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Instability of Hemoglobin Molecule : Unstable Hemoglobins with Substitutions at $\alpha_1\beta_1$ and $\alpha_1\beta_2$ Contacts, in Central Cavity, at External Surface, or with Deletions. — A Review. Part III.

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There are approximately 400 abnormal hemoglobins which have been reported to date and many out of these variants are of no clinical significance particularly those involving the substitution on the outside of the molecule. However, when an amino acid substitution takes place in a critical part of the interior of molecule or at the important functional contacts ($\alpha_1\beta_1$ or $\alpha_1\beta_2$) it can grossly affect the stability and functional property of the hemoglobin molecule as the oxygen affinity of the hemoglobin in the red cell depends upon an equilibrium between the high and the low affinity forms of hemoglobin.

The proline substitution has been known to disrupt secondary structure and so is the deletion in which loss of one or more amino acid residues leads to marked molecular instability and chronic hemolytic anemia. There are total of 78 unstable variants which belong to this group in which one of the above mentioned positions have either been affected or one or more amino acid have been deleted from the globin chain. The molecular pathology of these variants will be discussed in detail in the next few pages of this review article.

Key words : Hemoglobin molecule — unstable hemoglobin — hemolytic anemia

Substitutions at $\alpha_1\beta_1$ and $\alpha_1\beta_2$ Contacts

The $\alpha_1\beta_1$ and $\alpha_1\beta_2$ contacts are essential in maintaining the spatial arrangements when the oxygen is taken up or released from the hemoglobin molecule. The $\alpha_1\beta_1$ contact is more extensive as it involves 34 amino acid residues than $\alpha_1\beta_2$ which only involves 19 residues. The $\alpha_1\beta_2$ contact has its particular importance in the co-operative interaction, because if there is no heme-heme interaction no oxygen can be transported. This has been clearly observed in those pathological mutants in which the $\alpha_1\beta_2$ contact has either been affected or in those hemoglobins which are composed of four identical chains e.g. Hb H and Hb Bart's. Those hemoglobins have an increased oxygen affinity but no heme-heme interaction nor Bohr effect. Structural changes at the $\alpha_1\beta_2$ contact or in the central cavity may result in drastic changes in functional properties of molecule.

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At present there are 19 unstable hemoglobins in which either $\alpha_1\beta_1$ or $\alpha_1\beta_2$ contact has been affected and the clinical manifestations and other functional properties of these unstable variants have been summarized in Table 2A. Twelve out of 19 involve the $\alpha_1\beta_1$ contact which also includes Hb Leslie[79,80] in which β131 (H9) has been deleted (Table 2E). Out of the remaining 7, four have substitution at the $\alpha_1\beta_2$ contact, other 3 namely Hbs Köln,[40–52] Nottingham[55] and Djelfa[54,55] not only involve $\alpha_1\beta_2$ contact but heme contact, too (See Table 1). These hemoglobins are not only unstable but also have abnormal functional properties. Generally speaking, the substitution at the $\alpha_1\beta_1$ contact presents a mild instability of Hb molecule, whereas the substitution in the $\alpha_1\beta_2$ contact region will produce a hemoglobin molecule with an altered oxygen affinity as the instability of the molecule is rarely affected. No severe clinical symptom except a mild to slightly moderate hemolytic anemia has been reported in the carriers. A few hemoglobin variants owe their instability due to substitution at the $\alpha_1\beta_1$ interface, which permits the dissociation of $\alpha\beta$ dimer into monomers and allows hidden SH groups to become reactive. Hb Philly [β35 (C1) Tyr→Phe][81] is one of those unstable variants. The $\alpha_1\beta_1$ contacts are weakened when tyrosine is replaced by phenylalanine in Hb Philly and similar situation has been observed in Hb Tacoma [β30 (B12) Arg→Ser],[82,83] in which substitution by seryl residues lead to the loss of the stabilizing contacts thus accounting for the decreased stability of Hb Tacoma in the carrier.

Replacement of Amino Acids that Occupy either External or Surface Crevice or Central Cavity or Internal Position in the Hemoglobin Molecule

Several unstable hemoglobins have arisen due to the substitution in one of the above mentioned positions in the hemoglobin molecule (Table 2B and 2C). Replacement of amino acid that occupies an external position or located at the surface of hemoglobin molecule and does not involve in a specific contact but disturbs the secondary structure or that involves deletion of an amino acid usually results in mild instability of the hemoglobin molecule with no clinical manifestations. Several unstable variants have been listed under this category. They are Hbs Fort Worth [α27 (B8) Glu→Gly],[84] Hasharon [α47 (CE5) Asp→His],[85–90] Arya [α47 (CE5) Asp→Asn],[91] J-Rovigo [α53 (E2) Ala→Asp],[62] Pontoise [α63 (E12) Ala→Asp],[93] Hopkins-2 [α112 (G19) His→Asp],[94,95] Sögn [β14 (A11) Leu→Arg],[96] Strasbourg [β20 (B2) Val→Asp],[97] Henri Mondor [β26(B8) Glu→Val][98] and G-Ferrara [β57(E1) Asn→Lys].[99,100] Hb Shuanfeng [α27 (B8) Glu→Lys][101] also involves the replacement of an external amino acid residue B8, but is more unstable than Hb Fort Worth[84] and severe hemolytic anemia has been reported in the carriers. The replacement of glutamic acid by lysine affects the overall tertiary structure of the molecule. The glutamic acid (B8) neutralizes Arg (B12), a residue involved in the $\alpha_1\beta_1$ contact. When this is replaced by lysine, an extra positive charge is introduced in the molecule resulting in the modified orientation of B helix and disturbance of $\alpha_1\beta_1$ contact.

However, there are a few unstable variants in which the amino acid either occupying the external position or surface crevice has been affected but these produce a mild to variable degree of compensated hemolytic anemia because the substituted amino acid is either a charged residue or has a side chain of

different dimension, interferes with neighbouring amino acids thereby weakening the hydrophobic forces which hold the heme pocket together. These unstable hemoglobins include : Hbs Ann Arbor [α80 (F1) Leu→Arg],[102,103] Okaloosa [β48 (CD7) Leu→Arg],[104] Shepherds Bush [α74 (E18) Gly→Asp][105–107] and Bushwick [β74 (E18) Gly→Val].[108] In Hb Saki,[109] Hb Duarte[110] and Hb Saitama,[111] the affected residues are also those which are located at the surface of molecule but the clinical manifestations of these variants are severe because of proline substitution and these will be discussed in the later part of the text. There are 5 unstable hemoglobins in which central cavity of the hemoglobin molecule is affected. These include : Hbs Manitoba [α102 (G9) Ser→Arg],[112] Camperdown [β104 (G6) Arg→Ser],[113] Altdorf [β135 (H13) Ala→Pro],[114] Hope [β136 (H14) Gly→Asp][115] and Toyoake [β142 (H20) Ala→Pro].[116] Except those involving proline substitution these abnormal hemoglobins in the heterozygotes do not cause any clinical stigmata or abnormality in the red cells except a mild instability of the Hb molecule. This may be due to the replacement of the amino acid residues which is necessary in defining the hydrophilic nature of the central cavity (e. g. residue G6 (Arg) in Hb Camperdown) by another leading to decreased stability but not affecting the functional properties of the molecule. The situation is more concerning when an amino acid which occupies an internal position in the molecules is replaced by another (Table 2D). In certain case this internal residue is either part of heme or $\alpha_1\beta_2$ contact. It has also been observed that proline was newly substituted amino acid residue and it disturbed the helical arrangement. The mild severe hemolytic process has been observed when substitution involved an amino acid which occupied internal position in the molecule. The degree of anemia in most cases related to the type of amino acid substituted and the size of the side chain. A fully charged side chain can not exist in the interior of the molecule unless it is neutralized by an oppositely charged group. Severe hemolytic anemia has been observed in the following unstable variants in which an internal amino acid has been replaced. These are Hbs Savannah [β24 (B6) Gly→Val],[117] Volga [β27 (B9) Ala→Asp][118,119] Castilla [β32 (B14) Leu→Arg][120] and Buenos Aires [β85 (F1) Phe→Ser].[121] Brisk hemolysis with relatively high hemoglobin and red cell values and T/2 of 11 days was observed in the propositus of Hb Baylor [β81 (EF5) Leu→Arg).[122] Hb Pasadena [β75 (E19) Leu→Arg][123] shows an exceptionally mild instability of Hb molecule. A mild to moderate but compensated hemolytic anemia in many cases after splenectomy was noted in the following unstable hemoglobin in which the substitutions of an internal amino acid has taken place. These include : Hbs Tottori [α59 (E8) Gly→Val],[124] Etobicoke [α84(F5) Ser→Arg],[125] Suan-Dok [α109(G16) Leu→Arg],[126] Belfast [β15 (A12) Trp→Arg],[127] Riverdale-Bronx [β24 (B6) Gly→Asp],[128,129] St. Louis [β28 (B10) Leu→Gln],[130,131] Lufkin [β29 (B11) Gly→Asp],[132] J-Calabria [β64 (E8) Gly→Asp],[133,134] Burke [β107 (G9) Gly→Arg],[135] Peterborough [β111 (G13) Val→Phe],[136] Wien [β130 (H8) Tyr→Asp][137] and North Shore [β134 (H12) Val→Glu].[138,139] Both Hb Miyashiro [β23 (B5) Val→Gly][140] and Hb Brisbane [β68 (E12) Leu→His][141] are mildly unstable variants. Neonatal hemolysis has been observed in Hb F-Poole [γ130 (H8) Trp→Gly][3], a γ-chain variant found in an English newborn. A relatively severe hemolytic process was observed in carriers of those mutants in which an amino acid occupying

an internal position was replaced by proline, such as Hbs Genova [β28 (B10) Leu→Pro],[142,143] Perth [β32 (B14) Leu→Pro],[144–148] Mizuho [β68 (E12) Leu→Pro][149] and Atlanta [β75 (E19) Leu→Pro].[150] The mechanism for their instability and hemolytic process will be discussed under proline variants.

DELETION

There are 12 human hemoglobin variants in which one or more amino acid residues, sometime up to five, has been deleted (Table 2E). Hb Vicksburg [β75 (E19) Leu→0][151] is not unstable and therefore has been excluded. Hemoglobin Boyle Heights [α6 (A4) Asp→0][152] is the first example of a deletion in the α-chain involving the deletion of an external residue. The genetic mechanism involving these deletion is the loss of one or more triplets base pair from chromosome resulting from an unequal crossing over between the two normal regions representing sufficiently a great homology during meiosis. It is interesting to note that the deletion sometimes represent the area where the amino acid sequence is repeated and this causes a great deal of difficulty in establishing the structure of the deleted residues. The appropriate examples will be of Hb Leiden,[153] Hb Niteroi[60] and Hb Gun Hill.[61–63] As a result of deletion the contacts between the heme and the globin chains are weakened and thus bringing a variable degree of instability to hemoglobin tetramer. Since the heme is directly involved in the oxygen binding, its functional properties are impaired and some of these hemoglobins with deleted residues have an altered oxygen affinity. A mild to moderate sometimes compensated hemolytic anemia, elevated reticulocyte counts, change in RBC morphology and occasional jaundice are the few characteristics associated with these hemoglobinopathies.

The amino terminal of the β chains participates in 2,3DPG binding and the deletion of single residue from this N-terminal region of the β chains as in Hb Leiden [β6 or β7 (A3 or A4) Glu→0][153] alters the binding sites of these organic phosphates. Similar analogous phenomenon has been found in sheep hemoglobins which have a deletion near the N-terminal end of chain. According to Perutz in such hemoglobins the distance between the α groups of the β chain will be 6Å more than in Hb A.

There is a loss of two amino acids residues in Hb Lyon.[154] One is Lys β17 (A14), an external residue and the other is an internal residue β18 (A15), the valine. The Lys β17 (A14) is an important residue which forms a salt bridge with Glu β121 (GH4). Deletion of this lysyl residue (β17) destabilises the tertiary structure of the β chain. The increased oxygen affinity and impaired heme-heme interaction is due to the change in the spatial configurations.

The deletion of valyl residue β23 (B5) in Hb Freiburg[155] is due to the loss of a corresponding nucleotide triplet base from the gene which determines the structure of the normal chain producing defect in heme pocket and hemichrome formation. The absence of the threonyl residue β87 as in Hb Tours,[156] glutaminyl residue β131 in Hb Leslie[79,80] and leucyl residue β141 in Hb Coventry[64] are the further examples related to the deletion of single nucleotide base triplet from the gene which determines the structure of the β chain.

A repetitive sequence has been observed in Hb Niteroi[60] β42–44 (Phe–

Glu-Ser→0) or β43-45 (Glu-Ser-Phe→0). Both Phe β42 (CD1) and Phe β45 (CD4) form a bond with heme. Ser β44 (CD3) is also in heme contact with carboxyl group of propionic acid. The deletion of these important amino acid residues in the critical area would result into the lethal distortion of the hemoglobin molecule. The chronic hemolytic anemia associated with this hemoglobinopathy is absolutely compatible with the structural alternations in the hemoglobin molecule. The deleted amino acid residues (β56-59 or D7-E3) in Hb Tochigi[157] are those residues which are away from the distal histidine (7E) toward N-terminal and the structural abnormality in such a proximity of the heme molecule can impair the molecular stability of heme in the crevice E, F, G releasing heme from it. Hb St. Antoine [β74-75 (Gly-Leu→0)][156] is slightly unstable with a mild hemolytic anemia. The deleted segment in Hb St. Antoine [β74-75 (E18-19)] is far away from the binding site of 2,3DPG (Lys β82) and this structural change will have a minimal effect. There are three hydrophobic links between the invariable residue leucyl (F4) and the side chains of heme. The deletion of threonyl residue (F3) in Hb Tours[156] causes the leucyl residue (F4) move away from the heme molecule, consequently weakening these linkages. Hb Tours [β87 (F3) Thr→0][156] has no heme-heme interaction and greatly reduced stability of the molecule. The removal of an important section of the polypeptide chain in Hb Gun Hill[61–63] (3 lost residues of F helix and 2 first residues of FG helix) which forms essential heme contacts and linkages with α subunits is responsible for a major alternation in the primary structure of the β chain. His β92 (F8) forms a coordinated bond with heme iron; Lys β95 (FG2) makes a salt bridge with the propionic side chain of heme and Leu β96 (FG3) is in Van der Waals contact with one of CH groups of the main ring of the heme. As a result of alterations in the position of His (F8) and a loss of FG2 and FG3 residues, the β chain of Hb Gun Hill cannot form a stable bond with heme moiety, thus rendering the molecule unstable. Glutaminyl residue β131 (H9) is an internal residue located close to the middle of H-helix but also involves an $\alpha_1\beta_1$ contact. The deletion of this residue in Hb Leslie[79,80] disrupts the H-helix and weakens the $\alpha_1\beta_1$ contact. In several instances, the substitution at $\alpha_1\beta_1$ contact have resulted in the instability of the molecule and thus permitting the dissociation of the $\alpha\beta$ dimer into monomers allowing the -SH groups to become more reactive. The deletion in Hb Coventry[64] involves a hydrophobic heme contact leucyl residue β141 (H19). This is in homologous position with that of α chain unstable variant, Hb Biba [α136(H19) Leu→Pro].[65] The substitution or deletion of an important residue in the inside of heme pocket or interior of subunits can produce a gross molecular instability and Heinz body anemia.

PROLINE SUBSTITUTION

Proline, an imino acid, is usually found either at the commencement of the helix or at the inter-helical segment. Since 80% of the amino acid residues of the protein are in α-helical arrangement, the prolyl residue cannot participate in the helical formation. As a general rule it can only be accommodated in the first or last three positions of the α helix or in a non-helical segment of the globin chain. Its insertion thereafter would disrupt the helical formation

where the binding of amino acid with its preceeding residues is an essential requirement for a stable configuration. For this reason Hb Singapore [α141 (HC3) Arg→Pro][158] is not associated with instability and any clinical severity. But the substitution of prolyl residue for leucyl in Hbs Biba [α136 (H9) Leu→Pro],[65] Genova [β28(B10) Leu→Pro],[142,143] Yokohama [β31(B13) Lcu→Pro],[66] Perth [β32 (B14) Leu→Pro],[144–148] Mizuho [β68 (E12) Leu→Pro][149] and Southampton [β106 (G8) Leu→Pro][71,72] and replacement of alanine by proline as in Hb Madrid [β115 (G17) Ala→Pro][159] all occupying an internal position in the hemoglobin molecule, results in a profound distortion of the respective helices with an inevitable distortion of the tertiary structures of the subunits producing Heinz body hemolytic anemia. However, the heterozygotes of Hb Atlanta [β75 (E19) Leu→Pro][150] also involving the substitution of the internal residue are free of any clinical symptom.

At present there are 18 unstable hemoglobin variants listed in Tables 1 and 2 in which proline has been substituted. All these variants are associated with a variable degree of the instability and clinical severity. The loss of the heme contact and general disruption of molecular configuration that results from this proline substitution explains the molecular instability and consequent hemolytic anemia cases. In Hb Port Phillip [α91 (FG3) Leu→Pro][160] the steric role of leucine is lost. This leucine (α91) is involved in the movement of FG corner and also the side chain of Leu α91 (FG3) provides a support to the imidazole ring and prevents it from tilting backward during the deoxygenation. The hereditary non-spherocytic hemolytic anemia since the early childhood was reported in the propositus with Hb Biba [α136 (H19) Leu→Pro].[65] The leucyl residue α136, is not only the internal residue, but is also in heme contact. This substitution occurs in the C-terminal part of H-helix of the α chain and would disrupt the helix and change its direction. The heterozygotes of Hb Saki [β14 (A11) Leu→Pro][109] does not show any sign of hemolysis except a mild instability of Hb molecule because external residues is involved. This variant has been found in association with β-thalassemia gene and a gross splenomegaly and severe hemolytic process has been reported in the propositus. Similar situation has been observed in the patient who was doubly heterozygous for Hb Duarte [β62 (E6) Ala→Pro][110] and β-thalassemia. The presence of an allelic gene of β-thalassemia contributes to the increased production of the unstable hemoglobin variant. The replacement of β28(B10), an internal amino acid as in Hb Genova [β28(B10) Leu→Pro][142,143] results in the profound distortion of B helix with an inevitable distortion of the β-chain as a whole with possible alteration in subunit interaction within the hemoglobin molecule which is likely to follow after such a distortion of the β-chain.

It will be interesting to note that out of 18 unstable variants in which proline has been substituted, 11 involve the replacement of leucine residue. The side chains of the hydrophobic amino acids have specific purpose of binding on to the heme group ; they surround the heme group and produce a water repelling environment. Any change in size or type of amino acid side chain in this critical region would result in displacement of heme group much readily. Hbs Yokohama [β31(B13) Leu→Pro][66], Santa Ana [β88(F4) Leu→Pro][68,69], Sabine [β91(F7) Leu→Pro][19] and Southampton [β106(G8) Leu→Pro][71,72] are unstable. The heme group is directly affected due to this Leu to Pro substitution

as the introduction of proline at this position disrupts the heme contacts. The distal histidine (E7) keeps the heme iron in the reduced state and the proximal side of the heme plane is essential in heme globin binding. The variants of both distal (E7) and proximal histidine (F8) are not free of clinical symptoms. In both Hb Bicetre [β63(E7) His→Pro][67] and Hb New Castle [β92(F8) His→Pro][70], a chronic hemolytic anemia associated with these hemoglobinopathies is the product of multiple repercussions, namely loss of heme group responsible for stabilising the tertiary structure of the subunits and the introduction of a prolyl residue in the middle of α-helix. The disturbed helix will allow substantial entry of water into heme pocket. In Hb Altdorf [β135 (H13) Ala→Pro][114], the introduction of proline into internal positions of the H helix weakens the heme contacts of the residue Val β137(H15) and Leu β141(H19), thus conceivably causing loss of heme group from the β chain. Hb Toyoake [β142(H20) Ala→Pro][116] was found in a Japanese who had normal Hb level but decreased red cell survival. This abnormal Hb had an increased tendency to heme loss and subunit dissociation. The alanine β142 not only involves in an inter β-subunit contact but also connects the two functionally important residues, β141 Leu and β143 His, one of the binding sites for heme and 2,3DPG, respectively and there is a series of residues distal to it which play an important role in stabilising the molecule. The amino acid substitution at this site would affect the stability and easy dissociation subunits.

HEMOGIOBIN M-DISEASES

The M-hemoglobins are very important group of abnormal hemoglobins because they provide a lot of informations about the interplay of structure and function in the hemoglobin molecule. A mild to moderate instability of hemoglobin molecule has been associated with many M-Hbs. This is due to disturbance of bonding to the heme group as the firm binding of heme within the heme pocket is major requirement for the stability of each globin chain. Any process which causes the methemoglobin formation will tend to increase the amount of naked hemoglobin and subsequent precipitation within the red cells. Like those with enzymopenic methemoglobinemia the patients with Hb M are more or less cyanotic and lack functional impairment. The amino acid substitution in all the M-hemoglobins except Hb Milwaukee (β67 Val→Glu)[73] occurs either at the site of heme linked proximal histidine (α87 or β92) or distal histidine (α58 or β63). This substitution may be in the α-chain (Hb Boston, Hb Iwate[161] and Hb Iwata[4]) or in the β-chain (Hb Saskatoon, Hb Milwaukee and Hb Hyde Park). Except in case of Hb Iwata [α87(F8) His→Arg][4] in all other M-Hbs, the distal or proximal histidine has been replaced by tyrosine. The phenolic group of tyrosine forms a stable internal complex with sixth co-ordination position of heme iron, blocking it permanently in the ferric state which is in contrast to the readily exchangeable link with water in normal methemoglobin and this is the reason that in patients with M-Hb disease it is not possible to relieve cyanosis. The α chain abnormal M-hemoglobins produce cyanosis at birth while the β chain abnormal M-hemoglobins show their clinical manifestation after a couple of months of birth. The mode of inheritance is autosomal and the families with great number of cyanotic members in all

generations have heen reported.

Out of the presently known six M-hemoglobins (Table 1) which have been reported from all over the world Japan is the leader in the discovery of M-hemoglobins. With the exception of Hb Milwaukee[73] all other M-hemoglobins have been reported from Japan and interestingly their distribution is all over the country. (To be continued)

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Table 2A Unstable hemoglobins with substitutions either at $\alpha_1\beta_1$ or $\alpha_1\beta_2$ contacts

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 days	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Bohr effect	Clinical symptoms remarks	References
1. Prato α31(B12)Arg→Ser	$\alpha_1\beta_1$		4.4	12.2	41.0	93	27.7				+					22	Italian	Slightly faster than A					Mild hemolytic anemia.	162
2. *Port Phillip α91(FG3)Leu→Pro	$\alpha_1\beta_2$	E oxy		10.7	34	76			3-4		+					7	Chinese	Like F					Mild anemia.	160
3. Setif α94(G1)Asp→Tyr	$\alpha_1\beta_2$	C oxy	4.2	9.7	33	77		29	1.8		+			+		12-15	Algerian Iranian	Like S		Normal	↓	↑	Unstable. No functional disorders.	163,164
4. Petah Tikva α110(G17)Ala→Asp	$\alpha_1\beta_1$	I	4.9	9.4-10.7		75-77			2-3		+			+		31-32	Iraqu Jews	Like A	0.53-0.62				Unstable clinical course like Hb H disease.	165
5. Tacoma β30(B12)Arg→Ser	$\alpha_1\beta_1$		4.1	13.0	40	97	31.5	32.5			+			0		43	European	Slightly faster than A	1.21	Normal	↓	↓	Unstable. Hematological abnormality.	82,83
6. Philly β35(C1)Tyr→Phe	$\alpha_1\beta_1$			11-14.6					2-8	12	+	+	0	+		30-35	Italian	Like A					Chronic hemolytic state.	81
7. Vaasa β39(C5)Gln→Glu	$\alpha_1\beta_2$	E oxy		11.9-12.1		80-84			2-2.6		+					33.1	Finnish	Like K					Mild hemolytic anemia. Mildly unstable.	166
8. Williamette β51(D2)Pro→Arg	$\alpha_1\beta_1$	E	4.5-5.3	14.2-15.2	40-45				1.3-2.2		+	0				32-35	Black American	Like S		↑	Normal	↓	Unstable. No clinical symptoms.	167
9. Rush β101(G3)Glu→Gln	$\alpha_1\beta_2$	Facing to internal cavity HB to Asp(G1)(99) of the same β chain	3.7	11.9	35.5				3.0	19.5	+					35	Black American	Two bands Cathodal to A at pH 8.0		Normal			Mild hemolytic anemia.	168
10. Indianapolis β112(G14)Cys→Arg	$\alpha_1\beta_1$	I	2.5	4.7	16	75	26.2	29.4	14.0			+		+			European	Like A	1.96				Very unstable.	169
11. *Madrid β115(G17)Ala→Pro	$\alpha_1\beta_1$	I	3.4	9.7		94	28.3		33.5		+	+	+	+		23	Spanish	Like A Free α-chains					Moderately severe hemolytic anemia.	159
12. Fannin-Lubbock β119(GH2)Gly→Asp	$\alpha_1\beta_1$	E	3.9-5.2	11.6-14.2	35-41	81-88	27.2-29.7	33.2-33.8	1-2.2		+	0		0		41-45	Mexican	Between A and J		Normal	Normal	Normal	Slightly unstable; mild anemia.	170
13. Bougardirey Mali β119(GH2)Gly→Val	$\alpha_1\beta_1$	E	5.42	15.1	43	80	27.7	34.7			+					35	African	Like A					Slightly unstable.	171
14. *Khartoum β124(H2)Pro→Arg	$\alpha_1\beta_1$	E									+			0		30	Turkish	Like S					Slightly unstable. No clinical or hematological abnormalities.	172
15. Guantanamo β128(H6)Ala→Asp	$\alpha_1\beta_1$	I							3-4		+					36-38	Cuban	Like J					Mild hemolytic anemia. Slightly unstable.	173

Table 2B Unstable hemoglobin mutants involving either external or surface crevice residues

1. Fort Worth α27(B8)Glu→Gly	SB	E	4.8	11.3	33.1	68	23.3	34.2	2.6		+			0		5	Black American	Like A_2					Slightly unstable.	84
2. *Shuangfeng α27(B8)Glu→Lys	SB	E		4.5	21.0	117	25.0	21.4	5.0		+	+			1.3	13	Chinese	Slightly faster than A_2					Severe hemolytic anemia.	101
3. Hasharon (Sinai, Sealy, Ferrara) α47(CE5)Asp→His		E			37-49				1.2-3.9	19-25	+	+		0	0.6	16-19	Jew Italian Egyptian	Like S		Normal			Mild compensated hemolytic anemia.	85-90
4. *Arya α47(CE5)Asp→Asn		E									+			0		22	Iranian	Like S					Slightly unstable. No clinical symptoms.	91
5. J-Rovigo α53(E2)Ala→Asp		E									+					35-50	Italian	Like J					Unstable. Symptomless with normal hematological data.	92

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 days	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Bohr effect	Clinical symptoms remarks	References
6. *Pontoise α63(E12)Ala→Asp		SC									+			0		12	Spanish	Like J		↑	↓	Normal	Unstable.	93
7. *Ann Arbor α80(F1)Leu→Arg		SC									+	+		0			Caucasian	Slower than S	1.80				Hemolytic anemia compensated after splenectomy.	102,103
8. Hopkins-2 α112(G19)His→Asp		E			Normal	85			Normal		+					12-22	Caucasian			↑	↓		Unstable. No clinical abnormalities.	94,95
9. Sögn β14(A11)Leu→Arg		SC		12-13.3		90-100		30-32	0.2-0.4	22.0	+			0	0.4-0.5	30-32	Norwegian	Like S					Unstable without any clinical symptoms.	96
10. Saki β14(A11)Leu→Pro		SC	4.1	12.2	38	93					+					41	Yoruba Nigerian Greek	Like A	1.11	Normal	Normal	Normal	Unstable. No clinical abnormalities. But when found in association with β-thalassemia, a severe hemolytic process and gross splenomegaly is reported.	109
11. *Strasbourg β20(B2)Val→Asp		E	Normal	Normal	Normal	Normal	Normal	Normal	Normal		+					40	Portugese	Like A					Slightly unstable.	97
12. *Henri Mondor β26(B8)Glu→Val	SB to His	E	3.7	7.5	24				2.0		+					37.5	African	Slower than A					Slightly unstable.	98
13. Okaloosa β48(CD7)Leu→Arg		SC			38-49				2.4-4.1	↓	+					32-36	Caucasian American	Like S		↓	Normal	Normal	Mild hemolytic anemia. Reticulocytosis.	104
14. G-Ferrara β57(E1)Asn→Lys		E									+					32	Italian	Like G		Normal	Normal	Normal	Unstable. Symptomless.	99,100
15. Duarte β62(E6)Ala→Pro		E	6.95	15.1	47	68	21.9	31.9	10.4	16	+	+	+	+		8	German	Like A		↑	Normal		Chronic hemolytic state in combination with β-thalassemia.	110
16. Shepherds Bush β74(E18)Gly→Asp		E		13.0	41				5-8		+	+		+		24	S. African	Slightly faster than A		↑	↓	Normal	Well compensated hemolytic anemia. Persistent reticulocytosis. Improved after splenectomy.	105-107
17. Bushwick β74(E18)Gly→Val		E	3.4-3.8	10.5-11.1	33-36	96-97	30.1-31.6	30.7	4.2		+			+			Italian American	Slightly faster than A_2	0.85				Mild chronic compensated hemolytic anemia, Drug sensitive, Rapid post-synthetic destruction.	108
18. *Saitama β117(G19)His→Pro		E	3.4	9.8	31.8	94	29.3	31.0	33.6		+	+			2.8		Japanese	Like A		↓	↓		Hemolytic anemia and jaundice.	111

Table 2C Unstable hemoglobins involving residues occupying the central cavity of Hb molecule

1. Manitoba α102(G9)Ser→Arg		C	4.9	14.7	44				4.0		+					12-16	Canadian	Like F					Slightly unstable.	112
2. Camperdown β104(G6)Arg→Ser		C	3.9-5.4	11.1-18.8		82-87	27.7-29.0				+						Maltase	Fast fraction ahead of A		Normal	Normal		Slightly decreased stability.	113
3. Altdorf β135(H13)Ala→Pro		C		9.1-12.1					2-17		+					35	Italian	Like A		↑	↓		Mild to moderate hemolytic anemia	114
4. Hope β136(H14)Gly→Asp		C	4.7	12.4	40.5	86	26	31		Normal	+					40-45	Black American	Slightly anodal to A		↓	↓	Normal	Mild anemia. Unstable. Heinz bodies formation upon incubation with acetylphenylhydrazine.	115
5. Toyoake β142(H20)Ala→Pro		C	4.7	15.2	48.9	103	31.9	31.3	4.4	8	+	+	+			28	Japanese	Slightly faster than A		↑	↓	Normal	Chronic. Compensated hemolytic anemia.	116
Others																							
1. Grady (Dakar) α115-118 115 116 117 118 Ala-Glu-Phe-Thr-Glu 119 Phe-Thr-Pro (insertion)			4.5	13.4	41	88	29	33			+					8-18	Black American	Faster than A		↑	↓	Normal	Unstable. Symptomless.	174,175

Table 2D The unstable hemoglobin variants in which the amino acids occupying internal positions in the hemoglobin molecule has been affected

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 days	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Bohr effect	Clinical symptoms remarks	References
1. Tottori α59(E8)Gly→Val		I	3.6	12.1	36.1	100	33.5	33.5	12.0		+	+		+	1.2	10	Japanese	Like A		↑			Mild hemolytic anemia.	124
2. Etobicoke α84(F5)Ser→Arg		I		12.2-14.1				29-31	1.3-2.8		0			0		15.0	Irish	Like S					Less stable than HbA. Heat stability test negative. But the red cells stored at 20°C in glycerol show a positive heat stability test and inclusion bodies.	125
3. Suan-Dok α109(G16)Leu→Arg		I	5.6	12.3	40	72	22.1	30.7			+	+		+		9	Thailander	Slower than F	0.59				Hemolytic anemia. Clinical picture indistinguishable from Hb-H disease. Variant present in association with α-thalassemia.	126
4. Belfast β15(A12)Trp→Arg		I	6.5	13.7	44	68	20.5	30.6	4.0		+	+	0	+	Normal	27.5	Caucasian Irish	Slower than A	0.97	↑	Normal	Normal	Mild hemolytic process	127
5. Miyashiro β23(B5)Val→Gly		I	5.35	14.1	44	82	26.4	32.1	1.0		+					40	Japanese	Like A		↑			Unstable.	140
6. *Riverdale-Bronx β24(B6)Gly→Arg		I		11-12					10		+	+	0			30	Jew German	Like S	1.2				Compensated hemolytic anemia.	128,129
7. *Savannah β24(B6)Gly→Val		I	2.1	6.3	23	111	30	27	18.9		+	+		+	2.4	30	Caucasian American	Like F					Severe hemolytic anemia. No clear response to splenectomy.	117
8. *Moscva β24(B6)Gly→Asp		I									+			+		17	Russian	Like J		↓	Normal	Normal	Found in a patient with myeloid leukemia. Detailed information not available.	176
9. Volga β27(B9)Ala→Asp		I	3.0	9.1					20-30		+	+		+	↑	15-20	Russian Dutch	Like A					Severe chronic hemolytic anemia, compensated after splenectomy.	118,119
10. Genova (Hyogo) β28(B10)Leu→Pro		I		9.8							+			+	1.6	15	Italian, Canadian French	Like A	1.4	↑	↓	Normal	Severe hemolytic anemia. Improvement after splenectomy.	142,143
11. *St. Louis β28(B10)Leu→Gln		I	2.9	10.0	28.3	97.5	34.5	35.3	20		+	+		+		30	French	Like A	2.0	↑	↓	Normal	Severe hemolytic anemia improved after splenectomy. Cyanosis, 15% methemoglobin.	130,131
12. *Lufkin β29(B11)Gly→Asp		I	4.2	12.0	36	85	28	33	6.8		+			+	Normal	38.6	Black American	Like J		Normal	Normal	Normal	Hemolytic anemia but well compensated.	132
13. *Perth (Abraham Lincoln, Kobe) β32(B14)Leu→Pro		I	1.7	8.5	30	105	29.4	27.8	27	8	+	+	+	+	↑	33	Black American S.W. African	Like A	1.18	Normal	↓	Normal	Severe hemolytic anemia.	144-148
14. *Castilla β32(B14)Leu→Arg		I		9.6		110	34	33			+	+	+	+		22	Spanish	Like D		↓	↓	↓	Severe hemolysis.	120
15. J-Calabria (Bari, Cosenza) β64(E8)Gly→Asp		I	5.0-5.6	12.2-14.7	48-49	87-89	24.4-27.1	27.8-30.6	0.7		+				Normal	33-42	Italian	Like J		↑			Unstable, mild variable hemolytic anemia in heterozygotes. Doubly heterozygote for HbJ-Calabria/β-thalassemia shows moderate chronic hemolytic anemia with Heinz bodies and splenectomy.	133,134
16. *Mizuho β68(E12)Leu→Pro		I	1.5-2.8	5.2-8.5						2-6	+	+		+		6-10	Japanese	Like A					Relatively severe hemolytic anemia compensated after splenectomy.	149
17. Brisbane β68(E12)Leu→His		I		17.6	53						+						New Zealander	Like A		↑	↓	Normal	Mildly unstable with mild erythrocytosis.	141
18. Pasadena β75(E19)Leu→Arg		I		16.1	47	90	31.2	34.1	10.2		+	+				33	French Ancestory	Like S		↑	↓		Slightly unstable.	123
19. Atlanta β75(E19)Leu→Pro		I	3.4	9.6	31	91	29	31	7.5		+	+		+		33	Caucasian American	Like A					Mild hemolysis.	150
20. *Baylor β81(EF5)Leu→Arg		I	4.8	15.8	47					11	+	+			1.5	22	Italian Irish	Slightly faster than S		↑	↓	Normal	Brisk hemolytic process but with relatively high hemoglobin and red cell values.	122

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 days	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Bohr effect	Clinical symptoms remarks	References
21. Buenos Aires (Bryn Mawr) β85(F1)Phe→Ser		I	44	12.6	40				9.0		+	+	+	+		40-50	Argentenian American	Slightly slower than S to anode		↑	↓		Well compensated anemia.	121
22. *Burke β107(G9)Gly→Arg		I		10.6-12.3					8.1-14.7		+			+		30	Caucasian American	Like F		↓	↑	↑	Self compensatory hemolytic anemia.	135
23. *Peterborough β111(G13)Val→Phe		I	3.7	10.0	28.3	76	26.3	34.8	20.0		+	+		+			Italian	Like A		↓	Normal	Normal	Mild hemolysis compensated except during hemolytic crisis.	136
24. Wien β130(H8)Tyr→Asp		I		9.5							+	+	+	+			German	Like A					Hereditary hemolytic anemia.	137
25. North Shore β134(H12)Val→Glu		I	4.4-5.1	10.4-12.3		68-72	22.9-23.8	31.3-32.6			+					30	Australian Venezuelan	Slightly faster than A		↓	Normal	↓	Mild anemia.	138,139
26. F-Poole γ130(H8)Trp→Gly		I		13.1		103		37.4	5.0		+		+	+	15.9		English	Like F					Neonatal hemolysis.	3

Table 2E Unstable hemoglobins with deletion of one or more amino acid residues

Variant substitution	Contact	Position in molecule	RBC 10^{12}/l	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	Retics %	T/2 dyas	Instability test	Splenomegaly	Pigmenturia	Heinz bodies	Indirect serum bilirubin mg/dl	Abn. Hb %	Race or nationality	Electrophoretic mobility SG or CA	Ratio	Functional properties: O_2 affinity	Functional properties: n	Functional properties: Bohr effect	Clinical symptoms remarks	References
1. Boyle Heights α6(A4) Asp→0		E		Slight microcytosis							+					13	Mexican	Between F and S						152
2. Leiden β6 or β7 (A3 or A4) Glu→0		E		11.3-13.6					3-6	14	+	Int				25-33	Dutch Chinese	Like S	1.80	↑	Normal	Normal	Mild, hemolysis compensated except during crisis.	153
3. Lyon β17-18(A14-15) Lys-Val→0		E,SB to GLU GH4(121) I		11.0	37	77					+	0				37	French	Like J		↑	↓		Discrete anemia, minor instability.	154
4. Freiburg β23(B5) Val→0		I		13.1					9.0		+	+				27-32	German	Like F		↑			Mild cyanosis, hemolytic anemia.	155
5. *Niteroi β42-44(CD1-3) Phe-Glu-Ser→0 or β43-45(CD2-4) Glu-Ser-Phe→0	heme	SC,E,E E,E,E		6.8-10					12-20		+	+	+	+		25	Brazil Denmark	Between S and F		↓			Chronic hemolytic anemia with crisis induced by drugs and infections.	60
6. Tochigi β56-59(D7-E3) Gly-Asn-Pro-Lys→0		E							↑		+			+		20-25	Japanese	Faster than A					Moderate hemolytic anemia.	157
7. *St. Antoine β74-75(E18-19) Gly-Leu→0		E,I									+					25	French	Like A		Normal			Unstable. Mild hemolytic anemia. Slightly increased rate of spontaneous oxidation.	156
8. *Tours β87(F3) Thr→0		E									+					25	French	Like S		↑	0		Unstable. 45% heme loss.	156
9. Gun Hill β91-95(F7-FG2) Leu-His-Cys Asp-Lys→0	heme (Proximal His)	SC,E E, deoxy SB to His HC3(146)E		12.6-13.5	45-48	85-101	24-29	28	4-10	17	+	+	0	+	0.3	30	German English Black American	Slower than C	1.0	↑			Compensated hemolytic anemia. Half the globin chains in Hb GH lack heme group.	61-63
10. Leslie (Deaconess) β131(U9) Gln→0	$\alpha_1\beta_1$	I	5.1	9.3	31	61	18	29	9.2	17	+	+	+			85	Black American	Like F					Hemolytic anemia and no other clinical manifestations.	79,80
11. *Coventry β141(H19) Leu→0	heme	I		8.5		88			11.8		+	+				10	English	Like A					Hemolytic anemia.	64
Double Mutation																								
1. S-Travis β6(A3) Glu→Val β142(H20) Ala→Val		E C									+					14.1	Like S			↑	Normal	Normal	Mildly unstable, associated with sickling properties.	177
Elongated Chains																								
1. Cranston ^{144}Lys-Ser-Ileu-Thr-Lys-Leu-^{150}Ala- ^{155}Phe-Leu-Leu-Ser-Asn-Phe-Tyr-COOH (Frame Shift)				Normal	15-17		Normal	Normal	Normal	5-8		+			+	35	Italian American	Close to S	1.0	↑	Absent	Absent	Well compensated hemolytic anemia.	178

Key:
E = External; I = Internal; SB = Salt Bridge; SC = Surface Crevice; C = Central Cavity; HB = Hydrogen Bond; + = Positive; 0 = Absent; INT = Intermediate; ↑ = Increased; ↓ = Decreased; * = Not present in other family members