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Research Article

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Balanced Globin Chain Synthesis in Hereditary Persistence of Fetal Hemoglobin

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ABSTRACT In two black families with the hereditary persistence of fetal hemoglobin (HPFH) gene there are eight A-F heterozygotes and two double heterozygotes for sickle cell trait and HPFH. These patients are clinically asymptomatic and have homogeneous acid elution smears. Measurement of globin chain synthesis in peripheral blood demonstrates balanced production of a α and non-α (β plus γ) chains. In these patients, the balance is achieved by increased γ globin production and increased activity of the remaining β globin allele. In two patients, one A-F and the other S-F there is also balanced globin synthesis in the bone marrow. In a double heterozygote for HPFH and β-thalassemia, anemia (Hb: 11.5 g/100 ml) is associated with a moderate degree of globin chain imbalance. There is a correlation between balanced globin chain synthesis and the absence of anemia in patients with HPFH.

INTRODUCTION

Little is known of the mechanisms regulating human fetal hemoglobin (Hb F) synthesis during fetal and adult life. In patients with hereditary persistence of fetal hemoglobin (HPFH),[1] Hb F production persists in adult life. Homozygotes for HPFH have no anemia indicating that the Hb F completely compensates for the complete lack of Hbs A or A_2 (1). Unlike HPFH, in the homozygous form of β-thalassemia, where Hb A synthesis may be markedly reduced or absent, Hb F synthesis may increase but not to a level adequate to compensate for the reduced synthesis of Hb A. In so-called $\delta\beta$-thalassemia (2), in which Hb F is the only Hb component synthesized, the level of total hemoglobin production is also low. The molecular basis for the normal levels of hemoglobin in patients with HPFH is obscure, and studies of globin chain synthesis in these patients have been lacking.

We have studied two families with the HPFH gene. In four generations of the first family, individuals heterozygous for HPFH and for β-thalassemia and individuals doubly heterozygous for sickle-β-thalassemia (S-thal) and S-F were examined. In the second family, four HPFH heterozygotes were studied. The results indicate that the asymptomatic clinical course in patients with HPFH gene is associated with balanced globin chain synthesis in peripheral blood and bone marrow cells. In addition, in patients doubly heterozygous for a HPFH gene and either a sickle gene or a β-thalassemia gene, the severity of the anemia seems related to the degree of the imbalance in the synthesis of α globin relative to $\beta + \gamma$ globins.

METHODS

Clinical and hematological data. Hemoglobin levels and red cell indices were performed on a Model S Coulter Counter (Coulter Electronics, Inc., Fine Particle Group, Hialeah, Fla.). The distribution of Hb F in intact red cells was studied by the acid elution technique (3). Cellulose acetate electrophoresis using Tris-EDTA-borate buffer at pH 8.6 was routinely performed. The level of Hb F was measured by alkali denaturation (4). Starch gel electrophoresis was used to measure Hb A_2 (5). Agar gel electrophoresis was performed when Hb S was present and to confirm the presence and amount of Hb F.

The two families are black. The pedigree of the first family is shown in Fig. 1. Hematological data are in Table I.

Family I. III-3 (the proband), a 24-yr-old student nurse was studied because of hyperbilirubinemia found on a routine examination. She has had no hospital admissions in New York or in Haiti, where she was born and resided until age 19. Her physical examination reveals hepatomegaly, 3

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[1] *Abbreviations used in this paper:* HPFH, hereditary persistence of fetal hemoglobin; KRB, Krebs-Ringer bicarbonate; MCV, mean corpuscular volume; S-thal, sickle-β-thalassemia.

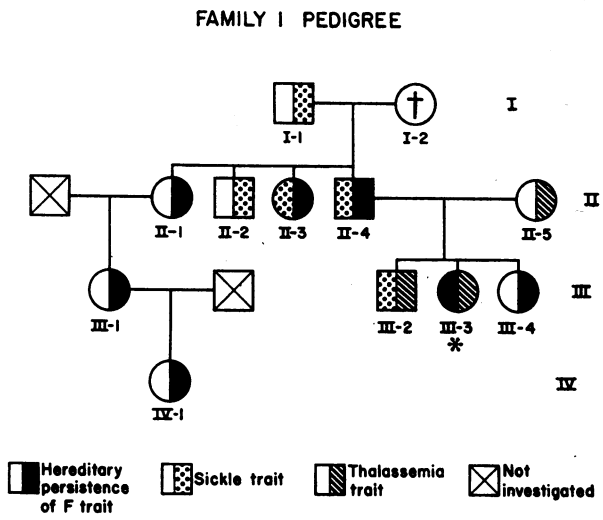

FIGURE 1 Family I pedigree. (*) indicates proband.

cm below the right costal margin, and a palpable spleen. She has a mild anemia. (Table I). Her smear shows a moderate number of target cells, moderate hypochromia, anisocytosis, poikilocytosis, and slight polychromasia. Hb F: 78%; Hb A: 19.5%, Hb A_2: 2.5%. By the acid elution technique, there is Hb F in every cell with a relatively homogeneous distribution. She is doubly heterozygous for high A_2-β-thalassemia trait and HPFH. The genetic findings in other family members, described below, are all consistent with this diagnosis.

II-4 (her father), age 52, is also asymptomatic. He has cholelithiasis. Physical examination reveals a 3-cm, firm, nontender liver and a 3-cm palpable spleen. His blood values include a low mean corpuscular volume (MCV) and a normal hemoglobin. The peripheral blood smear has moderate anisocytosis, slight polychromasia, and target cells. The cellulose acetate electrophoresis reveals an S-F pattern. Hb F is 24% by alkali denaturation, Hb A_2: 1.1%, and Hb S: 75%. There is no Hb A. The acid elution test reveals a relatively homogeneous distribution of Hb F in red cells, with over 70% of the red cells showing a high amount of Hb F, while the remainder of the cells show less Hb F.

II-3 (a paternal aunt), age 47, is clinically asymptomatic. Hb S is 76%, Hb F: 22.2% and Hb A_2: 1.9%.

II-3 and II-4 were diagnosed as double heterozygotes for sickle cell trait and HPFH because of the following: (*a*) absence of Hb A; (*b*) Hb F of 24% and 22%, respectively; (*c*) relatively homogeneous distribution of Hb F in the cells of II-4; (*d*) daughter of II-4 (III-4) with presence of HPFH gene and absence of sickle gene; and (*e*) sibling II-1 with HPFH. These latter findings also suggest the absence of atypical homozygous sickle cell disease in II-3 and II-4.

The 50-yr-old mother of the proband (II-5) has an unremarkable history and a normal physical examination. She is not anemic. Her peripheral smear shows slight anisocytosis and moderate numbers of target cells. Hemoglobin electrophoresis on cellulose acetate shows an A-A pattern. Hb F: 5.8%, and Hb A_2: 4.8%. Her acid elution test shows a heterogeneous pattern with 20–30% of the cells with large amounts of Hb F and the others with less or no Hb F. She has high A_2-β-thalassemia trait.

The brother of the proband (III-2), age 20, is clinically normal. His smear shows moderate anisocytosis, moderate hypochromasia, and poikilocytosis. Few target cells are present. Cellulose acetate electrophoresis reveals mainly Hb S. Starch gel electrophoresis shows 23% Hb A, 67.9% Hb S, 4.5% Hb A_2, and 4.7% HbF. By acid elution, there is a heterogeneous distribution of Hb F with 20% of the cells containing mainly Hb F and the remainder containing less to none. Patient III-2 is heterozygous for both sickle cell trait and β-thalassemia. In contrast to II-3 and II-4, Hb A is present, Hb A_2 is elevated, and the distribution of Hb F in red cells is heterogeneous.

The 22-yr-old sister (III-4) of the proband is also healthy. She is not anemic and shows 9.4% Hb F by agar gel electrophoresis and alkali denaturation. A paternal aunt (II-1), one paternal uncle (II-2), the paternal grandfather (I-1), paternal first cousin (III-1), and second cousin (IV-1) are all asymptomatic. The Hb F level is elevated to 16.0, 16.6, and 9.3% in II-1, III-1, and IV-1, respectively. The Hb A_2 is normal. Acid elution shows a relatively homogeneous distribution of Hb F in the red cells of II-1 and III-1. These findings are consistent with a diagnosis of HPFH in these subjects. I-1 and II-2 have sickle cell trait. The serum iron determinations are all in the normal range. Blood group determinations are compatible with the diagnoses.

TABLE I

Hematologic and Electrophoretic Data on Family I

Genotype	Family members	Hb	Hct	MCV	Hb S	Hb A	Hb F	Hb A_2	MCHC	Hb F distribution
A-S	I-1	ND	ND	ND	45	55	ND	ND	ND	ND
A-F	II-1	11.8	38	74	—	81.9	16.0	2.1	31	Homogeneous
A-S	II-2	ND	ND	ND	45	55	—	—	ND	ND
S-F	II-3	ND	ND	ND	76.0	—	22.2	1.9	ND	ND
S-F	II-4	12.5	38	79	74.7	—	24.2	1.1	33	Homogeneous
Thal trait	II-5	11.7	39	85	—	89	5.8	4.5	30	Heterogenous
A-F	III-1	12.3	36.4	75	—	81	16.6	2.3	34.7	Homogeneous
S-Thal	III-2	15.0	48.0	—	67.9	23	4.7	4.5	31.2	Heterogenous
Thal-F	III-3	11.5	35.2	66	—	19.5	78	2.5	33.4	Homogeneous
A-F	III-4	12.2	36.2	71	—	88.8	9.4	1.8	34	Homogeneous
A-F	IV-1	12.3	39	73	—	88.5	9.3	2.2	31	Homogeneous

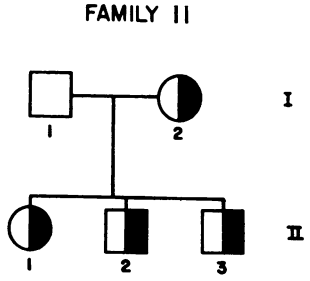

FIGURE 2 Family II pedigree. Solid black area indicates the presence of HPFH trait.

Family II. In the pedigree (Fig. 2), the proband, I-2 is a 24-yr-old woman found on population screening to have an A-F pattern on Hb electrophoresis. She is asymptomatic. Physical examination reveals cutaneous lesions of the upper eyelids and posterior neck, diagnosed as sarcoid. She has a palpable spleen 3 cm below the left costal margin. Her MCV is 71 (Table II). Starch gel electrophoresis shows Hb A of 72%, Hb F of 26.8%, and a slowly migrating mutant Hb A_2 (1.2%) with no detectable normal Hb A_2.

Her husband, I-1, is asymptomatic and clinically normal. Electrophoresis reveals A-A. Their three children, II-1, age 5, II-2, age 8, and II-3, age 9, are all asymptomatic and have normal physical examinations. Starch gel electrophoresis of II-1 reveals Hb A of 63.9%, Hb A_2 of 1.6%, and Hb F of 34.5%. II-2 and II-3 show slight microcytosis on smear, but are not anemic. Starch gel electrophoresis reveals Hb A of 80%, Hb A_2 of 1.5%, and Hb F of 18.5%, and Hb A of 79%, Hb A_2 of 2.0%, and Hb F of 19%, respectively. The acid elution is homogeneous, with Hb F in all cells. None of the children have the abnormal Hb A_2.

The study of the two generations of family II demonstrates that I-1, II-1, II-2, and II-3 are HPFH heterozygotes because of the asymptomatic clinical state, the electrophoretic findings, low Hb A_2, and homogeneous acid elution smears in addition to the elevated Hb F levels.

Globin chain synthesis. Red cells were washed with isotonic saline three times, and the buffy coat removed. Packed red cells were incubated with an equal volume of modified Krebs-Ringer bicarbonate (KRB) at pH 7.4 and 0.1 vol of [^{3}H]leucine (40 Ci/mmol) from New England Nuclear (Boston, Mass.) as reported previously (6). KRB (pH 7.4) is composed of 30 mM KCl, 7 mM KH_2PO_4, 7 mM $MgSO_4$, 160 mM $NaHCO_3$, 360 mM $FeCl_2$ $4H_2O$, 100 mM inosine, 340 mM glucose, 30 mM NaCl, and amino acids (520 mM) minus leucine. In the study of certain family members, bone marrow aspirated from the posterior iliac crest was similarly studied. After incubation, the KRB was removed, and the cells lysed with four vol of water. From the lysate, globin was precipitated by extraction with acid acetone and further purified by carboxymethyl cellulose chromatography using a modification of the method originally described by Clegg, Naughton, and Weatherall (7) and detailed elsewhere (8). The [^{14}C]carrier globin used was derived from either cord blood, normal, or sickle hemolysates. The $\alpha/\beta+\gamma$ ratios of the [^{14}C]hemolysates in 35 columns averaged 1.2 with an SD of 0.1. The ^{3}H $\alpha/\beta+\gamma$ ratios are calculated by correcting the ^{3}H ratios for the relative recovery of ^{14}C $\alpha/\beta+\gamma$ radioactivity. Samples were counted using a Packard Tricarb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The efficiency of counting with double label settings was 10% for ^{3}H and 30% for ^{14}C.

TABLE II

Hematologic and Electrophoretic Data on Family II

Genotype	Family members	Hb	Hct	MCV	Hb A	Hb F	Hb A_2	Hb F distribution
A-A	I-1	ND	—	—	—	—	—	ND
A-F	I-2	12.3	41	71	72	26.8	1.2*	ND
A-F	II-1	ND	ND	ND	63.9	34.5	1.6	ND
A-F	II-2	13.3	38.3	76	80	18.5	1.5	Homogeneous
A-F	II-3	14.0	38.7	77	79	19	2.0	Homogeneous

* All Hb A_2 is a mutant Hb A_2 migrating more slowly than normal A_2.

RESULTS

Family I. Reticulocytes from peripheral blood of the proband (III-3) produce 2.2-fold as many α as γ chains (α/γ ratio: 2.2), her α/β_A being 7.7 (Table III). As shown in Fig. 3, the reticulocytes show an excess of α chains ($\alpha/\beta+\gamma$: 1.73) of a moderate degree. This α/non-α ratio is in the range of β-thalassemia heterozygotes (6); the major non-α globin contribution is by γ chains (Table III).

II-3 and II-4, both S–F, show an α/β^S in peripheral blood of 1.0 and 1.4, respectively, and relatively balanced α to non-α chain synthesis (0.9 and 1.2, respectively). These data indicate less asymmetry in α and non-α synthesis than that reported for patients with sickle-β^0-thalassemia (6, 9). In addition, II-4 has balanced α/β^S synthesis in bone marrow. In contrast, III-2 with S-thal shows a greater excess of α over β^S globin synthesis with an α/β^S of 2.0 and an α/β of 4.6. However, he com-

TABLE III

Globin Chain Synthesis in Family I

Genotype	Family members	Cells	α/β^A	(α/β^S)	α/γ	$\alpha/\beta+\gamma$
A-S*	I-1	—	—	—	—	—
A-F	II-1	PB‡	1.4	—	5.0	1.1
A-S*	II-2	—	—	—	—	—
S-F	II-3	PB	—	(1.0)	7.3	0.9
S-F	II-4	PB	—	(1.4)	8.9	1.2
		BM	—	(1.1)	7.2	1.0
Thal trait	II-5	PB	1.4	—	5.3	1.1
A-F	III-1	PB	1.5	—	2.4	0.9
		BM	1.2	—	3.6	0.9
S-thal	III-2	PB	4.6	(2.0)	8.4	1.2
Thal-F	III-3	PB	7.7	—	2.2	1.7
A-F	III-4	PB	1.4	—	2.5	0.9
A-F	IV-1	PB	1.3	—	3.9	1.0

* No studies done on sickle trait patients.
‡ BM, bone marrow; PB, peripheral blood.

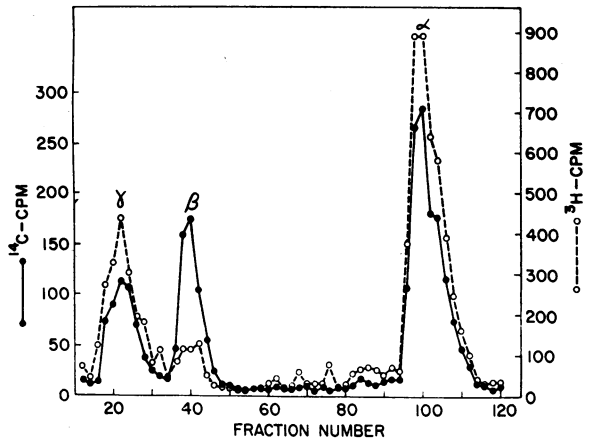

FIGURE 3 CMC column chromatography of globin prepared from reticulocytes of proband III-3. Cells were labeled with [^{3}H]amino acids by the procedures indicated in the text. (●——●) [^{14}C]leucine in globin from added labeled cord blood control. (○- - -○) [^{3}H]leucine radioactivity in globin from blood of patient III-3 of family I.

pletely compensates for total α and non-α production ($\alpha/\beta^A + \beta^S + \gamma$: 1.2). Simliar compensation has been reported in other mild β^+ S-thal patients (9). II-1 and III-1 with A-F show an α/β^A ratio in peripheral blood of 1.4 and 1.5, respectively, but an $\alpha/\beta + \gamma$ ratio of closer to unity (1.1 and 0.9, respectively). The chromatogram of patient II-1 (Fig. 4) illustrates the separation of γ, β, and α chains. In A-F patients, increased γ chain synthesis in addition to a relative increase in β synthesis leads to balanced α and non-α chain production. The bone marrow cells of III-1 show

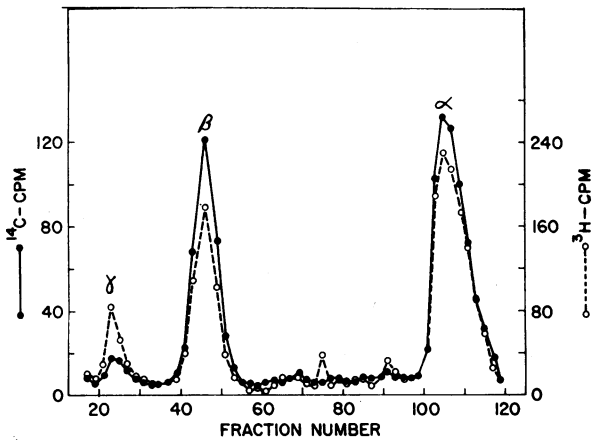

FIGURE 4 CMC column chromatography of globin prepared from reticulocytes of patient II-1. Cells were labeled with [^{3}H]amino acids by the procedures indicated in the text. (●——●) [^{14}C]leucine in globin from a mixture of added labeled cord blood and normal blood control. (○- - -○) [^{3}H]leucine radioactivity from blood of patient II-1 of family 2.

an α/β^A of 1.2 and $\alpha/\beta + \gamma$ of 0.9. II-5 with β-thalassemia trait has an α/β^A of 1.4 and achieves balanced total globin synthesis with an $\alpha/\beta + \gamma$ of 1.1. Similar results have been reported in some black β-thalassemia heterozygotes. III-4, another A-F heterozygote, has an α/β^A of 1.4 and completely compensates with $\alpha/\beta + \gamma$ of 0.9.

Family II. Three members of this family studied, II-1, II-2, and II-3, show balanced globin chain synthesis (Table IV). The $\alpha/\beta + \gamma$ ratios are 1.2, 1.1, and 1.1, respectively. I-2 has an $\alpha/\beta + \gamma$ ratio of 0.7 which is lower than normal and may represent the additional presence in this patient of an α-thalassemia gene.

DISCUSSION

The HPFH gene like the $\delta\beta$-thalassemia gene is associated with complete suppression of the synthesis of Hb A in most cases. This is the first study of globin chain synthesis in HPFH patients to date. The results show that there is balanced globin chain synthesis in peripheral blood of heterozygotes in the two families studied here. In addition, globin synthesis is close to balanced in the two patients with S-F in family I, who are also asymptomatic clinically and have close to normal Hb levels. In these patients, balanced globin synthesis is achieved by increased activity of the γ genes and the remaining β allele. The α/β ratios of 1.3–1.5 indicate compensation by the remaining β allele as well as by increased γ production.

The clinical and hematological data are most consistent with the interpretation that the high F values in these two families are due to the presence of the HPFH gene. The homogenous staining for Hb F using acid elution makes it unlikely that this high F gene is due to $\delta\beta$-thalassemia. HPFH is a benign state in which Hb F persists into adult life. Heterozygotes have been described from Uganda (10), Ghana (11), Jamaica (12), Puerto Rico (13), and the U. S. A. (14, 1), but not previously from Haiti. Homozygotes are rare and are usually asymptomatic (15). As can be seen from these family studies, individuals with HPFH trait or doubly heterozygous for Hb S and HPFH are asymptomatic. In the doubly heterozygous S-F patients studied, the percent of Hb F is higher than in most patients with

TABLE IV

Globin Chain Synthesis in Family II

Genotype	Family members	α/β	α/γ	$\alpha/\beta + \gamma$
A-A	I-1	ND	ND	ND
A-F	I-2	1.2	1.6	0.7
A-F	II-1	1.9	3.2	1.2
A-F	II-2	1.9	2.4	1.1
A-F	II-3	1.6	3.8	1.1

homozygous sickle cell disease; they have no crises and are generally asymptomatic. This has been attributed to the protective effect of the high Hb F in all cells as compared to the presence of high Hb F in only a small fraction of the cells in patients with sickle cell disease.

The S-F patients are also less symptomatic clinically than patients with S-thal who have no Hb A production. Globin chain synthesis in one black S-thal patient with no Hb A and in two patients of Italian origin with S-thal and no Hb A is unbalanced with relative α chain excess in peripheral blood (9, 16). This is in contrast to the balanced α and non-α synthesis in S-F patients described here. The high A_2-β-thalassemia gene in certain black patients produces a milder clinical disorder than in patients with β-thalassemia in other racial groups (17). As seen in the case of II-5 of the first family with the diagnosis of mild β-thalassemia trait, the α/β of 1.4 is well in agreement with such patients described by Braverman, McCurdy, Manos, and Sherman (18) and Schwartz (19). Balanced globin chain synthesis in some black β-thalassemic heterozygotes is described by Friedman, Hamilton, and Schwartz (16). The β-thalassemic gene in family I is a β^+-producing gene. In the S-thal patient, III-2, the mild clinical state is correlated with relatively balanced globin chain synthesis ($\alpha/\beta + \gamma$: 1.2). The mechanism for compensation in this S-thal patient differs from that of the S-F patients. In the S-F patients compensation is due to increased activity of both the β^S and γ genes, while in the S-thal patient β^A chain synthesis contributes significantly and γ synthesis is not as significant.

It is possible that the increased activity of the γ chain genes in every cell in HPFH is primarily responsible for balanced globin synthesis in patients with this disorder. If this is the only mechanism involved, why is the γ chain compensation so incomplete in β-thalassemia, especially $\delta\beta$-thalassemia? In the first family, the levels of Hb F vary from 9 to 23%, similar to that described by Schneider, Levin, and Everett (20). In the second family, the Hb F values are higher (20–30%) as described by Conley, Weatherall, Richardson, Shepard, and Charache (1). The two types of Hb F in black patients have been shown by Sukumaran et al. (21) to contain different ratios of glycine and alanine at position 136 of the γ chain. The presence of four γ-producing genes, two synthesizing 136 alanine-containing γ chains (γ^A) and two 136 glycine-containing γ chains (γ^G) has been postulated (22). There is evidence that each of these genes leads to a unique relative amount of γ chain synthesis; in addition, deletion of different of these γ chain genes may account for variations in the total amount of Hb F produced in different HPFH patients.

A recent report indicates that Hemoglobin Kenya, a γ-β fusion product is associated with a HPFH-like syndome with elevation of γ^G-chains primarily (23). These findings suggest that the absence of normal δ and β *cis* genes may permit the increased or continued expression of γ genes. In β-thalassemia, the persistence of activity of either structural or regulatory δ and β *cis* loci may prevent this continued or compensatory activity of γ genes.

In assessing the mild clinical state in these two families, one must also consider the possibility of the presence of an α-thalassemia gene that might account for balanced globin synthesis and the mild clinical state. There is no evidence for this in either family except for patient I-1 of family II. It is clear, however, that the mild clinical syndromes in the two families are associated with a lack of excess α chains. Excess α chains have been shown to contribute to the anemia in β-thalassemia by precipitating in erythrocytes as inclusion bodies, and decreasing the life span (24, 25). As a consequence of the increased γ globin synthesis and increased activity of the remaining β globin allele in the HPFH heterozygotes described in this report, no excess α chains are produced. This may explain, in part, the absence of anemia in these patients. In addition, in a series of patients with S-thalassemia, there is a correlation between the absence of anemia and relatively balanced globin synthesis in reticulocytes (9).

Alternatively, it is possible that the HPFH gene involves interaction of nonglobin genes with the globin-producing genes that affect the intracellular production of Hb in as yet unknown ways.

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