

ABNORMAL LABORATORY RESULTS

Screening for thalassaemia

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SYNOPSIS

The thalassaemias are the commonest single gene disorders in the world's population and are a common cause of hereditary anaemia. They should be suspected in any individual who has reduced red blood cell indices. A full blood examination and haemoglobin electrophoresis are the tests which should be used first to investigate a suspected carrier of a thalassaemia gene. Iron deficiency can confuse the interpretation of test results, so iron studies are also often required. DNA analysis may be needed to detect the carrier state, particularly in carriers of α -thalassaemia.

Index words: haemoglobinopathies, iron deficiency.

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Introduction

Functioning haemoglobin (Hb) molecules are tetramers made up of two pairs of globin chains, which bind oxygen at the iron porphyrin site attached to each chain. The different types of Hb are characterised by their globin chains, which in adults may be α , β , δ or γ . Normal adult Hb is made up of approximately 97.0% HbA ($\alpha_2\beta_2$), 2.5% of the minor adult Hb, HbA₂ ($\alpha_2\delta_2$) and less than 0.8% HbF ($\alpha_2\gamma_2$).

The thalassaemia syndromes are a heterogeneous collection of genetic disorders characterised by a reduced rate of production of one or more of the globin chains of haemoglobin. The α -globin genes are located in the α -cluster on chromosome 16 and are paired ($\alpha\alpha/\alpha\alpha$) whereas the single β -globin gene is found in the β cluster on chromosome 11. The thalassaemia syndromes are usually caused by point mutations or deletions in, or close to, these globin genes which reduce or abolish expression of the affected gene. The type of thalassaemia is named according to which gene is affected. Hence reduced production of α -chains is called α -thalassaemia and reduced production of β -chains is called β -thalassaemia. The resulting imbalanced globin chain production gives rise to the phenotype of thalassaemia, while the severity depends on which genes are affected and which mutation or combination of mutations is inherited.

A large number of thalassaemia mutations are now known and can all be characterised by DNA analysis. In a carrier these mutations may be silent or may result in the typical haematological phenotype characterised by red blood cell hypochromia and/or microcytosis. The inheritance of a β -thalassaemia mutation from each parent usually causes the severe disease called β -thalassaemia major.

The genetics of α -thalassaemia are more complex as one, two, three or all four genes may be affected. For example HbH disease is usually caused by the deletion of three α -globin genes ($--/\alpha$) as a result of the inheritance of a single gene deletion mutation ($-\alpha/$) from one parent and a two-gene deletion mutation ($--/$) from the other parent.

Other mutations cause the important, clinically significant structural Hb variants, such as Hb S, C, D, E, O and Lepore. Certain combinations of these mutations may cause severe disease as outlined in Table 1.

Testing in Australia

Australia's population is ethnically diverse and there have always been a significant number of carriers of β -thalassaemia mutations. If both parents are carriers there is a 1 in 4 chance in each pregnancy of them having a child with β -thalassaemia major.

Recent immigration to Australia, especially from South-East Asia, has introduced large populations of people from areas where α -thalassaemia is common. It has now become important in screening programs, particularly antenatal testing, to detect the carrier state for both α - and β -thalassaemia, in addition to the Hb variants which in the homozygous form, or in combination with β -thalassaemia, may cause severe disease.

The laboratory diagnosis of the thalassaemia carrier state is therefore of increasing importance both for antenatal diagnosis and for clinical management. Thalassaemias are common in Australia and are a significant public health problem. In Melbourne approximately 10% of women in their first pregnancy required DNA studies to adequately characterise their carrier state and to provide sufficient information to estimate the risk that their children would have severe disease (Table 1).

Table 1

Examples of the severe disease risk states identified in recent years in Melbourne's population

β -globin mutations	Homozygous β -thalassaemia
	Hb E/ β -thalassaemia
	Hb Lepore/ β -thalassaemia
	Sickle cell disease
	HbS/ β -thalassaemia
	HbS/HbC disease
α -globin mutations	HbS/HbD disease
	HbH disease (usually mild but occasionally severe)
	HbH hydrops syndrome (rare)
	Hb Bart's hydrops syndrome

Indications for testing

An accurate diagnosis may be needed to:

- explain haematological abnormality, such as reduced mean cell volume (MCV), mean cell haemoglobin (MCH), or anaemia
- confirm a diagnosis of the severe disorders such as sickle cell disease, or β -thalassaemia major
- characterise the mutation underlying a thalassaemia carrier state, particularly for α -thalassaemia where the molecular basis can only be determined and clarified by analysis of DNA
- test for silent mutations which might have clinical significance if inherited with a mutation from the other parent, for example silent α - or β -thalassaemia or coexistent α -thalassaemia in a β -thalassaemia or HbE carrier
- provide accurate genetic counselling to individuals and prospective parents
- identify serious disorders in the fetus and hence provide the additional option to couples of termination of pregnancy
- identify haemoglobins such as HbS preoperatively
- fully characterise a variant haemoglobin.

α -thalassaemia

Each individual normally inherits two pairs of functioning α -globin genes. These are designated as $\alpha\alpha/\alpha\alpha$. Mutations in this gene cluster causing α -thalassaemia most commonly delete one of the α -globin genes ($-\alpha/\alpha\alpha$). Point mutations (α^T) within one of the genes may also inactivate the gene. This usually causes a phenotype with a mild carrier state ($\alpha^T\alpha/\alpha\alpha$) equivalent to having single gene deletion mutation. Rarely a point mutation in one globin gene may reduce expression of both genes resulting in a more severe phenotype equivalent to the two gene deletion carrier. Single gene deletion mutations are found in most populations, but only occur naturally at high frequencies in areas of the world where malaria is or was endemic. The common deletional mutations can have slightly different effects on the patient's red blood cells (Table 2).

In South-East Asia up to 10% of the population are carriers of the more severe two gene deletion mutation ($--/\alpha\alpha$). These two gene deletion mutations are also found sporadically in other populations, including the Mediterranean region.

Table 2

Examples of mean values of mean cell volume (MCV) and mean cell haemoglobin (MCH) in adults according to α -thalassaemia genotype*

Genotype	MCV(fl)	MCH(pg)
$\alpha\alpha/\alpha\alpha$ (normal)	89±5	29±2
$-\alpha/\alpha\alpha$	84±6	27±2
$--/\alpha\alpha$, or $-\alpha/-\alpha$	75±4	23±2

* These figures are a guide only to illustrate typical values and are age and sex dependent. Precise values are given in the references cited.^{1,5}

The mutations of the α -globin genes may be inherited in any combination. The more severe clinical conditions arising from the inheritance of more than one α -thalassaemia mutation include HbH disease ($--/-\alpha$), Hb Bart's hydrops syndrome ($--/--$) and the rare HbH hydrops syndrome ($\alpha^T\alpha/\alpha^T\alpha$). HbH disease varies in severity, but is commonly a moderately severe chronic haemolytic anaemia associated with Hb in the 80–100 g/L range, with periodic exacerbations of anaemia because of infection or other oxidant stress.

Hb Bart's hydrops syndrome

This is a serious and significant clinical condition. It not only leads to the death of the baby, but may also adversely affect the health of the mother during pregnancy. In unsupervised pregnancies there is up to a 50% maternal mortality with a high incidence of hypertension and haemorrhage.¹ Affected babies usually die at delivery. These at-risk pregnancies should be recognised as early as possible and termination of affected pregnancies is advised on medical grounds.

β -thalassaemia

Each individual inherits, from each parent, a single β -globin gene located in the β -globin cluster on chromosome 11. The β -thalassaemia carrier state has been known for many decades. There are often typical hypochromic microcytic red blood cell changes. Hb electrophoresis reveals the diagnostic elevation of the minor adult HbA₂ ($\alpha_2\delta_2$).

Screening is not always straightforward. Some of the mutations are now known to have a less severe effect on gene expression.² Although they are capable of causing severe disease in homozygotes, the indices in carriers may be borderline or normal and the HbA₂ may be minimally elevated or even in the normal range.

In most populations where β -thalassaemia is present there is also a significant incidence of α -thalassaemia. Individuals therefore commonly inherit both α -thalassaemia and β -thalassaemia, an interaction which is usually benign and leads to a milder phenotype. The α -thalassaemia carrier state is masked in this setting and ultimately can only be excluded by DNA analysis. In our experience the HbA₂ level usually remains elevated in those who are carriers of both α - and β -thalassaemia.

Structural haemoglobin variants and thalassaemia

Many haemoglobin variants of clinical significance are known. For example, the substitution of one particular amino acid in the β -globin chain produces the HbS associated with the sickle cell disorders. In Australia we encounter only a small number of variants capable of causing severe disease in the homozygote or in compound heterozygotes. These include Hb E, S, C, D, O and Lepore which are all readily identified by Hb electrophoresis. It is common for there to be no other haematological change and hence the variants will be overlooked unless Hb electrophoresis is carried out.³ HbE behaves as a mild β -thalassaemia mutation and is common in

South-East Asia where more than 50% of the population are carriers in some areas.

Some of these variants, in combination with the β -thalassaemia gene, may cause severe disease. HbE/ β -thalassaemia is common in South-East Asia and varies clinically from a mild condition to the more common severe disease equivalent to β -thalassaemia major. β -thalassaemia combined with HbS or HbC usually results in sickle cell disease although the phenotype may vary considerably from mild to severe disease depending on which combination of mutations is inherited.

Laboratory diagnosis

The thalassaemias and structurally abnormal haemoglobins are common in Australia so accurate laboratory diagnosis is of growing importance. This is because of the increasing expectation of prospective parents to be offered antenatal diagnosis if there is an identifiable risk of them having a child with severe disease. There is also a need to characterise carrier states to provide an explanation of abnormal haematology, or to help clarify an otherwise confusing clinical picture, such as the coexistence of α -thalassaemia and iron deficiency anaemia.

Appropriate genetic counselling requires the detection and adequate characterisation of thalassaemia carrier states and the Hb variants. Local policies and practices on screening vary considerably.

Initial testing

The Melbourne working party on thalassaemia and haemoglobinopathies currently recommends that all suspected carriers have a full blood examination and Hb electrophoresis. Reduced red blood cell indices (MCV and MCH) are typical of the majority of carriers of β -thalassaemia, $\delta\beta$ -thalassaemia* and two gene deletion α -thalassaemia. Significant reticulocytosis is likely to be found in anyone with a chronic haemolytic anaemia such as in the HbS disorders and HbH disease. If the indices are reduced iron studies should be carried out to exclude iron deficiency or to identify it as a coexisting condition.

Electrophoresis is recommended as the majority of variant haemoglobins can only be detected by Hb electrophoresis and the indices are often normal in the carrier. High-performance liquid chromatography will identify variant haemoglobins, and also quantitate the HbA₂ level. Specialised laboratories may go on to carry out other tests on Hb variants in order to characterise them more fully, before deciding whether DNA analysis is required.

β -thalassaemia

Nearly all β -thalassaemia carriers have elevated concentrations of HbA₂, and reduced indices. The accurate quantitation of HbA₂ is of particular importance and concern. The upper limit of normal for HbA₂ is 3.5% of the total Hb. Any value above this should be regarded as diagnostic of the β -thalassaemia

carrier state, irrespective of the indices on the blood test. All but a few individuals, who are further studied by DNA analysis, will have a known mutation.

There are some clinically important β -thalassaemia mutations in which the indices may be normal, and the HbA₂ may also be normal, minimally elevated or borderline. Other mutations may also confuse the diagnosis of the carrier state. The HbA₂ level may be halved by the coexistence of a δ -gene mutation. These are uncommon but well known in some populations. Iron deficiency may lower a borderline HbA₂ into the normal range.⁴

Some of the less common mutations may have an entirely normal phenotype or normal indices with a mildly elevated HbA₂ level, in the 3.5–4.0% range. This is further complicated by the possibility of an individual having inherited both α - and β -thalassaemia in the carrier states where there may be an amelioration of haematological abnormality. In these situations, the MCV and MCH are variable and may be normal, or near normal. In our experience, however, the elevated HbA₂ is usually not changed by the coexistence of α -thalassaemia.

α -thalassaemia

The identification of an α -thalassaemia carrier is more complex and relies ultimately on DNA analysis to complete the testing and identify the mutation. Two gene deletion α -thalassaemia ($--/\alpha\alpha$), and homozygous single gene deletion α -thalassaemia ($-\alpha/-\alpha$) have a similar phenotype and typically show a moderate reduction in the MCV and MCH (Table 2).¹ Abnormality is more likely in the MCH rather than the MCV. This has been known for many years, however there seems to be a reluctance for laboratories to screen using the MCH as the primary critical reference value.^{2,3} Two gene deletion α -thalassaemia is most common in South-East Asia, but also occurs sporadically in other parts of the world. It should always be considered in anyone with suggestive indices, although DNA analysis is required for characterisation.

The majority of individuals with single gene deletion α -thalassaemia have entirely normal haematology.¹ In this situation, the carrier state can only be identified by DNA analysis. Therefore haematologically normal partners of an individual with a two gene deletion α -thalassaemia require DNA analysis to determine whether or not there is a risk of them having a child with HbH disease (Table 3).

Iron deficiency

Iron deficiency is common in adult women in Australia. In 1997 up to 40% of women attending their first antenatal appointment in Melbourne were iron deficient. This is not a universal figure, and is probably disproportionately high for the general population, however, it is a serious potential complicating factor when testing for a thalassaemia carrier state. Both iron deficiency and a thalassaemia carrier state may result in a low MCV and MCH. Erythrocytosis is more likely to be caused by thalassaemia, but it is not a diagnostic finding.

In pregnant women with a low MCV and MCH Hb electrophoresis should be carried out routinely, irrespective

* In $\delta\beta$ -thalassaemia the production of δ chains and β chains is impaired.

Table 3

Tests to be performed in the partner of a carrier to determine the risk of having a child with severe disease

Disorder found in one partner	Test to be carried out in other partner to exclude severe disease risk in a child
β-thalassaemia carrier	FBE, Hb electrophoresis, to exclude β-thalassaemia and δβ-thalassaemia carrier state, and Hb S, E, O, D, C and Lepore
Carrier of Hb Lepore, δβ-thalassaemia, Hb S, C, D, E or O	as for β-thalassaemia carrier
Two gene deletion α-thalassaemia (-/αα)	FBE, Hb electrophoresis, HbH inclusion prep, DNA analysis (to exclude risk of having a child with HbH disease or Bart's hydrops syndrome)
Single gene deletion α-thalassaemia (-α/αα or -α/-α)	as for two gene deletion α-thalassaemia to identify risk of having a child with HbH disease

FBE = full blood examination

of iron status. If possible the father should be tested. If a non-iron deficient partner has evidence of a thalassaemia carrier state or other haemoglobinopathy, then the woman should have full testing, including DNA analysis to adequately define the risk of them having a child with severe disease (Table 3).

Summary

The identification of carriers of thalassaemia and other clinically significant haemoglobinopathies is a two-stage process. Initially evidence for the carrier state is sought by carrying out a full blood examination and Hb electrophoresis. Iron deficiency

can be excluded as a complicating factor by iron studies in individuals who show a haematological abnormality consistent with this diagnosis. In this relatively simple way evidence for all but single gene deletion α-thalassaemia (and most non-deletional point mutations) will usually be obtained. Further studies including DNA analysis can then be carried out for final clarification of the carrier state. In this way it is usually possible to identify all but a few mutations and to provide informative counselling for individuals and couples.

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Self-test questions

The following statements are either true or false (answers on page 131)

- Thalassaemia and iron deficiency can cause microcytosis of red blood cells.
- Babies who carry mutations for both α-thalassaemia and β-thalassaemia usually die at birth.

Gardener's corner

Australians are increasingly using complementary medicines. *Australian Prescriber* will therefore be commenting occasionally on some of these medicines. This is not an endorsement of their effectiveness, but an attempt to provide health professionals with some information about the products their patients may be taking.

Agnus castus fruit

The fruits of the chaste tree (*Vitex agnus castus*) have historically been used as a remedy for gynaecological problems. While the active ingredient is uncertain, the fruits contain flavonoids and iridoids. Although the mechanism of action is unknown the active ingredient is thought to modulate the secretion of prolactin. It may also bind to opioid receptors.

A recent placebo-controlled trial studied an extract (Ze440) of agnus castus fruit in 170 women with premenstrual syndrome.¹ After three months, women who took the extract reported a greater reduction in symptoms than the women taking a placebo did. There were significant reductions in anger, irritability, headache, and breast fullness.

The fruit is not known to have serious adverse effects and none emerged in this trial. It is not known if there are any significant drug interactions with the extract.

Although significantly more women responded to the extract only 52% had an improvement of more than 50% in their symptoms. (24% of the women taking a placebo had a greater than 50% improvement.) The trial did not investigate if these benefits were maintained after the end of the study. Other formulations cannot be assumed to have the same efficacy as the extract used in this trial.

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