the expertise required and the expense of performing these tests. These tests are important for confirmation of previously undetected or extremely rare variants. This is particularly true of clinically important variants (unstable hemoglobins, high or low oxygen affinity hemoglobins, and M-

hemoglobins), many of which do not separate from Hb A because of a neutral charge substitution. Examples of these are Hbs Bethesda, Hammersmith, and Little Rock.

DNA sequencing usually involves polymerase chain reaction (PCR) amplification of one, two or three exons (coding regions) of each globin chain. This is followed by a second sequencing reaction to determine the nucleotide sequence of that segment that has been amplified. Three sets of primers will amplify the three exons of the ß chain. The nucleotide sequences of the two α-globin genes are very similar; however, sets of PCR primers have been developed that will sequentially amplify one or the other of the α chain genes.

DNA sequencing of both α or β genes may become less necessary for the identification of unusual hemoglobin variants due to the increase in use of mass spectrometry. However, these methods still have a role in the detection of thalassemia mutations, particularly point mutations. With the advent of automated sequencers, for those laboratories with sufficient volume, this has become a fairly routine procedure.

2. Multiplex Ligand-Dependent Probe Amplification (MLPA)

This method utilizes multiple DNA probe sets that hybridize at various places on the region of interest. Probe sets have been developed for both the α and β globin gene clusters. Probe sets that do not hybridize will not be amplified and thus indicate the absence or deletion of a particular stretch of DNA. These methods are very useful to determine the presence of large deletional mutations. Such methods are replacing older methods for detection of alpha thalassemia deletions such as gap PCR or Southern Blot. They also have a role in detecting large deletions of the ϭ β globin gene cluster which typically result in either hereditary or persistence of fetal hemoglobin or delta/beta thalassemia phenotypes.

F. Other methods

1. Globin chain electrophoresis

A RBC lysate is treated with a mixture of HCl and acetone which dissociates hemoglobin in to heme and globin. The heme ring is removed by repeated washings with acetone. Mercaptoethanol is incorporated into the solution because it protects the globin chains from oxidative denaturation. When the resultant preparation is run on cellulose acetate that contain 8M urea buffer, the α- and β-globin chains are clearly separated. Furthermore, any hemoglobin variants present will often show altered mobility of the affected chains. Globin chain electrophoresis is usually run at both an alkaline and acid pH, as some hemoglobins variants show slight differences in mobility. This method often gives additional information on hemoglobin variants that have similar mobilities by other methods. This method is not usually performed currently.

2. Amino Acid Sequencing

Amino acid sequencing is usually preceded by HPLC analysis of the hemoglobin molecule with trypsin digestion. Isolation of an abnormal tryptic peptide is useful because then only that portion of the protein needs to be sequenced.

Amino acid sequencing has been supplanted by mass spectrometry of tryptic peptide fragments to determine the amino acid substitution for hemoglobin variants.

3. Reverse-phase HPLC

In the past, this method was used more widely but now is typically only used to isolate tryptic peptides prior to analysis by mass spectrometry.

V. Alpha thalassemias.

α thalassemias are probably the most common genetic disorder worldwide. These occur in multiple ethnic groups around the world. Most α thalassemia mutations are due to deletions. These have arisen due to crossover events that have occurred between the α1 and α2 genes. This is due to the sequence hemology between the α1 and α2 globin genes. These events have occurred many times independently.

8 mutations so far describe the complete deletion of one of the α genes. Two very common types of α thalassemia are designated by the size of the deletion involved, namely –3.7 ("rightward") and –4.2 kb pairs ("leftward"). There are at least 50 known deletions which involve both of the α chains on the affected chromosome. Most are very rare. The 2 most common are the --SEA mutation which is found in Southeast Asians, the --MED-1 deletion found in Mediterranean area. There are at least 69 known types of non-deletional α thalassemia mutations. Most of these are extremely rare. These mutations are point mutations that involve various aspects of DNA transcription and translation. The most notable of these is Hb Constant Spring, which will be discussed below. The various combinations and clinical manifestations of the alpha thalassemias are given in Table 1.

1. Hemoglobin H disease.

This disorder results from deletion (or non function) of 3 of the 4 α globin genes. There is an imbalance of globin chain production with an excess of beta globin chains. These can form tetramers (β_4) which results in Hb H. Hb H is unstable, thus Hb H disease represents a form of hemolytic anemia. Clinically this is classified as a thalassemia intermedia. Hb H is seen as a fast band on cellulose acetate electrophoresis, isoelectric focusing, and capillary electrophoresis. Hb H inclusions can also be demonstrated by supravital stains such as variant cresyl blue.

A form of Hb H disease occurs when the –SEA deletion is seen in conjunction with Hb Constant Spring. Hb Constant Spring is due to a point mutation at codon 142 of the alpha gene. This changes the normal stop codon (TAA) to a codon that codes for glutamine (CAA). An additional 32

amino acids are added to the end of the alpha globin chain until the next stop codon is reached. This abnormal alpha globin is produced in very little amounts (1-2%) and in essence acts as a non-deletional alpha thalassemia mutation. Thus, in combination with a 2-chain deletion on the other chromosome 16, only 1 functional alpha globin chain is present, and Hb H disease is produced. Hb Constant Spring migrates behind Hb A2 on cellulose acetate and isoelectric focusing and is easily missed.

2. Barts hydrops fetalis

If a 2 alpha chain deletion is inherited from each parent, then no Hb A can be produced. This condition is incompatible with life. On electrophoretic methods, there is no Hb A or Hb F present, only Hb Barts (γ4) and a small amount of Hb Portland. On HPLC systems, a very distinct chromatogram is produced. On capillary electrophoresis, there are two types of Hb Portland seen, Hb Portland I ($\zeta_2 \gamma_2$), but also a different type called Portland II ($\zeta_2 \beta_2$). Hb Portland II is only seen in this pathologic condition.

VI. Beta thalassemias.

In contrast with the alpha thalassemias, of the at least 239 known beta thalassemia mutations, the vast majority are non-deletional. Deletional mutations are uncommon. Seven of these involve a large deletion that removes the entire beta globin gene. The remainder shows much smaller deletions of DNA, typically in the range of 1-10 nucleotides. An exception is the 619 base-pair deletion that is seen in the Indian subcontinent. Beta thalassemia mutations are due predominantly to point mutations within the beta globin gene, and may interfere with initiation, transcription, termination, or splicing of messenger RNA. Beta thalassemias are divided into those mutations which result in the total absence of beta chain production (β^0), and those which result in only a reduction of beta chain production (β^+). The beta thalassemias are also classified according to their clinical severity (Table 2).

Beta thalassemia major is usually due to 2 β^0 thalassemia mutations. Hemoglobin electrophoresis shows no Hb A, only Hb A_2 and Hb F. Beta thalassemia intermedia results from 2 β^+ mutations or a β^+/β^0 combination. On hemoglobin electrophoresis, there is a small amount of Hb A with the rest being Hb F and Hb A_2.

VII. Hereditary persistence of fetal hemoglobin and δ/β thalassemia.

These disorders are characterized by persistent elevation of Hb F into adult life. At the molecular level, most of these are due to large deletions that involve both the delta and beta globin genes. Some HPFH mutations are non-deletional and are due to point mutations in the promoter region of the gamma genes. HPFH and δ/β thalassemia mutations can be seen in conjunction with beta thalassemia mutations. These typically result in a clinical situation that is less severe than the hemoglobin electrophoresis results would suggest.

VIII. Hemoglobinopathies.

These refer to structural abnormalities in either the alpha, beta, delta, or gamma chains. Over one thousand hemoglobin variants have been described. Most of these are rare and do not cause clinical or hematologic effects. However, those that are clinically significant can cause a wide range of symptoms including the sickling disorders, hemolytic anemia, or a high or low oxygen affinity state. Other hemoglobin variants such as Hb E or Hb Lepore can also produce a thalassemic picture.

A. Hb S-related disorders.
Hb S results from a point mutation in the 6th codon of the beta globin gene, such that at the 6th position, glutamic acid is replaced by valine. Geographically, Hb S is found in highest concentration in Africa but is also found in areas of Saudi Arabia, India, and around the Mediterranean basin.

1.   Hb S trait.
In the U.S., Hb S trait is found in 8% of African-Americans.
In the heterozygous form, Hb S is almost entirely benign. Occasional patients with have rare bouts of hematuria or minimal concentrating defects (hyposthenuria). There is, however, a statistically significant increase in sudden death in military recruits undergoing basic training, or athletes in training camps under very extreme conditions.

2.   Sickle cell anemia (homozygous Hb S).
The prevalence of sickle cell anemia in the U.S. is 0.14% of African-Americans. The glutamic acid to valine substitution of Hb S promotes increased intramolecular contacts between hemoglobin molecules. In homozygous Hb S patients, Hb S will remain solution under normal conditions. However, under conditions of low oxygen tension, Hb S molecules will precipitate and polymerize to form long fibers (tactoids). These fibers make red cells less deformable, and so the sickled red cells tend to cause occlusions in the microcirculation. This problem is compounded by the fact that the red cells in patients with sickle cell anemia also show increased adhesiveness to the vascular endothelium. The myriad of manifestations of sickle cell anemia all can be traced back to the abnormal properties of the sickle red cells. These include a chronic hemolytic anemia, acute vasoocclusive events (acute chest syndrome, musculoskeletal crises, splenic sequestration crises, abdominal crises, neurologic crises) as well as chronic vasoocclusive sequelae which involves multiple organ systems (pulmonary, genitourinary, CNS, ocular, skeletal, skin). A notable effect is the phenomenon of autosplenectomy. Due to repeated microinfarcts from the spleen, by early adulthood the spleen in patients with sickle cell anemia has been reduced to a fibrotic nub.

3. Hb S/beta thalassemia.
   Hb S can be paired with a β^0 thalassemia mutation; this combination is clinically indistinguishable from homozygous Hb S. On hemoglobin electrophoresis, there is no Hb A seen, only Hb S, Hb A_2, and a minor elevation in Hb F. Hb S can also be seen in combination with a β^+ mutation. On hemoglobin electrophoresis there is some Hb A present. However, Hb S is always greater than Hb A. The severity of clinical symptoms is dependent on the amount of Hb A present. At least some sickling manifestations would be expected if the proportion of Hb A is low.

4. Hb S/C disease.
   The prevalence of Hb S/C disease in African-Americans is 0.13%. Hb S will co-polymerize with Hb C to produce a sickling disorder that is similar but no identical to homozygous Hb S. The degree of anemia and Hb S disease is usually not as severe, and autosplenectomy is uncommon. 50% of patients have splenomegaly. Hb S/C patients more often have a septic necrosis of the humeral head rather than the femoral head, and proliferative retinopathy, and renal papillary necrosis are more common than in homozygous Hb S.

5. Other sickling syndromes.
   There are a few other hemoglobin variants that will co-polymerize with Hb S to produce a sickling syndrome. These are Hb-D Los Angeles (beta121 glu→gln) Hb O-Arab (beta 121 glu→lys). Hb C-Harlem is a doubly substituted hemoglobin variant in which one of the substitutions is the Hb S mutation. Thus, Hb S in combination with Hb C-Harlem will also produce a sickling syndrome. Hb S does not co-polyermize with Hb E. However, the percentage of Hb S is much higher than Hb E, thus under extreme conditions some sickling manifestations have been reported in these patients.

6. Hb S/Hereditary persistence of fetal hemoglobin (HPFH).
   Hb S can be seen in combination with a deletional HPFH, as both disorders are found within the African American population. In this combination, Hb S accounts for 60-65% of the total hemoglobin, Hb F is approximately 30-35%, with the rest being Hb A_2. The high levels of Hb F inhibit polymerization of Hb S. Thus, patients with Hb S/HPFH are clinically benign, and do not show sickling manifestations.

B. Hb C disorders.
   The Hb C mutation occurs at the same codon as Hb S (codon 6) but involves a glutamic acid to lysine substitution.

1. Hb C trait.
   Hb C trait is benign and causes no clinical manifestations. Hb C trait is found in 2.4% of African-Americans. In Africa, Hb C is found almost exclusively in the western portion of Africa in the Ghana and Ivory Coast areas.

2. Homozygous C.
   This is a mild to moderate hemolytic anemia with associated splenomegaly. Apart from aplastic crises and cholelithiasis, patients homozygous for Hb C have few complications and do not suffer from vasoocclusive crises, sequestration crises, or infections prevalent among patients with sickle cell anemia.

3. Hb S/C disease.
   Hb S/C disease was discussed in the previous section.

4. Hb C/beta thalassemia.
   Similar to Hb S, Hb C can also be seen in combination with a beta thalassemia mutation. The clinical severity is dependent on whether this is a beta^0 or beta^+ mutation. A clue is an increased level of Hb F, and a greater degree of microcytosis.

C. Hb E disorders.
   Hb E results from a glutamic acid to lysine substitution at the 26th position of the beta chain. In the beta globin gene, the 26th codon is very near the junction of the first exon and first intron. The change in codon 26 from a GAG to AAG nucleotide triplet causes a nucleotide sequence that resembles the donor consensus sequence that is used to splice out the intron sequences and the resultant beta globin messenger RNA. If the normal donor consensus sequence is used in the splicing process, and intact beta globin chain with a Hb E mutation is produced. If the alternate splicing sequence is used, and abnormal and likely defective messenger RNA is produced. Thus, Hb E, in addition to a structural variant, is also a type of β^+ thalassemia.

   1. Hb E trait.
      These patients are clinically benign but have thalassemic red blood cell indices, including a low MCV and elevated RBC.

   2. Homozygous Hb E.
      Homozygous Hb E is also a benign disorder, but there may be mild anemia, and the red cells are more microcytic than in Hb E trait, mimicking beta thalassemia minor. The red cells contain over 90% Hb E and a slightly increased quantity of Hb F but no Hb A.

   3. Hb E/β thalassemia.
      Because the three most common β thalassemia mutations in Southeast

Asia are β^0 mutations, the combination of Hb E/β^0 thalassemia is occasionally seen. In contrast to the mild nature of Hb E disease, this combination presents as either a thalassemia intermedia or even a thalassemia major. This is the most significant β thalassemia syndrome that is found in Southeast Asia. There is a greater degree of anemia and microcytosis than in homozygous Hb E. On hemoglobin electrophoresis, the pattern is similar to homozygous Hb E. However, there is a greater degree of elevation of Hb F (20-30%).

D. Hemoglobins with altered functional properties
Certain point mutations located at critical points within the hemoglobin molecule can serve to alter its functional properties. This may result in a hemoglobin variant with either increased or decreased oxygen affinity, or perhaps unstable hemoglobin molecules. If the substitution produces a hemoglobin variant with increased oxygen affinity, the patient will manifest as erythrocytosis. An unstable hemoglobin variant will result in a hemolytic anemia. Many of these variants are difficult to identify, as they have a neutral charge substitution and will not separate from Hb A by many methods.

E. Other Noteworthy Hemoglobins

1. Hb D-Los Angeles (Hb D-Punjab) [β 121 Glu→Gln]
This is the fifth most common hemoglobin variant found worldwide. Hb D-Los Angeles (D-Punjab) results from a substitution of glutamine for glutamic acid at the 121st position of the beta chain (β 121 Glu→Gln). This abnormal hemoglobin demonstrates no functional abnormalities. Worldwide, it is found in highest proportion in the Punjab region of India and Pakistan, where the prevalence of Hb D trait is 3%. In the United States, it is found in persons of North European ancestry, particularly English, where the prevalence is 1 in 10,000. It is also found in African Americans, with a prevalence of 1 in 30,000. Its presence in these two groups indicates some genetic heritage from India or Pakistan. In the English it is felt to be a result of the long British occupation of India.

Hb D-Los Angeles trait is a harmless condition. Patients homozygous for Hb D-Los Angeles have normal hemoglobin levels, normal RBC indices, and no evidence of hemolysis. This condition should be distinguished from Hb D in combination with β^0-thalassemia, in which patients have mild anemia and minimal hemolysis. Hb D-Los Angeles copolymerizes with Hb S to produce a severe sickling syndrome. This is felt to be due to enhancement of polymerization due to the Glu→Gln substitution at β^{121}, an important contact point for Hb S polymerization.

2. Hb G-Philadelphia [ά(68)(E17) asn→lys]
This variant is common in African Americans and occurs in approximately 1 in 5000 individuals. It has also been reported in Italians from Northern Italy and Sardinia and a few Chinese families. It is the most commonly encountered ά-chain variant seen in the United States and is not associated with any clinical or hematologic manifestations. Because this variant is fairly common, it has many different synonyms, such as Hb Stanleyville-I, Hb G-Knoxville, Hb D-Washington, Hb D-St. Louis, Hb G-Bristol, Hb G-Azakouli, and Hb D-Baltimore.

In African Americans, this variant is frequently associated with a one ά-chain deletion (ά-thalassemia-2 trait) on the same chromosome 16. This results from a 3.7 kilobase pair deletion and will give a percentage of the hemoglobin variant of approximately 30%. If a one ά-chain deletion also occurs on the other chromosome 16 (which is seen in approximately 30% of African Americans), then only two of four ά-chains are present (*i.e.*, homozygous ά-thalassemia-2 trait). In this situation, the percentage of Hb G-Philadelphia is approximately 35-40% of the total hemoglobin and can be confused with a β-chain variant. Because Hb G-Philadelphia is common in African Americans, it will not infrequently be seen in combination with β-chain variants also common in African Americans, such as Hb S and Hb C.

VIV: Peripheral Blood Manifestations of Several Hemoglobin Disorders

1. Homozygous Hb S or Hb S/β^0 thalassemia.
   There are prominent red blood cell changes, including sickle cells (drepanocytes) as well as hyposplenic changes (Howell-Jolly bodies, target cells, acanthocytes).
2. Hb S/β^+ thalassemia
   The predominant finding is an increase in target cells. There are few, if any, sickle cells seen and hyposplenic changes are not present.
3. Hb S/C disease
   True sickle cells are usually rare; rather, one usually sees oddly shaped cells which have been called "clam" or "boat" shaped cells. Occasional cells may contain Hb C crystals, and target cells are increased.
4. Homozygous Hb C
   There are increased target cells and occasional cells contain Hb C crystals. These are dense polyhedral structures ("Washington monument crystals"). The surrounding red cell is often no longer intact. There are also pseudospherocytes. These are small dense cells which probably contain precipitated Hb C.
5. Homozygous Hb E
   There is usually an abundance of target cells with minimal anisopoikilocytosis.
6. Hb E/β thalassemia

The presence of other oddly shaped cells and basophilic stippling in addition to target cells likely indicates a diagnosis of Hb E with β thalassemia rather than homozygous Hb E.

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Table 1

Possible Combinations of α-thalassemias

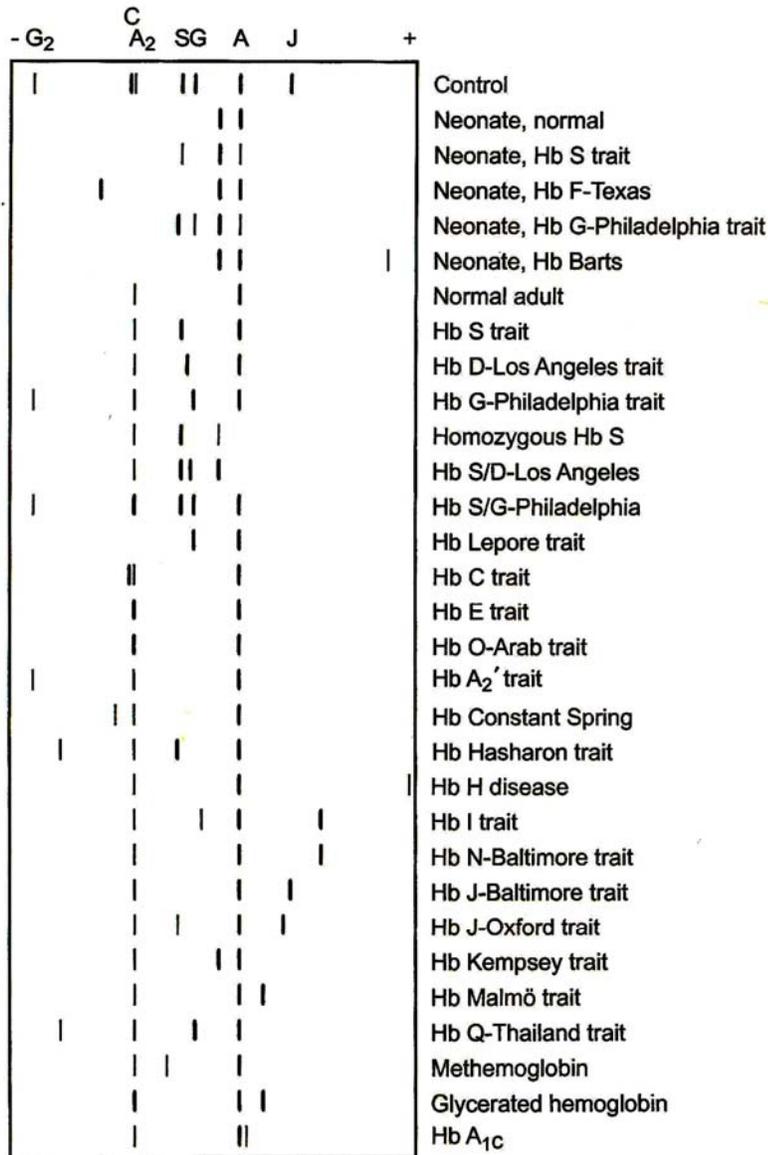| # of Genes Deleted	Configuration	Subtype	Clinical Manifestations
0	αα/αα	Normal	Normal
1	α-/αα	α-thalassemia 2 heterozygote	Clinically silent
2	α-/α-	α-thalassemia 2 homozygote	Microcytosis
2	--/αα	α-thalassemia 1 heterozygote	Microcytosis
3	α-/--	Hb H disease	Thalassemia intermedia
4	--/--	Barts hydrops fetalis (homozygous α-thalassemia)	Lethal

Table 2

Classification of β-thalassemia according to clinical severity

Subtype	Configuration	Clinical Manifestation
β-thalassemia major	β^0/β^0 or β^+/β^0	Clinical manifestation Severe anemia, red cell transfusions required to maintain life
B-thalassemia intermedia	β^0/β^+ or ↑ β^+/β^+	Moderate anemia. Red cell transfusion occasionally required
β-thalassemia minor (β-thalassemia trait)	β^0/β or β^+/β	Slight if any anemia Microcytic red cells, often with erythrocytosis

Figure 1 Examples of several Hb variants on IEF