

What influences Hb Fetal production in adulthood?

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*Human hemoglobin genes are located in α and β globin gene clusters in chromosomes 16 and 11, respectively. Different types of hemoglobin are synthesized according to the stage of development with fetal hemoglobin ($\alpha_2\gamma_2$) (Hb F) being the main hemoglobin in the fetal period. After birth, there is a reduction (to about 1%) in Hb F levels and adult hemoglobin, Hb A ($\alpha_2\beta_2$), increases to more than 96% of total hemoglobin. However, some genetic conditions whether linked to the β -globin gene cluster or not are associated with high Hb F levels in adults. Among those linked to β -globin are hereditary persistence of fetal hemoglobin, delta-beta thalassemia ($\delta\beta$ -Thalassemia) and the *XmnI* polymorphism (-158 C > T). Other polymorphisms not related to β -globin gene cluster are known to influence the γ -globin gene expression in adulthood. The most relevant polymorphisms that increase concentrations of Hb F are the HMIP locus on chromosome 6, the *BCL11A* locus on chromosome 2, the *Xp22.2* region of the X chromosome and the δq region on chromosome 8. Findings from our research group studying genetic factors involved in γ -globin gene regulation in adults without anemia in the northwestern region of São Paulo State showed that high Hb F levels are influenced by the presence of hereditary persistence of fetal hemoglobin mutations and the *XmnI* polymorphism, suggesting that both genetic alterations characterize the molecular basis of the evaluated population.*

Keywords: Fetal hemoglobin; Polymorphism, genetic; gamma-Globins

Organization of globin genes

Human globin genes are found in two gene clusters designated α (alpha) and β (beta) globin. The α -globin gene cluster is located on chromosome 16 with the resulting polypeptide chain totaling 141 amino acids. It consists of three inactive genes ($\psi\zeta$, $\psi\alpha$ and $\psi\alpha_2$), three functional genes (ζ , $\alpha 1$ and $\alpha 2$) and two genes with unidentified functions *in vivo* (μ and $\theta 1$).⁽¹⁾ The β -globin gene cluster is located on chromosome 11 with the synthesized products having 146 amino acids. This cluster consists of a pseudogene ($\psi\beta$) and five functional genes expressed at different development stages in the same order as they are arranged in the DNA segment (5' - ϵ γ^G γ^A δ β - 3').^(2,3)

During the ontogeny of globin chains, red blood cells express different genes belonging to the α and β -globin cluster; changes in their expression occur in two distinct stages of development: 1) from the embryonic to fetal period in early pregnancy which involves changes in the expressions of alpha-type globin (from ζ to α) and beta-type globin (from ϵ to γ) and 2) approximately six months after birth with just one change in the beta-type globin (from β to γ).⁽⁴⁾

Fetal Hemoglobin and its benefits for individuals with abnormal hemoglobins

Fetal Hemoglobin (Hb F), formed by two α and two γ -globin chains ($\alpha_2\gamma_2$), is produced at high levels in the fetal period due to a high expression of γ -globin genes.⁽⁵⁾

The γ -globin gene originates from a 5 kilobase (kb) tandem repeat. These genes differ from one another by only one amino acid [glycine (γ^G) or alanine (γ^A)] at position 136 of the polypeptide chain. At birth, γ^G chains are more abundant while γ^A chains predominate in adulthood.⁽⁶⁾

In adults, the β -globin gene is predominant; approximately 98% of all hemoglobin is comprised of adult hemoglobin, Hb A ($\alpha_2\beta_2$). Thus, γ -globin genes are poorly expressed; less than 1% of adult hemoglobin is made up of Hb F.⁽⁷⁾ Hb F levels can be evaluated by counting the number of F cells, that is, adult erythrocytes that contain measurable amounts of this hemoglobin. The Hb F and F cell levels vary considerably in healthy adults but usually there is a good correlation between the two.^(8,9)

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When γ -globin genes are highly expressed, higher Hb F levels in red blood cells may compensate defective β -globin products and significantly reduce the symptoms of hemoglobinopathies such as sickle cell anemia.⁽⁷⁾ The Hb F concentration and its distribution in red blood cells are major genetic modulators of disease and high levels of this hemoglobin dilute the amount of Hb S thereby inhibiting or delaying the polymerization process, the result of which is fewer harmful events.⁽¹⁰⁾ In beta thalassemia, an induced increase in gamma chain production has a beneficial effect on the clinical status of homozygotes, not only by reducing the imbalance in the α -type/non- α -type chains, but also by increasing the synthesis of total hemoglobin.⁽¹¹⁾ Thus, an increased γ -globin gene expression has clinical relevance in the treatment of diseases related to the β -globin gene.⁽⁷⁾ Some genetic conditions are known to influence γ -globin gene expression during adulthood, including hereditary persistence of fetal hemoglobin (HPFH) and delta-beta thalassemia ($\delta\beta$ -thalassemia).^(12,13)

Hereditary persistence of fetal hemoglobin

HPFH is usually a benign condition characterized by a lack of changes in the synthesis of β -globin resulting in a substantial increase in gamma chains and consequently high Hb F levels.⁽¹⁴⁾ This condition can occur due to single point mutations in the γ -globin gene promoter or β -globin gene cluster deletions, which remove the δ and β -globin genes.⁽¹⁵⁾ According to the literature, Hb F levels in deletional HPFH are, in most cases, higher compared to single point mutations. HPFH mutations are associated to different ethnic groups. Individuals that present HPFH mutations have normal erythrocytes.⁽¹⁶⁾ Studies by our research group on mutations related to increases in Hb F levels in adults without anemia in the northwestern region of São Paulo State show that 26.7% of cases were heterozygous for deletional HPFH (HPFH-1 - Black; and HPFH-2 - Ghanaian); HPFH-2 is the most common mutation. These mutations were found in individuals who had Hb F levels above 15% of the total hemoglobin (unpublished data). In relation to erythrocyte morphology, the presence of mild alterations was observed thus different to published findings.

Table 1 shows different Hb F concentrations for the point mutation and deletional mutations in HPFH and the associated ethnic groups.

Delta-Beta thalassemia [$(\delta\beta)^0$ -thalassemia]

Delta-beta thalassemia is characterized by a persistent expression of γ -globin genes during adulthood in association with a reduced or even absent expression of δ and β -globin genes resulting in increased Hb F levels and the presence of hypochromic and microcytic red blood cells.⁽¹²⁾ This genetic condition occurs due to deletions involving δ and β -globin genes [$\gamma^G\gamma^A(\delta\beta)^0$ -thalassemia] and also the γ^A gene

Table 1 - Mutations associated with high Hb F levels, ethnic groups and Hb F levels

Mutation type	Mutation	% of Hb F ⁽⁶⁾
Single point mutation	- 110 (A → C) γ^G (Czech)	0.6% - 0.8%
	- 114 (C → G) γ^G (Australian)	8.6%
	- 114 (C → T) γ^G (Japanese)	11.0% - 14.0%
	- 175 (T → C) γ^G	22.1%
	- 202 (C → G) γ^G	18.0% - 23.5%
	- 114 (C → T) γ^A (Georgia)	4.7% - 4.8%
	- 117 (G → A) γ^A (Black-Greek)	10.9% - 15.9%
	- 117 (G → A) γ^A (Greek-Italian)	7.1% - 19.0%
	- 158 (C → T) γ^A (Greek)	2.9% - 5.1%
	- 175 (T → C) γ^A (Black)	36.7% - 38.5%
	- 195 (C → G) γ^A (Brazilian)	4.5% - 7.0%
	- 196 (C → T) γ^A (Italian)	12.0% - 16.0%
	- 198 (T → C) γ^A (British)	1.8% - 13.0%
- 202 (C → T) γ^A	1.6% - 3.9%	
Deletional	HPFH-1 (Black)	21.4% - 28.2%
	HPFH-2 (Ghanaian)	21.6% - 27.2%
	HPFH-3 (Indian)	21.6% - 23.6%
	HPFH-4 (Italian)	21.0% - 30.0%
	HPFH-5 (Italian)	16.0% - 20.0%
	HPFH-6 (Thai)	17.2% - 20.0%

γ^G : gamma glycine gene; γ^A : gamma alanine gene; HPFH: Hereditary Persistence of Fetal Hemoglobin

Table 2 - $\delta\beta$ -thalassemia mutations associated with high Hb F levels

Mutation type	Ethnicity	% of Hb F ⁽¹⁶⁾
$\gamma^G(\gamma^A\delta\beta)^0$	Belgian	14.0% - 23.0%
	Malay-1	25%
	Black	17.3% - 21.6%**
	Chinese	9.3% - 15.7%
	German	9.9% - 12.5%
	Turkish	10.0% - 13.0%
	Macedonian	6.7% - 12.8%
$\gamma^G(\gamma^A\delta\beta)^0$	Thai	9.9%
	East European	13.0% - 18.0%
	Sicilian	4.0% - 18.6%
	Black	24.8%**
	Spanish	5.0% - 13.0%
	Japanese	7.8%
	Turkish	6.0% - 15.0%

** Mutations associated with Hb S

[$\gamma^G(\gamma^A\delta\beta)^0$ -thalassemia].^(1,16-19) Heterozygous individuals for $\gamma^G\gamma^A(\delta\beta)^0$ -thalassemia mutations present with variations in Hb F levels of between 4.0% and 18.6%, while for $\gamma^G(\gamma^A\delta\beta)^0$ -thalassemia mutations the Hb F concentrations range from 9.3% to 25% (Table 2).

Findings of our research group regarding the frequency of Sicilian $\delta\beta$ -thalassemia and Spanish $\delta\beta$ -thalassemia mutations in adults without anemia in the northwestern region of São Paulo State only showed the presence of the Spanish

mutation in three individuals with Hb F levels of 9.6%, 9.8% and 10.7% (unpublished data); these values are within the range expected for this mutation.⁽¹⁶⁾

Genetic polymorphisms involved in Hb F regulation

Family studies show that high Hb F levels tend to be inherited but the pattern of inheritance is often unclear and this trait does not seem to be correlated with the β -globin gene cluster.^(20,21) The persistence of Hb F and F cells in populations suggests that variability is not from the inheritance of just one gene locus, but from a combination of several genes on different chromosomes. Thus, in genetic terms, high Hb F levels in adulthood is regarded as a quantitative trait, in which the action of multiple genes combined with an environmental component may explain the absence of a clear pattern of Mendelian inheritance.⁽²²⁾

In some situations, it is not clear if genetic variability in the β -globin gene cluster plays a decisive role in Hb F variations. This variation is attributed to co-inherited polymorphisms in regulatory regions that affect the β -globin gene cluster or regions close to it. In this scenario, studies have been performed to identify candidate genes and also to analyze possible associations that might explain high Hb F levels in adulthood. It is believed that high Hb F levels are inherited as quantitative traits that are dependent on many gene loci.⁽²³⁾

Genome-wide association studies (GWAS) and family studies have shown that regions outside the β -globin gene cluster, including 2q16, 6q23, 8q and Xp22.2, are implicated in the regulation of Hb F levels.⁽²⁴⁾ Approximately 45% of variations in Hb F levels are associated with the presence of three main quantitative trait loci including the *XmnI* polymorphism on chromosome 11 (11p15), the *HMIP* locus on chromosome 6 (6q23) and SNPs (single nucleotide polymorphisms) for the *BCL11A* gene on chromosome 2 (2q16).^(9,25)

XmnI polymorphism (-158 C > T) – Chromosome 11

The *XmnI* polymorphism is known to influence the γ^G gene expression, predisposing carriers to increased Hb F concentrations in particular when they are under conditions of erythropoietic stress.⁽²⁶⁾ In patients with sickle cell disease and beta thalassemia, the presence of this polymorphism is associated with higher Hb F levels.⁽²⁷⁾ With this polymorphic site, there is an increase in the proportion of γ^G chains resulting in a $\gamma^G:\gamma^A$ ratio similar to that seen at birth (70:30).^(28,29)

This polymorphism is common in many populations with frequencies as high as 32% to 35% being reported.⁽²⁷⁾ Studies by Peri et al.⁽³⁰⁾ and Garner et al.⁽²²⁾ found similar frequencies for different populations of healthy Europeans. Our research group, on evaluating this polymorphic site in adults without anemia from the northwestern region of São Paulo, observed a frequency of 33.3%, with Hb F levels ranging from 2.0% to 33.3%. The *XmnI* polymorphic site was more frequent (60%) in individuals with Hb F below 15% of total hemoglobin (unpublished data).

For populations with changes in hemoglobin levels, the frequency of the *XmnI* polymorphism differs from that reported for the healthy population. Table 3 shows the literature reports regarding Hb F levels, the phenotype expressed and the presence of this polymorphism in different population groups.

8q region – Chromosome 8

Studies in India identified a quantitative trait locus (QTL) on chromosome 8, specifically in the 8q region linked to high Hb F levels and F cells. In these individuals, the presence of this locus is associated with the *XmnI* polymorphic site. Researchers suggest that 8q QTL may be responsible for encoding a regulatory factor that acts as a transcriptional activator or repressor which binds directly to the *XmnI* site or to regions close to it.⁽³⁶⁾

Table 3 - Frequency of the *XmnI* polymorphism and Hb F levels in healthy populations and individuals with hemoglobinopathies

Frequency of <i>XmnI</i> polymorphism	Population group	Hb F levels	Phenotype expressed	Reference
14%	Hong Kong	0.22 ± 0.03 g/dL	Heterozygote beta thalassemia	31
14%	Caucasian	0.34% - 1.07%	Healthy	28
39% - 41%	Iran	NA	Intermedia beta thalassemia	32
70.5%	Iran	NA	Major beta thalassemia	33
25%	India	NA	Intermedia and major beta thalassemia	34
3.3%	Saudi Arabia (Southwest)	9.3% ± 5.8%	Sickle cell disease	35
93.2%	Saudi Arabia (East)	11.3% ± 6.2%		
33.3%	Brazil	15.48% ± 11.69%	Without complains of anemia	(unpublished data)

NA = not available

6q23 region (*HMIP* locus) – Chromosome 6

Recent studies show that another QTL, *HMIP* at 6q23 in the *HBSIL*-*MYB* intergenic region, influences Hb F levels. The *HBSIL* gene is involved in the regulation of several cellular processes and is expressed in hematopoietic cells, whereas the *MYB* gene is involved in oncogenesis and plays a key role in the erythropoiesis process.^(25,37) It is believed that a gene in the 6q region is responsible for encoding a factor involved in the erythroid maturation pathway resulting in increased Hb F levels and F cell production.⁽³⁸⁾ The results obtained by Garner et al.⁽³⁹⁾ show the influence of 6q23 QTL on the modulation of Hb F levels and F cell production in Indian patients with beta thalassemia. However, it is not clear if the effect of this QTL is direct, that is, acting as a factor that interacts with γ -globin genes, or indirect.

In Caucasian populations, this region is presented in three blocks of linkage disequilibrium, where the second block is more strongly associated with the Hb F variation. The variability in block 2 of the *HMIP* region appears to play a pleiotropic effect on some hematological parameters in relation to the number of cells and erythrocyte content of healthy European individuals. This characteristic suggests that the *HBSIL*-*MYB* intergenic region contains distal regulatory sequences that act on precursor erythroid lineages.⁽³⁸⁾

Studies on Indian families identified that the 6q23 locus had a greater association with high Hb F levels in patients with beta thalassemia. These studies also demonstrated that for healthy subjects and individuals heterozygous for beta thalassemia, the homozygous presence of this site is linked to higher Hb F concentrations (from 1.1% to 3% for healthy individuals and 10% to 24% for patients with thalassemia).⁽³⁸⁾

In healthy individuals from Northern Europe, the presence of the 6q23 locus contributes to approximately 19% of the variation in Hb F levels.⁽²⁵⁾ In other populations this locus is present in healthy Afro-Americans and in patients with sickle cell disease from Britain and Brazil, contributing to about 3% to 7% of the variation in Hb F of these populations.⁽⁴⁰⁾

2q16 region (*BCL11A*) – Chromosome 2

Another QTL associated with high Hb F levels located in the 2q16 region of chromosome 2 is known as *BCL11A*. Studies by Menzel et al.⁽⁹⁾ found that, besides the *XmnI* polymorphism and *HMIP* locus, a third region, 2q16, was associated with Hb F concentrations accounting for approximately 15% of the variation. Published findings report that, for certain populations, the *BCL11A* QTL has the strongest association with Hb F variations.^(9,41) In patients with beta thalassemia, Uda et al.⁽⁴¹⁾ found that the *BCL11A* QTL was more frequent in intermedia beta thalassemia, which has milder clinical symptoms, compared to major beta thalassemia. Lettre et al.⁽⁴⁰⁾ reported similar results for patients

with sickle cell anemia in Afro-Americans and Brazilians, who had Hb F variations strongly associated to the presence of the *BCL11A* QTL.

From these findings, some researchers suggest that the *BCL11A* gene product, a zinc-finger transcription factor, is the key mediator in the process of silencing the γ -globin gene expression and in switching to the β -globin gene.⁽⁴²⁾ Studies using K562 cells showed that the *BCL11A* QTL binds to a central region of the γ -globin gene promoter to form a repressor thus acting as a silencing agent of the expression of this gene.⁽⁴³⁾ Studies by Xu et al.⁽⁴⁴⁾ showed that the silencing of γ -globin genes is the result of an interaction that occurs between the *BCL11A* transcription factor and the β -globin gene cluster interacting with chromatin to form a chromosomal loop in combination with the *SOX6* transcription factor in γ -globin gene promoter regions. These findings show that the *BCL11A* transcription factor may be a regulator in the development and genetic control of γ -globin gene silencing in adults.

Xp22.2 region – Chromosome X

Dover et al.,⁽⁴⁵⁾ on analyzing healthy subjects and sickle cell disease patients of African origin, showed that the percentage of F cells present in women was significantly higher than in men. It was thus suggested that regions located in the X chromosome could be responsible for this difference. These authors found that Xp22.2 was associated with F cell production and suggested that 70% of the variation in Hb F levels in sickle cell anemia patients is associated with variations in the percentages of F cells, which are partially controlled by the Xp22.2 locus on chromosome X.

Conclusions

Much evidence shows that co-inheritance of genetic determinants related to Hb F levels may play an important role in modulating the phenotype of patients with sickle cell anemia and beta thalassemia.⁽³⁶⁾ Thus, the identification of genes and genetic variants that contribute to the variability of Hb F production and the molecular mechanisms involved in this variation may help to discover new therapeutic strategies and develop pharmacological agents that increase the levels of this hemoglobin.⁽²⁵⁾ Our findings show that, for the population without anemia in northwestern region of São Paulo State, high Hb F levels are influenced by the presence of deletional HPFH mutations from African descendants and the *XmnI* polymorphism, showing that these two genetic alterations characterize the molecular base of this population.

In general, publications show the involvement of different loci in the regulation of Hb F, for example, quantitative trait loci on chromosomes 2 (2p16), 6 (6q23), 8 (8q) and X (Xp22.2).^(8,9,18,36,38,45) Besides these regions, recent studies have revealed the influence of some microRNAs in

the expression of the γ -globin gene, showing that high Hb F levels in adulthood may result from a range of genetic factors which may explain the observed variations in Hb F of healthy subjects and patients with hemoglobinopathies.^(46,47)

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