

Special Articles

HbF and F Cell Production in Human Adults

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After the switch from ϵ to γ -globin gene expression at about 6 weeks of gestation, HbF($\alpha_2\gamma_2$) becomes the major haemoglobin during the foetal stage of development, constituting 90-95% of the total haemoglobin. However, after the second switch from γ to β -globin chain production that occurs at about 34-36 weeks of gestation, the production of adult haemoglobin (HbA, $\alpha_2\beta_2$) rises dramatically while that of HbF decreases. At birth, HbF constitutes approximately $59 \pm 10\%$ of the total haemoglobin and continues to decline to 20% by 3 months, 7.5% at 6 months and less than 2% by the age of 1 year¹. These small amounts of HbF are not evenly distributed among the erythrocytes, but confined to a sub-population of red blood cells termed "F Cells or FCs"² Surveys have shown that HbF/FC values in normal individuals vary considerably (by > 20-fold) with the distribution strikingly positively skewed.³⁵

F cells are not similar to foetal red blood

cells, neither in the amount of HbF per cell nor in other characteristics which distinguish foetal and adult red cells; e.g. I antigen ("I" on FCs, but "I" on foetal red cell membrane), carbonic anhydrase enzyme (high in FCs, but undetectable in foetal red cells) and MCV (~80 fl for FCs, but >100 fl for foetal red cells)⁸. It was postulated from both clinical observations and the in vitro studies of erythroid progenitors that FCs and non-FCs are derived from the same stem cell population, and that the FCs represent the progeny of erythroid progenitors that had undergone an "accelerated" pathway of differentiation.^{6,7} Under normal circumstances, very few cells follow this "accelerated" pathway, resulting in the observed levels of FCs of < 4.5% which corresponds to < 0.6% HbF.^{4,5} However, it has clearly been shown that the HbF and F cell levels do correlate well.

Increased HbF and FC levels in adult lifes

Both HbF and FC levels may be increased in adults in various acquired and genetic disorders

1. Acquired increases of HbF and FC levels

In most acquired disorders, increased HbF production is due to the perturbation of eryth-

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ropoiesis; an increased demand for red cells leads to 'stress' erythropoiesis with preferential production of HbF. Increased HbF and FC production is thus often observed in situation of acute erythroid expansion as in severe iron deficiency following the treatment, in normal individuals following acute blood loss, in bone marrow regeneration after bone marrow transplants, in cytotoxic treatment and in pregnancy, particularly in early second trimester when blood volume increases sharply.⁸ High HbF levels can also be seen in patients with haemopoietic malignancies such as juvenile chronic myeloid leukemia (JCML), preleukemic syndrome and myelodysplastic syndrome (MDS). Some types of solid tumours are associated with high HbF levels, including choriocarcinoma, testicular tumour, bronchogenic carcinoma and hepatoma. High HbF levels can also be observed in patients with thyrotoxicosis.

2. Increased HbF levels in genetic disorders

Increased HbF peroduction may occur as a primary genetic disorder or secondary to other inherited disorders.

2.1 Genetic disorders associated with secondary increase of HbF level

A) Sick cell anaemia

Individualls heterozygous for the β^S -gene have normal HbF levels, but in sickle cell homozygotes, HbF levels may range from normal value of < 1% to the levels higher than 30%. Factors that account for this striking variation include the preferential survival of F cells and the variability in the intrinsic capacity for HbF

synthesis. HbF can effectively reduce the intracellular concentration of HbS so that the formation of HbS polymer becomes less likely.

B) β -thalassaemia

Slightly increased HbF levels are often observed in β -thalassemia heterozygotes as a result of the selective survival of F cells. In homozygotes, HbF level ranges from 10% in those with β^+ -thalassemia alleles to 100% in those with β^0 -alleles. This is due to the increased HbF production in response to an extreme erythropoietic stress and the selective survival of HbF -containing red cells.

2.2 Inherited disorders primarily associated with increased HbF levels

The inherited disorders in which the underlying mutation is a direct cause of increased HbF production has been traditionally classified into 2 groups; hereditary persistence of foetal haemoglobin (HPFH) and $\delta\beta$ -thalassaemia. Molecular analysis has demonstrated that HPFH and $\delta\beta$ -thalassaemia originate from both deletion and non-deletion of parts of the β -globin gene complex. The deletion of the β -globin cluster removing δ and β -globin genes results in deletional type of HPFH, while the extensive deletion removing δ and β -globin genes or the $A\gamma$, δ and β -globin genes causes the $\delta\beta$ - and $\gamma\delta\beta$ -thalassemia.

While only one evidence of non-deletional $\delta\beta$ -thalassaemia has been reported,⁹ sequencing of the γ -globin genes has revealed various single base substitutions in the promoter of the upregulated γ -globin gene in non-deletional

HPFH, including mutations at position -202, -175, -161, -158 and -114 of the γ -globin gene and -202, -198, -196, -195, -175, -117 and -114 of the δ -globin gene. These mutations are clustered in the γ -promoter regions that are potential binding sites for ubiquitous and erythroid-specific transcription factors. It seems likely that they alter the pattern of protein binding, resulting in increased affinity for positive regulatory factors or decreased affinity for inhibitory regulatory factors or a combination of both mechanisms.

In contrast to the $\delta\beta$ -thalassemia in which the HbF is unevenly distributed among the erythrocytes, the pattern of HbF distribution in the HPFH of the previously mentioned types (deletional and non-deletional) is evenly distributed, generating the so-called "pancellular" type of HPFH⁸. However, there is another type of HPFH which is more commonly seen in the normal individual namely the "heterocellular" HPFH. In the following section, the heterocellular HPFH will be discussed in detail.

Heterocellular HPFH

Heterocellular HPFH is another form of non-deletion HPFH which is characterised by modest increases in the levels of HbF (1-5%) in otherwise normal individuals. Because HbF is unevenly distributed among red cells, this form of HPFH is often referred to as heterocellular HPFH. It is also called Swiss-type HPFH, after the original report in which some members of a group of Swiss soldiers were found to have slight increases in HbF levels.¹⁰ Surveys of HbF and FC

levels among general population suggested that heterocellular HPFH is fairly common and is present in 10% to 15% of normal adults, representing the upper tail of the skewed HbF and FC distribution.

Inheritance of heterocellular HPFH

Molecular studies of heterocellular HPFH has demonstrated that in several families, the β -globin cluster is intact and no point mutations in the γ -globin gene promoter have been identified. Family studies showed that the high HbF and FC levels tend to be inherited and was likely to be polygenic and heterogeneous. In some families, the genetic determinant of heterocellular HPFH behaves as an allele of the β -globin complex^{11,12} whereas in others it segregates independently, implicating the involvement of *trans*-acting factors.¹³⁻¹⁵

In the recent study among the twins of the European background, HbF and FC production was shown to be overwhelmingly controlled by the genetic factors with the heritability of 89%¹⁶. The β -globin cluster is responsible for 37% of this heritability. The remaining genetic contribution is likely due to *trans*-acting genetic factors.

Evidence of *Cis*-acting genetic factors for heterocellular HPFH

Several sequence variations along the β -globin cluster have been shown to be associated with high HbF levels.^{17,18} They included;

1. Length polymorphisms within the $(AT)_x N_z (AT)_y$ motif of the β LCR-HS2.

2. A set of single nucleotide polymorphisms (SNP) in the 5' flanking region of the γ -globin gene, also referred to as the pre γ -framework.

3. The C-T substitution at the position -158 relative to the γ cap site, commonly referred to as the XmnI- γ site.

4. The ACGA-deletion at position -222 to -225 relative to the γ cap site, referred to as the γ -4bp site.

5. A length polymorphism of the (TG)_n motif within IVS2 of γ -globin gene.

6. The C-T polymorphism at the 3' enhancer sequence of the γ -globin gene.

Of all of these cis-regulatory sequences, the XmnI- γ site has been shown by both population and family studies to play an important role in controlling HbF and FC production, accounting for 35% of FC variance associated with the β -globin cluster.¹⁹ The rest seem to be in linkage disequilibrium with the XmnI- γ site in the control of HbF and FC production.²⁰ The XmnI- γ site was then postulated to be the quantitative trait locus (QTL) in the β -globin complex for HbF and FC production.

Evidence of trans-acting factors for heterocellular HPFH

High HbF production was also shown to be linked to chromosome X⁴ in which the F cell production (FCP) locus was mapped to the Xp22.2-22.3 interval in the investigation among the sickle cell anaemia.²¹ This FCP locus was found to account for 40% of FC variance in the subjects they studied.^{22,23}

Besides the FCP locus on the Xp22.2-22.3,

the QTL on chromosome 6 within the 6q23 interval was also shown to be associated with high HbF and FC levels in the investigation among the Asian Indian kindred with β -thalassaemia and heterocellular HPFH.²⁴ The candidate region was initially mapped to the 11 cM interval and was further refined to 1-2 cM interval between the microsatellite markers D6S270 and D6S1626 by the haplotype mapping to look for the key recombinant event in the family. The nucleotide sequences within this interval was completely constructed using the positional cloning strategy (screening BAC, PAC, YAC libraries followed by contig map generation).²⁵ The search for putative genes localised within this interval is currently being performed. At least four genes have been found including MYB (a proto-oncogene and a regulator for haemopoiesis), ALDH8A1 (an aldehyde dehydrogenase family protein), HBS1L (a putative translational elongation factor) and PDE7B (Phosphodiesterase 7B).

Another putative gene controlling HbF and FC production was most recently mapped to chromosome 8 at the region 8q11.²⁶ The protein product of this 8q11 gene was thought to interact directly or as a member of protein complex to the β -globin cluster via the XmnI- γ site. The candidate region was ~6.2 cM between the microsatellite markers D8S1803 and D8S1833.

Summary

Heterocellular HPFH is fairly common among otherwise normal human adults. It is now clear

that genetic determinants play major role in controlling HbF and FC production. However, the genetic background seems to be heterogeneous among different ethnic origins. This means that different genes for heterocellular HPFH could be localised in the studied subjects other than those published. It might also be possible that another putative QTL for HbF and FC production might be mapped in Thai population where β -thalassaemia and HbE are common and clinical modifying factors have not fully been elucidated.

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