Elevated foetal haemoglobin: a cause for concern?

At the Sickle Cell and Thalassaemia screening support service we frequently receive queries regarding antenatal screening results involving a raised level of Hb F, the foetal version of haemoglobin. Hb F is the predominant haemoglobin during foetal development, but levels drop sharply after birth as it is replaced by Hb A, the adult form of haemoglobin. Most individuals retain some degree of foetal haemoglobin expression even in adulthood, but this typically accounts for less than 1% of their total haemoglobin production. However, some people retain higher levels of Hb F expression throughout life (this is often termed hereditary persistence of foetal haemoglobin or HPFH). This "persistence" of foetal haemoglobin can vary from 1-100% Hb F in different individuals (although the level is typically fairly stable over the lifetime of a particular individual).

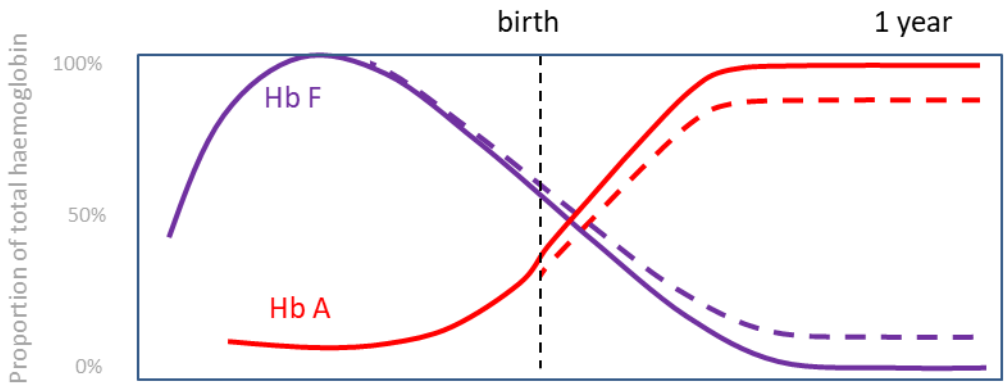


Figure 1: Haemoglobin gradually changes from the fetal form to the adult form. In most individuals (solid lines) the fetal form makes up less than 1% of the total by the time they are one year old. Individuals with HPFH (dashed lines) maintain higher levels of the fetal form of haemoglobin.

Hb F is functionally very similar to Hb A. In fact, adults that have no Hb A but that can express Hb F at sufficiently high levels are generally physically well and are not at increased risk of having children with health problems. Therefore, why would an increase in Hb F during antenatal screening be anything to worry about?

Well, often enough raised foetal haemoglobin is a benign finding. For instance, it is common to find small increases in Hb F during pregnancy. This increase tends to peak during the second trimester and is thought to result from the increased levels of red cell production required during pregnancy. Similar increases in Hb F are observed in other situations when the body needs to accelerate its production of red cells e.g. when recovering from blood loss or chemotherapy. Therefore, although the increased level of foetal haemoglobin is benign and in these individuals is actually helping them to deal with haematological stress, it can also be viewed as a disease marker because it can highlight the fact that the body may be responding to some kind of problem. In these circumstances, other abnormalities in the full blood count would be expected.

Another reason for increased foetal haemoglobin levels may be that there are mutations affecting the beta globin gene locus that alter the balance of expression of the genes within the cluster. Some of these mutations are associated with significant health issues whereas others are not. However,

even the mutations that are benign can cause confusion during neonatal screening once the baby is born.

(See information boxes for a summary of types of mutation)

So, when raised Hb F is identified during antenatal screening, what actions need to be considered?

As mentioned above, small elevations of Hb F of up to 10% are not uncommon in antenatal ladies. However, this range of Hb F overlaps with that seen in delta beta thalassaemia carriers (typically 5-15%). The elevated Hb F level is a key diagnostic indicator for these thalassaemia mutations because they are not associated with raised Hb A2 levels (see also COMMON MISCONCEPTION below). Although they are usually associated with some degree of hypochromia (paler coloured cells resulting from a reduction of the amount of haemoglobin present within the cell), this is variable, and its use as a diagnostic feature is complicated by the fact that the antenatal screening algorithm uses MCH (mean cell haemoglobin) to assess alpha thalassaemia risk, so a second diagnostic feature is required to ensure that further actions are triggered even if the lady is not from an ethnic group associated with increased risk of alpha zero thalassaemia. If the lady is suspected to be a carrier of delta beta thalassaemia then further action is required to assess the risk to her unborn baby, because if the baby also inherits a beta globin chain mutation from its father it is at risk of severe disorders such as transfusion dependent beta thalassaemia or sickle cell disease.

Two different thresholds have been established (5% Hb F with hypochromia and 10% without hypochromia) to avoid causing unnecessary alarm, because almost all antenatal ladies with an Hb F of between 5 and 10 and normal red cell indices will not have any clinically significant mutations, whereas an Hb F of 5-10% in combination with hypochromia is more suggestive of delta beta thalassaemia (although iron deficiency or alpha thalassaemia with coincidental elevation of Hb F are also possible explanations). An Hb F level of over 10% is more unusual so this warrants further investigation even in the absence of coexisting hypochromia.

If the partner is found to have abnormal screening results or is not available for testing, molecular analysis of the mother can be undertaken to establish whether she carries delta beta thalassaemia or whether her phenotype actually results from a combination of an HPFH mutation and alpha thalassaemia or iron deficiency, which would not confer significant risk to the baby unless she is a carrier of alpha zero thalassaemia.

If the lady is demonstrated to be a carrier of delta beta thalassaemia and her partner is either a carrier of a significant beta globin mutation or is unavailable, then she can be offered prenatal diagnosis to determine whether her baby is likely to suffer from a severe condition. If instead she is found to have a benign deletional HPFH mutation, this does not confer any significant health risk to her baby. However it is important that this information is noted and passed on to the team coordinating neonatal screening, because if the baby inherits a beta thalassaemia mutation or a beta variant such as Hb S from one parent and a deletional HPFH mutation from the second parent the newborn screening results may incorrectly suggest a diagnosis of beta thalassaemia major or sickle cell disease which could cause unnecessary alarm and confusion.

Other points to consider when noting raised Hb F is that Hb F may not actually be F but could be a rare variant (e.g. Hb South Florida) running in the Hb F position. In an antenatal screening situation you would still need to action partner testing either way so it is not usually necessary to trigger confirmatory testing in this scenario, although if this can be undertaken without significant delay it may help to provide a clearer lab report.

Also, if the lady's full blood count is abnormal, don't forget to consider whether the lady is suffering from a clinical condition which may require more care during her pregnancy.

COMMON MISCONCEPTION: Many people call us asking whether they have found a delta beta thalassaemia carrier when they see an HPLC trace with high Hb F and high Hb A2 in combination with hypochromia. In fact, delta beta carriers do not have a raised A2 value, because the delta globin gene is deleted which reduces the amount of the Hb A2 synthesised. Although delta beta thalassaemia mutations act clinically speaking as beta thalassaemia mutations, the phenotype of a carrier looks more like alpha thalassaemia with coexisting HPFH (although if this pattern is seen for example in a lady of African ethnic origin this scenario is considerably more likely than delta beta). An HLPC trace with both raised Hb F and raised Hb A2 is more likely to be explained by a beta thalassaemia carrier with high Hb F either as a result of the type of beta thalassaemia mutation (e.g. a mutation in the promoter region of the beta globin gene) or coexisting HPFH. The complexity of different possible scenarios makes it impossible to definitively diagnose delta beta thalassaemia on phenotype alone; if there are significant clinical or reproductive implications then genetic testing must be used to provide a definitive diagnosis.

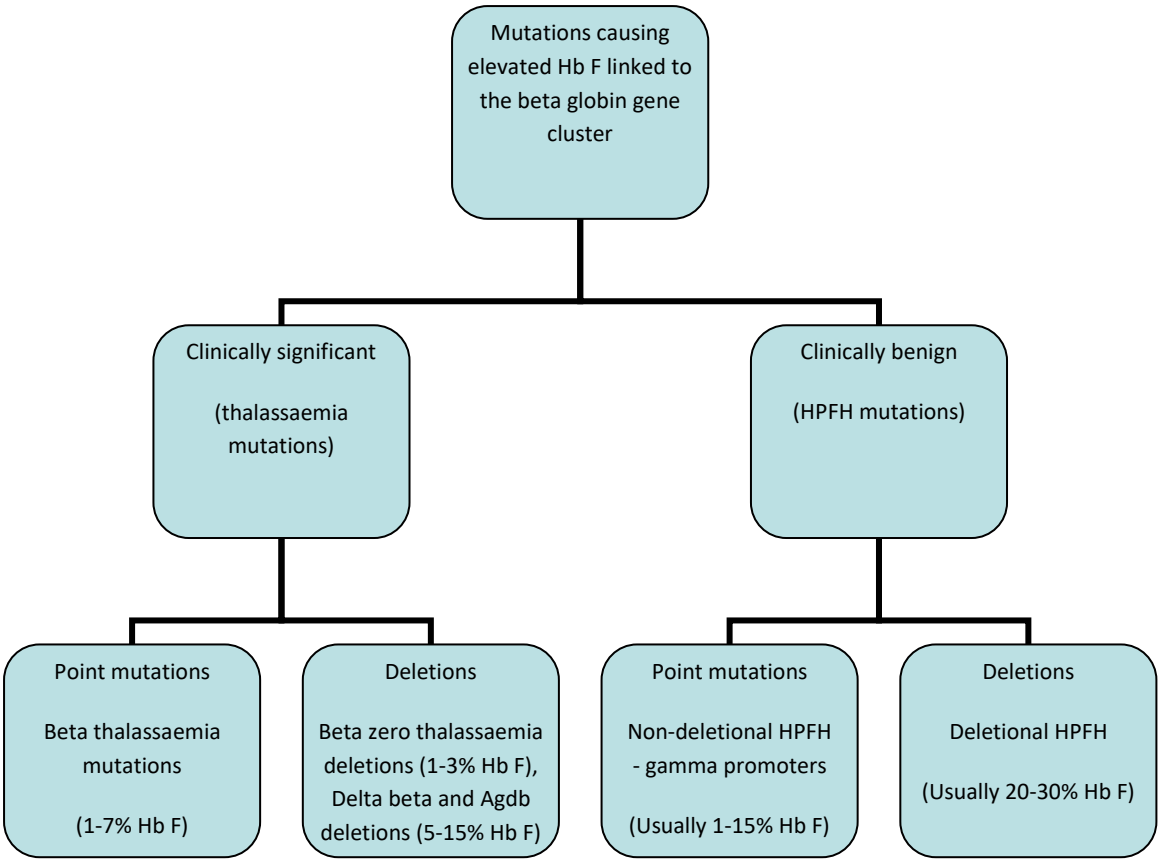


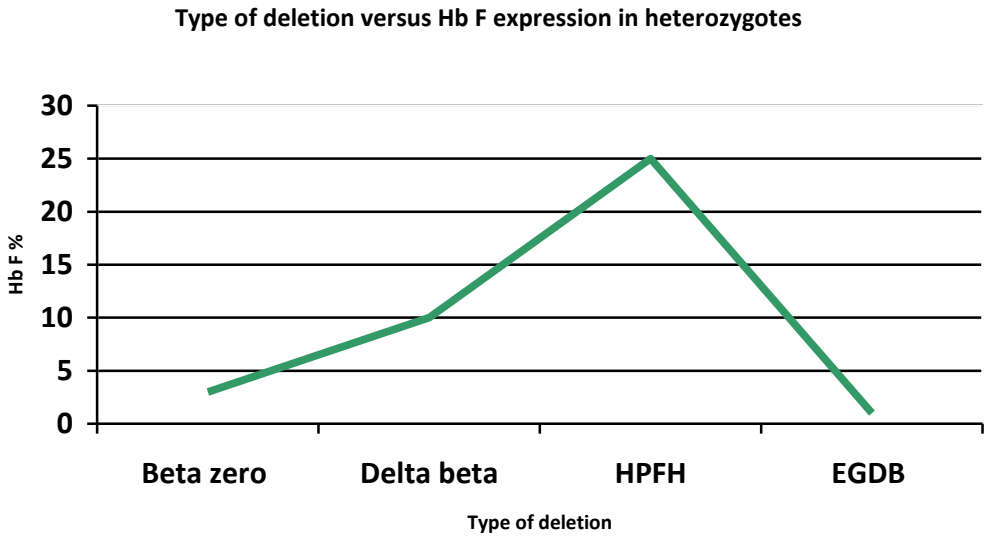
Figure 1: Types of mutations associated with raised Hb F - from a clinical perspective

Mutations of the beta globin cluster of genes that result in elevated Hb F fall into two categories; those that act as thalassaemia mutations and therefore typically result in the classical features of low MCV and MCH common to both alpha and beta thalassaemia, and those that only result in high Hb F but are not associated with any clinically severe conditions (classed as HPFH mutations).

- There are two main types of benign HPFH mutations linked to the beta globin locus:
    - HPFH deletions that remove the beta globin gene but enable expression of the gamma genes to be up-regulated in its place. Deletional HPFH mutations are typically associated with high Hb F % ranges of 20-30% in carriers (homozygotes usually have 100% Hb F due to deletion of both beta and delta genes). These deletions are most commonly observed in individuals of African ethnic origin.
    - Non-deletional HPFH mutations that often take the form of small mutations in the promoter region of the gamma globin genes that increase the expression of the fetal haemoglobin subunit (other HPFH mutations are not linked to the beta globin locus and involve mutations in other genes whose products then bind to gamma globin gene regulatory elements). Non-deletional HPFH mutations tend to have more moderate effects and are associated with variable degrees of elevated Hb F of up to around 15%, although some point mutations that can increase Hb F to over 20% have been described. These types of mutations have been described in many different populations.
- There are several different types of clinically significant thalassaemia mutations associated with increased Hb F.
    - Any beta thalassaemia mutation can cause small increases in HbF in carriers of a few %, although many carriers will not demonstrate this effect. Some beta thalassaemia mutations affecting the promoter of the beta globin gene are more consistently associated with raised Hb F although the levels are still typically under 8% in heterozygotes. This applies both to small mutations affecting the beta globin gene, and larger deletions that remove all or part of the beta globin gene but do not substantially affect the expression of other genes.
    - Rare dominantly acting beta thalassaemia point mutations that give rise to unstable variants resulting in beta thalassaemia intermedia or haemolytic anaemia may also be associated with raised Hb F, sometimes of up to 20%.
    - Hb F levels of 5-15% are often found in carriers of delta beta deletions. These deletions remove the beta and the delta gene (and sometimes one but not both gamma genes) causing partial up regulation of Hb F although this is insufficient to fully compensate for the loss of the beta gene in this case. The most common delta beta deletions are found in Mediterranean/Middle Eastern populations but they have also been described in other populations such as South East Asians.
    - Some deletions resulting in the formation of hybrid genes (e.g. Hb Lepore which is formed from the start of the delta globin gene fused to the end of the beta globin gene) are associated with elevated Hb F. The hybrid globin chain may also be detected via HPLC or other protein resolution methods.
    - See also "Clinical spectrum of beta deletions" below.

The clinical spectrum of beta globin gene deletions

Large deletions affecting the beta globin gene are on a spectrum sliding from classical beta zero thalassaemia mutation to pure HPFH with no thalassaemic effects, depending on the degree of F up regulation which in turn depends on the precise regulatory elements affected by the deletion. The precise location of these elements is not all known so when a new deletion is discovered it is not always simple to predict whether it will have any pathogenic effect. The main clue may come from the phenotype of the individual, but red cell indices can obviously be influenced by other factors (in particular it would be important to exclude alpha thalassaemia mutations and iron deficiency). Additionally, although some of these deletions seem to act fairly consistently, others are associated with very variable degrees of microcytosis and hypochromia in carriers (and variable clinical symptoms in patients who also have another significant beta globin mutation) for example the South East Asian HPFH deletion. Some deletions were originally designated as HPFH deletions because they were associated with raised F in carriers, but when they were later identified in combination with other beta mutations it became clear that they could result in a clinically significant phenotype, sometimes even transfusion dependency (e.g. HPFH3). Often the delta globin gene is involved in the deletion, which means that the A2 level in carriers is also not a reliable indicator of whether the deletion acts as a beta thalassaemia mutation. Some deletions at the beta locus extend to the foetal and/or embryonic globin chain genes. Those that only include one but not both gamma genes tend to act in a similar way to delta beta deletions and are often still associated with increased Hb F levels. Those that encompass both gamma globin genes obviously cannot upregulate Hb F on this allele. These deletions often also extend to the embryonic gene and are known as egdb deletions. They can cause haemolytic anaemia in heterozygous fetuses and babies, and are thought to be embryonically lethal in homozygotes due to complete absence of beta type globin chains.


Type of deletion versus Hb F expression in heterozygotes
30
25
20
15
10
5
0
Hb F %
Beta zero
Delta beta
HPFH
EGDB
Type of deletion