Hemoglobin $\alpha_2$: Origin, Evolution, and Aftermath

By Martin H. Steinberg and Junius G. Adams III

The melange of hemoglobins present in the erythrocytes of humans includes hemoglobin $\alpha_2$ (HbA$_2$), a tetramer of $\alpha$- and $\delta$-globin chains ($\alpha_2\delta_2$). Its unique characteristics reside in the non-$\alpha$-chain. HbA$_2$ is physiologically unimportant because it is normally less than 3% of the total Hb. The study of HbA$_2$ has provided insights into the evolution and phylogeny of globin genes and enhanced our understanding of gene expression and globin synthesis. HbA$_2$ has substantial clinical relevance. Its concentration fluctuates in the thalassemia syndromes and some acquired diseases, so that its measurement provides a useful diagnostic aid. This review will focus on the structure, function, and synthesis of the $\delta$-globin chain and HbA$_2$, as well as the features of this Hb that give it clinical utility.

CHARACTERISTICS OF THE $\delta$-GLOBIN GENE

Linkage relationships and chromosomal location of the $\delta$-globin gene. It has long been known that the $\beta$- and $\delta$-globin genes were closely linked. These initial linkage data were derived from the study of families in which both $\beta$- and $\delta$-globin variants were segregating. The location of the $\beta$-globin gene family on the short arm of chromosome 11 involved a variety of molecular techniques. The linear arrangement of the $\beta$-globin gene cluster was determined from data derived primarily from gene mapping and is shown in Fig 1. These genes have also been completely sequenced. The general form of the $\delta$-gene is akin to all other globin genes with three coding regions (exons) and two intervening sequences (introns).

Evolution of the $\delta$-globin gene. Because of the large body of sequence data that is available from the globin genes as well as the proteins that they produce, this gene family has provided an excellent opportunity to examine its molecular evolution. The globin genes are all thought to have arisen from a common globinlike heme protein (Fig 2). The earliest duplication of this ancestral gene led to the divergence of myoglobin and the globins that comprise Hb and most likely occurred approximately 700 million years before present (Mybp). The next duplication gave rise to the divergence of the $\alpha$-globin genes and occurred approximately 450 Mybp. The $\beta$-globin gene is thought to have duplicated about 180 to 200 Mybp into an ancestral gene for $\epsilon$- and $\gamma$-globin and an ancestral gene for $\delta$- and $\beta$-globin. Approximately 110 to 130 Mybp, the $\epsilon/\gamma$ parent gene diverged to establish the $\epsilon$ and $\gamma$ lineages.

The evolution of the $\delta$-globin gene was initially very confusing, because HbA$_2$ was present in humans, apes, and New World monkeys, but not in Old World monkeys. This finding was seemingly at variance with the evolutionary data which indicated that humans and Old World monkeys diverged after the divergence from New World Monkeys. This conundrum was ultimately solved with the finding that $\delta$-globin genes are indeed present in Old World monkeys, but have been inactivated by mutation.

After examination of the gene sequence of a number of primate species, the origin of the $\delta$-globin gene was first thought to have occurred relatively recently (about 40 million years ago). However, studies of the globin genes of mice, rabbits, and other primates make it more likely that this divergence occurred before the mammalian radiation approximately 85 to 100 Mybp, at about the same time as the $\epsilon$- and $\gamma$-globin genes diverged. It is clear from the comparisons of $\delta$-globin genes among mammals that the $\delta$-globin locus has not evolved as an independent lineage, but has evolved in concert with the $\beta$-globin gene. In each of the mammalian orders examined to date, the $\delta$-globin locus has acquired characteristics of the $\beta$-globin locus through gene conversion (a nonreciprocal exchange of genetic material between the two linked homologous genes). These gene conversions have most often occurred in the coding regions, rendering these regions useless in the quest for the primordial $\delta$-globin gene. Thus, the evolutionary origin of the $\delta$-globin gene has been performed using flanking and intervening sequence data (especially IVS-II).

Gene conversion and the $\delta$-globin gene. The nonallelic gene conversion events postulated to occur during the evolution of the $\delta$-globin gene are thought to have been rare when compared with the gene conversion between the two $\gamma$-globin loci. However, Petes has made an interesting observation concerning the structural variants of HbA$_2$. The evolution of the $\delta$-globin gene was initially very confusing, because HbA$_2$ was present in humans, apes, and New World monkeys, but not in Old World monkeys. This finding was seemingly at variance with the evolutionary data which indicated that humans and Old World monkeys diverged after the divergence from New World Monkeys. This conundrum was ultimately solved with the finding that $\delta$-globin genes are indeed present in Old World monkeys, but have been inactivated by mutation.

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were incubated with radioactive precursor amino acids, it
bone marrow erythroblasts into fractions of different levels
cytes. These experiments were extended by fractionating
molecular stability of these two molecules appears to be
hemolysate, while HbA, comprises only 2% to 3%. The
posttranslational survival of the two molecules accounts for
almost identical, making it highly unlikely that difference in
differences and 31 nucleotide differences. However, the
striking in the coding regions where there are 10 amino acid
human
low proportion of HbA,. In studies where reticulocytes
the low proportion of HbA,. In studies where reticulocytes
the &-globin gene in this system, &globin gene transcription was
expected CCAAT in the &globin gene. Because the CCAAT
the most striking differences between the
&globin genes approximates the synthesis of &globin in normal erythroid
cells. Humphries et al also made hybrid constructs of the &-
and &globin genes. When the 5' portion of the &globin gene was replaced by the homologous portion of the &globin
gene, transcription of this hybrid gene was equal to that of the
normal &globin gene. However, when the 5' portion of the
&globin gene was replaced by the homologous portion of the
&globin gene, the transcription of the hybrid gene was like that of the &globin gene. These findings suggested that
sequences in the &globin gene promoter were responsible for the decreased transcription of this gene. One of
the most striking differences between the &- and &globin genes is that the usually conserved CCAAT box is the 5'
 promoter region is CCAAC in the &globin gene and is the
expected CCAAT in the &globin gene. Because the CCAAT
box is required for normal transcription of the rabbit
&globin gene,67 this difference is thought to account for
most of the differences in the synthesis of &- and &globin synthesis. It has been shown that IVS-II of the &globin
gene also acts to reduce its synthesis by mechanisms that are unclear now.68

HbA, is synthesized in all erythroid progenitors and therefore its distribution in the blood is pancellular.61 This
contrasts with the expression of the &-globin gene in adults, where only a minor fraction of erythrocytes (F' cells)
contains HbF. This difference in the expression of these two minor Hbs found in adults may result from the restriction of
&-gene expression to a very few erythroid precursors. The special mechanisms that govern switching from fetal to
adult Hbs within the mammalian β-globin gene cluster inactivates the γ-globin genes as δ- and β-gene transcription is activated.62

Function of HbA, and the δ-globin chain. HbA, has functional properties that are nearly identical to those of HbA. It has similar oxygen affinity, Bohr effect, and cooperativity.63,64 Its response to 2,3-bisphosphoglycerate is also similar to that of HbA.65 The thermal stability is greater than that of HbA.66 HbA, inhibits polymerization of HbS.67 The residues 622 (Ala) and 687 (Gln) appear to be the important inhibitory sites. In instances where the HbA, is exceptionally high, and in the presence of elevated HbF levels, the combination of these two Hbs may modulate the phenotype of Hbs-β-thalassemia.68 The positive charge of HbA, may endow it with properties similar to other positively charged Hbs, such as Hbc, relative to its interaction with the erythrocyte membrane.69 HbA, has a higher affinity for erythrocyte membrane band 3 than does HbA.70 While the interaction of Hbc with the membrane is believed to determine the pathophysiologic properties of HbAC and HbCC cells,71 the concentrations of HbA, make it difficult that it can meaningfully affect cation transport and mean corpuscular Hb concentration.

Hybrids of the β- and δ-globin genes. The Lepore Hbs are the products of δβ hybrid globin genes and have provided insight into the mechanisms of decreased δ-globin synthesis. In 1958, Gerald and Diamond72 showed that the individuals who carried this variant exhibited hematologic manifestations identical to β-thalassemia, but had an electroforetically slow-moving Hb that comprised 10% to 15% of the total hemolysate. Subsequently, Baglioni73 showed that the non-α-globin chain of Hb Lepore had δ-globin sequences at its amino terminus and β-globin sequences at its carboxyl terminus. The Lepore Hbs most likely arose from a nonhomologous crossing over event in which the β-globin gene from one chromosome mispaired with the δ-globin gene of the other chromosome during synapsis. The result of this recombination is the deletion of a segment of DNA approximately 7 kb in length from a point within the transcribed portion of the δ-globin gene to a corresponding point in the β-globin gene. Therefore, the Lepore chromosome does not have a normal δ- or β-globin gene.

Current concepts of how the Lepore δβ fusion gene variants produce the phenotype of thalassemia are that the δ-globin gene sequences located at the 5′ portion of these fusion genes result in their characteristically reduced rate of synthesis.74 The major difference in this region, as mentioned previously, is that in the δ-globin gene, the CCAAT box promoter sequence is CCAAC. Another puzzling feature of the Lepore globin chain was that it was synthesized at a higher rate than the δ-globin chain of HbA. This increased synthesis of the hybrid globin is most likely due to the presence of the second intervening sequence of the β-globin gene.75 The rare homozygous forms of Hb Lepore, as well as combination of Hb Lepore with other β-thalassemia genes, are usually expressed clinically as moderately severe forms of thalassemia major.76

The reciprocal product of the crossover that produces the Lepore globin genes produces a chromosome with a δ-β-β configuration. Two of these “anti-Lepore” variants have been found, Hb Miyada77 and Hb P Nilotic.78 Because these individuals have a normal β-globin gene, they do not exhibit a thalassemic phenotype. The anti-Lepore globin gene has the β-globin gene promoter, so synthesis of these globins would be expected to be near that of the β-globin gene. In Hb P Nilotic heterozygotes, the variant Hb was found to comprise 21% to 28% of the hemolysate.77 This proportion closely approximates that expected if the P Nilotic globin chain was synthesized at nearly the same rate as the normal β-globin chain. It has been shown that the synthesis of Hb P Nilotic decreases in reticulocytes as compared with bone marrow cells,79 but this decrease in synthesis is not a major determinant of the total synthesis of this globin because it appears to accumulate in the expected amounts in red blood cell (RBC) precursors. The synthesis of the β-globin chain of Hb Miyada is apparently synthesized somewhat less efficiently than Hb P Nilotic, comprising only 17% of the hemolysate in heterozygous individuals.75 The synthesis of this abnormal globin was also found to be greatly decreased in reticulocytes when compared with bone marrow cells.78

The non-α-globin chain of Hb Parchman is also informative in this regard. If Hb Parchman arose a double crossover as initially suggested, it should contain the δ-globin gene promoter, IVS-I from the β-globin gene, and IVS-II from the δ-globin gene. The non-α-globin chain of Hb Parchman is synthesized at the same rate as the normal δ-globin chain, supporting the suggestion of Kosche et al80 that the decreased synthesis of the δ-globin chain is due to the presence of the CCAAC promoter and the δ-globin gene IVS-II.

δ-Globin variants. The known structural variants of the δ-globin gene are depicted in Table 1. Although these variants may be unstable or have elevated oxygen affinity, they are not associated with a clinically significant phenotype because, as mentioned previously, HbA, has no detectable effect on the oxygen transport of the RBC due to its low proportion. Because HbA, variants have no effect on phenotype, the number of structural variants of the δ-globin gene is far less than that of the α- or β-globin gene.

CLINICAL FEATURES OF HbA,

Measurement of HbA,. The δ-chain of HbA, contains two additional positive charges compared with the β-chain of HbA. This facilitates its separation by electrophoretic and chromatographic methods that rely on charge differences to resolve proteins from one another. Hb electrophoresis on starch or polyacrylamide gels or cellulose acetate membranes affords wide separation of HbA, from HbA and HbF and a means to quantitate accurately this minor Hb component.81,82 Acid gel electrophoresis does not resolve HbA, from HbA. The ease of working with cellulose acetate membranes, their commercial availability and reasonable cost, makes this the current electrophoretic method of choice.83 The low level of HbA, in erythrocytes, and the narrow range separating normal from abnormal, causes methods of measurement that are facile from the
theory technique and the choice of appropriate separative methods can circumvent this complication and permit the quantification of intact Hbs and contributes an effective way of assessing the 8-globin chain of HbA, from HbC or HbE. HbA, may be measured immunologically. This technique has the virtue of specificity. The levels obtained correlate well with the more traditional methods of measurement. To date, this procedure has not enjoyed widespread clinical application.

HbA, in health and disease. There is little a-chain synthesis in utero and the accumulation of HbA, does not become easily measurable until late in gestation. The HbA, level in normal newborns is 0.27% ± 0.02%. The amounts of HbA, vary with gestational age; they are lowest in the least mature infants. The "adult" HbA/HbA, ratio of about 32:1 is not reached until at least 6 months of age. This sluggish response of HbA, during maturation may reduce its value for the diagnosis of β-thalassemia in young infants. HbA, may be measured immunologically. This technique has the virtue of specificity. The levels obtained correlate well with the more traditional methods of measurement. To date, this procedure has not enjoyed widespread clinical application.

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Table 1. Structural Variants of the α-Globin Gene

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Amino Acid Residue No.</th>
<th>Abnormality</th>
<th>Hb Name</th>
<th>Population</th>
<th>Frequency</th>
<th>% Variant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTG → CGG</td>
<td>1 (NA1)</td>
<td>Val → Ala</td>
<td>A, Nilgata</td>
<td>Japanese</td>
<td>XR</td>
<td>—</td>
<td>184</td>
</tr>
<tr>
<td>CAT → CGT</td>
<td>2 (NA2)</td>
<td>His → Arg</td>
<td>A, Sphakia</td>
<td>Cretan Canadian</td>
<td>XR</td>
<td>—</td>
<td>185</td>
</tr>
<tr>
<td>AAT → AAA or AAG</td>
<td>12 (A9)</td>
<td>Asn → Lys</td>
<td>A, NYU</td>
<td>Eastern European</td>
<td>R</td>
<td>1.0</td>
<td>186</td>
</tr>
<tr>
<td>GGC → GGC</td>
<td>16 (A13)</td>
<td>Gly → Arg</td>
<td>A', Black American</td>
<td>q = .009</td>
<td>1.0</td>
<td>6.7,10</td>
<td></td>
</tr>
<tr>
<td>CTD → GAG</td>
<td>20 (B2)</td>
<td>Val → Glu</td>
<td>A, Roosevelt</td>
<td>Iraqi</td>
<td>XR</td>
<td>1.0</td>
<td>187</td>
</tr>
<tr>
<td>GCA → GAA</td>
<td>22 (B4)</td>
<td>Ala → Glu</td>
<td>A, Flatbush</td>
<td>Black American</td>
<td>R</td>
<td>—</td>
<td>188</td>
</tr>
<tr>
<td>GGT → GAT</td>
<td>24 (B6)</td>
<td>Gly → Asp</td>
<td>A, Victoria</td>
<td>Iraqi</td>
<td>XR</td>
<td>1.3</td>
<td>189</td>
</tr>
<tr>
<td>GGT → GAT</td>
<td>25 (B7)</td>
<td>Gly → Asp</td>
<td>A, Yokosha</td>
<td>Japanese</td>
<td>XR</td>
<td>44% of HbA,</td>
<td>190</td>
</tr>
<tr>
<td>GAG → AAG</td>
<td>43 (C22)</td>
<td>Glu → Lys</td>
<td>A, Melbourne</td>
<td>Italian</td>
<td>XR</td>
<td>1.2</td>
<td>191</td>
</tr>
<tr>
<td>GAT → GTT</td>
<td>47 (C66)</td>
<td>Asp → Val</td>
<td>A, Parkville</td>
<td>?</td>
<td>XR</td>
<td>—</td>
<td>192</td>
</tr>
<tr>
<td>CTD → GAG</td>
<td>51 (D2)</td>
<td>Pro → Arg</td>
<td>A, Adria</td>
<td>Italian</td>
<td>XR</td>
<td>1.6</td>
<td>193</td>
</tr>
<tr>
<td>GGT → GCT</td>
<td>59 (E13)</td>
<td>Gly → Arg</td>
<td>A, Indonesia</td>
<td>Malayman</td>
<td>q = .001</td>
<td>1.4</td>
<td>194,195</td>
</tr>
<tr>
<td>GAG → GGT</td>
<td>90 (F6)</td>
<td>GLu → Val</td>
<td>A, Honai</td>
<td>Japanese</td>
<td>XR</td>
<td>0.8</td>
<td>196</td>
</tr>
<tr>
<td>GTG → ATG</td>
<td>98 (F55)</td>
<td>Val → Met</td>
<td>A, Wrens*</td>
<td>Black American</td>
<td>XR</td>
<td>0.2</td>
<td>197</td>
</tr>
<tr>
<td>GAT → AAT</td>
<td>99 (G11)</td>
<td>Asp → Asn</td>
<td>A, Canada†</td>
<td>East Indian</td>
<td>XR</td>
<td>1.8</td>
<td>198</td>
</tr>
<tr>
<td>GGC → CAG or CAT*</td>
<td>116 (G16)</td>
<td>Arg → His</td>
<td>A, Coburg</td>
<td>Italian</td>
<td>XR</td>
<td>1.4</td>
<td>199</td>
</tr>
<tr>
<td>CCG → JGC</td>
<td>116 (G16)</td>
<td>Arg → Cys</td>
<td>A, Corfu</td>
<td>Greek</td>
<td>R</td>
<td>0.5–1.5</td>
<td>204</td>
</tr>
<tr>
<td>GAA → GTA</td>
<td>121 (H44)</td>
<td>Glu → Val</td>
<td>A, Manzanares*</td>
<td>Spanish</td>
<td>XR</td>
<td>0.4</td>
<td>200</td>
</tr>
<tr>
<td>CAA → GAA</td>
<td>125 (H53)</td>
<td>Gln → Glu</td>
<td>A, Zagreb</td>
<td>Yugoslavian</td>
<td>XR</td>
<td>1.1</td>
<td>201</td>
</tr>
<tr>
<td>GGT → GAT</td>
<td>136 (H14)</td>
<td>Gly → Asp</td>
<td>A, Babinga</td>
<td>Babinga pygmies</td>
<td>q = .0007</td>
<td>1.0</td>
<td>202</td>
</tr>
<tr>
<td>GGT → GAT</td>
<td>142 (H20)</td>
<td>Ala → Asp</td>
<td>A, Fitzroy</td>
<td>Greek</td>
<td>XR</td>
<td>1.4</td>
<td>203</td>
</tr>
</tbody>
</table>

Abbreviations: R, rare; XR, extremely rare.
*See text for details concerning possible gene conversion.
†High oxygen affinity.
‡See text for details concerning possible gene conversion.

standpoint of the clinical laboratory, such as densitometric tracings of electropherograms, to be inaccurate. For acceptable accuracy, the HbA, fraction must be eluted and measured spectrophotometrically. Refrigeration and freezing may reduce the HbA, percentage in stored hemolysates. The differential elution of HbA, in minicolumns is reliable, rapid, and inexpensive.

Both electrophoresis and conventional methods of column chromatography are incapable of separating HbA, from Hb variants that contain similar charge differences. Unfortunately, the very common HbC and HbE are in this group of positively charged Hbs. In the company of abnormal globins that contain only a single additional positive charge, like the sickle p-chain, the HbAz level has been reported to be higher than normal. However, good laboratory technique and the choice of appropriate separative methods can circumvent this complication and permit the use of electrophoresis and chromatography to measure HbA, in the presence of Hbs.

High performance liquid chromatography (HPLC) can separate HbA, from other Hb types as well as discriminate the α-globin chain of HbA, from α, β, and γ-globin chains. Cation exchange columns afford excellent resolution of HbA, from Hbs and HbC, but the time and expense of this method detract from its clinical use. The quantification of non-α-globin chains using a C, column (Vydac, Hisperia, CA) provides a useful surrogate for the measurement of intact Hbs and contributes an effective way of assessing HbA, in the presence of HbC or HbE.

HbA, may be measured immunologically. This technique has the virtue of specificity. The levels obtained correlate well with the more traditional methods of measurement. To date, this procedure has not enjoyed widespread clinical application.

HbA, in health and disease. There is little α-chain synthesis in utero and the accumulation of HbA, does not become easily measurable until late in gestation. The HbA, level in normal newborns is 0.27% ± 0.02%. The amounts of HbA, vary with gestational age; they are lowest in the least mature infants. The "adult" HbA/HbA, ratio of about 32:1 is not reached until at least 6 months of age. This sluggish response of HbA, during maturation may reduce its value for the diagnosis of β-thalassemia in young infants. The "adult" level of HbA, is 2.5% to 3.5%. In the presence of an α-globin variant, such as HbGPhiladelphia, the variant α-chain combines with the 8-chain to form an Hb tetramer with the structure, α variant β, α variant β. This tetramer, often called HbGp, usually comprises less than one half the total amount of HbA, in the presence of Hbs and HbC. Extra HbA, bands are a valuable clue to the presence of variant α-globin chains, although, depending on the charge of the α-variant, they may or may not separate from the major Hb bands.

Glycosylated forms of HbA, analogous to the minor
components of HbA, are present and can be quantified by HPLC and isoelectric focusing. As with HbA, these glycohemoglobins are elevated in poorly controlled diabetics.

**Low HbA,** The causes of reduced levels of HbA, are shown in Table 2. Other than the age-related decrement from "normal" found in infants and very young children, low HbA, values are in most instances the result of either reduced synthesis of the δ-globin chain (thalassemia), or posttranslational modifications in the assembly of the HbA, tetramer (Table 2). Reduced HbA, tetramer assembly can result from either acquired or genetic disorders. In either case, the proximate cause is the same; a reduction in the synthesis of α-globin chains.

**Posttranslational causes of reduced HbA,** Hb tetramer assembly follows rapidly on the formation of dimers consisting of α- and non-α-globin chains. Dimer formation, in turn, is dependent on the charge of the non-α-chain. Normal α- and β-monomers have nearly equivalent positive and negative charges, and are united by electrostatic attraction. The δ-chain is more positively charged than the β-chain (or γ-chain). Under normal conditions when there is α-chain sufficiency or slight excess, HbA is formed in priority to HbA, because αβ dimers form in preference to αδ dimers. When the supply of α-globin chain is limited, the effect of charge is exaggerated, as the β-chain (and γ-chain) compete more effectively than the δ-chain, for the limited quantity of α-globin.

Acquired conditions causing low HbA, A number of acquired conditions are capable of reducing α-globin synthesis relative to that of non-α-chains. Most seem to have their effect through the common mechanism of absolute or functional iron deficiency. In the absence of sufficient iron, a repressor of initiation of protein synthesis is formed. This may preferentially affect α-, rather than non-α-globin chain initiation, resulting in a relative deficiency of α-chains. Patients with iron deficiency anemia have reduced levels of HbA, This is most apparent in individuals with the most severe iron deficiency. Individuals with anemia, microcytosis, and low levels of HbA, on the basis of iron deficiency might be mistaken for carriers of one of the more severe forms of α-thalassemia. When iron deficiency and β-thalassemia coexist, the HbA, level has been reported to decrease, although it may remain within the range expected for thalassemia heterozygotes. Iron deficiency may not affect the HbA, in all patients with β-thalassemia. We recently studied two patients heterozygous for the −88 C → T β-thalassemia who also had iron deficiency anemia. Their HbA, levels were appropriately elevated while they were iron deficient and did not change during iron repletion. The iron utilization defect associated with sideroblastic anemias may also reduce HbA, levels.

There may be profound effects on α-globin synthesis in certain myeloproliferative disorders. The expression of all α-globin genes is affected and the phenotype can mimic the genetically determined HbH disease. While these instances are uncommon, they can be associated with low HbA, values. The HbA, level in acute myeloid leukemias (AML) is lower than in acute lymphocytic leukemias. This, and other hematologic differences between these groups, suggests that the AML clone involves the erythroid lineage. In juvenile chronic granulocytic leukemia a pattern very similar to fetal erythropoiesis may develop. Fetal Hb levels may soar, accompanied by low HbA, values, recapitulating normal neonatal findings and the reciprocity of γ- and δ-globin gene expression. Erythroleukemia has also been associated with very low levels of HbA, in the absence of HbH. Conceptually, these acquired "α-thalassemias" bridge the difference between the reduced HbA, secondary to acquired disease and low HbA, associated with genetic abnormalities of α-globin synthesis.

**Genetic conditions associated with low HbA,** α-Thalassemia is extraordinarily common in certain populations. The deficit in α-chain synthesis ranges from trivial to extreme and the level of HbA, varies commensurately with the deficit in α-globin synthesis. With the mildest types of α-thalassemia, HbA, values in individuals may be indistinguishable from normal. When α-globin production is impaired significantly, the reduction in HbA, is dramatic. In 21 patients with HbH disease, the HbA, ranged from 0.5 to 1.8% and averaged 0.8%. Homotetramers of δ-chains have been reported in α-thalassemia hydrops fetalis.

**The δ-thalassemias.** Thalassemia-inducing mutations may affect the δ-globin gene. Uncomplicated δ-thalassemia has no clinical repercussions. In δ⁺-thalassemia, both heterozygotes and homozygotes have reduced HbA, levels. When the δ-gene is totally inactivated (δ⁻-thalassemia), the heterozygote has half normal HbA, levels, while HbA, is absent in the homozygote.

When δ-gene expression is abolished as a result of large DNA deletions that remove the β-, and at times the γ-globin genes, as well as the δ-gene, the resulting phenotype is a consequence of impaired β- or γ-gene expression. Heterozygotes for δβ-thalassemia and gene deletion hereditary persistence of fetal Hb (HPFH), have half-normal HbA, levels; homozygotes have no HbA,. With Lepore Hbs there is a 50% reduction of HbA, level in heterozygotes and no HbA, in homozygotes. While the percentage of HbA, may be low when δ-globin gene expression is abolished, the absolute level, expressed as picograms of HbA, per cell, is slightly elevated. This reflects increased synthesis

### Table 2. Causes of Reduced Levels of HbA,

<table>
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<tr>
<th>Reduced δ-Globin Synthesis</th>
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<tbody>
<tr>
<td>Neonatal period</td>
</tr>
<tr>
<td>δ-Thalassemias</td>
</tr>
<tr>
<td>δβ and γδβ-Thalassemias</td>
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<tr>
<td>Lepore Hbs</td>
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<tr>
<td>δ-Globin chain variants</td>
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<tr>
<th>Reduced HbA, Tetramer Assembly</th>
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<tbody>
<tr>
<td>Genetic</td>
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<tr>
<td>α-Thalassemia</td>
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<tr>
<td>Acquired</td>
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<tr>
<td>Iron deficiency</td>
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<td>Lead poisoning</td>
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<td>Sideroblastic anemia</td>
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<td>Myeloproliferative disorders</td>
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115-117: Reduced HbA, Tetramer Assembly
119: Acquired conditions causing low HbA, A number of acquired conditions are capable of reducing α-globin synthesis relative to that of non-α-chains. Most seem to have their effect through the common mechanism of absolute or functional iron deficiency. In the absence of sufficient iron, a repressor of initiation of protein synthesis is formed. This may preferentially affect α-, rather than non-α-globin chain initiation, resulting in a relative deficiency of α-chains. Patients with iron deficiency anemia have reduced levels of HbA, This is most apparent in individuals with the most severe iron deficiency. Individuals with anemia, microcytosis, and low levels of HbA, on the basis of iron deficiency might be mistaken for carriers of one of the more severe forms of α-thalassemia. When iron deficiency and β-thalassemia coexist, the HbA, level has been reported to decrease, although it may remain within the range expected for thalassemia heterozygotes. Iron deficiency may not affect the HbA, in all patients with β-thalassemia. We recently studied two patients heterozygous for the −88 C → T β-thalassemia who also had iron deficiency anemia. Their HbA, levels were appropriately elevated while they were iron deficient and did not change during iron repletion. The iron utilization defect associated with sideroblastic anemias may also reduce HbA, levels.

There may be profound effects on α-globin synthesis in certain myeloproliferative disorders. The expression of all α-globin genes is affected and the phenotype can mimic the genetically determined HbH disease. While these instances are uncommon, they can be associated with low HbA, values. The HbA, level in acute myeloid leukemias (AML) is lower than in acute lymphocytic leukemias. This, and other hematologic differences between these groups, suggests that the AML clone involves the erythroid lineage. In juvenile chronic granulocytic leukemia a pattern very similar to fetal erythropoiesis may develop. Fetal Hb levels may soar, accompanied by low HbA, values, recapitulating normal neonatal findings and the reciprocity of γ- and δ-globin gene expression. Erythroleukemia has also been associated with very low levels of HbA, in the absence of HbH. Conceptually, these acquired "α-thalassemias" bridge the difference between the reduced HbA, secondary to acquired disease and low HbA, associated with genetic abnormalities of α-globin synthesis.

**Genetic conditions associated with low HbA, α-Thalassemia is extraordinarily common in certain populations. The deficit in α-chain synthesis ranges from trivial to extreme and the level of HbA, varies commensurately with the deficit in α-globin synthesis. With the mildest types of α-thalassemia, HbA, values in individuals may be indistinguishable from normal. When α-globin production is impaired significantly, the reduction in HbA, is dramatic. In 21 patients with HbH disease, the HbA, ranged from 0.5 to 1.8% and averaged 0.8%. Homotetramers of δ-chains have been reported in α-thalassemia hydrops fetalis.**

**The δ-thalassemias.** Thalassemia-inducing mutations may affect the δ-globin gene. Uncomplicated δ-thalassemia has no clinical repercussions. In δ⁺-thalassemia, both heterozygotes and homozygotes have reduced HbA, levels. When the δ-gene is totally inactivated (δ⁻-thalassemia), the heterozygote has half normal HbA, levels, while HbA, is absent in the homozygote.

When δ-gene expression is abolished as a result of large DNA deletions that remove the β-, and at times the γ-globin genes, as well as the δ-gene, the resulting phenotype is a consequence of impaired β- or γ-gene expression. Heterozygotes for δβ-thalassemia and gene deletion hereditary persistence of fetal Hb (HPFH), have half-normal HbA, levels; homozygotes have no HbA,. With Lepore Hbs there is a 50% reduction of HbA, level in heterozygotes and no HbA, in homozygotes. While the percentage of HbA, may be low when δ-globin gene expression is abolished, the absolute level, expressed as picograms of HbA, per cell, is slightly elevated. This reflects increased synthesis
of HbA, from the chromosome in trans to the gene deletion (see below).

Early descriptions of δ-thalassemia were based on hemato-

logic and family studies. In the context of our present
ability to define the thalassemias at the molecular level,
some of these reports are unreliable. The mutations de-
scribed in the δ-thalassemias are shown in Table 3. There
have been a few surprises among these mutations and they
semble the defects that have been found to cause other
thalassemias like frameshift mutations, splicing defects,
gen...
reciprocal fashion with that of the γ-globin gene. This reciprocity is evident as Hbf levels decrease rapidly during the last trimester of gestation and is also observed in the β-thalassemia syndromes. In homozygous β-thalassemia, cells with the highest Hbf levels have the lowest Hba2 concentrations. The relationship between Hba2 and Hbf was strikingly illustrated in a patient with β-thalassemia trait and the Swiss type HPFH receiving chemotherapy. The Hbf level increased dramatically, from 4.5% to 26%, accompanied by a decrease in Hba2 from 4.5% to 2.4%.

The cause of increased Hba2 in heterozygous β-thalassemia appears to reside at both the transcriptional and posttranslational level of Hb synthesis. There is an increase in both the percentage and absolute amount of Hba2 present, with the former about twice as great as the latter. Reduced production of β-globin, with a relative excess of α-globin chains, favors the formation of α6 dimers and the assembly of HbA6 tetramers. If part of the cause of elevated Hba2 was due to posttranslational perturbations, the product of each δ-globin gene should contribute equally to this increase; ie, the effect should be present both in cis and in trans to the β-thalassemia gene. This has been shown directly in the study of families where a structural variant of the δ-globin chain segregates independently from the β-thalassemia-causing mutation. Increased δ-gene transcription, as a result of a β-thalassemia-causing mutation, might be expected to occur only in cis. The mechanism for the “compensatory” increase in δ-globin synthesis is not totally clear but may result from a “competition” among the β-globin-like gene promoters for transcription factors.

Exceptionally high Hba2. The mean level of Hba2 in 879 carriers of β-thalassemia of diverse ethnic backgrounds was 5.08% ± 0.39%. The highest observed value was 6.8%. In 184 black patients with β-thalassemia trait the mean Hba2 level was 4.97% ± 1.07%. Some individuals with β-thalassemia trait have Hba2 concentrations that are significantly higher than these mean levels. The exceptionally high Hba2 levels are usually the result of a unique and informative class of small deletions of DNA that usually begin within the β-globin gene and extend 5', removing the gene promoters. A summary of the mutations so far described that are associated with high Hba2 are shown in Fig 3. These deletions may have direct repeats, partial homologies, purine-rich regions, AT-rich sequence, topoisomerase II recognition sites, and homologies to donor splice sequences in proximity to their 5' and 3' ends. These have been postulated to lead to nonhomologous recombinations by several mechanisms. In contrast to the very high Hba2 present with these 5' deletions, the 600-bp deletion in the 3' portion of the β-globin gene, found in Asian Indians with β-thalassemia, is associated with typical Hba2 levels. A 3.4-kb deletion has its 5' terminus between nucleotides −910 and −128 while the 3' breakpoint is located between the 4va II and Xmn I sites that lay 3' to the β-gene. This deletion removes both the β-gene promoters and the 3' enhancer. The Hba2 level in the single heterozygote examined was 6.7%. Perhaps the loss of the 3' enhancer element modulates the increase of Hba2 expected from the removal of the proximal promot-
heterozygous for both HbS and a 5' deletion β3-thalassemia would then appear to be homozygous for the β3 gene.

The HbA2 levels in heterozygotes for these 5' β-gene deletion thalassemias have been reported to range from about 7% to 12%. We are unaware of the description of instances of bona fide HbA2 levels that exceed the top of this range. Laboratory reports of HbA2 values more than 15% or 20% are apt to be spurious or represent instances of Hbc or HbE heterozygosity complicated by α-thalassemia. Another theoretical possibility for exceptionally high level of HbA2 is the presence of a Miyada-type Hb, or PG-fusion, it was not documented. Recent follow-up of this indistinguishable from normal.

Megaloblastic anemias have been associated with HbA2 concentrations that exceed normal levels. The most severely anemic patients have the highest HbA2 values. However, the incidence of this finding seems low, the magnitude of the increase above normal is slight, and, in one study, the means of the HbA2 levels in the normal and megaloblastic anemia groups were similar. Perhaps the high HbA2 of megaloblastic anemia is a result of more Hb synthesis occurring in less mature erythroid precursors.

The HbA2 levels appear to be increased in some instances of unstable Hbs. This is likely to be a post-translational event where the unstable β-chain has difficulty forming β-β dimers.

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