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Molecular basis for dominantly inherited inclusion body β-thalassemia

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ABSTRACT **Analysis of the molecular basis of dominantly inherited β-thalassemia in four families has revealed different mutations involving exon 3 of the β-globin gene. It is suggested that the phenotypic difference between this condition and the more common recessive forms of β-thalassemia lies mainly in the length and stability of the abnormal translation products that are synthesized and, in particular, whether they are capable of binding heme and producing aggregations that are relatively resistant to proteolytic degradation.**

The thalassemias, the commonest genetic diseases, result from a wide variety of different mutations of the α- and β-globin genes that direct synthesis of adult hemoglobin ($\alpha_2\beta_2$) (1). Imbalanced globin chain production is a major factor in determining the severity of anemia in β-thalassemia. The absence or severe reduction of β chains in homozygotes or compound heterozygotes for β-thalassemia results in a large excess of α-chains that precipitate in erythrocyte precursors causing their premature destruction in the bone marrow and, hence, in severe anemia. Heterozygotes for β-thalassemia are, on the other hand, usually symptomless and have mild anemia, poorly hemoglobinized erythrocytes, and minimal α-chain precipitation in their precursors. Therefore the β-thalassemias are, in the main, recessive disorders in which the inheritance of two abnormal β-globin genes is required to produce a clinically detectable phenotype.

In 1973 we described an Irish family with an unusual form of β-thalassemia that was characterized by anemia, enlargement of the spleen, and gross abnormalities of the erythrocytes and their precursors; the disorder was transmitted through several generations in an autosomal dominant fashion (2). Subsequently we have studied three similarly affected kindreds, all of Anglo-Saxon origin, and several others have also been reported.

Here we describe the molecular pathology of these dominantly inherited forms of β-thalassemia. The findings, taken together with previous studies of the mutations that underlie the common recessive forms of the condition, suggest that subtle differences in the lengths and sequences of abnormal translation products of the β-globin gene may be reflected in major variations in the associated heterozygous phenotypes.

MATERIALS AND METHODS

Blood Samples. Blood samples were collected with EDTA as anticoagulant and hematological data were obtained using an automated cell counter. Hemoglobin (Hb) analyses and Hb A_2 and F levels were measured using standard techniques.

RNA and DNA Analyses. Total cytoplasmic RNA was prepared from peripheral blood reticulocytes by phenol/chloroform extraction and the relative α- and β-globin mRNA levels were determined by hybridization to purified cDNAs specific to α- and β-globin genes (3). DNA was extracted from peripheral blood leukocytes and analyzed by Southern blotting (4). Seven restriction fragment length polymorphisms (RFLPs) in the β-globin gene cluster—*Hin*dII-ε, *Hin*dIII-$^{G}\gamma$, *Hin*dIII-$^{A}\gamma$, *Hin*dII-ψβ, *Hin*dII-3′ψβ, *Ava* II-β, and *Bam*HI-β—were studied and the β haplotypes were determined. The β-globin gene probe used was either the *Pst* I 4.4 β or the 0.9-kilobase (kb) *Bam*HI/*Eco*RI IVS-2 β fragment. The number of α-globin genes was determined by hybridizing *Bam*HI- and *Bgl* II-digested DNA with α- and ζ-globin gene probes, respectively. Probes were radiolabeled with [^{32}P]dCTP by the random hexamer printing method (5).

β-Globin Gene Cloning and Sequence Analysis. The β-globin gene from the Irish family, family 1 (Fig. 1), was directly cloned into plasmid according to a protocol previously described (6). Briefly, 120 μg of DNA isolated from peripheral blood leukocytes from III2 was digested with seven enzymes (*Taq* I, *Pvu* II, *Hin*dII, *Bst*E1, *Bcl* I, *Sst* I, and *Stu* I), all of which cut outside the 3.7-kb *Bgl* II/*Pst* I β-globin gene fragment. The bulk digest was phenol/chloroform extracted, ethanol precipitated, and digested to completion with *Bgl* II and *Pst* I. An aliquot was checked for complete digestion by blot hybridization. The digested DNA was fractionated by electrophoresis in a 0.8% agarose gel and the genomic DNA fraction of size 3.5- to 3.9-kb fragments was isolated by electroelution and cloned directly into *Bam*HI/*Pst* I-digested pUC18 vector. The genomic library was screened with a *Bam*HI/*Eco*RI β-globin gene probe. Four positive colonies were detected, of which two β-globin clones were from the thalassemia allele identified by the presence of the *Ava* II-β RFLP. These clones were then constructed in M13 for sequencing by the dideoxy chain-termination method (7) using dATP[^{35}S] (Amersham) and Sequenase (United States Biochemical). Nine synthetic 19-mer oligonucleotides complementary to the regions around nucleotide −925 (primer 1), nucleotide −478 (primer 2), nucleotide −300 5′ to the β-globin gene (primer 3), nucleotides 12–31 3′ to the Cap site (primer 4), codon 71 in exon 2 (primer 5), intervening sequence 2 (IVS2) nucleotide 360 (primer 6), IVS2 nucleotide 654 (primer 7), codon 114 in exon 3 (primer 8), and nucleotide 300 base pairs (bp) downstream from the termination site (primer 9) were used as primers for sequencing the entire β-globin gene extending from 1560 bp upstream from the Cap site to 670 bp downstream from the termination site.

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Abbreviations: RFLP, restriction fragment length polymorphism; PCR, polymerase chain reaction; IVS, intervening sequence; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

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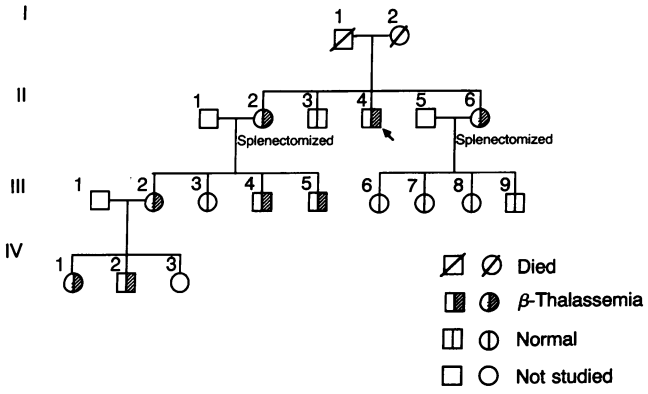


FIG. 1. Family 1: Pedigree of the Irish family with inclusion body β-thalassemia (2). II4 (arrow) is the propositus.

DNA Amplification and Direct Genomic Sequencing. The β-globin genes from the propositus in family 2 were enzymatically amplified by the polymerase chain reaction (PCR) using heat-stable Taq DNA polymerase from *Thermus aquaticus* (8) (Cetus) and a DNA thermal cycler (Perkin–Elmer/Cetus) (see Fig. 2). One microgram of genomic DNA was subjected to 30 cycles of PCR with a 1-min denaturation period at 94°C, a 2-min annealing period at 55°C, and a 3-min extension period at 72°C according to a protocol previously described (9). After amplification the products were examined in a 1.2% agarose gel and the fragment of expected size was isolated by electroelution onto a DEAE membrane (NA45 Schleicher & Schuell) followed by two phenol/chloroform extractions and ethanol precipitation. The amplified material was directly sequenced by the dideoxy chain-termination method using the sequencing primers as shown in Fig. 2. One-half to 1.0 pmol of the purified double-stranded template was annealed to 1.0–5.0 pmol of sequencing primer by heating to 98°C for 5 min and immediately placing it in dry ice. Reactions were then carried out using dATP[^{35}S] and Sequenase (United States Biochemical).

RESULTS

Family 1. The clinical and hematological details of the Irish family have been described elsewhere (2); a revised family pedigree is illustrated in Fig. 1. Detailed hematological and hemoglobin investigations did not show any evidence of hemoglobin instability or detectable β-chain variants. All affected members have a moderate anemia with splenomegaly, increased levels of Hb A_2 and F, and increased α/β chain synthesis ratios. Two family members, II2 and II6, underwent splenectomy. Since the initial report in 1973, II2 has died. At autopsy, extensive extramedullary hemopoiesis with marked erythroid hyperplasia of the bone marrow was demonstrated in the ribs, long bones, and skull. There was also extensive hemosiderosis of the pancreas, kidneys, lymph nodes, ovaries, thyroid, and bronchus. The distribution of iron in this case occurred mainly in parenchymal tissues, which is typical of overload derived from excessive iron absorption rather than from transfusion. This pattern of iron overload together with the extensive extramedullary hemopoeisis are typical of a hematological disorder characterized by ineffective hemopoeisis.

Family members II4, II6, III2, III4, IV1, and IV2 all have a normal α-globin genotype (αα/αα). RFLP analysis showed that the β-thalassemia gene in family 1 was associated with +−−−−++ β haplotype. In III2 the β-thalassemia chromosome could be differentiated from the normal β chromosome (−++−+−+) by the presence of the *Ava* II-β RFLP; the β-thalassemia gene was cloned and sequenced in the M13 system.

DNA sequence analysis of 3703 bp of the β-thalassemia gene from III2, extending from 1560 bp upstream of the Cap site to 670 bp downstream from the termination site, revealed a complex rearrangement in the third exon that involved two deletions, one of 4 bases in codons 128 and 129 and the other of 11 bases in codons 132–135. The deletions were interrupted by an insertion of 5 bases, CCACA, followed by the normal sequence of 8 nucleotides (Fig. 3 *Upper* and *Lower*). The modification results in a frameshift reading through to codon 153 and should result in the synthesis of a variant β-globin that is seven residues longer than normal. This complex rearrangement was confirmed by M13 sequencing of both strands and by oligonucleotide hybridization with normal and mutant probes specific for the sequence modification in this region (data not shown). This rather complex rearrangement was observed in three other affected members (II4, II6, and III4), including the propositus.

Hybridization of cDNA to total cytoplasmic RNA of the propositus, II4, showed a normal α/β mRNA ratio of 1.4 (3).

Family 2. The propositus is a 30-year-old British female who presented at the age of 21 years with a flu-like illness and gave a long-standing history of intermittent ill-health characterized by pallor, lassitude, and jaundice. Physical examination showed mild icterus and splenomegaly. Investigations revealed the following: Hb, 8.3 g/dl; mean corpuscular volume (MCV), 64.0 fl; mean corpuscular hemoglobin (MCH), 20.0 pg; reticulocytes, 12.0%; Hb A_2, 4.3%; Hb F, 3.9%; α/non-α-globin synthesis ratio, 2.0; serum ferritin, 3690 mg/liter. A bone marrow examination showed marked dyserythropoiesis and large inclusion bodies in the erythroid

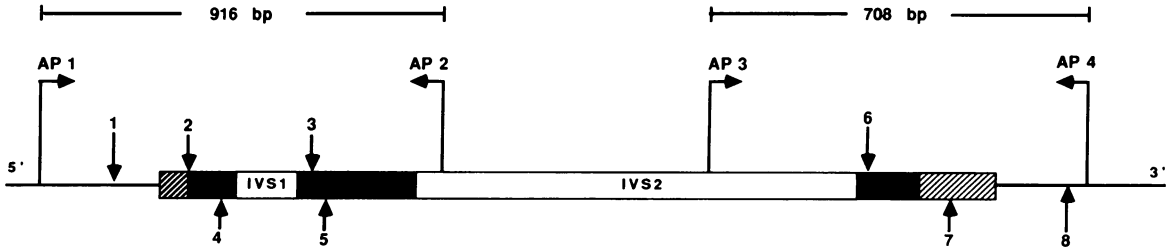
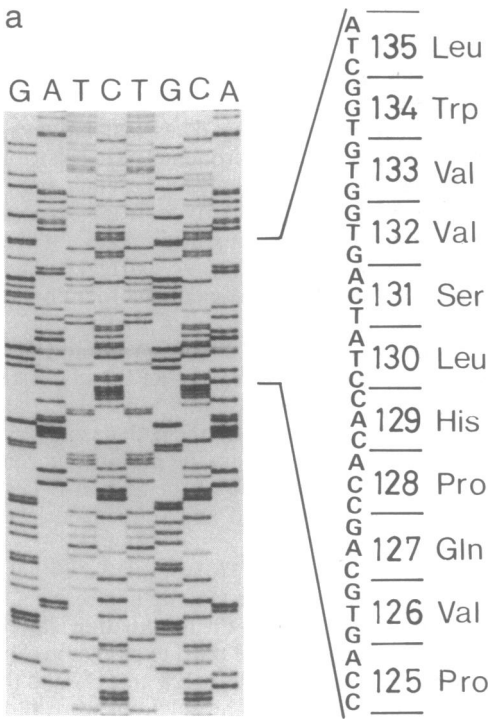


FIG. 2. Representation of the β-globin gene with the position of the primers used in amplification by PCR and in direct genomic sequencing. PCR primers AP1 and AP2 encompass a 916-bp fragment from position −307 to nucleotide 113 in the IVS2 of the β-globin gene, and AP3 and AP4 encompass a 708-bp region from nucleotide 592 in the IVS2 to 320 bp downstream from the termination codon. Arrows above the horizontal line represent locations of the primers used to sequence in the forward direction and arrows below the line represent locations of the primers used to sequence in the reverse direction. The sequences of these primers are 5′ → 3′: AP1, CGATCTTCAATATGCTTAC; AP2, CATTCGTCTGTTTCCCATTCTA; AP3, CAATGTATCATGCCTCTTTGCAC; AP4, GGCATAGGCATCAGGGCTG; 1, GAGCCACACCCTAGGGTTG; 2, ATGGTGCACCTGACTCCTGAGG; 3, CCCAGAGGTTCTTTGAGTCCTTT; 4, CAACTTCATCCACGTTCACC; 5, CAGCATCAGGAGTGGACAGATC; 6, GTCTGTGTGCTGGCCCATC; 7, CAGTTTAGTAGTTGGACTTA; 8, GCAGCCTCACCTTCTTTCATGG. AP1, AP2, AP3, and AP4 can also be used as sequencing primers.



b

Codon	125	126	127	128	129	130	131	132	133	134
Normal	CCA	CTG	CAG	GCT	GCC	TAT	CAG	AAA	GTG	GTG
	Pro	Val	Gln	Ala	Ala	Tyr	Gln	Lys	Val	Val
III2	CCA	GTG	CAG	CCA	CAC	CTA	TCA	GTG	GTG	TGG
	Pro	Val	Gln	Pro	His	Leu	Ser	Val	Val	Trp

Codon	135	136	137	138	139	140	141	142	143	144
Normal	GCT	GGT	GTG	GCT	AAT	GCC	CTG	GCC	CAC	AAG
	Ala	Gly	Val	Ala	Asn	Ala	Leu	Ala	His	Lys
III2	CTA	ATG	CCC	TGG	CCC	AGA	AGT	ATC	ACT	AAG
	Leu	Met	Pro	Trp	Pro	Thr	Ser	Ileu	Thr	Lys

Codon	145	146								
Normal	TAT	CAC	TAA							
	Tyr	His	Term							
			147	148	149	150	151	152	153	
III2	CTC	GCT	TTC	TTG	CTG	TCC	AAT	TTC	TAT	TAA
	Leu	Ala	Phe	Leu	Leu	Ser	Asn	Phe	Tyr	Term

FIG. 3. DNA sequence of β-thalassemia gene of III2 in Fig. 1. (*Upper*) Sequence analysis of part of the β-thalassemia gene in the region of codon 127 showing the complex insertion and deletions. (*Lower*) Presumed abnormal β-chain variant with 153 residues resulting from the complex rearrangement that involved deletions of 4 and 11 bases (boxed) and an insertion of 5 bases (underlined).

precursors. The patient became transfusion dependent at the age of 27 years and, because of increasing transfusion requirements, splenectomy was carried out 2 years later. Postsplenectomy, inclusion bodies could be detected in peripheral erythrocytes on incubation with methyl violet. Her father was clinically asymptomatic, although her mother has a long history of "anemia." Investigations showed the mother to be heterozygous for β-thalassemia: Hb, 11.7 g/dl; MCV, 60.0 fl; MCH, 19.5 pg; Hb A_2, 3.8%; Hb F, 2.4%, with bizarre morphological changes and marked basophilic stippling of the peripheral erythrocytes. α-Globin gene mapping showed that, though the mother has a normal α-globin genotype ($\alpha\alpha/\alpha\alpha$), the propositus and her father have five α-globin genes ($\alpha\alpha\alpha/\alpha\alpha$).

Direct genomic sequencing of all of the three exons, the exon/intron boundaries, and the 5′ and 3′ flanking regions of the β-globin gene from the propositus revealed a G → T (GAA to TAA) substitution in codon 121 of exon 3 (Fig. 4). The sequence modification, G ↓ AATTC to TAATTC abolishes the cleavage site for *Eco*RI in exon 3, so that *Eco*RI analysis of the 708-bp fragment amplified using primers AP3/AP4 results in three fragments—a 708-bp fragment from the mutant allele and a 307-bp fragment and 401-bp fragments from the normal β allele (data not shown). This mutation could also be detected by Southern blot hybridization of *Eco*RI-digested DNA with a β-globin gene probe. Using both methods of detection, the β 121 G → T mutation was confirmed in the propositus and her mother.

Family 3. The propositus is a 42-year-old British male who presented at the age of 36 years with lethargy and was found to be icteric with splenomegaly. The hemoglobin findings were as follows: Hb, 11.6 g/dl; MCV, 77.0 fl; MCH, 26 pg; reticulocytes, 4%; Hb A_2, 5.0%; Hb F, 3.0%; and serum ferritin, 620 mg/liter, with bizarre morphological changes and marked basophilic stippling of the peripheral erythrocytes. Bone marrow was markedly dyserythropoietic with large inclusion bodies in erythrocyte precursors. Both of his parents have died but the mother had a long history of "anemia." His wife is normal; they have two sons: one is normal and the other is heterozygous for β-thalassemia with a hematological profile similar to that of his father.

Family 4. The propositus is a 25-year-old British female who presented with anemia during pregnancy. Hematological investigations were as follows: Hb, 9.0 g/dl; MCV, 64 fl; MCH, 19.5 pg; reticulocytes, 4.3%; Hb A_2, 4.8%; and Hb F, 1.1%. The spleen was not palpable. Blood film showed grossly abnormal erythrocytes with marked basophilic stippling. No other family members were available for study.

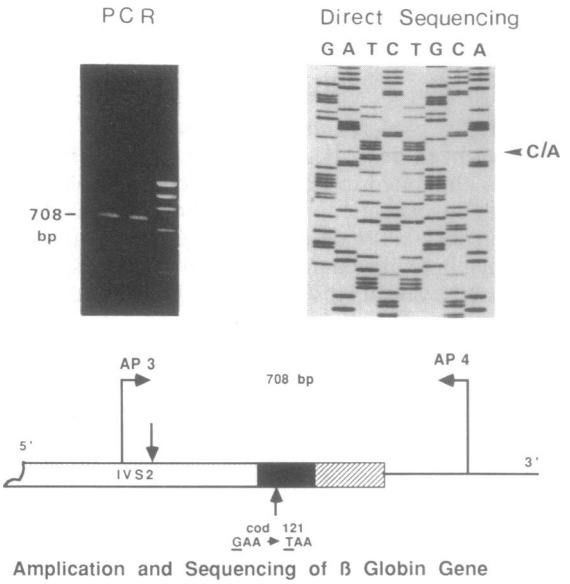


FIG. 4. Sequence analysis of part of the β-globin gene of the propositus in family 2. A specific region of the β-globin gene was enzymatically amplified by PCR using primers AP3 and AP4 and directly sequenced by the dideoxynucleotide chain-termination method. The PCR simultaneously amplifies the wild-type and the β-thalassemia alleles so that both alleles are sequenced. In this case the reverse strand was sequenced; if both alleles were normal, only a C should be present in the first position of codon 121 (arrow). Presence of an A as well as a C in this position indicates an A substitution in the mutant allele.

In families 3 and 4, all members have a normal α-globin genotype (αα/αα) and the β 121 GAA → TAA mutation was detected in affected members by *Eco*RI restriction analysis of PCR-amplified genomic DNA and Southern blot hybridization.

DISCUSSION

Although β-thalassemia primarily affects people of Mediterranean, Asian, and African ancestry in the malaria-endemic regions, sporadic cases have been reported in many different ethnic groups from other areas. Typically, heterozygotes for β-thalassemia, including β^0-thalassemia in which no β-globin chains are synthesized, are clinically asymptomatic with minimal hematological abnormalities (1). However, there are reports of β-thalassemia heterozygotes who coinherited triplicated α-globin gene loci (10, 11) and, presumably with the greater globin chain imbalance, manifested a more severe clinical syndrome. Four families, of North European descent, came to our attention because of the unusually severe clinical phenotype associated with heterozygous β-thalassemia. With the exception of the propositus in family 2, none of the affected individuals in these families has inherited extra α-globin genes.

Cloning and sequence analysis of the β-thalassemia gene in the Irish family showed a complex rearrangement in the third exon involving an insertion of 5 bp and two minor deletions of 4 and 11 bp confirmed by synthetic oligonucleotide hybridization to genomic DNA from four family members. The net result of this rearrangement is a loss of 10 bp, resulting in a frameshift and synthesis of an abnormal and most probably unstable β-chain variant of 153 residues, 7 longer than normal. The amino acid sequence of the variant β chain is normal up to residue 127 but continues as an abnormal stretch of 26 residues that contains 15 hydrophobic residues, including three prolines at positions 128, 137, and 139. This difference should result in a variant β chain that is more acidic than normal and that would probably cochromatograph with the γ chain in CM-cellulose fractionation for biosynthesis studies. However, extensive hemoglobin biosynthesis studies on the propositus, including short-term incubation and pulse–chase experiments (data not shown), did not detect the abnormal β-chain variant but showed a considerable degree of imbalanced synthesis of α and β chains with a significant pool of free α chains (2). Furthermore, it had previously been shown that both α- and β-chain peptides were present in the inclusion bodies (2). The normal α/β mRNA ratio in reticulocytes indicates that the defect occurs posttranscriptionally. It is likely, therefore, that this β-chain variant is extremely unstable and incapable of forming a tetramer with α chains and, together with the resulting excess α chains, precipitates in the erythrocyte precursors to form inclusion bodies. The most likely cause of the instability of this variant β chain is the proline residue at position 128, which disrupts helix H and removes the essential heme contacts Val H15 and Leu H19 and all of the stabilizing interactions with helix G.

The clinical features of a dominant dyserythropoietic anemia associated with inclusion bodies in normoblasts, first described in the Irish family (2), were similar to those observed in β-thalassemia heterozygotes of a Swiss–French family (12, 13). Hence, the term "inclusion-body β-thalassemia" was proposed (12). Recently the mutation in the β-thalassemia gene in the Swiss–French family has been characterized (13) and found to be a G → T substitution in codon 121, which is identical to that causing the severe β-thalassemia in three of the families reported here. The same change has also been reported as a spontaneous mutation in a person of Greek–Polish descent (14); this individual had a clinical picture of moderately severe thalassemia that was interpreted as a compound heterozygosity for β 121 G → T and another β-thalassemia mutation. Interestingly, biosynthesis studies on reticulocytes of this patient demonstrated a truncated β-globin corresponding to the translation product of the β 121 Glu → term mRNA. The truncated β-globin comprised between 0.05% and 0.1% of the total globin (15).

Several other families with a phenotypically dominant form of β-thalassemia have been reported (16–20), some of which are due to highly unstable variant β chains—e.g., Hb Indianapolis (18), which are rapidly catabolized and lead to erythroblast destruction within the bone marrow. These differ significantly from the classical unstable hemoglobin variants where most damage occurs to erythrocytes in the circulation resulting in a hemolytic anemia rather than an ineffective erythropoiesis (21).

The translation products that result from different mutations of the β-globin gene, together with the associated heterozygous phenotypes, are summarized in Fig. 5. Nonsense or frameshift mutations that result in premature termination early in the β-chain sequence, and that give rise to truncated β chains of up to 72 residues, are associated with

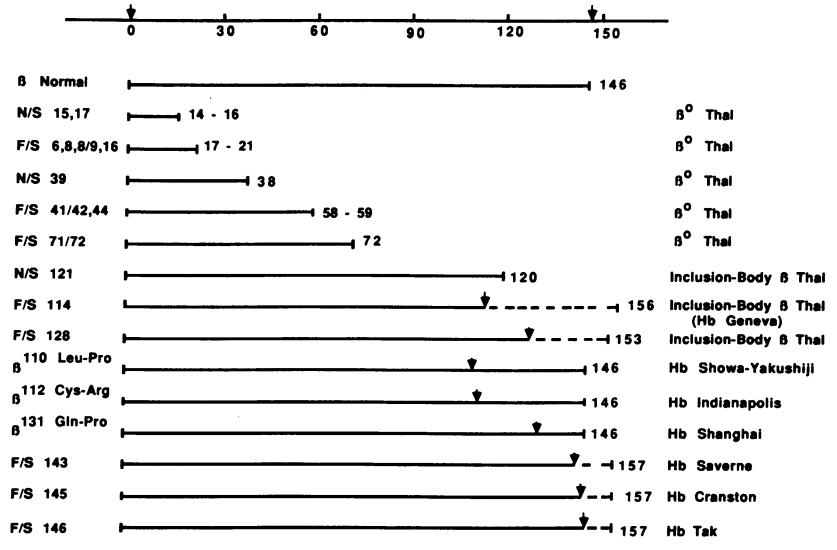


FIG. 5. Mutations in the β-globin locus with lengths of translation products and the heterozygous phenotype. N/S and F/S refer to nonsense and frameshift mutations, respectively. N/S 121 and F/S 128 are the β-thalassemia mutations reported in this paper; Hb Saverne, Hb Tak, and Hb Shanghai are described in refs. 23, 24, and 25, respectively.

a phenotype of typical heterozygous β-thalassemia—that is, mild hypochromic anemia with minimal dyserythropoiesis. No β chains are produced as a result of these mutations, and in heterozygotes only half the normal amount of β-globin is synthesized; the resulting excess α chains are presumably degraded by proteolytic enzymes in erythrocyte precursors. However, mutations that produce an in-phase termination later in the β sequence lead to longer truncated products—for example, 120 residues in the case of the nonsense β 121 mutation. Here the heterozygous phenotype changes dramatically to that of dominantly inherited β-thalassemia. It is probable that this remarkable transformation reflects the heme binding properties of the truncated products. Those with only 72 residues cannot bind heme, whereas those truncated to residue 120 should bind heme since only helix H is missing. Such a heme-containing truncated product should have some secondary structure and hence be less susceptible to proteolytic degradation. However, the lack of helix H would expose one of the hydrophobic faces of helix G and also the hydrophobic patches of helices E and F, a phenomenon that is likely to lead to aggregation. It is probable, therefore, that these heme-containing aggregations of truncated β chains, together with the excess of α chains that result from the inability of these β chains to form tetramers, form the inclusion bodies that are characteristic of this condition and that are responsible for the major dyserythropoietic component of the anemia. In the case of the frameshift insert at position 128 described in this paper, the new proline at H6 would disrupt the H helix; this is followed by the hydrophobic sequence Val-Val-Trp-Leu-Met-Pro-Trp-Pro and later by Leu-Ala-Phe-Leu-Leu. These hydrophobic sequences would also lead to aggregation and precipitation of the abnormal β chain. On the other hand, frameshift mutations such as Hb Cranston (22), which are close to the C terminus of the β chain, still allow the formation of viable tetramers and hence are associated with minimal phenotypic changes.

The picture that is emerging from these studies is that many exon 3 mutations are associated with a dominant phenotype for heterozygous β-thalassemia. As pointed out recently, they are rare and usually found in North Europeans (17). Presumably because of their severity they would not have come under selective pressure as is the case for the recessive forms of β-thalassemia and hence, unlike the latter, will not have reached high gene frequencies in malarious populations.

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1. Weatherall, D. J. & Clegg, J. B. (1981) *The Thalassemia Syndromes* (Blackwell).
2. Weatherall, D. J., Clegg, J. B., Knox-Macaulay, H. H., Bunch, C., Hopkins, C. R. & Temperley, I. G. (1973) *Br. J. Hematol.* **24**, 681–702.
3. Hunt, D. M., Higgs, D. R., Old, J. M., Clegg, J. B., Weatherall, D. J. & Marsh, G. W. (1980) *Br. J. Hematol.* **45**, 53–64.
4. Old, J. M. & Higgs, D. R. (1983) *Methods in Hematology* (Churchill Livingstone, Edinburgh), pp. 74–92.
5. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **1232**, 6–13.
6. Nicholls, R. D., Hill, A. V. S., Clegg, J. B. & Higgs, D. R. (1985) *Nucleic Acids Res.* **13**, 7569–7578.
7. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5469.
8. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
9. Thein, S. L., Hesketh, C., Brown, J. M., Anstey, A. V. & Weatherall, D. J. (1989) *Blood* **73**, 924–930.
10. Galanello, R., Ruggeri, R., Paglietti, E., Addis, M., Melis, M. A. & Cao, A. (1983) *Blood* **62**, 1035–1040.
11. Thein, S. L., Al-Hakim, I. & Hoffbrand, A. V. (1984) *Br. J. Haematol.* **56**, 333–337.
12. Stamatoyannopoulos, G., Woodson, R., Papayannopoulou, T., Heywood, D. & Kurachi, S. (1974) *N. Engl. J. Med.* **290**, 939–943.
13. Fei, Y. J., Stoming, T. A., Kutlar, A., Huisman, T. H. J. & Stamatoyannopoulos, G. (1989) *Blood* **73**, 1075–1076.
14. Kazazian, H. H., Jr., Orkin, S. H., Boehm, C. D., Goff, S. C., Wong, C., Dowling, C. E., Newburger, P. E., Knowlton, R. G., Brown, V. & Donis-Keller, H. (1986) *Am. J. Hum. Genet.* **38**, 860–867.
15. Adams, J. G., III, Morrison, W. T., Steinberg, M. H., Kazazian, H. H., Jr., & Orkin, S. H. (1986) *Blood* **64**, Suppl. 69 (abstr.).
16. Friedman, S., Ozsuylu, S., Luddy, R. & Schwartz, E. (1976) *Br. J. Haematol.* **32**, 65–77.
17. Kazazian, H. H., Jr., Dowling, C. E., Hurwitz, R. L., Coleman, M. & Adams, J. G., III (1989) *Am. J. Hum. Genet.* **45**, Suppl. 0950.
18. Adams, J. G., III, Boxer, L. A., Baehner, R. L., Forget, B. G., Tsistrakis, G. A. & Steinberg, M. H. (1979) *J. Clin. Invest.* **63**, 931–938.
19. Beris, P., Miescher, P. A., Diaz-Chico, J. C., Han, I.-S., Kutlar, A., Hu, H., Wilson, J. B. & Huisman, T. H. J. (1988) *Blood* **72**, 801–805.
20. Kobayashi, Y., Fukumaki, Y., Komatsu, N., Ohba, Y., Miyaji, T. & Miura, Y. (1987) *Blood* **70**, 1688–1691.
21. Bunn, H. F. & Forget, B. G. (1986) *Hemoglobin: Molecular, Genetic and Clinical Aspects* (Saunders, Philadelphia).
22. Bunn, H. F., Schmidt, G. J., Haney, D. N. & Dluhy, R. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3609–3613.
23. Delanoe, J., North, M. L., Arous, N., Bardakjian, J., Pflumio, F., Brunagel, M. L., Lacombe, C., Poyart, C., Galacteros, F., Rosa, J. & Blouquit, Y. (1984) *Blood* **64**, Suppl. 56 (abstr.).
24. Lehmann, H., Casey, R., Lang, A., Stathopoulou, R., Imai, K., Tuchinda, S., Vinai, P. & Flatz, G. (1975) *Br. J. Haematol.* **31**, Suppl., 199–131.
25. Zeng, Y. T., Ren, Z. R., Chen, M. J., Zhaom, J. Q., Qiu, X. K. & Huang, S. Z. (1987) *Br. J. Haematol.* **67**, 221–223.