Genetics and blood

Haemoglobinopathies and clotting disorders

Sylvia A Metcalfe
BSc(Hons), PhD, is Group Leader, Genetics Education and Health Research, Murdoch Childrens Research Institute, and Associate Professor in Medical Genetics, Department of Paediatrics, The University of Melbourne, Victoria. sylvia.metcalfe@mcri.edu.au

Kristine Barlow-Stewart
FHGSA(GenCounsel), PhD, is Director, The Centre for Genetics Education of NSW Health, and Associate Professor, Faculty of Medicine, University of Sydney, New South Wales.

Janine Campbell
MBBS, FRACP, FRCPA, PhD, is a haematologist, Clinical Haematology, Royal Children's Hospital, Melbourne, Victoria.

Jon Emery
MA, MBBCh, MRCGP, FRACGP, DPhil, is Professor, Discipline of General Practice, University of Western Australia.

BACKGROUND
Genetic disorders of the blood are common inherited conditions of global impact. The haemoglobinopathies and clotting disorders represent two areas of significance to Australian primary care practitioners.

OBJECTIVE
This article describes the haemoglobinopathies and thrombophilias and their relevance to primary care practitioners. In particular it describes the role of the general practitioner in identifying who is at risk of being a carrier of, or at risk of developing, these conditions.

DISCUSSION
Global migration patterns to Australia have meant that the carrier frequency of haemoglobinopathies has increased in recent years. General practitioners play a key role in carrier screening and ideally should consider screening of couples in pre-pregnancy situations wherever possible. Genetic predisposition to thrombophilias is an important factor regarding the risk of thrombophilias and should be considered as part of the indications for screening.

Genetic conditions of the blood, which include the haemoglobinopathies and clotting and bleeding disorders, represent the most common of the inherited disorders. This article is based on *Genetics in family medicine: the Australian handbook for general practitioners*.[1] This resource also includes patient information and reference to additional sources of information.

Haemoglobinopathies

The haemoglobinopathies result in anaemia of varying severity due to reduced levels of globin synthesis (thalassaemias) or alterations to the structure of haemoglobin (structural variants, eg. sickle cell anaemia).[2] Worldwide, about 300 000 infants are born each year with a haemoglobinopathy and an estimated 5% of the world's population are carriers of a gene mutation for autosomal recessive conditions.[3] Distribution of carriers and the disease state varies according to the population and country of origin. The carrier frequency in the overall Australian population is not known. However, due to global migration, population groups in which the haemoglobinopathies are common are increasingly encountered in the Australian health care system. General practitioners play a significant role in recognising and identifying patients at high risk of being a haemoglobinopathy carrier[4] (*Figure 1*, *Table 1*).

Thalassaemias

The thalassaemias are caused by decreased production of a specific globin chain:

- absent/decreased α-globin chain synthesis in α-thalassaemia, and
- absent/decreased β-globin chain synthesis in β-thalassaemia.

The relative imbalance in the globin chains found in thalassaemias (*Table 2*) leads to inclusions in the red blood cells (RBC) and haemolytic anaemia with subsequent bone marrow expansion. Splenectomy may be performed and management of severe anaemia involves life long blood transfusions, typically every 3–4 weeks, resulting in excess iron build up. This excess iron can be eliminated from the body using iron chelating agents (eg. desferrioxamine infusion subcutaneously by pump or, more recently, through oral chelators). Despite chelation therapy, complications arise from iron deposition in endocrine and other organs leading to diabetes, cardiomyopathy, liver fibrosis and cirrhosis. There is also the risk of blood borne infections from transfusions, although currently this is less relevant in Australia. While bone marrow transplantation may cure thalassaemia, there is significant risk of complications and mortality. The life expectancy of well treated, compliant patients is not known, but is likely to be normal or near normal.

α-thalassaemia

α-thalassaemia can manifest in the fetus during pregnancy

or in the child after birth, as α-globin production usually starts during fetal development. Severity of symptoms depends on the extent of the mutations involved. Unlike the genes for other globin chains, there are two copies of the α-globin genes on each specific chromosome, one or more of which may be deleted in α-thalassaemia with varying outcomes (*Table 3*).

Hydrops fetalis due to deletion of all four α-globin genes is fatal in the fetus or neonate, but is also life threatening to the mother due to the high risk of developing early pre-eclampsia, antepartum or postpartum haemorrhage, and preterm delivery. Significantly, only carriers of the αα/-- genotype are at risk of having a baby with hydrops fetalis: a one in 4 risk for every pregnancy when both parents are carriers with this genotype. This genotype is most commonly found in people of Southeast Asian origin. When the genotype is detected in both parents, risk of hydrops fetalis must be explained and counselling provided. If only one parent has the genotype and the other has α-/α or αα/α- genotype, then the most severe outcome will be having a baby with HbH disease (α-/- genotype (*Table 3*), a condition that may require blood transfusion therapy. As the specific genotypes of the parents determine the outcome for the baby, it is important that DNA testing is carried out on couples at risk of being carriers.

β-thalassaemia

The common clinical features of β-thalassaemia manifest after birth, usually within 6–12 months and include: pallor, lethargy, poor appetite, failure to thrive, irritability and difficulty settling, developmental delay and haemolytic anaemia. Splenomegaly, hepatomegaly, growth failure with

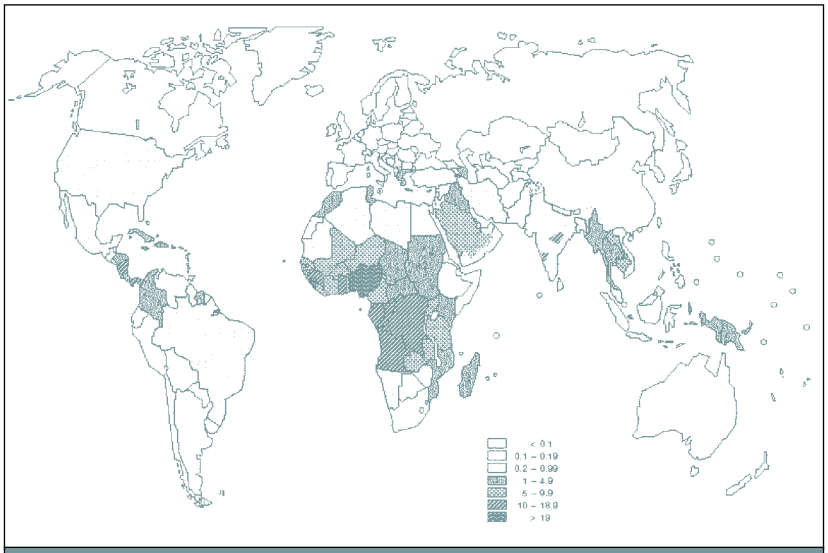

Figure 1. Global distribution of haemoglobin disorders, in terms of births or affected infants per 1000 births[12]

Table 1. Prevalence of haemoglobinopathies and populations at high risk

Haemoglobinopathy	High risk ethnic populations	Carrier frequency
β-thalassaemia	• Middle Eastern • Southern European • Indian subcontinent • Central and Southeast Asian • African	One in 5 to one in 12
α-thalassaemia	• Chinese • Southeast Asian • Southern European • Middle Eastern • Pakistani • African • Pacific Islander • Maori • Some Indigenous Australians in the Northern Territory and northern Western Australia	Approximately one in 20
Sickle cell disease	• African • African American • Middle Eastern • Southern European • Indian subcontinent • South American • Caribbean	Up to one in 4

bone changes, fractures and leg ulcers can be seen in childhood. These features vary in severity according to the nature of the mutations involved and whether both copies of β-globin genes are affected. When no β-globin chains are produced (ie. homozygous β^0, often referred to as β-thalassaemia major) severe anaemia results. When there is reduced (10–30% of normal) synthesis of β-globin chains (homozygous β+) moderate anaemia results. Having a mutation in only one copy of the β-globin gene is the carrier state (β-thalassaemia trait or β-thalassaemia minor), with some changes seen in haematology results (*Table 4*). Carriers are essentially healthy and should not be treated with iron unless they are iron deficient. However, carrier women should take 5 mg of folate per day throughout all pregnancies.

Sickle cell disease

The most common type of sickle cell disease, HbS, is due to a mutation that alters the structure of the β-globin chain. Having only one mutation is the carrier state (sickle cell trait). Being homozygous for HbS leads to a chronic haemolytic anaemia when RBC form an irreversible sickle shape after repeated cycles of deoxygenation.

Sickle cell crises involve intermittent episodes of vascular occlusion and tissue ischaemia causing pain and resulting in acute and chronic damage to virtually every organ, especially the spleen, brain, lungs and kidneys. People with sickle cell disease often autosplenectomise within the first 10 years of life. Recognition of the warning signs of a sickle cell crisis, such as fever, respiratory symptoms, pallor, lethargy, splenic enlargement, and neurologic changes, is important so that the patient

Table 2. Types of haemoglobins and globin chains present in normal adult blood and in haemoglobinopathies

Haemoglobin (Hb)	Globin chains	Percentage found in normal adult blood	Clinical state
HbA	$\alpha^2\beta^2$	~97%	Normal
HbA2	$\alpha^2\beta^2$	2–3%	Normal
HbF	$\alpha^2\beta^2$	<1%	Normal
HbH	β^4	0%	α-thalassaemia
HbBarts	γ^4	0%	α-thalassaemia
	α chain aggregates – insoluble	0%	β-thalassaemia
HbS	$\alpha^2\beta s^2$	0%	Sickle cell disease

Table 3. Outcome of mutations to α-globin genes

Number of α-globin genes affected	α-globin chains produced	Genotype*	Outcome
0	100%	αα/αα	Normal – healthy
1	75%	αα/α–	Silent carrier (one gene deletion α-thalassaemia minor) – healthy
2	50%	αα/-- or α–/α–	Carrier – α-thalassaemia trait (two gene deletion α-thalassaemia minor) Changes seen in haematology results (mild hypochromic microcytic anaemia)
3	25%	α–/--	Haemoglobin H (HbH) disease – mild to severe haemolytic anaemia May require blood transfusions
4	0%	--/--	Haemoglobin Barts (HbBarts) – hydrops fetalis (fatal before or around the time of birth)

* As there are two α-globin genes on each chromosome 16, the genotype is represented by the number present on one chromosome/the number present on the second chromosome – indicates deletion/mutation

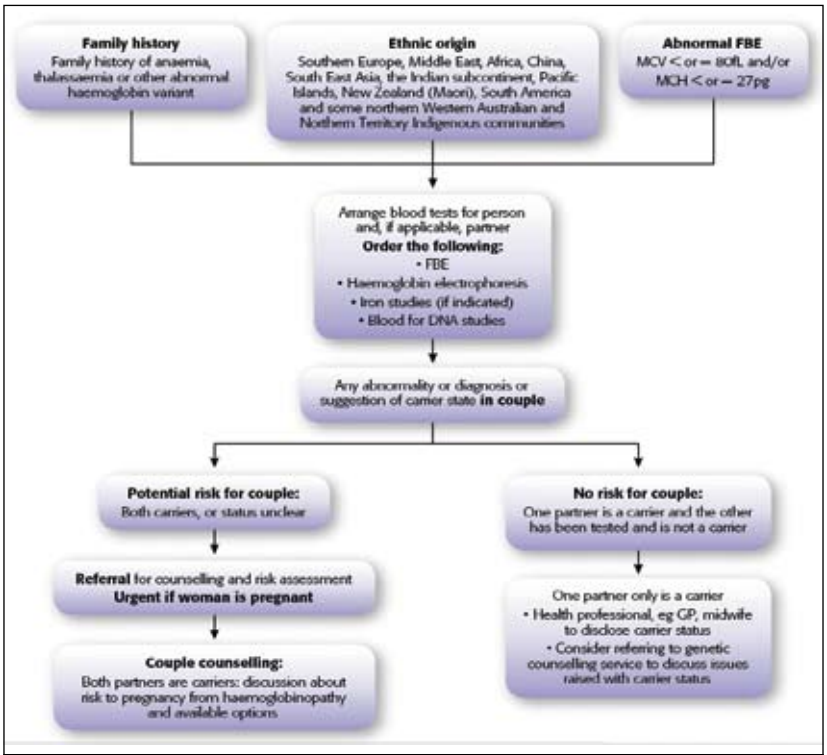

Figure 2. Suggested protocol for targeting carrier testing of high risk populations, for patients in any one of the three categories shown[1]

Table 4. Interpretation of haemoglobinopathy carrier testing results

MCH (pg)	Ferritin	Haemoglobin electrophoresis	Interpretation
≥27	Normal	Normal	Thalassaemia unlikely but one gene deletion α-thalassaemia not excluded
	Normal	HbS present	Carrier for sickle cell disease
	Low	Normal	Reduced iron stores or iron deficiency, thalassaemia unlikely but one gene deletion α-thalassaemia not excluded
<27		HbA_2 increased HbF increased	Carrier for β-thalassaemia
	Normal	HbA_2 normal HbH present	Carrier for α-thalassaemia
		HbS present	Carrier for sickle cell disease
			Possible co-existent thalassaemia carrier state
		Normal	Possible carrier for α-thalassaemia DNA testing indicated
<27	Low	Normal	Iron deficiency Thalassaemia may coexist If woman is pregnant, seek advice about DNA testing; test partner for full haemoglobinopathy screen

can be sent immediately to the emergency department of a hospital for intravenous fluids, pain relief and other treatment if indicated. Some patients will require blood transfusions and iron chelation. While carriers are usually healthy, often with no changes seen on full blood examination (FBE), HbS is present in haemoglobin electrophoresis and, in some rare cases (eg. anaesthesia or long distance air travel) the RBC can undergo partial sickling.

Other forms of sickle cell disease can be due to co-inheritance of sickle cell trait with another mutation in the β-globin gene (either for a different haemoglobin structural variant or for β-thalassaemia), known as compound heterozygosity. As there are many combinations of mutations, either leading to sickle cell disease or thalassaemia phenotype, referral to a specialist service such as a haematology clinic, thalassaemia clinic or genetics service is advised.

Carrier testing – the role of the GP

General practitioners play an important role in offering haemoglobinopathy carrier testing, comprising:

- FBE
- iron studies (ferritin), and
- haemoglobin electrophoresis[5,6]

to patients in any one of the three high risk categories shown in *Figure 2*. Note that changes to FBE are not necessarily seen in carriers of HbS (*Table 3*), and therefore at risk individuals should always have haemoglobin electrophoresis performed.

DNA testing is then indicated for:

- possible carrier for α-thalassaemia (low-borderline mean cell/corpuscular volume [MCV] or mean cell/corpuscular haemoglobin [MCH], normal ferritin and normal haemoglobin electrophoresis, *Table 3*)
- proven carrier for β-thalassaemia and partner is also a carrier for thalassaemia or other haemoglobinopathy
- confirmation of carrier status for a haemoglobin variant.

Whenever possible, carrier testing should be discussed and performed before conception, as DNA testing can be time consuming. DNA testing of couples who are both carriers is necessary for prenatal diagnosis. If the woman is pregnant when found to be a carrier, her partner should be tested urgently. Couples who are both carriers should be referred to a specialist service.

Clotting disorders

The hereditary thrombophilias represent a group of conditions with a genetic predisposition to develop thrombosis.[7] This may be due to:

Table 5. Major hereditary thrombophilia conditions

Category	Condition	Clinical state
Group 1 conditions Due to a defect or deficiency of an anticoagulant protein	Antithrombin deficiency	• Severe thrombophilia • 60% of heterozygotes develop VTE by age 60 years • Homozygosity generally incompatible with life
	Protein C deficiency	• Moderate to severe thrombophilia • Up to 50% of heterozygotes develop VTE by age 60 years • Homozygotes develop severe thrombophilia: neonatal purpura fulminans; disseminated intravascular coagulation
	Protein S deficiency	• Moderate thrombophilia • 30% of heterozygotes develop VTE by age 60 years • Homozygotes develop severe thrombophilia: neonatal purpura fulminans
Group 2 conditions Due to genetic mutations that result in an increased tendency toward thrombosis	Activated protein C (APC) resistance/factor V Leiden*	• Mild thrombophilia • Factor V Leiden: – 6% of heterozygotes develop VTE by age 65 years – homozygotes develop moderate thrombophilia
	Prothrombin gene variant	• Mild thrombophilia • <5% of heterozygotes develop VTE by age 60 years • Homozygotes develop moderate thrombophilia
	Elevated levels of factors VIII, IX and XI	• Mild thrombophilia

* APC inactivates factors Va and VIIIa to bring about anticoagulation. Approximately 90% of cases of APC resistance are due to a single mutation in the factor V gene known as factor V Leiden

- a deficiency of anticoagulant factors (group 1, *Table 5*)
- an excess of procoagulant factors (group 2), or
- abnormal fibrinolysis.[8]

The risk of thrombosis is higher for patients with group 1 conditions than group 2, however group 2 conditions occur approximately five times more frequently than group 1 conditions (*Table 6*).

Screening for thrombophilia is recommended when there is:

- deep vein thrombosis (DVT) in a person <50 years of age
- spontaneous thrombosis in the absence of recognised risk factors
- recurrent thrombosis
- a family history of thrombosis
- thrombosis in unusual sites (eg. central nervous system, abdominal veins, upper limb).[9]

DNA testing for factor V Leiden and prothrombin gene variant is available on the Medicare Benefits Schedule only if the patient has a personal history of DVT or a family history of a diagnosed inherited thrombophilic condition.

Management of hereditary thrombophilia

Primary prophylaxis for individuals with hereditary thrombophilia is not recommended, as the lifetime risk of death from bleeding on anticoagulants is greater than the risk of death from thrombosis in previously asymptomatic individuals. However, there are acquired risk factors that increase the risk of venous thromboembolis (VTE):

- obesity
- increasing age
- prolonged immobilisation
- surgery or trauma
- pregnancy and the puerperium
- smoking
- combined oral contraceptive pill or hormone therapy (see below)
- active cancer
- antiphospholipid antibodies
- acquired activated protein C resistance.

Opinion is divided over the role of thromboprophylaxis for individuals with known thrombophilia who are travelling for long periods. There is virtually no information available for road travel, with all publications relating to air travel. All authorities recommend frequent mobilisation, avoidance of alcohol and sedatives, and below the knee graduated compression stockings.[10] Thromboprophylaxis may be required during or after pregnancy in asymptomatic

Table 6. Prevalence of hereditary thrombophilias in the general population vs. prevalence in individuals with VTE

Thrombophilia	General population (heterozygous frequency)	Individuals with a VTE	Individuals with a VTE and risk factor(s)*
Antithrombin deficiency	1 in 500	1 in 25	1 in 20
Protein C deficiency	1 in 500	1 in 50	1 in 25
Protein S deficiency	3–13 in 1000	1 in 50	1 in 25
Factor V Leiden	1 in 20**	1–2 in 10	3–5 in 10
Prothrombin gene variant	2–3 in 100**	5–10 in 100	1.5 in 10

* Age <50 years, family history of VTE, recurrent VTE and absence of acquired risk factors except pregnancy or COCP use

** In caucasian population (rare in people of Asian or African descent)

women with hereditary thrombophilias and decisions regarding management in pregnancy should be made in consultation with a specialist haematologist, obstetric physician or obstetrician.

Contraception and thrombophilia

Oestrogen containing oral contraceptives or hormone therapy increase the risk of VTE by 2–4 fold and are relatively contraindicated in women with hereditary thrombophilia. Third generation combined oral contraceptive pills (COCP) are more thrombogenic than second generation preparations. Screening all women for hereditary thrombophilias before commencing oestrogen has not been shown to be cost effective.[11] Before prescribing oestrogens, a careful history with respect to past medical, family and additional risk factors for thrombosis should be taken. Decisions regarding oestrogen use should be made on an individual basis, after a risk benefit analysis has been performed. Factors that should be considered when determining the use of the COCP include:

- past or family history of VTE
- other risk factors for VTE
- suitability of alternative means of contraception
- patient preference and compliance.

Advice should be sought from a specialist haematologist and/or family planning expert if required.

Summary of important points

- Wherever possible carrier testing for haemoglobinopathies should be carried out, preferably before planning a family, for those with a reduced MCH/MCV, family history, from specific ethnic backgrounds, or partner of known or identified haemoglobinopathy carrier.
- Investigations should include FBE, iron studies (ferritin), haemoglobin electrophoresis, and where indicated, DNA testing.
- Specific indications for screening for risk of developing thrombophilias include DVT in a person <50 years of age, spontaneous thrombosis in the absence of recognised risk factors, recurrent thrombosis, family history, and thrombosis in unusual sites.

Conflict of interest: none declared.

Acknowledgment

Thanks to Yasmin Bylstra, Laura Forrest, Luke Morphett, Gabrielle Reid and Dominic Ross.

References

1. Barlow-Stewart K, Emery J, Metcalfe S, et al. Genetics in family medicine: the Australian handbook for general practitioners. Canberra: Biotechnology Australia, 2007.
2. Old J. Haemoglobinopathies and thalassaemias. In: Rimoin DL, Connor JM, Pyeritz RE, Korf BR, editors. Emery and Rimoin's principles and practice of medical genetics. Churchill Livingstone 2002;1861–98.
3. World Health Organisation. Thalassaemia and other haemoglobinopathies. Report by the Secretariat. Available at www.who.int/gb/ebwha/pdf_files/EB118/B118_5-en.pdf.
4. Berdoukas V. Haemoglobinopathies: how much should the GP know? Modern Medicine of Australia 1997;March:126–34.
5. Bowden D. Screening for thalassaemia. Australian Prescription 2001;24:120–3.
6. Trent R, Webster B, Bowden D, et al. Complex phenotypes in the haemoglobinopathies: recommendations on screening and DNA testing. Pathology 2006;38:507–19.
7. Seligsohn U, Lubetsky A. genetic susceptibility to venous thrombosis. N Engl J Med 2001;244:1222–31.
8. Crowther MA, Kelton JG. Congenital thrombophilic states associated with venous thrombosis: a qualitative overview and proposed classification syndrome. Ann Int Med 2003;138:128–34.
9. Baglin T. Thrombophilia testing: what do we think the tests mean and what do we do with the results? J Clin Path 2000;53:167–70.
10. The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;(Suppl):September.
11. Sass AE, Neufeld EJ. Risk factors for thromboembolism in teens: when should I test? Curr Opin Pediat 2002;14:370–8.
12. World Health Organisation. Available at www.who.int/genomics/public/Maphaemoglobin.pdf [Accessed May 2007].

afp CORRESPONDENCE email: afp@racgp.org.au