

HAEMOGLOBIN DISORDERS (HAEMOGLOBINOPATHIES)

BETA (β) THALASSAEMIA ALPHA (α) THALASSAEMIA SICKLE CELL DISORDERS

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FOREWORD BY THE PRESIDENT

This book contains basic information about β -thalassaemia, α -thalassaemia and sickle cell disease. Whether you are a carrier or a patient, or simply interested in finding out more, we encourage you to read this book. Every effort has been made by the authors to include all important information regarding these diseases, their inheritance, prevention and treatment.

If you need to know more details on any aspect described in this book, we advise you to consult your physician, genetic counsellor or national health authority. The authors of this book will also be very happy to answer your questions inasmuch as possible.

TIF is greatly indebted to the authors, Dr. Androulla Eleftheriou and Dr. Michael Angastiniotis, both of whom are members of TIF's Scientific Advisory Panel, for their invaluable contribution to the preparation of this educational book. Sections One (about β -thalassaemia), Two (about α -thalassaemia), and Three (about sickle cell disorders - SCD) of this book, aim to provide basic information about haemoglobin disorders in a simple manner to the community at large.

I hope that this book, which constitutes part of our educational material, will contribute significantly to TIF's efforts in spreading awareness across the world about haemoglobin disorders.

PANOS ENGLEZOS

PRESIDENT, THALASSAEMIA INTERNATIONAL FEDERATION

ABOUT THALASSAEMIA INTERNATIONAL FEDERATION

The **Thalassaemia International Federation** (TIF) was established in 1987 with the mission to support the establishment and promotion of national control programmes for the effective prevention and appropriate clinical management of haemoglobin disorders including thalassaemia and sickle cell disease in every affected country of the world. TIF is today an “umbrella” Federation, comprising to-date 117 national thalassaemia associations from 56 countries worldwide, safeguarding the rights of hundreds of thousands of patients worldwide to quality healthcare.

TIF works in official relations with the World Health Organization (WHO) since 1996. It collaborates closely with scientific and medical professionals from more than **70 countries**, of the world with the most relevant international and European health bodies, pharmaceutical companies and agencies, and, very importantly, with other disease-orientated patients’ organizations: a network of collaboration that contributes immensely to the achievement of TIF’s mission and vision.

TIF’s educational programme is one of its **most important and successful activities**. It includes the organization of local, national, regional and international workshops, conferences and seminars, as well as the preparation, publication and translation of leaflets, magazines and books for health professionals, patients/parents and the community at large, distributed to more than **60 countries in the world**.

OUR MOTTO

“UNITY IS OUR STRENGTH”
“KNOWLEDGE IS OUR POWER”

OUR MISSION

“EQUAL ACCESS TO QUALITY
HEALTH CARE FOR EVERY PATIENT
WITH THALASSAEMIA ACROSS
THE WORLD”

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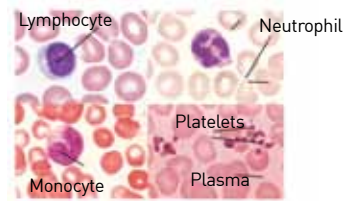
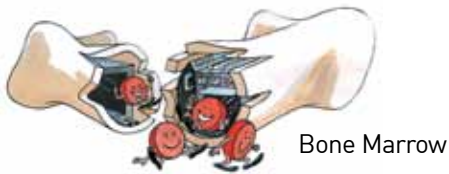
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GENERAL INTRODUCTION

INTRODUCTION

Haemoglobin disorders or haemoglobinopathies are a group of conditions affecting human blood - more specifically an important substance or protein called haemoglobin contained in the red blood cells, hence the name haemoglobin disorders or haemoglobinopathies. This protein constitutes an important part of the human blood, which is the vital fluid that brings nourishment, such as oxygen (O₂), hormones, proteins, fats and carbohydrates to the body's organs and tissues and carries away waste substances such as carbon dioxide (CO₂), urea and uric acid.



BLOOD

In adults, blood is exclusively produced in a special tissue called marrow, which is found in the central cavity of the bones (bone marrow). Blood consists of two major components:

- i the plasma, which is the yellow liquid that constitutes about 55% of the volume of blood and contains water, salts and important proteins, and;
- ii three types of cells which are microscopic building blocks, trillions of which make up the human body. The cells found in blood are:
 - The white cells or leucocytes, which are classified to: Granulocytes, Neutrophils, Eosinophils, Basophils, Monocytes and Lymphocytes
 - The platelets or thrombocytes, and
 - The red cells or erythrocytes

Each type of blood cell has specific functions and each contributes, in its own special way, to the well-being of the human organism, including protection against infection (white cells); limiting blood loss when a vessel is damaged (platelets) and provision of oxygen to tissues and vital organs (red cells).

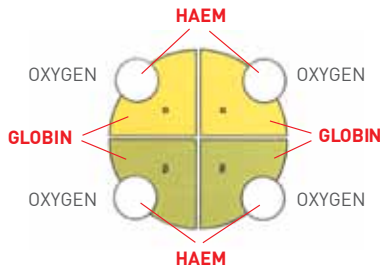
Many diseases in humans are caused by abnormalities in the blood and these are categorized according to the component of the blood which is affected: white cell diseases, platelet diseases and red cell diseases.

Red cell diseases include amongst others the haemoglobinopathies or haemoglobin disorders, the most severe of which are alpha (α -) and beta (β -) thalassaemia and sickle cell disease (SCD).

HAEMOGLOBIN

Each cubic millimeter of human blood contains 4,500,000 - 5,000,000 red cells and each one of them is packed with 300 million molecules of haemoglobin - the substance that gives blood its red colour (because of the iron it contains), and most importantly has oxygen carrying capacity (oxygen being essential for the growth and performance of the cells and organs).

of the human organism). The haemoglobin molecule itself consists of two major parts: **(i) the globin** and **(ii) the haem**:



Haemoglobin

- i **Globin** is a protein made up of smaller units, referred to as chains – the alpha (α) and the non-alpha such as beta (β), gamma (γ), and delta (δ) chains. The alpha (α) chains couple with equal amounts of beta (β) chains to make up the haemoglobin A (HbA), which is the main type of haemoglobin in adults (and will be referred to thereafter in this text as “adult normal haemoglobin (HbA)”) and up to 10% of the haemoglobin of the foetus. Alpha chains also couple with other chains, making up the haemoglobins found at various stages of human life, from conception, through foetal life, to birth and after birth.
- ii The ‘haem’ part of the haemoglobin molecule contains iron – the metal that is essential for the growth and normal functioning of the cells and that gives blood its red colour. Iron has the capacity to easily bind and lose oxygen, providing the haemoglobin molecule with the capacity to carry and distribute oxygen to tissues and organs of the human body. Adults have about 4g of iron in their body, 75% of which is used to synthesize the haemoglobin molecules of the red cells.

INHERITANCE

Haemoglobinopathies are genetic or hereditary disorders that are passed on from parents to children according to a specific pattern of inheritance known as “Mendelian recessive autosomal pattern of inheritance”. They are called genetic because all characteristics are passed on from parents to children through genes, which are the biological units of inheritance that provide all the information needed for controlling growth and development throughout human life. In the case of haemoglobinopathies, the contribution of genes is necessary from both parents (recessive inheritance) for the inheritance of these disorders, which can affect male and female children alike (autosomal).

Deoxyribonucleic Acid is a chemical substance often referred to by its abbreviation, DNA, which constitutes the key component of genes, of which a great number are needed to carry out the many and complex biological functions of the human organism. Genes are linked together in the cell on long piles of DNA called chromosomes, of which there are 23 pairs, half inherited from one and half from the other parent.

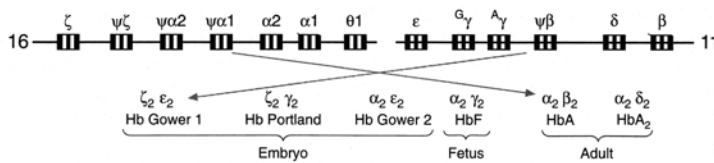
NOTE: The words alpha, beta, gamma and delta, wherever met in this text will be referred to with their respective abbreviations: α , β , γ , δ .



DNA



Chromosomes



The α - and β -globin gene clusters on chromosomes 16 and 11 respectively. In the extended α - and β -globin genes the introns are shaded dark, the 5' and 3' non-coding regions are hatched, and the exons are unshaded.

In the case of adult haemoglobin, the production and synthesis of its α - and β -chains, which constitute its major component, is controlled by genes on specific chromosomes. Four α -globin genes on chromosome 16 and two non- α -globin genes, such as β , γ and δ , on chromosome 11 are responsible for the production, in exactly equal numbers, of α - and β - chains respectively.

The individuals who have a defect in one of the α -globin genes responsible for the production of α -chains, which may cause reduced production of these chains, are called α -thalassaemia carriers. If the defect involves more of the α -globin genes, then fewer α -chains are produced and the individual may be affected more significantly.

Similarly, a defect in the gene responsible for the production of β -chains may cause a small or a severe reduction of β -chains.

The degree of β -chain reduction will determine whether an individual is only a β -thalassaemia carrier, or a patient with β -thalassaemia major or intermedia.

In contrast to the thalassaemias (alpha and beta), in which the defect in the genes results in reduced production of α - and β -globin chains, (α or β), there are conditions in which the defect in the genes results in the production of "wrong" kinds of proteins whose structure, as well as their function, are different from that of the adult normal haemoglobin (HbA). These are called abnormal or structural haemoglobin variants. The most common of these variants are known as haemoglobin S (HbS) and haemoglobin E (HbE), although many others have been identified. Reference is made to the inheritance and clinical outcome of these in all three sections (1, 2 and 3), accordingly.

THE MAJOR HEAMOGLOBIN DISORDERS ARE:

α-chain disorders	β-chain disorders
α-thalassaemias	Sickle cell disorders
HbH disease	Sickle cell anaemia (HbSS)
α-thalassaemia Hydrops Fetalis	HbS/β-thalassaemia
(=Hb Bart's Hydrops Fetalis)	HbSC disease
α-chain variants	HbSD disease
	HbS/EHbS/O Arab
	Other rare sickling disorders
	β-thalassaemias
	β-thalassaemia major
	β-thalassaemia intermedia
	HbE/β-thalassaemia
	Other rare thalassaemias

This book provides information on β- thalassaemia, α-thalassaemia and the abnormal hamoglobin S (HbS), in this order in sections One, Two and Three respectively.

ABOUT
BETA (β)
THALASSAEMIA

SECTION 1



HAEMOGLOBIN DISORDERS (HAEMOGLOBINOPATHIES)

BETA (β)-THALASSAEMIA (THALASSAEMIA) OR MEDITERRANEAN ANAEMIA OR COOLEY'S ANAEMIA

INHERITANCE PATTERNS OF β-THALASSAEMIA

Pattern A

Both parents with unaffected β-globin genes: when in both parents the β-globin genes are unaffected, i.e. fully functional, all their children will inherit two unaffected, fully functional β-globin genes, and all children will thus have adult normal haemoglobin (HbA).

Pattern B

When one of the parents is a carrier of β-thalassaemia, i.e. has one affected β globin-gene with reduced function, and the other parent carries 2 unaffected, fully functional, β globin-genes, then each child born to these parents (i.e. at every pregnancy) has a one-in-two (50%) chance of inheriting the affected β-globin gene from the carrier parent. (FIG. 01)

One may also come across other names describing a carrier of β-thalassaemia, such as:

- Carrier of the β-thalassaemia trait
- Individual heterozygous for β-thalassaemia
- Individual with β-thalassaemia minor
- Carrier of the stigma
- β-thalassaemia carrier

Pattern C

Partners who both carry one affected β-globin gene are referred to as an “at-risk” couple. As described below, although being a carrier of β-thalassaemia has no adverse health effects, if he/she plans to make a family with another carrier, then at each and every pregnancy, the risks involved are as follows:

- a one-in-four (25%) chance that the child will have two affected (non or partially functional) β-globin genes. This child will have β-thalassaemia major/intermedia, the full-blown disease. β-thalassaemia intermedia/major is also known as Mediterranean Anaemia or Cooley's Anaemia. The patient may also be referred to as homozygous for β-thalassaemia
- a one-in-two (50%) chance that the child will be a carrier of β-thalassaemia, i.e. inherit one affected and one unaffected globin gene, and
- a one-in-four (25%) chance that the child will have both β-globin genes completely unaffected, i.e. fully functional. (FIG 02)

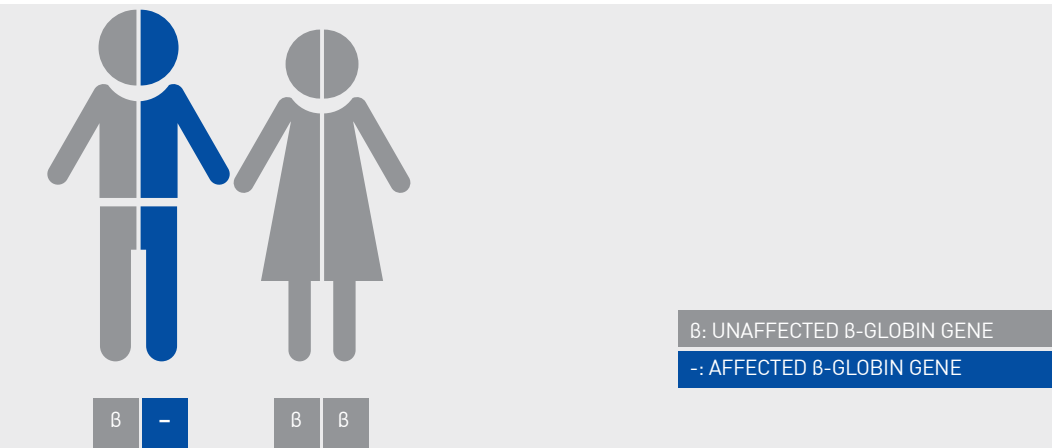
ABOUT CARRIERS OF β-THALASSAEMIA

A carrier is a person who has an unaffected, fully functional β-globin gene on one chromosome and an affected β-globin gene, i.e. one with reduced function, on the other. In this text, the fully functional β-globin gene will be referred to as “unaffected” β-globin gene and the one with reduced function as “affected” β-globin gene. Carriers of β-thalassaemia do not have a disease.

They have no physical or mental symptoms and do not require a special diet, medical advice or treatment. Carrying β -thalassaemia has no effect on health, length or quality of life. Carriers through do have smaller red blood cells than non-carrier individuals. This is because a carrier has inherited an affected β -globin gene from one parent, which, as a result, makes less

FIG. 01 INHERITTANCE PATTERNS

ONE PARENT IS A CARRIER OF β -THALASSAEMIA (HAS ONE UNAFFECTED AND ONE AFFECTED β -GLOBIN GENE) AND THE OTHER HAS UNAFFECTED β -GLOBIN GENES



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

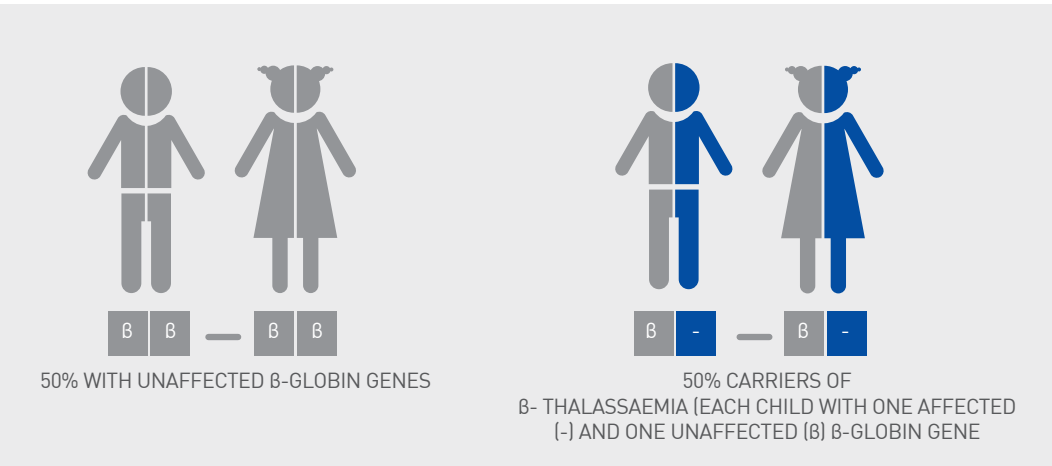
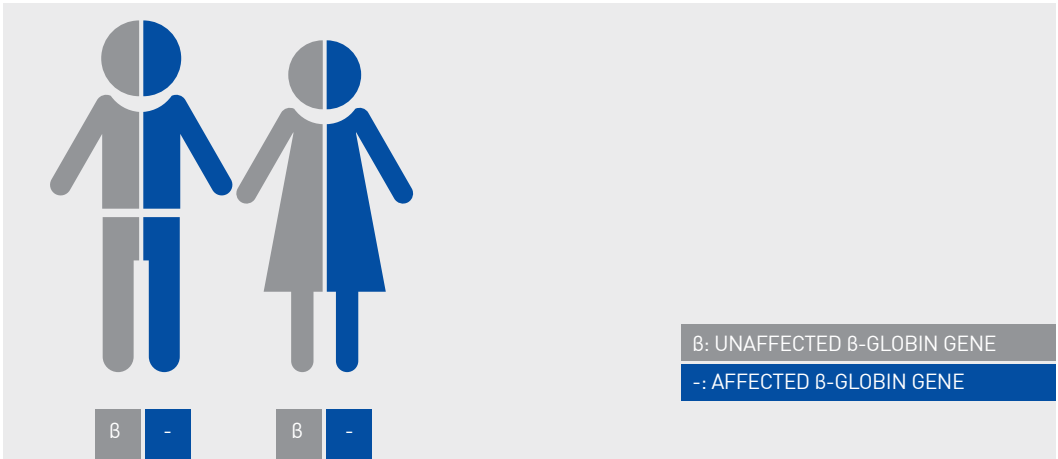
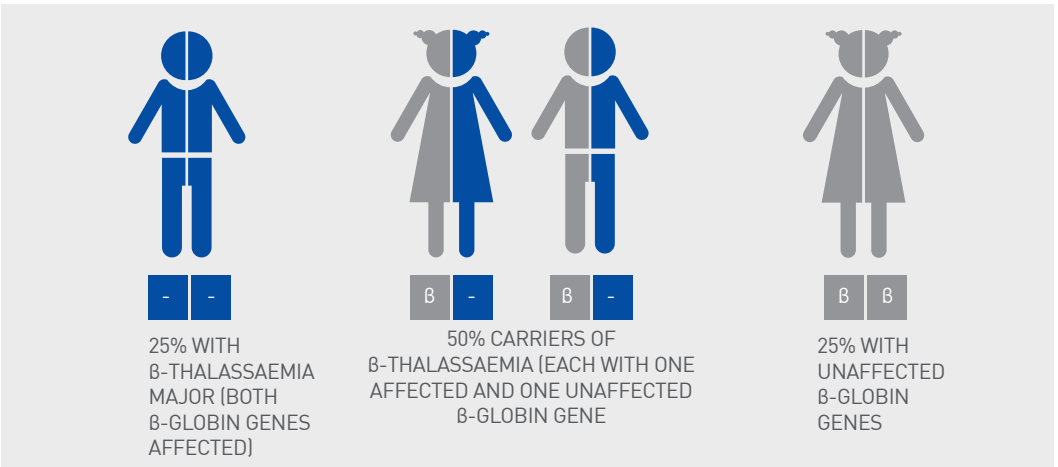


FIG. 02

BOTH PARENTS ARE CARRIERS OF β -THALASSAEMIA (i.e. EACH WITH ONE AFFECTED AND ONE UNAFFECTED β -GLOBIN GENE)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:



adult normal haemoglobin (HbA). His/her red cells thus contain less haemoglobin than usual and consequently their size is smaller and their colour paler than the red cells of non-carriers. Carriers, however, make up for this by producing more red cells, and in this way the blood continues to function and serve the human organism normally.

The carrier status cannot become a disease over time. Indeed, most individuals will be unaware that they are carriers unless they are specifically tested. Some carriers may experience mild anaemia, which can be inaccurately diagnosed as iron deficiency anaemia. Laboratory tests, however, can differentiate between the two and physicians may then advise the individuals or carriers accordingly.

FREQUENTLY ASKED QUESTIONS ABOUT CARRIERS

What about pregnant women who are carriers?

Like other pregnant women, those who carry β -thalassaemia can also become iron deficient and may need extra iron. The mild anaemia due to carrying β -thalassaemia can, however, become more severe during pregnancy and a pregnant carrier may, though very rarely, need a blood transfusion. The anaemia will improve after the baby is born.

Is there any treatment or intervention to stop being a carrier?

A person who is born carrying β -thalassaemia will always carry it in his/her genes throughout life.

Can the β -thalassaemia carrier status be transmitted or acquired at a later stage in life?

The β -thalassaemia carrier status cannot be acquired or transmitted through the environment, transfusion or other means by which people become infected. However, carriers who have inherited β -thalassaemia from their parents could pass it on to their children.

Can carriers donate blood?

Carriers may be suitable blood donors if their haemoglobin level meets the national inclusion criteria for donating blood.

What should carriers do if they are thinking of having children?

Carriers should encourage their partners to have a special blood test which ideally should be done before they start a pregnancy. In this case, partners who are both carriers should see a genetic counsellor or a physician to obtain further information and take the time to consider and decide what is best for them.

Is there anything else that a carrier should do?

A carrier should let their brother or sister know about their status and advise them to also have a blood test for haemoglobin (Hb) disorders.

$\delta\beta$ -Thalassaemia

In this form of thalassaemia, both the δ -genes and the β -globin genes are not functioning, a situation partly balanced by increased production of other chains, especially the γ -chains, since the γ -gene is fully functional.

Like the β -thalassaemia carrier, the $\delta\beta$ -thalassaemia carrier is a healthy individual and needs no medical treatment, but the affected gene can be passed on from the parent to his/her children. A child inheriting either one $\delta\beta$ -thalassaemia gene from each $\delta\beta$ -carrier parent or one $\delta\beta$ -thalassaemia gene from one parent and one β -thalassaemia gene from the other parent will develop a severe haemoglobin disorder, similar in its clinical outcome to β -thalassaemia major or β -thalassaemia intermedia, and will thus require similar clinical management.

“Abnormal Haemoglobins”

A number of “abnormal” types of adult haemoglobin have been identified, which differ both in their

structure and clinical outcome. These are also known as “structural haemoglobin variants” and include **HbS**, which is the haemoglobin responsible for sickle cell disease (**SCD**), haemoglobin E (**HbE**), haemoglobin C (**HbC**), haemoglobin D (**HbD**) and haemoglobin Lepore (**Hb Lepore**). These are passed on from parents to their children and are inherited in exactly the same way as β -thalassaemia. However, only those individuals that inherit from both parents Hb Lepore or HbS will develop clinically significant conditions that require medical care.

Inheritance of the other abnormal haemoglobins, i.e. HbC, or HbD or HbE from both parents is not related to any significant clinical outcome, and so these do not require any medical attention.

However, inheritance of an “abnormal” haemoglobin, Lepore, E or S from one parent and β -thalassaemia from the other parent, results in compound haemoglobinopathies, such as Hb Lepore/ β , HbE/ β , and HbS/ β , which are clinically significant blood disorders similar to β -thalassaemia major/intermedia or sickle cell disease and require appropriate medical care. More details associated with the most common of the “abnormal” haemoglobins, HbE, are provided below.

Haemoglobin E (Hbe)

HbE is one of the most common abnormal haemoglobins, particularly prevalent amongst people of South East Asian origin. Like the β -thalassaemia carrier, the HbE carrier is a healthy individual and needs no medical treatment. However, the affected genes can be passed on from the parent to his/her children according to the inheritance pattern described earlier for β -thalassaemia. A child inheriting two HbE genes, one from each of his/her parents, will still be healthy and not require medical care.

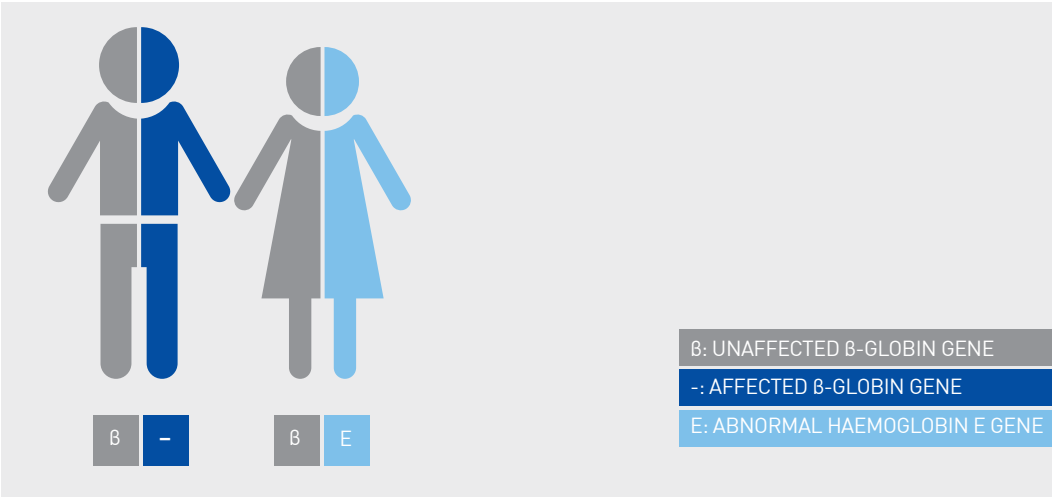
HbE/ β -thalassaemia

HbE becomes important only when the child inherits from one parent the affected β -globin gene and from the other parent the abnormal HbE gene. This will result in HbE/ β -thalassaemia which is a serious disorder, the clinical symptoms of which are similar to those seen in β -thalassaemia intermedia, but which may sometimes be as severe as those seen in β -thalassaemia major. (FIG 03)

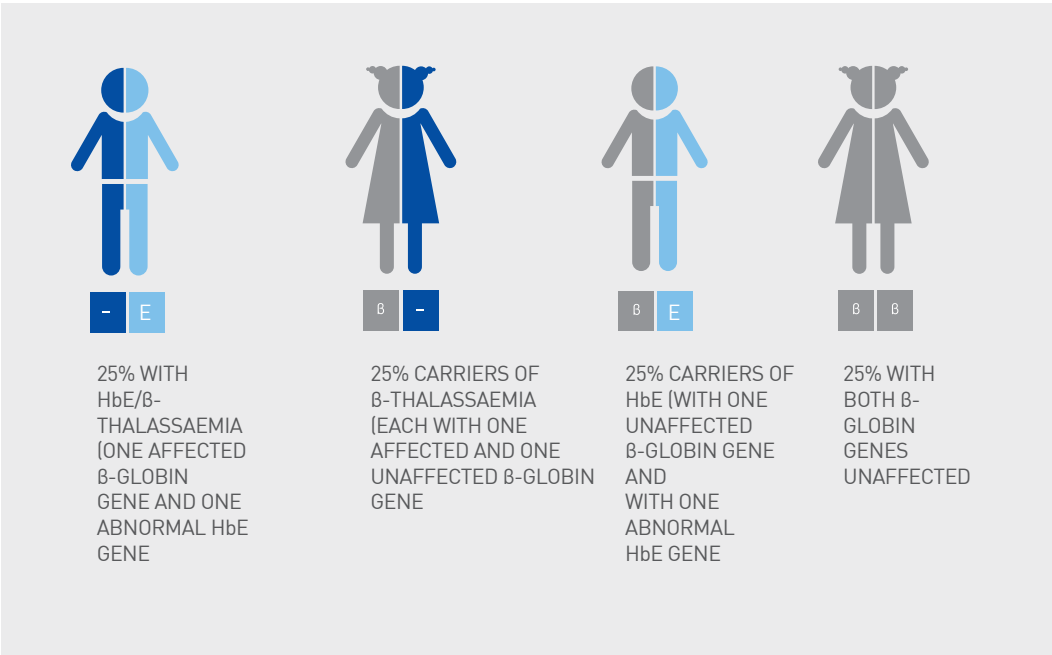
Sickle cell disorders are different in cause and clinical outcome from β - and α -thalassaemia, and these are described in detail in Section 3. Inheritance of the β -thalassaemia gene from one parent and the HbS gene from the other will result in a haemoglobin disorder called HbS/ β -thalassaemia. The clinical outcome of this compound haemoglobin disorder is similar to sickle cell disease, which is very different from β -thalassaemia major/intermedia and which consequently is different in its clinical management (see Section 3 for more detailed information).

FIG. 03

ONE PARENT IS A CARRIER OF THE ABNORMAL HbE GENE AND THE OTHER IS A B-THALASSAEMIA CARRIER (HAS ONE AFFECTED B-GLOBIN GENE)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:



HOW DOES ONE KNOW WHETHER ONE IS A β -THALASSAEMIA CARRIER?

In most cases, simple but specific laboratory tests can identify whether a person is a carrier of β -thalassaemia or any other haemoglobin disorder.

Laboratory testing to establish whether one is a carrier of β -thalassaemia

Laboratory testing for thalassaemia includes a routine blood test known as a Complete Blood Count (CBC), which involves measuring the content of haemoglobin inside the red cells, and other parameters such as the Mean Corpuscular Volume (MCV) and the Mean Corpuscular Haemoglobin (MCH), both of which will be lower in carriers of β -thalassaemia.

Red blood cells are also seen under a microscope in order to examine their size and shape. The red cells of a thalassaemia carrier will be a paler shade of red (hypochromic), of various shapes (poikilocytosis), and smaller (microcytosis) compared to the red blood cells of a non-carrier, which are a darker red in colour, round and concave in shape.

Another test that is used to determine the presence of β -thalassaemia carrier state includes a laboratory process known as haemoglobin electrophoresis, which enables measurement of the quantity of the major HbA and minor HbA₂ components of adult normal haemoglobin, of which the latter (i.e. HbA₂) is always raised in the carrier. Other haemoglobins present in adult red cells, such as foetal haemoglobin, HbF, and abnormal haemoglobin S (HbS), may also be measured by electrophoresis. In most cases, the above tests are sufficient to determine whether an individual is a carrier of β -thalassaemia.

Other ways to diagnose thalassaemia carrier state include iso-electric focusing (IEF), which is another kind of electrophoresis, High Pressure Liquid Chromatography (HPLC for short) and Capillary Electrophoresis, which are considered today as methods of reference both for screening and for confirming the diagnosis of haemoglobin disorders.

Where the above tests are inconclusive and do not allow the laboratory scientists to provide a confirmed diagnosis, other more specialized tests are available, such as genetic tests, i.e. tests that are based on the examination of the individual's DNA. For this test, blood is often required from other members of the family for a definite diagnosis to be made.

On occasions where iron deficiency exists, it may obscure the diagnosis and so it must be excluded as the cause of the red cell abnormalities. It may be necessary to correct the iron deficiency first by giving iron tablets to the individual and repeating the test in at least one month's time, after which the diagnosis may be confirmed.

THE CHOICES AVAILABLE TO AN 'AT RISK COUPLE'

Today, partners in a relationship who are aware that they are carriers of β -thalassaemia have a number of choices with regard to having a family. These should be discussed as early as possible with an expert health professional, a genetic counsellor if available.

If a couple decides to proceed with pregnancy and test the foetus

There are three methods of prenatal diagnosis that can determine whether a foetus has β -thalassaemia major or intermedia:

i. Amniocentesis

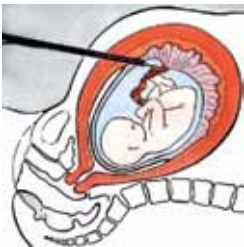
This test is performed in the second trimester of pregnancy, after about 15 weeks' gestation. Using ultrasound as a guide, a trained obstetrician inserts a very thin needle through the mother's abdomen. A small amount of amniotic fluid, containing cells from the foetus, is withdrawn. This is then analysed in the laboratory to determine whether the foetus has β -thalassaemia (major or intermedia). The specialist obstetrician will be able to explain and discuss in detail all aspects of this procedure, including potential risks for the couple involved.

ii. Cordocentesis

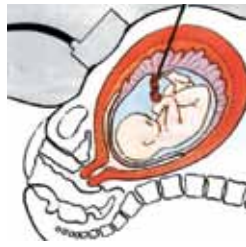
This is a test performed after 18 weeks of pregnancy. Under ultrasound guidance, a fine needle is inserted through the abdomen into the foetal umbilical cord, through which a small volume of blood from the foetus is aspirated. The blood is analyzed in the laboratory. The specialist obstetrician will be best able to explain and discuss with the parent causes of failure in obtaining pure foetal blood, as well as any other potential risks when undergoing the procedure. Early and specific diagnosis with molecular methods has almost completely replaced cordocentesis, which is now mainly indicated in pregnant women who report late, when CVS is inconclusive.

iii. Chorionic Villus Sampling (CVS)

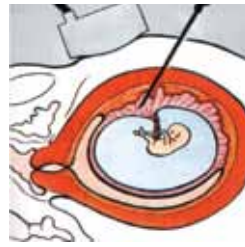
This is another method of diagnosing haemoglobin disorders in the foetus and can be performed earlier than both amniocentesis and cordocentesis, at about 10-11 weeks' gestation. Using ultra-sound as a guide, the specialist obstetrician removes a small sample of cells from the chorionic villi, i.e. cells that contain the same genetic information as the foetus and which will eventually form the placenta. The cells are removed either with a thin needle inserted through the mother's abdomen (transabdominal) or a thin catheter inserted through the vagina (transcervical). The cells are then analysed and a diagnosis made. As with other prenatal diagnosis methods, information on potential risks and benefits of using this procedure is provided to the couple by the specialist obstetrician.



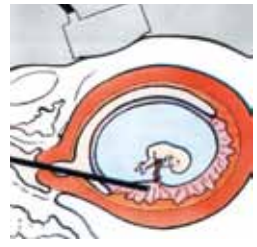
Amniocentesis



Cordocentesis



CVS



How is the diagnosis of the foetus made after obtaining samples using the above methods of prenatal tests?

Amniocentesis and CVS, both based on DNA analysis, are otherwise known as genetic tests, and

involve identifying the genetic abnormality (mutation) present in the parents. This kind of testing constitutes the most accurate means of diagnosing inherited diseases. As with all tests, there is a possibility of laboratory error, albeit a very small one.

These tests confirm whether the foetus is healthy, a carrier or affected. The couple should be made aware and decide how to proceed.

Other approaches

Prenatal diagnosis and the termination of pregnancy are methods that may not be acceptable to every couple at risk due to religious, cultural or personal beliefs.

Prevention cannot rely on the identification of carriers alone. If prenatal diagnosis and pregnancy termination are not acceptable, other methods of prevention that minimise intervention and psychological stress have been developed, while others are still in the research stage. For example, pre-implantation genetic diagnosis (PGD) is a refined, established laboratory procedure whereby:

- i. cells from a very early embryo (resulting from in-vitro fertilisation) are analysed in the laboratory to confirm the presence or absence of a haemoglobin disorder. Unaffected embryos are then selected for implantation into the womb of the carrier woman;
- ii. unfertilised ova (eggs) which do not carry a haemoglobin disorder or mutation, detected by polar body diagnosis, are selected from the carrier woman and then fertilised using her husband's sperm. The selected fertilised ova are then introduced into her womb.

PGD is, however, a costly and technologically demanding procedure and several attempts are often necessary for a successful pregnancy.

β-THALASSAEMIA (MAJOR OR INTERMEDIA, OTHERWISE KNOWN AS MEDITERRANEAN ANAEMIA OR COOLEY'S ANAEMIA)

Patients with β-thalassaemia major, the most severe form of thalassaemia, cannot make adult normal haemoglobin, and as a consequence cannot produce normal red blood cells. In these individuals, each red blood cell contains much less haemoglobin, and there are far fewer red cells than the normal range. This causes anaemia, which is severe in these patients.

A child with β-thalassaemia major does not, however, develop severe anaemia until three months to one year of age. If left untreated, affected children have a very poor quality of life and most will die at a very young age. The patients who have β-thalassaemia intermedia, which is considered in the majority of cases to be a "milder" condition than β-thalassaemia major, can manage without regular blood transfusions, at least in the early years of life, although they still have a serious anaemia. Nevertheless, the anaemia may get worse with age, and they may need to start regular blood transfusions later in childhood or in adult life. Many other medical complications, including endocrine, cardiac and hepatic, are seen in both β-thalassaemia major and intermedia. Because of this, multidisciplinary medical care is required.

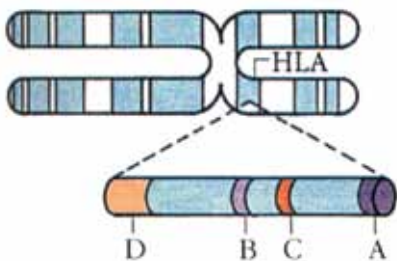
WHAT IS THE TREATMENT OF β -THALASSAEMIA MAJOR?

The basic treatment is regular blood transfusion, usually every two to four weeks. Children who are transfused appropriately grow well and have a normal life. However, to live past their twenties, patients with β -thalassaemia major also need to start a specific treatment from early childhood to remove the excess iron, which is released from the haemoglobin of the transfused red cells, as these continuously break down. As a result, iron builds up in the body and causes overload, which can ultimately damage vital organs, such as the heart, liver and endocrine glands. Iron can be removed by specific drugs, called iron chelating agents, that have the capacity to bring it out in the urine and/or the stools. The traditional and first iron chelating agent to be used with efficiency and safely is Desferrioxamine, which is injected under the skin for many hours almost every day, using a small pump. Although difficult and cumbersome, this treatment has proved to be a life-saving one for decades.

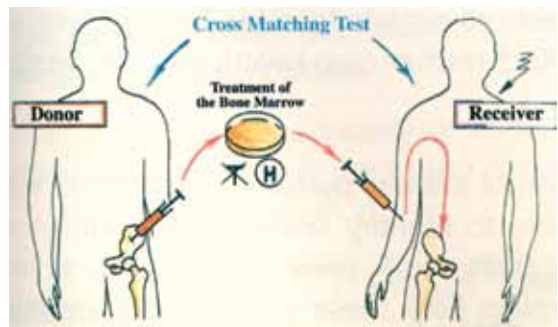
The outlook is, however, steadily improving as new iron chelating agents that can be taken by mouth are increasingly becoming available. Already, two orally taken drugs, Deferiprone (L1) and Deferasirox, are available in many countries and are in use by many thousands of patients across the world.

Children born today with thalassaemia major or intermedia can be expected to live an almost normal length of life, provided that they can obtain all the appropriate treatment according to consensus guidelines. Considerable research is focused on further improving medical care of thalassaemia major through pharmaceutical substances that may ultimately lead to a reduction in the need for blood transfusion therapy and iron chelation.

Cure of β -thalassaemia can only currently be achieved by haemopoietic stem cell transplantation (HSCT), which includes bone marrow transplantation (BMT). This has been shown to be successful in the majority of cases, provided that a fully matched (HLA compatible) sibling (brother or sister) can be identified, and the patient is in a very good condition clinically, i.e. he or she has been receiving appropriate clinical management from the early years. However, only a relatively small percentage of patients (about 20%) will have a fully matched family donor, and considering that this procedure is still an expensive one, BMT cannot be the solution to the total cure of this disease for the majority of patients.



HLA - TYPING



PROCEDURE OF BMT

Pre-implantation Genetic Diagnosis (PGD-HLA)

One possible answer to the limitations associated with BMT, particularly with regard to finding a suitable donor, is for the parents to have another child who will be fully compatible with the living, affected one.

In order to ensure that this new child will be a compatible donor, a special process known as PGD-HLA has been developed in recent years. According to this process, the embryo to be implanted into the woman's womb is selected in the laboratory not only to be free from thalassaemia, as in the PGD technology described previously in the context of prevention, but also to have the same tissue characteristics (HLA type) as the living, affected child. In such a case, blood from the umbilical cord will be taken at birth, the cells of which may be used for transplanting into the patient. In other words, the "new" baby is selected from the beginning to be a perfect donor for his/her living affected brother or sister.

This is a laborious process, and not ethically approved by all. Nevertheless, it may provide a better chance of a matched donor for curing patients with β -thalassaemia major and other severe hereditary disorders.

Gene therapy

Another final cure for thalassaemia could be provided by means of another new and promising scientific process, called gene therapy. This is still experimental and it involves correcting the affected gene outside of the body, in the laboratory, and then replacing it into the patient's blood-forming tissues, making them capable of producing new red cells, which will contain adult normal haemoglobin (HbA). The world thalassaemia medical community and patients/parents' communities very much look forward to the success of this method which does not depend on donors as in the case of BMT, but, on the contrary, on the patient's own cells. This procedure does not have the limitations encountered in bone marrow transplantation. It is hoped that science will soon overcome the many current technical difficulties and make the dream of a total cure from thalassaemia a reality for all. A specialist physician or genetic counsellor or TIF will be able to provide more information and update you on all the above curative procedures.

HOW IS β -THALASSAEMIA MAJOR/INTERMEDIA DIAGNOSED?

A child newly born with β -thalassaemia major/intermedia will show no visible signs of the disease. Even laboratory tests may fail to diagnose thalassaemia, particularly if the parents have not been tested, if no prenatal tests were carried out, and if there is no other affected child in the family. Laboratory testing at this early stage will be of value in diagnosing the presence of "abnormal" haemoglobins, such as HbE or HbS.

In most cases, β -thalassaemia major can be diagnosed in the first few months and before the age of 2 years.

β -thalassaemia intermedia, which in the majority of cases is a "milder" clinical condition than β -thalassaemia major, can, however, remain undiagnosed for longer periods.

DIAGNOSING THALASSAEMIA MAJOR/INTERMEDIA IN THE LABORATORY

Haematological parameters

In thalassaemia major/intermedia, the size and volume of red blood cells and the concentration of haemoglobin inside them are significantly reduced. Haemoglobin levels are very low. Some haematological indices most commonly found in patients with thalassaemia (major) are shown below:

- Haemoglobin (Hb) g/dl 6.8 (range 3.9-9.3)
- Mean Corpuscular Haemoglobin (MCH) pg 20.9 (range 15-26)
- Mean Cell Volume (MCV) FL 65.8 (range 57-75)
- Mean Corpuscular Haemoglobin content (MCHC) g/dl 30.9 (range 26-34)

Morphology of RBC

Observed under a microscope, the red blood cells appear paler (hypochromic) and smaller (microcytic) than normal and, very importantly, the majority have abnormal sizes and shapes – anisocytosis and poikilocytosis respectively – changes which are more marked than those seen in the carrier.

Haemoglobin electrophoresis

This is a laboratory process that separates the different proteins that make up a haemoglobin molecule – HbA, HbA₂, and HbF. A diagnosis of thalassaemia major/intermedia is indicated where:

- levels of foetal haemoglobin are higher than normal and may vary between 20-90%,
- HbA₂, which usually contributes to up to 3% of adult normal haemoglobin (HbA), may be non-existent, reduced, slightly elevated, or even within normal range.

Molecular methods

These are laboratory methods used to confirm or obtain more specific information with regard to the diagnosis. They are based on DNA technology, which identifies the mutations (the genetic changes) that cause the disorder – information that, in addition to confirming a diagnosis, may also provide an indication of the clinical severity of the disease.

Although the laboratory and clinical diagnosis of β -thalassaemia major/intermedia is usually fairly straightforward, difficulties may arise, particularly in developing countries where the prevalence of other diseases such as malaria can complicate the diagnosis.

For example, malaria can cause anaemia and splenomegaly, and, although the haematological laboratory findings are quite different from those seen in thalassaemia major/intermedia, it may be necessary to treat the patient with anti-malarial drugs before reassessing his/her condition and diagnosis.

Other conditions may also cause anaemia and splenomegaly as well as raised HbF levels and additional clinical laboratory tests are necessary to differentiate and confirm the diagnosis of thalassaemia major/intermedia. An accurate diagnosis of thalassaemia is essential before the initiation of treatment.

CAN PATIENTS WITH THALASSAEMIA MAJOR/INTERMEDIA HAVE CHILDREN?

Yes, many adult patients are now married and have become parents or even grandparents (in some countries). The possibilities of passing on their genes to their children are as follows:

- i.** Children from parents of whom one has β -thalassaemia major/intermedia and the other has unaffected, fully functional β -globin genes, will all be carriers of β -thalassaemia (in which case, they will be free of thalassaemia, and will not need any medical treatment or monitoring). (FIG. 04)
- ii.** Children from parents of whom one is a β -thalassaemia carrier and the other is a patient with β -thalassaemia major/intermedia will have a one-in-two chance (50%), to be carriers of β -thalassaemia (and be free of the disease) and a one-in-two (50%) chance to be patients with β -thalassaemia major/intermedia. (FIG. 05)
- iii.** When both parents are patients with β -thalassaemia major/intermedia, all children will also be patients with β -thalassaemia major/intermedia. (FIG.06)

FIG. 04

ONE PARENT WITH β -THALASSAEMIA MAJOR/INTERMEDIA (WITH BOTH β -GLOBIN GENES AFFECTED) AND THE OTHER WITH BOTH β -GLOBIN GENES UNAFFECTED (β/β)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

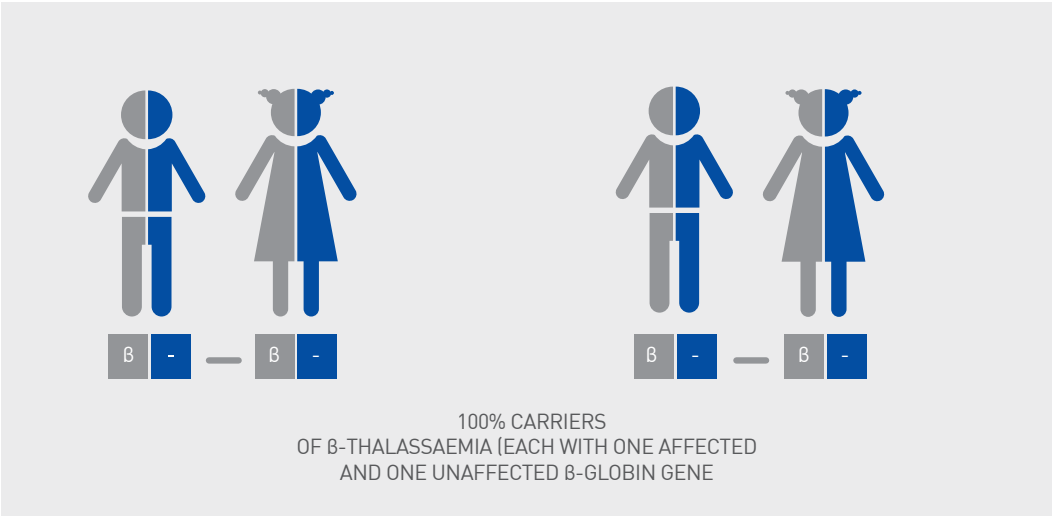


FIG. 05

ONE PARENT WITH β -THALASSAEMIA MAJOR/INTERMEDIA (BOTH β -GLOBIN GENES AFFECTED) AND THE OTHER A CARRIER OF β -THALASSAEMIA (WITH ONE AFFECTED AND ONE UNAFFECTED β -GLOBIN GENE)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

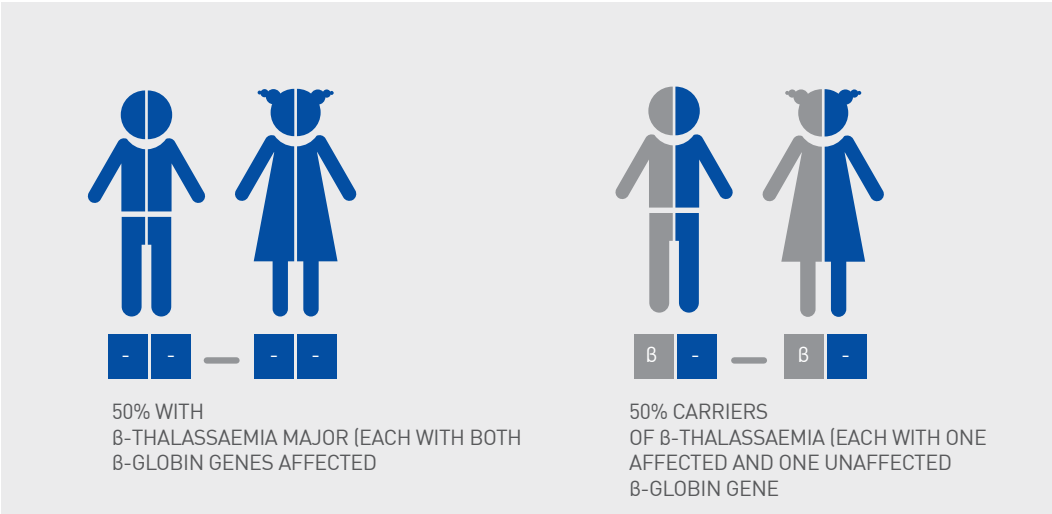
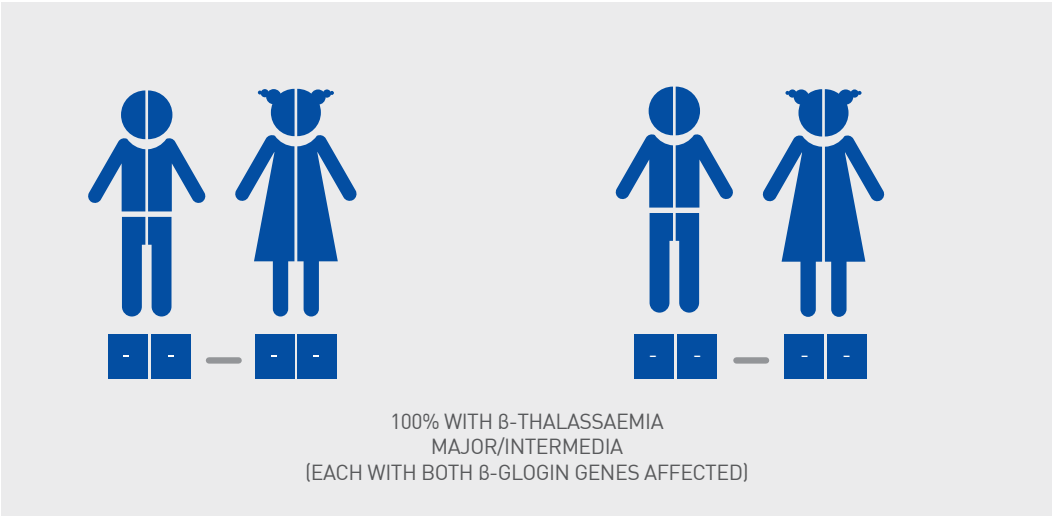


FIG. 06

BOTH PARENTS ARE PATIENTS WITH β -THALASSAEMIA
MAJOR/INTERMEDIA (BOTH PARENTS HAVE BOTH β -GLOBIN GENES AFFECTED)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:



ABOUT
ALPHA (α)
THALASSAEMIA

SECTION 2



HAEMOGLOBIN DISORDERS (HAEMOGLOBINOPATHIES)

ALPHA (α)-THALASSAEMIA

Haemoglobin molecules inside the red blood cells are comprised of an equal number of two kinds of proteins called α -globin chains and β -globin chains. There are four genes controlling the production of α -globin chains on chromosome 11 and two non-alpha globin genes controlling the production of other chains including the β -globin ones on chromosome 16.

INHERITANCE PATTERNS OF α -THALASSAEMIA

Figure 07 demonstrates the various types of alpha thalassaemia resulting from the possible combinations of α -globin genes.

Silent carrier of α -thalassaemia or α^+ thalassaemia carrier

The individual who has only one (out of the four) α -globin genes affected (non-functional or defective) is called a silent carrier of α -thalassaemia, also referred to as an α^+ thalassaemia carrier. The other three α -globin genes, which are unaffected, i.e. fully functional, produce nearly normal amounts of the α -globin chains and when coupled with β -globin chains produce adult normal haemoglobin (HbA). The affected α -globin gene may or may not result in slightly smaller red cells. For this reason, diagnosis by simple microscopic examination of the blood in the laboratory may be very difficult to be established, hence the term "silent" used to describe these carriers. Only very specific laboratory tests, based on DNA analysis, can accurately diagnose a silent carrier of α -thalassaemia (see below).

When one parent has all four α -globin genes unaffected and the other parent is a carrier of α -thalassaemia (silent carrier)

In this case, at each pregnancy there is a one-in-two (50%) chance that the children will have all four of their α -globin genes unaffected and produce adult normal haemoglobin (HbA) and one-in-two (50%) chance that the children will have α^+ thalassaemia (silent carriers), like their carrier parent.

When both parents are carriers of α^+ thalassaemia (silent carriers)

At each pregnancy, there is a one-in-four (25%) chance that a child will have the adult normal haemoglobin (HbA), a one-in-two (50%) chance that a child will be a carrier of α^+ thalassaemia (silent), and a one-in-four (25%) that a child will be a carrier of α^0 thalassaemia. (FIG. 08)

Carrier of alpha zero (α^0) thalassaemia

The individual who has two (out of four) α -globin genes missing or affected is called a carrier of alpha zero (α^0) thalassaemia. These individuals may also be referred to as carriers of α -thalassaemia minor or carriers of α -thalassaemia trait. The two missing, or affected, α -globin genes may be on the same chromosome (cis position) or on different chromosomes (trans position) as shown in Figure 07, types 3 and 4, respectively.

ABOUT SILENT CARRIERS OF α^+ THALASSAEMIA AND CARRIERS OF α^0 THALASSAEMIA

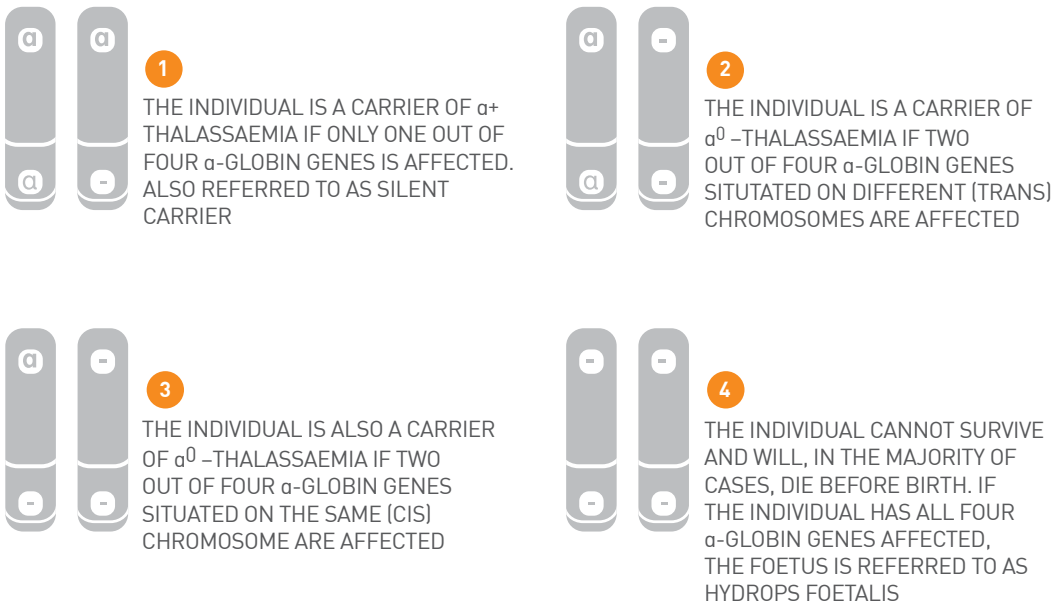
Carriers of α -thalassaemia (α^+ and α^0), like carriers of β -thalassaemia or sickle cell disease, do

*Functional: with normal capacity to produce α -chains / Non-Functional: lacking normal capacity to produce α -chains, defective

not have a disease. They have no physical or mental symptoms and do not require a special diet, medical advice or treatment. Therefore, carrying α -thalassaemia (α^+ or α^0) has no effect on health, length or quality of life.

FIG. 07

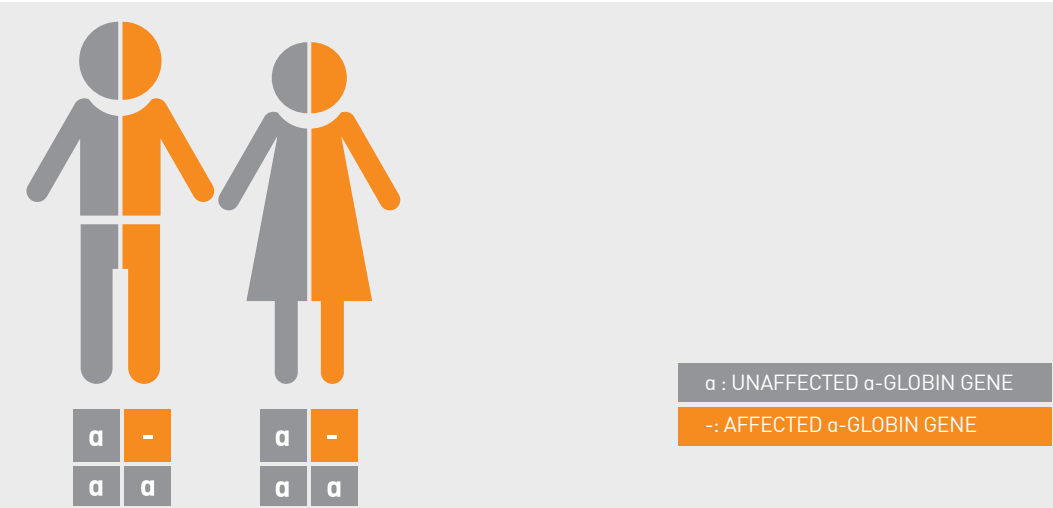
ALPHA THALASSAEMIA TYPES RESULTING FROM POSSIBLE COMBINATIONS OF α -GLOBIN GENES



The red blood cells are usually smaller, more so in the case of α^0 thalassaemia, since the quantity of α -globin chains and hence the adult normal haemoglobin that they produce is reduced. When the red cells are examined under the microscope, apart from being smaller (microcytic), they may be paler (hypochromic) with unequal size (anisocytosis) and shape (poikilocytosis), compared to the red cells of non-carriers.

FIG. 08

BOTH PARENTS ARE CARRIERS OF α^+ THALASSAEMIA (SILENT CARRIERS)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE :



The carrier status cannot become a disease over time. Indeed, most will be unaware that they are carriers, unless specifically tested. However, some carriers may experience mild anaemia, which can be inaccurately diagnosed as iron deficiency anaemia. Laboratory tests, however, can differentiate between the two states.

Frequently asked questions about carriers (α^+ or α^0)

The answers are the same as those concerning β -thalassaemia carriers and the reader can refer to that section (page 14).

Combinations of α^+ and α^0 thalassaemias

The various types of α -thalassaemia genes, as described above, combine in different ways to produce blood disorders that range from mild to severe in their effect on the human body.

They are inherited in exactly the same way as all haemoglobin disorders, according to the Mendelian recessive autosomal pattern of inheritance, as described earlier in the general introduction of this booklet.

When both parents are carriers of α^0 thalassaemia, with the two affected α -globin genes on different chromosomes (trans position), at each pregnancy all children will be carriers of α^0 thalassaemia, exactly as their parents. (FIG. 09)

i. "At-risk couples"

When both parents are carriers of α^0 thalassaemia with two affected α -globin genes on the same chromosome (cis position), at each pregnancy there is:

- one-in-four (25%) chance that a child will have unaffected α -globin genes, and will thus have adult normal haemoglobin (HbA)
 - one-in-two (50%) chance that a child will be a carrier of α^0 thalassaemia (cis type) and
 - one-in-four (25%) chance that the child will have the full blown disease, α -thalassaemia major, otherwise known as Hydrops Foetalis, in which all four α -globin genes are affected (see below for more details) (FIG. 10)
- ii. When one parent is a carrier of α^0 thalassaemia, i.e. with two affected α -globin genes on the same chromosome (cis position) and the other parent is a carrier of α^+ thalassaemia (silent), i.e. has one affected α -globin gene on one chromosome, then at each pregnancy there is:
- one-in-four (25%) chance that the child will be born with a clinically significant condition, Haemoglobin H disease (see below for more details), where 3 out of 4 α -globin genes are affected,
 - one-in-four (25%) chance to have all four α -globin genes unaffected and have adult normal Haemoglobin (HbA),
 - one-in-four (25%) chance to be a carrier of α^+ thalassaemia (silent),
 - one-in-four (25%) chance to be a carrier of α^0 thalassaemia. (FIG. 11)

INDIVIDUALS WITH HbH DISEASE AND INHERITANCE PATTERNS

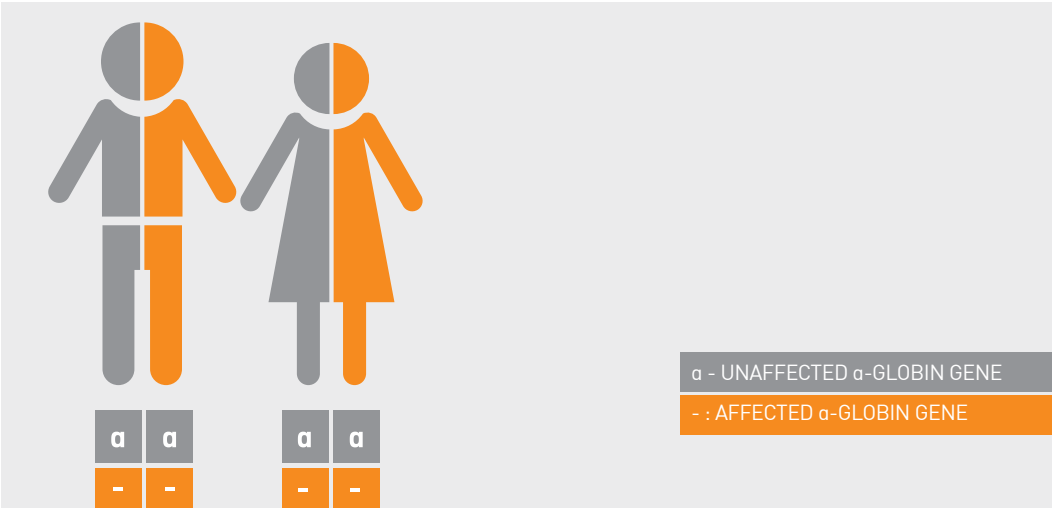
i. When one parent has HbH disease and the other is a carrier of α^+ thalassaemia (silent)

At each pregnancy there is:

- one-in-four (25%) chance of the child to be a carrier of α^+ thalassaemia (silent)
- one-in-two (50%) chance to be a carrier of α^0 thalassaemia, and
- one-in-four (25%) chance to be affected by HbH disease. (FIG. 12)

FIG. 09

BOTH PARENTS ARE CARRIERS OF α^0 THALASSAEMIA
THALASSAEMIA GENES ON DIFFERENT CHROMOSOMES (trans)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

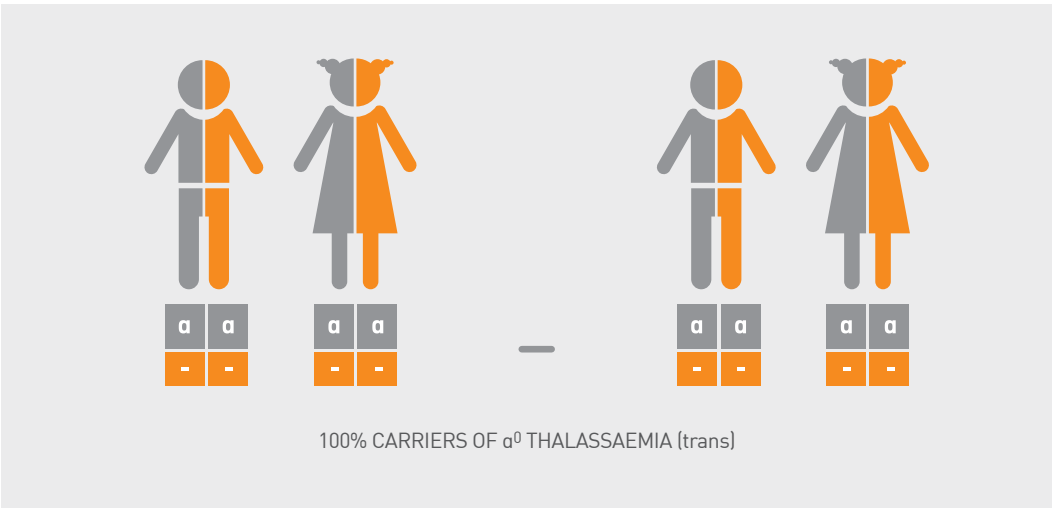
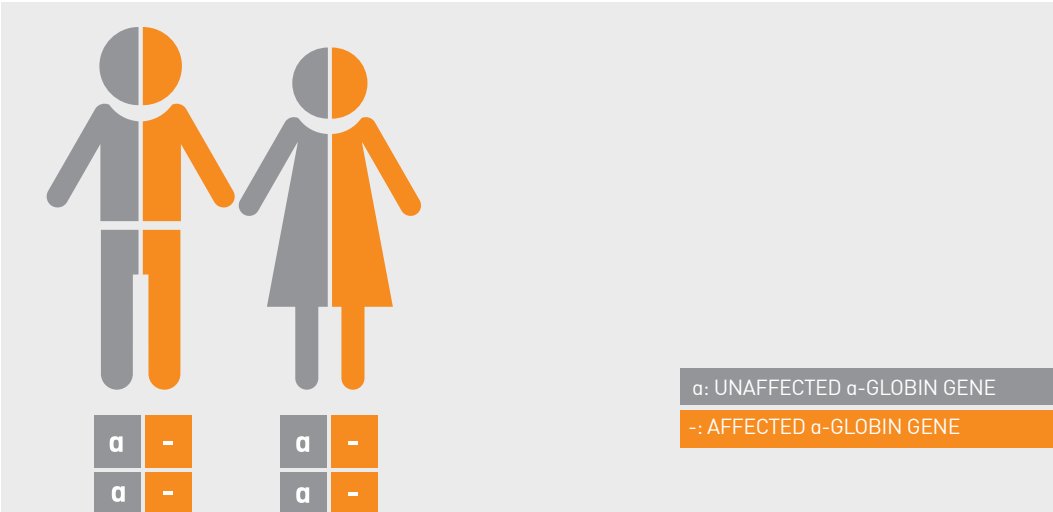


FIG. 10

BOTH PARENTS ARE CARRIERS OF α^0 THALASSAEMIA (cis)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

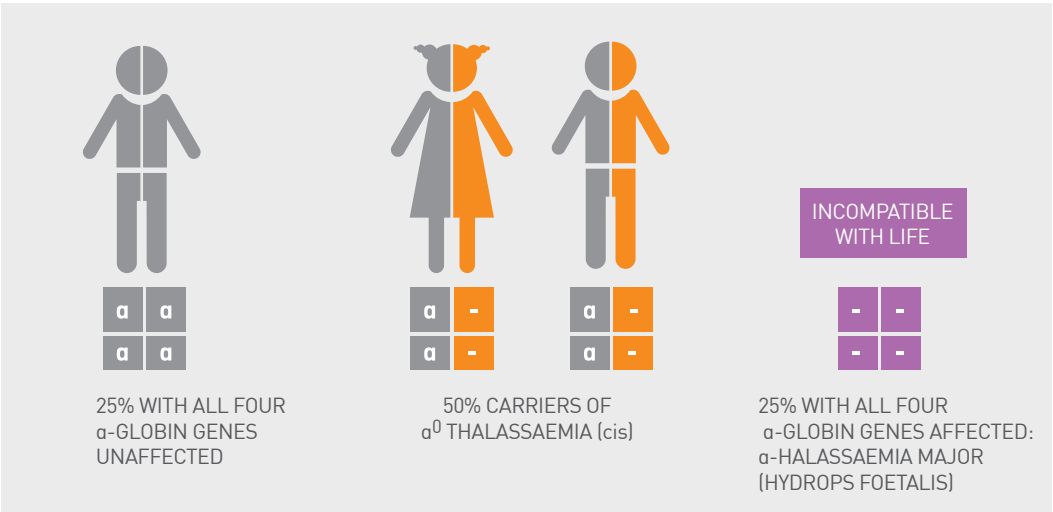
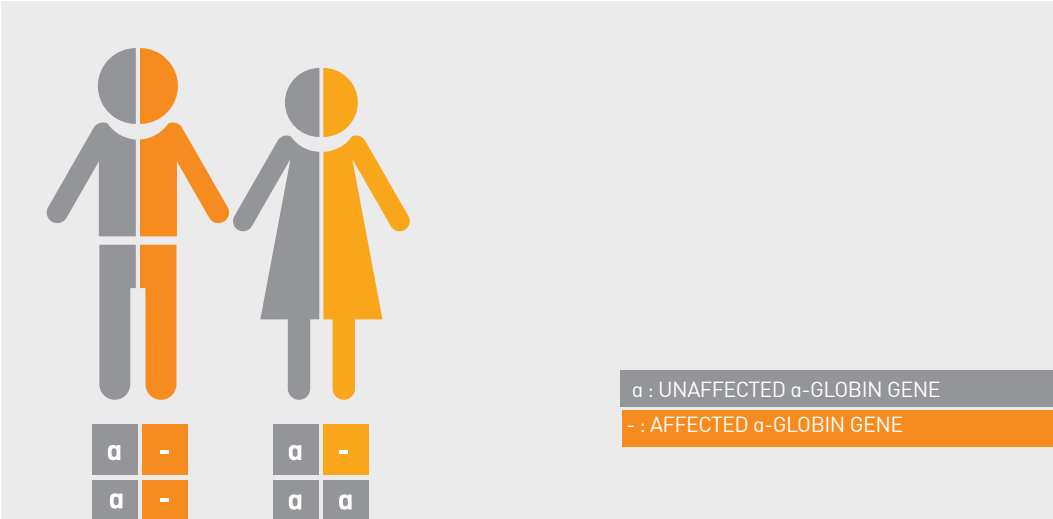


FIG. 11

ONE PARENT CARRIER OF α^0 THALASSAEMIA (cis) AND THE OTHER CARRIER OF α^+ THALASSAEMIA (SILENT)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

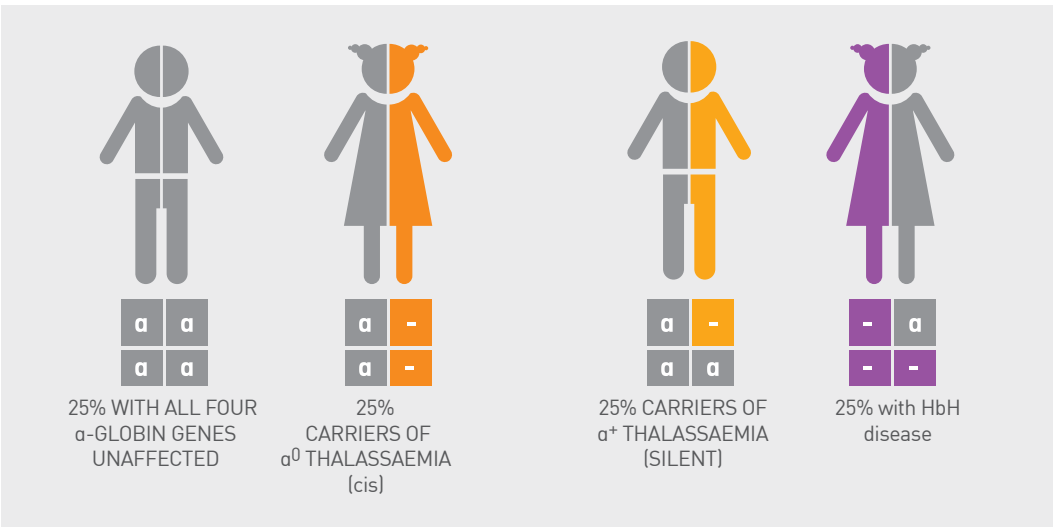
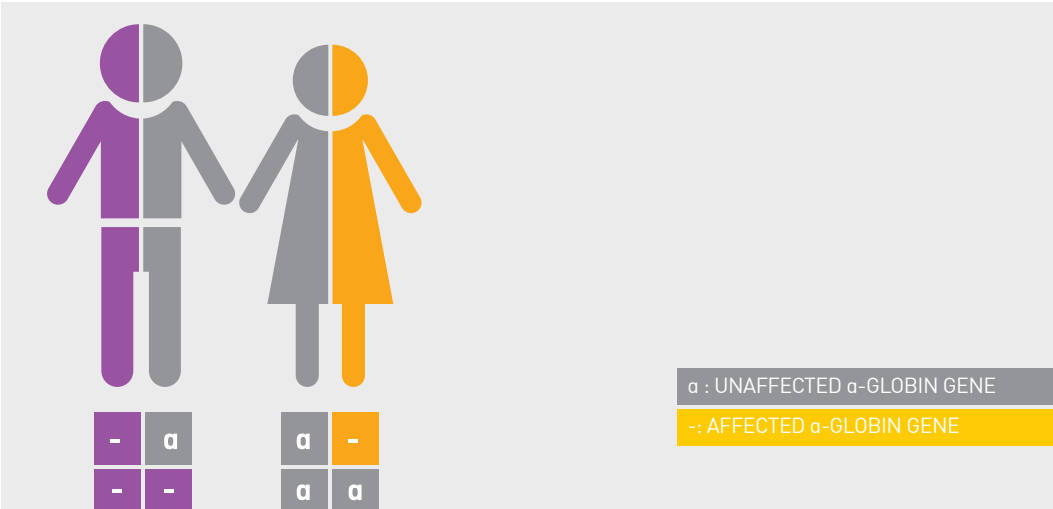
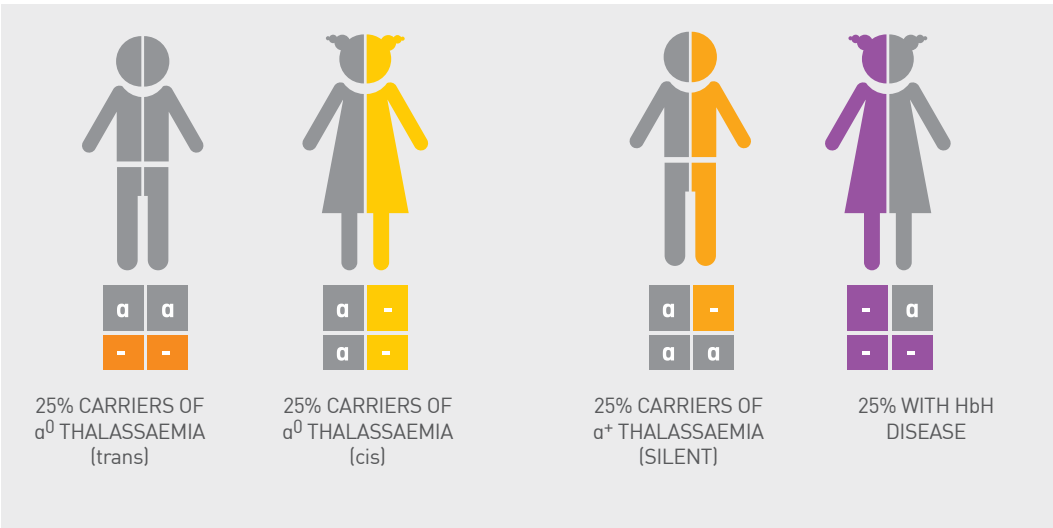


FIG. 12

ONE PARENT WITH HbH DISEASE AND THE OTHER A CARRIER OF α^+ THALASSAEMIA (SILENT)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:



ii. When one parent has HbH disease and the other is a carrier of α^0 thalassaemia (cis)

At each pregnancy there is:

- one-in-four (25%) chance for the child to be a carrier α^+ thalassaemia (silent)
- one-in-four (25%) chance to be a carrier of α^0 thalassaemia
- one-in-four (25%) chance to be affected by HbH disease, and
- one-in-four (25%) chance to have α -thalassaemia major (Hydrops Foetalis). (FIG. 13)

iii. When one parent has HbH disease and the other one is a carrier of α^0 thalassaemia (trans)

At each pregnancy there is a one-in-two (50%) chance of being a carrier of α^0 thalassaemia or being affected by HbH disease. (FIG. 14)

iv. When both parents have HbH disease

At each pregnancy there is:

- a one-in-four (25%) chance that their child will have all four α -globin genes affected and will be born with α -thalassaemia major (Hydrops Foetalis),
- a one-in-two (50%) chance to have HbH disease with three out of four α -globin genes affected, and
- a one-in-four (25%) chance to be a carrier of α^0 thalassaemia.

It is also possible for α -thalassaemia to combine with other rare variants affecting the α -globin gene. Consult your physician or genetic counsellor, or write to TIF in case you need information about any combinations that are not described in this book.

HbH DISEASE

The condition whereby a child inherits from its parents only one unaffected α -globin gene and three (3) affected ones is associated with a greatly reduced production of α -chains.

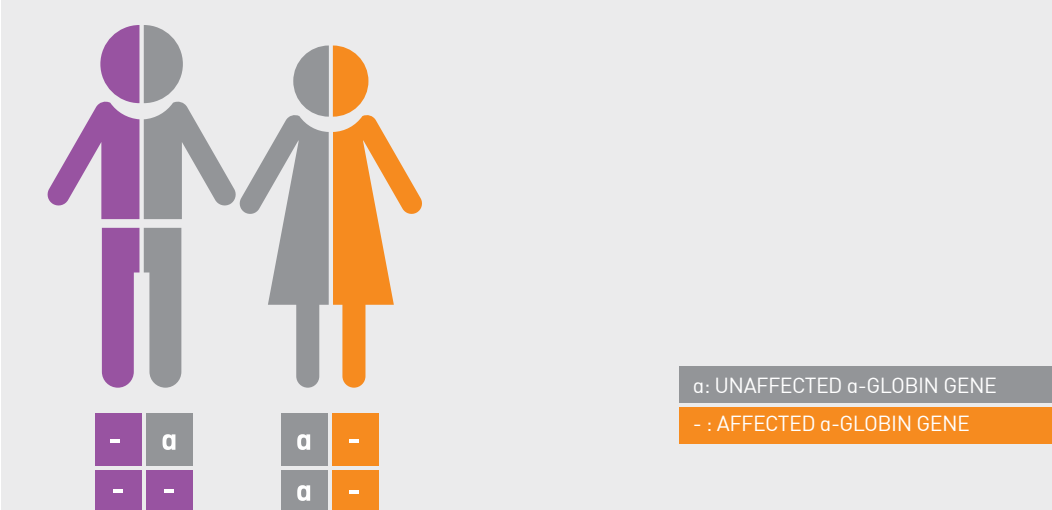
In this situation, the excess β -chains, which continue to be produced by the unaffected β -globin genes, cannot pair up with α -chains to produce adult normal haemoglobin (HbA). Instead, the free β -chains join together to form a new haemoglobin (β_4) in the patient's blood, known as HbH.

Although this is not the adult normal haemoglobin found in human adult red cells, HbH has the capacity, like adult normal haemoglobin (HbA), to deliver oxygen efficiently to the tissues. It is, however, a relatively unstable molecule, and its continuous breakdown results in early death (breakdown) of red cells (haemolysis), which can lead to a moderate to severe anaemia in the affected individual and to other related health problems, such as bone deformation, fatigue, formation of gall stones and enlargement of the spleen, which may vary from mild to severe.

The anaemia is mild in most patients with HbH disease. They generally require no treatment however, such as blood transfusion, and they usually lead a normal life. In some cases, the anaemia may be severe and aggravated by certain factors, such as infection, pregnancy or when taking specific medication. As a chronic disorder, HbH disease requires a general annual check-up, follow-up, and, if and when needed, medical treatment in a specialized centre.

FIG. 13

ONE PARENT WITH HbH DISEASE
AND THE OTHER A CARRIER OF α^0 THALASSAEMIA (cis)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

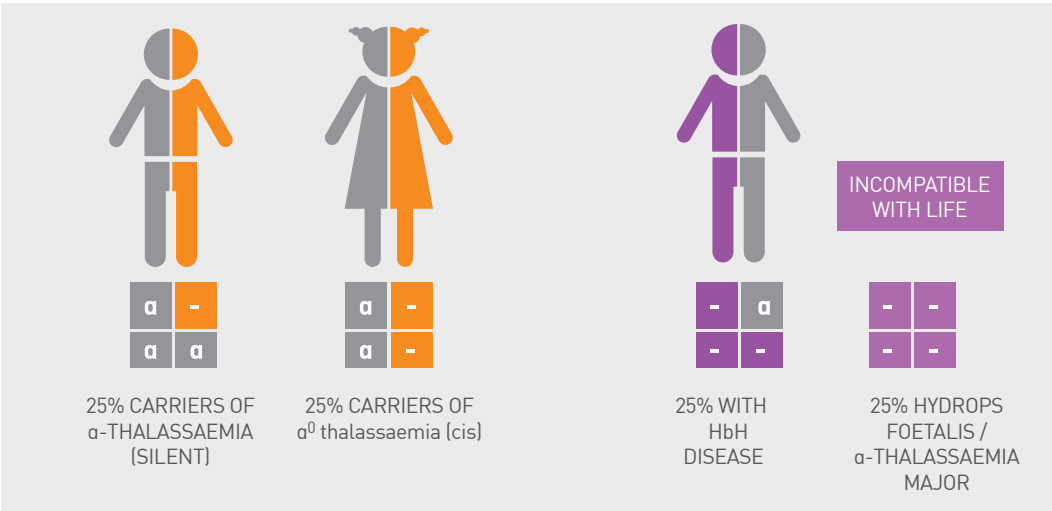
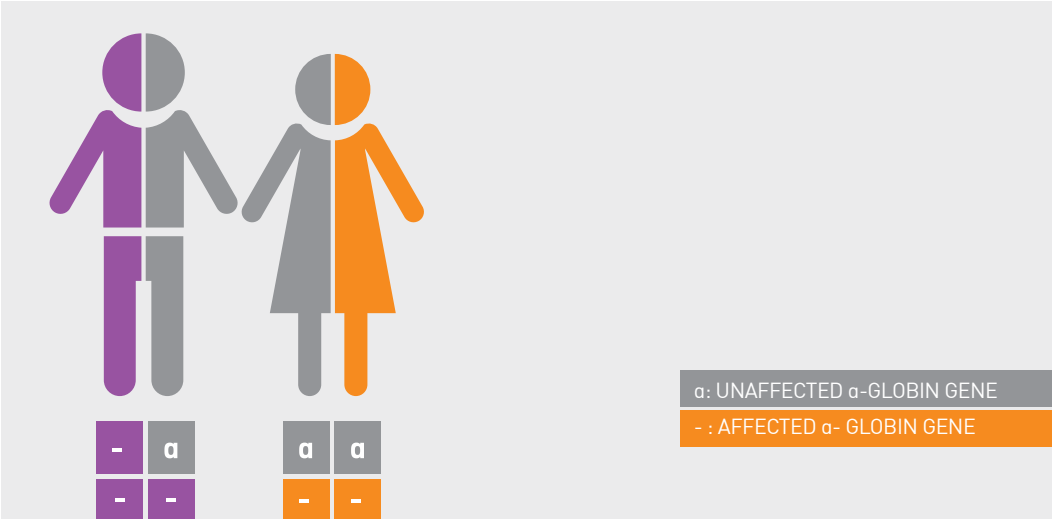


FIG. 14

ONE PARENT WITH HbH DISEASE
AND THE OTHER A CARRIER OF α^0 THALASSAEMIA (trans)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:



HYDROPS FOETALIS (α -THALASSAEMIA MAJOR)

In this situation, the body cannot produce any α -chains and therefore adult normal haemoglobin (HbA), which requires the coupling of both α - and β -chains, cannot be produced. Instead, a different type of chains is produced, called γ -chains. These chains make up the haemoglobin of the embryo (HbF), and when joined together they form another type of haemoglobin, called HbBarts. This, however, does not have any oxygen-carrying capacity and thus cannot sustain life.

Hydrops foetalis results in severe anaemia that affects the baby while in the womb, before birth. An affected foetus appears initially to grow normally, but gradually in the course of pregnancy develops heart failure, which leads to the swelling of the foetus and the placenta and to a marked increase in the volume of the amniotic fluid (hydramnios). This often leads to premature birth and the baby is usually dead (stillborn) at the time of delivery. The mother may also develop high blood pressure and may have difficulty in delivery. She also has a danger of bleeding after birth, if the contents of the womb are retained. As the life and wellbeing of the mother are at risk, early detection and prevention is essential in this case.

In some extremely rare cases, in-utero blood transfusion has allowed the birth of children with Hydrops Foetalis, who then, however, require lifelong blood transfusion and special medical care.

INHERITED α - AND β -THALASSAEMIA GENES

Alpha thalassaemia may be inherited along with β -thalassaemia and abnormal haemoglobins (structural variants) from carrier parents. In particular, the presence of α -thalassaemia carrier status in patients with β -thalassaemia major may contribute positively to the clinical outcome of β -thalassaemia major.

Can serious haemoglobin disorders be prevented?

Carrier couples who know of the risk for their children can obtain reliable, detailed and up-to-date information from specialized centres, national health authorities and patients/parents' support groups on all severe forms of Haemoglobin disorders to discuss their prevention and today's treatment requirements as well as inform them on any other aspect.

For the majority of couples at risk for HbH disease, prevention is not necessary, since this condition, despite possible complications such as mild anaemia and gallstones, is compatible with a long and good quality life. If there are reasons to expect a more severe condition, then the doctor will discuss preventive measures with the parents.

However, if there is risk of Hydrops Foetalis, prevention based on prenatal diagnosis and termination of pregnancy is imperative, because of the risk to the mother's health.

LABORATORY TESTING TO ESTABLISH WHETHER ONE IS A CARRIER

Laboratory testing for thalassaemia and other haemoglobinopathies includes a routine blood test, known as a Complete Blood Count (CBC), which involves measuring the level of haemoglobin and other parameters, such as Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH). These will be lower in individuals carrying the α and β -thalassaemia genes and some other abnormal haemoglobins.

However, the α -thalassaemia carrier status is difficult to confirm using the above-mentioned laboratory parameters, and even when using Haemoglobin Electrophoresis, which is the method that separates and measures the various types of haemoglobin and which in the majority of cases is used to diagnose and confirm β -thalassaemia and SCD. Diagnosis of α -thalassaemia is frequently made by exclusion of other possible causes. Genetic or DNA-based testing alone can give a definitive diagnosis and is also essential for the differentiation between the different kinds of α -thalassaemia carrier states (α^+ or α_0 – trans/cis)

HOW IS HbH DISEASE DIAGNOSED?

A child born with HbH disease will show no visible signs of the disease but may develop jaundice early in life. The baby may be diagnosed if a neonatal screening programme is available in the country where the family lives. The diagnosis is difficult to suspect if the parents have not been tested, if no prenatal tests were carried out, and if there is no other affected child in the family. It is possible to diagnose HbH disease at a very early stage, but only by means of genetic, DNA-based tests that can identify the genes the child has inherited from each parent (see further below). Other methods include:

- i. **Measurement of common haematological parameters by electronic equipment – red cell counters.** HbH disease is suggested where the size and volume of red blood cells and the concentration of haemoglobin inside them are significantly reduced, and the haemoglobin levels are low.
- ii. **Blood film and RBC morphology.** Observed under a microscope, the red blood cells of an individual with HbH disease appear paler (hypochromic), smaller (microcytic) and significantly variable in size (poikilocytosis). These changes are more marked in individuals with HbH disease than in carriers of α -thalassaemia.
- iii. **Chromatographic methods (Electrophoresis or HPLC).** These are laboratory processes that separate the different proteins that make up a haemoglobin molecule – such as HbA, HbA₂, and HbF. HbH forms a separate fraction and is detected at levels which may vary between 1-40% (usually 8-10%). HbA₂, which usually accounts for up to 3% of common adult haemoglobin, will be reduced (by 1-2%).
- iv. **Genetic or DNA-based methods.** These are specialized laboratory methods that can confirm the diagnosis and provide more specific information concerning the mutations (the genetic changes) that cause HbH disease.

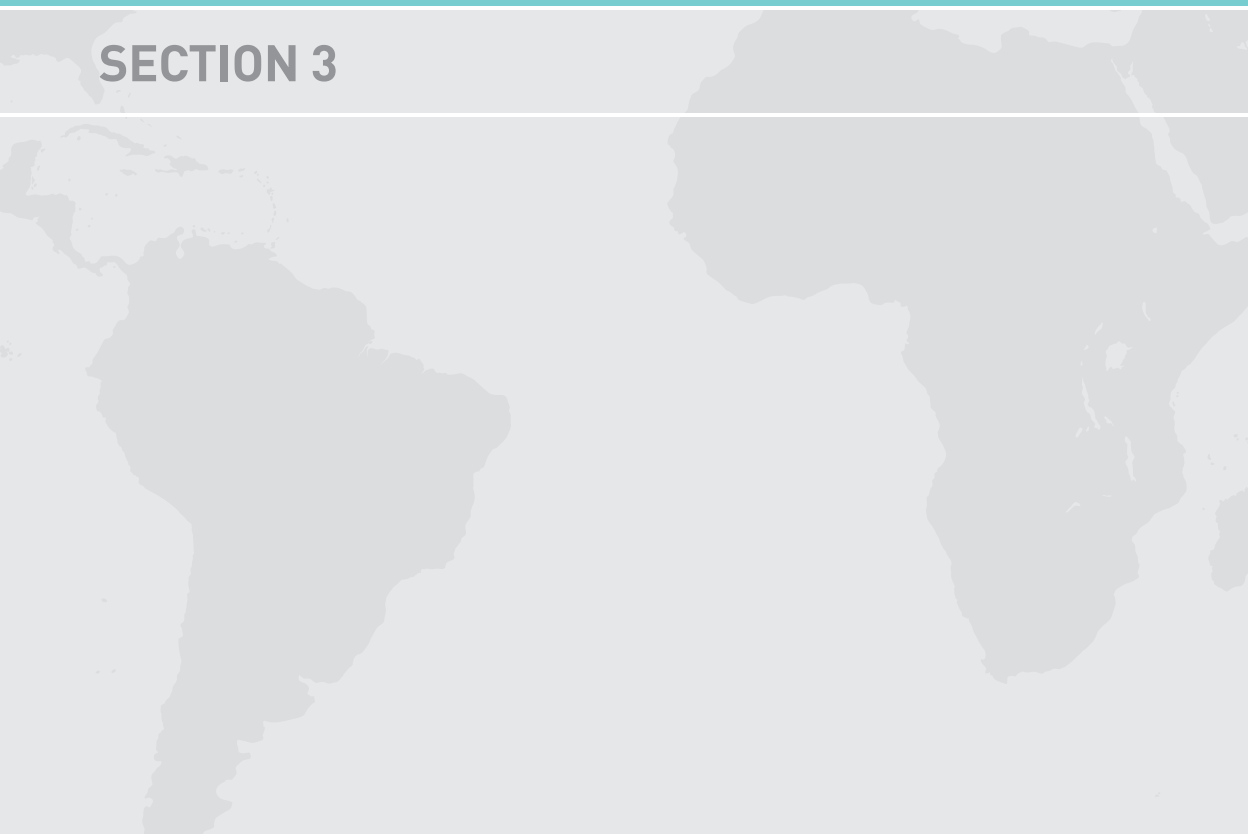
LABORATORY TESTING OF FOETUS FOR Hb-BART'S – HYDROPS FOETALIS (α -THALASSAEMIA MAJOR)

Apart from the types of tests described in previous sections (page 18) that can determine whether a foetus has a severe form of thalassaemia, ultrasound examination of a pregnancy “at-risk” at regular intervals is able to detect the changes characteristic of Hydrops Foetalis. Diagnosis will however be confirmed by either amniocentesis or CVS. These tests confirm whether the foetus is healthy, a carrier or affected. The couple may decide then how to proceed.

Analysis of the sample usually takes about a week. Termination of pregnancy can only be recommended if the life of the mother is endangered.

ABOUT
SICKLE CELL
DISORDERS

SECTION 3



HAEMOGLOBIN DISORDERS (HAEMOGLOBINOPATHIES)

SICKLE CELL DISORDERS

In contrast to the thalassaemias (alpha and beta), in which the defect in the gene results in reduced production of α - or β -globin chains, the sickle cell conditions result from gene defects which produce a different kind of haemoglobin protein, whose structure and function differ from the normal adult haemoglobin (HbA). It is thus referred to as a structural variant or abnormal haemoglobin. Moreover, because of the shape the red blood cells with this haemoglobin assume (i.e. crescent/sickle shape) in the homozygous state, i.e. the full blown disease, it is widely known as Sickle Cell Haemoglobin, or, in abbreviation form, HbS.

INHERITANCE PATTERNS OF HbS

HbS / HbA

When one of the parents is a carrier of HbS and the other parent carries the adult normal haemoglobin (HbA), there is at each pregnancy a one-in-two (50%) chance that the child will inherit HbS, i.e. he/she will be a carrier of HbS, just like one of his/her parents.

Other names describing an individual who carries the HbS include:

- Carrier of Haemoglobin S (HbS)
- Carrier of the sickle cell trait
- Individual heterozygous for HbS
- Sickle cell carrier
- Carrier of Sickle Cell Disease

HbS/HbS

If both parents are carriers of HbS, there is at each pregnancy a one-in-two (50%) chance that the child will also be a carrier of HbS, a one-in-four (25%) chance that the child will be completely unaffected, i.e. have adult normal haemoglobin (HbA) and a one-in-four (25%) chance that the child inherits HbS from both of its parents (HbSS). This child will develop the full-blown disease known as sickle cell disease (SCD), or otherwise known as sickle cell disorder or sickle cell anaemia or homozygous sickle cell disease. (FIG. 15)

HbS/HbSS

If one parent is a carrier of haemoglobin S (HbS) and the other is a patient with sickle cell disease (HbSS), at every pregnancy there is a one-in-two chance (50%) that the child will be an HbS carrier, and a one-in-two chance (50%) that the child inherits the full blown sickle cell disease (HbSS or SCD).

HbS/ β -thalassaemia

If one parent is a carrier of HbS and the other parent is a carrier of β -thalassaemia, at each pregnancy there is a one-in-four chance (25%) that their child inherits unaffected, fully functional haemoglobin in its genes and have adult normal Haemoglobin (HbA), a one-in-four chance (25%) that the child will be a carrier of HbS, a one-in-four chance (25%) that the child will be a carrier of β -thalassaemia and a one-in-four chance (25%) that the child inherits HbS from one parent and a β -globin gene from the other; this child will have what is referred to as compound haemoglobin disorder Haemoglobin S/ β -thalassaemia (HbS/ β -thal). (FIG. 16)

Other abnormal Haemoglobins: Haemoglobin C (HbC), Haemoglobin D Punjab (HbD Punjab), Haemoglobin E (HbE) and Haemoglobin O Arab (HbO Arab) are amongst the most common Haemoglobins, which, like HbS, differ in structure and function from the common normal adult

haemoglobin (HbA). These do not (i.e. in the homozygous state) result in clinically significant conditions, but when inherited with HbS, some of these may have significant clinical outcome.

HbS/HbC disease

If one parent is a carrier of HbS and the other is a carrier of another abnormal haemoglobin, haemoglobin C (HbC), at each pregnancy there is a one-in-four chance (25%) that the child inherits unaffected genes, an equal chance (25%) that the child will be a carrier of HbC, an equal chance (25%) that the child will be a carrier of HbS, and a one-in-four chance (25%) that the child inherits a compound haemoglobin disorder called haemoglobin S/haemoglobin C (HbS/C).

HbS/HbD Punjab

If one parent is a carrier of HbS and the other is a carrier of the abnormal variant haemoglobin D Punjab, at each pregnancy there is a one-in-four chance (25%) that the child inherits, as above, unaffected genes, a one-in-four chance (25%) that the child will be a carrier of haemoglobin D Punjab, an equal chance (25%) that the child will be a carrier of HbS and an equal chance (25%) that the child inherits the compound haemoglobin disorder referred to as haemoglobin D Punjab/haemoglobin S (HbS/HbD Punjab).

HbS/HbE

If one parent is a carrier of HbS and the other is a carrier of HbE, at each pregnancy there is a one-in-four chance (25%) that the child will inherit, as above, unaffected, fully functional genes, a one-in-four chance (25%) that the child will be a carrier of HbE, an equal chance (25%) that the child will be a carrier of HbS and an equal chance (25%) that the child inherits the compound haemoglobin disorder called Haemoglobin S/Haemoglobin E (HbS/HbE).

HbS/HbO Arab

If one parent is a carrier of haemoglobin S and the other of haemoglobin O Arab, at each pregnancy there is a one-in-four chance (25%) chance that the child will inherit, as above, unaffected genes an equal chance (25%) chance that the child will be a carrier of haemoglobin O Arab, an equal chance (25%) that the child will be a carrier of sickle cell disease and an equal chance (25%) chance that the child inherits the compound haemoglobin disorder called Haemoglobin S/Haemoglobin O Arab (HbS/HbO Arab).

ABOUT HbS CARRIERS

Carriers of HbS do not have a disease. They have no physical or mental symptoms and do not require a special diet, medical advice or treatment. Their red blood cells are usually similar in size to those of non-carriers, since the quantity of haemoglobin is not reduced. Smaller red cells (microcytic) are sometimes seen in those carriers of HbS who have co-inherited α -thalassaemia, a combination common in many populations. Otherwise, the red cells examined under the microscope may be indistinguishable from red cells of non-carriers. Sometimes, altered shapes (poikilocytosis) and cells with pointed ends are seen, but the typical sickle-shaped cells, characteristic of this disorder in the full blown disease (HbSS), are not often seen in HbS carriers. The carrier status cannot become a disease over time. Indeed, most individuals will be unaware that they are carriers unless specifically tested. Carrying haemoglobin S has no effect on health, length or the quality of life. The rare exception to this occurs when a carrier is in a situation of severe lack of oxygen, in which case, pain and blood vessel blockage may be experienced.

FREQUENTLY ASKED QUESTIONS ABOUT CARRIERS

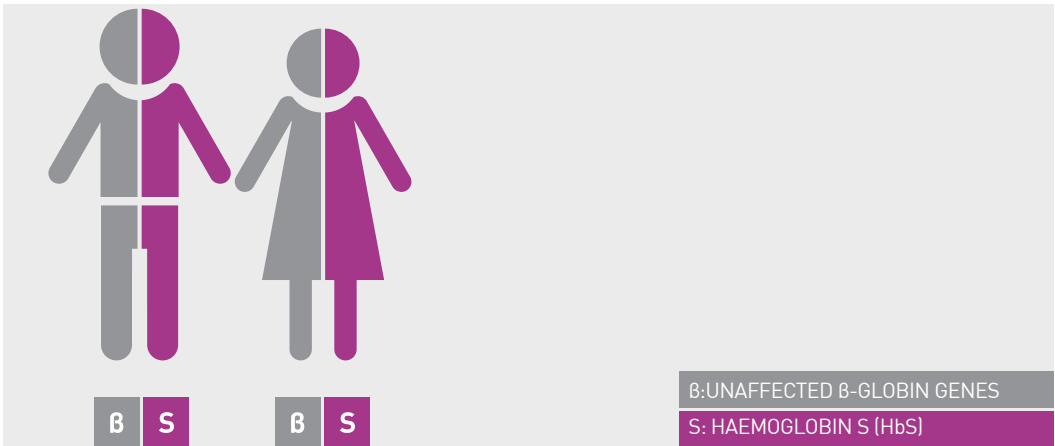
What about pregnant women who are carriers?

Like other pregnant women, those who carry HbS can become iron deficient and may need extra iron. The anaemia will improve after the baby is born. HbS carrier pregnant women are also more prone to urine infections, as compared to non-carriers or carriers of other haemoglobin disorders.

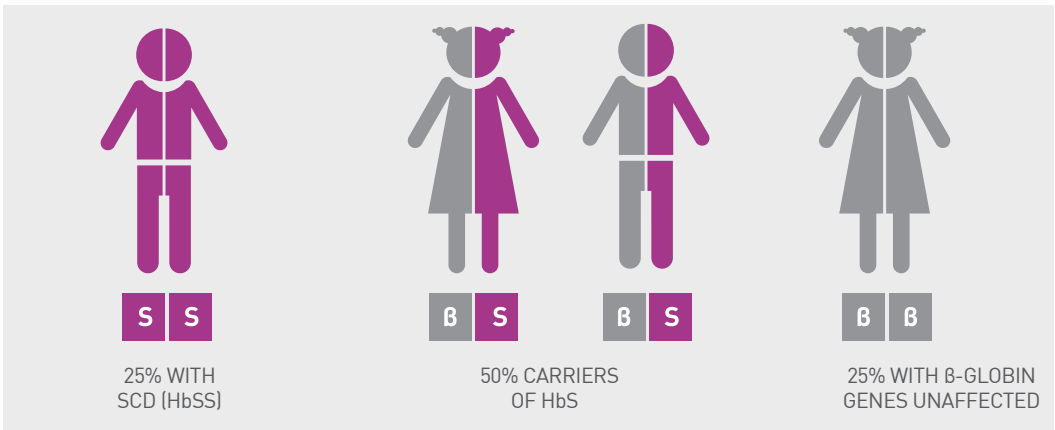
Like β - & α -thalassaemia carriers, a person who carries HbS will always carry it in his/her genes throughout his/her life, and the carrier status cannot be acquired or transmitted through the environment, transfusion or other means by which people become infected. However carriers who have inherited HbS from their parents could pass it on to their children through their genes.

FIG. 15

BOTH PARENTS ARE CARRIERS OF HAEMOGLOBIN S (HbS)



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:



Can carriers donate blood?

Carriers may be suitable blood donors if their haemoglobin level meets the national donor inclusion criteria.

How does one know whether he/she is a HbS carrier?

In most cases, simple but specific laboratory tests can identify whether a person is a carrier of HbS or of any other haemoglobin disorder.

LABORATORY TESTING

Laboratory testing for sickle cell carrier state, as for other haemoglobinopathies, includes a routine blood test known as a Complete Blood Count (CBC), which involves measuring the level of haemoglobin and other parameters, such as the Mean Corpuscular Volume (MCV), and the Mean Corpuscular Haemoglobin (MCH), both of which may be within normal range in HbS carriers, in contrast to those carrying α - or β -thalassaemia.

Another test that is used to determine the carrier state includes a laboratory process known as haemoglobin electrophoresis, which enables the measurement of the quantity of the major (HbA) and minor (HbA₂) components of adult haemoglobin, foetal haemoglobin (HbF) and HbS. In the case of HbS carriers, the HbS fraction will constitute up to 40% of the total haemoglobin. The presence of HbS may be confirmed by very simple tests in a test tube (e.g. Solubility Test).

Other ways to diagnose HbS include Isoelectric Focusing (IEF), which is another kind of electrophoresis, High Pressure Liquid Chromatography (HPLC) and Capillary Chromatography, all of which are considered today as methods of reference for screening as well as for confirming the diagnosis of haemoglobinopathies.

If the above tests are inconclusive, especially if combinations with other abnormal haemoglobins or thalassaemias are involved, and do not allow the laboratory scientists to provide a confirmed diagnosis, other more specialised tests are available. These are genetic tests, i.e. tests which examine the genetic material – the DNA of the blood of the individual but also often of other members of the family.

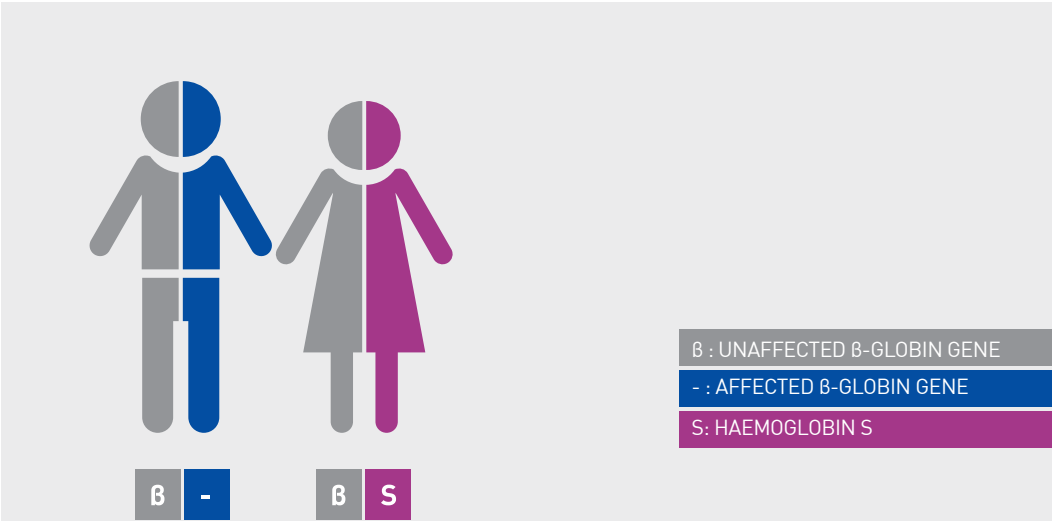
CLINICAL OUTCOME OF SICKLE CELL DISEASE AND THE COMPOUND HAEMOGLOBIN DISORDERS

What is sickle cell disease?

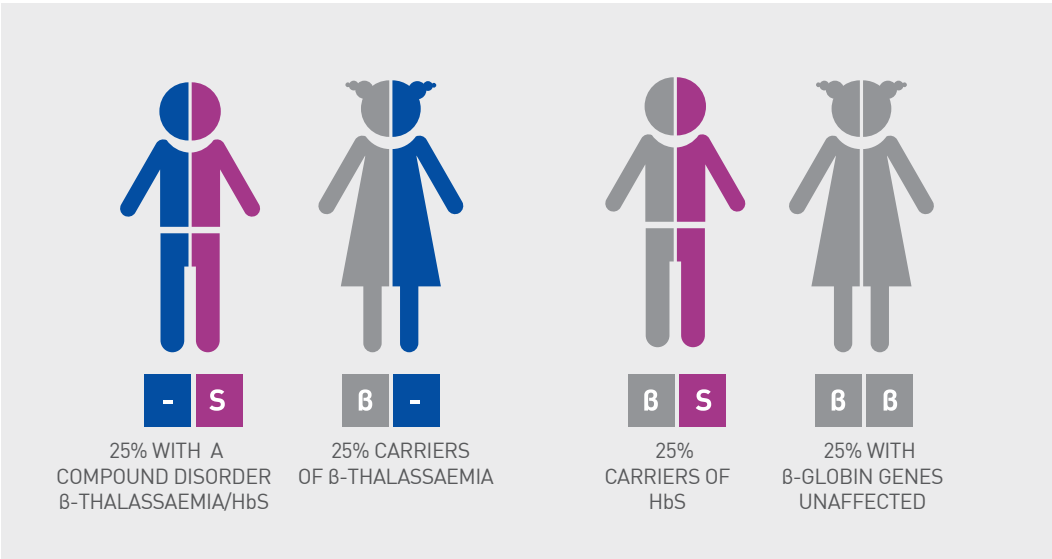
In this condition, almost all of the haemoglobin in the patient's blood is HbS. The red cells which contain this abnormal haemoglobin will change in shape from a biconcave disc to a crescent, or sickle shape, but will also lose flexibility, becoming considerably more rigid. This means that they cannot change their shape easily, as red cells with adult normal Haemoglobin (HbA) do. As a consequence, passage through small blood vessels is difficult. Loss of flexibility can become serious in conditions of lack of oxygen or in the case of an infection with fever, or when patients with SCD become dehydrated. In these situations, the sickle red cells greatly increase in number and can block the passage of blood (sickling crisis). As a result, the patient feels pain in the area of the body which is not receiving blood. Such events can be severe enough to damage tissues in joints, spleen, kidneys and even the brain.

FIG. 16

ONE PARENT IS A CARRIER OF HbS AND THE OTHER OF β -THALASSAEMIA



FOR EVERY PREGNANCY THE CHANCES ARE FOR THE CHILDREN TO BE:

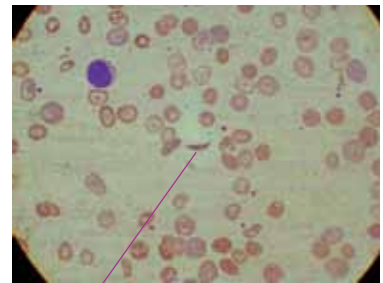


In addition, as these altered red cells (sickle cells) do not survive in the circulation of blood for as long as normal cells do, and are continuously destroyed, the patients experience a degree of anaemia, which may become severe under certain circumstances, leading to a need for blood transfusion.

Basic indications for the need of red cell transfusion in Sickle Cell Disease are the severity of the anaemia and vascular occlusion. Patients with Hb (Haemoglobin) levels above 7g/dl do not require transfusion. In the case of a vascular occlusion, the aim of transfusion is to prevent or abort intravascular sickling of red cells by means of increasing blood volume or by replacing circulating sickled cells with normal red cells. Exchange transfusion is indicated in programmed major surgery, in eye operations and for the prevention or management of acute vascular occlusion. If given regularly, transfusion will suppress the production of sickle cells by the patients' bone marrow.

As a chronic disorder, sickle cell disease requires treatment in specialized centres aimed at both preventing and managing complications, including the prevention of infections by means of immunizations and the management of pain, which may be severe enough to require hospitalization.

In order to prevent some of the complications effectively, it is necessary to have the patient under continuous observation from early childhood, and in this context a policy of newborn screening is recommended, so that affected children may be identified and followed up from birth.



Sickle Cell

HbS in combination with β -thalassaemia & other abnormal Haemoglobins

HbS/ β -thalassaemia is the most common compound haemoglobin disorder. It has a clinical outcome similar to sickle cell disease, which is very different from β -thalassaemia major/intermedia, and which consequently is managed in the same way as sickle cell disease and as described above. The clinical severity of HbS/ β -thalassaemia varies just as in SCD.

HbS/HbC, HbS/HbD Punjab, HbS/HbE and HbS/HbO Arab are sickle cell disorders. They are rare and are generally of a mild type. Some persons, however, may develop various degrees of anaemia and may occasionally have attacks of severe pain in joints or other parts of the body. A few may even experience severe health problems and may need frequent admissions to hospital. People with these disorders should attend a sickle cell clinic regularly for a check-up and advice.

At present, it is not possible to predict whether a particular couple will have children with mild, moderate or severe HbS/HbC, HbS/HbD Punjab, HbS/HbE or HbS/O disorder. It is possible, however, to test a foetus for these disorders early in pregnancy. It is best if "at-risk" couples see a specialist or genetic counsellor to discuss their options. This should ideally be before starting a pregnancy, or at least as early in pregnancy as possible.

DEVELOPMENTS IN THE MANAGEMENT OF SICKLE CELL DISEASE

Sickle cell disease affects people in different ways and with variable severity. Some people are mildly affected, whilst others are severely affected, variability occurring even amongst members of the same family.

The reasons for this are not always clear but several contributing factors have been linked with the severity of sickle cell disease. For example, the level of foetal haemoglobin (HbF), which some people continue to produce well into adulthood, is important. Normally, the level of haemoglobin F falls to about 1% by the end of the first year of life and stays at this level right through adulthood. Children with sickle cell disease, however, may go on producing higher than usual levels of haemoglobin F for a longer time. Levels of HbF above 7% appear to be related to fewer sickling crises and fewer complications of sickle cell disease. The level of HbF may be boosted by the use of certain drugs, and this is currently considered as the most promising method for reducing sickling of the blood cells. The advantage of foetal haemoglobin is that it does not have the capacity to sickle and its presence prevents red blood cells from sickling.

Hydroxyurea

Several drugs have been shown to increase the production of foetal haemoglobin. Of these, Hydroxyurea is the most promising and the one that is currently being prescribed. There is good evidence now that Hydroxyurea reduces sickling crises and the need for blood transfusions, although it may not be suitable for all patients. Hydroxyurea though has the potential to reduce bone marrow activity, and this as a consequence increases the risk of infection. It should not be used for patients who are likely to become pregnant, or for those who have difficulty following or adhering to the instructions given for their treatment.

Careful follow-up is needed, with regular visits to the clinic. Hydroxyurea is not a cure for sickle cell disease, but it is an effective treatment for preventing or reducing sickling crises. Its effect will only last for as long as the person is taking the drug.

Haemopoietic stem cell (HSCT) including bone marrow transplantation (BMT)

This is the only cure for sickle cell disease at the moment. Transplantation involves taking normal stem cells from a 'matched' relative or donor and giving them to the person with sickle cell disease. This is done after the patient has had treatment with strong medicines to wipe out the patient's own bone marrow, which is responsible for producing the abnormal sickle red blood cells.

Whilst this treatment has been performed successfully on many patients with sickle cell disease, there are several problems involved. It is best done using a matched brother or sister, ideally before the patient develops any complications. As the severity of sickle cell disease can be so variable, it is difficult to justify a high-risk treatment like BMT, where some patients may die, and some may survive but with long term problems. It is essential to carefully select for BMT those that are likely to benefit most or those that are at higher risk of developing serious complications. For example, one test which measures the blood flow in the brain (Transcranial Doppler) can identify patients at-risk for developing a stroke, and so may be used to identify potential candidates for transplantation. The risks and benefits of transplantation must be assessed in each individual case.

As in the case of β -thalassaemia major, it is often difficult to find a relative with a fully-matched bone marrow, making this curative method applicable only to a relatively small proportion of patients (around 20-25%). Rates of success and complication-free survival vary and depend mainly on the patient's good clinical condition prior to BMT and the expertise of the centre.

Gene Therapy

In the future, it is likely that gene therapy will be offered for SCD, in the course of which the defective gene is replaced by a normal one. Research in this area has made significant advancements in recent years, but application of gene therapy to humans for a final cure has still a long way to go. To find out more about these developments, talk to your counsellor or doctor or write to TIF.

HOW IS SICKLE CELL DISEASE DIAGNOSED?

A child born with sickle cell disease will show no visible signs of the disease. The baby may be diagnosed if a neonatal screening programme is available in the country where the family lives. Early diagnosis is particularly important to prevent complications. Screening of newborns is the best way to achieve this, if the parents have not been tested, no prenatal tests were carried out, and there is no other affected child in the family.

It is possible to diagnose sickle cell disease at this very early age by means of simple but specific laboratory tests, such as those described earlier in this booklet for the diagnosis of the sickle cell carrier state (page 45).

Special features seen in the laboratory diagnosis of SCD include the presence of sickle-shaped red cells, which constitute around 10% of all the cells, and the presence of HbS reaching levels of around 90-95% in one of the laboratory analyses. When more specific information is needed to clarify or confirm diagnosis, genetic tests are used (as described earlier for confirming the carrier state).

CAN SICKLE CELL DISEASE AND OTHER HAEMOGLOBIN DISORDERS BE PREVENTED?

Carrier couples who know of the risk for their children have today a number of choices. They can take steps to make sure that they have healthy children, and that affected children have the best possible care from birth. Their choices are certainly not simple. Every couple, and every citizen in general can obtain reliable, up-to-date information from national health authorities, reference/academic centres and patients/parents support groups. Parents who are both carriers, referred to as "at-risk" couples, should know well their risk as early as possible, so that they have enough time to make the decisions that they feel are right for them.

Such couples need to see a haemoglobinopathies specialist (or a specialist genetic counsellor), who will inform them of the exact nature of the risk and what choices are available to them, including prenatal diagnosis in case of a pregnancy to establish whether the foetus is affected or not. An obstetrician specializing in prenatal diagnosis may also provide more detailed information on the procedures available, their risks and benefits, and the possible choices that a couple may make to avoid having an affected child.

The methods used for prenatal diagnosis of Sickle cell disease are the same as those described for β -thalassaemia.

GENERAL EPIDEMIOLOGICAL INFORMATION

Thalassaemia was originally considered to be a disease limited to the Mediterranean region, hence its names Mediterranean Anaemia and Thalassaemia. The latter is a compound Greek word from thalassa (meaning sea) and anaemia (meaning poor blood), and describes the anaemia most commonly observed on individuals living by the sea and more specifically the Mediterranean Sea. The first report came from Thomas Cooley in 1925, a paediatrician from USA who published his observations for patients with origin from countries around the Mediterranean Sea. Hence the name Cooley's Anemia is also used for this disorder. It is now known that thalassaemia syndromes occur widely throughout many parts of the world, across southern Europe from Portugal to Spain, Italy and Greece, in a number of Eastern European countries, the Middle East through to Iran, Pakistan, India, Bangladesh, Thailand, Malaysia, Indonesia and southern China, as well as countries along the north coast of Africa and South America. Thalassaemia is particularly prevalent in areas in which malaria is or was endemic.

It is believed that, in these areas of the world, some people underwent a slight change in their genes – what we refer to in science as a genetic adjustment, or a mutation, which led to important changes in the environment of the red cells. These changes prevented malaria parasites from growing and multiplying in the red blood cells which constitute their hosts, thus giving these people a survival advantage over those in whom this genetic change did not occur. Carriers of thalassaemia (α and β), as well as carriers of other Hb disorders, such as sickle cell, were thus better able to survive malaria than non-carriers. The number of carriers increased significantly over the years in malaria-endemic regions of the world, as large numbers of non-carriers died as a result of severe malaria infections.

For sickle cell disease, it is believed that the sickle cell abnormal haemoglobin originated in Africa, where it is most commonly encountered, while India is considered as an additional place of origin. From these areas, population migrations have taken the gene to almost all regions of the world. SCD is most prevalent in the indigenous population of Africa, and some Middle East and Mediterranean countries. Thalassaemia and Sickle Cell syndromes are collectively known as Haemoglobin Disorders or Haemoglobinopathies, reflecting the origin of their cause: the Haemoglobin (Hb) molecule in the red blood cells.

Population migrations and intermarriages between different ethnic groups have introduced thalassaemia and SCD in almost every country of the world, malaria-endemic or not, including northern Europe and other countries where thalassaemia and SCD did not previously exist and were not known in the indigenous population. According to current epidemiological data, about 7% of the global population carries an affected haemoglobin gene, with between 300,000 and 500,000 affected children born annually. More than 80% of these are born and live in the developing part of the world. About 70% of them have a sickle disorder, and the rest have thalassaemia syndromes. A significant number of affected children born in developing countries even today die undiagnosed, are misdiagnosed, receiving sub-optimal treatment, or left untreated altogether.

There is an urgent need to develop and improve national control programs of prevention and management, especially since today there is ample evidence, knowledge and experience for effective strategies.

National control programmes are urgently needed to improve prevention, reduce affected births and provide appropriate clinical management to improve the survival and quality of life of the patients with Hb disorders across the world.



Map of haemoglobin disorders over the years in malaria-endemic countries worldwide
 “Guidelines to the clinical Management of Thalassaemia 2000”



Countries affected by malaria before the establishment of control programmes

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17. Guidelines for the Management of non Transfusion Dependent Thalassaemia (NTDT) (2013)



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