Transition of Thalassaemia and Friedreich ataxia from fatal to chronic diseases

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Abstract

Thalassaemia major (TM) and Friedreich's ataxia (FA) are autosomal recessive inherited diseases related to the proteins haemoglobin and frataxin respectively. In both diseases abnormalities in iron metabolism is the main cause of iron toxicity leading to increased morbidity and mortality. Major efforts are directed towards the prevention of these diseases and also in their treatment using iron chelation therapy. Both TM and FA are endemic in Cyprus, where the frequency per total population of asymptomatic heterozygote carriers and patients is the highest worldwide. Cyprus has been a pioneering nation in preventing and nearly eliminating the birth of TM and FA patients by introducing an organized health structure, including prenatal and antenatal diagnosis. Effective iron chelation therapy, improved diagnostic methods and transfusion techniques as well as supportive therapy from other clinical specializations have improved the survival and quality of life of TM patients. Despite the tiresome clinical management regimes many TM patients are successful in their professional lives, have families with children and some are now living well into their fifties. The introduction of deferiprone led to the elimination of cardiac failure induced by iron overload toxicity, which was the major cause of mortality in TM. Effective combinations of deferiprone with deferoxamine in TM patients caused the fall of body iron to normal physiological ranges. In FA different mechanisms of iron metabolism and toxicity apply to that of TM, which can be targeted with specific iron chelation protocols. Preliminary findings from the introduction of deferiprone in FA patients have increased the hopes for improved and effective therapy in this untreatable condition. New and personalised treatments are proposed in TM and FA. Overall, advances in treatments and in particular of chelation therapy using deferiprone are transforming TM and FA from fatal to chronic conditions. The paradigm of Cyprus in the prevention and treatment of TM can be used for application worldwide.

Core tip: Thalassaemia major (TM) and Friedreich's ataxia (FA) are inherited diseases related to iron toxicity, with high morbidity and mortality rates. Cyprus has the highest frequency of TM and FA worldwide. Prenatal diagnosis and other health policies almost abolished the birth of TM and FA patients in Cyprus. Deferiprone has increased the survival and quality of life of TM patients, who are now reaching normal life expectancy and it is also promising for FA patients. Personalised treatments are proposed for TM and FA. The Cyprus experience can be used as a paradigm for the prevention and treatment of TM worldwide.

INTRODUCTION

Thalassaemia major (TM) and Friedreich's ataxia (FA) are autosomal recessive inherited diseases with serious pathological complications, morbidity and mortality. Although the two diseases are genetically different they are both related to abnormalities in proteins of iron metabolism namely frataxin in FA and haemoglobin in TM[1-5].

Adult haemoglobin is composed of two alpha and two beta globin chains, each containing an iron molecule embedded in a protoporphyrin ring, which is responsible for the transport of oxygen to all cells of the body[1]. Frataxin is a mitochondrial matrix protein which functions in iron-sulfur cluster containing enzymes within the chain assembly responsible for respiration and energy transduction[3,4].

While frataxin is encoded by the gene of chromosome 9, the beta globin chains of haemoglobin are encoded by a single gene on chromosome 11 and the alpha globin chains of haemoglobin are encoded by two genes which are closely linked on chromosome 16[1,5,4].

In patients with TM, insufficient or no beta globin chains of haemoglobin are produced and the abnormal haemoglobin cannot deliver oxygen efficiently to the tissues. TM is a fatal disease if it is not treated with regular blood transfusions every 1-4 wk and chelation therapy[5].

FA is a progressive neurodegenerative disease with significant morbidity and has no effective treatment[6]. In FA patients the production of frataxin is severely reduced. Frataxin is a highly conserved mitochondrial matrix protein composed of 130 amino acids, has MWt 14.2 kDa and weakly binds iron[3,4]. In almost all FA patients there is an expansion of the guanine-adenine-adenine (GAA) trinucleotide in the first intron of both alleles of the frataxin gene. While in normal individuals the alleles of the frataxin gene have 36 or fewer GAA repeats, in FA disease the alleles have approximately 70 to more than 1200 to 1700 GAA repeats[3,4,6].

Major efforts have been taken worldwide for the control and reduction of births of TM and FA patients. Prenatal and antenatal diagnoses are increasingly being used in certain endemic areas and in ethnic groups for the prevention of these and other inherited diseases. Cyprus has the highest rate of asymptomatic heterozygote carriers and patients per population of both TM and FA by comparison to any other nation[7-11]. There is a 25% chance of TM or FA child being born from a pairing of asymptomatic heterozygote carrier parents. Within this context an effective prevention programme is needed for the containment of TM and FA in endemic areas like Cyprus.

Regarding patient treatment, recent developments involving primarily iron chelation therapy using the iron chelating drug deferiprone (L1) suggest that major advances have taken place in reducing morbidity and mortality in TM and also promising steps towards improvement in the treatment of FA[5,12]. The strategy responsible for these advances and the transition of these diseases from the fatal to the chronic stage can better be described using the paradigm of Cyprus[9]. Within this context the recent developments in the past few years in TM and FA in Cyprus have been reviewed with particular emphasis on the strategies regarding the health organisation structures, educational efforts, prevention measures and the overall clinical management. Related epidemiological and socioeconomic consequences are also described.

Special emphasis is also given to the mechanisms and pathogenesis of gross or focal iron overload toxicity in both TM and FA[5]. The treatment of gross or focal iron overload toxicity using chelation appears to be the major therapeutic option for decreasing the associated high mortality and morbidity observed in these two categories of iron metabolic diseases. The role of L1 is highlighted, since it is considered as one of the major factors in the transition of TM from a fatal disease to a chronic disease and also increases the prospects of effective treatment in FA[5].

EPIDEMIOLOGY, TREATMENT AND SURVIVAL OF THALASSAEMIA PATIENTS

TM is classified as a haemoglobinopathy, the most common group of inherited diseases in humans. More than 200 mutations of the haemoglobin genes have been reported, causing a range of pathological abnormalities from asymptomatic to fatal states[1,2]. Patients with TM can only survive if they are regularly transfused with normal red blood cells from appropriate blood donors. Iron accumulated from regular long term red blood cell transfusions is toxic to many organs and becomes fatal unless removed by chelating drugs. Iron overload toxicity in TM has one of the highest metal related morbidity and mortality rates globally[13].

The geographic distribution and prevalence of thalassaemia is mostly in developing countries found in the Mediterranean, Middle East and South East Asia, where over 90% of thalassaemia patients are born. More than 100000 thalassaemia babies is estimated to be born annually and there are 100 millions of thalassaemia heterozygote asymptomatic carriers worldwide[2]. In India alone the annual birth rate of TM is estimated at 9000[14].

Thalassaemia is considered as an orphan disease in the European Union (EU), the United States and other developed countries due to the low number of patients compared to the total population, which is mainly Caucasian[2,15-17]. The treatment of TM patients in EU countries (*e.g.*, Cyprus, United Kingdom, Greece and Italy) is supported by the state, while in most developing countries insufficient funds are available for their treatment.

Organisation structure and collaborating bodies involved with the coordination for the prevention diagnosis and treatment of thalassaemia in Cyprus
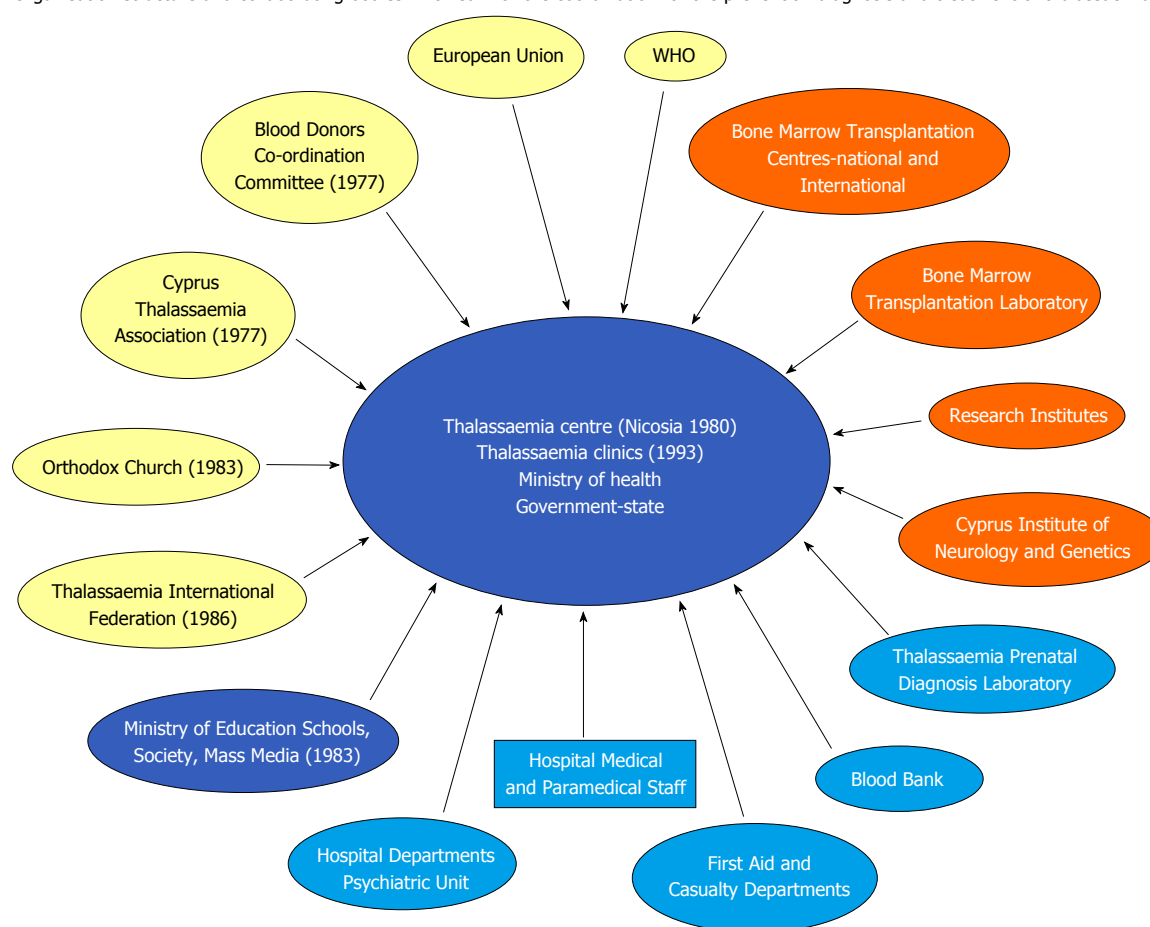

Figure 1 The organisational health structure and the chronological development of Thalassaemia in Cyprus. WHO: World Health Organization.

In the case of Cyprus, epidemiological data suggest that the indigenous population is numbered at around 800000 thousand and an extra 200000 thousand are mostly immigrants from developing countries working in Cyprus. The thalassaemia heterozygote frequency, which is associated mainly with the beta globin chain trait is 1 in 6 Cypriots. There are about 800 TM and thalassaemia intermedia patients ie (1 in 1000) who are regularly transfused and treated in specialist thalassaemia clinics in the main cities of Cyprus (Nicosia, Limassol, Larnaca, Paphos)[9].

Historically, the introduction of government policies to control thalassaemia came about after Cyprus gained independence in 1960. Before this period TM patients in Cyprus had a similar fate to TM patients in developing counties, where there is inadequate treatment including blood transfusions and chelation therapy[14,18,19].

Estimates from the health budgets for the treatment of TM patients were colossal for the size of Cyprus at the time of independence. Projections for the rate of birth of TM children and the associated expenditure on treatment predicted the collapse of the national health service or abandonment of the treatment due to unaffordable costs. The socioeconomic consequences for the families with TM children were devastating.

Based on these dilemmas the government of Cyprus

introduced a new policy about 35 years ago for the set up of a thalassaemia centre, an educational campaign for population screening for thalassaemia trait, for the need of prenatal diagnosis of affected mothers, in addition to better clinical management for existing TM patients. An outline of the organization health structure, the collaborating bodies involved in the prevention, diagnosis and treatment of thalassaemia in Cyprus as well as a historic perspective is shown in Figure 1.

The plan for the prevention of births of TM babies includes the option of prenatal diagnosis, which provides an opportunity to the pregnant mother to decide about the course of the pregnancy, usually before the 3rd month of pregnancy. In the case of birth of a TM child, the survival prospects are directly related to the treatment options available at the country of residence. Non transfused TM patients die from ineffective erythropoiesis of normal red blood cells and related complications usually by the age of 2-7 years. If regular red blood cell transfusions are available from matched blood donors, survival is expected to increase to about 15-20 years. At this stage TM patients usually die from excess iron accumulation and iron overload toxicity induced organ damage. Cardiac failure due to excess iron deposition in the heart is the major cause of death in regularly transfused TM patients

who do not receive adequate iron chelation therapy[20-23].

Life expectancy in TM increases following the application of effective chelation therapy within a period of a few years having started transfusions. A few compliant TM patients who adhered to the recommended protocol of sc deferoxamine (DF) (40-60 mg/kg per day, at least 5 d per week) were treated successfully since birth and have now exceeded 50 years of age. Compliance with sc DF for the majority of TM patients is poor and the average life expectancy is much shorter. Within this context, before deferiprone (L1) was introduced the mean life span of TM patients in the United Kingdom, was estimated to be 30-35 years[24]. In contrast, following the introduction of L1 in 1999, an improvement in compliance and efficacy as well as a reduction of cardiac deaths was observed in Cyprus and many other countries[25-29]. The prospects of compliance in relation to chelation therapy have also increased following the introduction of deferasirox (DFRA) in 2007. Chelation therapy with DFRA may mostly benefit TM patients who are intolerant or have other complications with both DF and L1 chelation therapy[18]. Several aspects are influencing the morbidity and mortality of TM patients worldwide including the efficacy, toxicity, availability and cost of all chelating drugs. In particular, access to these drugs may especially influence the overall survival of TM patients most of who live in the developing countries[18].

EPIDEMIOLOGY, TREATMENT AND SURVIVAL OF FRIEDREICH ATAXIA PATIENTS

FA is the most common autosomal recessive ataxia condition. It was identified by the German physician Nikolaus Friedreich in 1863 and only recently in 1996 the genetic mutation as well as the affected protein namely frataxin was identified and characterized[30-32]. This neurodegenerative inherited disease related to low production of frataxin appears to cause abnormalities in iron metabolism including the accumulation of iron in the mitochondria and consequential pathological complications of different severity in various organs. In particular, it causes progressive damage to both the central and peripheral nervous system resulting in a variety of symptoms ranging from gait disturbance and speech problems to diabetes and heart disease[3,4,33,34].

The geographic distribution and prevalence of FA is mainly in countries with Caucasian populations. It accounts for almost 50% of all the cases of hereditary ataxia. The FA carriers in Europe are estimated to be about 1:90, where in the United States it affects 1 in 50000 people. Apart from Europe and the United States, cases of FA have been recorded in the indigenous population of Mexico, Iran and India[35-37]. However, FA appears not to exist among the indigenous populations of sub-Saharan Africans, American Indians, Chinese, Japanese and Southeast Asians[38].

The FA carrier rate in Cyprus is estimated to be about 1:10, where in a cluster in the villages Kathikas and Arodhes in the Paphos district of Cyprus it is 1:5 or 1:6[10,39]. A total of 26 patients with FA were identified in Cyprus in a study in 2009[11].

There are individual variations in the onset and the severity of FA, which is characterised by progressive tissue and organ damage and significant morbidity. The onset of FA is early and almost always present before the age of 20 years. Loss of ambulation occurs about 15 years after the onset of the disease. More than 95% of patients are wheelchair bound by the age of 45 years. Frequent clinical findings of FA include the incoordination of limb movements, impairment of position and vibration, dysarthria, nystagmus, diminished or absent tendon reflexes, scoliosis, *etc*. In general, FA has progressive multipathological effects with a life span of approximately 30-40 years and the most frequently reported causes of death are diabetes mellitus and cardiomyopathy[3,4,33,34].

The socioeconomic consequences for the FA patients and their families and the costs associated with the treatment and care of these patients requires state intervention including steps for its prevention, diagnosis and treatment at the local level and worldwide. Within this context, preliminary scientific and epidemiological evidence prompted the introduction of government measures for the prevention or decrease in the number of births of FA patients in Cyprus similar to the adopted model of TM. These prevention measures were concentrated in the Paphos district where most of the patients and carriers of FA were previously identified and also to descendants from the Paphos district living elsewhere in Cyprus[11].

THE ROLE OF DIAGNOSIS AND PREVENTION OF THALASSAEMIA AND FRIEDREICH ATAXIA IN CYPRUS

Prenatal and antenatal diagnoses are widely used for the prevention of many inherited diseases with serious or fatal clinical complications, which are endemic in certain areas or ethnic groups. Both TM and FA belong to this category of diseases and Cyprus has the highest rate of asymptomatic heterozygote carriers of these diseases in comparison to any other nation. Within this context the prevention programme for TM started about 35 years ago whereas for FA it was recently adopted by the government in 2011 using the same guidelines as for TM.

In Cyprus the fate of TM patients prior to independence in 1960 was similar to that of a large majority of TM patients in the developing countries today, namely early death at 2-7 years due to lack of blood transfusions and other treatments. Following the independence of Cyprus and the initiation of a transfusion programme the survival rate of the TM patients increased. However, blood supplies were insufficient due to the high demand and the high annual birth rate of TM children at that period which was about 70 new cases per year[7].

The alarming rate of TM births and the socioeconomic consequences for the small population of Cyprus at that period prompted the initiation of new measures by the government in conjunction with institutions and organisations that could facilitate the process of prevention and the improvement of the treatment of patients.

Several initiatives including educational and publicity measures were taken to inform the general public of the campaign for the prevention of births of TM children and its implications in the families affected and society in general. Within this context a programme for the prevention of TM has been operating in Cyprus since 1973[7]. However, the major and most effective strategy for prevention was founded on thalassaemia trait compulsory screening for premarital couples. The strategy of compulsory screening was introduced in 1983, after an agreement between the government of Cyprus and the church, which carried out almost all weddings at that time. Accordingly a medical certificate was required by the church from all the premarital couples for confirming their thalassaemia status before proceeding to marriage.

In the meantime, the doctor managing the thalassaemia clinic where the diagnosis was carried out had to screen and inform the premarital couples of the possibility of having children with TM if they were both thalassaemia carriers and also to inform them of the available options to them including the option to decide the course of the pregnancy. Following marriage the prevention programme included genetic counselling and screening tests which were offered only for couples with thalassaemia trait, including chorionic villus sampling at about 11 wk of gestation of the pregnant mother[40,41]. If the test indicated a TM foetus, then the various options available were discussed and the final choice made.

Recent TM preventive measures for couples with thalassaemia trait included other possibilities, for example the pre-implantation diagnosis procedure[40]. This procedure was brought in to effect in Cyprus in the last 17 years and involves *in vitro* fertilisation for the analysis of early embryos and also the selection of an egg free of haemoglobin disorders. Following selection, further procedures include fertilisation of the egg in the laboratory and implantation into the womb[40,42]. Another possible option for future application is *in utero* allogeneic bone marrow transplantation.

The screening for thalassaemia trait is in most cases relatively simple and inexpensive, involving the electrophoresis of haemoglobin from blood samples[43-46]. Similarly, a blood sample is used in the case of FA screening but a more expensive DNA analysis is required to identify an FA carrier[47-49]. The strategy adopted for screening for the FA trait status involves in the first instance only persons with origins from the areas in Paphos where high prevalence of FA was identified[11].

The screening for thalassaemia carriers and the introduction of prenatal and antenatal diagnosis resulted in a significant drop in the number of births of patients with TM. The birth rate of TM children in Cyprus between

1970 and 1980 was estimated to be about 30-50 per year and a total of about 400 TM births were recorded. Following the implementation of the government and church policy on thalassaemia in 1983, the birth rate of TM babies between 1984 and 1994 dropped to about 25, *i.e.*, a rate 2-3 per year[7-9].

However, new trends have arisen in the last 20 years in Cyprus including major demographic changes, which had a major influenced in the birth rate of TM patients. It is estimated that 20% of the people residing in Cyprus are foreign workers and the total population has increased to approximately 1 million. Cyprus became a member country of the EU in 2004 and as a result of implementation of EU laws a medical certificate for thalassaemia is not obligatory for civic weddings, unlike church weddings[9]. Similarly, many couples are deciding to have TM children instead of abortion, because of emotional, religious and ethical reasons and also as a result of the newly improved treatments, which lead to an increase in the life expectancy of TM patients. As a result of these trends and also the marriage of Cypriots with non-Cypriot carriers the number of births of TM children has increased slightly in the last few years[50]. It is estimated that there were about 25 births of TM children from 2005 to 2011, *i.e.*, a rate of 3-4 per year. The trend on the birth rate of TM patients is set to persist at present, unless the law on civic weddings is changed and one similar to that of church weddings is adopted[9]. In the meantime, further reduction in the birth rate of TM children in Cyprus is expected following the recent introduction of other techniques such as that of the pre-implantation diagnosis. This procedure is costly but allows the selection of a non TM foetus by parents with thalassaemia trait or by parents where one is a TM patient and the other has the thalassaemia trait[42].

The prevention programme for FA is similar to TM but with slight modifications. The major difference is that the screening programme for FA carriers is concentrated in premarital couples in the Paphos district and individuals living in other areas of Cyprus but originating from the Paphos district. In a previous study involving the Paphos district a total of 1050 persons aged 18 and over were screened and 98 (9.33%) FA carriers were identified[11]. More FA carriers have been identified since 2011, when the government prenatal screening programme for FA on premarital members of couples originating from Paphos was initiated. In this case the partners of those identified as FA carriers were also screened.

While major efforts have been taken in Cyprus for the diagnosis of carriers of TM and FA and the prevention of births of TM and FA patients, there are still a small number of births with these inherited diseases and especially of TM patients every year. The small number of births of TM patients is related to cases of non prenatal screening and the choice of parents to have a TM child. Within this context the treatment of the newly born and existing patients is another continuous challenge in the day to day running of the thalassaemia clinics and the

Table 1 Biochemical and clinical monitoring of Thalassaaemia major patients
Determination of thalassaemia mutation and blood group (ABO, rhesus)
Vaccination: Flu, pneumonia, haemophilous influenza, meningitis a + c and hepatitis B
Cardiac: Physical examination, echocardiography, holter, stress test, muga scan, (once a year or earlier if required)
Liver and kidney function tests, urate, cholesterol, triglycerides, Ca, PO4, fasting glucose test and prothrombin time (every six months)
Blood glucose tolerance test and screening for hepatitis B antibodies, hepatitis C and HIV (every year)
Bone densitometry, chest X-ray, Endocrine (T3, T4, TSH, PTH), ophthalmology, ear, nose, throat and echo of the abdomen examination (every year)
Iron load estimation: Serum ferritin and Zn (every 3-4 mo), serum ferritin for patients with normal physiological iron range (every month), MRI T2 and T2* of the heart and liver (once a year)
Full blood count (every 1-1.5 wk for those treated with L1 and L1/DF combination)
Supporting specialist therapy team: Cardiologist, endocrinologist, haematologist, paediatrician, dietician, gynaecologist, psychologist. Other clinical faculties if required

The table was adapted from ref. [9]. HIV: Human immunodeficiency virus; MRI: Magnetic resonance imaging; DF: Deferoxamine; TSH: Thyroid-stimulating hormone; PTH: Parathyroid hormone.

government health structure adopted in general.

ORGANISED HEALTH STRUCTURES FOR THE TREATMENT OF THALASSAEMIA AND FRIEDREICH ATAXIA PATIENTS IN CYPRUS

The developmental model of an organised health structure for the prevention, diagnosis and treatment of TM in Cyprus was primarily based on an initiative of the parents of TM children and also the physicians in charge of the patients, other scientists, the church, the government and other non governmental organisations (Figure 1). The cornerstone of the health structure was based on the organisation of the Cypriot thalassaemia society and the blood donors association in 1977. Further milestones in the development of an organised health structure was the organisation in Nicosia (the capital of Cyprus) of the thalassaemia centre in 1980 and thereafter the gradual evolution of the thalassaemia clinics in each of the districts of Cyprus in 1993.

In the postnatal phase an organised health structure for the treatment of TM patients in Cyprus involving many health sectors and other organisations has been designed and developed over many years with the centre of activity based in the district thalassaemia clinics (Figure 1). The structure was based on the main form of treatment of TM patients, namely chronic red blood cell transfusions and iron chelation therapy. A continuous programme of biochemical and clinical monitoring involving many common laboratory tests and other clinical specialists was organised by the thalassaemia clinics (Table 1).

A major aspect of the treatment and the survival of TM patients is blood transfusion. Blood donation and collection in Cyprus is organized successfully by the volunteer blood donor co-ordination committee in conjunction with the blood bank of the ministry of health. The blood donors co-ordination committee in Cyprus was initiated in 1977 by the local thalassaemia society and a number of clinicians with the slogan "give blood and save lives" with a reference to the TM patients[7-9].

Blood donation and collection was coordinated and progressively covered all towns and villages in all districts and community sectors in Cyprus. It involved both state and private organisations such as the army, schools, universities, police, banks, businesses and many others. Blood donation is strictly on a volunteer basis and is organised and carried out in each district by the local blood donor coordination committee involving thousands of blood donors. It is a purely volunteer organization with no financial budget or treasurer. Blood supplies are generally sufficient but in the summer holidays for example problems may arise in blood donation and blood supply may be at a shortage. In the latter case the state media call for an emergency blood donation of the relevant blood groups in shortage to be donated at the nearest district hospital.

Continuous biochemical and clinical monitoring as well as supportive specialist care involving a multi-disciplinary team is available in all the thalassaemia clinics in Cyprus for the follow up treatment of TM patients (Table 1). The multi-disciplinary clinical team include almost all clinical sectors such as specialists in cardiology, endocrinology, haematology, paediatrics, nutrition, gynaecology, psychology, *etc*.[9].

Iron chelation therapy introduced within a few years of beginning the red blood cell transfusions is essential for the survival of TM patients. In the absence of iron chelation therapy the life expectancy of TM patients is less than 20 years. Under these circumstances TM patients usually die from excess iron accumulation and organ damage. In particular cardiac failure due to excess iron deposition in the heart is the major cause of death in regularly transfused TM patients who do not receive effective iron chelation therapy[20-24].

Iron chelation therapy is widely available for both the use of DF and L1 and also in many cases for DFRA in different countries including Cyprus[51]. The T2 and T2* relaxation time magnetic resonance imaging techniques have been used over the past decade for the routine diagnosis of excess iron load in the liver, the heart and also other organs[52-56]. Monitoring of serum ferritin levels is also routinely used for estimating body iron overload.

Organ function and other biochemical tests are also routinely performed at different intervals as shown in Table 1.

Bone marrow transplantation has also been considered as a therapeutic option especially for young patients (usually under 7 years) when a matched human leukocyte antigen sibling is identified by the bone marrow transplantation laboratory. It is estimated that a total of about 20 Cypriot TM patients received bone marrow transplantations and in most cases were successful except for one death and two graft rejections[9]. Similar results were reported in TM clinics in other countries[57-60].

Treatment problems and other complications associated with the life of TM patients are frequently assessed by arranging interviews between the clinician in charge of the thalassaemia clinic and each TM patient around every other week[9].

The small number of FA patients by comparison to the large number of TM patients in Cyprus required the adoption of a smaller different organisational health structure for treatment provision in FA. Despite the abscence of effective therapy in FA, major efforts are directed towards the therapy of the symptoms of FA patients. Clinical and biochemical monitoring, as well as supportive therapy for the FA patients is carried out in a specialised neurology centre in Nicosia the capital of Cyprus[11,39,61].

In relation to treatment it appears that gross body or focal iron overload toxicity is the main cause of death in both TM and FA. A better understanding of the normal iron metabolic pathways and the detection and characterisation of the iron abnormalities and iron overload toxicity in both TM and FA can lead to effective treatments and reduction of the morbidity and mortality in these diseases.

IRON METABOLISM IN NORMAL AND ABNORMAL CONDITIONS INCLUDING THALASSAEMIA AND FRIEDREICH ATAXIA

Iron is an essential metal required by all mammalian cells for many biological processes and normal physiological functions. The solubility of ferric (Fe^{3+}) iron in aqueous solution at pH 7.4 is negligible (10^{-18} mol/L) and iron precipitation rapidly occurs in biological media in the absence of chelating proteins or low molecular weight (LMwt) chelators. Ferric iron is deposited in cells in a polynuclear form as ferritin, which is water soluble and haemosiderin which is not water soluble. Ferrous (Fe^{2+}) iron is more soluble than ferric iron in physiological conditions. In biological systems ferrous iron is found in mononuclear form bound to proteins but not in polynuclear form as precipitates or deposits. Ferrous iron is a major catalyst of free radical reactions. In contrast, ferritin and other protein bound iron does not appear to facilitate the catalysis of free radical reactions. However, haemosiderin and haemosiderin-like ferric iron deposits

(focal iron), as well as other forms of labile, LMwt ferric iron are considered to be toxic. The latter provide a continuous source of catalytic iron which under certain conditions can readily give rise to free radical reactions and cascades leading to oxidative stress damage.

There are many conditions associated with iron metabolic imbalance, which are caused by genetic, nutritional, iatrogenic, environmental, disease and other factors and their combinations[5,13]. These factors can cause deficiency or overload or abnormal distribution of iron and a corresponding pathological effect from the molecular to tissue levels[5,13,62]. Examples of such changes in iron metabolic disorders is nutritional iron deficiency anaemia, idiopathic haemochromatosis which is a genetic iron overloading condition, Hallervorden-Spatz syndrome where iron accumulation in the brain is observed, anaemia of chronic disease where iron is diverted and deposited in macrophages of the reticuloendothelial system, sideroblastic anaemia where iron is deposited in ring sideroblasts, FA where excess iron is deposited in mitochondria, atransferrinemia where the iron transport protein transferrin is not produced leading to abnormal iron distribution and many others[5,13]. Abnormalities in iron metabolism can also be observed in relation to changes in the many proteins involved in the regulation of iron metabolism and of changes involving iron containing proteins including the haemoglobinopathies[1,5,13].

Body iron levels and iron distribution in the organs are governed under normal conditions by homeostatic controls of iron uptake, distribution and storage. In general, these levels are regulated by dietary iron absorption in the gastrointestinal tract, the erythropoietic activity of the bone marrow and the rate of iron excretion[5,13,62]. The absorption of iron mostly takes place from the intestine and is regulated by several metabolic pathways involving regulatory proteins such as hepcidin and ferroportin[63]. Following the uptake of iron into the enterocytes from the gut lumen, iron is then taken by plasma transferrin and transported in blood for delivery to all the cells of all tissues[63,64].

The total body iron of normal adult humans is estimated at 3-5 g and is present in different forms mainly as haemoglobin (58%), which is found in red blood cells, myoglobin (9%) which is found in muscle tissue and also as intracellular ferritin/haemosiderin (30%) which is found mainly in the liver but also other organs (15-17). The uptake, transport and distribution of iron is tightly controlled. For example, the uptake of iron from transferrin by cells and its storage intracellularly is regulated by specific iron regulatory proteins based on the translational control of the transferrin receptor synthesis at the cell surface and also that of intracellular ferritin, as well as by hepcidin[5,13].

The amount of iron delivered to cells is mainly determined by the number of transferrin receptors and also the iron saturation of transferrin. Transferrin iron saturation under normal physiological conditions is 25%-35%. However, in transfusional iron overload or primary hae-
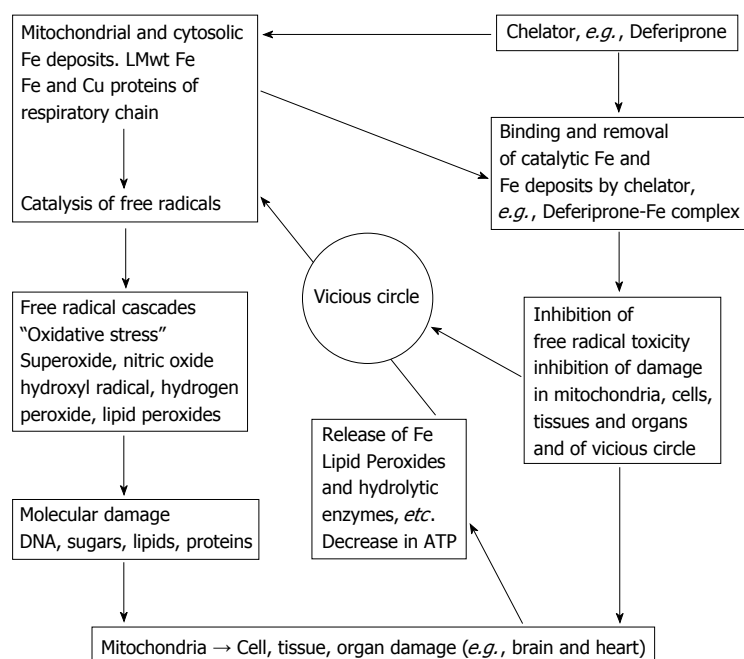
Figure 2 The mode of chelating and antioxidant activity of deferiprone in Friedreich Ataxia. Deferiprone can chelate intracellular and intramitochondrial iron deposits and labile low molecular weight (LMwt) iron, which are responsible for the catalytic formation of toxic free radicals and toxic byproducts. It can inhibit iron toxicity related damage to the heart and brain of Friedreich ataxia patients.

mochromatosis the saturation of transferrin with iron is much higher and usually exceeds 100%. At this stage where transferrin is saturated with iron, non-transferrin bound iron can be detected in the plasma[65-67].

The iron released from the continuous breakdown of effete red blood cells and the catabolism of haemoglobin, as well as of other cells of the body is transported in the blood by transferrin, which in conjunction with hepcidin and other regulatory proteins of iron metabolism maintains equilibrium between the sites of iron absorption from the gut, intracellular storage and also utilisation. Iron uptake by cells is accomplished by the binding of transferrin iron to a transferrin receptor on the cell surface. Usually two molecules of monoferric or diferric transferrin is bound to each transferrin receptor, which subsequently is incorporated into the cell within an endosome. The release of iron from transferrin is carried out through acidification of the endosome from pH 7.4 to 5.6. The iron molecules released are in a LMwt form and are incorporated in the "transit iron pool". Iron is then transported to all parts of the cell including the mitochondria, for storage in ferritin or haemosiderin and for incorporation into apoproteins for the formation of iron containing proteins.

The LMwt intracellular transit iron pool is thought to be composed mainly by LMwt chelator iron complexes[62,68]. Some LMwt naturally occurring chelators are found in cells, *e.g.*, citrate, ATP, ADP, glutathione, amino acids and some absorbed from food eg phytic acid, polyphenols and ascorbate. These and other similar molecules are thought to be involved in the transfer of iron in different intracellular compartments affecting intracellular iron metabolism. For example, they can form ternary

metal complexes with apo-proteins or facilitate the incorporation of iron into the metal domain of proteins. The mechanisms and processes of iron uptake, release and exchange are governed by the same kinetic and thermodynamic parameters as for those applied to other chelators and their iron or other metal complexes[68]. Within this context, it is anticipated that chelators present at high concentration**s** eg citrate (10 mmol/L, in plasma) and glutathione in cells (5 mmol/L in liver cells) may play a significant role in these processes.

One of the most important functions of iron and some iron containing proteins is the catalysis of free radical reactions, and formation of related byproducts which are essential for physiological processes[69]. Free radicals and other nitrogen and oxygen activated products such as the nitrogen oxide, superoxide, hydroxyl radical, lipid peroxides and hydrogen peroxide are constantly and continuously generated in aerobic organisms during normal metabolism and physiological functions and also in response to both internal and external stimuli. However, if free radical reactions and related by-products are continuously overproduced and are not regulated or controlled by the antioxidant pathways and molecules, this can cause free radical toxicity and damage from the molecular level to the organ level as shown in many diseases including TM and FA[69-71].

The catalytic effects of iron can cause free radical toxicity cascades and biomolecular damage including damage to sugars, lipids, proteins, DNA and also widespread subcellular, cellular, tissue and organ damage (Figure 2). Such free radical cascades are not sufficiently neutralized by antioxidants but can usually be prevented by iron binding or chelation[69,70].

Iron absorption and transferrin iron delivery appears to be normal in FA patients, with the general iron metabolic parameters such as serum ferritin and transferrin iron saturation to be within the normal physiological ranges. The frataxin abnormality appears to affect selectively certain organs and tissues and the damage to be manifested slowly over many years, in a time scale similar to the case of iron overload in hereditary haemochromatosis patients[13,62,63]. In the affected cells the frataxin abnormality appears to cause the increased uptake and deposition of iron in mitochondria.

The transport of iron from the cytosol to the mitochondria and its incorporation into the apoproteins for the formation of mitochondrial iron containing proteins or incorporation into mitochondrial ferritin is not well characterised or understood in FA[72-74]. Similarly, there are controversies as to the presence of only mitochondrial iron deposits or both cytosolic and mitochondrial iron deposits in the affected cells. Accordingly, some investigators have reported the presence of excess iron in mitochondria but not the cytosol and others excess iron deposits in both sites[75,76].

A possible pathway can be suggested to explain these differences. Within this context and under normal conditions a pathway is operating involving the transport of LMwt iron complexes from the cytosol or protein bound iron, across the mitochondrial membrane and the formation of "intra-mitochondrial transit LMwt iron pool" before iron incorporation into the mitochondrial apoproteins or mitochondrial ferritin.

Incorporation of iron into ferritin including mitochondrial ferritin is usually in the reduced mononuclear ferrous form which is then oxidised and polymerised inside ferritin in an oxohydroxide iron phosphate polynuclear form[77,78]. Polynuclear iron cannot be incorporated inside ferritin but can be deposited as a haemosiderin-like polynuclear iron in the cytosol or mitochondria. In the latter case deposits of iron oxyhydroxy phosphate and sulphur polymers were identified in an animal model of FA, which were different from the forms of iron incorporated into mitochondrial ferritin[73]. Similarly, haemosiderin-like iron can also be formed from the breakdown of the ferritin protein shell and exposure of the intra-ferritin iron core[77,78]. Based on these observations two different stages of iron deposition appear to be taking place in FA in a time dependent manner. In the initial stages there is an increased uptake of cytosolic iron into mitochondria, where cytosolic iron deficiency may also occur[75]. In the second stage, saturation of mitochondria with iron can cause progressive increase in cytosolic iron accumulation and the formation of iron deposits in the cytosol[76].

The iron deposition process in FA may be facilitated by several factors including the low production of ATP in mitochondria which decreases both the intra-mitochondrial LMwt transit iron pool and iron incorporation in mitochondrial ferritin, thus leading progressively to the increased polymerisation and deposition of haemosiderin-like iron in mitochondria[79]. Similarly, decreased production of ATP could cause a decrease in the cytosolic LMwt leading to progressive iron polymerisation and deposition in cytosol[78].

An additional factor that may facilitate excess iron deposition in mitochondria is the presence of oxidative environment which causes iron oxidation and precipitation. Ferrous iron is more water soluble than ferric iron and the former can be oxidised and precipitate forming a haemosiderin-like polynuclear iron inside the mitochondria. Other similar factors may possibly be involved in the intra-mitochondrial recycling of iron, where for example iron re-utilisation from the turnover of mitochondrial iron containing proteins is blocked and it undergoes polymerisation and accumulation as iron deposits in the mitochondria[80].

Another possibility for the excess iron deposition in mitochondria is the presence of a mechanism of iron loading of mitochondria similar to that observed in primary or hereditary haemochromatosis involving the malfunction of regulatory proteins like herceptin and ferroportin. In the case of chronic iron overload disorders, iron deposition progressively results in increased formation of haemosiderin polynuclear iron complexes which are difficult to mobilise. In such cases polynuclear iron removal from ferritin, haemosiderin and other forms of iron deposits follows the "last in-first out" general principle. Similarly, newly formed polynuclear iron formations and precipitates can be mobilised faster and more efficiently by chelation than older ones[81].

In addition to the toxic iron deposits and the increased oxidative stress observed in the mitochondria of FA patients, low frataxin levels can also cause other metabolic abnormalities such as the insufficient production of iron-sulfur clusters that are required for mitochondrial electron transport and energy transduction, as well as the functioning of aconitase and other iron containing enzymes or iron related metabolic pathways[79]. The distortion of these processes and in particular of mitochondrial electron transport is an additional powerful source of free radical production, which if not controlled can cause free radical cascades, toxicity and damage (Figure 2).

Organs such as the heart and the brain with high energy consumption and increased numbers of mitochondria appear to be particularly sensitive to these forms of abnormalities. Cardiac, brain and other organ damage, resulting in insufficient organ functioning levels is the overall cause of increased morbidity and mortality in FA.

PATHOGENIC EFFECTS OF THE IRON METABOLIC ABNORMALITIES IN THALASSAEMIA AND FRIEDREICH ATAXIA

There are many differences between TM and FA in relation to the iron metabolic pathways involved, such as the cellular and body distribution of iron, the origin and quantity of iron accumulated, the biochemical parameters

related to iron imbalance, the target organs of iron toxicity and many others. In assessing these differences it is important to specify some of the pathways, properties and characteristics of iron metabolism which are relevant to the diagnosis and treatment of each of these two conditions. Within this context the characterisation of the molecular pathways involved under normal physiological conditions and the pathological effects observed in both TM and FA, including the pathogenesis of gross or focal iron overload toxicity are considered essential in the understanding of the mechanisms involved in both diseases and also for the proposed treatment options.

Gross body iron overload is one of the most common metal overload toxicity condition which can be caused by increased iron absorption from the gut (primary haemochromatosis) or regular transfusions of red blood cells (secondary haemochromatosis) or a combination of both these two processes. Thalassaemia patients or patients with other refractory anaemias are regularly transfused every 1-4 wk, usually with 1-3 units (1 unit = 200 mg of iron) of red blood cells for maintaining haemoglobin levels above 9-10 mg/dL. Iron overload in refractory anaemias including TM has the highest morbidity and mortality rate worldwide in comparison to other iron or other metal overloading condition. One of the most seriously affected group of iron overloaded transfused patients are the TM patients.

The accumulated iron from transfusions cannot be excreted and is stored intracellularly as ferritin and especially as haemosiderin. The latter protein increases in concentration in some main organs, particularly in the liver as well as the heart and spleen of the transfused TM patients. Previous electron microscopy studies in cardiomyocytes and hepatocytes of TM patients have shown that in iron overloaded conditions iron loaded ferritin arrays are formed intracellularly mainly in primary lysosomes and haemosiderin iron aggregates in secondary lysosomes[21,82,83]. Further ultrastructural observations suggest that in heavy iron overload in TM there are some iron-laden lysosomes which are ruptured into the cell sap and may be a cause of toxicity. Other forms of subcellular damage which are identified for example in cardiomyocytes of TM who suffered congestive cardiac failure, include the presence of large cytoplasmic vacuoles, increase in the electron density of nuclei and increased amounts of heterochromatin, substantial loss of myofilaments, swollen mitochondria with loss of their cristae but with no iron deposits within them[84].

The damaging effects of iron overload and the rupture of iron-laden lysosomes into the cell sap can also cause the release of hydrolytic enzymes and potentially toxic forms of labile iron. The latter can catalyse the production of free radical cascades and cause further free radical damage which can progressively lead to a vicious cycle of further cellular, tissue and organ damage (Figure 2)[69,70].

Iron overload and toxicity causes organ damage, which is generally detectable when approximately 50-100 units of red blood cells have been transfused. This damage is reversible in the early stages but in many cases is so extensive that it can become irreversible[84]. Regularly transfused iron loaded TM patients usually die from iron overload related cardiomyopathy[20,23,26]. The possibility of congestive cardiac failure due to iron overload deposition in the heart is observed in TM patients with cardiac magnetic resonance imaging (MRI) T2* values of lower than 9 ms[53-55]. In addition to heart complications, other forms of iron overload toxicity include liver fibrosis and cirrhosis, splenomegaly, endocrine organ damage leading to diabetes, retarded growth and sexual immaturation. A major difference between TM and FA is that there is no iron accumulation in the brain or related toxic side effects involving the nervous system in TM patients.

Different molecular factors and pathological effects due to iron toxicity are observed in FA patients. Friedreich ataxia is an autosomal recessive disease like TM, which is caused by a mutation involving homozygous GAA trinucleotide intronic repeat expansions in the gene in chromosome 9, which encodes the mitochondrial matrix protein frataxin[3,4]. The repeat expansions interfere with transcription of the protein frataxin. About 97% of patients with FA have expansions of a GAA repeat in the first intron of both alleles of the frataxin gene. In normal individuals the alleles of the frataxin gene have 36 or fewer GAA repeats, while in the FA disease the alleles have from approximately 70 to about 1700 GAA repeats. It appears that regarding pathogenesis the age of onset correlates inversely to the size of the GAA repeat of the expansions, and directly with the rate of disease progression.

Frataxin appears to play an important role in mitochondrial iron homeostasis and is involved in the biosynthesis and structural assembly of iron-sulfur cluster proteins and also in haem synthesis. It is severely reduced to about 5%-20% of normal in FA patients but the level of its deficiency needed to cause disease is still unknown. The decreased production of frataxin in FA patients appears to be associated with many clinical abnormalities[3,4].

Variations in the levels of frataxin are observed between different tissues and the tissues mostly affected are expected to have severely reduced levels. A detailed description of the symptoms or signs and the anatomic localization involved is reviewed elsewhere[85]. The severity of the disease in FA and other inherited diseases seems to also depend on many other factors. Variations in compensatory mechanisms and alternative metabolic pathways which are in operation in affected cells and tissues may contribute to minimising the severity of FA[85]. Similar variations are observed in other inherited and metabolic diseases including TM and hereditary haemochromatosis[13,62,85-87].

Deficit of frataxin is mostly related to many abnormalities associated with mitochondrial structure and function. The main and characteristic abnormality in FA is an increased iron accumulation and deposition in the mitochondria matrix and a corresponding increase in

oxidative stress[3,4,88,89]. There is also deficit of mitochondrial respiratory chain complex activities and impairment of tissue energy metabolism, decrease in production of ATP, effects on the function of aconitase and other abnormalities which lead to mitochondrial misfunction, including also to a further increase in the production of free radicals and other reactive oxygen species. Increased cytosolic iron in cells of specific tissues is observed despite that the general body iron stores and metabolic parameters are within the normal physiological range in FA[90].

Histopathological and MRI studies have shown that iron primarily accumulates in heart muscle, dentate nuclei (spinocerebellar tracts) and spinal cord of patients with FA[76,91,92]. In these studies, focal localised iron deposits have been detected by MRI T2* in the brain and the heart of FA patients[76,91]. In histopathological studies stainable positive iron deposits have been identified in the cardiomyocytes in autopsy and biopsy specimens of FA patients[76]. It is important to note that patients with short GAA repeat expansions and long survival have neither heart disease nor focal localised iron deposits.

Many contributory factors appear to be involved in the observed variation in the onset and progression of disease in FA patients. In addition to the size of the GAA repeat expansions and the focal localised iron deposits in the heart and brain, other such factors include the possibility of frataxin isoforms, distribution of iron in other tissues or organs, dietary habits and many other[62,63,93]. The pathogenesis of the disease and the high level of toxicity observed mainly in the heart and the brain of FA patients may also be related to the high requirements and consumption but insufficient utilization of oxygen due to mitochondrial malfunctioning in these main target organs. Other organs appear not to be affected to the same extent because of reduced requirements, consumption and utilisation of oxygen.

There are many differences in the distribution and pathogenic effects of iron in TM and FA. In both conditions gross or focal iron overload deposits appear to be a major factor in the cause of the toxicity. Furthermore, in TM iron overload is in the form of ferritin and more so in the form of haemosiderin and situated in lysosomes in the cytosol and not the mitochondria[84]. In contrast, polynuclear iron deposits of mainly ferritin and haemosiderin have been identified in both the mitochondria and cytosol of cardiac and other biopsy samples of FA patients[76,88,89].

Despite that cardiomyopathy is the main cause of death in both diseases, iron accumulation in the hearts of patients with FA is overall much lower in comparison to TM patients. However, cardiomyocytes in FA appear to be more susceptible to toxicity and damage arising mainly from focal iron deposition, mitochondrial iron overload and respiratory chain protein malfunction in comparison to mainly cytosolic gross body iron overload deposition and toxicity in TM.

Iron deposition in the pancreas may also be partly the cause of the increased incidence of diabetes in both the TM and FA categories of affected patients[34,94,95]. Pancreatic damage due to iron overload is well documented in TM and in some cases it may be reversible with chelation therapy at the early stages, whereas in FA it is still under investigation[96].

Iron deposition in the cerebellum in the brain of FA patients, as well as in other parts of the central and peripheral nervous system may play a major part in the cause of the observed pathology of FA and especially the ataxia[85]. Neurons appear to be more susceptible to iron toxicity than other cells. In contrast iron accumulation in the hypophysis (pituitary gland) in the brain of TM patients affects body growth and delays puberty. No other parts of the brain or of the nervous system in general appear to accumulate and deposit iron in TM and no neurological effects have been reported in severely iron loaded TM patients.

In general, the clinical problems arising from the abnormalities of frataxin are not equally affecting the FA patients or the various organs with the exception of the heart, the brain and other parts of the nervous system[97]. For example the serum iron and haemoglobin levels of FA patients appear to be within the normal physiological range despite that haem synthesis is carried out in mitochondria mainly in the bone marrow and spleen[90]. This suggests that the haemopoietic tissues are not affected or targeted by the toxicity mechanisms related to low levels frataxin and also of other related abnormalities including iron deposition observed in the mitochondria and cytosol of FA patients[97].

Overall, the iron deposition rate in the mitochondria of the tissues affected in FA patients appears to be very slow. Similarly, the process of iron toxicity may take years to reach the stage for pathological symptoms to be expressed and identified. Similar time scales are observed in the deposition of excess gross body iron load, as well as the expression of iron toxicity and pathological symptoms in hereditary haemochromatosis patients[13].

Whatever the rate or mechanisms of iron accumulation and deposition in TM and FA, it appears that gross body or focal localised iron overload is the major source of pathogenesis and the major factor of the increased morbidity and mortality observed in both diseases. Within this context iron chelation is considered at present as the first line therapy for the treatment of TM and FA patients.

THE ROLE OF DEFERIPRONE IN CHANGING THALASSAEMIA FROM FATAL TO A CHRONIC DISEASE

Regularly transfused TM patients not receiving iron chelation therapy have a poor prognosis and usually die from congestive cardiac failure and other iron overload complications by the age of 20 years[20-24].

There are three main iron chelating drugs which are regularly used for the treatment of transfusional iron overload in TM and other conditions namely DF, L1
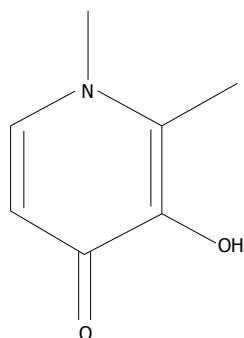
Figure 3 The chemical structure of the iron chelating drug deferiprone (L1). Deferiprone can bind iron through the two molecules of oxygen. It is a bidentate chelator and at physiological pH three molecules of L1 are used for binding one molecule of iron.

and DFRA. Both DF and L1 are generic drugs, whereas DFRA is a relatively new patented drug and all three are marketed in many countries worldwide[13].

Deferiprone was invented in 1981 in the United Kingdom and selected as a leading chelating compound out of about 150 related analogues and other chelating compounds (Figure 3)[13,98-101]. Many preclinical studies have shown promising results in the ability of L1 to mobilise iron from iron containing proteins, cells and animals and to prevent iron induced free radical toxicity[13,99-101].

The first clinical trials in iron loaded myelodysplasia and TM patients in 1987 in London, United Kingdom showed that oral L1 can cause equivalent iron excretion to subcutaneous DF[102,103]. The academic initiatives in the development of L1 continued and multicenter clinical trials were initiated by academic departments in many European countries, in Canada and in India, confirming the earlier results in London, United Kingdom[104-108]. The multicenter clinical trials involved mainly thalassaemia patients who were unable to receive DF due to toxicity, low compliance or both. Deferiprone was first registered in India in 1994 and then in the EU, Asian and other countries in 1999 and the United States in 2011[109].

The introduction of iron chelation therapy has increased the survival of TM patients. In a United Kingdom report prior to the introduction of L1 and DFRA the mean life expectancy of TM patients treated with DF was reported to be 35 years[24]. Deferoxamine was freely available to all TM patients in the United Kingdom and the main reasons of fatalities were iron overload cardiac failure due to non-compliance with the daily subcutaneous administration of DF and the inability of DF to remove effectively iron from the heart[24]. Similar outcomes with the DF treatment were observed in other countries such as Italy, Greece and Cyprus where DF was also freely available from the state to all TM patients[24-26].

Substantial reduction in morbidity and mortality has been observed since the introduction of L1 due to increased compliance and the effectiveness of L1 to remove excess cardiac iron. This improvement has been observed in many countries such as Cyprus, Greece, Italy, United Kingdom and Hong Kong where monotherapy

of L1 or combinations with DF were used[25-29]. This was also encouraging evidence that iron overload toxicity is reversible and can be prevented in most cases especially when appropriate chelation therapy protocols using L1 and DF are introduced at an early age. Recent evidence suggests that the use of the appropriate chelation protocols and especially of specific combinations of L1 and DF could achieve the main aim of iron chelation therapy, namely the complete elimination of excess iron load and the associated iron overload toxicity complications[110].

However, the progress in the treatment of TM patients in developed countries including iron chelation therapy is not reflected in developing countries. It is estimated that more than 90% of TM patients worldwide, who mostly live in developing countries do not receive regular red blood transfusions and iron chelation therapy due to the high cost of treatment. Similar problems were encountered in Cyprus prior to 1977, when regular transfusions or chelation therapy were not accessible to the majority of patients due to the high cost, lack of facilities and lack of an organised health structure system.

Much progress has taken place in the treatment of TM and particularly regarding iron chelation therapy in the last 20 years in Cyprus that has changed the prognosis of TM from a fatal to a chronic disease. Since the introduction of L1 in Cyprus in 1999 many patients (70%) with poor compliance and adverse toxic effects to DF have either included L1 as a part of a combination protocol or switched over to L1 monotherapy[25]. The advances in chelation therapy with the introduction of L1 has led to the overall decrease in iron overload including serum ferritin levels, in improved compliance with chelation therapy, which was previously characterised as poor, as well as improved quality of life for patients[9,25].

The transition from a fatal to a chronic disease can be illustrated by the follow up of TM patients in the thalassaemia clinics of Cyprus. The TM patients undergo regular clinical and biochemical monitoring related to the complications of the underlying disease and the associated therapeutic applications including chelation therapy (Table 1)[9]. Similarly, the introduction of clinical studies and clinical trials involving TM patients in some of the clinics has played an important role in the development of general and personalised optimal therapies for the benefit of all patients[9,51].

Within this context clinical studies have shown that the most effective treatment of transfusional iron overload is the use of L1/DF combination protocols and particularly the International Committee On Chelation (ICOC) protocol (L1 at 75-100 mg/kg per day and DF at 40-60 mg/kg at least 3 d per week). This protocol appear to reduce serum ferritin and to increase MRI T2* of the heart and liver progressively to physiological normal levels[110-114]. Furthermore L1 monotherapy (85-100 mg/kg per day) appears to be sufficiently effective for maintaining the body iron stores at normal physiological levels in most TM patients[112,114]. This development is a major breakthrough in iron chelation therapy because it allevi-

ates TM patients from all the toxic side effects associated with iron overload toxicity and reduces substantially the overall morbidity and mortality to levels similar to those observed in the normal population[110,113].

An increase in compliance in TM patients was noted following the introduction of L1, in addition to the efficacy in iron removal. The majority of TM patients appear to receive satisfactory treatment with L1 in Cyprus either in combination with DF (> 50%) or as monotherapy (< 20%)[9,25]. Increase in compliance is also observed in TM patients receiving DFRA, despite that in some reports the efficacy in the overall iron removal and in particular iron removal from the heart is substantially reduced in comparison to the L1/DF combination[115,116]. Similarly, improved compliance and reduced episodes of local allergic reactions were also noted in many TM patients using an elastomeric pump as opposed to an electronic pump for the subcutaneous infusion of DF (< 20%)[9].

Important progress has also been observed following the introduction of the MRI T2* and T2 relaxation time techniques which have shown to be very valuable for monitoring excess iron load and for improving iron chelation therapy for the TM patients in Cyprus and other countries[9,52-56].

The most important finding in relation to chelation therapy in TM is that since the introduction of L1 the quality of life for most patients has greatly improved and most adult TM patients in Cyprus have developed professional careers, got married and had families. Characteristically about half of the 50 adult TM patients attending the thalassaemia clinic in Paphos have married, had in total 29 children and of note one female patient treated with L1 has successfully given birth to 4 children and also has two grandchildren[9].

The paradigm of Cyprus and the adopted organisational health structure for the prevention, diagnosis and treatment of TM including the protocols on iron chelation therapy with L1 in particular, could be used as an example to be followed by many countries where there is a high incidence of TM. Similarly, the high safety profile of L1 prompted its clinical application in many other non iron loaded conditions with focal localised iron deposits such as acute kidney disease, neurodegeneration with brain iron accumulation, Parkinson's disease and FA[117-120].

THE ROLE OF DEFERIPRONE IN THE TREATMENT OF FRIEDREICH ATAXIA

The general clinical care of FA patients is primarily concentrated on the treatment of the symptoms of the underlying disease. There is no established or effective treatment available for FA at present and no clear therapeutic targets or strategies developed to reverse the pathogenesis of the disease. Most efforts until recently were concentrated on the use of antioxidants for the treatment of the oxidative stress related pathology of FA. The most widely used antioxidants in FA are vitamin E, idebenone, coenzyme Q10, selenium and N-acetyl cysteine[121,122].

Despite that these antioxidants are used at random and in many cases for prophylaxis by FA patients the results are not encouraging and in most cases do not delay the onset or the progression of the disease[121,122].

The suggestion of the use of specific iron chelation therapy and especially L1 for the removal of excess iron from mitochondria for the prevention of pathogenesis and the treatment of FA in general was originally proposed in 2003[5]. The proposed mechanism for the mode of action of L1 was removal of localised excess deposited iron and inhibition of the iron induced catalytic activity and free radical cascades which caused increase in oxidative stress and cellular damage. This proposal was based on *in vitro* findings, animal studies and clinical results suggesting that L1 had access to all major organs and tissues including the heart and the brain[5,69,70]. The ability of L1 to remove excess deposited iron from the hearts of FA patients similar to TM patients, was also suggested since focal or gross iron overload appears to be the main cause of mortality in both diseases[5,69,70]. Similarly, it was also suggested that the application of L1 was a more appropriate therapy than the use of antioxidants for the toxicity arising from focal or localised excess deposited iron and also that the therapeutic options included drug combinations[5,69,70].

The suitability of L1 for clinical use in FA was until recently questioned, mainly because of lack of experience and knowledge on L1 chelation by those involved with the care of FA patients. Within this context a number of concerns were expressed regarding possible toxicities.

One of the major concerns in the use of iron chelating drugs in non iron loaded conditions including those with focal or localised excess deposited iron such as FA, Parkinson's disease, Alzheimer's disease and Hallevorden-Spatz syndrome is the induction of iron deficiency anaemia and other related toxicity. Despite that such hypotheses may be theoretically valid, each case should have been evaluated separately and background information on the clinical use of chelators thoroughly investigated. Within this context many wrong assumptions, expectations and conclusions can be made that may effect future investigations. In particular, the physicochemical, pharmacological and toxicological properties as well as the mode of action of chelators need to be taken into consideration for targeting each one of such conditions.

The specific properties of iron chelating drugs required for targeting iron toxicity and the risk/benefit assessment for use in each clinical condition have been previously reviewed[5,13]. Similarly, the suitability of L1 in FA and other non iron loaded conditions with focal or localised excess deposited iron as well as the concerns in relation to iron deficiency and related toxicity have also been previously addressed[5,69,70]. In such cases the interactions of chelators and especially L1 with tranferrin is essential in understanding the mechanisms and pathways involved[64,123].

In vitro and *in vivo* studies have previously shown that L1 can donate iron to unsaturated or partially saturated

tranferrin provided L1 is in the form of an iron complex and is present at a concentration of about the same level or lower than what is required to saturate tranferrin with iron[64,123]. Monitoring of plasma samples of normal individuals have shown that the oral administration of L1 causes a progressive increase of tranferrin iron saturation from the normal physiological range level (20%-35%) to a level of up to 80% over a period of 7 h[124]. This coincides with the results of pharmacokinetic studies where L1 has been shown to be cleared from plasma over a period of 6-7 h[67]. These findings suggest that L1 can mobilise iron from cytosolic intracellular storage sites or in the case of FA from focal iron deposits in mitochondria also and donate it to apotransferrin or monoferric transferrin in plasma, resulting in an increase of tranferrin iron saturation[64,124,125].

The above study suggests that the iron mobilised by L1 in individuals with normal body iron store levels including focal iron deposits in FA patients could be redistributed in the body *via* transferrin instead of being excreted. Measurement of urinary iron excretions have shown that usually only about 1-3 mg iron can be excreted by normal individuals of about 75 kg body weight, per a 3 g dose of L1[67,124-126]. In comparison, in iron loaded TM patients of approximately the same body weight, substantial increases in iron excretion of up to 71.5 mg can be observed by using the same dose of L1[67,123-126].

Despite that the net amount of iron excreted by L1 in normal individuals is small, continuous administration over prolonged periods of many months and years may cause iron deficiency, unless iron can be replaced from dietary sources or from iron supplements[64,123]. Monitoring of the biochemical parameters related to the iron status is particularly important for FA patients treated with L1 or other chelating drugs. The same monitoring applies for Parkinson's disease and other non iron loaded groups of patients, as well as for individuals using L1 as an antioxidant or anti-ageing drug, where administration of L1 may continue for many months and years[69,127].

Following the original proposal for the use of L1 in the treatment of FA several studies and clinical trials have been carried out confirming the selection and suitability of L1 as a promising therapeutic agent in this condition[5]. In cell studies using the HEK-293 FA model in which frataxin mRNA was knocked down, L1 (50 μmol/L) caused an increased in ATP production, mitochondrial membrane redox potential and resistance to staurosporine-induced apoptosis[128]. In the same model L1 outperformed DFRA, DF and salicylaldehyde isonicotinoyl hydrazone confirming its role as an effective iron chelator and antioxidant. The superiority of L1 in this model is based on its higher iron binding potential (log $\beta3 = 36$), the increased access to intracellular and intramitochondrial compartments, the iron mobilising and antioxidant properties and the efficient cellular exit of the L1 iron complex[13].

A number of clinical trials examining possible therapeutic effects of L1 in FA patients have been reported in the last few years. In one clinical trial nine Friedreich ataxia patients were treated with a dose of L1 of 20-30 mg/kg per day for 6 mo. In this study L1 has been shown to reduce excess toxic iron in the brain as determined by MRI T2*, and also to reduce ataxic gait and neuropathy in general[118]. Similar encouraging results were observed in patients with FA using a combination therapy of L1 with the antioxidant drug idebenone[129]. In another study 13 patients were treated with triple therapy using deferiprone at 5-25 mg/kg per day, idebenone at 10-20 mg/kg per day and riboflavin at 10-15 mg/kg per day for 15-45 mo[130]. Both neurological and heart function benefits were noted in some patients. Some adverse effects due to L1 were observed in four of the patients[130]. Furthermore, a double-blind, randomised, placebo-controlled phase 2 trial of L1 in a group of 80 individuals with FA was reported[6]. Full details of this study have not yet been published[6]. No significant overall changes in the ataxia scale scores in this study were observed, despite that improvement in posture, gait, and kinetic function were observed in some patients. Similarly, the L1 treatment was associated with cardiac improvement such as a decrease in the left ventricular mass. Decrease in serum ferritin levels was observed in a few patients. There was also one case of neutropenia which was resolved on L1 withdrawal[6].

Overall major therapeutic outcomes have been generally observed in FA patients treated with L1 during the clinical trials with both neurological and heart function improvements. It should be emphasised that complete clearance of excess cardiac iron in TM and brain iron in neurodegenerative disease patients has been previously achieved using higher doses of L1 over longer periods and the same result is expected in FA patients[112-114,119,131]. These optimistic results offer hope to FA patients who otherwise have no other effective treatment.

There are several concerns on the protocols used so far in FA patients including the low doses of L1 and the combinations with other antioxidant drugs. In particular, there is a scope of major improvements in the use of L1 in FA patients, including the prospects of personalised medicine. In the latter case the therapeutic targets have to be clearly defined and the appropriate L1 dose protocols selected based on the focal iron load in the brain, heart, pancreas and other organs, as well as the overall risk/benefit assessment in each patient. In general, the elimination of focal or localised iron deposits in the heart, the brain and other organs of FA patients are considered as a major therapeutic target in FA because it is a source of continuous toxicity and cause of pathological symptoms.

Previous clinical studies with L1 in non iron loaded patients or normal volunteers can serve as an example for designing appropriate treatment protocols of FA patients. For example, focal or labile iron deposits have also been targeted in kidney diseases patients. In clinical studies using doses of L1 of 50-75 mg/kg per day in about 50 non iron loaded patients with acute kidney disease for up to 9 mo, L1 improved kidney function, caused a de-

crease in proteinuria and no serious toxic side effects[117]. Overall L1 was well tolerated despite that the doses used were two or three times higher than those used in the FA studies. It is anticipated that similar higher doses of L1 at 50-75 mg/kg per day can also be used in most FA patients, especially those with increased focal iron deposits in the brain, heart and pancreas. The prospects of other improved therapies including chelator combination therapies, combinations with other drugs and the introduction of personalised medicine based on the focal iron deposits and the stage of the underlying disease should also be considered for future clinical investigations.

FUTURE PROSPECTS IN THE TREATMENT OF THALASSAEMIA AND FRIEDREICH ATAXIA

Major progress has been achieved in the diagnosis, prevention and treatment of TM and FA in the last two decades. The paradigm of Cyprus shows the way forward for the prevention and treatment of these pathological inherited diseases. However, these achievements have not yet been realised for most patients and in particular the vast majority of TM patients who live in developing countries. Similar developments have occurred in FA, but the lack of clear approaches and suitable strategies may undermine the efforts for prevention and effective therapeutic interventions, especially in relation to the removal of focal toxic iron deposits in the heart and the brain.

One of the major achievements of the last two decades is the substantial reduction in the births of TM children in Cyprus and many other countries worldwide using similar birth prevention measures. Public education policies and especially consultations with the heterozygote couples at risk of giving birth to TM children played a major role in this achievement. Similarly, the availability of prenatal and antenatal diagnoses substantially reduced the rate of births of TM children.

The quality of life and survival of TM patients have increased substantially in the last 20 years as a consequence of the introduction of effective therapeutic postnatal measures and especially diagnostic advances in the estimation of iron overload using the MRI T2* and T2 techniques and especially the effective treatment of iron overload with L1 and its combinations[86,132]. These changes and related improvements are more obvious in Cyprus and other countries where government health authority intervention is significant and appropriate by-laws as well as appropriate procedures are implemented using effective organisational health structures.

The organisational health structure adopted in Cyprus led to the decrease and almost the total elimination of births of TM children. The birth of a few TM children in Cyprus by those not adhering to the prenatal and antenatal screening programmes has highlighted the importance of the implementation of health policies related to the government's strategy for the prevention of thalassaemia.

The introduction of other interventions such as pre-implantation diagnosis has also shown in many cases an extra effective measure in reducing the birth of TM children[42]. However, it is a more difficult and risky procedure than prenatal diagnosis with low success rate and is also more expensive. The prospects of use of *in utero* allogeneic bone marrow transplantation in the future may decrease further the birth of TM children but this still requires further research[60,133].

The treatment of TM patients is also entering a new phase. Bone marrow transplantation offers a cure for TM patients but the risk of rejection of the transplant and of fatalities is still high[57-60]. The vast majority of TM patients follow the treatment of regular red blood cell transfusion and chelation therapy. Complete treatment of iron overload in TM patients is now envisaged from the use of the ICOC L1/DF combination protocol, which is leading progressively to the elimination of all excess toxic iron load and the attainment of normal iron levels, which are within the physiological range. This outcome increases the prospects of living free of iron related toxicity complications and with longer-term survival, similar to that observed in the general normal population[110,134].

In addition to the increased survival, the quality of life of TM patients has drastically changed in Cyprus and other countries adopting similar policies. The improvement in treatment, which mainly involved the introduction of L1 and other advances such as the introduction of elastomeric pumps for the delivery of subcutaneous DF and also the set up of a specialist multi-disciplinary team for the clinical follow up of TM patients has also contributed to this transition. For example, some of the TM patients have been attending tertiary education and many are employed in different professional sectors. In addition the marital and family status of the TM patients has also changed and most adults are married and have children. For example, in a fertility report of Cypriot TM women in 2004, improved attainment of reproductive capacity has been observed with a total of 358 successful pregnancies[134]. An increasing number of TM patients are now over 50 years of age and have become grandparents. This development followed the introduction of L1 and the L1/DF combination, where there was a significant drop in the mortality rate of TM patients from cardiac complications and also the reversal of iron overload toxicity in other organs[9,25-29,113,134].

The apparent increase in survival of TM patients is observed in Cyprus, and also many other countries using related chelation therapy and similar supportive therapy protocols[25-29]. The transition leading to the longer term survival outcome appears to be related to the improved compliance in the oral chelation therapy and more importantly to the ability of L1 to mobilise rapidly excess iron deposits from the heart, which has also been repeatedly confirmed in several thalassaemia centers using the T2* and T2 relaxation time MRI techniques[54,55,111,116].

The ability of L1 to reverse cardiac, liver, kidney and possibly other organ or tissue iron related damage in dif-

ferent categories of patients and its antioxidant properties provide further evidence of its potent therapeutic effects[69,70]. This therapeutic option may also apply to FA patients at present where so far no effective treatment has become available and L1 appears to offer the most promising therapeutic option, especially if used at the appropriate doses and protocols.

The exceptional therapeutic properties of L1 by comparison to other therapeutics in FA include several advantages such as its ability to enter most tissues and organs including the heart and the brain and also subcellular organelles such as mitochondria and cytosolic compartments at high therapeutic concentrations and to remove progressively and effectively toxic iron deposits. The efficacy of L1 in targeting iron toxicity arising from labile toxic iron forms and also from gross body or focal localised iron deposits, as well as its ability to redistribute iron *via* transferrin and reach iron balance in tissues, can make L1 a universal iron detoxifier and iron metabolic regulatory drug in many diseases including FA[64,69,117,135,136]. In addition, L1 can also act as an antioxidant by inhibiting iron catalysed free radical reactions and cascades arising from mitochondrial malfunction, which is considered a major cause of toxicity in the pathology of FA and also of other diseases (Figure 2)[5,69,70].

There are many other therapeutic advantages in the clinical use of L1 in FA and other conditions[64,69,70,137]. For example, the role of other metals in addition to iron in the cause and acceleration of neurodegeneration in FA and also other diseases is an expanding area under investigation[138-141]. Within this context, L1 can chelate and mobilise aluminium and copper and eliminate their associated toxicity as shown in cell and animal studies and also in clinical conditions[64,142-144].

Future prospects regarding the treatment of FA include the identification of all the molecular targets associated with the pathological mechanisms, the design of new therapeutics and protocols, as well as the optimisation of the existing experimental treatments. Within this context the importance of mitochondrial and cytosolic iron deposition and its significance in the neuropathy, cardiomyopahy as well as other organ damage needs to be further investigated, qualified, quantified and the associated toxicity defined similar to the iron overload toxicity in TM[54-56].

The optimisation of chelation therapy in FA needs to be further investigated. More effective removal of iron in FA can be achieved using higher doses of L1 than those used in the reported clinical trials. In this case two or three divided doses to a total of 50-75 mg/kg per day of L1 can be used depending on the level of focal iron deposition, which can be determined by the MRI T2 and T2* techniques[54-56]. Similarly, prophylactic use of lower doses of L1 (*e.g.*, 10 mg/kg per day) can also be used at the early stages in young diagnosed patients to delay or prevent focal iron load deposition and the onset of pathological symptoms.

Alternative and complimentary experimental treatments to that of iron chelation therapy could also be considered. For example, regular venesection treatment at an early age can be investigated in FA patients not only for prophylaxis but also as a form of main treatment at later stages. This treatment may be more appropriate for FA patients with excess mitochondrial and cytosolic iron deposition. Chronic venesection treatment is simple and inexpensive and can be well tolerated and used before and after the onset of the symptoms in FA patients. The rate of venesection can vary from that of regular blood donors to a more intensive one similar to that of hereditary haemochromatosis patients[13]. Reduction in the rate of organ and mitochondrial focal iron load deposition and associated toxicity as well as delay in the onset of pathological symptoms is expected using this form of treatment.

The therapeutic use of hyperbaric oxygen as a prophylactic measure in asymptomatic FA patients before the onset of mitochondrial focal iron load could also be considered for improvement of neuronal function[145]. Relative improvements were previously observed following the therapeutic use of hyperbaric oxygen in some stroke patients who had focal neuronal damage and substantial reduction of ATP production[145]. This method can also be applied to FA patients with focal iron deposits and pathological symptoms but special safety procedures may be required because of possible exacerbation of the mitochondrial induced oxidative stress. Combination therapies of chelating drugs, antioxidants, hyperbaric oxygen and venesection could provide an alternative investigational approach to the treatment of FA patients.

Gene therapy is the ultimate target for the complete treatment of both TM and FA[146,147]. However, such experimental therapies have been tried in many diseases with no optimistic outcome so far[146,147]. In all cases of the proposed introduction of new protocols or new treatments for FA or TM patients, a risk/benefit assessment is necessary for comparison of the existing and the new treatments. This assessment is particularly important for FA patients where no effective treatments are available at present.

The recent progress in the prevention, diagnosis and treatment of TM and FA patients in Cyprus can serve as a model by other countries and worldwide organisations such as the World Health Organization for minimising the births of TM and FA children and for improving the treatment of these and other inherited diseases worldwide. In particular, the organisational health structures adopted and the new treatments provided are shifting the disease profile of TM from a fatal disease to chronic one, in Cyprus and other countries using similar models. It is also hoped that the same model could be adopted for FA, including the introduction of effective new treatments.

Further efforts are needed for the development and improvement of organisational health structures for the prevention, diagnosis and treatment of TM and FA patients in developing countries, where health resources are extremely limited. Similarly, further advances in the de-

sign of personalised medicine which can provide optimal therapies for individual patients are also in progress and are the subject of in depth investigation.

CONCLUSION

The experience of the Cypriot model of organisational health structure implies that prenatal and antenatal diagnoses can be successfully used for reducing substantially the birth rate of TM and FA patients and the overall prevention of the incidence of these inherited pathological diseases worldwide. The introduction of new treatment methods, particularly the use of effective therapeutic protocols of L1, resulted in many improvements including better compliance with chelation therapy and improved the quality of life, as well as reduction or reversal of iron overload related organ damage and an overall increase in the survival of the TM patients in Cyprus and other countries using similar iron chelation protocols.

Similarly, the introduction of L1 in the treatment of FA patients increases the prospects of reducing the morbidity and mortality rates observed in this disease. New possible treatments for FA patients such as improved chelation dose protocols of L1, venesection and hyperbaric oxygen could be investigated and selected on individual patient cases based on a risk/benefit assessment. Improved diagnostic techniques for the detection of stored iron such as MRI and of iron toxicity could improve the therapeutic targeting methods for both TM and FA.

It appears that recent advances in the diagnosis of the pathogenesis and the treatment are changing TM and FA from fatal to chronic diseases.

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